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Is the Recovery of Stepping Following Spinal Cord Injury Mediated by Modifying Existing Neural Pathways or by Generating New Pathways? A Perspective

The recovery of stepping ability following a spinal cord injury may be achieved by restoring anatomical connectivity within the spinal cord. However, studies of locomotor recovery in animals with complete spinal cord transection suggest that the adult mammalian spinal cord can acquire the ability to generate stepping after all descending input is eliminated and in the absence of neuronal regeneration. Moreover, rehabilitative gait training has been shown to play a crucial role in teaching existing spinal pathways to generate locomotion and appropriately respond to sensory feedback. This brief review presents evidence that neural networks in the mammalian spinal cord can be modulated pharmacologically and/or with task-specific behavioral training to generate weight-bearing stepping after a spinal injury. Further, the role that spinal learning can play in the management of humans with spinal cord injury is discussed in relation to interventions that are designed primarily to enhance neuronal regeneration. [de Leon RD, Roy RR, Edgerton VR. Is the recovery of stepping following spinal cord injury mediated by modifying existing neural pathways or by generating new pathways? Phys Ther. 2001;81:1904–1911.]

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Recently, there has been a surge in the efforts to regenerate the injured spinal cord as a primary means of regaining locomotor function in the adult mammal. Indeed, restoring some anatomical and perhaps functional connectivity appears to be possible based on studies that have used, for example, peripheral nerve grafts, the administration of antibodies that block growth-inhibiting protein activity, or the implantation of engineered cells. On the other hand, a remarkable degree of locomotor recovery achieved by the mammal with spinal cord injury can be attributed to a reorganization of spared neural pathways. For instance, it has been estimated that if as little as 10% of the descending spinal tracts are spared, some voluntary control of locomotion can be recovered. If the loss of supraspinal input to the spinal cord is complete, locomotor networks in the spinal cord are still capable of generating stepping.

Cats that have a complete spinal cord transection at a low thoracic level and receive locomotor training on a treadmill can regain the ability to perform full weight-bearing treadmill stepping. In addition, these cats are able to adjust their rate of walking to the speed of the treadmill belt and to respond appropriately to an external load that is applied to the hind limbs. The research suggests that the spinal cord is able to integrate and adapt to sensory information during locomotion. Because neurons in the adult mammal do not normally regenerate across a complete spinal cord lesion, the recovery in locomotor ability cannot be mediated by descending pathways nor can adaptations in muscle properties account for all of the observed recovery characteristics. Instead, these neural networks that exist below the level of the lesion learn to generate stepping in the absence of supraspinal input.

In this article, we will review findings that indicate that the plasticity in existing spinal pathways mediates locomotor recovery following a complete spinal cord injury in adult mammals. Although studies of nonhumans will be our primary focus, the role of pharmacological interventions and rehabilitative training interventions in managing paralysis in humans with spinal cord injury will be discussed. It appears that a key to restoring motor function following spinal cord injury in humans will not only involve enhancing anatomical connectivity but will also require preserving and restoring synaptic function in neural networks within the lumbosacral spinal cord.
Motor Tasks Can Be Learned by the Spinal Cord After a Spinal Injury

The idea that the spinal cord can learn how to step after a spinal injury is supported by studies of spinal reflex conditioning. Simple hind-limb motor responses to cutaneous or electrical stimulation were enhanced in animals with completely transected spinal cords via classical conditioning (i.e., pairing the stimulus with another stimulus that evoked a stronger motor response). The findings that these reflex responses were enhanced within minutes of conditioning indicated that sprouting or regeneration could not account for the learned motor responses. Rather, the conditioned spinal reflexes were mediated by changing synaptic efficacy along the reflex arc, perhaps through long-term potentiation or by changing motoneuronal properties (e.g., firing threshold), which appears to mediate some forms of synaptic plasticity.

If simple motor responses can be acquired by the spinal circuits, can the spinal cord also acquire the ability to perform complex motor behaviors? We performed a series of experiments to examine whether the spinal cord of the adult cat could be trained to execute hind-limb stepping and standing after a complete spinal cord transection at a low thoracic level. Hind-limb locomotor recovery was compared between cats with spinal cord transection that received daily treadmill training and cats that were not trained following the spinal cord transection. In the absence of training, the cats executed successful steps with both hind limbs, but they frequently stumbled. Treadmill-trained cats, however, executed 3 times more weight-bearing steps than the nontrained cats and thus were able to maintain longer episodes of stepping. During the early period of locomotor recovery, low levels of electromyographic (EMG) activity were recorded from the soleus (ankle extensor) and tibialis anterior (ankle flexor) hind-limb muscles in the step-trained cats (see 2 weeks post-spinal, Fig. 1). After the cats acquired the ability to step following 12 weeks of training, the soleus and tibialis anterior muscle activity increased relative to the levels observed in the earlier period (see 12 weeks post-spinal, Fig. 1). The recovery of hind-limb standing after spinal cord transection also improved with training.

Improvement in standing performance was associated with an enhanced hind-limb extension, which was particularly evident in increased levels of EMG activity in the soleus muscle. Together, these findings suggested that the spinal cord of the cat was capable of acquiring the ability to perform complex motor tasks, such as stepping and standing, following the elimination of descending input from the brain. The activity levels within existing spinal pathways controlling hind-limb extension and/or flexion appear to have been modified through hind-limb motor training in a use-dependent manner.

Pharmacologically Modifying Activity in Existing Spinal Neural Networks Improves Stepping After Spinal Cord Injury

Several researchers have demonstrated that stepping in animals with spinal transection can be induced by pharmacological agents that enhance the activity in the locomotor-generating spinal circuits. For example, the administration of the noradrenergic agonist clonidine elicited stable, full weight-bearing stepping patterns in adult cats within the first week after spinalization. Prior to the administration of the drugs, the lumbar spinal cord was unable to generate locomotion, but 2 minutes after intrathecal administration of clonidine, sufficient flexion and extension movements were generated to execute the swing and stance phases of the step cycle. The effects lasted for approximately 5 hours, after which the pre-drug levels of performance reappeared, indicating that the improved locomotor performance was initiated by the temporary activity-enhancing effects of clonidine.

Figure 1. Rectified electromyographic waveforms from the soleus (Sol) and tibialis anterior (TA) muscles during one step cycle (0.4 m/s) at 2 weeks and 12 weeks after spinalization. Electromyographic bursts from 10 consecutive step cycles were averaged to generate the waveforms shown. The data are from one cat that was trained to perform hind-limb stepping after spinalization. Horizontal calibration, 1 s; vertical calibration, 1 mV.
The inability of cats to step early after spinal cord transection may be due, in part, to a generalized decrease in the net level of excitation in those spinal pathways that control stepping. For example, complete spinal cord transection eliminates all of the descending noradrenergic terminals, and it has been estimated that the amount of noradrenaline that is present in the lumbar spinal cord below the lesion site is decreased by 90%. Theoretically, pharmacologically activating the noradrenergic receptors in the lumbar spinal cord pathways restores activity in those pathways. Interestingly, the effects of clonidine were different when administered to cats that were trained for several months after transection of the spinal cord (ie, after the cats had regained weight-bearing stepping at a range of treadmill speeds). At this stage, stepping was initiated easily on the treadmill, and the primary effect of clonidine was to increase stride length. This was in contrast to the administration of clonidine at an earlier stage after transection of the spinal cord when clonidine was necessary to induce stepping. Thus, over the several months of training, compensatory plasticity appeared to develop within the spinal cord to sufficiently activate the locomotor-generating circuitry.

Use of pharmacological agents to reduce neuronal inhibition also has been shown to facilitate locomotion in animals with spinal transection. Intrathecally administered bicuculline, a gamma-aminobutyric acid (GABA) antagonist, improved locomotor performance in nontrained adult cats several months after spinalization. For example, before bicuculline was given, few full weight-bearing steps could be executed, but 30 minutes after its administration, continuous stepping occurred over a range of treadmill speeds and this facilitory effect lasted for over 2 hours. Similarly, the administration of strychnine, a glycnergic receptor antagonist, to dogs with spinal transection resulted in an improved ability to walk overground within 30 minutes after the drug was given. Furthermore, intrathecal administration of strychnine to the lumbar region of the spinal cord in rats with spinal transection initiated weight-bearing hind-limb stepping on a treadmill, whereas prior to strychnine administration, no hind-limb movements were observed.

Biochemical analyses also indicate an increased level of inhibitory transmitters in the spinal cord following injury, which suggests an increased level of inhibition. GABA and glycine messenger ribonucleic acids, proteins, and receptors were upregulated in the lumbar spinal cord several months after transection of the spinal cord based on analyses performed in adult cats and rats. These findings suggest that GABAergic and glycnergic inhibition of spinal networks suppressed the generation of locomotion in animals with chronic spinal transection. The lack of recovery observed in some nontrained animals, therefore, may have been related to long-term adaptations occurring in inhibitory neural pathways in the spinal cord that inhibited locomotor activity. Furthermore, reducing the levels of inhibition appeared to have a net facilitory effect on the spinal networks that control hind-limb stepping.

Inhibition in the Spinal Cord Is Reduced by Rehabilitative Training

We performed a series of experiments to examine whether the evidence for inhibition in the spinal cord could be modified by hind-limb motor training following a complete spinal cord transection. In one experiment, 4 cats were trained to stand after spinalization, and their ability to perform treadmill stepping was tested before and after the administration of strychnine. During treadmill tests performed after 12 weeks of training, 3 of the 4 cats failed to produce any stepping movements, and their hind limbs dragged with no weight-bearing steps performed on the moving treadmill belt. The fourth cat stepped only at slow treadmill speeds with frequent stumbling. Reducing inhibition with strychnine improved stepping performance (ie, the number of steps executed at a range of treadmill speeds increased from 30 to 45 minutes after strychnine was administered).

Before strychnine was administered, disorganized EMG burst patterns were recorded from flexor (tibialis anterior) and extensor (soleus) hind-limb muscles, whereas more consistent EMG burst patterns occurred after strychnine was administered (Fig. 2A). The facilitatory effects of strychnine were not long-lasting, however, and stepping performance returned to baseline (prestrychnine) levels during treadmill tests performed 1 to 3 days later. The cats subsequently were trained to step, and the ability to step was retested. After receiving step training, the cats not only acquired the ability to step at a range of treadmill speeds, but the pattern of locomotion was no longer affected by strychnine (Fig. 2B). These findings suggest that inhibition in the spinal cord was reduced via treadmill training when the cats learned to step. Further evidence comes from preliminary biochemical analyses performed on the lumbar spinal cords of trained cats with spinal transection. These data indicate that the levels of inhibitory neurotransmitters (ie, glycine and GABA) within those pathways that execute stepping were decreased by step training and increased by stand training.

Hind-limb motor activity following strychnine administration was influenced by sensory feedback from the hind limbs. During treadmill stepping, reciprocal activity was observed in the soleus and tibialis anterior muscles, with little coactivation before and after strychnine administration (Fig. 3). In contrast, when cats with transected spinal cords performed full weight-bearing hind-limb standing, tonic EMG activity was recorded in...
the soleus muscle, whereas no EMG activity was observed in the tibialis anterior muscle (before administration of strychnine, Fig. 4). After the cats received strychnine, this pattern of muscle activity was maintained (after administration of 0.1 mg of strychnine, Fig. 4). During unloaded cyclical activity in the hind limbs (ie, “air stepping” elicited by lifting the cats above the ground so that their bodies were aligned perpendicular to the ground with the hind limbs positioned below the trunk), minimal activity was elicited in the soleus or tibialis anterior muscle (before administration of strychnine, Fig. 5).

After strychnine was administered, the amplitude of the movements was greater, as was the amount of EMG burst activity in both muscles (after administration of 0.1 mg of strychnine, Fig. 5). However, unlike the reciprocal bursting pattern observed during full weight-bearing stepping, the soleus and tibialis anterior muscle EMG bursts were largely overlapping when the hind limbs executed air stepping (after administration of 0.1 mg of strychnine, Fig. 5). These findings suggested that the effect of strychnine did not simply excite the spinal networks and induce stepping. Instead, the ability of the spinal networks to integrate sensory input appeared to be improved after reducing inhibition with strychnine.

**Implications of Spinal Learning for Treatment Strategies Following Spinal Cord Injury in Humans**

The findings reviewed thus far demonstrate that neural networks below the level of a complete low thoracic spinal cord injury in cats can mediate effective locomotion. Stepping can be initiated if the net excitability of the locomotor networks is increased pharmacologically. Noradrenergic and serotonergic agonists, which are known to induce stepping in animals with spinal transection, have been tested in people with spinal cord injury. The success observed after administration of these drugs in facilitating locomotor recovery, however, has been limited. Clonidine, for instance, has been reported to improve, have little effect on, or even impair stepping ability in people with spinal cord injury. Differences in the dosages and methods of drug delivery (ie, oral or intrathecal administration) may partially explain these discrepancies. In addition, it is not clear from any of these studies whether the kind and amount of proprioceptive feedback was controlled before and after drug administration. Based on the studies of locomotor recovery in animals, it is likely that the functional state of the synapses in the spinal cord (ie, the synaptic milieu) influences the sensitivity of the spinal circuits to pharmacological manipulation. Noradrenergic agonists, such as clonidine, may be of more benefit during the initial stages of recovery based on the experiments performed in cats with spinal transection. Other factors such as the level of recovery and training history clearly affect the responsiveness of locomotor-generating networks to agonists or antagonists of spinal neurotransmitter systems known to play a role in locomotion.

Other pharmacological agents that may improve stepping in people with spinal cord injury are drugs that reduce inhibition in the spinal cord. Based on biochemical and pharmacological studies in animals with chronic spinal transection, inhibitory influences on the spinal neural networks are elevated after injury to the spinal cord, and weight-bearing stepping can be triggered by
reducing inhibition with glycinergic or GABAergic antagonists. However, if the effects of inhibitory blockers are only temporary, how can these drugs be utilized in order to improve long-term recovery? One possibility is that inhibitory blockers can be used to facilitate locomotor training. Pharmacologically reducing inhibition increases the responsiveness of the spinal circuits to proprioceptive input generated when the limbs are manipulated during training. Flexor and extensor reflexes can be elicited more easily after reducing inhibition, thereby improving the execution of limb movements.

Based on studies of animals, it appears that gait training can accomplish the same behavioral goals as pharmacological treatments, and, more importantly, can induce long-term effects on the locomotor-generating capabilities of the spinal cord. Gait training that is based on the techniques used in studies of animals has in recent years been applied to the rehabilitation of humans with spinal cord injury. A key component of the locomotor training in humans is a body weight support system that controls the amount of loading on the legs. Leg movements are assisted manually by therapists while the load on the legs is mechanically adjusted to facilitate stepping. Wernig and colleagues examined the effect of this type of gait training in 89 people with incomplete chronic or acute spinal cord injury. Many of the people had received traditional interventions prior to gait training and failed to recover walking. In contrast, after 3 to 20 weeks of training, 76% of the people with chronic spinal cord injury and 92% of the people with acute spinal cord injury acquired the ability to walk independently over-ground with the assistance of walkers or canes. These and similar findings raise the possibility that the human spinal cord can acquire the ability to generate stepping after spinal cord injury. Moreover, using treadmill training to provide the appropriate sensory and motor patterns associated with full weight-bearing stepping seems essential, at least in some cases, to regain the ability to walk.

**Combining Multiple Therapies to Improve Recovery After Spinal Cord Injury**

Combinations of interventions, we believe, have a greater potential to affect recovery than relying on one method of treatment alone. Training, in our opinion, can improve the functional outcomes that can be expected from interventions that facilitate neural regen-
operative interventions may increase the likelihood that reparied descending pathways gain control of functional spinal networks.

**References**


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