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Recovery of control of posture and locomotion after a spinal cord injury: solutions staring us in the face

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Abstract

Over the past 20 years, tremendous advances have been made in the field of spinal cord injury research. Yet, consumed with individual pieces of the puzzle, we have failed as a community to grasp the magnitude of the sum of our findings. Our current knowledge should allow us to improve the lives of patients suffering from spinal cord injury. Advances in multiple areas have provided tools for pursuing effective combination of strategies for recovering stepping and standing after a severe spinal cord injury. Muscle physiology research has provided insight into how to maintain functional muscle properties after a spinal cord injury.

Understanding the role of the spinal networks in processing sensory information that is important for the generation of motor functions has focused research on developing treatments that sharpen the sensitivity of the locomotor circuitry and that carefully manage the presentation of proprioceptive and cutaneous stimuli to favor recovery. Pharmacological facilitation or inhibition of neurotransmitter systems, spinal cord stimulation, and rehabilitative motor training, which all function by modulating the physiological state of the spinal circuitry, have emerged as promising approaches. Early technological developments, such as robotic training systems and high-density electrode arrays for stimulating the spinal cord, can significantly enhance the precision and minimize the invasiveness of treatment after an injury.

Strategies that seek out the complementary effects of combination treatments and that efficiently integrate relevant technical advances in bioengineering represent an untapped potential and are likely to have an immediate impact. Herein, we review key findings in each of these areas of research and present a unified vision for moving forward. Much work remains, but we already have the capability, and more importantly, the responsibility, to help spinal cord injury patients now.

Keywords

spinal cord injury; rehabilitation; robotic motor training; pharmacological intervention; skeletal muscle adaptation; proprioception; epidural stimulation; locomotion

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Why does spinal cord injury result in a loss of movement control?

Movements are defined by the combination of motor pools activated, the level at which they are recruited, and the effectiveness with which the corresponding muscles generate force. The diminished level of movement that follows a spinal cord injury has been attributed generally to an inability to activate motor pools. For most individuals with a spinal cord injury, however, this is less of a factor than typically assumed. Three more salient issues are linked to the impaired ability to recruit appropriate ensembles of motor units in a manner that yields effective movement. First, a significant portion of movement loss is attributable to functional alterations of the spinal circuitry that disrupt the coordination of the motor pools. Second, when a person with a severe, incomplete spinal cord lesion attempts to perform a movement, the level of recruitment is insufficient for some motor pools, while actually exceeding normal levels for others. Finally, it is well known that chronic spinal cord injuries lead to a progressive decline in muscle function. All three of these impairments must be addressed to realize the maximal potential for recovering functional locomotion.

Aberrant synapse formation leads to inappropriate muscle recruitment and poor coordination

The loss of most, if not all, descending neural control after a spinal cord injury rapidly triggers adaptation of circuits in the brain and spinal cord. In particular, the neural circuitries responsible for posture and locomotion undergo major reorganization, a process that can continue to evolve for years (Humphrey et al., 2006). These adaptations include the formation of new functional connections. Yet while a large number of new synapses are formed, there is overwhelming evidence that many of these are abnormal connections that misdirect neurons to inappropriate downstream motor networks. The development of such aberrant connections (between the brain and the spinal cord for incomplete injuries, and within the spinal cord circuitry for complete injuries) generally results in poor coordination, unintended movements, and spasticity. For example, when individuals with a severe mid-thoracic spinal cord injury attempt to flex or extend the ankle on one side, often the entire lower limb will flex or extend, or the movement will occur bilaterally (Maegele et al., 2002). Such stimulus-evoked activation of abnormally large numbers of muscles is very common, and may correspond to the widespread synapsing of locomotor network neurons onto multiple nonspecific targets (Calancie et al., 1993). This lack of specificity in synapse formation leads to coactivation of circuits that are not normally activated synchronously, which is a major determinant of step failure.

Changes in the excitability of the spinal locomotor networks render some synapses hyperexcitable and others hypoexcitable

In the literature, the spasticity and other functional deficits associated with spinal cord injury are often attributed to hyperexcitability of the spinal circuitry (Nance, 2003). It seems unlikely, however, that this explanation is sufficient to explain the complex cadre of neural changes that accompany spinal cord injury. Hyperexcitability is not always detrimental. In some cases, a higher-than-normal level of recruitment can actually serve as a significant positive adaptation: cooperation between motor pathways, wherein hyperexcitation of one motor pool helps to compensate for hypoexcitation of a related motor pool, can be an important mechanism and strategy for regaining motor function. Furthermore, although there is an increase in the number of aberrant connections after a complete spinal cord injury, some of the spinal circuits are hypoexcitable. Up-regulation of the inhibitory neurotransmitter systems, i.e., the GABAergic and glycinergic systems, depresses the excitability of the spinal circuitry after injury (Edgerton et al., 2001). Strategies targeted at reversing this hypoexcitable state have been very effective. Within minutes, pharmacological treatment with antagonists of these inhibitory neurotransmitters dramatically enhances the locomotor capability of complete spinal cats, taking them from being completely unable to step, to being able to execute successful weight-

bearing stepping over a range of speeds (De Leon et al., 1999b). In addition, locomotor training can reverse the depression of the spinal circuits by reducing the number of glycine receptors and the level of GAD_{67} expression (Edgerton et al., 1991, 2001; Tillakaratne et al., 2002). After a spinal cord injury, pathways can be rendered either hyper- or hypoexcitable. Successful rehabilitation requires properly managing the level of excitability, as necessary, of each of the critical locomotor circuits. Excitatory treatments are needed for some circuits, while inhibition is required for others.

Progressive deterioration of muscle properties diminishes the ability to generate movements

The skeletal musculature is highly sensitive to the level of neuromuscular activity, i.e., the levels of activation and loading imposed on the muscles (Edgerton and Roy, 1996; Roy et al., 1991). The chronic decrease in both the activation and loading levels of the muscles below the level of a spinal cord injury results in atrophy, a concomitant loss of force generating potential, and a decrease in fatigue resistance (Castro et al., 1999; Gerrits et al., 2002, 2003; Shields and Dudley-Javoroski, 2006). In other words, the muscles become weak and easy to fatigue in the absence of any countermeasure interventions. The functional consequence of these muscle adaptations is that the individual must recruit a higher percentage of motor units from the muscles involved in performing any given task. Regaining the ability to stand or step most likely will be adversely affected if the muscles are allowed to deteriorate over any prolonged period. A number of rehabilitative strategies have been implemented in attempts to prevent muscle deterioration associated with a chronic decrease in muscle use. The most effective intervention has been the use of electrical stimulation under loaded conditions. With the appropriate use of this countermeasure, it seems feasible that the skeletal musculature can be maintained in a state that will provide the optimum conditions for regaining standing and stepping ability via epidural stimulation and/or pharmacological interventions (see below).

The motor deficits associated with spinal cord injury arise from multiple deficiencies in the neuromuscular system. In this review, we will identify how appropriate interventions involving activity-based therapies can be used to improve posture and locomotion by restoring muscle properties and reinforcing appropriate synaptic connections.

Initiating, sustaining, and stopping movements: sources of control

“Conscious” control

A common, but incorrect, assumption is that control of movement occurs almost exclusively in the motor cortex. Likewise, it is a misconception that most movements are controlled consciously. On the contrary, there is overwhelming evidence that the details of most movements are performed routinely, with little conscious or voluntary effort. Shik and Orlovsky (1976) proposed the concept of “automaticity” in movement control, suggesting that many movements are executed by parts of the brain and spinal cord that are not commonly associated with “voluntary” or “conscious” control. For over a century, it has been known that even tasks as complex as weight-bearing locomotion can be executed rather effectively in animals after the cerebral cortex is ablated (Grillner, 1981). This evidence of subcortical control of posture and locomotion suggests that there are potential sites in the brainstem and spinal cord that can generate complex movements in response to general stimulating signals without requiring detailed, millisecond-to-millisecond conscious control.

“Brainstem” control

Shik et al. (1966) convincingly demonstrated in decerebrate cats that a region within the mesencephalon caudal to the pons (now commonly referred to as the mesencephalic locomotor region) can be tonically electrically stimulated to induce stepping. When either the voltage or frequency of stimulation is increased, animals step faster. These authors recognized, however,

that the speed of stepping also is regulated strongly by the speed of the treadmill belt. Mori et al. (1991) demonstrated that an awake, sitting cat can be induced to rise and to begin stepping by stimulating chronically implanted electrodes placed in the ventral tegmental field of the caudal pons along its midline. In addition, cats can be induced to stop stepping and to sit on their hindquarters by stimulating the dorsal tegmental field. These results demonstrate that very complex motions can be triggered with relatively non-specific stimulation parameters, but at specific sites within the brainstem.

"Spinal" control

Multiple sites within the spinal cord can be stimulated to induce or facilitate stepping movements. Stepping can be initiated in cats by providing relatively nonspecific tonic stimulation to a region of the spinal cord referred to as the "locomotor strip" (Kazennikov et al., 1983). This strip is located just lateral to the dorsal boundary of the dorsal horn, lying at a depth of approximately 1–2 mm, and extending from C1 caudally to approximately the L1 spinal cord segment. The capability of the spinal cord to convert rather non-specific stimulating signals into functional motor activity is even more remarkable than in the brainstem. For example, stimulation via epidural electrodes placed anywhere between the T12 and the L6 spinal cord segments can induce locomotor-like and standing-like movements in both cats and rats (Gerasimenko et al., 2002, 2003, 2008; Ichiyama et al., 2005; Kazennikov et al., 1983). Nevertheless, regional differentiation does exist, and it dictates how the spinal cord responds to electrical stimulation. For example, in rats, electrodes placed along the midline of spinal cord segments L2 and S1 seem to be more effective in facilitating stepping compared to electrodes placed at other levels (Ichiyama et al., 2005), whereas stimulation of the L2 and L5 spinal levels is most effective in humans and cats, respectively (Gerasimenko et al., 2002, 2008). In addition, stimulating at spinal cord segments L2 and S1 seem to be more effective in facilitating stepping compared to stimulating at either segmental level alone in the rat (van den Brand et al., 2007).

The ability of epidural stimulation to generate effective stepping is attributed frequently to the activation of neural circuits in the spinal cord responsible for central pattern generation, i.e., circuits that generate coordinated alternating flexor-extensor neuromotor patterns in the absence of supraspinal or sensory modulation. Central pattern generation certainly has an important role in locomotion, but when the injury spares the locomotor circuitry and afferent inputs, sensory information, e.g., proprioceptive, cutaneous, etc., can be equally, if not more, important in shaping the recovered locomotor patterns. Effective execution of weight-bearing stepping in spinal subjects appears to be accomplished when the spinal locomotor circuits are modulated by stepping-associated sensory input. The important point is that the stimulation parameters can be rather nonspecific if the sensory input associated with stepping is available to provide the fine tuning.

"Sensory" control

The intact spinal cord has a remarkable ability to utilize cutaneous and proprioceptive sensory information to adapt to different environmental conditions during locomotion (Buford and Smith, 1993; Forssberg, 1979). A series of experiments reported over the last few years demonstrate that these capabilities are retained after spinal cord injury (Cai et al., 2006; Cote and Gossard, 2004; Musienko et al., 2007; Timoszyk et al., 2002, 2005). For example, it is well known that complete, low-thoracic spinal cats can modify their stepping kinematics during the swing and stance phases to adapt to changes in treadmill speed, making essentially the same adjustments as intact animals (De Leon et al., 1998). These complete spinal cats can modulate the excitatory levels of appropriate motor pools during different levels of weight bearing, and they can even walk backwards when the treadmill is reversed (Musienko et al., 2007). Essentially the same responses have been observed in human subjects with complete spinal

cord injuries when they are partially assisted to walk on a treadmill (Harkema et al., 1997). These experiments demonstrate that it is the combination of the intrinsic ability of the spinal circuitry to execute rhythmic motor patterns with the accessibility to activity-specific sensory information that allows the spinal cord to function with effective automaticity and minimal or no control from the brain. The realization that the locomotor circuits can function independently from brain control opens the door to new paradigms for recovering posture and locomotion in individuals with severe spinal cord injuries.

Treatment paradigms for preserving muscle function after a spinal cord injury

Spinal cord injury leads to the degradation of muscle properties

One of the primary effects of a spinal cord injury on the motor system is a loss of mass and function of the muscles below the level of the injury. These effects appear to be muscle type specific. Muscles that function as extensors, i.e., those that are heavily involved in weight support and propulsive functions and show the highest daily activity levels, are the most affected after the injury. For example, in chronic complete spinal cats, the soleus and medial gastrocnemius muscles (primary plantarflexors) show a greater loss in mass and maximum force potential than the tibialis anterior (a primary dorsiflexor) (Roy et al., 1991). In addition, the predominantly slow soleus is affected more than the predominantly fast medial gastrocnemius. Spinal cord injury also has a severe impact on the muscle phenotype, i.e., there is a general shift toward an increase in the percentage of fibers having a faster phenotype within the affected muscles (Talmadge, 2000). This is particularly evident in muscles that normally not only have a high percentage of type I (slow) fibers such as the soleus, but also involve a shift to the fastest phenotypes in normally predominantly fast muscles such as the medial gastrocnemius and tibialis anterior. In effect, the muscles below a spinal cord lesion become smaller, weaker, and more fatigable after the injury. Similar effects generally are observed in human subjects after a spinal cord injury: atrophy, loss of maximum force potential, slow to fast fiber type conversion, and increased fatigability (Burnham et al., 1997; Castro et al., 1999; Gerrits et al., 1999; Shields, 1995).

Activity-based treatments help to maintain muscle properties

A number of interventions have been attempted to prevent the loss of muscle function associated with spinal cord injury. Several exercise modalities have been used with varying results. For example, we have shown that training complete spinal cord transected cats to step on a treadmill ameliorates, but does not prevent, the loss of mass and force potential of the soleus muscle (Roy et al., 1998), but has a minimal effect on these properties in the medial gastrocnemius and tibialis anterior muscles (Roy et al., 1999). Training the cats to support their weight (stand training), on the other hand, had a positive effect in both the soleus (Roy et al., 1998) and the medial gastrocnemius (Roy et al., 1999). A “passive” cycling exercise regime in spinal rats also had a muscle-specific effect: the total cross sectional area of all fiber types was maintained near control levels in the soleus, but not in the extensor digitorum longus (a predominantly fast synergist of the tibialis anterior for dorsiflexion) (Dupont-Versteegden et al., 1998). This same cycling paradigm in combination with fetal spinal cord tissue implant was more effective in maintaining the mass of the soleus and plantaris (a predominantly fast plantar flexor) muscle than either intervention alone (Dupont-Versteegden et al., 2000), highlighting the importance of combinatory strategies for rehabilitation.

Even limited amounts of daily muscle stimulation can help to maintain muscle properties. The effectiveness of stimulation depends, in part, on how the subjects are trained. We have used the model of spinal cord isolation, which results in neuromuscular inactivity, to examine these issues. The advantage of the spinal cord isolation model is that the baseline level of

neuromuscular activity in the muscle is known, i.e., the muscles are virtually inactive (Roy et al., 2007), and thus the effects of imposing known patterns and/or amounts of activity can be determined. For example, we found that the same amount of activation (electrical stimulation through the lateral gastrocnemiussoleus nerve mimicking the EMG pattern observed during treadmill walking) imposed during repeated isometric contractions was more effective in maintaining mass and phenotype of the cat soleus muscle closer to normal than when stimulating with either lengthening or shortening contractions (Roy et al., 2002). Using the rat model of spinal cord isolation, we have initiated studies to determine the minimum number of contractions required to maintain the properties of a muscle. As little as 1 min of brief, high-load isometric contractions per day was sufficient to significantly ameliorate the loss of mass and maximum tetanic tension, and the shift from slower-to-faster phenotypes in the otherwise inactive medial gastrocnemius muscle (Kim et al., 2007). In addition, delivering the same amount of activity during two sessions per day was more effective than one session per day (Fig. 1). Furthermore, a recent study in our laboratory using the same two per day stimulation protocol indicates that a total of 4 min of stimulation per day maintained the mass of the medial gastrocnemius at normal control values. In effect, these data from animal studies indicate that a minimal amount of high-load activity is very effective in maintaining skeletal muscle properties (Kim et al., 2008).

In humans, the most common rehabilitative strategy used to maintain skeletal muscle mass after a spinal cord injury has been functional electrical stimulation, usually administered during cycling activity. Functional electrical stimulation ameliorates muscle loss if the muscles are allowed to produce significant forces during the stimulation. For example, Crameri et al. (2004) highlighted the importance of the loading characteristics in paraplegic subjects: both fiber size and phenotype were maintained closer to control values in the vastus lateralis (a knee extensor) using an isometric loading paradigm as opposed to a dynamic, concentric, minimally loaded paradigm. In addition, this group also showed that an isometric electrical stimulation regime could largely prevent the adverse effects of spinal cord injury on the fiber size and fiber type of paralyzed human muscle if initiated soon after the injury (Crameri et al., 2000). Similarly, Stein et al. (1992) stimulated the tibialis anterior of spinal cord injured subjects for 6 weeks under conditions where the muscle was allowed to shorten (low load) and found an increase in fatigue resistance but no change in tetanic force. The importance of load in maintaining muscle properties also was demonstrated for the wrist extensors of tetraplegic subjects (Hartkopp et al., 2003). A high resistance protocol (30 Hz, maximum load) improved muscle strength and fatigue resistance, whereas a low resistance protocol (15 Hz, 50% of maximum load for the same total work as the high resistance protocol) improved only the fatigue resistance over a 12-week period. A potential complication with functional electrical stimulation, however, is that electrically evoked isometric contractions can cause muscle damage in long-term spinal cord injured patients (Bickel et al., 2004). Whether or not the muscles become more damaged or nonfunctional, however, is probably determined by the specific parameters of the stimulation paradigm.

Functional electrical stimulation also can ameliorate the adaptations in fiber phenotype associated with spinal cord injury. For example, long-term electrical stimulation (1 year) of the vastus lateralis can prevent the shift from slow to fast phenotypes usually associated with spinal cord injury (Andersen et al., 1996). Harridge et al. (2002) reported that a fast-to-slow conversion can occur in muscles of human spinal cord injured subjects if the stimulation is intensive enough: they showed an up-regulation of type I myosin heavy chain in the tibialis anterior when stimulated at 10 Hz, 2–6 h/day for 4 weeks. Martin et al. (1992) reported an increase in the oxidative capacity and type I (slow) fibers in the tibialis anterior muscle of spinal cord injured patients after 24 weeks of functional electrical stimulation under no load conditions (the muscles were free to shorten with no external loading of the foot). Other interventions also have been shown to be effective: Stewart et al. (2004) reported that 6 months

of body weight support training in incomplete (ASIA C) spinal cord injured subjects resulted in an increase in the mean cross sectional area of type I and IIa fibers, an increase in the percentage of type IIa fibers, a concomitant decrease in type IIa/IIx fibers, and an increase in the oxidative capacity of the vastus lateralis muscle. There also was an improvement in ambulatory capacity and fatigue resistance (time on the treadmill). These data are particularly intriguing and begin to address the important issue of whether the maintenance of muscle mass has a positive effect on the recovery of locomotor ability.

One of the primary deleterious effects of a spinal cord injury in humans is an increase in the fatigability of the muscles. It appears that the decrease in fatigue resistance after an injury is progressive: the soleus muscle of chronic paralyzed subjects (3.7±2.05 years) is more fatigable than in acute paralyzed subjects (4.6±1.1 weeks) (Shields, 1995). Functional electrical stimulation, under either loaded or unloaded conditions, has been effective in restoring or maintaining fatigue resistance. For example, Gerrits et al. (2000) reported an increase in fatigue resistance in the quadriceps muscles of motor-complete spinal cord injured subjects after 6 weeks of functional electrical stimulation cycle ergometry training. Subsequently, they showed that low-frequency stimulation (10 Hz) was more effective than high frequency stimulation (50 Hz) in increasing fatigue resistance while having similar effects in increasing maximum tension capability (20%) over a 12-week period (Gerrits et al., 2002). When initiated within 6 weeks of injury, a unilateral plantar flexion electrical stimulation protocol applied under high-load conditions resulted in the following: compared to the non-stimulated limb, the stimulated limb was less fatigable and produced higher torques under the same testing conditions (Shields and Dudley-Javoroski, 2006). Some possible mechanisms involved in regaining fatigue resistance in muscles after a spinal cord injury include: (1) an increase in the percentage of high oxidative fibers and/or fibers containing the slow isoform of sarco(endo)-plasmic reticulum calcium ATPase (SERCA2) (Talmadge et al., 2002), (2) an improved oxidative capacity of the muscles, e.g., increased succinate dehydrogenase activity (Gerrits et al., 2003), and (3) an increase in fiber size, thus decreasing the number of activated motor units required to perform a given task.

Neurotrophic factors help to maintain muscle properties even in the absence of neuromuscular activity

Thus far we have emphasized the role of neuromuscular activity, i.e., the amount and pattern of loading and activation, in maintaining the homeostatic level of skeletal muscles. It is important to realize, however, that several other factors must be considered. For example, in the absence of neuromuscular activity, the presence of an intact neuromuscular connectivity has a beneficial effect on the muscle properties. After 60 days of inactivity, the relative mass, maximum tetanic tension, specific tension (tension/physiological cross sectional area), and fatigue resistance of the rat soleus is significantly higher in spinal cord isolated (neuromuscular connectivity intact) than in denervated (no neuromuscular connectivity) rats (see Table 1 in Roy et al., 2002). Similarly, the motoneurons associated with the affected muscles are differentially affected: even after prolonged periods of inactivity the motoneurons in spinal cord isolated animals maintain their size and succinate dehydrogenase activity level near control values, whereas axotomized motoneurons have decreased succinate dehydrogenase levels (Chalmers et al., 1992; Roy et al., 2007). The differences in the effects on the muscle properties in these two models of inactivity are most likely related to the presence of activity-independent neurotrophic influences between the muscle and the innervating motoneurons. The recent report by Lee et al. (2007) showing that a long-term peripheral nerve graft in combination with acidic fibroblast growth factor repair (6 months) in complete spinal transected rats was effective in partially restoring the mass and slow phenotype composition of the soleus muscle is consistent with a possible beneficial role of neurotrophic (growth) factors. Spinal cord injury depresses the mRNA and/or protein levels of brain-derived

neurotrophic factor and neurotrophin-3 in the spinal cord and/or skeletal muscles, and exercise subsequently elevates these levels to or above control values (Dupont-Versteegden et al., 2004; Gomez-Pinilla et al., 2004; Ying et al., 2005). Thus, these activity-independent neurotrophic factors may be significant contributors to the amelioration of muscle fiber atrophy and phenotype shifts observed with the functional electrical stimulation and exercise countermeasures described above. For injuries such as denervation, where the endogenous supply of neurotrophic factors is lost, exogenous administration may serve as an effective treatment for maintaining muscle function. This may be particularly true for cauda equina lesions where, despite massive denervation, functional electrical stimulation can recover some of the lost muscle mass, even after prolonged periods of injury (Kern et al., 2004).

Significance of the concept of "physiological state" of the spinal circuitry in relearning to step

Phase-dependent modulation of proprioceptive input during stepping

A clear example of the dynamic ability of the spinal locomotor circuitry to process and adapt to sensory information is the enhanced activation of flexor motor pools in response to a mechanical tripping stimulus. When an obstacle is placed in front of the paw of a spinal cat during the swing phase of stepping, there is enhanced flexion of the tripped limb (Forssberg, 1979). If this same mechanical stimulus is applied during the stance phase, however, there is enhanced excitation of the ipsilateral extensor motor pools. In other words, the same stimulus will cause opposite effects depending on the phase of the step cycle.

The functional importance of phase-dependent modulation of sensory input to the spinal cord has been reinforced by recent experiments demonstrating a very predictable suppression or potentiation of monosynaptic and polysynaptic responses when electrically evoked stimuli are applied to the dorsum of the spinal cord. The amplitudes of these responses are increased during the normal active bursting phases of a given muscle and suppressed during the inter-burst intervals. In other words during the stance phase, the net effect of sensory input is potentiated in the extensor musculature phase, while during the swing phase the sensory input is potentiated in the flexor musculature. This type of modulation occurs in both uninjured and complete spinal rats, cats, and humans (Gerasimenko et al., 2007; Lavrov et al., 2006, 2008). A similar modulation of responses has been reported in uninjured human subjects during treadmill locomotion (Courtine et al., 2007) (Fig. 2). From these data, it is clear that the spinal circuitry processes sensory information in a strongly cyclic, phase-dependent manner whether or not the spinal cord is injured.

Although the mechanisms for these dynamic responses are unknown, the results reflect a level of "smartness" and decision making capability of the spinal cord circuitry, and provide some insight into how sensory information combines with central pattern generation to generate such remarkably effective locomotion after a spinal cord injury. For instance, these observations make it obvious that phase-dependent processing of proprioceptive input provides a remarkable means of coordinating massive amounts of dynamic sensory information projecting to the motor pools that generate stepping.

More long-term changes in "state dependence"

Another kind of state-dependent modulation of the motor output that can be achieved with a longer time constant can be mediated pharmacologically in the spinal animals. Administration of a number of agonists and antagonists of each of the neurotransmitter systems within the spinal circuitry can readily improve or depress locomotor function. The direction and magnitude of this modulation depends, of course, on dosage, but each pharmacological intervention will depend in large part on the functional state of the locomotor circuitry. For

example, administration of a modest dose of strychnine, a relatively specific blocker of glycine-mediated inhibition, can have a dramatic effect in facilitating effective weight-bearing stepping within a matter of minutes, whereas the same dosage administered to a spinal animal that has been trained, and that therefore can step well, will have minimal effect (De Leon et al., 1999b). Similar responses have been observed while modulating serotonergic, noradrenergic, and GABAergic systems. These observations provide examples of pharmacological modulation that can be accomplished within a timeframe of minutes. Step training or stand training can change the physiological state of the spinal circuitry that generates stepping over a period of weeks. In general, the efficacy of the spinal pathways can be modulated with time constants ranging from almost instantaneous (mechanical tripping model), to minutes (pharmacological treatments), and even up to weeks or months (step training) (De Leon et al., 1999a).

Treatment paradigms for restoring locomotor control after a spinal cord injury

As discussed above, two of the fundamental elements for controlling movement are (a) regulating the levels of activation of the appropriate motor pools, and (b) managing how these motor pools are coordinated, i.e., controlling the relative amplitude and timing of activation among muscles. Since it becomes more difficult to control these factors after a spinal cord injury, pharmacological and spinal cord stimulation strategies that increase the excitability of the locomotor circuits, as well as activity-based training techniques that reinstate functional motor pool coordination, can be highly effective in helping subjects regain the ability to step.

Pharmacological treatments

Pharmacological treatments can have an important role in restoring the chemical environment of critical locomotor circuits after a spinal cord injury. Many of the central nervous system neurotransmitters, including the monoamines, are synthesized in isolated regions of the brain (e.g., 5-HT is synthesized in the raphe nucleus) and then transported to the spinal cord. Spinal cord injuries that disrupt the descending flow of neurotransmitters can severely hinder synaptic communication caudal to the lesion, which translates into motor function loss. The damage caused by diminished supraspinal input is aggravated by a significant up-regulation in the inhibitory potential of spinal neurons that mediate locomotion. This causes the locomotor circuitry to become less responsive to excitation from peripheral afferents, which normally provide important proprioceptive triggers that can control many of the details of locomotion.

Despite the disruption of critical neurotransmitter systems, the spinal locomotor circuits retain the capability to respond to sensory-driven pre-synaptic excitation. For example, serotonergic receptors remain functional after a spinal cord injury and under some circumstances are even up-regulated (Fuller et al., 2005; Kim et al., 1999; Otoshi et al., 2009). Since reversing the chemical changes caused by spinal cord injury relates to increasing neurotransmitter supply rather than regenerating lost receptors, pharmacological treatments that supplement the spinal cord with an exogenous supply of neurotransmitter agonists can help regulate synaptic communication and coordinate the activation of stepping-related motor pools. A number of studies have shown that the responsiveness of the locomotor circuitry to sensory input can be readily tuned by “bathing” the lumbosacral spinal cord with various neurotransmitter agonists and antagonists (De Leon et al., 1999b; Edgerton et al., 1997a, b; Kiehn et al., 2008; Rossignol and Barbeau, 1993; Rossignol et al., 1998, 2001). The effectiveness of such blunt pharmacological presentation is quite remarkable.

Low-dose pharmacological treatments, including monoaminergic, glycinergic, and GABAergic agonists, help to supplement the neurotransmitter environment of the post-injury

spinal cord and thus can partially restore synaptic communication (Parker, 2005; Rossignol and Barbeau, 1993). Treatments using quipazine (Antri et al., 2002; Barbeau and Rossignol, 1990; Feraboli-Lohnherr et al., 1999; Fong et al., 2005; Guertin, 2004a, b), clonidine (Barbeau and Rossignol, 1991; Chau et al., 1998; Cote et al., 2003), L-DOPA (Barbeau and Rossignol, 1991; Guertin, 2004b; De Mello et al., 2004; Doyle and Roberts, 2004; McEwen and Stehouwer, 2001), strychnine (De Leon et al., 1999b; Edgerton et al., 1997a, b), and/or bicuculline (Edgerton et al., 1997a, b; Robinson and Goldberger, 1986) have been shown to facilitate locomotor recovery. The enhanced synaptic transmission generated by using these drugs potentiates other treatments, including spinal cord stimulation and locomotor training, by lowering the activation threshold of the neurons associated with locomotion. When quipazine, a broad-spectrum serotonin agonist, is combined with step training, it removes the "ceiling" on locomotor recovery that occurs when training is applied alone, resulting in much higher levels of performance in spinal mice (Fong et al., 2005) (Fig. 3). Similarly, treatment with both quipazine and 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)-tetraline], a 5-$HT_{1A/7}$ agonist, increases the stepping performance in spinal rats above and beyond that which can be elicited using spinal cord stimulation or either drug alone (Antri et al., 2005). Cells engineered to secrete serotonin also can enhance locomotion when transplanted into the lumbosacral spinal cord, presumably by making a continuous source of the neurotransmitter available during training (Feraboli-Lohnherr et al., 1997; Gimenez et al., 1998; Ribotta et al., 2000; Hains et al., 2001; Majczynski et al., 2005). When used to facilitate rather than directly generate locomotion, low-dose pharmacological treatments potentially provide an effective solution for enhancing stepping in spinal cord injured subjects.

Repeated administration of quipazine also facilitates cellular modification. Immunohistological analysis of the lumbar spinal cord of spinal mice that were given various combinations of quipazine and locomotor training showed that while quipazine had no discernable cellular effect when administered alone, when combined with step training it significantly increased 5-HT_{2A} receptor expression, as well as the levels of AMPA GluR1 and pCREB, which are markers for early and late long-term potentiation, respectively (Otoshi et al., 2005). These results suggest that exogenously administered neurotransmitter agonists may have an important role in facilitating learning and memory during activity-based treatments. If this is indeed the case, spinal cord injury could provide a useful model for examining which neurotransmitter receptors are involved in memory formation.

Advancing current pharmacological paradigms will require development in several key areas including: determining the most effective drug "cocktail" for facilitating various treatments, identifying specific regions of the spinal cord to target, and defining the optimum timeframe for administration. Hochman et al. (2001) and Jordan and Schmidt (2002) have shown that 5-HT_{1A} and 5-HT_7 receptors, both of which have been implicated in the control of stepping, are centered in different regions of the lumbar spinal cord. Ichiyama et al. (2005) has demonstrated that activating each of these pools affects different aspects of stepping. These findings provide examples of the differential concentration of neurotransmitter receptors within the spinal cord, and highlight the need for spatially specific treatments. Furthermore, since the spinal chemical environment is highly dynamic during the first few months after a spinal cord injury, due largely to the antagonism between recovery processes and spreading secondary damage, a different pharmacological intervention will likely be required at each stage of injury progression. Since synaptic communication is essential for all motor functions, pharmacological treatments are a critical area of ongoing spinal cord rehabilitation research.

Locomotor training

It is well established that locomotor training can enhance the recovery of stepping (Edgerton et al., 1997a, b, 2001, 2008) after a spinal cord injury in mice (Cai et al., 2006; Fong et al.,

2005), rats (Cha et al., 2007; Timoszyk et al., 2005), cats (Barbeau and Rossignol, 1987; De Leon et al., 1998, 1999a; Edgerton et al., 1991; Lovely et al., 1986, 1990) and, excitingly, even human subjects (Dietz and Harkema, 2004; Harkema et al., 1997; Van De Crommert et al., 1998; Wernig et al., 1998). Engaging the spinal circuitry with sensory input associated with weight-bearing stepping is essential to activating the locomotor circuitry so that effective locomotion can be regained. Using this information, the spinal cord likely performs functional pruning of the many aberrant pathways that form after a spinal cord injury, strengthening those circuits that are relevant to the trained stepping patterns (Ahn et al., 2006; Ichiyama et al., 2008). Traditional manual training involves supporting the subject in a harness over a moving treadmill while a team of therapists/researchers repeatedly guides the legs through a step cycle. In complete injured individuals remarkable levels of recovery can be attained if training is provided persistently over a period of weeks to months.

Experience has helped define a set of critical requirements for effective step training. First, the stepping pattern used to train must have kinematics and kinetic parameters that are stable and appropriate to the training conditions. Second, it is important for training to provide sensory stimuli that closely match normal conditions. The spinal cord circuitry is highly sensitive to proprioceptive and cutaneous inputs: “good” stimuli are processed with exquisite efficiency, whereas “bad” stimuli can lead to failure. Using the same stepping pattern, when spinal rats are trained on an elliptical-like device that maintains continuous contact with the hindpaw, recovery is poorer than on a standard treadmill, where paw contact is broken during swing (Timoszyk et al., 2003). In this example, even seemingly innocuous application of plantar stimulation during stance is enough to detract from the training effect. Third, weight bearing is essential for maximizing recovery. In addition to helping maintain muscle properties (Roy et al., 1991; Stewart et al., 2004), subjects who are challenged to bear increasing amounts of their weight are more likely to achieve better stepping than subjects who are fully supported during training (Edgerton et al., 1991). Finally, although repetitive and consistent application of training paradigms is essential to recovery, there should be a small degree of variability in the parameters that are used to train to prevent locomotor performance from becoming dependent on a single set of stimuli. A controlled amount of variability in training enables subjects to benefit from experiential learning (Cai et al., 2006). This important concept will be discussed in greater detail below.

Several variables affect the extent of recovery that can be attained using step training. Recovery is partially dependent on the severity of the injury and the developmental stage of the subject when it occurs. Training is particularly effective in subjects with an incomplete injury, who typically recover more easily than subjects with a complete injury (Coleman and Geisler, 2004; Marino et al., 1999; Waters et al., 1995). Subjects injured at an early age exhibit better neurologic recovery than those who are older at injury due to the ability to develop alternative neural pathways during the formative period of the central nervous system (Scivoletto et al., 2003). The method of training also affects recovery. While traditional forms of therapist/researcher-assisted training have shown tremendous benefit, there are limitations on the extent of recovery that can be achieved using manual approaches. For example, the size disparity between human hands and small mouse legs makes it difficult to control the hindlimb movements of spinal mice with sufficient consistency and precision to be effective. In a study using spinal mice, after more than a month of training, it was clear that a manual approach was unable to provide efficient training. In contrast, statistically significant improvement was attained when training was carried out using a high-precision robotic system (Fong et al., 2005).

From a research perspective, an inability to replicate training movements day-to-day, or even step-to-step, makes it impossible to evaluate training techniques rigorously. Inconsistency and the lack of precision inherent to manual training were the impetus for developing robotic

training systems. Diagrammed in Fig. 4, our robotic step-training system for rodents consists of two motor-driven arms (one for each leg), a weight-support device, and a computer-controlled treadmill. The first robotically assisted training algorithm examined used the robot arms to train a single stepping pattern repetitively during alternate periods for 15 min per day in mice. During the intervening periods, robotic control was turned off to allow the mice to step freely. Compared to the manual method, daily robotic training generated visible and statistically significant improvement within 2 weeks, although the quality of stepping remained poorer than in uninjured control mice (Fong et al., 2005). Using this technique, we observed a plateau in the level of improvement, which we interpreted as saturation in the amount of benefit that the spinal circuitry could extract from a single training pattern. To overcome this, the next set of robotically assisted training algorithms were designed to give subjects the opportunity to experience multiple viable stepping patterns, and even to expose them to occasional failure. The general idea behind these "assist-as-needed" paradigms was to provide sufficient variability to enable the subjects to learn from several good and bad stepping patterns, yet enough control to prevent catastrophic failure. The results highlight the importance of experiential learning and demonstrate that, while it is important to enforce proper interlimb coordination, mice that are exposed to a continuum of stepping patterns recover stepping more quickly and more robustly than those locked into a fixed trajectory training pattern (Fig. 5): essentially, how you train does matter (Cai et al., 2006).

Technological development has provided a quantum leap in the understanding of how locomotor training produces recovery after a spinal cord injury, and continued advancement will enable novel treatment paradigms to be conceived and tested (Winchester and Querry, 2006). Currently, ongoing research is testing the capability of robotic systems to train animals to stand (Bigbee et al., 2007; Liang et al., 2006). Another important study is examining how learning algorithms can utilize robotically sampled stepping data to design optimal training protocols for specific injuries (Cai et al., 2006). This is particularly important as advanced training paradigms make the transition from the laboratory to the clinic, since the wide variability in spinal cord injuries is certain to require customized treatment for each patient. At the same time, robotic training systems for humans are being developed in earnest to assist therapists in overburdened clinics and to give more patients access to the highest standard of care (Aoyagi et al., 2007; Hesse et al., 2003; Ohta et al., 2007; Reinkensmeyer et al., 2006). Beyond spinal cord injury, robotically assisted training has broad applicability to stroke, Parkinson's disease, and many other conditions that involve loss of motor function. The convergence of technology and science has tremendous potential, not yet fully explored, that can rapidly translate into better patient outcomes.

Spinal cord stimulation

The discovery that spinal cord stimulation can be used in multiple ways to facilitate locomotion has opened new avenues for locomotor rehabilitation. Currently, there are two major strategies for spinal cord stimulation. First, in its more common usage, low-level stimulation is applied to broad areas of the spinal cord to increase the general excitability of the locomotor circuits. Typically, fine-wire (dimensions: ~300 ×1000 μm), ball (diameter: ~900 μm), or spring (dimensions: 1000 ×3600 μm) electrodes are placed on the epidural surface of the spinal cord, and square wave pulses (amplitude: 1–10 V or 10–200 μA, duration: 100–250 μs, frequency: 1–100 Hz) are applied (Gerasimenko et al., 2003; Ichiyama et al., 2008). Stimulation over this range of parameters is below the threshold for direct motor activation, but is sufficient to facilitate sensory-triggered movements. Used in this manner, the effect of spinal cord stimulation is similar to that of the pharmacological treatments: by lowering the activation threshold of locomotor neurons, spinal cord stimulation makes it easier for proprioceptive and cutaneous signals to enable stepping. The type of motor output elicited by stimulation is affected by both extrinsic and intrinsic parameters. Extrinsically, the effect of stimulation is

activity-dependent: during stimulation, complete spinal cats and rats will adapt their stepping pattern, stepping forwards or backwards in accordance with their orientation on the treadmill (Musienko et al., 2007). Intrinsically, stimulation-evoked movement is frequency-dependent in humans, stimulation between 5 and 15 Hz preferentially induces standing, whereas stimulation between 25 and 50 Hz favors stepping movements (Jilge et al., 2004). A second approach to spinal cord stimulation involves direct generation of muscle movement by supra-threshold stimulation of motoneurons. Rather than facilitating locomotor circuits at the sensory or inter-neuronal levels, penetrating cylindrical electrodes (diameter: 25–30 μm, height: 60–100 μm) are inserted into the ventral horn at sites that are specific for particular muscles (amplitude: 20–300 μA, duration: 200–300 μs, frequency: 1–50 Hz) (Gaunt et al., 2006; Mushahwar et al., 2002, 2004). Given a sufficiently large number of implanted electrodes, this approach bypasses the intrinsic circuitry and allows for external (e.g., computer-driven) control of sequences of muscle movements. The disadvantages of this method are: penetrating electrodes can damage the spinal cord tissue, circumventing the locomotor control circuitry eliminates the benefit of intrinsic synergies that coordinate agonist and antagonist muscles, and real-time control of the many muscles necessary to generate smooth and stable locomotion is computer intensive. In general, an advantage of spinal cord stimulation over direct muscle stimulation is that it typically recruits muscle fibers in a more normal physiological order, i.e., fatigue resistant (slow oxidative) before fast fatiguing (fast glycolytic) fibers, and thus helps to maintain endurance (Bamford et al., 2005). Both epidural stimulation, which facilitates the locomotor circuitry, and intraspinal stimulation, which triggers movements directly, are active areas of research.

Our research in spinal cord stimulation is focused on developing high-density epidural electrode arrays to extend the potential of the epidural stimulation approach (Fig. 6). These arrays consist of platinum electrode contacts and wire lines that are embedded in a parylene-C substrate, and are fabricated using techniques borrowed from semiconductor and microelectromechanical systems processing (Rodger et al., 2007,2008). The high biocompatibility of the constituent materials makes these arrays well suited for chronic implantation: platinum has a long history of biocompatibility and meets both Tripartite and ISO 10993 standards, while parylene-C is certified as a United States Pharmacopeia Class VI plastic. Parylene-based electrode arrays offer numerous advantages over existing technologies. Parylene arrays allow for stable implantation: formed as thin films (~20 μm thick), they are highly flexible and conform to the spinal cord surface, resisting displacement. Furthermore, the close fit to the spinal cord promotes encapsulating connective tissue growth, which further secures the array and effectively precludes movement. Postimplantation migration is the most common cause of clinical electrode failure (Barolat, 2000;LeDoux and Langford, 1993;North et al., 2005;Renard and North, 2006), in one study forcing 23% of implanted patients to have corrective, follow-up operations (Andersen, 1997). Thus, thin-film parylene arrays provide a critical advancement. Microfabrication processes enable the design of arrays that have novel electrode configurations that can be used to test advanced stimulation algorithms. Using multilayer fabrication techniques, it is now technically possible to build arrays with densities up to 1024 electrodes in a 5 mm ×6 mm area (although, currently, practical application of such high densities is limited by connector and stimulator technologies). Additionally, electroplating can be used to increase the surface area of each electrode by around 40-fold, which provides two important advantages: it enables higher levels of charge transfer from small electrodes, and it expands the current range over which charge can be transferred capacitively, reducing the occurrence of tissue and electrode damage associated with high current, Faradaic charge transfer (Merrill et al., 2005).

Our electrode arrays provide several key benefits over existing technologies. Access to a large number of small electrodes makes possible selective, high-precision stimulation of focal regions of the spinal cord. Electrode arrays are enabling the identification of specific regions

of the spinal cord that are responsible for different components and phases of the step cycle. As our knowledge of the somatotopic organization of the locomotor circuitry increases, the selectivity provided by electrode arrays will make it possible to target stimulation appropriately to address the specific deficiencies of individual injuries. When stimulation is targeted directly at the tissue of interest, less current is needed to generate a desired effect than with bulk stimulation methods. Moreover, by confining the stimulating current to the regions that require it, high-density electrode arrays eliminate unnecessary stimulation of neural tissue, which reduces the potential for long-term damage due to repeated pulsed electrical stimulation. High-density electrode arrays are thus making spinal cord stimulation more effective and safer. Finally, the electrode arrays can be used as a diagnostic tool to measure the properties of spinal cord evoked potentials to assess the locomotor circuitry at different stages of injury and recovery.

While it is remarkable that relatively non-specific stimulation can promote stable, state-dependent treadmill locomotion in the absence of supraspinal control, this represents just the beginning of what can be achieved using spinal cord stimulation. Since the effect of spinal cord stimulation can change dramatically when the electrode site is moved by as little as 200–300 μm (Kazennikov et al., 1983), targeted stimulation approaches can be leveraged to control different components of stepping. In spinal rats, stimulation of the L2 spinal segment induces a general enhancement of the locomotor rhythm, while stimulation of the S1 segment activates the extensor muscles during stance (van den Brand et al., 2007). Stimulation applied to each of the spinal segments between T12 and L6 produces different locomotor effects (Ichiyama et al., 2005). The effects of stimulation also vary in the medial-lateral direction, a finding that provides rationale for pursuing 2-D array designs. As the spatial resolution of the electrode arrays continues to improve, spinal cord stimulation will evolve from providing nonspecific excitation of the spinal circuits to fine tuning very specific aspects of the locomotor pattern. What is most exciting about spinal cord stimulation is that it can be used effectively at both of these levels.

Future stimulation approaches will examine the implementation of biologically inspired stimulation patterns, as well as patterns that involve simultaneous or sequential stimulation of multiple electrode sites. Other applications for multielectrode spinal cord stimulation include management of chronic pain, stroke, and other conditions involving motor function loss. The continued development of electrode array technology is providing unparalleled access to the interneuronal circuitry, and may serve as the best technique for fine-tuning gross motor behaviors after a spinal cord injury.

Integrating neuroengineering and biological concepts to regain posture and locomotion

Based on the successful recovery that we have attained using muscle stimulation, spinal cord stimulation, pharmacological interventions, and activity-based training, the potential for enhancing locomotor recovery by aggressively pursuing complementary and synergistic strategies is clear and represents a logical direction for translating some of the basic biological concepts to the clinical setting. While it cannot always be assumed that multiple interventions will be complementary (Maier et al., 2009) with careful consideration of their interactive effects, multi-intervention approaches truly are the obvious solutions that are staring us in the face.

We already have observed significant positive interaction when multiple modes of treatment are combined (Fig. 7). Optimal recovery of locomotion requires two important factors: the damaged spinal cord must be provided with adequate information that it can use to relearn to step, but, before that, it must be prepared to receive that information. This explains why the

recovery of locomotion using robotically assisted training, which provides information on functional stepping patterns, is significantly enhanced by coadministration of pharmacological agonists that improve synaptic signaling. In mice, e.g., while robotic training restores gross stepping function, pharmacological modulation with quipazine further improves locomotion by facilitating the recovery of movements that are difficult to access with training alone, e.g., activation of the distal extensor muscles during weight-bearing stance (Fong et al., 2005). We also have observed substantial recovery in rats from a combination of locomotor training, two serotonergic drugs, and multiple-site epidural stimulation, and have shown that selective combinations of these treatments lead to very different locomotor effects (van den Brand et al., 2007). The next step is to optimize the combination treatment parameters to maximize the synergies between the constituent interventions. All evidence suggests that engaging complementary approaches may result in the greatest functional gains.

Combinations of paradigms can be effective when each component treatment focuses on repairing a different aspect of motor function loss. Figure 8 depicts the recovery of stepping after spinal cord injury as a multicomponent process. Although this diagram to some degree over-simplifies the complexity of the underlying mechanisms, it provides a didactic representation of how the different treatments interact to promote locomotor recovery. First, muscle stimulation initiated early after injury helps to maintain muscle properties at a functional level. Insufficient muscle tone and/or lack of a normal complement of fatigue-resistant fibers make it more difficult for subsequent treatments to generate appropriate movements, increase the likelihood of injury, and may result in poor endurance. Second, both pharmacological facilitation and spinal cord stimulation can be used to increase the general excitability of the spinal circuits and to strengthen the efficacy of synaptic transmission. Neither low-dose drug treatment nor subthreshold spinal cord stimulation can generate movement independently or produce long-lasting recovery, but they can sufficiently lower the activation threshold of relevant sensory neurons and interneurons to enable even small amounts of stepping-associated stimuli to trigger and sustain locomotion. Third, activity-dependent training helps to reinforce kinematically appropriate stepping patterns. Repetition of these facilitated movements improves muscle recruitment and coordination, and enables the spinal cord to learn to perform new tasks. Over time, the persistent activation of specific spinal pathways results in changes that strengthen those synaptic connections and provide the structural basis for motor learning. The most effective training techniques promote locomotion that is robust to disturbances by exposing the spinal cord to a range of viable stepping patterns. With minimal invasiveness, rather simple methods of locomotor training can be used to recover gross motor function. Fourth, spinal cord stimulation can be used in a secondary role to fine-tune the locomotor pattern. Since high-density electrode arrays provide access to focal regions of spinal cord tissue, they can be used to target selectively the circuits that control particular components of a movement, and thus to address more specific motor defects. In some cases, targeted stimulation approaches may supplant specialized training techniques, which can be both invasive and resource-intensive.

Continued technological advancement in pharmacological treatment, spinal cord stimulation, and activity-based training offers great potential. Pharmacological therapies will improve with the arrival of sophisticated drug delivery systems that enable treatment of focal regions of the spinal cord. Spinal cord stimulation will continue to progress with electrode array development. Activity-based treatments will advance in conjunction with the development of learning algorithms that will help define optimal training protocols that adapt dynamically with the constantly evolving state of the recovering spinal circuitry. With the aggressive pursuit of the combination therapies “staring us in the face,” the expectations for recovery of locomotion are now significantly higher for individuals with spinal cord injury, their family, friends, therapists, and physicians.

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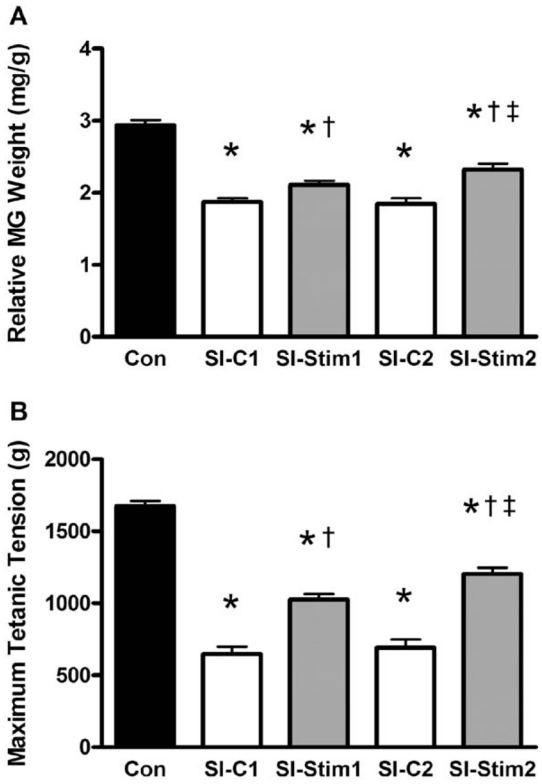

Fig. 1.

Brief periods of daily, high-load isometric contraction reduce the loss of muscle function after spinal cord injury. Spinal cord isolated rats (complete spinal cord transections at a mid-thoracic and a high-sacral level, plus dorsal rhizotomy performed between the two transection sites) that were administered one (SI-Stim1) or two (SI-Stim2) bouts of muscle stimulation daily exhibited less atrophy (A, muscle mass normalized to body mass) and a smaller loss of force generation capability (B, maximum tetanic tension) in the stimulated medial gastrocnemius (MG) muscle (gray bars) compared to the non-stimulated contralateral muscle (white bars, SI-C1 and SI-C2) after 30 days of treatment. Stimulation was applied at twice the minimum amplitude necessary to generate a maximum tetanic contraction. The stimulation algorithm applied a 1-s-duration, 100-Hz pulse train that was repeated every 30 s for 5 min. For Stim1, this algorithm was repeated six times over a 1-h period, with 5-min rest periods between repetitions. Stim2 also was stimulated six times, but a 9-h rest period was imposed between the third and fourth cycles. While the daily amount of stimulation was the same for both groups, rats that were stimulated twice/day versus once/day maintained muscle properties that were more similar to those of uninjured controls (black bars). Values are reported as mean±SEM. *, †, and ‡, significantly different from uninjured control (Con), from non-stimulated contralateral muscle, and from Stim1, respectively. Adapted with permission from Kim et al. (2007).

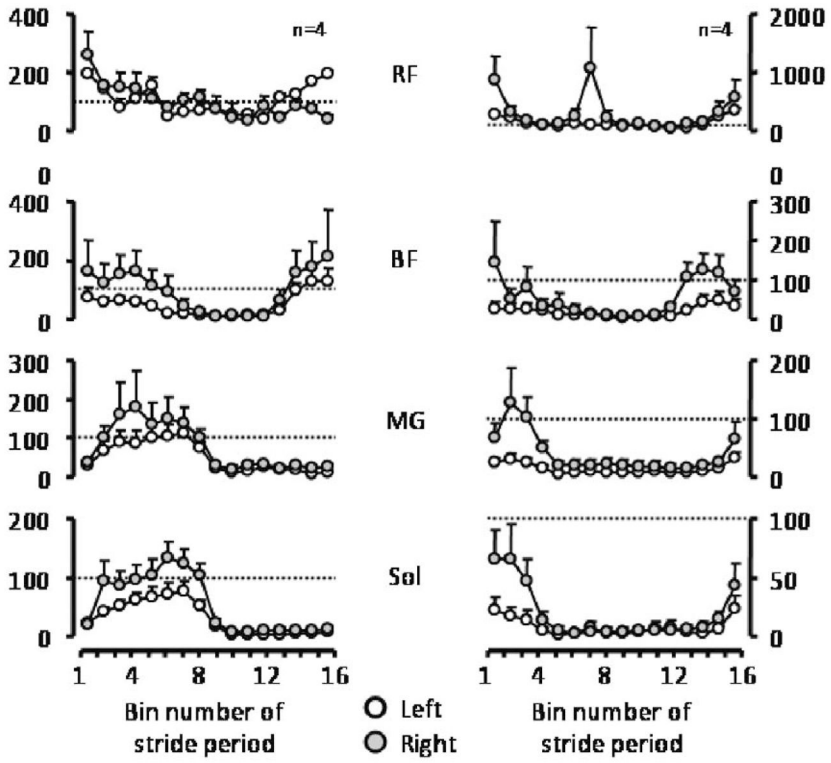

Fig. 2.

Monosynaptic muscle responses evoked by spinal cord stimulation are task and phase dependent. Phase-dependent modulation of the multi-segmental monosynaptic response (MMR) amplitude was observed throughout the gait cycle in the leg muscles studied in uninjured subjects. The MMR modulation pattern also was motor-task specific, differing during walking compared to running. Transcutaneous spinal cord stimulation was applied using a AgCl cathode placed on the skin overlying the T11 and T12 spinous processes during walking (left panels: 3.5 km/h) and running (right panels: 8.0 km/h). The resultant MMR responses were recorded bilaterally from selected leg muscles in eight individuals (open and shaded circles depict the left leg and right leg, respectively). Ten step cycles were analyzed for each subject. The data were discretized into 16 time bins corresponding to different periods of the step cycle, beginning with heel strike. Each data point represents the mean±SD of the MMR amplitude, reported in each individual as a percentage of the MMR amplitude recorded during standing (dashed horizontal line). All evoked potentials are recorded in millivolts. Muscles recorded: RF, rectus femoris; BF, biceps femoris; MG, medial gastrocnemius; Sol, soleus. Adapted with permission from Courtine et al. (2007).

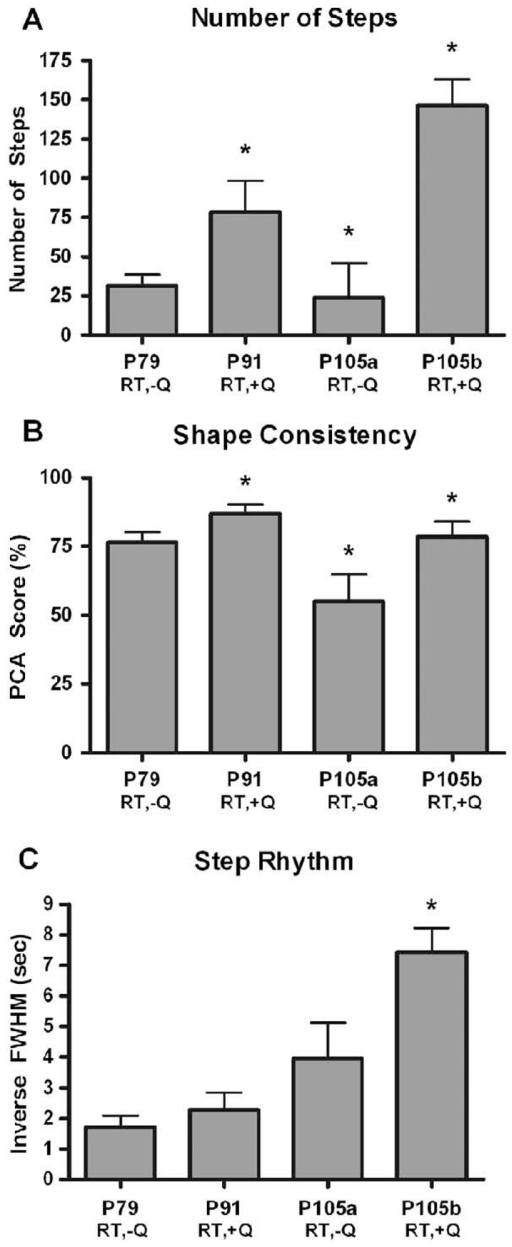

Fig. 3.

Pharmacological treatment complements robotic training in enhancing spinal locomotion. In robotically trained spinal mice ($n = 8$), coadministration of quipazine increased the number of steps performed (A) and improved step shape consistency (B), but did not affect step rhythm (C). After an initial period of robotic training, which ended at 79 days post-lesion (P79), increases in the number of steps performed and in step shape consistency were observed when quipazine was used to supplement training (P91). This effect was reversed when quipazine was withdrawn, demonstrating that the improvement in locomotion was attributable to the quipazine treatment (P105a). An additional bolus dose of quipazine immediately restored the pharmacologically mediated enhancement (P105b). These results suggest that quipazine and robotic training have complementary effects. Step rhythm, on the other hand, improved steadily throughout the course of robotic training, which is consistent with previous results that suggest

that robotic training has a greater effect on step rhythm than quipazine. *, Significantly different from P79 (RT, −Q). RT, robotically trained; +Q, treated with quipazine; −Q, not treated with quipazine. Adapted with permission from Fong et al. (2005).

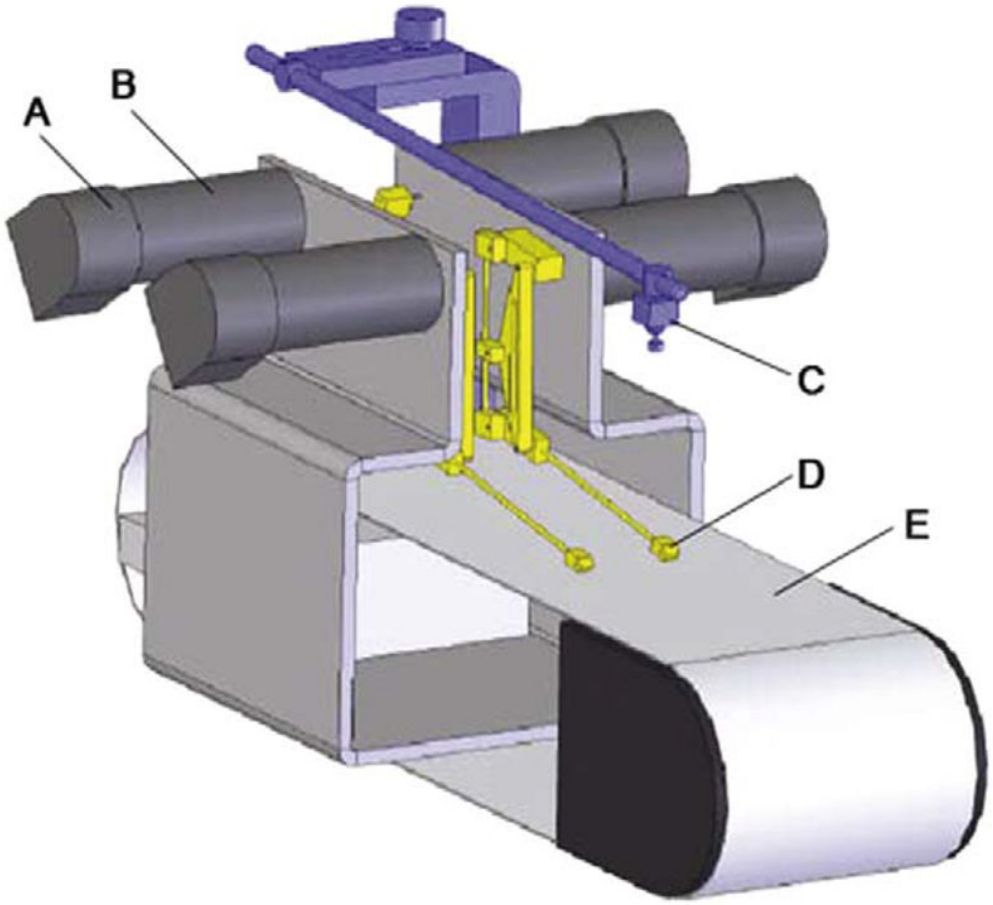

Fig. 4.

Diagram of rodent robotic step training and evaluation system. The rodent robotic system consists of the following major components: (A) four optical encoders, (B) four DC motors, (C) a weight-support device, (D) two 5-bar parallelogram linkages, and (E) a motor-driven treadmill. When used in an active mode, the system applies step-training algorithms that are commanded by an external motion controller. In a passive mode, the optical encoders record the trajectories of the legs during free stepping. Robotics thus enables quantitative monitoring of both training and recovery. Adapted with permission from Cai et al. (2005).

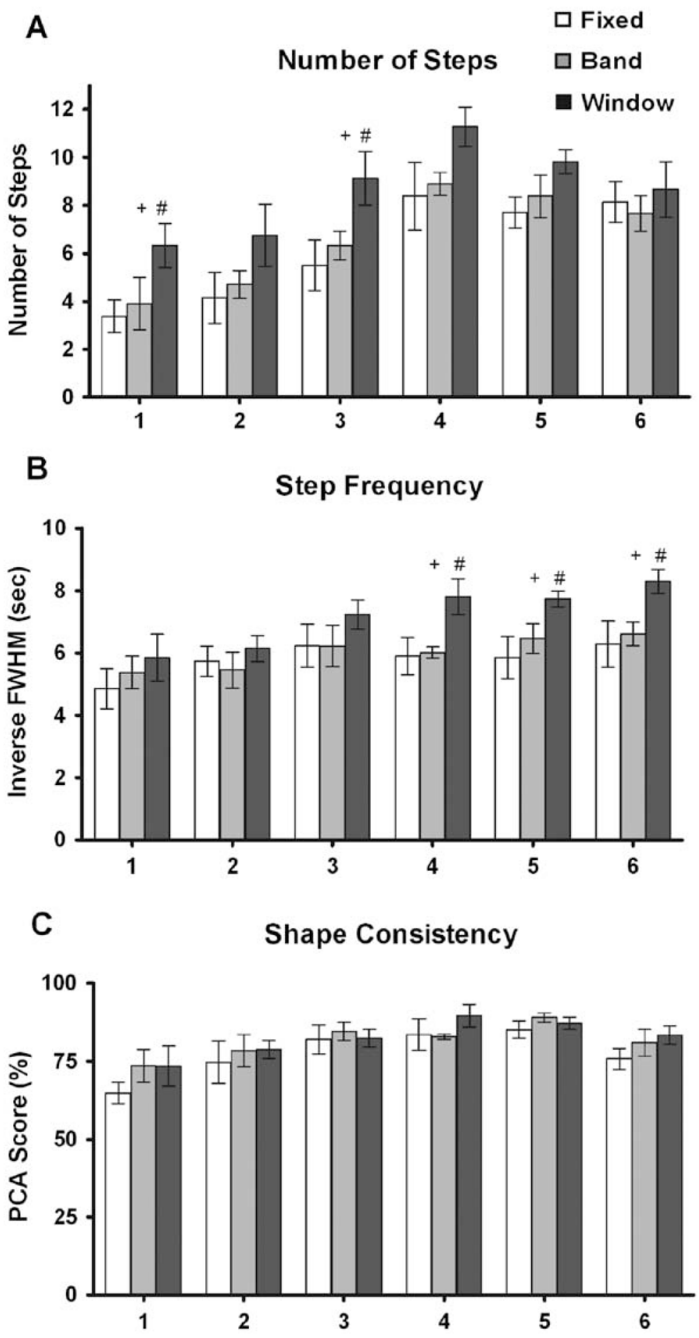

Fig. 5.

Variability in robotic step training promotes robust locomotor recovery. After 4 weeks of robotically assisted step training, complete spinal mice that were trained using a "window" control algorithm (black bar) were able to execute more steps (A), and displayed better step rhythm (B), than mice hat were trained with either a "band" algorithm (gray bar) or using traditional, continuous-assistance, "fixed" trajectory training (white bar). Contrasted with fixed trajectory training, "window" and "band" training i.e., assist-as-needed paradigms allow the hindlimb to deviate to some degree away from the nominal trained trajectory: the robotics only exert corrective action when the position of the hindlimb moves beyond a set limit, at which point a restoring force is generated (force magnitude encoded by an error-dependent velocity

field). “Window” training enforces alternating interlimb coordination, whereas “band” training does not. The data suggest that the additional sensory information provided to the spinal circuitry during “window” training enhances locomotor recovery, but that interlimb coordination should be controlled when training an alternating gait. Values are reported as mean±SEM. + and #, significantly different from “fixed” and “band” training group, respectively. Adapted with permission from Cai et al. (2006).

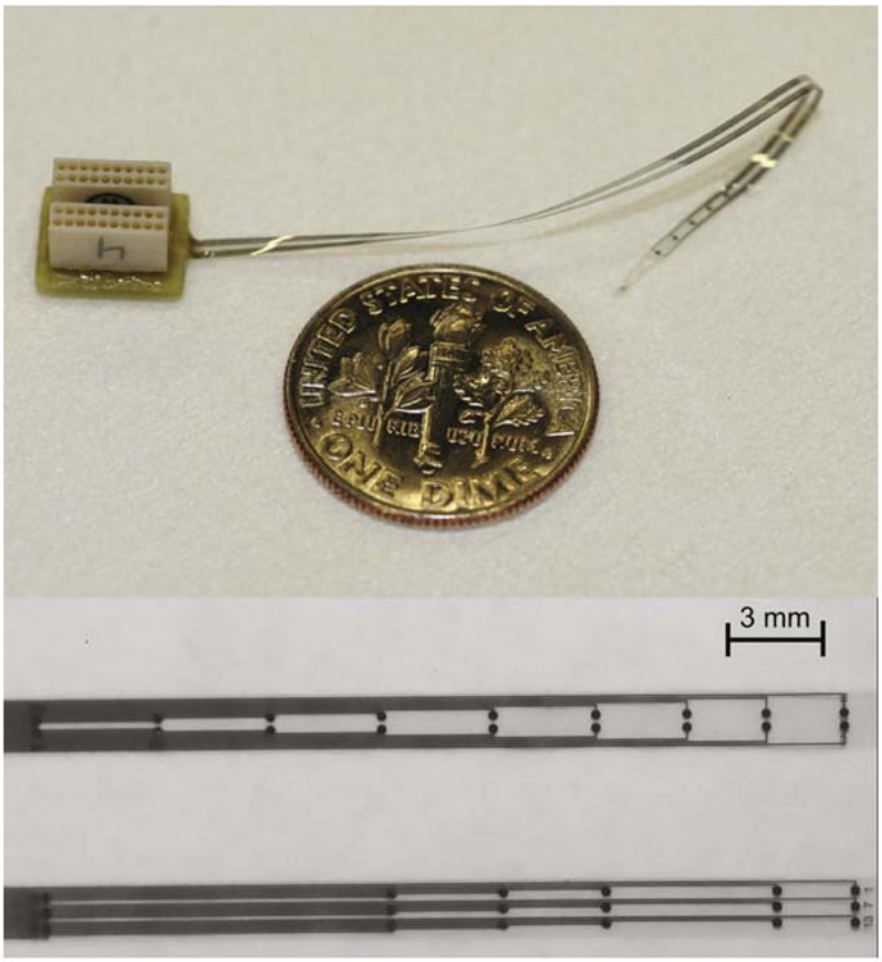

Fig. 6.

Photographs of spinal cord electrode arrays. (Top) Photograph of a spinal cord electrode array and 36-pin head connector juxtaposed against a small coin for size comparison. (Bottom) Close-up photograph of the 18-electrode contacts of a 3 × 6 electrode array with physiologically determined rostrocaudal interelectrode spacing.

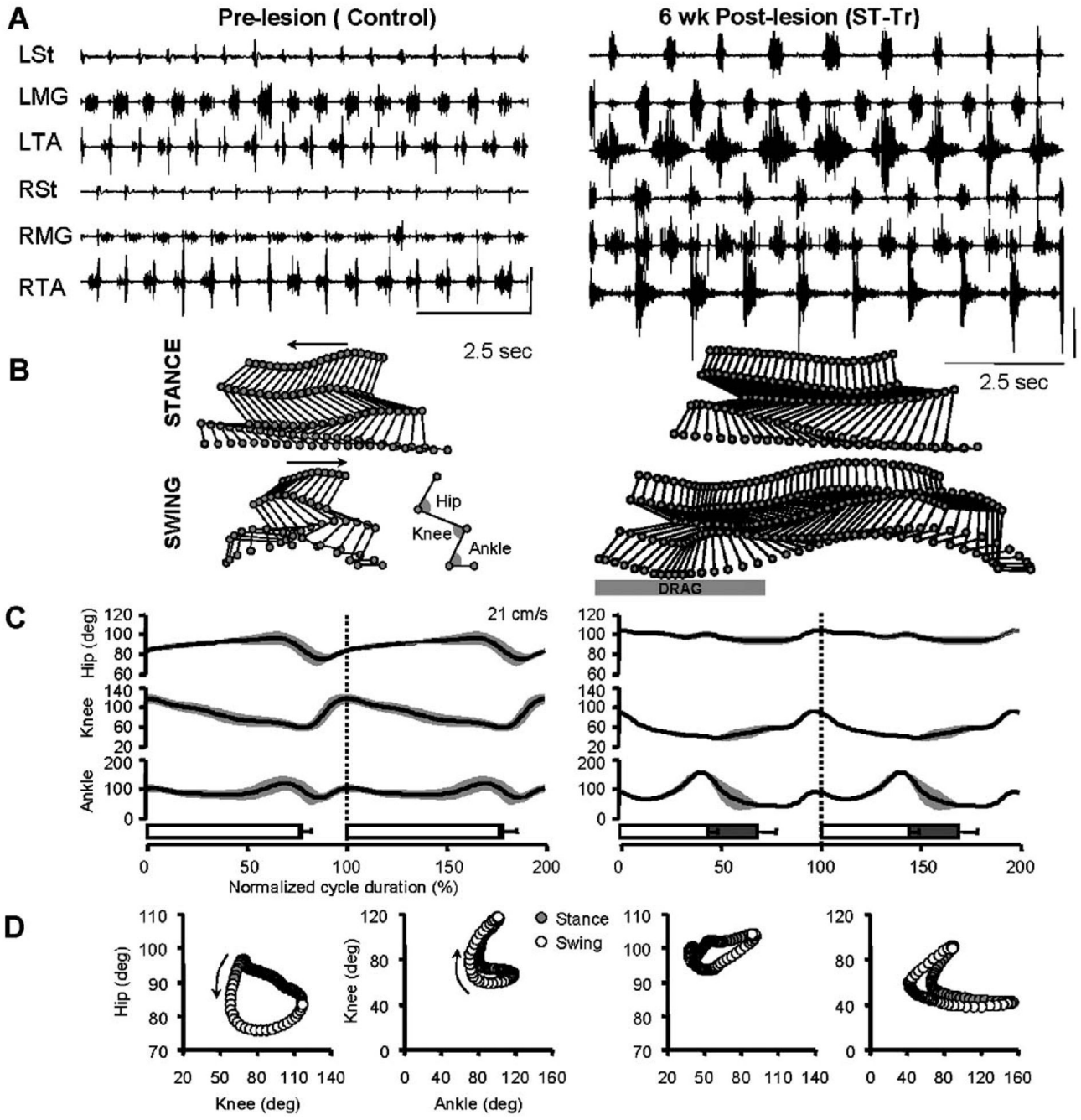

Fig. 7.

Bipedal stepping approximating that observed pre-lesion can be recovered after a complete spinal cord transection with the aid of epidural stimulation and pharmacological facilitation. EMG (A) and kinematic (B–D) data are shown for a rat before and 6 weeks after receiving a complete mid-thoracic (~T9) spinal cord transection while stepping on a treadmill at 21 cm/s. After the transection, the rat was administered quipazine and epidural stimulation. Representative stick diagram decompositions of the left hindlimb movements during the stance and swing phases of gait are shown in B. Mean waveforms of the hip, knee, and ankle joint angle for the left hindlimb are plotted for a normalized gait cycle duration in C. Each trace is an average of 15 (control) and 18 (spinal transected-trained, ST–Tr) successive steps.

Horizontal bars at bottom indicate mean value of stance phase (blank) and foot drag duration (shaded). Angle–angle plots showing coupling between hip and knee (left) and knee and ankle (right) from the same data shown in C are shown in D. Filled and empty circles represent stance and swing phases of gait, respectively. Arrows indicate direction along which time is evolving. Shaded portion of the lines in C shows SEM. Adapted with permission from Gerasimenko et al. (2007).

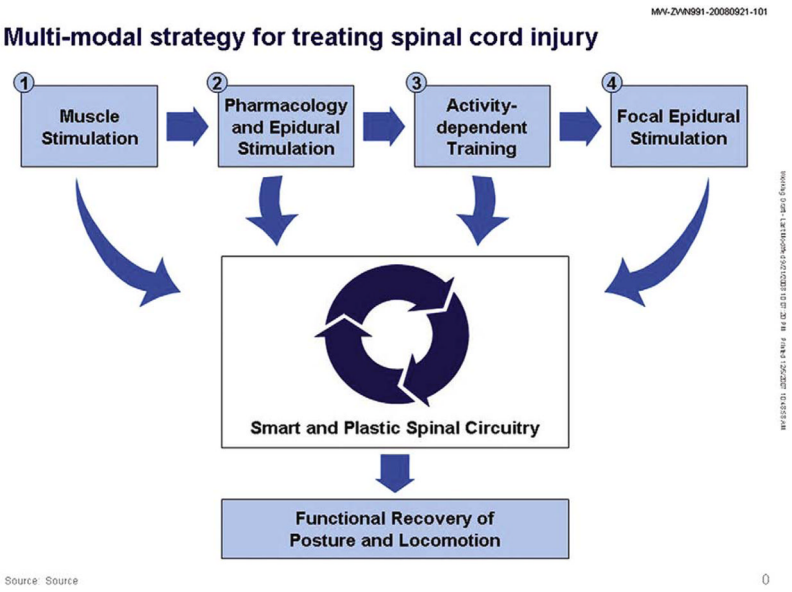

Fig. 8.

A multimodal approach to spinal cord rehabilitation. It is becoming increasingly clear that combining multiple treatment paradigms can produce enhanced recovery. This diagram depicts a promising four-step approach to recovering locomotor function: (1) application of muscle stimulation to maintain normal properties of the muscles; (2) use of pharmacological treatments and epidural stimulation to recreate an electrochemical environment conducive to spinal learning; (3) administration of activity-dependent motor training to provide the appropriate cues necessary to teach the spinal cord to walk; and (4) delivery of focal epidural stimulation to refine and facilitate functional stepping patterns. All of these treatments modulate sensory input to the lumbosacral spinal circuitry, which processes the information and uses it to recover functional posture and locomotion.