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Full-length review

# Tapping into spinal circuits to restore motor function

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**Abstract**

Motivated by the challenge of improving neuroprosthetic devices, the authors review current knowledge relating to harnessing the potential of spinal neural circuits, such as reflexes and pattern generators. If such spinal interneuronal circuits could be activated, they could provide the coordinated control of many muscles that is so complex to implement with a device that aims to address each participating muscle individually. The authors' goal is to identify candidate spinal circuits and areas of research that might open opportunities to effect control of human limbs through electrical activation of such circuits. David McCrea's discussion of the ways in which hindlimb reflexes in the cat modify motor activity may help in developing optimal strategies for functional neuromuscular stimulation (FNS), by using knowledge of how reflex actions can adapt to different conditions. Michael O'Donovan's discussion of the development of rhythmogenic networks in the chick embryo may provide clues to methods of generating rhythmic activity in the adult spinal cord. Serge Rossignol examines the spinal pattern generator for locomotion in cats, its trigger mechanisms, modulation and adaptation, and suggests how this knowledge can help guide therapeutic approaches in humans. Hugues Barbeau applies the work of Rossignol and others to locomotor training in human subjects who have suffered spinal cord injury (SCI) with incomplete motor function loss (IMFL). Michel Lemay and Warren Grill discuss some of the technical challenges that must be addressed by engineers to implement a neuroprosthesis using electrical stimulation of the spinal cord, particularly the control issues that would have to be resolved. 

*Keywords:* Locomotion; Central pattern generator; Spinal cord injury; Functional neuromuscular stimulation; FNS; FES

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## 1. Introduction

During the past 30 years, researchers have developed motor prostheses to restore functional movement to paralyzed limbs by electrically activating skeletal muscle. These devices typically require one electrode, one lead connection between the electrode and the stimulator, and one channel of a multichannel stimulator for each muscle, and complex algorithms to coordinate the excitation of multiple muscles for each stereotypical motion. Implementation of these neuroprosthetic systems has been successful and has created a demand for the restoration of even more function. However, more function requires activating more muscles, and thus, an additional electrode, lead and stimulator channel for each added muscle. If ways could be found to harness the potential of spinal neural circuits, such as reflexes and pattern generators, coordinated contractions of many muscles could be initiated with far less hardware and far less surgery to implant it than present systems require.

Over the same 30-year span, researchers from the basic science community have learned a great deal about interneuronal circuits in the spinal cord. When activated, these circuits provide stereotyped movements that employ coordinated control of many muscles. Some of these movements are executed only once in response to a single stimulus, and effect novel movements, while others, that employ pattern generators, provide rhythmic coordinated contractions. Researchers have investigated these movements, studied their properties for a wide range of external stimuli and are beginning to map the anatomical connections of many spinal circuits. Some researchers have also begun to think about how such neural circuits might be used in humans and some have even carried out experiments on humans with paralysis.

In an effort to share the accumulated knowledge and motivated by the need to improve the performance of motor prostheses, the authors have addressed the problem of gaining access to spinal circuits. Their goal is to identify candidate spinal circuits and areas of research that might

open opportunities to effect control of human limbs through electrical activation of such circuits.

## 2. Can use be made of spinal reflex circuits following spinal cord injury (SCI)? (David McCrea)

We will examine features of some spinal reflex systems that may be useful for movements assisted by functional neuromuscular stimulation (FNS). The focus will be on recent observations of cat hindlimb reflexes elicited during locomotor activity. While it is obvious that motor activity results from the combined actions of descending pathways, intrinsic spinal cord circuitry and segmental reflexes, the way in which reflex systems exert their regulatory control is only beginning to be appreciated. Although it has long been recognized that reflex gain is controlled by such things as motoneuron excitability and afferent fiber transmitter release, reflexes are often incorrectly assumed to be ''fixed function'' modules in which their actions simply add to or subtract from centrally generated movements. Rather, spinal reflex systems modify motor activity both by direct actions on motoneurons and indirectly through the interneurons that form the spinal pattern generating circuitry for locomotor (and other) movements. Spinal interneurons that mediate reflexes are also the targets of descending motor control systems. Descending regulation of the excitability of reflexes, according to the current motor state, could switch from inhibitory to excitatory reflexes [131]. The result is a flexible control system in which not only the gain, but the sign and distribution of reflex actions can be modified to adapt motor output to changing conditions. An understanding of how reflex systems function under different conditions is, therefore, essential to optimizing FNS strategies. We begin with a brief survey of the classical spinal reflexes and outline some properties for consideration in FNS assisted movements.

### *2.1. Monosynaptic excitation by primary muscle spindle afferents*

The most thoroughly studied spinal reflex is the monosynaptic excitation of homonymous and close synergist motoneurons with a disynaptic inhibition of antagonist motoneurons evoked by activation of group Ia (primary) muscle spindle afferents. Group Ia afferents can be activated by low strength electrical stimulation, and are naturally activated by muscle length increases or activity in the gamma motoneuron system that regulates spindle stretch sensitivity [117]. Although these features seem ideal for use in an FNS strategy, the relatively low gain and the narrow distribution of excitatory and inhibitory effects [64] limit the use of this reflex system for FNS. Furthermore, the concomitant inhibition of antagonists during activation of Ia fibers has the potential to interfere with postural stability.

### *2.2. Cutaneous reflexes*

Cutaneous reflexes involve several interneuron systems and complex spinal pathways. This results in a pattern of reflex actions that is state-dependent, changing in different motor tasks and under different conditions, for example, during locomotion [165]. Without detailed knowledge of the factors that can modify the operation of cutaneous reflex pathways, cutaneous FNS is limited to well-controlled conditions. Another problem in the use of cutaneous FNS is that electrical activation of cutaneous afferents usually results in weak reflex effects unless repetitive stimulation is used. In patients with intact spinal sensory systems, this may be painful, limiting the applicability of cutaneous FNS. Another consideration is that cutaneous reflexes are exerted both locally (i.e., to muscles innervating surrounding areas) and extensively throughout the limb. Under some conditions, cutaneous stimulation may elicit a flexor withdrawal response [65]. Such a general excitation of flexors may destabilize posture. On the other hand, there may be certain circumstances in which the activation of specialized, local cutaneous reflex pathways could be useful for FNS. For example, the activity of cutaneous afferents might be useful to sense foot contact [104] or cutaneous stimulation could be used to reinforce muscle activity at particular times during a motor task [74].

### *2.3. Reflexes evoked from spindle secondaries*

Reflexes evoked from the group II (secondary) muscle spindle afferents remain the least understood lumbar spinal reflex system. Early discussion of their actions noted the similarity of effects evoked from group II and cutaneous afferents, i.e., both evoked flexion reflexes [65]. Interneurons contacted by group II afferents also have other reflex actions including disynaptic excitation or inhibition of motoneurons [110] and relays between descending motor commands and motoneurons [129]. Recent studies suggest that an increase in the gain of group II reflexes is a component of human spasticity [67]. This observation is part of the growing evidence that group II reflex systems form an essential part of the continuous regulation of movements. The ability of group II afferents to evoke the flexion reflex is likely to be of less importance. At present, however, the difficulty in selective activation of group II afferents and our rudimentary knowledge of their role in proprioception complicates the development of FNS strategies targeted to group II afferents. As mentioned, the state-dependent ability of group II and high threshold cutaneous afferents to evoke strong limb flexion may further limit the use of these reflex systems when the goal is to activate anti-gravity muscles.

### *2.4. Reflexes evoked from tendon organs; non-reciprocal group I reflexes*

The final reflex system to be considered here is that evoked from Ib (tendon organ) muscle afferents. This

system serves as a good example of multi-sensory convergence in spinal reflex systems since low threshold joint and cutaneous afferents, and Ia spindle afferents also activate those reflexes originally ascribed to Ib afferents [109]. In this paper, we will refer to these reflex systems as ''group I non-reciprocal'' reflexes to distinguish them from the Ia reciprocal inhibition of antagonists and to emphasize the combined input from group Ia and Ib afferents to the interneurons interposed in the spinal reflex pathways. Non-reciprocal group I reflexes evoked from ankle extensor afferents have received the most attention because, at least in the cat, they are the most widely distributed. In preparations that are not engaged in an organized motor program (i.e., decerebrate, anaesthetized, or ''resting'' preparations), group I strength stimulation (defined as stimulation at intensities twice that recruiting the lowest threshold fibers in the nerve) of ankle extensor nerves produces mixtures of di- or tri-synaptic latency reflexes that are predominately inhibitory to ankle, knee and hip extensor motoneurons and excitatory to bifunctional flexors such as posterior biceps. The inhibitory actions are unlikely to be of great use for FNS designed to increase weight support by extensor motoneuron recruitment. An important recent observation, therefore, is that non-reciprocal reflexes are reconfigured during locomotion into a system that excites extensor motoneurons. As will be discussed, these actions are a part of a natural strategy for motor control and appear to be an ideal candidate for use in FNS.

### *2.5. Reconfiguration of group I reflexes*

Until recently most reflex studies were carried out in the absence of locomotion where non-reciprocal reflexes evoked from ankle extensor groups Ia and Ib afferents are predominately inhibitory to extensors [109]. The dominance of these actions was the basis for discussing non-reciprocal group I reflexes as being organized into a negative feedback system. Accordingly, muscle activity during movements would activate group I afferents which in turn would evoke a reflex inhibition of extensors [109]. We now appreciate that during locomotion, a reorganization of these reflexes results in a system that is excitatory to extensors. These excitatory actions can result in a termination of ongoing flexor activity and an initiation of extensor activity, if extensor group I afferents are activated during the flexor phase of the step cycle (i.e., a resetting of the step cycle to extension) [52,79,88]. More importantly for the present discussion is the fact that activation of ankle extensor group Ia or Ib afferents during extension increases ongoing extensor activity [52,88,155]. Stimulation of ankle extensor group I afferents results in larger and longer bursts of activity in ipsilateral hip, knee and ankle extensors [88].

The high sensitivity of groups Ia and Ib afferents to muscle stretch and tendon tension respectively results in their rhythmic activity during even low intensity locomotion on a flat surface [159]. This activity provides the opportunity for reflexes evoked by these afferents to interact with the central locomotor pattern generating circuitry to provide step to step regulation even during normal, unperturbed locomotion. Support for the idea that group I afferents are an important part of normal step cycle regulation comes from a number of studies and in particular those from Hiebert et al. [99], Pearson [156], Whelan et al. [198], and Whelan and Pearson [199] in decerebrate or conscious cats (also see Rossignol's section of this paper). In the absence of ankle extensor group I afferent feedback, there is inadequate extensor activity and an increased yield at the knee during treadmill walking [199]. This implies that a significant amount of extensor motoneuron drive during locomotion originates from excitatory group I reflexes. The term 'extension enhancement' has been introduced to describe the reflex excitation of extensors produced by group I afferents during locomotion [88].

The ability of ankle extensor group I afferents from one hindlimb to control stepping behavior depends upon the preparation and the locomotor task. The largest effects are observed during fictive locomotion in decerebrate cats [e.g., Ref. [88]]. Not surprisingly, with more of the neuraxis intact, the ability of one source of afferent feedback to control the entire stepping behavior is diminished. In conscious animals, stimulation of ankle extensor afferents is less effective than in decerebrate preparations and also less effective during quadrupedal than during bipedal locomotion [199]. Evidence suggests, however, that the same circuitry expressed during locomotion in decerebrate cats is also present in the adult human. Thus, training to re-establish locomotor capabilities following spinal cord lesions is significantly enhanced by body support that permits limb loading and presumably an activation of load receptors (i.e., group Ib tendon organ afferents) [94] (see Barbeau's section of this paper). This is an important observation since it suggests that manipulations such as FNS that are marginally effective in normal subjects, may be very effective in promoting weight support and locomotion following disruption of descending control of the spinal cord.

### *2.6. Spinal pathways responsible for extension enhancement*

Animal studies have revealed at least four reflex mechanisms contributing to group I evoked enhancement of ipsilateral extensor activity during locomotion. The first is the monosynaptic reflex described above. While activation of muscle spindles with stretch will monosynaptically excite motoneurons, the limited distribution of this excitation [64] restricts its contribution to extension enhancement to homonymous and close synergist motoneurons. Monosynaptic excitation from ankle extensor afferents is thus unlikely to contribute significantly to the excitation of hip,

knee and ankle extensors observed during locomotion [88]. The other mechanisms underlying extension enhancement result from the reorganization of group I reflex pathways during locomotion. They are: a suppression of the group I evoked non-reciprocal inhibition that occurs at rest; an activation of excitatory interneurons that receive input from group I afferents and project directly to extensor motoneurons; and a longer latency group I excitation that is evoked through extensor portions of the locomotor circuitry. Fig. 1 summarizes the changes in group I reflexes to extensor motoneurons during locomotion.

Intracellular studies show that the non-reciprocal, inhibitory post-synaptic potentials (IPSP) produced by extensor nerve stimulation at rest disappear during locomotion [79,133]. The disappearance of non-reciprocal inhibition is caused by an unknown mechanism that tonically inhibits the interneurons responsible for evoking non-reciprocal inhibition [13,133]. In addition to the inhibition of interneurons in non-reciprocal inhibitory pathways, two types of group I evoked extensor motoneuron excitation emerge during locomotion. The first is a very short latency excitation (disynaptic, 1.6 ms latency) evoked through a pathway consisting of a single interneuron between group I afferents and extensor motoneurons. Such excitation was originally reported during fictive locomotion induced by L-DOPA in decerebrate, high spinal cats [177]. Disynaptic excitation has been more extensively characterized recently during fictive locomotion evoked by midbrain stimulation. These disynaptic excitatory post-synaptic potentials (EPSP) are evoked from either Ia muscle spindle or Ib tendon organ afferents and only during the extensor phase of locomotion [14,133]. They are evoked most readily from ankle extensor group I afferents which produce excitation of ankle, knee and hip extensor motoneurons. Stimulation of hip and knee extensor afferents evokes a more limited distribution of disynaptic excitation [14].

A population of interneurons has been found in caudal lumbar segments that are candidates for mediation of disynaptic extensor excitation during locomotion [13]. These cells project to motoneurons and are monosynaptically activated by group I afferents during the extension phase but not at rest. They are also rhythmically active during extension in the absence of peripheral nerve stimulation. No attempt has been made yet to verify that the candidate interneurons are excitatory. If so, their rhythmic locomotor activity would contribute to the depolarization of extensor motoneurons as part of the central locomotor network as well as being involved in the reflex regulation of extensor motoneurons. Because locomotion can occur in preparations without disynaptic group I EPSPs [133], the disynaptic excitatory system appears to be organized in parallel to other excitatory outputs from the central pattern generator (CPG) and is not an essential component of the CPG [133]. Thus, these interneurons reinforce extensor motoneuron excitation during locomotion. The regulatory mechanisms that permit expression of disynaptic excitation

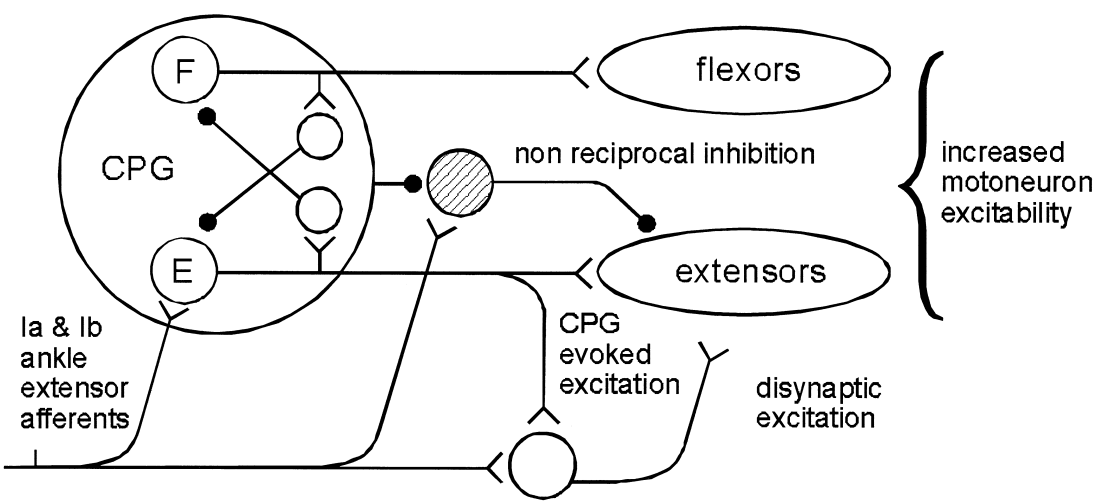



Fig. 1. Changes in group I reflexes to extensor motoneurons during locomotion. Inhibitory synapses are indicated by small filled circles and excitatory synapses by the forked lines. Extensor group I (Ia muscle spindle and group Ib tendon organ) afferents excite three populations of spinal interneurons: two contacting extensor motoneurons and one within the extensor portion of the spinal locomotor pattern generator (CPG). The hatched circle represents the population of interneurons that in the absence of locomotion, mediate the non-reciprocal inhibition of extensor motoneurons. During locomotion these interneurons are inhibited and another population of group I activated interneurons (open circle) is released from inhibition. Phasic excitation of these excitatory interneurons by the central locomotor circuitry (CPG) allows group I afferents to produce disynaptic excitation of hindlimb extensors during the extension phase of locomotion. Group I excitatory actions mediated though the CPG include the extension enhancement discussed in the text. During locomotion, motoneuron excitability is increased by a reduction in motoneuron threshold and rheobase as well as a reduction in the post-spike after hyperpolarization which results in faster motoneuron firing rates. The reorganization of reflex pathways and increased motoneuron excitability allows feedback from extensor group I afferents to contribute substantially to limb extension during locomotion. The monosynaptic excitation of homonymous motoneurons and the disynaptic (reciprocal) inhibition of flexor motoneurons by group Ia afferents are not shown in the diagram.

during extension or prevent its appearance at rest remain unknown.

It is noteworthy that there is both the reduction in non-reciprocal group I inhibition and the presence of a short latency excitation following the monosynaptic H reflex in some human subjects during treadmill walking [191]. It is thus reasonable to conclude that these pathways characterized during fictive locomotion in decerebrate cats also operate in humans.

Several observations indicate that extensor group I afferents exert powerful control over the locomotor circuitry. The cycle can be reset to extension, i.e., the ongoing flexor phase is terminated and a premature onset of extension is initiated by group I strength stimulus trains to ankle extensor nerves during flexion [52,88]. The same stimulation delivered during extension increases hindlimb extensor motoneuron discharges and may prolong the step cycle [52,155,88]. In addition, the step cycle period can be entrained by group I strength stimulation [52,157,155] and continued extensor group I afferent activation can prevent the initiation of flexion [63]. These observations illustrate strong control of the locomotor circuitry by extensor group I afferents. Intracellular recording shows that, in addition to the disynaptic excitatory pathway, there is another and more powerful source of extensor excitation during locomotion. Group I stimulation of ankle extensors also evokes an oligosynaptic excitation with a minimum latency of 3.5–4 ms [79] indicating a relatively short (but not disynaptic pathway) from group I afferents to extensor motoneurons. These longer latency effects may be evoked through the extension part of the locomotor circuitry [79,37]. This suggestion is strongly supported by the simultaneous distribution of this excitation to all the hindlimb extensors [88]. Simultaneous intracellular recordings from pairs of motoneurons show that extensor group I stimulation produces depolarization of extensors and hyperpolarization of flexor motoneurons with identical latencies [89]. This, in turn, indicates that group I afferents can access flexor and extensor portions of the locomotor circuitry through equal length pathways.

### *2.7. Summary: altered non-reciprocal group I pathways*

Clearly, the powerful effects of extensor group I actions on locomotion are produced by relatively direct access to locomotor circuitry, and with latencies suggesting relatively few interneurons between the afferents and motoneurons [52,79,88,89]. The reconfiguration of group I reflex pathways during locomotion offers great potential for FNS-assisted movements. Stimulation of relatively few group I afferents can take advantage of the existing reflex circuitry to reinforce extensor activity and control the step cycle. The key is to ensure that the spinal reflex pathways are in the appropriate state so that the reflex output can be predicted. The reversal to excitatory group I reflexes results in a positive instead of a negative feedback control system during locomotion. Using models of the reflex arc that include the compliant and non-linear properties of muscle, Prochazka [159] discusses how positive feedback can operate in a stable reflex system without oscillation. The efforts of Barbeau, Rossignol and others to facilitate the operation of the spinal locomotor pathways may be crucial to future FNS strategies that take advantage of non-reciprocal group I reflexes.

### *2.8. Reconfiguration of motoneuron properties during motor tasks*

The motoneuron is no longer considered a passive player in the operation of reflex systems. From the original view that motoneuron action potentials occurred by a simple summation of inhibitory and excitatory input crossing a fixed threshold, has come the recognition that intrinsic motoneuron currents can be manipulated to amplify and transform synaptic input during movement. Furthermore, the firing properties of motoneurons are altered to facilitate firing during locomotion.

### *2.9. Voltage-dependent conductances during locomotion*

A plateau potential is a maintained depolarization following a brief depolarizing synaptic input or intracellular current injection. In cat motoneurons, these potentials are facilitated by 5-HT and other transmitters and are due to the activation of intrinsic motoneuron conductances [107]. Plateau depolarizations can be maintained for prolonged periods until terminated by a brief hyperpolarization. It now seems clear that the rhythmic depolarizations of motoneurons during locomotion (i.e., the locomotor drive potentials, LDP) are due in part to plateau-like potentials. Motoneuron LDPs show a voltage dependency, increasing in amplitude with membrane depolarization [37]. Voltage-dependent depolarizations allow the possibility for amplification of excitatory reflexes during locomotion. Recent studies during fictive locomotion show that if the membrane potential of a motoneuron is within a critical range where the plateau potential is enabled but not fully expressed, excitatory group I reflexes can produce larger depolarizations by facilitating these voltage-dependent conductances [37,132].

### *2.10. Motoneuron threshold and repetitive firing during locomotion*

Recent evidence obtained during fictive locomotion shows that the membrane potential at which a motoneuron action potential is evoked is more hyperpolarized (i.e., decreased) during locomotion than at rest [122]. The lowering of spike threshold makes motoneurons more excitable by reducing the amount of synaptic current needed to

initiate spiking. During fictive locomotion, in 13 motoneurons studied, the threshold voltage for producing an action potential was hyperpolarized by 3–17 mV (mean 7.4 mV) [122]. The analysis has now been extended to more than 30 motoneurons; all of which showed a lowering (hyperpolarization) of voltage threshold during fictive locomotion. The results are robust and reproducible. Recently, an observation in Aplysia provided another example of a similar state-dependent decrease in action potential threshold [51]. At present, the pharmacology and neural pathways involved in motoneuron threshold reduction remain unknown. For future FNS efforts, an ability to induce threshold reduction and increase motoneuron excitability could greatly increase the effectiveness of any excitatory spinal reflexes.

In resting preparations, the relationship between motoneuron firing and injected intracellular current (the F–I plot) is well characterized and consists of a shallow primary firing range followed by a steeper secondary (and sometimes tertiary) range. The summation of currents underlying the after-hyperpolarization (AHP) is responsible for the shape of the F–I curve. During locomotion, motoneuron repetitive firing properties are altered with much higher rates of firing being found during locomotion than during other conditions [36,66,176]. Some of this alteration is due to a reduction in the AHP which contributes to both the higher rates and a flattening of the F–I curve during locomotion [36,122]. Thus, locomotion appears to involve both decreased threshold for action potential initiation and increased repetitive firing capabilities. In some cases, firing rates during fictive locomotion can be produced with about half the intracellular required current during resting conditions [122]. The alteration of motoneuron conductances during locomotion undoubtedly contributes to the excitation produced by reorganized group I reflexes. Transformation of the spinal cord into a locomotor-like state that includes increase in motoneuron excitability could greatly increase the effectiveness of FNS.

### *2.11. Summary*

Spinal cord reflex systems can affect entire populations of motoneurons and not just small groups. The widespread excitation of extensors produced by activation of group I proprioceptors has obvious applications in step-cycle regulation and weight support during locomotion with FNS. These reorganized group I pathways are more effective in motoneuron recruitment than monosynaptic reflexes and are an example of stable motor control using a positive feedback system. Their contribution to motor control is task- and state-dependent and appears to be important during natural overground locomotion. The recruitment of motoneurons during movement is governed by many factors including the reconfiguration of intrinsic membrane currents. During some motor tasks motoneurons become more excitable, thereby increasing the efficiency of premotoneuronal circuitry in generating muscle tension.

State-dependent reflexes offer both a blessing and a curse. The ability to use spinal reflex mechanisms in conjunction with FNS to produce movements will depend critically on a knowledge of the current ‘‘reflex state’’ of the spinal cord. Appropriately timed, reflexes could be used to facilitate ongoing motoneuron activity to a previously unrecognized extent. State-dependent changes in reflex gain, distribution and sign could, however, make the design of reflex-assisted control strategies difficult. In my opinion, our immediate challenge is to understand the control of the various ‘‘states’’ of the spinal reflex systems. To this end, we need to know how to activate and control spinal pattern generation circuitry and how to supplement missing descending control with augmentation of spinally located control systems. Further work on the neuropharmacology of spinal interneurons with intrathecal drug application may quickly bring significant advances that can then be used in FNS control strategies.

## 3. Are embryonic motor activity and adult locomotion produced by similar mechanisms? (Rhythmic activity in developing spinal cord) (Michael J. O’Donovan)

The neural mechanisms responsible for the genesis of mammalian and avian locomotion are not fully understood because adult neural networks are complex and experimentally inaccessible. To circumvent these limitations, my laboratory has been analyzing the spinal networks that produce embryonic movements. The utility of this approach is predicated on the assumption that adult and embryonic motor networks operate in a similar manner. In this paper, we will review this question using evidence gathered from studies of the developing chick embryo.

Several studies have provided us with a comprehensive description of the development of motor activity in the chick embryo and its maturation into locomotion [2,32, 92,93,108]. This behavioral and electromyographic (EMG) work has shown that the basic synergies of muscle activation that characterize locomotion — alternation of antagonists and co-activation of synergists — emerge early in development and become progressively refined thereafter. These observations have led to the idea that the circuitry controlling locomotion is assembled quite early in development. However, in contrast to locomotion — which is under voluntary control — embryonic motor activity is generated autonomously by spinal circuits in the absence of descending inputs [125].

Because of the experimental difficulties associated with experiments in ovo, my laboratory has employed an isolated preparation of the chick spinal cord that expresses spontaneous rhythmic activity in vitro [125,146]. This activity comprises recurring episodes in which spinal neu-

rons (motoneurons, and interneurons), are activated synchronously and rhythmically. Although we have concentrated most of our efforts on the hip flexor sartorius (a flexor) and the knee extensor femorotibialis external (an extensor), we believe that results from these motoneurons can be generalized to all the motoneurons supplying the hindlimb. This is because the activation pattern of hindlimb motoneurons is highly stereotyped in the isolated cord preparation [125].

Sartorius and femorotibialis motoneurons are co-activated briefly at the beginning of each cycle. Following this, sartorius motoneurons stop firing at a time when the femorotibialis motoneurons peak in their discharge — leading to a period of alternation [125,146]. Sartorius motoneurons fire a second burst in each cycle which can overlap with the decaying femorotibialis burst. During an episode, this pattern is a repeating unit that recurs with each cycle. The only exception to this generalization is at the beginning of an episode when the cycles can recur very rapidly resulting in tonic femorotibialis discharge coupled with minimal sartorius firing. An episode of this activity can last up to 1 min and recur every 10–15 min, although the actual duration and frequency depend on the embryonic age and experimental conditions [49,145,183].

To understand how this activity is produced by the spinal cord requires answering three questions. First, what determines the timing and phasing of discharge between different pools of motoneurons during episodes of activity? Second, what controls and regulates the spontaneous occurrence of episodes? And finally, what processes are responsible for the rhythmic activity within an episode?

### *3.1. Timing of motoneuron discharge during bursts of motor activity*

Several experimental approaches have been used to resolve the factors that control the timing of discharge in motoneurons. The results have been surprising because the mechanisms controlling the timing of flexor/extensor alternation in the embryo appear to differ from those presumed to operate during adult locomotion [for review, see Ref. [85]]. In adult animals, it is generally thought that flexor and extensor alternation is the result of excitatory and inhibitory synaptic drive that alternates between the respective motoneurons [158]. Thus, while flexors are being excited, extensors are being inhibited and vice versa. This mechanism is not responsible for the timing of flexor and extensor activity in the developing chick cord because all of the motoneurons are depolarized synchronously during each cycle of activity [49,146]. Nonetheless, flexor sartorius motoneurons stop firing at the peak of their depolarization whereas femorotibialis motoneurons do not. The pause in sartorius discharge appears to depend on a synaptically induced shunt that is mediated primarily by gamma aminobutyric acid ($GABA_A$) receptors [146,179]. Evidence in support of this idea was obtained by injecting small quantities of the $GABA_A$ antagonists bicuculline or picrotoxin within the sartorius motor column during an episode [179]. This procedure abolished the sartorius pause, presumably by blocking premotor $GABA_A$ terminals on the soma and proximal dendrites of sartorius motoneurons [179]. In contrast to this result, we found that *bath-applied* $GABA_A$ antagonists *do not abolish* the sartorius pause. Instead, the pause was lengthened and a pause was induced in each cycle of femorotibialis activity [179]. Under these conditions, both pauses could be abolished by local injection of excitatory amino acid antagonists within the respective motor pools.

These apparently contradictory results can be reconciled by recognizing that the equilibrium potentials of all the major transmitters are above rest potential in the early chick cord. As a result, functional inhibition results from shunting or voltage clamping the membrane by the active conductance [146]. Following *bath-application* of $GABA_A$ and glycine antagonists, the excitatory drive increases to motoneurons, as manifest by increased muscle nerve discharge. One result of this increased drive is that the motoneurons are now shunted by an excitatory conductance rather than by GABA- or glycine-gated channels. Strikingly, however, even though the pause is now generated by glutamatergic (and other) synaptic inputs, it is still longer in sartorius motoneurons than in femorotibialis motoneurons. This suggests at least two possibilities. The first is that sartorius motoneurons receive a greater number or a stronger set of synaptic inputs than femorotibialis motoneurons — for both glutamatergic and GABAergic inputs. Alternatively, some feature of the sartorius motoneurons themselves makes them more susceptible to shunting by their synaptic inputs than femorotibialis motoneurons.

### *3.2. What accounts for the occurrence of spontaneous episodes of activity?*

Our evidence suggests that the hyperexcitability of developing networks coupled with activity-dependent network depression can account for the production of spontaneous episodes of activity. These findings, which are discussed below, have allowed us to construct a model which accounts for behavior of developing spinal networks [for a fuller description, see Refs. [144,148,149,194]).

The occurrence of spontaneous activity is controlled, in part, by a periodic variation in network excitability (Fig. 2). The origin of this variation is an activity-dependent depression of network excitability that recovers in the interval between episodes. The inhibition of network excitability by an episode takes two forms. The first is a long-lasting reduction of synaptic transmission that may be a combination of pre- and post-synaptic factors [68]. The second mechanism is a post-episode hyperpolarization of

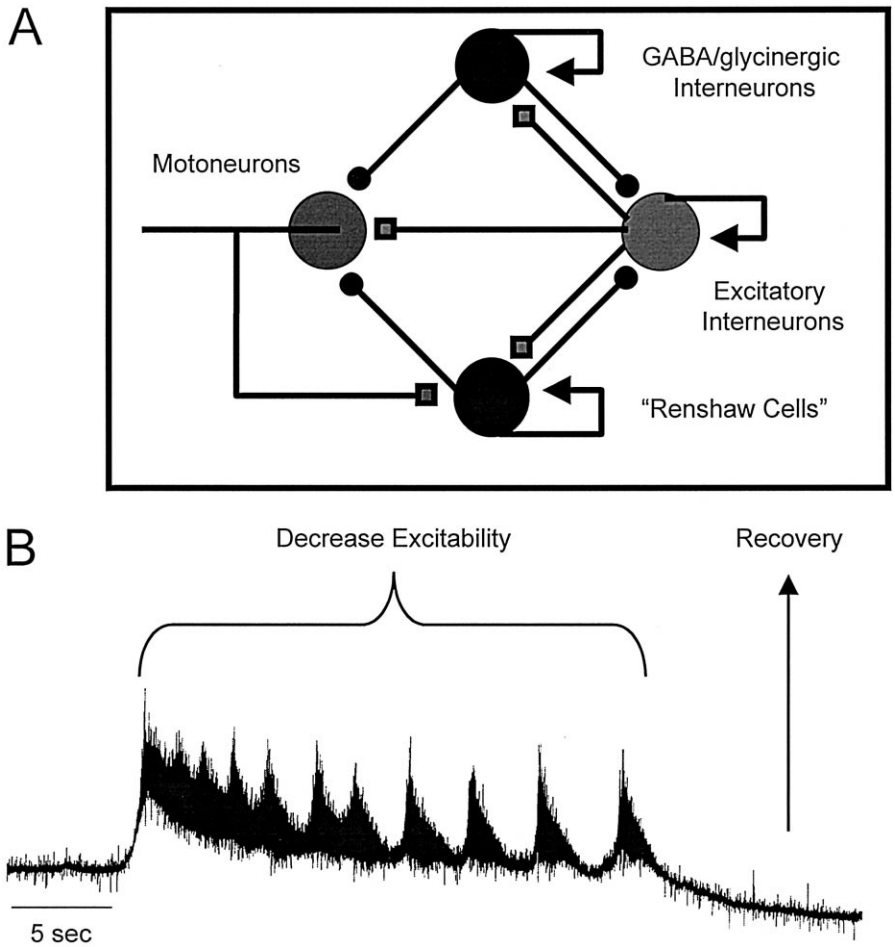



Fig. 2. Schematic of the spinal circuitry underlying spontaneous activity in the developing spinal cord and its modification by spontaneous activity. (A) Circuit diagram illustrating the connectivity of various types of neuron in the ventral spinal cord of the embryonic chick embryo at E10. Four types of neuron are identified: motoneurons, ''Renshaw cells'' mediating recurrent connections between motoneurons and other interneurons, excitatory interneurons and GABA/glycinergic interneurons that may mediate the sartorius pause. At this stage of development, all of the synapses are depolarizing. The arrows on the interneurons indicate that the population is recurrently interconnected and can be recruited by positive feedback. (B) Electrical recording of femorotibialis muscle nerve activity during an episode of spontaneous activity showing the effects of the activity on network excitability.

spinal neurons that recovers as a depolarizing ramp in the interval between episodes [48]. We do not yet fully understand the mechanism of the ramp but have evidence that a modulation of GABAergic synaptic transmission is involved [48].

To understand how a spontaneous episode occurs, we will describe the recovery of the network from a previous episode of activity. Just after an episode, network connectivity is too low to support activity. As the efficacy of synaptic transmission recovers (due to the absence of activity), network connectivity progressively increases. Concomitantly, individual spinal neurons progressively depolarize during the inter-episode interval. Eventually, some interneurons begin to fire as their membrane potential crosses threshold. With further increases in excitability, a point will be reached where the network connectivity is high enough to allow the discharge of isolated groups of neurons to propagate throughout the whole network. It is this explosive recruitment of the network that constitutes an episode. Once the episode occurs, it induces several activity-dependent mechanisms that limit firing. The process will recur as the membrane potential and synaptic transmission recover in the next inter-episode interval.

### *3.3. What is the mechanism of rhythmicity?*

The mechanism of rhythmicity during an episode is unknown. Our results allow us to exclude some of the mechanisms that have been implicated in the genesis of locomotor rhythms in other species. For example, the excitatory amino acid agonist *N*-methyl-D-aspartate (NMDA) can induce rhythmic oscillations of membrane potential in motoneurons and interneurons of several species in the absence of synaptic interactions [84,101, 102,162,185]. This cellular rhythmicity has been proposed to play a role in the genesis or maintenance of locomotor

activity [84,164]. Spinal neurons in the chick cord also express rhythmic, voltage-dependent membrane potential oscillations in the presence of bath-applied NMDA [50]. However, such oscillations do not appear to be essential, because rhythmic activity can occur in the presence of NMDA receptor antagonists after a short period of block [29,49]. Nonetheless, such properties could still play an important role — perhaps locally in dendrites where they could serve to amplify synaptic inputs.

Another mechanism of rhythmicity originates from hyperpolarization-activated conductances, such as the low threshold t-channels or the mixed cationic conductance $I_h$ [53,128,130]. These conductances underlie post-inhibitory rebound and can set up oscillations when the neurons containing them are reciprocally connected to inhibitory neurons. This mechanism has been proposed to be important in the genesis of swimming in the *Xenopus* embryo [163]. Although post-inhibitory rebound can be observed in embryonic chick motoneurons following hyperpolarizing current injection, it seems unlikely that these currents play a critical role in rhythmicity. This is because all synaptic potentials we have detected in embryonic chick neurons younger than day 12 ($<$ E12) are depolarizing. As a result, it is difficult to see how hyperpolarization activated currents could be recruited in the course of synaptic activity.

Our current hypothesis is that the mechanism controlling the cycling within an episode is similar to that regulating the occurrence of episodes, albeit on a much shorter (cycle to cycle) time scale. The most likely candidate is activity-dependent depletion of transmitter. Such short term synaptic depression has been shown to generate cyclical activity in modeling studies of cultured spinal neurons [178] and has been implicated in the epileptiform bursting of hippocampal slices [186]. Of course, short-term synaptic depression is not the only mechanism that could operate to limit firing within a cycle. Other cellular processes, such as spike frequency adaptation or summation of $Ca^{2+}$ activated $K^+$ conductances, could also be involved.

At present, these ideas for the rhythmicity within the episode remain hypothetical. To establish if some form of short term depression is responsible for the rhythm will require monitoring transmitter release in the interval between cycles and establishing the existence or otherwise of other cellular processes that depress firing.

### *3.4. Comparison between the genesis of locomotion and embryonic motor activity*

We have suggested elsewhere [147] that the spinal networks active during embryonic development may include the precursors of locomotor circuits. While this may be true, several important differences exist between the mechanisms of embryonic motor activity and the production of adult locomotion.

Probably the most important distinction between the two types of behavior, is in the way the networks are activated. In the adult, locomotion is initiated and controlled by descending commands from the brain. In the embryo, the properties of the spinal networks themselves determine how, and when, they are activated. Central to the autonomous generation of spinal activity in the embryo, are two properties that change during development. This is depolarizing early in development and becomes hyperpolarizing later [140,143,179]. As a consequence, these classically inhibitory neurotransmitters can be functionally excitatory during development [for review, see Ref. [47]].

A second important difference between embryonic and mature spinal networks is the existence of a ventral set of GABAergic spinal interneurons in the embryo that disappear later in development [15]. The fate of these neurons is unknown but we have proposed that they experience a transmitter switch, during development, from GABA to glycine [35]. Although the cells could die, this seems unlikely because developing spinal interneurons do not appear to experience a period of naturally occurring cell death [136]. This transient population of GABAergic interneurons appears to be responsible, in part, for controlling the occurrence of spontaneous activity in the embryo. The autonomous activation of embryonic spinal networks is clearly not locomotor in nature and it is presumed to be necessary for normal limb and network development [for reviews, see Refs. [69,148]].

### *3.5. Conclusion*

One of the important lessons to emerge from these studies is that spontaneous activity generated by spinal networks is a highly robust mechanism. By this, we mean that the generation of activity does not depend on the details of circuit organization. Periodic rhythmic activity can be produced by circuits of glutamatergic and cholinergic neurons or alternatively, by networks of GABAergic and glycinergic neurons. At present, we do not know which components and properties of the network are robust and which are not. Nevertheless, the presence of robustness provides embryonic rhythm generating networks with a mechanism that is resistant to major perturbations, generated either internally or externally (see Ref. [150] for further discussion of these ideas). It remains to be determined, whether locomotor networks in the adult spinal cord also exhibit robustness. If so, then it will be important to identify which network components and properties can tolerate variations, and which are tightly controlled.

## 4. Triggering, modulating and adapting the spinal pattern generator for locomotion in cats (Serge Rossignol)

What have we learned from studies on locomotor control mechanisms in cats and other animals that could be useful to therapeutic approaches in humans with spinal-cord

injuries? Our basic assumption is that some of the studied control mechanisms have been conservatively preserved through evolution and that the formulation of related basic concepts can at least help to outline a general framework of understanding that will guide therapeutic approaches, namely, in the field of locomotor rehabilitation. Some of the work reported by Barbeau below, which was initially based on work in animals, indeed illustrates the basic validity of this assumption.

This section will first define the concept of CPG (its importance and limitation) and then summarize some of the control mechanisms related to triggering, modulating and adapting this centrally generated locomotor pattern. The topic of locomotor control has been the subject of several general reviews during the last 15 years [18,87, 154,165,189].

### *4.1. The central pattern generator*

Rhythmic patterns can be generated in the lumbosacral spinal cord in the absence of phasic (paralyzed preparations) or tonic (deafferented preparations) afferent inputs as well as in the absence of command signals from supraspinal structures. The notion that neuronal circuits in the spinal cord are capable of generating such alternating reciprocal patterns of activity with the characteristics of coupling and frequency typical of locomotion, scratching, or fast paw shake is well established in the cat [86,153,167] as well as in other vertebrates (i.e., lampreys, tadpoles. chicks, neonatal rats and even in some primates such as the marmoset [reviewed in Ref. [165]]). The evidence for such a spinal CPG has been, up to now, less compelling in humans, although rhythmic alternating activity in the lower limbs after complete spinal cord section in humans has been described [Refs. [55,56,105,123], reviewed in Ref. [165]] and seen after epidural electrical stimulation of the spinal cord [57,180]. Recent work in normal humans using vibration of tendons also suggests the presence of a CPG in humans [90].

Although the notion of CPG suggests a relative autonomy of function, it should be understood that this CPG is, in normal conditions, in continuous interaction with afferent and descending signals. The notion of CPG is important because it suggests that many of the rhythmic patterns themselves are organized as spinal motor subroutines. These can be triggered by peripheral afferents and descending commands, modulated by the same signals and most probably, modified in the long term when there are permanent changes in these signals after central and peripheral lesions. Such interactions will now be considered.

### *4.2. Triggering the CPG: supraspinal initiation of locomotion*

In high decerebrate cats, locomotion on the moving treadmill, called ''fictive'' locomotion, after paralysis, can be triggered by electrical stimulation (around 100 μA at 20–60 Hz) of a region of the mesencephalon located below the inferior colliculus (the cuneiformis nucleus). As first described by Shik et al. [182], the speed of locomotion as well as the mode of coupling between limbs (walking, trotting, galloping) can be adjusted by modifying the strength of the brainstem stimulation as well as the speed of the treadmill. This mesencephalic locomotor region (MLR) and its closely located structure, the pedunculopontine nucleus (PPN) (see Fig. 3), probably exert their action through projections to the medial reticular formation, which in turn projects to the spinal cord via tracts in the ventral and ventro-lateral funiculus (VLF in Fig. 3) [115,116,165,181]. In acute decerebrate cats, lesion of these pathways prevents the activation of locomotion by MLR stimulation [187].

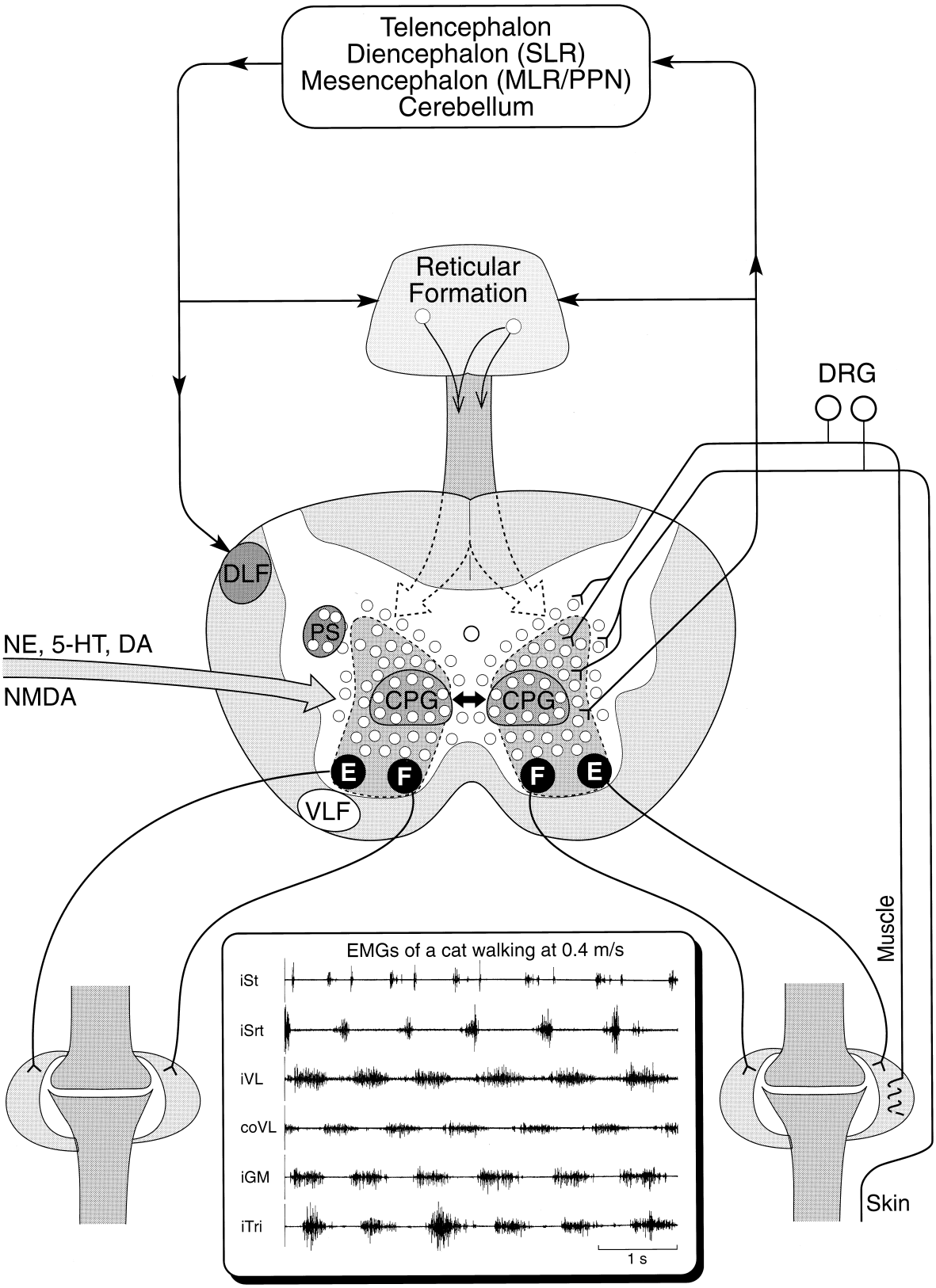
Massive chronic lesions of the ventral and ventro-lateral pathways in cats suggest that other descending pathways can also be used to trigger locomotion since such cats can regain voluntary four-legged locomotion overground after a few weeks, although some deficits such as decreased weight support and unstable forelimb–hindlimb coupling can persist [34,38,77,78,169,203]. It is probable that pathways coursing through the dorsolateral funiculi (DLF, pathways from the brainstem or the telencephalon or else propriospinal pathways) are responsible for the trigger of locomotion in such lesioned cats. Indeed, it has been suggested that there exists a strip of cells in the brainstem (pontomedullary locomotor strip) whose locomotor initiating capabilities are exerted through a dorsolateral system (DLF in Fig. 3) [119,120]. Chronic lesions of the dorsal columns and DLF may result in foot dragging and an inability to properly negotiate obstacles placed on the treadmill belt [114] but does not prevent the ability to generate locomotion [78].

From the above studies, we conclude that there appear to be several descending tracts capable of triggering locomotion in cats (Fig. 3). Similar conclusions can also be reached from study of partial spinal lesions in humans [139]. This conclusion is important in the present context since it suggests the possibility that electrical stimulation of different tracts could ''tap'' the spinal cord and initiate the locomotor pattern.

### *4.3. Pharmacologic triggering of locomotion*

The pioneering work of the Swedish school [86,111,112] has laid the foundation for pharmacologic activation of spinal locomotor circuitry. They have shown that L-DOPA, a noradrenaline precursor, especially after potentiation by a monoamine oxidase inhibitor, can activate a fictive locomotor pattern in the paralyzed spinal cat. Similarly, it was shown that clonidine, an α2-noradrenergic agonist [71], could trigger locomotion of the hindlimbs in acute spinal cats. We have also shown that, within the first week after

spinalization, clonidine can induce locomotion and that the characteristics of the pattern evoked varies with time after spinalization [23,25,27,28,44,45,153,168]. Fig. 4 gives a dramatic example of locomotion being initiated by an i.t. injection of clonidine in a cat 8 days after a complete spinal transection at T13. The figure represents the kine-



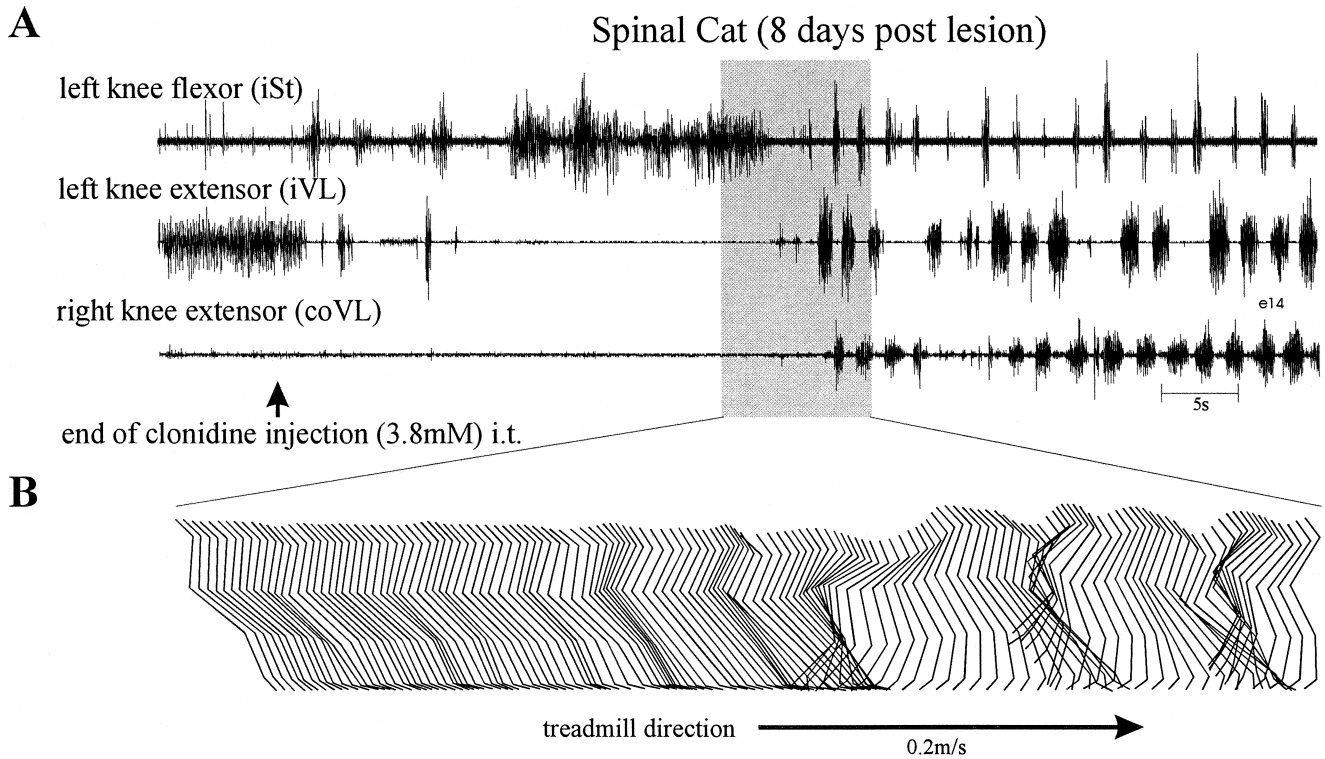



Fig. 4. I.t. injection of a bolus of 100 μl (3.8 mM) of clonidine in a spinal cat 8 days post-lesion. See text for description. Muscle abbreviations are as in Fig. 3.

matics and associated EMG activity of one flexor muscle and two extensor muscles of the knee, one on each side. Before the shaded rectangle, there is no organized EMG activity nor any rhythmic movements of the leg facing the camera. The leg is actually dragged from left to right by the moving treadmill belt. The arrow points to the time of the end of the clonidine injection. About 1 min after the end of the injection, the cat starts to walk as evidenced by the movements of the limb and the rhythmically organized EMG.

We have been unable in the cat to trigger locomotion in the first post-spinalization week with agonists of other systems such as serotonin, dopamine or NMDA. This does not imply that these cannot trigger locomotion in other species or are not important in other aspects of locomotor control.

### *4.4. Sensory evoked locomotion*

Sensory stimuli can trigger locomotion in high decerebrate cats. The proximity of the pontomedullary locomotor strip to the trigeminal sensory nuclei has led some [141] to hypothesize that the trigger of locomotion by stimulation of this area might be related to a stimulation of the trigeminal nucleus. In decerebrate paralyzed cats, tonic stimulation of the dorsal roots can evoke fictive locomotion [41]. In chronic spinal cats, simply moving the treadmill belt is sufficient to initiate locomotion [26]. In less

Fig. 3. Summary of structures involved in the initiation and control of locomotion (for more details see text and Ref. [165]). This diagram illustrates how spinal circuitry (CPG) is thought to produce the basic locomotor pattern by exciting and inhibiting groups of interneurons as well as flexor (F) and extensor (E) motoneurons (enclosed in the shaded area), which in turn activate muscles, each of which has its own pattern of activity or its ''signature''. This spinal circuitry can be activated by supraspinal structures located in the telencephalon, diencephalon (SLR, subthalamic locomotor region); mesencephalon (MLR, mesencephalic locomotor region; PPN, pedunculo-pontine nucleus) and perhaps the cerebellum. The MLR exerts its effect through the reticular formation which projects through the VLF (ventrolateral funiculus shown in transparency). Other structures may exert their effect through the DLF coursing dorsolaterally (DLF, dorsolateral funiculus) and through propriospinal interneurons (PS). Afferents originating from the skin and muscles, with their cell bodies in the DRG (dorsal root ganglion) can influence the CPG. Various neurotransmitter systems can also trigger or modulate the locomotor pattern (NE, noradrenaline; 5-HT, 5-hydroxy-tryptamine (serotonin); DA, dopamine; NMDA, *N*-methyl-D-aspartate). Other abbreviations: co, contralateral; EMG, electromyogram; GM, gastrocnemius medialis (ankle extensor); i, ipsilateral; m/s, meters/second; Srt, sartorius (hip flexor and knee extensor/flexor); St, semitendinosus (knee flexor and hip extensor); Tri, triceps (elbow extensor); VL, vastus lateralis (knee extensor).

active cats, especially early after spinalization, perineal stimulation is a powerful stimulus to induce locomotion.

### 4.5. Modulating the CPG: supraspinal stimulation

The characteristics of the locomotor pattern can be changed by stimulation of the various descending pathways (see Fig. 3) to accommodate for changes in speed, direction and posture. This subject has been reviewed extensively in various papers cited earlier but some general principles will be recalled because these might be important in the present context where electrical stimulation may be envisaged as a therapeutic avenue. First, responses evoked during locomotion are quite different from those evoked at rest. For instance, stimulation of the medial reticular formation may evoke at rest some generalized excitatory responses involving several muscles, even antagonistic muscles [60,61]. During walking, the same stimulus can yield well-organized reciprocal responses in several muscles that would tend to enhance the ongoing locomotor coupling of muscles at the time of stimulation [62]. These responses are not only state-dependent, but also phase-dependent so that the same stimulus applied to the reticular formation may yield an excitatory response in an extensor during one phase and an excitatory response in the antagonist flexor in the opposite phase of the locomotor cycle. Altogether, this suggests that the spinal pattern generator has some degree of autonomy and that the responses to descending stimuli are a function of the state of the spinal circuitry. This principle has been well established now since the work of Orlovsky [151].

On the other hand, it is also becoming clear that certain stimuli given to the reticular formation during fictive locomotion [151] or to the MLF or Deiter's nucleus [172], as well as stimuli to the motor cortex during locomotion in intact cats, can also reset the locomotor cycle [16,17] and thus have more than a modulating effect, but a rather profound effect on the timing of the pattern itself.

### 4.6. Pharmacologic modulation

While in the spinal cat the noradrenergic system is mainly implicated in triggering locomotion, all neurotransmitter systems exert modulating effects on some characteristics of the locomotor pattern and cutaneous reflex excitability [23,25,28,166,168]. For instance, in the spinal cat, noradrenergic agonists will tend to increase duration of the bursts as well as the step length and duration for a given treadmill speed. Serotoninergic agonists, on the other hand, will tend to increase markedly the amplitude of discharge of all muscles but especially extensor muscles. Dopaminergic agonists (Apomorphine) appear to increase the excitability of the flexor muscles. Although NMDA agonists have been shown to initiate locomotion in high decerebrate cats [59], we have been unable to initiate locomotion with these drugs in the very early period after the spinal lesion [46]. However, in late spinal cats, locomotion can be reinstated by NMDA after previously blocking it with AP5, a specific NMDA blocker.

The effects of various agonists on locomotion can depend on the type of preparation. For instance, whereas clonidine can initiate locomotion in the complete spinal cat, it can be deleterious to locomotion in cats with massive ventral and ventrolateral lesions [38,39]. Although this might be due to a different overall effect of the agonist in the presence of both pre- and post-synaptic receptors, there is also the possibility that the drug may act on the excitability of reflex pathways, which could be essential to maintain locomotion in cats with partial lesions. These observations are undoubtedly important for the evaluation of drugs in humans [54].

### 4.7. Sensory modulation

The subject of interaction between sensory inputs and locomotion is vast, has been reviewed extensively [165,171] and is covered in the section by McCrea above. Fig. 3 illustrates some of the afferents originating from the skin or from muscles that may impinge on the CPG or else terminate on neurons and interneurons which are under the influence of the CPG activity (darker shaded area). During fictive locomotion, the CPG can be entrained by cutaneous stimuli [170] as well as proprioceptive stimuli [165, 170,171], indicating that they may have a powerful influence on the timing of the centrally generated pattern. This has been directly studied during fictive locomotion by analyzing the effect of static limb positioning on the centrally generated pattern [154,174]. From these studies, we can conclude that retraction of the forelimb or extension of the hip greatly accelerates the basic rhythm frequency whereas protraction of the forelimb or flexion of the hindlimb can altogether arrest locomotion.

Brief electrical or mechanical stimuli to the skin or skin nerves can produce various responses depending on the phase of the step cycle. Thus, a stimulus during swing can activate flexor muscles while the same stimulus during stance may have no effect or may excite or inhibit ongoing extensor activity [165,171]. An important aspect for the focus of this paper is that these responses are well-integrated within the step cycle and therefore can be used to enhance the ongoing phase of the movement.

The importance of proprioceptive afferents during locomotion has been assessed in various experiments. In cats, if the foot is placed in a hole instead of firmly on ground, the precontact EMG activity is the same (presumably of central origin) but the subsequent normal stance activity is turned off, suggesting that the propriospinal inputs at foot contact may be important in maintaining such activity in the ankle extensors [98]. This has also been more directly studied in humans by evaluating the gain of the stretch reflex during the early stance phase, leading to the conclu-

sion that stretch reflexes may contribute substantially to the muscle activity during stance [190,201].

A further important consideration here is that such proprioceptive reflexes are state-dependent. As McCrea has detailed above, afferent inputs from some proprioceptors like the Golgi tendon organ which are inhibitory at rest, become excitatory during locomotion [79,88,98], furthering the importance of such proprioceptive inputs during locomotion.

### *4.8. Adapting the CPG*

One of the key questions in therapeutic strategies for rehabilitation is the question of plasticity of locomotor mechanisms. Such strategies must take into consideration the fact that the organism has already adapted perhaps optimally to the central or peripheral lesion of the nervous system. Secondly, one of the hopes in these strategies is that external devices and/or other forms of stimulation can modify the generating and control mechanisms of locomotion on a long-term basis. Some evidence that there is some degree of plasticity has been described recently, as discussed below.

### *4.9. Locomotor training*

After spinalization, cats progressively recuperate locomotor functions of the hindlimbs. This progression suggests that the generating locomotor circuitry is being progressively modified. Intensive locomotor training appears important to achieve optimal locomotor recuperation [26,33,103,152]. As mentioned above, the effect of some drugs varies with time after spinalization, suggesting that the spinal circuitry changes functionally with time after injury. A recent study has shown that the locomotor pattern evoked with clonidine within the first 10 days post-spinalization improves daily [44]. Furthermore, after about 10 days of intensive training, with daily injection of clonidine, there is expression of a well-organized locomotor pattern with hindquarters weight support and proper foot placement [44]. Such results are important for the training studies done in humans as will be summarized by Barbeau.

### *4.10. Adaptation to lesions*

The mechanisms of locomotor adaptation form an emerging field in studies of locomotion. Whereas there are probably some major hardwired components of the CPG, such as those related to the strict alternation between antagonist muscles at a joint [72], there might be other plastic adaptation related to muscles used in compensating strategies in the case of peripheral lesions. In a recent study [43], we have shown that normal cats can adapt very rapidly within 1–2 days to the neurectomy of the ankle flexors tibialis anterior and extensor digitorum longus by producing a small but sufficient increase in knee and/or hip flexion during the swing phase. After spinalization, the fine compensation seen in the otherwise intact cat is lost but the spinal locomotion which is normally regular and well organized, is now quite disorganized and dominated by knee hyperflexions as if the spinal cord had ''learned'' part of the mechanisms used to overcome the deficit before spinalization. Other studies of this kind are now underway to investigate the spinal capabilities to adapt locomotion to cutaneous denervation of the paws.

### *4.11. Conclusions*

From this brief overview of animal experiments, we can extract some concepts which may be important for designing therapeutic strategies for humans with SCIs.

- There is most probably in humans a spinal (or brainstem–spinal) component of the locomotor generating mechanism which could be accessed through various types of sensory or descending inputs or by pharmacologic stimulation.
- All types of inputs to these locomotor control mechanisms have to be evaluated within the context of locomotion because the effects of these stimuli are state-dependent and phase-dependent. Knowledge of responses in static conditions only may not be adequate.
- These stimuli also have to be evaluated in various types of spinal lesions and at various times after the onset of the lesion because these stimuli may interact with control mechanisms which have themselves been changed (quantitatively or qualitatively) as a result of the lesion.

Based on the knowledge obtained from animal experiments, a combination of (1) locomotor training with the addition of adequate pharmacotherapy (to reduce spasticity or enhance the activity of locomotor generating circuits) and (2) FNS to mimic the action of some central or peripheral pathways will probably constitute the best approach to locomotor rehabilitation in patients with spinal-cord injury.

## 5. Can locomotor training enhance the recovery of walking following SCI? (Hugues Barbeau)

SCI with an incomplete motor function loss (IMFL) is associated with multiple motor problems that lead to altered walking behavior, which is reflected by a reduced walking speed, postural problems related to bearing weight, maintaining balance and hyperactive spinal reflexes. This section presents some of the recent developments and concepts emerging from both animal and human studies aimed at enhancing recovery of walking following SCI.

New locomotor training and pharmacological interventions, used individually or together, have been identified as important approaches that modify the recovery process following SCI in humans. We conclude that the nervous system continues to manifest a great deal of plasticity even several years after SCI.

### 5.1. Locomotor training approaches

Animal findings (see Rossignol's section above) and clinical observations that neurologically impaired subjects cannot adequately bear weight on the more affected lower limb have led to the introduction of locomotor training, using a treadmill and body weight support (BWS). This technique was proposed by Finch and Barbeau in 1985 [70] and was introduced in SCI subjects with IMFL [22]. BWS reduces the load borne by the lower limbs and can be provided by various means: pneumatic [75], pulley [22,142], spring [95] and robotic systems [184]. Supporting a percentage of the body weight (up to 40%) was associated with an increase in comfortable walking speed, single-limb support time, stride length, and endurance [195]. The kinematic pattern revealed a straighter trunk associated with improved hip, knee and ankle joint angular excursion. Furthermore, EMG activity throughout the cycle showed more normal timing.

There are many clinical implications and advantages of such locomotor training strategies. The different components of gait may be retrained simultaneously under dynamic conditions. Locomotor retraining may be initiated early in the rehabilitation period, with BWS provided as needed for the subject to assume an upright position and to allow assisted or unassisted stepping by the lower limbs. As subjects walk on the treadmill with a reduced load on their lower extremities, equilibrium demands are reduced and gait deviations may be alleviated. The use of BWS can also be combined with other strategies. Recent clinical studies suggest that such locomotor training exercise combined with BWS is important in optimizing walking patterns and in achieving full weight bearing (FWB) [21,196].

Our laboratory has investigated the effectiveness of such a locomotor training approach in subjects with SCI or stroke. One study examined the effect of 6 weeks of interactive locomotor training with BWS on the kinematic pattern in nine people with chronic SCI-IMFL. The locomotor training was performed on a treadmill, with the subject mechanically supported in a comfortable overhead harness at different percentages of BWS, monitored with a strain gauge transducer [22]. At the end of 6 weeks of training, overground walking capability improved in subjects who had reached FWB on the treadmill; and weight support capability improved in subjects who had not yet reached FWB. (*FWB group*: overground speed: 0.11 m/s $\pm$ 0.11; treadmill speed: 0.24 m/s $\pm$ 0.7 vs. *non-FWB group*: overground speed: 0.04 m/s $\pm$ 0.04; treadmill speed: 0.13 m/s $\pm$ 0.11).

In a randomized clinical trial, stroke patients who received 6 weeks of gait training with BWS ($n = 50$) recovered better balance and walking abilities than those who received similar gait training while bearing their full weight ($n = 50$). Fourteen sitting and standing activities were each scored on a five-point scale [196] (see Table 1).

A 3-month post-training follow-up revealed that those who trained with BWS continued to have significantly higher scores for overground walking speed and lower limb motor recovery (0.52 m/s compared to 0.30 m/s, $p = 0.006$). The effect of locomotor training using BWS is presently being investigated in subjects with various neurological disorders of varying severity [21,58,97,197,197a], and has been compared to a conventional physiotherapy program [96,97].

FNS was introduced more than 35 years ago by Liberson et al. [127] as an orthotic system that replaced conventional braces with motor responses elicited by stimulating the common peroneal nerve during walking. While Liberson applied FNS to preventing footdrop in hemiplegic subjects, Kantrowitz [118] was the first to implement the new technology in SCI subjects. FNS systems have become increasingly complex; some have up to 50 channels of stimulation that operate under closed-loop-control based on signals from foot switches or goniometers. FNS has been used to restore a variety of movements including walking [202] and was first reported for SCI-IMFL subjects in 1989 [19]. However, this approach has not been widely used in rehabilitation for a number of technical reasons. Early stimulators were bulky, unreliable, prone to breakage and expensive [188]. Recent studies of simple systems for FNS-assisted walking (common peroneal nerve stimulator) showed a limited increase of the walking speed ($< 0.1$ m/s) and a reduction of oxygen consumption during gait [80,188].

In our laboratory, the combination of a simple FNS system with locomotor training has been effective for improving walking in subjects with SCI-IMFL [124a]. Within the first year, a gradual improvement of the maximal walking speed could be observed, with the mean magnitude of the increase being 0.26 m/s $\pm$ 0.24 ($N = 14$). But, more remarkably, the walking speed of people with SCI-IMFL increased even when the stimulator was temporarily turned off. This retained increase of walking speed without FNS is called the therapeutic effect and has the same magnitude as reported earlier (0.25 m/s $\pm$ .20, $N = 14$). This therapeutic effect on walking has been reported

Table 1
Effects of 6 weeks of gait training

| | BWS group | Non-BWS group |
|---|---|---|
| Balance score (maximum of 70) | 37.2 $\pm$ 2.1* | 29.4 $\pm$ 3.1* |
| Walking speed | 0.38 m/s** | 0.27 m/s** |

* $p = 0.001$; ** $p = 0.02$.

anecdotally for hemiplegic patients [127] and for incomplete spinal injury patients [81,124]. Changes in their assistive ambulatory devices from crutches to canes and changes in the ergonomic efficiency of the walking behavior were also observed. From these results, we conclude that increases in maximal walking speed during training with the FNS orthosis are mostly due to a therapeutic effect, which implies that plasticity occurred during training, as seen in the spinal cat. Finally, we conclude that BWS and FNS types of locomotor training stress different prerequisites for walking to achieve recovery of functional walking and, thus, their combination should be investigated.

### *5.2. Pharmacologic approaches to enhance locomotion in people with SCI-IMFL*

Based on animal findings [25,166,168], we believe that a rationale for locomotor pharmacotherapy can be developed for incomplete SCI. In a placebo-controlled study of the effects of clonidine on the walking pattern of SCI subjects, those with clinically complete SCIs were supported and assisted in stepping and their ability did not change with clonidine [54,192]. The inability to trigger a locomotor pattern in complete SCI subjects could possibly be explained by several factors such as the chronic state of the patients (see discussion in Ref. [192]).

In contrast, the most severely impaired SCI-IMFL subject showed a marked improvement in walking ability, as revealed by EMG and kinematic pattern [192]. More normal activation patterns of the tibialis anterior and soleus muscles have also been observed in two cases treated with clonidine [30] and with tizanidine [121].

Recently, a brief report was published on the acute effect of an i.t. injection of a bolus of clonidine on the overground walking pattern of an SCI-IMFL subjects [160]. Within a half-hour of clonidine injection, three of the 10 SCI-IMFL subjects doubled their maximal overground walking speed. The speed gains were maintained or increased over successive evaluations during the subsequent 6 h. During the same time period, the flexor reflex amplitude and the resistance to passive movement were reduced whereas the H-reflex amplitude remained unchanged. In the same subjects, the changes in walking speed, flexion reflexes and spasticity score were minimal on days when placebo injections were given. The reduction of signs of spasticity concurrent with the increase of walking speed suggests that i.t. clonidine may be a powerful medication for SCI-IMFL. Furthermore, these results are consistent with findings of changes in locomotor pattern and reduced excitability of spinal reflexes with clonidine in chronic spinal cats. However, the relationship between change in reflex and change in locomotor behavior is still unclear.

### *5.3. Locomotor training combined with pharmacologic approaches*

The combination of noradrenergic drugs and locomotor training has proven powerful in accelerating the recovery of locomotion in spinal cats [23–25,166,168]. In our laboratory, we have conducted a similar investigation with human subjects. The combined effects of clonidine and cyproheptadine together with a locomotor training program using BWS and treadmill walking exercise were investigated in chronic SCI-IMFL subjects. They had completed standard rehabilitation but were unable to walk at entry,

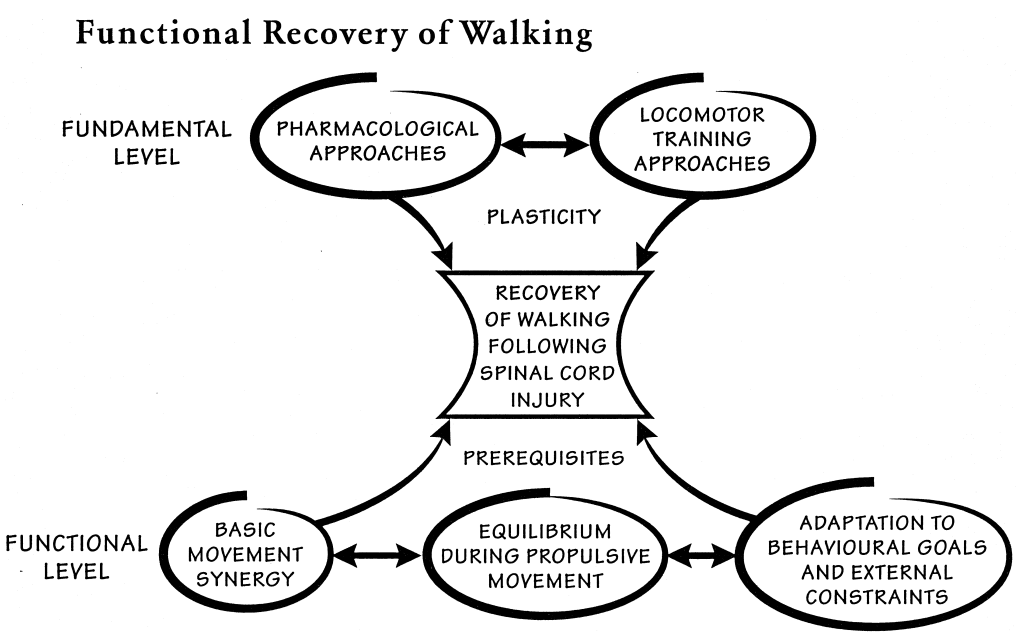



Fig. 5. Schematic representing fundamental and functional levels of recovery of locomotion.

into the study [73]. Both subjects were able to initiate walking on the treadmill while taking these drugs, and 6 weeks of locomotor training with continued drug therapy further improved their locomotor capabilities. The subjects achieved walking with FWB on the treadmill, and some limited overground walking was achieved with walking aids. On the treadmill, they showed a higher walking speed associated with a greater stride length and greater angular excursions at the hip, knee, and ankle. This has also been observed when medications have been combined with locomotor training using FNS devices [124]. These findings suggest that locomotor training using BWS or FNS could be powerful approaches to neurological rehabilitation, after the locomotor pattern has been expressed with medication.

### *5.4. Conclusion*

From fundamental studies in both animals and humans following SCI, we can conclude that it is important to understand the recovery process following SCI as well as underlying factors that can influence this recovery process. New locomotor training, pharmacological approaches and their combination have been identified as important factors to modify the recovery process following SCI in both animal and human. These results suggest that a great deal of plasticity is possible following SCI, and that changes could take place for many years following the injury (see Fig. 5). These new approaches are still in experimental stages in SCI-IMFL subjects: several studies are underway to evaluate (a) efficacy of locomotor training approaches using walking exercise and BWS, (b) functional electrical stimulation, (c) pharmacological approaches and (d) combinations of (a), (b) and (c) at different times following SCI. In addition, treatment strategies should address three important prerequisites to performing the locomotor task adequately and efficiently; (1) basic or stereotyped movement synergy, required to achieve propulsion; (2) maintenance of equilibrium during propulsive movement; (3) adaptation of the locomotor pattern to the behavioral goals of the person as well as to the constraints introduced by the environment such as obstacles, change of direction and speed (see Fig. 5). We strongly believe that a combined approach can provide a comprehensive and integrated program for the enhancement of locomotion in individuals with SCI or with other neurological impairment.

## 6. Control issues in using spinal circuits to restore functional movement (Michel Lemay)

### *6.1. Cyclic vs. static movement*

It is very likely that the circuitry involved in the control of posture is part of the spinal cord circuitry responsible for cyclic motion, and it too may be activated via microstimulation. For example, Giszter et al. [76] stimulated the paralyzed frog's spinal cord at the lumbar–sacral level at the level where McCrea (see above) showed the existence of disynaptic interneurons that had an extension function during gait. Giszter et al. [76] measured the forces produced at the ankle, and found that microstimulation produced a coordinated behavior that often showed a convergence to a single point in the work space. He found four types of force patterns that can be thought of as being parts of a swimming cycle. Later, Saltiel et al. [173] used NMDA in the frog in the same area and was able to elicit tonic responses and cyclic responses. The directions of the static force vector measured at a single limb position were found to be a subset of the directions of the force plateaus produced during cyclic responses. This result seems to indicate that the static force fields that could be combined to produced novel movements may be a subset of the circuitry used during rhythmic motion. This raises the question as to whether spinal circuitry for reaching exists since the upper extremities are generally not involved in cyclic movements. A series of papers by Alstermark and Sasaki [3–5] and Alstermark et al. [6–11] indicate that there is a spinal circuit involved in the control of the forelimb in cat, although the effect of the circuit on the limb mechanical properties has not been characterized yet.

### *6.2. Duration of activation*

Nerve and muscle activation in FNS systems is of an on/off nature: with stimulation on, muscle is activated; when stimulation is off, muscle is passive. Spinal cord circuitry seems to be very different. Under pharmacological influence, activation of spinal circuits can be fairly prolonged. With electrical activation the duration of the activation varies. Some sites produced long plateaus that outlasted the stimulation by up to 3 s, while other sites were of an on/off nature [126]. With better mapping of the cord, we may be able to sort out which spinal circuits are best suited to producing posture-maintaining contractions, and which circuits are best suited to producing fast motion contractions. Using both electrical stimulation and pharmacological treatment, we may be able to selectively control the spinal circuits suited for movement (stronger, shorter duration) and the ones better suited for the control of posture (weaker but longer duration).

### *6.3. Modification of the response during motion*

Rossignol refers to the fact that the response obtained during a portion of the gait cycle may not be the response obtained during another portion. This raises the question of how relevant our standard measurements of isometric force will be to actual muscle output during motion. Isometric measurements may not be sufficient. In an ongoing investi-

gation, we are taking isometric force measurements during microstimulation of the spinal cord in frogs, and comparing the isometric forces measured (correlated in time and space with the trajectory) to the forces obtained via an inverse dynamic analysis of the motion produced by the microstimulation. We are finding the forces' magnitude to be an order of magnitude apart, differences that are too large to be accounted for by unmodeled dynamic elements such as friction or damping.

This indicates that the measurements required to implement a functional neuroprosthesis will need to be matched to the desired functional tasks; for example, recruitment characteristics for a walking system will need to be measured during walking.

Finally, spinal circuits have evolved to facilitate the control of multi-joint motion — walking or reaching — and they can be evoked to facilitate the functional control of those limbs. We will have to think differently about controlling stimulation through spinal circuits than we do about directly controlling muscle contractions to produce torques at desired times. We will probably have to use electrical stimulation in combination with pharmacological agents to modify the excitability of tissue at times. We will have to deal with the fact that responses will be state-dependent. However, the level of coordination provided by the spinal cord will facilitate the control of multi-joint movements, tasks that with traditional FNS require a sophisticated timing of the activation pattern, timing that is sensitive to the different rates of muscle fatigue.

## 7. Technical challenges to using spinal circuitry in neural prostheses (Warren Grill)

A central characteristic of spinal neural control circuits is state-dependence; meaning that the output generated by a particular stimulus is dependent upon the state of the physiological system. McCrea discusses state-dependent excitation of extensor motoneurons by group I afferents via an interneuronal pathway, and also state-dependent increases in motoneuron excitability. Rossignol reinforces the fact that modulatory segmental and descending inputs have state-dependent effects on ongoing motor behaviors. Since segmental reflexes are largely preserved after chronic SCI, they may influence, in a state-dependent manner, motor behaviors evoked by activation of spinal circuitry. These state-dependent effects will complicate the engineering task of generating a behavior using electrical activation of the nervous system. Classical engineering control theory is built on the premise of linear, time invariant input–output properties. The state dependence of spinal input–output systems challenges classical engineering techniques, and will require innovative solutions, perhaps including adaptive control and fuzzy logic.

There are several other practical and technological issues which must be addressed. First, the location of the spinal neurons that regulate the behavior of interest must be identified. In animals, there are a number of techniques by which the locations of neurons may be identified including conventional retrograde and anterograde tracers, as well as a relatively new retrograde transneuronal viral tracer (psuedorabies virus) that allows identification of higher order neurons after injection into an end organ [175,193]. In addition, expression of the immediate early gene c-*Fos* enables identification of neurons that were active during a particular behavior [20,83,113,138]. Thus, there is a variety of powerful tools to identify neuronal locations in experimental animals, but it is not clear how this would be accomplished in humans. If the resolution of non-invasive imaging techniques such as positron emission tomography (PET) or functional magnetic resonance imaging (f-MRI) improves to the submillimeter range, then these techniques will allow location in the spinal cord of the neurons that control specific behaviors.

Second, implementing a neural prosthesis by electrical activation of neural circuits within the spinal cord requires a long-term interface between the electrodes and the biological tissue. High density arrays of silicon microelectrodes are presently being developed [12,42,106]. However, the mechanical properties of silicon are dramatically different from those of the spinal cord. This mismatch in mechanical impedance will create differential motion under acceleration and may lead to mechanically induced tissue damage and instability. A new generation of devices, perhaps incorporating flexible, conducting polymers is needed to build long-term stable interfaces with the spinal cord [e.g., Ref. [137]].

Third, chronic electrical activation of spinal neurons will require knowledge of what stimulation levels can be tolerated by spinal cord tissue. Although non-damaging levels for stimulation of the cortex have been identified under certain conditions [1], recent studies have found that non-damaging levels of stimulation within the brainstem (cochlear nucleus) are different from those in the cortex [134]. Thus, similar differences may also exist between the non-damaging levels for stimulation of the spinal cord and other CNS tissues. Previous data suggest that the non-damaging levels of stimulation will also depend on the electrode material, surface area, and the pattern of the applied stimuli. Thus, an important question is how much stimulation can spinal cord tissue tolerate on a chronic basis [200].

Finally, if we have identified a group of neurons using imaging or tracing, then techniques are required to localize activation to a small group of cells without activating neighboring axons and cells, which may generate antagonist functions. The spinal cord is a complex inhomogeneous, anisotropic volume conductor, and the pattern of neuronal excitation generated by extracellular stimulation is strongly influenced by the electrical properties of the surrounding tissue [82]. Further, spinal neurons are geometrically complex with extensive dendritic arbors, vary-

ing somal dimensions, different electrical properties, and tortuous axons. The pattern of excitation generated by a particular stimulus depends on the spatial relationship between the electrode and the neuronal structure as well as the stimulus parameters [91,135]. Thus, new electrode geometries and stimulus parameters are required to produce controlled and selective activation of targeted neuronal populations.

The resolution of these scientific and technological questions will make possible a new generation of neural prostheses, which use relatively simple implanted devices, coupled to biological neural networks, to achieve control of complex behaviors. Microstimulation of the spinal cord with implanted electrodes in people with neurological disorders gives the possibility of targeted and controlled release of neurotransmitters and, thus, the possibility of exploiting the mechanisms used by the nervous system to regulate complex functions.

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