Probing the Human Spinal Locomotor Circuits by Phasic Step-Induced Feedback and by Tonic Electrical and Pharmacological Neuromodulation

Ursula S. Hofstoettera, Maria Knikoua, Pierre A. Guertinta and Karen Minassiana,*,d

aCenter for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria; bThe Graduate Center, City University of New York, NY, USA; cDepartment of Psychiatry and Neurosciences, Laval University, Quebec City, Canada; dCenter for Neuroprosthetics and Brain Mind Institute, School of Life Sciences, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland

Abstract: The mammalian lumbar spinal cord experimentally isolated from supraspinal and afferent feedback input remains capable of expressing some basic locomotor function when appropriately stimulated. This ability has been attributed to spinal neural circuits referred to as central pattern generators (CPGs). In individuals with a severe spinal cord injury, rhythmic activity in paralyzed leg muscles can be generated by phasic proprioceptive feedback during therapist- or robotic-assisted stepping on a motorized treadmill. Here, we critically review to what extent the resulting motor output represents locomotor-like activity, and whether these motor patterns are the result of activation of CPGs, as commonly suggested in the literature. Attempts will be made to further delineate the pivotal roles played by mechanisms such as spinal proprioceptive reflexes and their alterations after spinal cord injury, the central excitability level, and by neurotransmitters critical for spinal locomotor activity. We will discuss the view that the muscle activity produced during assisted passive treadmill stepping is resulting from the entrainment of spinal reflex circuits by the cyclically generated proprioceptive feedback. We suggest that the activation of CPG circuits depends on the presence of a sustained tonic excitatory drive, as can be provided by electrical spinal cord stimulation, or by specific combinations of dopaminergic agonists, adrenergic/dopaminergic precursors and/or 5-HT receptor agonists. Novel rehabilitation strategies using spinal cord stimulation and rhythmic-activity producing drugs during locomotor therapy will pave the way for clinically relevant advances in restoration of motor function in people with severe spinal cord injury.

Keywords: Central pattern generator, locomotion, neuromodulation, rehabilitation, spinal cord injury, spinal cord stimulation, spinal reflexes, treadmill stepping.

1. INTRODUCTION

Locomotion is probably one of the most complex motor functions, normally involving virtually all areas of the central and peripheral nervous system. Under normal conditions, full expression of locomotion requires inputs from cortical areas (visual detection, planning and decision-making), mesencephalic areas (trigger on/off signals), as well as cerebellar and vestibular structures (equilibrium and tonus) [1–3]. The final integration and interpretation of these supraspinal signals takes place in the spinal cord. Indeed, locomotion critically depends on the complex interaction with several neural circuits that are located for most parts within the lumbar area of the spinal cord [4–10]. Monoaminergic and catecholaminergic terminals from descending pathways and their respective receptor targets in the spinal cord as well as spinal glutamatergic, GABAergic and glycinergic systems have all been shown to play modulatory, excitatory, and inhibitory roles in spinal circuitry-driven locomotor activity [11,12]. Both, segmental spinal reflexes and distributed CPGs (networks of spinal neurons that can produce rhythmic activity autonomously because of single-cell properties and specific connections between cells; [13]) are believed to control locomotor rhythm and pattern generation [8,9]. Peripheral inputs from proprioceptors (muscle spindles, Golgi tendon organs, and joint receptors) provide sensory feedback capable of enhancing, resetting or adapting each step to external factors [14–19]. A severe cervical or thoracic spinal cord injury (SCI) classified clinically as complete (American Spinal Injury Association Impairment Scale A; AIS A) or motor complete/sensory incomplete (AIS B) is associated with an integral loss of signaling activity and communication between supraspinal structures and the lumbar spinal circuits. As expected, an immediate and generally irreversible loss of the ability to generate and control locomotion occurs after such SCI. Another consequence associated with SCI is the adaptive transformation, including gene expression and receptor expression level changes, of sublesional structures in the spinal cord [20,21]. Following the isolation of the lumbar spinal cord from supraspinal control and the injury-related plasticity [21–24], proprioceptive afferent input processing [25–27] and rhythmic motor output generating capabilities [8,28–31] have been shown to enable, if appropriately stimulated, the expression of basic rhythmic leg muscle activity in paralyzed individuals.

One approach used to investigate the ability of the spinal circuits to produce rhythmic activity in individuals suffering of AIS A or AIS B injuries is analysis of the effects associated with assisted and body-weight supported (BWS) stepping on a treadmill. This method involves a motorized treadmill system where partial BWS is provided using a harness to a patient placed in upright position while bilateral alternating leg movements on the moving treadmill belt are manually assisted or executed by therapists, mechanical devices, or robotic exoskeletons [25,32–37]. Another method that can probe the involvement of spinal circuits in the generation of rhythmic leg motor activity in humans is epidural spinal cord stimulation (ECS) [28,38–41]. When applied over the lumbar and upper sacral spinal cord (located between the 11th thoracic and 1st lumbar vertebral levels), epidural stimulation has been shown to predominantly activate large-to-medium diameter afferent fibers entering the spinal cord through the posterior roots (dorsal roots) [30,42–46].
Additional neural structures that may be recruited by epidural stimulation are longitudinally running myelinated fiber branches in the most superficial layers of the dorsal and dorsolateral columns of the spinal cord white matter [47,48]. SCS may thus excite groups of afferents that are also activated mechanically during treadmill stepping, but in a tonic rather than phasic manner. The neural activation pattern that SCS provides when applied continuously at a constant stimulation frequency can be viewed indeed as a bilateral tonic drive to several spinal cord segments simultaneously. In contrast, the kinematic- and load-related feedback input to the spinal cord generated by assisted stepping movements on a treadmill can be viewed as phasic input to specific spinal cord segments. Hence, SCS and treadmill stepping may provide complementary approaches to study some of the spinal mechanisms involved in rhythmic motor pattern generation after SCI. In addition to probing the human lumbar spinal circuits through afferent projections activated mechanically or electrically, recent findings introduced the possibility of their pharmacological activation (clinicaltrials.gov/ct2/show/NCT01484184). Pharmacological stimulation using a specific combination of orally-active molecules (buspirone + levodopa and carbipoda) generated involuntary rhythmic muscle activity and left-right alternating leg movements in paralyzed individuals lying supine, thought to be mediated by CPGs (see companion paper, [49]).

In this article we discuss in-depth the results of studies that focus on mechanisms underlying rhythmic motor pattern generation by the human spinal cord isolated from supraspinal inputs by a severe lesion and probed by different types of stimulation. Pertinent information derived from animal experimental studies is provided on the mechanisms underlying the generation of locomotor activity and its regulation by afferent input.

2. AFFERENT STIMULATION TO PROBE SPINAL PATTERN GENERATING MECHANISMS: ANIMAL EXPERIMENTAL STUDIES

2.1. Afferent Input can Modify Ongoing Fictive Locomotion Induced by Tonic Stimulation

One method in classical physiology experiments to study sensorimotor interaction during locomotion is to centrally induce fictive locomotion (rhythmic activity recorded without any movement generated), and then test whether selective afferent stimulation disturbs or resets the regular locomotor rhythm [50,51]. For instance, in cat preparations, fictive locomotion is induced either by intravenous administration of L-DOPA (and the monoaminoxidase inhibitor nialamid) in curarized, acutely spinalized animals, or by tonic electrical stimulation of the mesencephalic locomotor region (MLR) in the decerebrate model. Under these conditions, rhythmic alternating activity is recorded from the nerves supplying flexor and extensor muscles. The spinal circuits underlying the generation of such rhythmic patterns of fictive locomotion in absence of phasic descending or peripheral inputs are referred to as a CPG [13]. A disturbance of the rhythm of fictive locomotion by specific afferent stimulation is typically considered as evidence for direct access of the stimulated fibers to the CPG.

Normally, in absence of locomotor activity, stimulation of group Ib afferents from extensor muscles evokes a short-latency, nonreciprocal inhibition among homonymous and close synergistic extensor motoneurons [52,53]. The same stimulation given during locomotor activity produces instead an oligosynaptic excitation in homonymous and synergistic extensor motoneurons throughout the limb [17,50]. Specifically, brief electrical stimulation of group I afferents from knee and ankle extensors effectively resets fictive locomotion during swing (to stance) or enhances extensor activity during stance. A contribution of muscle spindle Ia afferents and Golgi tendon organ Ib afferents has been demonstrated using different types of stimulation such as muscle vibration and low-intensity nerve stimulation [17]. These findings support the notion that extensor group I afferent have excitatory connections to the extensor-related elements of the CPG [17,54–56].

Resetting of an otherwise steady fictive locomotor activity can also be produced by brief trains of stimulation of the flexor reflex afferents (FRA) that include cutaneous, joint, and groups II and III muscle afferents. In curarized spinal cats, FRA stimulation delivered during the extension phase of fictive locomotion terminates the extensor activity and initiates an abrupt transition to a phase-advanced flexor burst, while the same stimulation delivered during the flexion phase results in prolongation of this phase [57]. It was earlier observed by Lundberg and colleagues that trains of FRA stimulation could induce short sequences of self-sustained rhythmic, alternating discharges in afferents to flexors and extensors in L-DOPA treated spinal cats [58,59]. These effects are state-dependent, occurring only under the same conditions (i.e., injection of L-DOPA and monoaminoxidase inhibitors) during which fictive locomotor activity may develop in the acute spinal cat [4]. Together, these results are interpreted such that at least part of the interneurons intercalated within the FRA pathways are elements of the CPG networks [58–60].

Natural stimulation of the proprioceptors from the hindlimbs can also alter the timing of induced fictive locomotion. Frigon and Gossard demonstrated that sensory input from load and stretch-sensitive receptors in ankle extensors influence the centrally generated rhythm during fictive locomotion in adult decerebrate cats [61]. Sustained slight dorsiflexion applied during episodes of spontaneous fictive locomotion significantly decreased the burst frequency of fictive locomotion by increasing the extension phase durations. Andersson and Grillner investigated the effect of proprioceptive feedback from hip movements on the spinal circuits underlying fictive locomotion in the acute spinal cats [62]. Fictive locomotion was pharmacologically elicited by intravenous administration of L-DOPA, and curarization prevented generation of actual movements. Imposed passive sinusoidal hip movements entrained the rhythmic motor pattern, even after the entire hindlimb including the skin was denervated, except for the hip joint and surrounding muscles.

The studies in cats reviewed in this section demonstrate that proprioceptive inputs from ankle and hip act phase-specifically upon the CPG to elicit powerful effects onto several muscle groups in a coordinated and synergistic manner. Afferent projections have parallel connectivity to motoneurons through segmental reflex pathways, but also through more complex, multi-segmental circuits, including the CPG. The coordinated effects of specific afferent inputs clearly require ongoing locomotor activity to be centrally generated. Indeed, afferent input was found in those studies to modulate CPG activity, but not to cause the locomotor activity. The CPG circuits were rather activated by a tonic drive provided by electrical stimulation of the MLR or by pharmacological neuro-modulation.

2.2. Afferent Feedback Input can Produce Stepping on the Treadmill Under Specific Conditions

In the experimental animal studies reviewed in section 2.1, the CPGs producing fictive locomotion were uncoupled from producing any movements. However, movement-induced afferent feedback can be utilized to assist generation or maintenance of functional rhythmic motor activity. Cats spinalized as adults can step involuntarily and bear weight through their hindquarters on a treadmill after intense training [63]. The stance and swing phase durations, the joint angular excursions, and electromyographic (EMG) activity of hindlimb muscles are similar to those of non-injured cats stepping actively at comparable speeds [63]. However, even in these cases some additional excitatory inputs, such as perineal stimulation, tail pinching/creeping, or plantar pressure is required during step training, at least at the initial stages of injury and
step training [64,65]. This suggests that although the adult spinal cat can perform weight-bearing hindlimb stepping on a treadmill after task-specific training [66], additional tonic excitatory inputs are required to initiate or maintain locomotor activity. Further, even after intense step training and tonic stimulation, locomotor abnormalities persist in the adult spinal cat, involving a later onset of EMG bursts, especially that of the medial gastrocnemius muscle, reduced EMG amplitude and muscle force, changes in synchronization of flexor muscles acting at various joints which are related to foot drag at the onset of the swing phase, clonic EMG activity, and absence of equilibrium control [67–69].

Rhythmic capacity is also present in other mammalian models used for studies of spinal locomotor circuits, such as mice and rats. A recent study showed that spinal cats can recover involuntary hindlimb stepping capability by treadmill training, but when pinching of the perineal area was applied throughout the training sessions [70]. Without such additional tonic stimulation, both untrained and trained rats produced only occasional hindlimb flexion movements, likely in response to stretch when the limb was drawn backwards by the motorized treadmill belt. Other means of stimulation are normally used in these species to augment the central excitability to a level that allows lumbar circuits to respond to step-related feedback with the generation of coordinated stepping movement on a treadmill. For example, robotic devices that mechanically modulate afferent input to the lesioned spinal cord [71], tonic epidural SCS that interacts with phase- and task-specific afferent feedback [72,73], and pharmacological agents such as serotonon and noradrenaline agonists [74,75] are used to increase locomotor activity of these chronic spinalized animal models for studying restored stepping on a motorized treadmill.

Thus, step-specific sensory feedback generated on a treadmill can induce hindlimb stepping in spinal cats, mice, and rats. Yet, in order to translate the afferent input into useful motor output, the lumbar circuits require additional activation by different types of tonic stimulation.

2.3. Drug Combinations can Induce CPG Activity with or without Significant Afferent Inputs

Smaller animal models such as mice and rats have been used to demonstrate in-vivo that putative CPG-mediated locomotor-like movements using pharmacological aids in non-curarized, spinalized animals are inducible. Air-stepping with mice or rats completely suspended with a harness has been used to remove most significant load-related group I inputs. No other stimulation, such as tail pinching, is applied in such studies. McEwen and colleagues have shown that neonatal rats with mid-thoracic spinal transection can express locomotor-like movements following L-DOPA (adrenergic/dopaminergic precursor) and quipazine (5-HT2A/2C/3 agonist) administration subcutaneously [76]. Comparable effects found in adult mice were shown to critically depend upon spinal dopaminergic, serotonergic, and glutamatergic systems [77–80]. Effective CPG activation is best obtained using specific combinations of molecules [79,81]. Different types of agonists or precursors (for example 5-HT1A/7 receptor agonists, D1/5 receptor agonists, L-DOPA, 5-HT2A receptor agonists) can trigger some CPG activity, often mixed with non-locomotor movements, in air-stepping or treadmill conditions but only specific combinations with these molecules have been reported to elicit full weight-bearing stepping on a treadmill in untrained, non-otherwise stimulated mice following spinal transection [75,82–84]. This is somehow reminiscent of findings made earlier in isolated spinal cord preparations showing that dopamine and 5-HT with or without NMDA induce superior CPG-activating effects than either molecule administered separately [85,86]. When drawing conclusions from these studies, we should consider that the cells on which the drugs are acting and their exact mechanism of action are not completely defined, yet no neural system below a complete spinal transection other than the CPG is known to trigger rhythmic motor activity in response to such pharmacological stimulation.

3. TREADMILL STEPPING AFTER SPINAL CORD INJURY IN HUMANS

It has been suggested that “... on a moving treadmill, individuals with spinal cord injury are enabled to perform rudimentary stepping movements. These movements evoke an appropriate afferent input to the spinal cord leading to leg muscle activation comparable with that during walking ...” [87]. The aim of this section is to critically review these assumptions.

3.1. Neural Activation Patterns Produced by Treadmill Stepping in SCI Individuals

In the generation of rhythmic leg muscle activity on a treadmill, proprioceptive feedback from the moving legs becomes one of the major sources for phasic modulation of spinal circuits after motor complete SCI [25,34,35,72]. In paralyzed individuals, each step is produced by external assistance under BWS conditions [88], either by physiotherapists [89] or robotic-driven gait orthosis systems [35]. The rhythmic, alternated loading and unloading of the legs together with the multi-joint flexion and extension movements result in the mechanical activation of peripheral receptors in the hip and lower-limb muscles, tendons, and joints. This stimulation produces gait-phase dependent sequences of afferent inflow to the spinal cord with complex temporal and spatial patterns (cf. [90,91]). When external assistance is withdrawn, the phasic EMG activation patterns cease and the legs drag or collapse [92,93].

3.1.1. Stretch-Mediated Neural Activation Patterns

The role of muscle spindle afferents in the control of movement, and the contribution of stretch reflexes in the generation of motor output during assisted treadmill stepping in individuals with motor complete SCI have received much attention for more than two decades [25,92,94,95]. The muscle spindle is a sensory organ that detects muscle length and the velocity of muscle stretch [96]. There are large-diameter group Ia and medium-diameter group II muscle spindle fibers. The primary endings are sensitive to both the static and dynamic components of muscle stretch, while the secondary endings are mainly sensitive to the static component. Thus, the steady-state length of a muscle is signaled by the discharge of both group Ia and II muscle spindle afferents, while the group II afferents are highly sensitive to the velocity of stretch during the period of the actual joint movement. Further, muscle spindle activity is controlled by gamma motor innervation through corticospinal pathways that can increase the firing rate of the sensory endings [96]. Firing rates of muscle spindle afferents increase with increasing muscle lengths during passive stretch and active eccentric muscle contractions. During passive shortening of the muscle, firing pauses because of the unloading of the spindles. However, during concentric muscle contractions gamma motoneurons are activated together with alpha motoneurons and spindle afferents discharge despite the shortening of the muscle.

The consequences of the disruption of descending gamma motoneuron activation after severe SCI are not well studied. Information gained from stroke patients [97,98] and from one study in individuals with chronic motor complete SCI and spasticity [99] suggest that firing rates of muscle spindles recorded at rest and during passive stretch are not different from that of control subjects with intact nervous system. Nevertheless, during rhythmic muscle shortening and lengthening movements, the absence of descending gamma modulation will inevitably affect the muscle spindle firing patterns. In individuals with intact nervous system, the gamma motor system is activated in different types of voluntary concentric contractions, resulting in substantial spindle activation during muscle shortening [100]. In paralyzed individuals, muscle spindles will not be activated during the shortening phases of a movement and their firing patterns during imposed flexion-extension movements.
may be similar to those generated by passive movements in individuals with intact nervous system. Burke and colleagues studied the responses of muscle spindles of the tibialis anterior in individuals with intact nervous system during passive and voluntary movements of the ankle joint [101]. Passive stretching and shortening movements of the relaxed muscle produced afferent firing only during the stretching phase. When the same movement was reproduced actively, particularly against an external load, the muscle spindles also discharged throughout the concentric phase of the movement. The voluntary activation under external load thus decreased the contrast between the afferent firing frequencies during the shortening and lengthening phases of the movements (Fig. 1). In addition, the firing frequency of the muscle spindle afferents was lower during passive stretch than during active eccentric contractions due to the gamma modulation occurring in parallel with the muscle activity.

During active stepping, activity of group Ia and II fibers from muscle spindles is influenced by gamma drive with tonic and phasic components that can have different contributions in different muscles, as suggested in cats [18,20,102,103]. Thus, the exact firing patterns of the stretch sensitive receptors will be modulated during passive treadmill stepping in the absence of volitional-contraction related modulation of gamma drive. Passive stretch of a muscle during imposed stepping movements on the treadmill would increase the firing frequencies of the muscle spindle afferents, while firing frequencies will decrease and drop to zero during the passive shortening phases of that muscle. This means that the spindle firing frequencies during alternated lengthening and shortening movements would become more phasic when compared to active walking of an individual with intact nervous system. Spindle firing would only contribute to muscle activation during the lengthening phases of the receptor-bearing muscle. The contribution of stretch reflexes to muscle activation may hence decelerate forward movements of the leg during swing or increase extensor-muscle and joint stiffness during stance of imposed stepping movements on the treadmill. Stewart and colleagues observed that when the EMG activity during assisted treadmill stepping in individuals with SCI was reduced (following the administration of clonidine), “... the research assistants reported a reduction in the resistance previously felt when pulling the leg forward ...” [92].

Physiological muscle spindle firing patterns would be further related to the exact kinematics of the externally imposed stepping-like motions on the treadmill. Some potential differences between the ankle- and knee-joint movement during assisted passive stepping of individuals with SCI and physiological stepping of individuals with intact nervous system on a treadmill shall be mentioned here. When the patient makes contact with the ground through the heel first, a rapid passive plantar flexion movement can follow due to lack of controlled eccentric contraction of the dorsiflexors at the early stance phase, resulting in a sudden stretch of the tibialis anterior. The occurrence of this non-physiological muscle spindle firing is specifically likely when using exoskeletons that do not permit active control of the ankle-joint movements. When the patient makes ground contact through the footrest first, ankle extensor muscle spindle afferents will fire earlier than during normal locomotion. Last, during manually-assisted stepping and minimum BWS of the patient, the knee joint at the end of the stance phase sometimes gives away, when the therapist stops supporting extension while preparing to initiate the swing phase of the stepping movements, resulting in a sudden stretch of the knee extensors and thus to another phase-inappropriate muscle spindle discharge.

Many spinal pathways control the synaptic effectiveness of afferent inputs and stretch reflex excitability. Specifically, sensory information is gated by presynaptic inhibition [104–107] depending on the state of the reflex and locomotor circuits [108]. When presynaptic inhibition is exerted at the afferent axon terminals on motoneurons, it can reduce the amount of transmitters that will be released by these afferents following their activation. Presynaptic inhibition is under descending control [95] and is modulated in a phase-dependent manner during stepping in humans [109,110], as well as in absence of any movement during induced fictive locomotion in the cat [111–114]. Presynaptic inhibition is thus regulated by descending control, sensory feedback as well as by the operation of the CPG. At the onset of selective voluntary contractions of individual muscles, presynaptic inhibition of Ia fibers to motoneurons of the contracting muscle is decreased, while presynaptic inhibition of Ia afferents on motoneurons not involved in the contraction is increased [115]. Thus, presynaptic inhibition at the onset of movement is organized in a way to increase selectivity of muscle activation. During locomotion, presynaptic inhibition filters afferent inputs according to the phase of the step cycle before the input can affect post-synaptic neural targets, and can thus reduce the impact of afferent inputs in phases where it could produce unwanted motor outputs. The absence or dysfunction of any supraspinal or spinal control mechanisms of presynaptic inhibition could thus result in the generation of non-functional, non-timely appropriate muscle activity during passive movement.

Indirect methods to study presynaptic inhibition in humans include the assessment of the soleus H-reflex size during specific motor tasks, and the amplitude modulation of the soleus H-reflex following conditioning stimulation of the femoral or common peroneal nerve, or mechanical tendon vibration [116]. The H-reflex size depends on the excitability state of motoneurons, as well as on the level of presynaptic inhibition acting upon the Ia afferent terminals responsible for the monosynaptic depolarization of alpha motoneurons [116]. When the H-reflex size alone is used in studies of presynaptic inhibitory control during a motor task or movement, it is based on the assumption that at similar background EMG activity
levels, postsynaptic inhibition remains largely constant and thus changes in the H-reflex size that are not in accordance to the background EMG modulation can be attributed mostly to presynaptic inhibition [116].

Based on this assumption, the task-dependent modulation of presynaptic inhibition was described in humans. During the transition from seated to standing, and from standing to walking, there is a rapid reduction of the H-reflex size even when there is little change in the EMG level during the initiation phase of the movement [117,118]. Capaday and Stein compared soleus H-reflex amplitudes and modulations in individuals with intact nervous system during active stepping on a treadmill and standing [14]. During stepping, the soleus H-reflex was strongly modulated in amplitude throughout the step cycle. The soleus H-reflex was small at the time of foot contact but increased rapidly to a maximum amplitude during late stance, and then decreased to a low value or was completely absent after toe-off and throughout the swing phase. This amplitude modulation of the soleus H-reflex was not always closely correlated to the background EMG modulation. Further, at similar soleus EMG levels, the soleus H-reflex was always much larger during tonic contractions while standing than during rhythmic activity while walking. Since the H-reflex amplitude was not always closely related to the EMG produced during stepping and was task-dependent (standing vs. walking), it was suggested that the modulations were not simply a reflection or function of motoneuronal excitability, but that presynaptic inhibition was a major contributor to these observations. A subsequent study of the same group found that while peak EMG activity of soleus during running was considerably higher than during walking on a treadmill, the maximum amplitude of the H-reflex was significantly smaller during running than during walking [14]. A tonic increase in the amount of presynaptic inhibition of the Ia terminals to alpha motoneurons during running was suggested as the most likely mechanism accounting for the difference in H-reflex gain in the two motor tasks. Knikou and colleagues compared the soleus H-reflex following conditioning stimulation to the antagonist nerve at an interval that the soleus H-reflex depression is associated with the presynaptic inhibitory network [110]. At similar background EMG levels, the conditioned soleus H-reflexes were modulated in a manner similar to the unconditioned soleus H-reflexes, and it was suggested that presynaptic inhibition was upregulated at heel contact and downregulated at late stance and early swing phases [110].

The pronounced locomotor-task specific presynaptic inhibition suggests a downregulation of Ia monosynaptic feedback contribution to muscle activation during active stepping in individuals with intact nervous systems [95]. There is a general understanding that presynaptic inhibition of Ia afferent terminals is decreased in spastic SCI patients [119–122]. Knikou and colleagues studied the modulation of the soleus H-reflex and of presynaptic inhibition during treadmill walking in people with motor complete and incomplete SCI [36,123]. The soleus H-reflexes showed considerably less modulation compared to healthy subjects during stepping, but the modulation patterns varied among patients. The most common pathological patterns observed were lack of or reduced soleus H-reflex depression during the swing phase and absent progressive facilitation of the soleus H-reflex from mid- to late-stance phases (Fig. 2).

In patients with more severe spasticity, the H-reflexes elicited at the different phases of the step cycle showed little or no modulation. Further, presynaptic inhibition exerted on Ia afferents, assessed via a conditioning soleus H-reflex protocol, was absent in motor complete and incomplete SCI individuals when tested at rest, and its modulation during assisted stepping was different to that of healthy control subjects [122]. It was suggested that a combination of mechanisms had resulted in the abnormal behavior of the soleus H-reflex during stepping, including impaired function of reciprocal inhibition that contributed partly to co-contractions between ankle antagonistic muscles (Fig. 3) and clonic EMG bursts in soleus [124], reduced levels of presynaptic inhibition, reduced levels of postsynaptic inhibition, and abnormal excitability of soleus motoneurons.

![Fig. (2). Step-related modulation of spinal pathways underlying the stretch reflex alters after spinal cord injury.](image)

![Fig. (3). Coactivation of ankle antagonistic muscles during stepping in individuals with spinal cord injury.](image)
In conclusion, a basic correlation of muscle spindle firing patterns with shortening and stretching of the receptor-bearing muscle during passive joint movements on the treadmill will remain after SCI. The exact firing patterns, however, will change in absence of supraspinal-controlled gamma modulation, probably resulting in a more phasic pattern synchronized to the muscle lengthening phases of movement. Additional differences of the muscle spindle firing patterns will result from differences between the exact kinematics of the assisted joint movements on the treadmill and voluntary stepping. Considering enhanced transmission of impulse trains at the Ia-motoneuron-synapse due to reduced homosynaptic depression in SCI individuals [108,122], and reduced functional presynaptic inhibition of Ia monosynaptic feedback normally associated with an active locomotor task [125,126], the effects of stretch-induced muscle spindle afferent volleyes will play a major role in the generation of the motor outputs as observed during assisted treadmill stepping. Yet, stretch reflex pathways may not contribute to a concentric muscle contraction, and altered proprioceptive-input processing following SCI can result in stretch-related muscle activity in physiologically inappropriate phases of the stepping movements.

3.1.2. Load-Mediated Neural Activation Patterns

Load-sensitive feedback provides another important contribution to the muscle activity during assisted treadmill stepping in SCI individuals [25,35]. The Golgi tendon organ, a proprioceptive receptor that senses changes in muscle tension and transmits this information via the Ib afferent pathway, is normally thought to provide the major load-related feedback, but primary and secondary muscle spindle afferents and cutaneous afferents also have the capacity to signal load-related feedback information [127].

Load-related afferent feedback facilitates the ankle extensor EMG activity in individuals with intact nervous system at the stance phase [128,129]. Studies applying sudden perturbations of the ankle angle during treadmill stepping have been conducted to identify the contributions of the afferent modalities to the soleus EMG activity during locomotion. An abruptly imposed ankle plantar flexion during the stance phase of walking unloads the muscle-tendon complex of soleus and produces a transient spinal-mediated drop in the extensor EMG activity. The amount of EMG activity removed by the rapid plantar flexion is then interpreted as the proprioceptive contribution to the extensor activity during stance.

It was shown that the soleus EMG activity and its unloading response to a rapid plantar-flexion perturbation were not influenced by an anaesthetic block of feedback from the foot and ankle [130]. Thus, cutaneous or proprioceptive afferents from intrinsic muscles of the foot likely did not contribute to the extensor EMG activity during the stance phase of stepping. The unloading effect remained unchanged after ischemic depression of the group la afferents from the lower leg, as well as following common peroneal nerve block [129]. These results imply that short-latency soleus stretch reflexes, or reciprocal la inhibition following a stretch of the tibialis anterior by the perturbation did not contribute to the unloading response in the soleus EMG. Grey and colleagues studied the effects of imposed rapid plantar flexion perturbations on the unloading response in the soleus EMG at the late stance phase in subjects during stepping on an inclined, level, or declined treadmill [131]. It was shown that Ib afferent feedback was modulated differently from the muscle spindle feedback during the different stepping conditions. The soleus background activity and its unloading response were related directly to the Achilles tendon tension and inversely to the ankle displacement and angular velocity. These observations suggest that force feedback contributes predominantly to the late stance phase enhancement of the soleus EMG during locomotion. af Klint and colleagues showed that the amount of depression in soleus activity following plantar-flexion perturbation decreased with reduced limb load during treadmill stepping with transient alterations of the amount of BWS provided [132]. The pharmaceutical depression of the secondary muscle spindle afferent pathway using the α2-adrenergic agonist tizanidine hydrochloride, however, did not affect the unloading response [132].

These series of studies suggest that Golgi tendon organ feedback constitutes an essential part of the sensory feedback to the extensor activity, providing reinforcing force feedback during the late stance phase of locomotion via an excitatory group Ib pathway. It is not clear to what extent these observations are related to the results in experimental animals, where afferent activity from extensor nerves during the stance-like phases of fictive locomotion acts upon several muscles synergistically probably through the extensor part of the CPG circuits (cf. Section 2.1). Studies in humans were largely confined to the soleus muscle only. Grey and colleagues studied the unloading response also in the medial gastrocnemius, however, the response was not present in most subjects and when present, it was of very short duration compared with that of the soleus muscle [131].

The importance of limb-load related afferent feedback in the generation of EMG activity during assisted and BWS treadmill stepping in individuals with motor complete SCI has been repeatedly reported. Dobkin and colleagues observed that the EMG activity in soleus and medial gastrocnemius muscles increased with decreasing BWS and suggested that load and joint kinematics were among the components that modulated the motor output [89]. Harikema and colleagues showed that EMG activity in soleus and medial gastrocnemius muscles were tightly correlated with the limb peak loading at the stance phase during BWS and manual assisted stepping in motor complete SCI [25]. The correlation between soleus mean EMG amplitude and soleus muscle-tendon stretch during the lengthening EMG activity of the muscle was lower than the correlation between the soleus mean EMG amplitude and peak load, and lower than the correlation between the soleus mean amplitude and muscle-tendon length changes during the entire step cycle in a person with motor complete SCI [25]. These results were interpreted such that the level of loading is a sensory feedback from the periphery that can modulate efferent activity in a manner similar to people with full supraspinal control. However, co-contraction between dorsiflexors and plantarflexors was apparent during the step cycle, and the non-physiological EMG activity of tibialis anterior that occurred during stance was related to the limb load as well [25]. This finding might thus suggest that limb loading was not simply triggering a critical feedback mechanism for ankle extensor activity, but enhanced the overall responsiveness of the spinal circuits (including non-functional activity) to the step-induced afferent feedback. In a similar context, it was suggested that the generation of EMG activity on a treadmill in persons with motor complete SCI required load-sensitive feedback [35]. Simulated stepping-movements produced by a robotic exoskeleton during 100% BWS without ground contact generated no or low-amplitude EMG activity in the paralyzed legs. This was interpreted such that pathological stretch reflexes induced by the movements contribute little to the leg muscle activation during walking. Robotic-driven treadmill stepping with 70% BWS on the other hand was reported to generate EMG activity “which in several aspects was similar to that observed in the healthy subject” [35]. Close inspection of the reported EMG data however suggests that the medial gastrocnemius was active at both stance and swing phases, and tibialis anterior showed only a short period of activation at the stance-to-swing transition phase, following the unloading foot-off movement when the muscle was passively stretched. Further, biceps femoris was active during swing and rectus femoris briefly during late stance, in both cases during the respective phases when the muscles were passively stretched, and both activities were absent in a healthy control group. In an attempt to modify the stretch-related feedback while continuing to provide alternating limb load, the knee joints of the robot were blocked during the imposed step-like movements and the results were reported to be “about the same as seen with normal leg
movements” [35]. The published data however show the absence of activity in rectus femoris and tibialis anterior, and alterations in the activation of other muscles as well. A careful interpretation of these findings – also within the context of the studies discussed in Section 3.1.1 – then might be that both, muscle-stretch related as well as load related feedback input contribute to the EMG activation on the treadmill individuals with motor complete SCI. One effect of the load sensitive feedback may be to add to the central state of excitability of the lumbar spinal circuits [33, 133]. To what extent the resulting EMG activation pattern represents ‘locomotor’ activity is further discussed in section 3.2.

3.1.3. Step-Related Neural Activation Patterns and Sensory-Input Processing are Altered After SCI

In conclusion of section 3.1, a severe SCI results in disruption of the supraspinal activation of alpha and gamma motoneurons, alternations in the neural firing patterns of muscle-stretch related proprioceptive feedback during movement, impaired modulation of incoming afferent inputs, and impaired function of the premotor neuronal spinal circuits. The spinal lesion transforms the effect of stretch-induced feedback to such an extent that it becomes crucial for the muscle activation during assisted locomotor movements as compared to its more limited contribution to muscle activation during active walking in individuals with intact nervous system. Load-sensitive feedback additionally contributes to muscle activity generated on the treadmill. The absence of the supraspinal activation of neuronal circuits underlying the reciprocal organization of antagonistic muscles, and of the supraspinal task- and phase-dependent modulation of presynaptic inhibition results in altered sensory-input processing after SCI and hence may alter the integrated actions of the afferent input upon the motoneurons and generate pathological as well as phase-inappropriate motor activity.

3.2. Rhythmic Motor Activity Patterns During Treadmill Stepping After SCI in Humans

The human spinal cord circuits below a severe cervical or thoracic SCI have the ability to produce motor activity during BWS and assisted stepping. To what extent this motor activity is ‘locomotor-like’ and resembles the voluntary muscle activity observed during normal human walking and whether this motor output is due to the activation of CPGs, as suggested by some researchers [134–136], are issues that warrant an in-depth discussion.

In Fig. 4, the muscle activation profiles from people with motor complete (AIS A, B) and motor incomplete (AIS C, D) SCI and healthy subjects (controls) during robotic-assisted stepping at 50% BWS are indicated [37,123]. In motor incomplete SCI, the muscle activation profiles have some similarities to those observed in healthy subjects, but the activation profiles of both ankle and knee muscles are greatly impaired in motor complete SCI. For example, the medial hamstrings activity at the swing-to-stance transition and the prolonged activity in tibialis anterior during swing associated with muscle contraction for foot clearance as produced during normal walking are absent in the motor complete SCI subjects. Both activities are largely occurring in the absence of muscle lengthening in healthy individuals.

A common explanation of the EMG patterns produced in individuals with severe SCI on the treadmill is that the activity is due to partial or full operation of CPGs, but this cannot explain the patho-

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**Fig. (4).** Leg EMG activation profiles during stepping in individuals with motor complete SCI (AIS A-B), motor incomplete (AIS C and AIS D) SCI, and intact spinal cord (controls). Mean average normalized EMG from the left and right leg muscles during robotic-assisted treadmill stepping at 50% BWS from one subject with AIS A, one with AIS B, four with AIS C, and eight with AIS D. Average normalized EMG for controls are from 13 healthy subjects. The EMG is normalized to the maximal homonymous EMG recorded during stepping. Grey background denotes the stance phase duration. Data adopted and modified with permission from [37,123].
logical EMG patterns that are apparent in motor complete spinal lesions. Further, the EMG patterns produced do not regularly evoke phases of ‘automatic’ or ‘spontaneous’ involuntary movement on the treadmill, such as the hip-stretch induced synergy to initiate the swing phase. Such observations are rather the exceptions. The activation of spinal circuits underlying flexion and extension synergies in response to step-induced feedback input indeed seems to require a combination of some degree of spastic muscle tone (background excitability) and residual descending connectivity, as well as specific movement-mediated afferent cues. Wernig and Müller reported that individuals with severe SCI could induce a few unaided bipedal steps after prolonged periods of treadmill training [33]. Specifically, five individuals with motor incomplete SCI with overall low motor scores and with one leg demonstrating complete functional paralysis (when tested in a resting supine position) could elicit alternating flexion-extension synergies when in an upright position over a motorized treadmill belt. Initiation and maintenance of stepping was possible only when the hip joint was extended beyond 180° after mid-stance to stretch the iliopsoas muscle, the knee joint was fully extended during the stance phase, and the leg was fully unloaded after bearing full weight. The stepping patterns induced in this study may suggest the activation of spinal circuits incorporating ipsilateral flexor or crossed extensor reflexes. In a study of Dobkin and colleagues, patients with incomplete SCI, yet with non-functional residual motor function, were encouraged to contribute actively to the stepping movements while manual assistance was continuously provided [89]. The voluntary facilitation not only augmented the EMG activity as generated by passive movements alone, but also produced additional EMG bursts, like a burst in iliopsoas, medial hamstrings, and tibialis anterior muscles in the swing phases. After a few training sessions, these patients became able to self-initiate the swing phases on the treadmill when the hip was moved into extension. These observations suggested that supraspinal inputs clearly added functional components to the EMG patterns during stepping that could not be generated by step-induced afferent feedback alone.

We may consider at this point that EMG activities timed to the gait cycle are not necessarily locomotor-like, or step-like, just because they are rhythmic in nature. In the following, we review in detail the characteristic activation patterns of various thigh and lower leg muscle groups with respect to the phases of the gait cycle as produced during assisted and BWS treadmill stepping in individuals with motor complete SCI (Fig. 5).

The timing of the activation in rectus femoris most frequently coincided with the stretch applied to the muscle in the late stance phase when the knee was passively flexed as the leg was drawn backward [35,88,92,137–141]. A similar activation during the stance-to-swing transition was described for the knee extensor vastus lateralis by Harkema [88], while Dobkin and colleagues [89] observed its activation from swing-to-stance transition, possibly during weight acceptance with knee flexion.

Activity generated in biceps femoris and medial hamstrings were found to be timed to phases within the gait cycle when the leg was moved forward during swing phases, that is, when the biarticular muscles of the posterior thigh were stretched [35,92,93].

![Fig. (5). Timing of EMG activity in leg muscles during BWS and assisted stepping in individuals with motor complete SCI. The duration of activity is shown on a normalized time scale from heel strike to heel strike; shaded areas mark stance phases. RF, rectus femoris; VL, vastus lateralis; BF/MH, biceps femoris/medial hamstrings; TA, tibialis anterior; MG, medial gastrocnemius; SOL, soleus. Hip and knee goniometric data illustrated below RF, VL, and BF/MH derived from robotic-assisted stepping; knee and ankle data below lower-leg muscle groups derived from motor complete SCI subjects during manually assisted stepping (black lines) and actively stepping non-injured controls (gray lines).](image-url)
137,138,140–142]. One study showed that the hamstrings EMG activity increased by increasing the amplitude and the velocity of muscle stretch [141].

Tibialis anterior activity frequently occurred during the stance phase of the gait cycle [25,88,89,138,142], also in the form of clonus [92,141]. The onset and waning of the EMG activity typically coincided with that of medial gastrocnemius [25,89,92,138]. A brief activation at the stance-to-swing transition [35,88,137] or no activation throughout the gait cycle [140] was reported as well.

The main activation of medial gastrocnemius occurred during the stance phase, and often started well before the onset of loading of the leg [25,35,89,137,140]. It should be noted that the gastrocnemius is a biarticular muscle that is also stretched by the knee extension during late swing. Activity produced in soleus consistently started during mid- or late-swing phases and terminated before the end of stance [25,34,88,92].

Clonic EMG activity was frequently observed across the various muscles [25,89]. Particularly, clonic EMG patterns were described at or following foot contact, throughout the stance phase and stance-to-swing transition during stepping [25,88,92,141–144]. Clonus was also found to occur before swing phase initiation and during mid-to-end swing in the gastrocnemius muscle [142,145].

Characteristically, muscle activation in motor complete SCI in response to afferent feedback on a moving treadmill belt occurs during the lengthening phases of the respective muscles and can be related to immediate stretch. One exception is the non-functional tibialis anterior activity during stance. This type of activity may be clonus triggered by the initial foot contact (cf. section 3.1.1) or reflect impaired reciprocal inhibition.

3.3. Influence of Spinal Circuits’ Excitability and Residual Supraspinal Input on the Generation of Rhythmic Motor Output

Increasing the central state of excitability of the lumbar spinal circuits caudal to an SCI by pinching of the leg, reducing BWS, electrical SCS, or reduced antispasticity medication, facilitates the motor output during assisted treadmill stepping [33,133,141,146,147]. Specifically, some degree of spastic muscle tone in the lower limbs is critical for the generation of rhythmic EMG activity in response to the externally induced stepping movements [33,87,92,143]. In this context, spasticity may be interpreted as an increased excitability of the spinal circuits, also reflected by an increase in their susceptibility to respond to afferent input. Inter-individual differences in the relative excitability of various spinal pathways may also be the cause of the variability in motor patterns generated during assisted treadmill stepping with relatively similar kinetics and kinematics across the population of motor complete SCI individuals [88]. Stewart and colleagues investigated the effects of clonidine, a noradrenergic agonist, on spasticity and on the EMG activity generated during treadmill stepping in people with SCI, of whom six had no motor function below the lesion [92]. The stepping movements in the paraplegic patients were manually induced under BWS. The EMG patterns seen in the placebo sessions in most paraplegic subjects during assisted treadmill stepping were stretch-related activity in the medial hamstrings in the swing phase, and clonic activity in tibialis anterior and gastrocnemius evoked at foot contact. Similarly, when tested in a sitting position at rest, hamstrings activity was consistently produced during passive extension of the knee, and clonus in the lower leg muscles could be evoked as well. During the clonidine sessions, the manually imposed leg movements on the treadmill largely failed to generate any muscle activity. In the sitting position, the tonic stretch reflexes and clonus were abolished while on clonidine.

The responsiveness of the spinal circuits may be further influenced by some residual descending neural connections. Even after a motor complete SCI according to standard neurological classification [148], a small amount of white matter crossing the lesion is commonly preserved [149–151], and propriospinal connections [152] may also survive and pass neural information around the lesion site [153,154]. Conduction along such preserved systems is clearly compromised, but may provide some translational inhibition or excitation to the lumbar spinal circuits [33,147,155,156].

In conclusion, motor complete SCI individuals require a certain excitability level of the lumbar spinal circuits on which step-related peripheral feedback can act to produce rhythmicity during assisted treadmill stepping. Such ongoing activity within the circuits can result from some degree of spinal spasticity. Most of the leg muscle activation patterns described in motor complete SCI occur during the muscle-lengthening phases of the stepping movement and can be related to immediate stretch. Sensory-driven flexion synergies across multiple joints that produce muscle contraction and actual leg movements seem to require some motor-incompleteness of the SCI.

4. SPINAL CORD STIMULATION AFTER SPINAL CORD INJURY IN HUMANS

4.1. Neural Activation Patterns Produced by Spinal Cord Stimulation

The spinal locomotor network studied in reduced animal preparations is normally activated by tonic pharmacological or electrical stimulation, that is, by sustained excitatory inputs with rather invariant temporal and spatial aspects. It has been assumed that such excitation mimics an unpatterned descending drive from the brainstem that spinal cord networks convert into rhythmic outputs [157]. With the application of SCS in motor disorders [158,159] and the specific placement of epidural electrodes over the lumbar spinal cord [160], a method that could deliver a tonic excitatory drive to spinal cord segments that are essential for locomotion was developed in humans. The spinal circuits are likely activated through a combination of afferent stimulation of posterior roots (also termed dorsal roots), containing axons of the dorsal root ganglion neurons, and of posterior columns (dorsal columns), containing the ascending intraspinal continuations of these axons. Afferent orthodromic action potentials in the posterior roots [29,45,46,161] and antidromic action potentials in the posterior columns [47,48], in turn, will result in excitation of spinal interneurons and motoneurons through collateral projections. Due to the compact arrangement of the lumbar and upper sacral roots in the dural sac, as well as of the afferent branches in the posterior columns, each electrical pulse normally activates sensory fibers associated with several spinal cord segments bilaterally [30,48]. Therefore, tonic SCS generates a series of highly synchronized multi-segmental excitatory inputs. The resulting repeated release of neurotransmitters by the stimulated afferents may maintain a relatively continuous level of excitation of a wide range of spinal circuits, including those with pattern generating capability [162,163].

4.2. Rhythmic Motor Activity Patterns Produced by Spinal Cord Stimulation

Tonic electrical stimulation of the lumbar spinal cord was shown to generate rhythmic motor outputs and movements in cats and rats, with or without pharmacological facilitation [4,73,164,165]. By applying epidural stimulation to the corresponding spinal cord segments in humans, locomotor-like activity can be generated in the paralyzed legs of individuals with complete SCI studied in the supine position [28,38,166,167]. The term locomotor-like in these studies denoted rhythmic EMG patterns with reciprocal activation in antagonistic lower-limb muscle groups and the generation of involuntarily flexion-extension movements. The finding that the lumbar spinal cord isolated from supraspinal control could convert a tonic excitation into coordinated rhythmic motor activity was interpreted as evidence for the existence of CPGs in humans [8,28]. Notably, SCS with appropriate parameters immediately induces rhythmic movements on the examination bed without manual assis-
tance provided to the legs. Such activity ceases after stimulation is stopped.

The characteristics of the spinal pattern generating circuits were analyzed by Danner and colleagues [162]. In individuals with motor complete SCI lying supine, ongoing tonic SCS (at intensities three-fold the threshold for evoking EMG responses in the legs, and frequencies around 30 Hz) generated various rhythmic multi-muscle activation patterns in the lower limbs, including coactivation, mixed muscle synergies, and locomotor-like patterns (Fig. 6). Such rhythmic activity present in the main thigh and lower leg muscle groups were elicited in seven out of ten subjects with (motor-) complete SCI studied. Statistical decomposition of the EMG data identified three basic temporal components shared across the different EMG patterns, a flexor and an extensor basic activation pattern, and one that was characteristically associated with the activation of the bi-articular hamstrings muscle group during the rhythmic activity. The basic activation patterns were interpreted as excitatory neural drives provided by spinal burst generators, each imposing a specific spatiotemporally organized activation on the motoneuron pools [162]. The flexor and extensor basic patterns corresponded to the output of a CPG in reduced animal preparations [5,7].

The EMG burst activities produced by lumbar SCS are composed of a series of stimulus-synchronized responses, each representing an afferent-induced compound muscle action potential, at short latencies [29,168]. Successively evoked responses are rhythmically amplitude-modulated, resulting in the burst-like EMG envelopes. It was suggested that the rhythmic motor patterns had resulted from the integrated activities of repetitively activated spinal reflex circuits, as well as of the operation of multisegmental patterns-generating circuits, the latter being activated due to the tonic nature of the afferent input [30,41]. Experiments in rats have come to the same conclusion [169,170]. The stimulus-triggered EMG composition under SCS could be the result of synchronized excitatory postsynaptic potentials and excitation of motoneurons and interneurons at multiple spinal segments.

Because of the functional integrity of neuronal circuits and peripheral nerves below the lesion site, the contribution of movement mediated afferent feedback in the generation of rhythmic activity in the studies reviewed above cannot be excluded. However, in the supine position, significant contribution from proprioceptors mediating loading and hip extension, being restricted by the examination table, is unlikely. The switch between extensor- and flexor-like phases likely was controlled centrally in the absence of proprioceptive feedback cues, otherwise critical for phase transitions during stepping. It should though be noted that proprioceptive feedback during or after contraction and relaxation of leg muscles, and joint movements, could have added phasic activation to the spinal cord circuits. This said, SCS also generates antidromic action potentials propagating in the electrically stimulated sensory fiber toward the periphery [171,172]. The antidromic activity generated by repetitive SCS, such as at 30 Hz, may thus partially cancel the sensory feedback related to the induced rhythmic activities, and overall reduce the influence of sensory input on the rhythmic motor outputs. The burst frequency of the SCS-induced rhythmic output patterns must then have been largely determined by phasic changes innate in the spinal circuits. Under tonic drive, these circuits generated a wide range of physiologically relevant burst frequencies, including those corresponding to slow and fast gait [162].

The specific spinal circuits underlying the central generation of the rhythmic motor patterns remain unknown. In principle, a simple network with appropriate pattern of interconnections between neurons can generate rhythmic activity under ongoing tonic stimulation. A network consisting of two populations of flexor and extensor motoneurons, reciprocally coupled by Ia inhibitory interneurons, and Renshaw cells that provide a delayed inhibition of the Ia inhibitory interneurons, can produce oscillatory output in response to tonic input [173]. There are some observations to suggest that circuits involving Ia inhibitory interneurons and Renshaw cells are activated by SCS [47,163]. However, such inhibitory circuits alone cannot explain the burst-like patterns of rhythmic activities as produced by SCS. For instance, non-modulated responses in the tibialis anterior muscle in response to low-frequency SCS (2–10 Hz) have relatively low EMG amplitudes, but the responses in the same muscle can attain large amplitudes in the form of rhythmic bursts of activity when SCS frequency is close to 30 Hz, without changing the intensity of the stimulation [168]. This observation suggests that during the rhythmic activity additional excitation acted upon the

Fig. (6). Rhythmic patterns of EMG activity and envelopes of the rectified EMG in response to tonic multi-segmental driving input provided by epidural SCS. Data derived from three motor complete SCI individuals lying in the supine position. Tonic stimulation was continuously applied with parameters given at the top of the figures; electrode pairing (‘0’, most rostral and ‘3’, most caudal electrode along a linear array, ‘+’ and ‘-’, polarity of the active electrodes), stimulation frequency and intensity. Gray background denotes flexion-like phases defined by the activity of tibialis anterior muscles. Q, quadriceps; Ham, hamstrings; TA, tibialis anterior; TS, triceps surae. Modified with permission from [162].
tibialis anterior motoneurons. Furthermore, the stimulus-synchronized responses constituting the burst-shaped activities during the flexion phases under SCI delay with respect to the shortest-latency reflexes [29,30,166]. This may be the result of the time needed for facilitation/disfacilitation of excitation spinal circuits with intercalated interneurons and their synaptic delays. Similar burst-phase related delayed responses were also identified during rhythmic activity induced by SCS in rats and were suggested to be related to the flexor reflex [174], or to be mediated through some elements of the spinal locomotor networks [169].

4.3. Tonic Excitation of the Human Lumbar Spinal Cord Activates Circuits with Pattern Generating Properties

In conclusion of section 4, it is obvious from the differences of the inputs provided and motor output patterns generated, that tonic SCS and treadmill stepping involved different mechanisms of rhythm generation. In the studies using SCS, the rhythmic motor output was not entrained by the excitatory inputs provided. Rhythmicity was rather generated by spinal circuits that switched between flexion- and extension-like phases. To become and be maintained operational, the rhythm generating networks seem to require a continuous excitation, as provided by tonic stimulation. While some of the rhythmic motor activity patterns produced by SCS in humans resemble those of fictive locomotion in reduced animal preparations, further studies are required to clarify to what extent the circuits engaged by SCS in humans correspond to CPG networks. Clearly, the leg muscle activation patterns produced by SCS are not the same as during active walking. Bipedal human locomotion involves more than just a simple pattern of flexion and extension. One question for future work would be to address to what extent task-specific sensory feedback could tune the rhythmic patterns evoked by SCS to become more functional.

5. PHARMACOLOGICAL STIMULATION AFTER SPINAL CORD INJURY IN HUMANS

5.1. Cellular and Intracellular Mechanisms Underlying Drug-Induced, CPG-Mediated Leg Movements

As mentioned earlier, animal studies (in-vitro isolated spinal cord and in-vivo spinal transected mice) have shown that specific combinations of drugs using dopaminergic agonists, adrenergic/dopaminergic precursors and/or 5-HT receptor agonists most effectively elicit putative CPG-mediated hindlimb locomotor movements (see section 2.3). Our understanding of the synergistic action of drugs on the CPG enabled by such combinations of different molecules is incomplete. Using animals pretreated with selective receptor antagonists or knock-out models with missing specific receptor subtypes, Guertin and colleagues have obtained evidence suggestive that 5-HT1A, D1, and NMDA receptors are critically involved in pharmacological activation of CPGs [21,78–80].

Stepping induced by subcutaneous, intraperitoneal or oral administration of CPG-activating drugs in low-thoracic spinal transected mice is likely centrally-mediated by lumbar CPG elements since well-coordinated movements are found. This conclusion is further supported by the similar effects that can be achieved when animals are suspended during air-stepping conditions as on a motorized treadmill, without requiring additional excitatory inputs, such as tail or sexual organ pinching. At the transmembrane level, it is well documented that buspirone is a 5-HT1A receptor agonist whereas L-DOPA is a noradrenaline/dopamine precursor. Carbidopa does not cross the blood brain barrier, it is a decarboxylase inhibitor typically used to augment by fivefold the central bioavailability of L-DOPA.

The corresponding targets (e.g., 5-HT1A and D1 receptors) are expressed in lumbar spinal cord segments [175–178] and dopamine release (mainly from descending tracts originating supraspinally in A11 nuclei; [179]) exists also in the spinal cord (D cells; [180]). L-DOPA is converted to dopamine and noradrenaline in catecholaminergic axon terminals and, in absence of descending dopaminergic terminals due to spinal transection, D cells may compensate by upregulation of aromatic L-amino acid decarboxylase (AADC; [180–183]).

However, the mechanisms at an intracellular level are not well understood. For example, how L-DOPA and buspirone act together for effective CPG activation in spinal transected animals remains largely unclear. At least two possible explanations exist. Firstly, since CPG neurons belong to various classes (VO-V3, HB9, etc.) with different properties and neurotransmitters, it may be that L-DOPA and buspirone act on different classes of CPG elements that, once activated, can trigger overall activity in the entire network. Secondly, both compounds may act upon the same key CPG neurons (e.g., pacemaker HB9 cells) for better activation of G-coupled protein cascades involving cAMP and adenylyl cyclase-induced [Ca$^{2+}$], increase leading to robust NMDA-dependent pacemaker property expression [75,78,79,184]. This is supported by results obtained in lampreys showing various converging actions upon intracellular Ca$^{2+}$-dependent mechanisms including Ca(V)1.3 channel-mediated robust post-inhibitory rebounds [185].

5.2. First Tests of CPG-Activating Drugs in Patients with Complete or Motor-Complete SCI

For clinically relevant use in humans, it is imperative to identify drugs that can mimic the CPG-activating effects of comparable cocktails in animal models, but cross the blood brain barrier and are centrally active upon oral administration. According to these criteria, Guertin and colleagues developed Spinalon™, a drug that is composed of L-DOPA, carbidopa and buspirone for activation of both spinal dopaminergic and serotonergic mechanisms, and demonstrated its efficacy in mice [82]. This combination was also chosen because each of the molecules were shown to be relatively safe in patients with Parkinson’s disease or anxiety, respectively. Moreover, co-administration of L-DOPA, carbidopa (or other decarboxylase inhibitors) and 5-HT1 agonists (buspirone or comparable agents) were already tested in patients with Parkinson’s disease for improved levodopa-induced effects without increasing side effects [186].

A phase I/IIa study on the effects of Spinalon™ was recently completed in 45 individuals with (motor) complete SCI (see companion paper, [49]). The study was a randomized, placebo-controlled, double-blind dose escalation study. The participants received Spinalon™ in the form of over-encapsulated buspirone and Sinemet® (levodopa/carbidopa), or a placebo, an over-encapsulated cornstarch powder. The tests were performed in the supine position. Blood samples before and at regular intervals after treatment were collected for hematological and pharmacokinetic analyses. EMG activity of vastus lateralis, biceps femoris, tibialis anterior, and gastrocnemius muscles bilaterally was monitored before and several time points after drug administration. Buspirone/levodopa/carbidopa (10-35 mg/100-350 mg/25-85 mg) showed no sign of safety concerns. Only mild nausea was reported by 3 participants. At higher doses (50 mg/500 mg/125 mg), buspirone/levodopa/carbidopa was considered to have reached maximal tolerated dose, since 3 out of 4 subjects experienced related adverse events including vomiting.

The Spinalon™-treated groups displayed significant EMG activity with locomotor-like characteristics, that is, with rhythmic and bilaterally alternated EMG bursts. The onset of the rhythmic activity was spontaneous and occurred within 15 and 120 minutes post-administration while the subjects were lying supine, without manual assistance provided by the examiners. The study therefore provided preliminary evidence on safety and efficacy following a single administration of a rhythmic-activity producing drug that putatively acted upon the CPG in people with SCI.
Guerin and colleagues are currently planning further tests to provide evidence on the potential of this approach in triggering stepping movements on a motorized treadmill in people with severe SCI, without or with additional means of applying excitatory input to the spinal cord, such as muscle vibration or electrical stimulation. It will be also of interest whether repeated administration of Spinalon® over a longer period of time would increase the activity of the spinal CPG and the induced-motor output.

6. CONCLUSION AND FUTURE DIRECTIONS

Experimental studies have demonstrated CPG capabilities of the isolated mammalian lumbar spinal circuits and the interaction of specific afferent inputs with the CPG activity. In-vivo animal experiments have further shown that proprioceptive feedback can produce hindlimb stepping on a motorized treadmill, but additional sources of excitatory drive provided to the spinal cord are normally required to initiate or maintain effective weight-bearing stepping.

Externally assisted treadmill stepping in individuals with motor complete SCI can produce leg muscle activity that is synchronized to the phases of stepping, yet the activation patterns differ from those during normal walking. Much of the EMG activity produced can be explained by the entrainment of spinal reflex circuits by proprioceptive feedback related to cyclically appearing muscle stretch. SCI alters the rhythmic patterns of muscle spindle firing during passive stepping movements on a treadmill, and an altered input-processing by spinal cord circuits is apparent. This leads to an increased contribution of stretch-induced afferent volleys to the generation of the motor outputs in these patients. Some critical level of sustainable state of excitability of the circuits, as can result from spinal spasticity, is required to respond to the feedback inputs with the generation of motor output. Load-related proprioceptive input is probably further increasing the central excitability state of the circuits.

SCS can provide a tonic excitation to the human lumbar spinal cord, a type of sustained drive also used in animal experimental studies to activate spinal CPGs. The SCS-induced muscle activity occurs largely within two alternating phases of rhythmicity, thus being more similar to fictive locomotion in reduced animal preparations than to the complex muscle activation patterns of active bipedal human walking.

Preliminary evidence suggests that specific combinations of pharmacological substances that can effectively activate CPGs in animal models can also generate rhythmic activity in the paralyzed legs in individuals with a motor complete SCI studied in the supine position. It remains to be shown whether and how proprioceptive inputs generated during stepping interact with SCS and pharmacologically-driven CPG activity.

The development of new therapeutic strategies for restoring motor function of paralyzed muscles after SCI is a continuous endeavor from both the scientific and clinical community. While significant advances have been made in understanding better the pathological expression of muscle tone and paralysis in humans, it is apparent that rehabilitation requires the simultaneous utilization of different strategies. Locomotor training may be administered along with SCS and CPG-activating drugs to restore motor function in patients who remain wheelchair bound after standard-of-care rehabilitation. Thus, there is a great need for clinical research studies that utilize neurophysiological, clinical, and pharmacokinetic biomarkers under conditions during which training of the motor system is multidimensional.

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CONFLICT OF INTEREST

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Human Spinal Locomotor Circuits


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