Review

The Human Central Pattern Generator for Locomotion: Does It Exist and Contribute to Walking?

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Abstract

The ability of dedicated spinal circuits, referred to as central pattern generators (CPGs), to produce the basic rhythm and neural activation patterns underlying locomotion can be demonstrated under specific experimental conditions in reduced animal preparations. The existence of CPGs in humans is a matter of debate. Equally elusive is the contribution of CPGs to normal bipedal locomotion. To address these points, we focus on human studies that utilized spinal cord stimulation or pharmacological neuromodulation to generate rhythmic activity in individuals with spinal cord injury, and on neuromechanical modeling of human locomotion. In the absence of volitional motor control and step-specific sensory feedback, the human lumbar spinal cord can produce rhythmic muscle activation patterns that closely resemble CPG-induced neural activity of the isolated animal spinal cord. In this sense, CPGs in humans can be defined by the activity they produce. During normal locomotion, CPGs could contribute to the activation patterns during specific phases of the step cycle and simplify supraspinal control of step cycle frequency as a feedforward component to achieve a targeted speed. Determining how the human CPGs operate will be essential to advance the theory of neural control of locomotion and develop new locomotor neurorehabilitation paradigms.

Keywords

CPG, central pattern generator, human, locomotion, modelling, neuromodulation, spinal cord, spinal cord injury, spinal cord stimulation

Introduction

Is there a spinal central pattern generator (CPG) for locomotion in humans? More than 20 years after the first modern report on spontaneous rhythmic activity in an individual following spinal cord injury (SCI; Bussel and others 1988) and the first review articles addressing this very question (Bussel and others 1996; Illis 1995), we have still no definite answer. While some scientists *believe* in its existence, others are convinced of the opposite—a situation that is unsatisfactory for both groups because without direct evidence, either opinion remains inconclusive. The problem also has roots in the definition of a CPG, which stems from reduced animal preparations and requires the generation of rhythmic motor activity by spinal circuits after the experimental elimination of supraspinal control and movement-related afferent feedback (Grillner 1985). No human injury model exists that can fully meet these requirements. The debate has also been fueled by the initial lack of convincing evidence for the existence of CPGs in non-human primates (Eidelberg and others 1981) and by the limited recovery of gait in individuals with severe SCI (Dietz and Fouad 2014), which could be interpreted as a cortical dominance over spinal automatisms in human locomotion (Duysens and van de Crommert 1998). Furthermore, spinal contribution to muscle activity during human gait could be related to feedback activation through spinal reflexes rather than to feedforward activation by CPGs. Some

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neuromuscular models of human locomotion relying on interconnected spinal reflexes demonstrated that human-like gaits could be obtained without CPGs (Geyer and Herr 2010; Song and Geyer 2015) and hence questioned their functional relevance. We critically address these points in the present review.

Following background information on CPGs gained from animal experiments, we will discuss studies suggesting that similar circuits also exist in humans. We will present neurophysiological studies of individuals with SCI in whom involuntary rhythmic leg activity emerged spontaneously (Calancie and others 1994) or was generated by electrical spinal cord stimulation (Dimitrijevic and others 1998). Common to these observations are the smoothness and almost wave-like appearance of the involuntary multijoint leg movements occurring in the supine position, the high degree of coordination of the associated electromyographic patterns, and the presence of a neural drive to the lumbar segments of the spinal cord. Another focus will be on the knowledge gained from a cutting-edge neuropharmacological study in SCI individuals (Radhakrishna and others 2017). The systemic administration of a particular combination of orally active neuromodulators, previously demonstrated to elicit temporary episodes of automatic weightbearing walking in spinal mice (Guertin and others 2010; Guertin 2014), can also trigger rhythmic muscle activity and left-right alternating movements in the legs of paralyzed individuals lying supine. We will elaborate on whether the neuropharmacological modulation of the human lumbar spinal cord circuits might have acted through pattern generating mechanisms comparable to those in quadrupedal mammals.

Finally, we will discuss whether CPG-associated activity in patients only reflects a non-physiological condition, when phylogenetically old networks in the spinal cord become autonomous after severe lesions have released them from supraspinal motor centers. We will present the alternative view that CPGs might indeed contribute to normal locomotion. Some elements of primitive step-like movement patterns in newborns that base on innate spinal locomotor networks have been proposed to be used in mature human walking (Dominici and others 2011; Grillner 2011). Furthermore, novel neuromechanical simulations suggest that the integration of CPGs may in fact have physiological advantages in motor control of bipedal human locomotion (Dzeladini and others 2014).

The Central Pattern Generator for Locomotion in Animal Studies

Fictive Locomotion and the Central Pattern Generator

It is sometimes stated in the literature that CPGs are neural circuits in the spinal cord capable of producing rhythmic *movements*. As a matter of fact, the experimental demonstration of a CPG is performed with the spinal cord circuits decoupled from any movement. In vitro isolated (brainstem-) spinal cord preparations of the lamprey, an ancient lineage of swimming vertebrate that lack paired appendages, or of the newborn rat are the typical reduced models used in such experiments (Cazalets and others 1992; Grillner 1985). Another model is the decerebrated or spinalized cat, with movement abolished and therefore rhythmic proprioceptive feedback eliminated, for instance, by paralytic agents (Andersson and others 1978; Hultborn and others 1998). The output produced by CPGs is recorded as electroneurographic activity with cyclic alternation between nerves to left and right axial muscles in swimming vertebrates or to extensor and flexor hindlimb muscles in quadrupedal mammals. Such motor patterns are termed fictive locomotion, wherein the term *fictive* refers to the fact that the rhythmic activity is recorded without any actual movement. Fictive locomotion is induced by various types of tonic pharmacological or electrical stimulation, and can also occur spontaneously after decerebration (Frigon 2012). Hence, CPGs can be viewed as spinal neural circuits that can autonomously generate fictive locomotion in the absence of connections from the brain and movement-related peripheral feedback information.

Organization of the Mammalian Hindlimb Central Pattern Generators

The adult cat spinal cord in in vivo experiments relying on electrophysiological methods and pharmacological manipulations was the classical model for many decades in studies of mammalian hindlimb CPGs (Graham Brown 1914; Grillner and Zangger 1979; Jankowska and others 1967). In recent years, combined electrophysiological and molecular genetic approaches in the developing mouse spinal cord have allowed to manipulate identified classes of spinal interneurons and test the consequences on circuit function and locomotor behavior (Kiehn 2011, 2016). Together, these studies have led to a profound understanding of the organization of mammalian CPGs. The CPG circuits consist of multiple interneuron cell types, many of which are derived from four major subclasses of ventral interneurons, called V0, V1, V2, and V3 interneurons (Goulding 2009; Grillner and Jessell 2009). The organization of the hindlimb CPGs can be functionally subdivided into rhythm-generating and pattern-generating circuits (Kiehn 2016). Rhythm-generating kernels drive the CPG network and set the pace of the locomotor rhythm. There is strong evidence that they are comprised of excitatory, ipsilaterally projecting interneurons (Grillner 2006; Hägglund and others 2013; Kiehn 2006), likely a heterogeneous population of glutamatergic neurons overlapping with several of the developmentally defined spinal interneuronal groups, which are currently partially identified

(Dougherty and others 2013). Different glutamatergic interneurons of the V2a class located downstream of the rhythm-generating kernels drive ipsilateral motoneurons or project to commissural interneurons that have axons crossing the midline (Dougherty and others 2013; Kiehn 2016). The pattern-generating circuits distribute the centrally generated rhythmic drive to the different motoneuron pools. Two aspects of pattern generation can be distinguished. Flexor-extensor pattern-generating circuits control intralimb coordination, and left-right pattern-generating circuits coordinate the activity between the hindlimbs. Two groups of ipsilaterally projecting inhibitory interneurons, V1 and V2b neurons, are involved in flexor-extensor alternation (Zhang and others 2014), organized in layers with circuits between flexor- and extensor-related excitatory rhythm-generating kernels (Kiehn 2016), and circuits directly upstream the motoneurons, including Ia-inhibitory interneurons, which reciprocally inhibit antagonist motoneurons and each other (Talpalar and others 2011). Commissural interneurons are the essential components of the circuits controlling the relationship of motor output on the left and right sides of the spinal cord (Kiehn 2011, 2016). Crossed inhibition is accomplished by a dual pathway composed of inhibitory commissural interneurons ($V0_D$) that project directly to contralateral motoneurons, and excitatory commissural interneurons ($V0_V$) which provide inhibition via premotor inhibitory neurons. Crossed excitatory left-right synchronizing circuits likely include glutamatergic commissural interneurons that project directly to motoneurons. The different pathways are active during different types of gaits that require alternating (walk, trot) or synchronous (bound) activity in the hindlimbs. An important advancement in our understanding of the CPG organization is that reciprocal inhibition between antagonistic flexor- and extensor-related excitatory kernels is not a prerequisite for rhythmicity, as originally assumed in the "half-center" model (Graham Brown 1914). Excitatory neurons alone can generate rhythmic activity and burst generators driving individual flexor or extensor motoneuron pools can be activated in isolation (Hägglund and others 2013). Changes in the coupling of the burst generators allow for the variation in the coordination between antagonists and muscles across joints during locomotion.

Are Central Pattern Generators Used during Normal Locomotion in Mammals?

The similarity of the electroneurographic activation patterns during fictive locomotion to the electromyographic activity during real locomotion is generally interpreted such that part of the motor pattern during normal stepping in intact animals is generated by CPGs (Frigon 2012; Grillner 1985). Nevertheless, evidence for the partial or full operation of CPGs during active walking remains indirect, since experiments involving real stepping cannot eliminate supraspinal control and peripheral feedback in the animal at the same time. For instance, adult spinalized cats can be made to perform involuntary hindlimb stepping on a motorized treadmill belt after intensive task-specific training (Barbeau and Rossignol 1987; Lovely and others 1986). While supraspinal involvement is removed in this model, spinal reflex circuits, usually regarded as extrinsic to the CPGs, are cyclically entrained by the natural stimulation of leg proprioceptors during the stepping movements and contribute to the generated rhythmic muscle activation. Furthermore, although locomotion of these spinalized cats displays the essential characteristics of normal walking patterns, it is not identical to stepping of intact cats or even of decerebrated cats, in which the brain stem locomotor regions remain connected to the spinal cord (Grillner 1985). The use of CPGs during functional walking can be deduced from another classical physiological study. Electrical stimulation of specific brainstem areas at a constant frequency, for instance, 30 Hz, can make a decerebrated cat step on a treadmill with body-weight support (Shik and others 1966; Shik and Orlovsky 1976). By solely increasing the stimulation intensity, the speed of locomotion increases, actually accelerating the freely moving treadmill belt, and the gait changes from a slow walk to trot and finally gallop. The likely unspecific descending neural drive generated by such stimulation determining complex locomotor behaviors implies that spinal circuits must have been causative in orchestrating and changing the precise timing and pattern of the limb movements to achieve different speeds and gaits.

Rhythmic Locomotor-Like Activity Spontaneously Originating from the Human Spinal Cord

Spontaneously emerging and highly regular rhythmic involuntary leg movements have been repeatedly observed in individuals following SCI. Bussel and others (1988) reported on a patient with a clinically complete low cervical SCI who developed rhythmic activity widely distributed to extensor muscles of the trunk and lower limbs below the level of the lesion. The electromyographic bursts of activity were synchronous across all muscles bilaterally, with extensors and flexors rhythmically co-activated at a frequency of 0.3 to 0.6 Hz. Evoking a flexion reflex induced automatic alternating flexion-extension activity for several cycles. Calancie (2006) described spontaneous rhythmic leg movements in two individuals with chronic incomplete and four with complete cervical SCI examined in the supine position. The involuntary contractions were smooth and graded and spanned multiple leg muscles bilaterally, with highly reproducible rates within 0.3 and 0.5 Hz. In the incomplete SCI subjects, muscles were activated reciprocally between

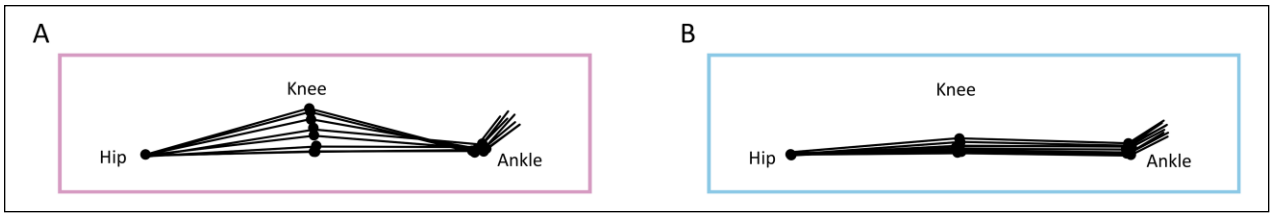

Figure 1. Spontaneous involuntary locomotor-like movement after spinal cord injury. (A) Joint movements of the right leg during an episode of involuntary rhythmic activity in an individual with incomplete spinal cord injury lying supine. The movement onset was spontaneous. The stick diagram illustrates the movement during one rhythmic cycle. Not shown is the alternating movement between right and left leg that looked like stepping. (B) The subject's voluntary attempt to perform stepping-like movements. The illustrations are constructed based on joint angle recordings from Calancie (2006).

agonists and antagonists, and in an alternating manner between left and right leg, such that the movements resembled stepping (Fig. 1). In the complete SCI subjects, contractions occurred simultaneously in all muscles bilaterally, but shared similar burst frequencies as those seen in the incomplete subjects. The onset of involuntary muscle contractions only occurred when the hip was extended. In experimental animal studies, proprioceptive input from the muscles around the hip joint has been shown to modulate CPG activity (Andersson and Grillner 1983). A further common factor across all subjects was the presence of additional pathologic conditions, including cases affecting the hip joint, or soft tissues around the pelvis or thigh, causing nociceptive inflow to the spinal cord and thought to be the reason for the increased spinal cord excitability at the time when the spontaneous activities were expressed. Involuntary movements were abolished when these pathologies were treated. A report of Nadeau and others (2010) is of supplementary interest because the subject studied had a complete SCI at the midthoracic level and showed alternating activity between some muscles during the episodes of spontaneous rhythmic activity. Notably, continuous intrathecal delivery of baclofen, a $GABA_B$ receptor agonist capable of inhibiting mono- and polysynaptic reflexes, did not abolish the rhythmic activity, whereas it disappeared after bilateral infiltrations of the hip joint with corticosteroids, used medically in the treatment of joint pain or inflammation. Overall, the spontaneous expression of rhythmic activity, the rate and distribution of smooth contractions, the reliance on stretch-sensitive receptors of the hip and increased spinal cord excitability were taken as indications that these patterns reflected the activity of some elements of CPGs in humans.

Rhythmic Locomotor-Like Activity Generated by Spinal Cord Stimulation

Human Lumbar Spinal Cord Circuits Can Convert Tonic Input into Rhythmic Output

Spontaneous rhythmic activity occurs only in a few percentage of SCI individuals, likely because it depends on a specific combination of conditions increasing the excitability of the lumbar spinal cord under reduced supraspinal control (Calancie 2006). When epidural spinal cord stimulation (Fig. 2A) became available as a clinical tool to alleviate spinal spasticity, Dimitrijevic and others (1998) recognized that the same method could be used to apply an excitatory drive to the lumbar spinal cord to provoke rhythmic movement in paralyzed legs. Indeed, when stimulation was applied to the posterior lumbar spinal cord structures (Fig. 2B and C) with frequencies of 25 to 50 Hz and intensities producing visible contractions in the legs, involuntary rhythmic flexion-extension movements (Fig. 2D) were generated in individuals with complete low cervical or thoracic SCI (Dimitrijevic and others 1998). Rhythmic activity was produced in all six participants studied. The movements were initiated while the subjects were lying in the supine position, that is, in a position with minimal sensory feedback related to axial limb load and hip extension angle otherwise essential for signaling phase transitions during locomotion (Rossignol and others 2006). Some of the electromyographic activity captured from the paralyzed legs demonstrated locomotor-like patterns with reciprocity between antagonists (Fig. 2E). The finding that the lumbar spinal cord could convert a sustained excitatory input into a coordinated rhythmic motor output was interpreted as evidence for the existence of CPGs in humans (Dimitrijevic and others 1998).

Flexible Organization of Pattern Generation

Danner and others (2015) investigated, in detail, rhythm and pattern generation by epidural stimulation in ten individuals with motor complete SCI lying supine. Coordinated rhythmic activity across thigh and lower leg muscles was most readily elicited with the stimulating cathode localized over the upper lumbar spinal cord segments (see also Shapkova 2004), suggesting that the circuits controlling hip flexors are essential elements of the rhythm-generating network entraining circuits controlling knee and ankle movement (Grillner 2006). Most of the rhythmic patterns were generated

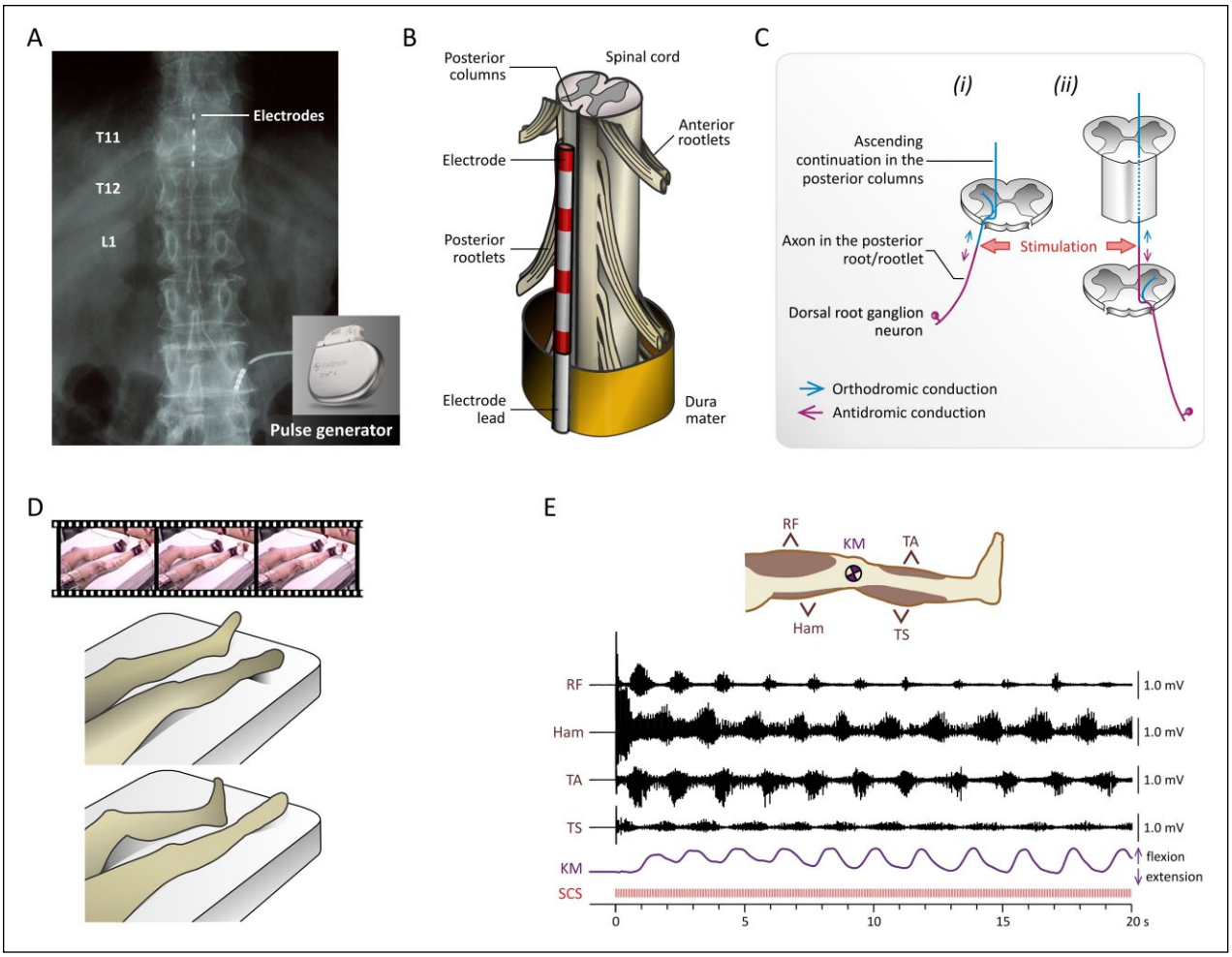

Figure 2. Locomotor-like activity generated by epidural spinal cord stimulation. (A) An epidural spinal cord stimulation system is composed of a lead with several electrodes, connected to an implantable pulse generator. Shown in the X-ray is a lead model with four electrodes (white rectangles) at the T11 vertebral level. (B) Drawing of a linear epidural lead with four electrodes (red cylinders) relative to the neural structures within the dural sac. The electrodes are located inside the vertebral canal but outside the dura mater covering the spinal cord, within a distance of a few millimeters to the posterior roots (dorsal roots) and the posterior columns (dorsal columns) of the spinal cord. Note that the real anatomy at the lumbar spinal cord levels is more complex, with the posterior aspect of the spinal cord being densely surrounded by long posterior rootlets and roots of several segments. (C) Sketch illustrating the electrically stimulated neural structures. The most proximal portions of the axons of dorsal root ganglion neurons (afferents transmitting sensory information to the spinal cord) with large-to-medium diameters within the posterior rootlets are primarily stimulated (i), and their ascending continuations in the posterior columns might be stimulated as well (ii). Each electrical pulse with sufficient intensity generates orthodromic and antidromic action potentials. Spinal circuits and motoneurons are activated trans-synaptically through the collateral projections of the stimulated afferents. (D) Drawing representing involuntary rhythmic movements induced by continuous spinal cord stimulation in an individual with motor complete spinal cord injury lying on an examination bed. The movements shown in the example were of small amplitudes, but were distinctly smooth and coordinated at hip, knee, and ankle joints. (E) Electromyographic activity of rectus femoris (RF), hamstrings muscle group (Ham), tibialis anterior (TA), and triceps surae muscle group (TS) with locomotor-like pattern induced by epidural spinal cord stimulation (SCS; onset of application at time "0"). The muscle activation patterns were effective in producing alternating flexion and extension movements, as documented by an angle measurement device (electronic clinometer) at the knee. Modified with permission from Tator and others (2012).

unilaterally, on the side of stronger stimulation, implying that the generation of rhythmic motor output does not depend on the presence of oscillating activity in the contralateral half of the spinal cord (Kiehn 2016). Within a leg, only 10% of all rhythmic activities analyzed had a locomotor-like pattern (Fig. 2E), while the most common pattern (38%) was a synchronous rhythmic activation across muscle groups. The remaining cases were different combinations of

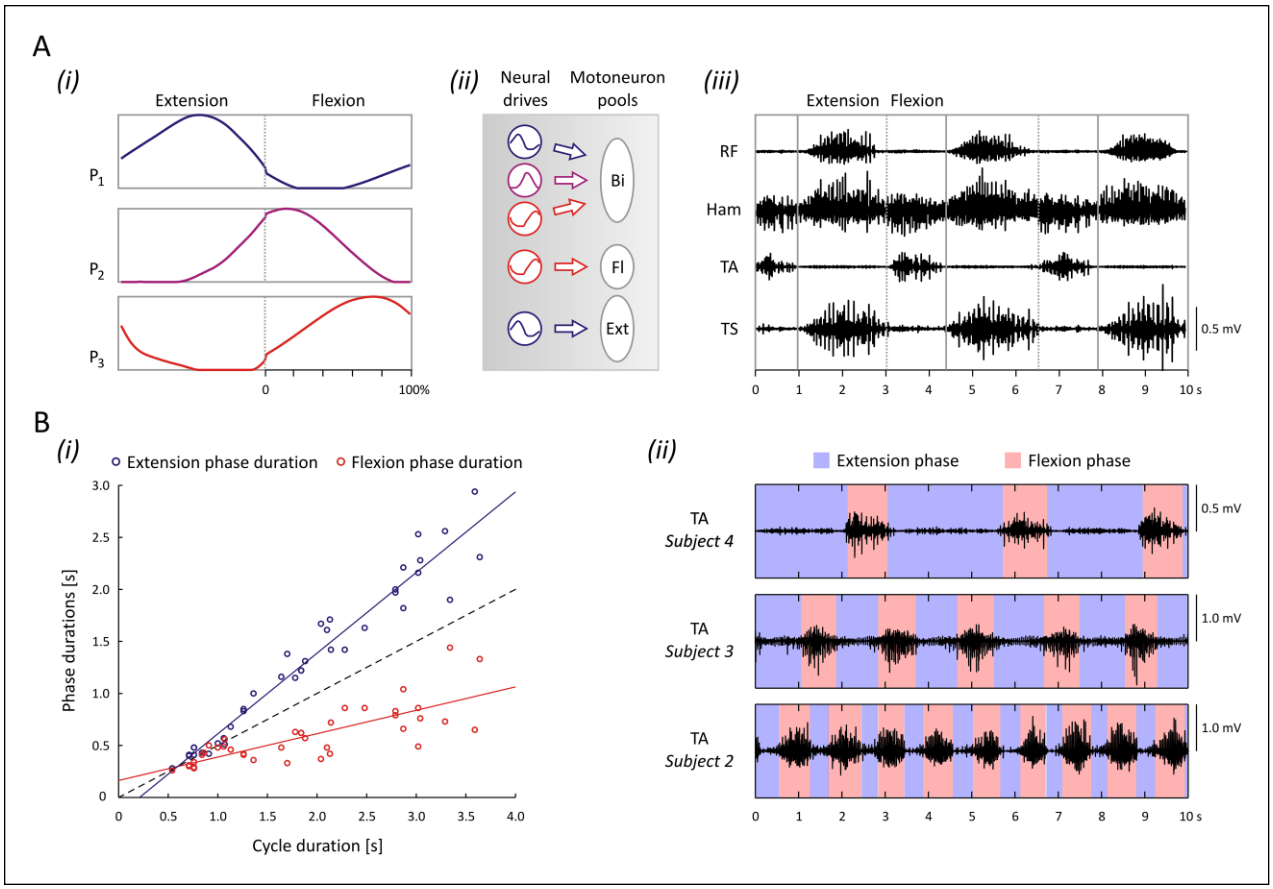

Figure 3. Flexible organization of burst generation and asymmetric control of cycle period. (A) (i) Linear combinations of three basic activation timing profiles (p_1, p_2, p_3) closely reproduce a large set of rhythmic electromyographic data acquired from different muscles and individuals with motor complete spinal cord injury during epidural spinal cord stimulation (Danner and others 2015). (ii) Two of these basic activation patterns represent alternating bursts of activity during extension- (Ext) and flexion-like (Fl) phases, while a third one characteristically contributed to the activation of the bifunctional (Bi) hamstrings muscle group. (iii) In the exemplary electromyographic recording shown, the extensor basic activation pattern p_1 drove the activity of rectus femoris (RF), the hamstrings muscle group (Ham), and triceps surae muscle group (TS), the flexor basic activation pattern p_3 largely loaded on Ham and tibialis anterior (TA), and Ham activity required the largest contribution of p_2. The example indicates how seemingly complex activation patterns can be reproduced by three basic activation patterns with different contributions. (B) Asymmetric regulation of the cycle period of spinal cord stimulation-induced rhythmic activity. (i) Scatterplots and regression lines for extension and flexion phase durations expressed relative to the corresponding cycle duration of rhythmic activity. Data are derived from 39 examples of multimuscle rhythmic electromyographic activity with various cycle durations collected from seven individuals with (motor) complete spinal cord injury lying supine. With longer cycle durations, the extension phase durations increased with a steeper slope. The dashed line with a slope of 0.5 is a reference indicating changes of cycle duration, which would have equal extension and flexion phase durations. Modified with permission from Danner and others (2015). (ii) Three examples of electromyographic recordings from tibialis anterior (TA) during spinal cord stimulation-induced rhythmic activity from the same data set as in (i), showing only a minor decrease in the TA-burst durations (indicating the flexion phases) in spite of a considerable increase in burst frequencies; subjects are numbered as in the original study (Danner and others 2015). Such asymmetric regulation of the of extensor and flexor phases with changes in speed is characteristic for terrestrial locomotion.

synchrony or alteration of rhythmic activity in the thigh and lower leg muscles. Factorization revealed that a statistical model based on only three basic activation patterns best represented the process that had generated the electromyographic data (Fig. 3A(i, ii)). Seemingly diverse and complex rhythmic activities (Fig. 3A(iii)) could be reproduced by differently weighted sums of these basic activation patterns, that is, by their flexible combination. The basic activation patterns were interpreted as neural drives of spinal burst generating elements. This interpretation suggests a versatile organization of human spinal motor control, combining the output of excitatory rhythm-generating kernels (Dougherty and others 2013) in various ways to produce different patterns of rhythmic activity (Kiehn 2016). Similarly, animal

experiments have shown that the mammalian lumbar spinal circuits can produce different rhythmic motor behaviors, such as scratch and locomotion, as well as different types of locomotion, such as walk, trot, gallop, and bound, thought to be produced by tuning parallel inhibitory and excitatory pathways interconnecting burst generating elements or within the pattern-generating circuits (Frigon 2012, Kiehn 2016). Clearly, the rhythmic motor patterns generated by epidural stimulation (Danner and others 2015) were more similar to those of fictive locomotion in animal experiments than to the complex patterns of erect human walking, where the activity across flexors and extensors, respectively, of hip, knee and ankle are not synchronous.

Asymmetric Regulation of Stance and Swing Phase Durations

The speed of locomotion is related to the burst frequency or, inversely, to the cycle of rhythmic activity. During walking with different speeds in humans (Murray 1967) as well as quadrupedal mammals, the cycle period is regulated primarily by changes of the stance phase duration. Supraspinal control and proprioceptive feedback can alter the cycle duration and the timing of extension and flexion phases, but it is thought that the extensor-dominated asymmetry in cycle period regulation is an intrinsic property of CPGs (Frigon 2012). The epidural-stimulation induced rhythmic electromyographic activity analyzed by Danner and colleagues (2015) had a wide range of burst frequencies of 0.3 to 1.8 Hz, including those corresponding to slow and fast gait. The relative extension-phase duration was 65.6% ± 10.4% of the cycle period, a value similar to normal walking. Increasing burst frequencies were accompanied by a decrease of the relative extension phase durations (Fig. 3B). This may suggest that the speed-dependent asymmetric modulation of stance and swing phase during normal walking might be at least partially controlled by the lumbar locomotor circuits in humans as well. Interestingly, similar results were obtained when the stepping reflex was studied in newborns (another model to study human locomotion with weak descending input from the brain, see the section Infant Stepping below) on a treadmill. The cycle duration of the stepping reflex changed with changes of the treadmill belt speed, and this adaptation resulted almost entirely from a change in the extensor burst duration, whereas the flexor burst duration remained nearly constant (Yang and others 1998).

Is a Central Pattern Generator Required to Explain the Observed Rhythmic Activity?

The neural underpinning of the spinal rhythm and pattern generator for locomotion in humans is unknown. Hence, it would be compelling to explain the generation of rhythmic activity under tonic epidural stimulation entirely based on circuits composed of motoneurons and spinal interneurons identified in humans. In simulations using an electronic circuit model, an inhibitory network of two populations of motoneurons, reciprocally coupled by Ia inhibitory interneurons, and Renshaw cells that provide a delayed inhibition of the Ia inhibitory interneurons, was shown to be sufficient to produce rhythmic flexor-extensor alternation (Miller and Scott 1977). In a recent experimental study using a mouse model (Talpalar and others 2011), the gene encoding the vesicular glutamate transporter 2 was inactivated, therefore physiologically inactivating all glutamatergic ventral spinal interneurons (including the presumed core elements of central rhythm generation). Bath application of neuroactive drugs to the isolated in vitro spinal cord nevertheless induced rhythmic activity, with alternation in ipsilateral flexor- and extensor-related ventral roots. A purely inhibitory interneuronal network must have generated the rhythmicity, and additional experiments suggested a network of reciprocally connected Ia inhibitory interneurons. However, the maximum rhythm frequencies were restricted to 0.4 Hz, while the obtainable frequency range was 0.2 to 1.4 Hz in the control mice. Further, electrical stimulation of the lumbar posterior roots, which produced fictive locomotion in the control mice, failed to generate any rhythmic activity in the knockout mice. This finding indicates that the glutamatergic neurons within the spinal circuits were essential for centrally evoking rhythmic activity in response to electrical stimulation of afferent pathways that project onto the lumbar locomotor networks.

There are some characteristics of the rhythmic electromyographic activity generated by epidural stimulation in SCI individuals that cannot be explained by inhibitory networks alone. When the frequency of epidural stimulation is increased to around 30 Hz (Danner and others 2015; Dimitrijevic and others 1998) without changing the site and intensity of the stimulation, responses in some muscle groups can attain larger electromyographic amplitudes during rhythmic activity than during unmodulated motor output (e.g., at a stimulation frequency of 10 Hz), or in response to single stimuli (Fig. 4A; Jilge and others 2004). This finding clearly suggests that during bursts of rhythmic activity, some additional excitatory influence acted on the motoneurons. Furthermore, the defined relation between stimulus pulse and response during epidural stimulation (Minassian and others 2004; Minassian and others 2007) gives information about the spinal pathways that had transmitted the response (Fig. 4B). Responses constituting rhythmic electromyographic activity peaking during flexion phases can have latencies delayed by up to 10 ms with respect to the shortest-latency responses to single stimuli (Minassian and others 2004; Minassian and others 2007), indicating that the activity was transmitted

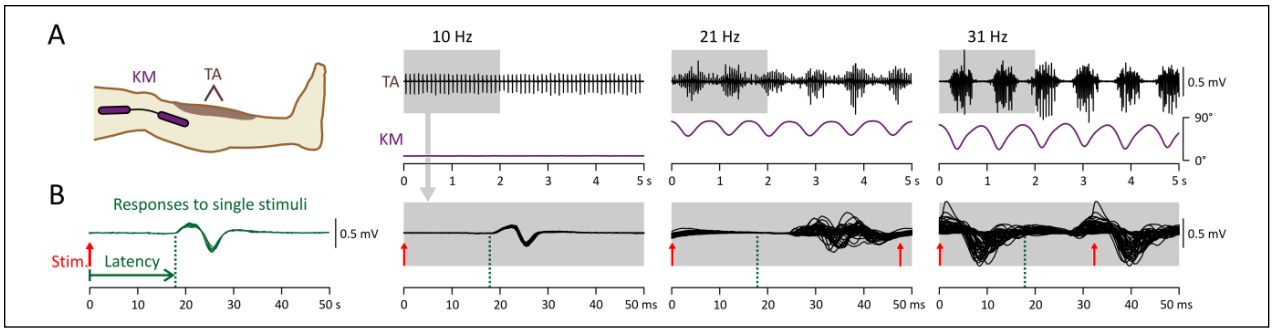

Figure 4. Characteristics of spinal cord stimulation-induced rhythmic electromyographic activity of a flexor muscle. (A) Continuous electromyographic recordings from tibialis anterior (TA) and goniometric recordings of stimulation-induced movements from the knee (KM). In the given example, responses in TA attain larger amplitudes when rhythmic activity is generated in the muscle with stimulation frequencies of 21 and 31 Hz compared with the unmodulated responses to 10 Hz with unchanged site and intensity of stimulation. Recordings derived from a complete spinal cord injured individual lying supine (Jilge and others 2004). (B) Five responses to single stimuli (panel on the left) with same stimulation site and intensity as in A and stimulus-triggered representation of the first 2 seconds of the continuous recordings (gray backgrounds) from A shown in enlarged time scale. During epidural lumbar spinal cord stimulation, each pulse of sufficient intensity evokes a posterior root-muscle reflex (Minassian and others 2004; Minassian and others 2007) that is electromyographically detected as an afferent-induced compound muscle action potential. Rhythmic activities are composed of a series of such evoked potentials, which are amplitude-modulated resulting in the burst-like shapes. The posterior root-muscle reflexes evoked by single stimuli have a short latency corresponding to a monosynaptic reflex. During rhythmic activities, these short-latency potentials are suppressed while delayed responses emerge, reflecting the involvement of central processing and additional synaptic delays. Modified with permission from Jilge and others (2004).

through spinal pathways with intercalated interneurons. These excitatory interneurons could be potential elements of the spinal rhythm or pattern generating circuits (Dougherty and others 2013; Kiehn 2016; Lavrov and others 2008).

Rhythmic Locomotor-Like Activity Generated by Neuropharmacological Stimulation

Rhythmic neural activity underlying locomotion critically depends on neurotransmitters used and released by neurons of the brain and spinal cord (Guertin 2014). Various inhibitory and excitatory neurotransmitters, such as γ-aminobutyric acid (GABA), glycine, glutamate, serotonin (5-HT), dopamine (DA), and noradrenaline (NA), were shown to significantly influence the activity of the CPG in different ways, demonstrated generally via in vitro isolated spinal cord preparation (Grillner and El Manira 2015; Guertin 2013; Harris-Warrick 2011; Sharples and others 2015). Specific roles of some of these neurotransmitter systems in spinally generated locomotor activity have been identified and further investigated in vivo using mainly spinalized cat models. Precursors such as 5-HTP and L-DOPA as well as small molecule ligands acting on corresponding receptors (e.g., α2-adrenergic receptor agonist, clonidine, or 5-HT2 serotonergic receptor antagonist, cyproheptadine) were shown to acutely alter locomotor activity in immobilized spinalized cats (Grillner and Zangger 1979; Jankowska and others 1967) or in spinalized cats stepping on a treadmill (Barbeau and others 1987).

These experiments paved the way to the first pilot studies with so-called "pro-locomotor" drugs in people with SCI. In SCI individuals displaying some voluntary control over leg muscles, clonidine, cyproheptadine and baclofen were shown to improve the results of intensive rehabilitation approaches combining treadmill stepping, body-weight support and manually assisted leg movement for stepping execution, but enhanced locomotor function was mainly attributable to anti-spastic effects of the drugs (Barbeau and others 1999; Fung and others 1990; Wainberg and others 1990). No locomotor activity was elicited by clonidine in completely paraplegic patients, and weight support and physiotherapist assistance remained pivotal at all times during treadmill stepping (Stewart and others 1991).

The minor effects induced pharmacologically in humans motivated the launch of several additional studies on the pharmacological control of spinal locomotion. Several studies of Stehouwer in 1997 (e.g., McEwen and others 1997) and Guertin in 2004 (e.g., Guertin 2004) demonstrated that small molecules capable of penetrating the blood brain barrier such as quipazine (5-HT2/3 receptor agonist) could trigger, within minutes post-administration, locomotor-like air-stepping movements in the hindlimbs of spinal rats or mice suspended in a harness, without further assistance, repeated training (and related

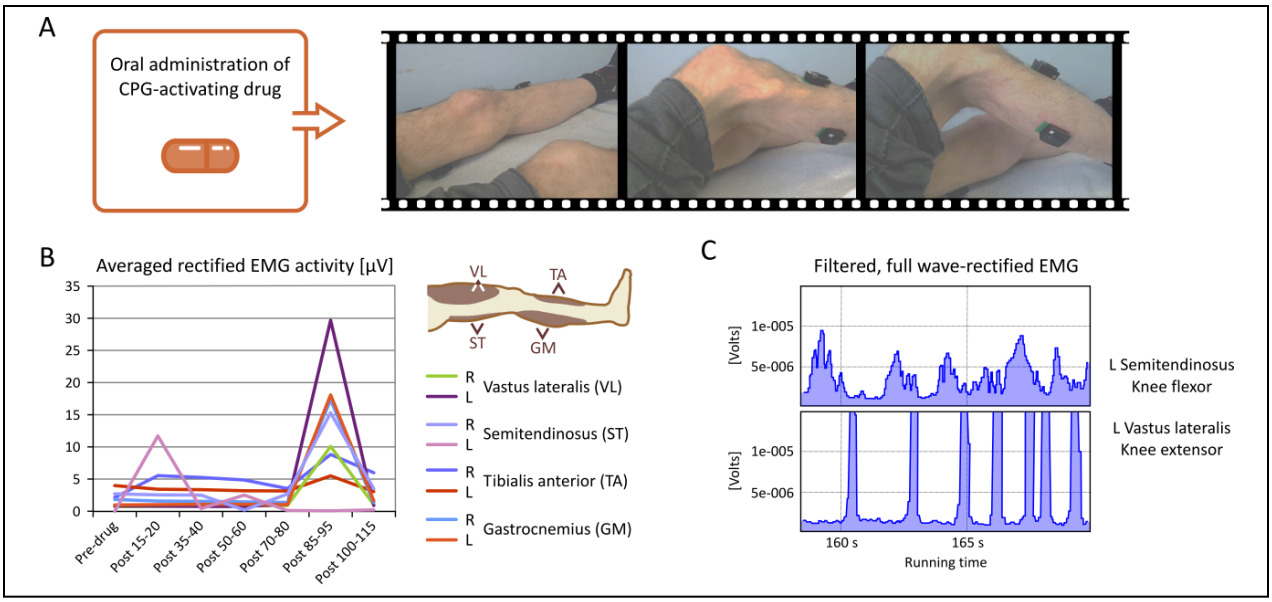

Figure 5. Rhythmic leg movement and muscle activation induced acutely in paraplegic individuals by neuropharmacological stimulation. (A) Successive frames from a video showing the effects of Spinalon 100 minutes post-administration in a subject who received orally the highest dose tested (500/125 mg levodopa/carbidopa + 50 mg buspirone); approximately 1 second between frames. A large-amplitude movement from an initially extended position occurred spontaneously in the individual with a motor complete spinal cord injury. (B) Amplitudes averaged from 5-minute periods of electromyographic activity of right (R) and left (L) leg recorded for 115 minutes post-administration of a lower dose of Spinalon (250/67 mg L-DOPA/carbidopa + 25 mg buspirone) in another subject. Electromyographic activity in all monitored muscles increased significantly compared with pre-drug administration levels near 90 minutes post-administration. (C) Envelopes of raw electromyographic activity in a subject who also received 250/67 mg L-DOPA/carbidopa + 25 mg buspirone, showing the rhythmic character of the induced activity at 45 minutes post-administration as well as the flexor-extensor out-of-phase relationship. Modified with permission from Radhakrishna and others (2017).

plasticity-induced changes spinally), or intense sensory stimulation such as tail pinching. This was taken as evidence for CPG-activating effects given that no stimuli other than quipazine on central spinal neurons could have been involved in movement initiation.

The synergistic effects of specific drug combinations in activating the CPG were the focus of subsequent studies in mouse models of SCI. Guertin and colleagues found that L-DOPA (NA/DA precursor) combined with benserazide or carbidopa (decarboxylase inhibitors enhancing central actions of L-DOPA) and buspirone (5-HT1A receptor agonist), induced episodes of weight-bearing stepping on a treadmill in spinal mice requiring no other form of external stimuli, assistance or training (Guertin and others 2010; Guertin and others 2011). They concluded that this drug combination may be suited for a therapeutic pro-locomotor drug for individuals with SCI and developed the drug product called Spinalon (levodopa + carbidopa + buspirone). In addition to its efficacy in animal experiments, its clinical relevance lies in the facts that it is orally-active, each active molecule administered separately is relatively safe and approved for the treatment of different neurological disorders (Parkinson's disease and anxiety), and that there is no drug-drug interaction between its two main molecules, L-DOPA and buspirone.

A phase I/IIa double-blind randomized clinical study on the effects of Spinalon (clinicaltrials.gov/ct2/show/NCT01484184) was recently completed in 45 individuals with an SCI clinically classified as complete or motor complete, sensory incomplete for at least 3 months (Radhakrishna and others 2017). For regulatory purposes, the clinical study aimed at assessing safety and the maximal tolerated dose of Spinalon as a spinal rhythm generating tritherapy in SCI patients. From a neuroscientific standpoint, electromyographic recordings from several leg muscles with the subjects placed in the supine position also allowed to evaluate the existence of a CPG in humans and the feasibility of activating it solely with pharmacological aids. A single dose of either Spinalon, levodopa/carbidopa (ratio 4:1), buspirone or a placebo was administered using a dose-escalation design. Indeed, below maximal tolerated doses, electromyographic activity and involuntary movement were triggered within 15 and 120 minutes post-administration in Spinalon-treated individuals (Fig. 5). Key locomotor characteristics such as rhythmicity, flexor-extensor reciprocity and left-right alternation were clearly identifiable in some cases

(Radhakrishna and others 2017). However, some subjects who had received Spinalon remained unresponsive for unclear reasons, while others displayed low-level muscular contractions only, and their legs, in the initially extended position, did not move with large amplitudes. The individuals who received the placebo, buspirone alone, or levodopa/carbidopa alone did not display a significant increase of electromyographic activity, or rhythmic flexor-extensor activity.

In summary, the clinical study showed for the first time that rhythmic contractions and locomotor-like movements could be acutely elicited pharmacologically in individuals with motor complete SCI. With the spontaneous onset of rhythmic activity, it is unlikely that sensory inputs from leg proprioceptors contributed to movement initiation or signaled extension-flexion transitions in the supine position and without manual assistance by the examiners. These findings, taken together with the fact that similar neuromodulators were also effective to elicit spinally generated locomotor activity in animals, provide further important and complementary evidence for the existence of CPGs in humans. Strategies combining supported stepping protocols with neuromodulation using comparable drug combinations and epidural spinal cord stimulation have triggered re-expression of hindlimb locomotion after complete SCI in adult rats (Courtine and others 2009) and unprecedented levels of sprouting and regeneration of the spinal cord circuits (van den Brand and others 2012). With the availability of an effective pharmacotherapy for humans, similar combinatorial therapies can be now tested in patients with SCI.

Is the Central Pattern Generator Used during Normal Walking in Humans?

The observation of rhythmic activity in individuals with SCI does not give information on whether the underlying circuits are utilized during active walking. In the following, studies will be discussed suggesting that these functional networks may indeed play a physiological role in pattern and rhythm control during normal walking in humans.

Infant Stepping

The stepping reflex is a stereotyped leg movement that can be evoked in newborns when held upright and inclined forward with the feet touching the ground. As a response, the legs lift and descend rhythmically. The stepping reflex mainly reflects spinal and brainstem control and is normally lost within the first months of development and growing interaction of supraspinal and spinal circuits. Forssberg (1985) characterized the stepping reflex as a forceful synchronous flexion at hip and knee at the end of stance and through early swing, followed by a synchronous extension through the end of swing, with a high degree of co-activation across muscles of different joints. It was suggested that infant stepping was produced by innate pattern generators in the spinal cord, yet that the plantigrade locomotion of adult humans (generation of loading responses and propulsive forces, asynchronized activity of extensor and flexor muscles), cannot be produced exclusively by the same circuits. Based on the gradual transformation of infant stepping to adult locomotion, Forssberg (1985) further concluded that the same spinal pattern generators are also utilized during adulthood, and that gradual development of the nervous system transforms the circuits' activity to the specific human locomotor patterns. Dominici and others (2011) applied factorization to a large set of electromyographic data recorded from reflex stepping in infants and locomotion in toddlers, preschoolers, and adults. During reflex stepping, the electromyographic activity was modulated rhythmically, with coactivation of extensors during stance, and of flexors during swing. The activity of the multiple muscles could be explained by two sinusoidal-like basic activation patterns, one peaking during stance, the other during swing, nearly identical to the basic extensor and flexor patterns identified by Danner and others (2015) in SCI individuals (p_1 and p_3 in Fig. 3A(i)). These two basic patterns of infant stepping were retained and refined through development, and were complemented by two new patterns, timed on foot contact and lift-off. This suggests that the two original extensor and flexor patterns and the circuits that generated them, likely CPGs, are not suppressed during development, but rather retained, integrated and adapted in the neural control of adult human locomotion (Dominici and others 2011; Grillner 2011; Yang and Gorassini 2006).

Neuromechanical Simulations of Human Locomotion

Under normal conditions, locomotion depends on the complex interactions between descending commands, spinal circuits that are largely located within the lumbar area of the spinal cord, the musculoskeletal system, and the environment (Rossignol and others 2006). Sensory feedback from proprioceptors, including muscle spindles, Golgi tendon organs, and joint receptors, is continuously integrated to adapt the motor output to external conditions (Frigon and Rossignol 2006). Neuromechanical simulations that combine numerical models of the musculoskeletal system with numerical models of neural circuits can help investigating the roles of individual components and in particular those of CPGs and different

sensory feedback pathways during locomotion (Ijspeert 2008; Ijspeert 2014). Such models cannot provide definite proofs of the existence or absence of CPGs, but they can be very useful for testing hypotheses about the interaction between central and peripheral rhythm generation, making predictions, and suggesting new experiments.

For instance, Taga and colleagues developed neuromechanical models of bipedal walking to demonstrate how stable locomotion could be obtained by coupling an abstract CPG model, spinal reflexes and a simple two-dimensional (2D) mechanical model of the body (Taga and others 1991; Taga 1998). These models showed that synchronized rhythmic behavior could be obtained between the neural activity of the CPG and the steps of the mechanical system, and that the whole neuromechanical system could exhibit stable limit cycle behavior, that is, robustness against (small) perturbations. The possibility to generate stable locomotion without considering CPGs was demonstrated by Geyer and colleagues in similar 2D and 3D models but with more realistic muscle models (Geyer and Herr 2010; Song and Geyer 2015). The models could generate rhythms and stable locomotion without CPGs by relying only on a set of muscle reflexes. The simulated joint angles, ground reaction forces, and electromyographic signals closely resembled those from human recordings.

The fact that purely reflex-driven models could replicate several features of human locomotion questioned the physiological benefit that a CPG would add to neural control of bipedal locomotion (Geyer and Herr 2010; Song and Geyer 2015). A generally accepted biological role of CPGs, studied in different animals such as lampreys and cats, is their capacity to be controlled by simple descending inputs to modulate their rhythm frequency to change locomotor speed and induce changes in their synchronization state for gait transitions (Kiehn 2006; Shik and others 1966). Another role is suggested from the understanding that CPGs from the viewpoint of control engineering can be seen as generators of feedforward signals, they are therefore by definition more resistant to sensory noise than pure feedback controllers. Consequently, two hypotheses for their contribution to bipedal human locomotion come to mind—first, CPGs can simplify the supraspinal control of speed of locomotion, and second, CPGs make locomotion more robust against the inherent noise of sensory signals and sensory failure. Indeed, the reflex-driven neuromuscular models (Geyer and Herr 2010; Song and Geyer 2015) do not allow simple control of speed, but rather require adjusting the strengths of multiple sensory feedback pathways to change the speed of locomotion. Such complex control strategy to be executed by supraspinal centers appears neurophysiologically questionable (Grillner 1985). The reflex-based models also suffer from sensitivity to sensory noise, and cannot produce stable locomotion when too much noise is added to sensory signals.

A recent neuromechanical modeling study tested whether CPGs could simplify the neural control of speed of locomotion in humans (Dzeladini and others 2014). Based on a hypothesis that a CPG could be also viewed as an observer of the sensory system (Kuo 2002), the reflex-driven network of Geyer and Herr (2010) was extended by a CPG network able to produce rhythmic activity and reproduce the patterns of sensory information observed during steady-state walking. The CPG network was composed of two layers, a rhythm-generating kernel, the "oscillators," and a pattern-generating layer of "CPG interneurons" that could reproduce the sensory inflow as an actual output signal (Fig. 6). The output of the CPG interneurons and sensory signals were then combined with an open set of parameters, determining their relative contributions for each single sensory pathway. The best results, in terms of robustness and the ability to modulate the walking speed, were obtained when the relative weights of the CPGs vs. sensory signals contributing to the activity of the motoneurons followed a proximo-distal gradient, with the CPGs driving more the hip muscles, and sensory signals controlling more the distal muscles. With these settings, the addition of the CPG network allowed the variation of the gait in a large range of speeds by modulation of a single parameter only through supraspinal influence, the oscillation frequency of the CPGs, without reducing the robustness of locomotion when compared to the purely reflex-driven model.

Conclusions

The existence of CPGs across vertebrates, including mammals such as mice, rats, rabbits, and cats, is firmly established (Guertin 2009). In the context of human spinal motor control, the demonstration of fictive locomotion in a non-human primate model, in acutely spinalized marmoset monkeys in the absence of phasic afferent input, was of essential relevance (Fedirchuk and others 1998). Some elements of these intrinsic networks should be conserved in humans and integrated with evolutionary bipedality, with a higher dependence on descending control and sensory feedback due to the upright gait (Duysens and van de Crommert 1998; Grillner 2011). Yet the same degree of evidence as in animals cannot be provided in humans, as there is no direct equivalent for fictive locomotion in humans. Still, the studies reviewed here showed that in the absence of a volitional motor task and under reduced influence from step-specific proprioceptive feedback (alternating lower limb loading and unloading together with backward-stretch at the hip), the human spinal circuits can produce rhythmic multimuscle activation patterns that closely resemble CPG-induced fictive

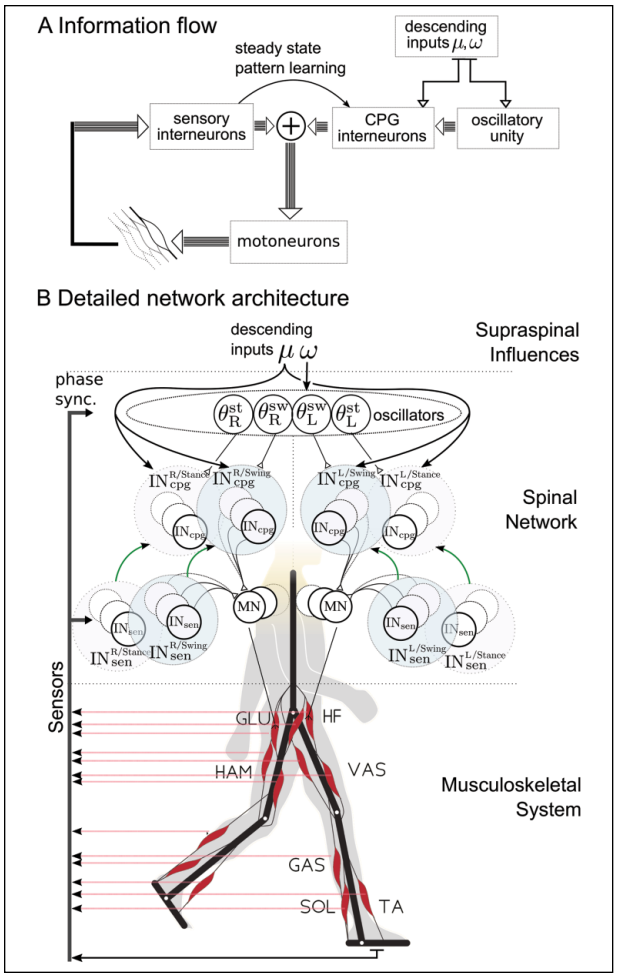

Figure 6. Neuromuscular model to study the relative contribution of central pattern generators (CPGs) and proprioceptive feedback to human locomotion, as proposed in Dzeladini and others (2014). (A) Information flow in the network. Each connection is modeled as a constant delay to account for the latency in neural communication. Proprioceptive feedback information from stretch-sensitive muscle spindles and muscle-tension sensitive Golgi tendon organs of the moving legs are fed back into the so-called "sensory interneurons" of spinal reflex circuits. CPG interneurons learn to mimic the pattern of activity of the sensory interneurons in steady state walking. The CPG interneurons are further driven by oscillator elements of the CPGs. The activity of the motoneurons is then given as a weighted sum of the feedforward drive through the CPG interneurons and feedback via the sensory interneurons. The relative weights of sensory and CPG interneurons for any given pathway can be tuned. (B) Detailed network architecture. Ground sensors are used to detect stance phases. Then, depending on whether the limb is in a stance or swing phase, different sensory pathways are active. The network is symmetric: Left/right parts of the figure correspond to the parts of the network acting on left/right leg muscles, respectively. The network comprises a three-level architecture: (1) Supraspinal influences: μ represents the activity-modulation pathway, scaling the output of each CPG, and ω is the frequency imposed on the CPG network. (2) Spinal network: There are four oscillators sharing the same ω, with two oscillators controlling each leg. On each side, one oscillator is synchronized with the touchdown event (and serves as a clock for the INcpg active during stance) and one with the lift-off event (and serves as a clock for the INcpg active during swing). The motoneuron activity is the result of the cumulated activity of sensory pathways and their corresponding CPGs. (3) Musculoskeletal system: composed of a two-dimensional articulated body, with seven muscles per leg implemented with Hill-like models as in Geyer and Herr (2010). Muscle activity is defined as a delayed and filtered motoneuronal activity, accounting for the neural communication latency and excitation-contraction coupling, respectively. IN_{cpg}, CPG interneurons; IN_{sen}, sensory interneurons; MN, motoneurons; GLU, gluteus; HF, hip flexor; VAS, vastus lateralis; HAM, hamstring; GAS, gastrocnemius; SOL, soleus; TA, tibialis anterior. Modified with permission from Dzeladini and others (2014).

locomotion in isolated animal spinal cord preparations. Furthermore, similarly as in animal experiments, the activity of these circuits can be expressed spontaneously, provoked by a sustained neural drive under electrical stimulation, or triggered by pharmacological neuromodulation with specific drug combinations. Our interpretation is that, taken together, these facts underpin the view that CPGs do exist in the human spinal cord.

The human CPGs are likely distributed throughout the lumbar and upper sacral spinal cord, with essential elements localized within the upper lumbar cord segments, and autonomous circuits in each side for the left and right leg. The capability to generate rhythmic patterns with a variety of combinations of synchronous or reciprocal activation of muscles implies an architecture of flexibly interconnected burst-generating elements (Danner and others 2015). Neuromechanical simulations suggest that CPGs might have a bigger influence on proximal joints, and that distal joints might rely more on proprioceptive feedback during stepping (Dzeladini and others 2014). The recent resurgence of interest in epidural stimulation in individuals with SCI (Angeli and others 2014) and novel clinical pharmacotherapies (Radhakrishna and others 2017) will provide new opportunities to reveal some physiological characteristics of the human CPGs.

It seems plausible that under normal conditions, the motor system would make use of an available functional spinal system, which could reduce the complexity of neural control of locomotion. Being only a part of the motor system composed of many autonomous and interdependent subcircuits, and with an important regulative role of feedback mechanism during movement, it is obvious that CPGs alone would not produce all neural control components essential for bipedal human locomotion. The course of development of locomotor patterns from newborns to adults suggests that at least part of multimuscle flexion

and extension synergies are controlled by CPGs (Grillner 2011; Yang and Gorassini 2006). Another important role of CPGs in human locomotion would be to allow a simple control of speed and regulation of stance and swing phases of the cycle period (Danner and others 2015; Dzeladini and others 2014; Yang and others 1998). Based on findings from neuromechanical simulations, one could speculate that relatively autonomous rhythm generators, which could ensure synchronization with the musculoskeletal system and sensory feedback, would be essential to achieve a target walking speed planned by supraspinal centers. Interestingly, the concept of CPGs is used in locomotion controllers for many robots, not only for swimming and multilegged robots, but also for bipedal robots (Ijspeert 2008). We envision that neuromechanical simulations and robots will increasingly be used to help investigating how neural control mechanisms interact with body and environment (Ijspeert 2014), and the role of CPG circuits in locomotion control.

Independent of the relative importance of CPG-activity in normal human locomotion, CPGs are an obvious target for neurorehabilitation strategies to improve locomotor function in patients following SCI. Functionally triggering the spinal circuits to re-express locomotion in individuals after severe SCI with standard-of-care rehabilitation and treadmill therapy alone has not been successful to date (Dietz and Fouad 2014). We have now essential elements available for developing new CPG-modulating clinical therapies, supported overground-walking protocols in combination with pharmacotherapy and spinal cord stimulation (Minassian and Hofstoetter 2016; Minassian and others 2016).

Declaration of Conflicting Interests

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