



Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions

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An ability to produce rhythmic activity is ubiquitous for locomotor pattern generation and modulation. The role that the rhythmogenesis capacity of the spinal cord plays in injured populations has become an area of interest and systematic investigation among researchers in recent years, despite its importance being long recognized by neurophysiologists and clinicians. Given that each individual interneuron, as a rule, receives a broad convergence of various supraspinal and sensory inputs and may contribute to a vast repertoire of motor actions, the importance of assessing the functional state of the spinal locomotor circuits becomes increasingly evident. Air-stepping can be used as a unique and important model for investigating human rhythmogenesis since its manifestation is largely facilitated by a reduction of external resistance. This article aims to provide a review on current issues related to the “locomotor” state and interactions between spinal and supraspinal influences on the central pattern generator (CPG) circuitry in humans, which may be important for developing gait rehabilitation strategies in individuals with spinal cord and brain injuries.

Keywords: central pattern generator, sensory input, rhythmogenesis, locomotion, humans

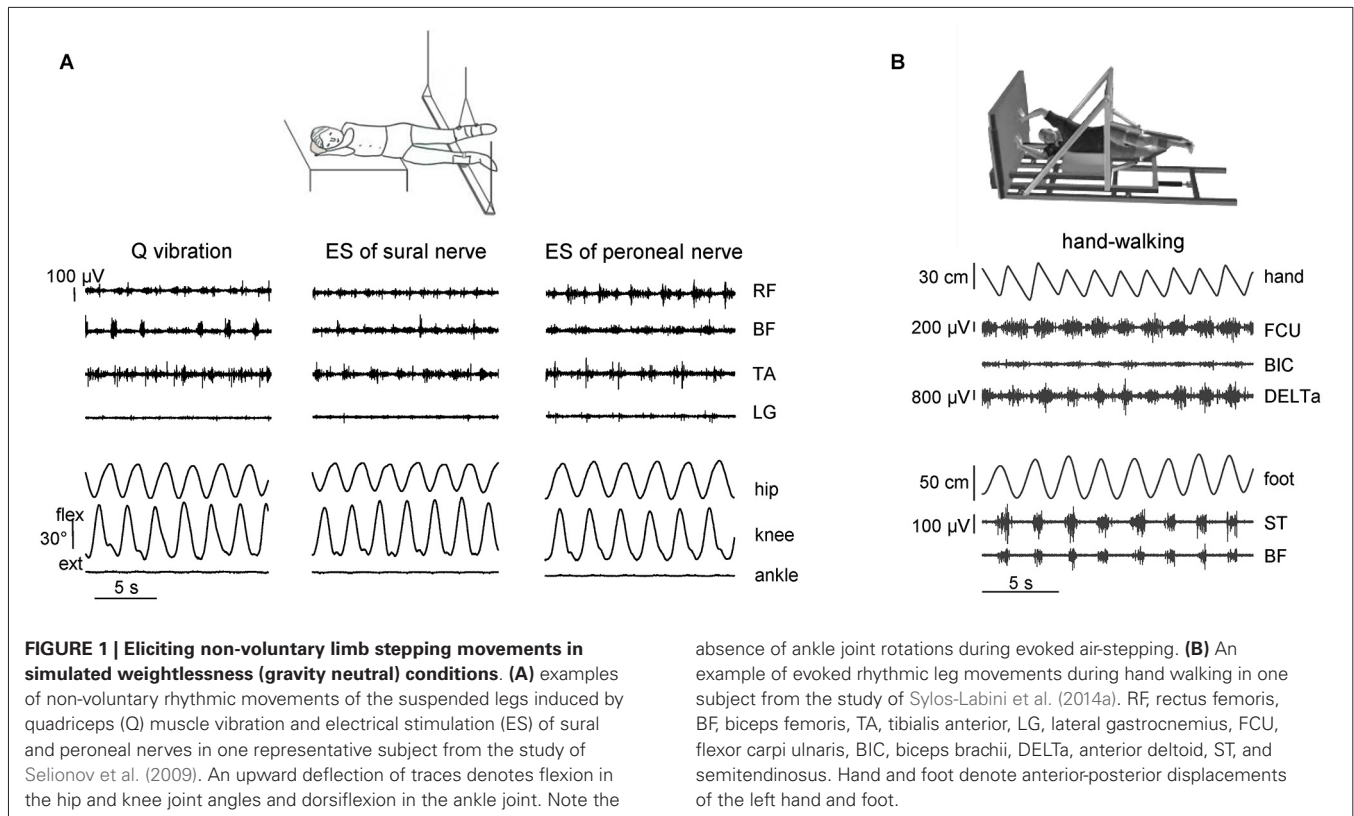
INTRODUCTION

It is now largely accepted that the neural circuitry controlling locomotion involves a central pattern generator (CPG; Grillner, 1981). CPG functioning depends on supraspinal inputs and sensory feedback (Shik, 1997; Orlovsky et al., 1999; Pearson, 2004; Jordan et al., 2008). Most CPGs are quiescent under resting condition and become recruited by supraspinal pathways with command function (Grillner, 2006). Sensory activity establishes the timing of major phase transitions and contributes to the production of motoneuronal drive (Nielsen and Sinkjaer, 2002; Pearson, 2004), and may also trigger a stepping-like output (Sherrington, 1910; Gurfinkel et al., 1998; Gerasimenko et al., 2010).

The capacity of the mammalian lumbosacral spinal cord to generate rhythmic activity in the absence of input from the brain is firmly established in animal models (Sherrington, 1910; Graham Brown, 1912; Grillner, 1981) and there is indirect evidence that CPGs may also be a feature of the human spinal cord (Bussel et al., 1996; Minassian et al., 2004; Shapkova, 2004; Dominici et al., 2011; Hubli and Dietz, 2013; Ivanenko et al., 2013). The available evidence suggests that many locomotor-related movements that humans perform routinely (walking, running, cycling, swimming, crawling, backward walking, etc.) use similar rhythm circuitry but additionally require specialized control circuits (Zehr, 2005; Patrick et al., 2009; Hoogkamer

et al., 2014). In fact, the capacity of neural circuits to generate rhythmic activity represents the common core for various locomotor tasks (Zehr, 2005). The aim of this article is to provide a review on current issues related to the excitability of spinal CPG circuitry in humans. Under normal conditions, it is sometimes difficult to investigate impairments in the CPG functioning due to interference with the ongoing task of body weight and balance control (including intense feedback). Therefore, one might examine the rhythmogenesis capacity of spinal circuitry in conditions not-complicated by these two factors.

Body weight support systems coupled with robotic devices or pharmacologic treatments are now often used in the rehabilitation practice to assist locomotor recovery in individuals with neuromotor disorders (Dietz, 2009; Sale et al., 2012; Hubli and Dietz, 2013; Valentin-Gudiol et al., 2013; Meyns et al., 2014; Moraru and Onose, 2014). There is still limited evidence of the efficacy of treadmill interventions with body weight support in some injured populations due to the complex nature of the control of locomotion, compensatory strategies, and plasticity of neuronal networks (Grasso et al., 2004; Picelli et al., 2013; Valentin-Gudiol et al., 2013; Swinnen et al., 2014; Sylos-Labini et al., 2014b). We will not review here any detailed analysis of clinical outcomes for ambulation when using locomotor training with body weight support systems and refer to other reviews (e.g.,



Wirz et al., 2005; Sale et al., 2012; Valentin-Gudiol et al., 2013; Scivoletto et al., 2014). The main focus here is to give emphasis to a facilitatory effect of simulated weightlessness on rhythmogenesis and its potential for assessing the state of the CPG circuits and for gait recovery after spinal cord injury and other neuromotor disorders.

LOCOMOTOR "STATE" OF THE SPINAL CIRCUITRY

Historically, Goltz and Freusberg (1874) were the first to report spontaneous air-stepping of the hindlimbs of the spinal dog before voiding the distended bladder, presumably due to some excitatory state of the spinal circuitry. In decerebrated animals exhibiting spontaneous fluctuations in their level of rigidity, rhythmic movements can be evoked by peripheral stimulation, provided there is an appropriate level of background extensor tonus and that the tonus is neither too low nor too high (Beritoff, 1915). In addition, an increase in tonus precedes the initiation of locomotion (Mori et al., 1982). The excitability status or state of the spinal network is thus of particular importance (Edgerton et al., 2008). Air-stepping can be used as a unique and important model for investigating human rhythmogenesis since its manifestation is largely facilitated by a reduction of external resistance, such as that resulting from body weight unloading (Gurfinkel et al., 1998; Selionov et al., 2009). Below we consider various experiments and observations in conditions of reduced gravity effects that help revealing the intrinsic properties of locomotor pattern generators and making evident the facilitation of non-voluntary limb stepping in humans.

The spinal CPG circuitry can be activated in healthy humans by applying tonic central or peripheral sensory inputs. As we previously mentioned, in addition to the control of the timing of major phase transitions and muscle activity production (Nielsen and Sinkjaer, 2002; Pearson, 2004), sensory activity has access to the functional state of CPG and may initiate a stepping-like output (Sherrington, 1910; Gurfinkel et al., 1998; Gerasimenko et al., 2010). **Figure 1A** illustrates different examples of stimulation techniques that were explored for eliciting non-voluntary air-stepping: continuous muscle vibration (40–60 Hz, ~ 1 mm amplitude), and electrical stimulation of the superficial peroneal or sural nerves (0.3 ms duration pulses, 2–3 mA, 60 Hz) (Selionov et al., 2009). To minimize interference with the ongoing task of body weight and balance control, stepping movements are elicited during air-stepping in the absence of gravity influences and reduced external resistance. The subjects were tested while lying on their side with the legs supported using long ropes attached to the ceiling (**Figure 1A**) or using an exoskeleton (**Figure 1B**) so that they provided low-friction pendulum-like leg motion in the horizontal plane with a limited vertical motion component. The afferent signals due to vibration or electrical stimulation of peripheral nerves may increase the excitability of several segments of the spinal cord, which may facilitate triggering of locomotor-like movements. The latency of the elicited cyclic movements varied significantly across subjects and conditions (range 1–25 s). The delay in the onset of leg movement likely reflects the general property of the pattern generation circuitry and transition from tonic activation to

the phasic CPG output. Generally, cyclic movements increased monotonically for 2–10 cycles until they reached a relatively constant amplitude of angular oscillations (Gurfinkel et al., 1998; Selionov et al., 2009; Gerasimenko et al., 2010). The characteristics of non-voluntary air-stepping (amplitude, cycle duration) were similar to the voluntary stepping in the same conditions.

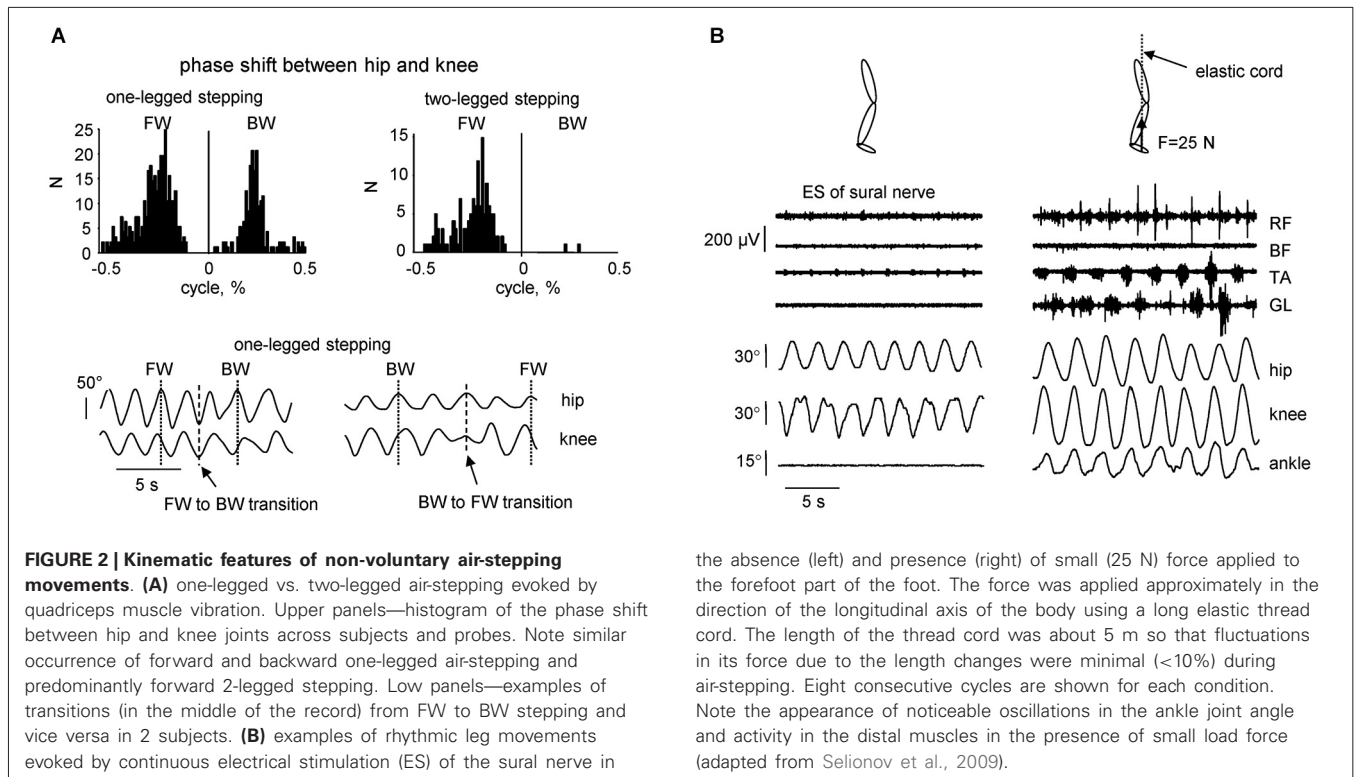
In addition to peripheral sensory stimulation, central tonic facilitatory influences may be used for eliciting rhythmic leg movements, such as the Jendrassik maneuver and the Kohnstamm phenomenon (Gurfinkel et al., 1998; Selionov et al., 2009). An intriguing approach related to the role of tonic influences is the Kohnstamm phenomenon (Kohnstamm, 1915), which consists in the appearance of involuntary tonic activity and a particular sensation of “lightness” after the cessation of a long-lasting (30–40 s) isometric effort. Post-activation phenomena can therefore be used as a tool to study tonic influences. After-effects of a voluntary, long-lasting contraction in the leg muscles featured alternating rhythmic leg movements that lasted for about 20–40 s (Selionov et al., 2009), corresponding roughly to a typical duration of the post-contraction activity (Craske and Craske, 1986; Duclos et al., 2004; Ivanenko et al., 2006b). The difference in the effects of the two techniques (the post-contraction phenomenon and the Jendrassik maneuver) may point to the importance of tonic activation of the lumbosacral enlargement, since voluntary arm contractions (due to the Jendrassik maneuver) are weaker in evoking stepping movements: they act primarily on the cervical spinal cord and are not sufficient to evoke air-stepping unless the experimenter triggers them (Selionov et al., 2009).

Other techniques for triggering stepping movements are based on the more direct stimulation of the spinal cord by electromagnetic (Gerasimenko et al., 2010), transcutaneous or epidural electrical stimulation (Shapkova and Schomburg, 2001; Gorodnichev et al., 2012), which can initiate and sustain movements more robustly than by stimulation of sensory afferent fibers. Transcutaneous electrical spinal cord stimulation (at 5–40 Hz) is applied over T11–T12 vertebrae and presumably activates the locomotor circuitry through the dorsal roots (Gorodnichev et al., 2012; Gerasimenko et al., 2014), while epidural stimulation is based on an implanted array of electrodes directly placed over the back portion of the lower thoracic-upper lumbar spinal cord (Figure 4A, upper panel). Rhythmic locomotor-like leg movements in a gravity neutral position can be evoked in ~10–50% of healthy subjects, and the degree of activation may depend on supraspinal influences and the state and the rhythmogenesis capacity of the spinal circuitry (Gurfinkel et al., 1998; Selionov et al., 2009; Gerasimenko et al., 2010). The common feature of all stimulations described above is that they are tonic. In this respect, they corroborate earlier pioneering observations in decerebrate cats that stepping can be induced using a simple tonic stimulation pattern applied to the mesencephalic locomotor region (Shik et al., 1966), but they also show that this type of control can be initiated at the lumbosacral spinal cord level. Overall, the findings suggest that nonspecific tonic excitability may elicit or facilitate CPG activity.

Finally, automatic, alternating movements of the legs can be initiated by upper limb movements by asking participants to move their arms rhythmically, as in hand-walking (Figure 1B; Sylos-Labini et al., 2014a). The idea is grounded on the evidence that the coordination between arms and legs during human locomotion shares many features with that in quadrupeds (Falgairolle et al., 2006; Zehr et al., 2007; Patrick et al., 2009; Dietz, 2011; Kutz-Buschbeck and Jing, 2012). For instance, inter-limb coupling in humans has previously been demonstrated by evoking reflexes in one limb and observing the extent to which the movement of another limb modulates reflex expression during walking (Haridas and Zehr, 2003; Mezzarane et al., 2011; Massaad et al., 2014). The coupling between the activity of cervical motoneurons underlying hand-walking and the activity of lumbosacral motoneurons underlying leg movements (Figure 1B) is presumably indirect, delayed and asynchronous (e.g., leg stepping is often characterized by a non-integer ratio between arm and leg movements frequency). These variable features suggest that signals related to arm movements do not directly entrain the motor commands to leg muscles, but affect the state of the lumbosacral locomotor circuitry, consistent with a facilitatory effect of arm swinging on cyclic leg muscle activity (de Kam et al., 2013). In addition, it has been recently shown that cervical transcutaneous stimulation of the spinal cord significantly facilitates non-voluntary air-stepping leg movements and the lumbosacral locomotor-related neuronal circuitry (Gerasimenko et al., 2014). One possible route for these trigger signals is through the intrinsic spinal pathways (propriospinal interneurons) linking cervical to lumbosacral regions in humans (Nathan et al., 1996). However, considering the latency of the leg responses relative to arm oscillations, supraspinal contributions cannot be excluded. Rhythmic arm movements imitating those during running or walking can also evoke prominent modulation of leg muscle EMGs during standing (Danna-Dos-Santos et al., 2009). Whatever the exact mechanism, these findings (Figure 1B) reinforce the idea that there exists a functional coupling between arm and leg CPGs.

INTERACTION BETWEEN RHYTHM-GENERATION ACTIVITY AND SENSORY INPUT

The previous studies, which aimed to activate the CPG circuits using the “air-stepping” paradigm (Gurfinkel et al., 1998; Selionov et al., 2009; Gerasimenko et al., 2010, 2014; Sylos-Labini et al., 2014a), also revealed some essential features of the intrinsic rhythm generation in humans. The evoked cyclic movements share many of their characteristics with animals. For instance, given the extensive evidence for the presence of commissural interneurons driving the contralateral locomotor circuitry (Kiehn, 2011), oscillator mechanisms and tonic influences may not be limb-specific. We found, for example, that treating one limb (e.g., applying electrical stimulation of the peroneal or sural nerves of one leg) can have its output transferred to another limb, even if the treated limb is kept stationary (Selionov et al., 2009). Also, although pattern generators for each limb have the potential to produce relatively autonomous rhythmic patterns (Forssberg et al., 1980; Yang et al., 2005), right



the absence (left) and presence (right) of small (25 N) force applied to the forefoot part of the foot. The force was applied approximately in the direction of the longitudinal axis of the body using a long elastic thread cord. The length of the thread cord was about 5 m so that fluctuations in its force due to the length changes were minimal (<10%) during air-stepping. Eight consecutive cycles are shown for each condition. Note the appearance of noticeable oscillations in the ankle joint angle and activity in the distal muscles in the presence of small load force (adapted from Selionov et al., 2009).

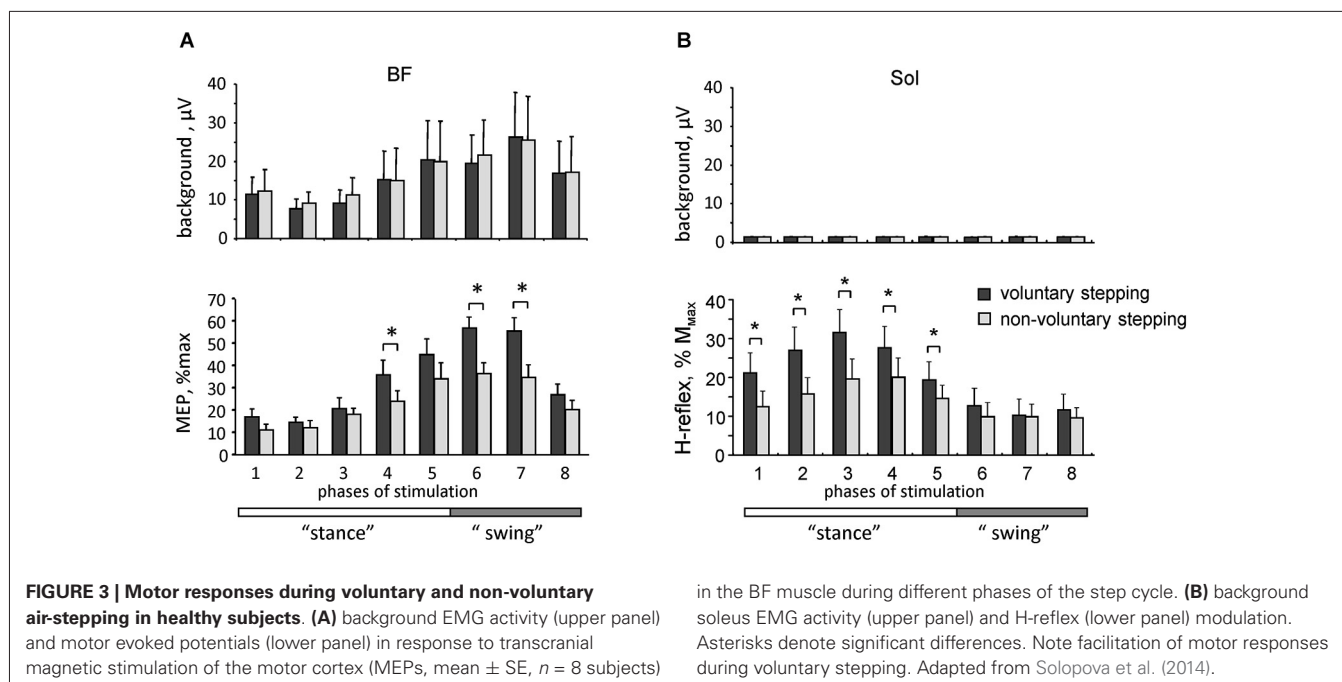
and left sides are strongly coupled under most natural conditions (Orlovsky et al., 1999; Ivanenko et al., 2006a; Maclellan et al., 2014). Further evidence of the importance of bilateral coupling is demonstrated by the finding that two-legged stepping was more stable (and predominantly forward, **Figure 2A**, upper panels), whereas one-legged stepping in some subjects displayed frequent spontaneous transitions from forward to backward direction and vice versa (**Figure 2A**, lower panels).

Air-stepping tends to involve prominent movements in the hip and knee joints, whereas the ankle joint is typically not involved, unless minimal loading forces are applied to the foot (**Figure 2B**). The facilitatory effect of forces is often accompanied by modulation of the EMG activity, consistent with phase-dependent contribution of sensory activity to the pre-programmed motoneuronal drive of the distal muscles during human walking (Duysens et al., 2000; Nielsen and Sinkjaer, 2002). Even individuals with clinically motor complete paralysis demonstrate modulated activity of distal leg muscles during assisted stepping with body weight support (during locomotion with 100% body unloading, no EMG activity was present) (Harkema et al., 1997; Dietz et al., 2002). It can be concluded that afferent input from load-related receptors (including Golgi tendon organs, spindles, cutaneous receptors, and various load mechanoreceptors in the foot arch, Duysens et al., 2000; Pearson, 2004; Gravano et al., 2011) contributes to the generation of locomotor activity in the isolated human spinal cord. Therefore, the sacral pattern generation circuitry (Cazalets and Bertrand, 2000) might be inactivated when the input from the support surface is lacking. The more direct stimulation of the spinal

cord locomotor circuitry using repetitive electromagnetic stimuli can evoke ankle joint oscillations (Gerasimenko et al., 2010). However, in this case it likely involves stimulation of the dorsal roots, and thus load-related afferents. Overall, the lack of ankle joint movements during non-voluntary air-stepping (**Figures 1A, 2B**) supports the hypothesis that the upper lumbar pattern generator activity may constitute the major oscillator “pacemaker,” whereas the sacral generator could play a subordinator role for adaptation to specific foot-support interactions. Also, minimal contact forces during air-stepping may significantly improve accurate foot trajectory control, suggesting that the support surface represents an importance reference frame and is included in the locomotor body scheme (Ivanenko et al., 2002).

ENGAGEMENT OF SUPRASPINAL MOTOR AREAS

Better understanding of interactions between spinal and supraspinal influences on the state of CPG circuitry may be important for developing gait rehabilitation strategies in individuals with spinal cord and brain injuries. In addition, there is an increasing consensus that motor centers in the brain, and the motor cortex in particular, play an essential and greater role in human walking compared to other mammals (Capaday, 2002; Yang and Gorassini, 2006; Petersen et al., 2012; Beloozerova et al., 2013). For instance, the coherence analysis demonstrated significant coupling between EEG recordings over the leg motor area and EMG from the tibialis anterior muscle prior to heel strike during the swing phase of walking, suggesting that the motor cortex and corticospinal



tract contribute directly to the muscle activity observed in steady-state human walking (Petersen et al., 2012). Recently, we compared motor evoked potentials (MEP) in response to transcranial magnetic stimulation of the motor cortex and the H-reflex during voluntary and vibration-induced air-stepping movements in healthy humans (Solopova et al., 2014). Both the MEPs and H-reflex were significantly smaller during vibration-induced cyclic leg movements at matched amplitudes of angular motion and muscle activity (Figure 3). One may suppose that in both cases the locomotor-like leg movements are evoked via activation of the spinal pattern generation circuitry. The greater responsiveness to central inputs during voluntary CPG activation (Figure 3) may be related to facilitation of transcortical reflex pathways (Christensen et al., 1999), increased depolarization of motoneurons, and/or an overall facilitatory effect on spinal motoneurons and interneurons. Interestingly, modulation of the H-reflex was observed in the absence of noticeable background EMG activity of the soleus and tibialis anterior muscles (likely due to the absence of limb loading and ankle joint movements), and occurred during the hypothetical stance phase of the step cycle (Figure 3), consistent with a CPG phase-related modulation of spinal reflexes.

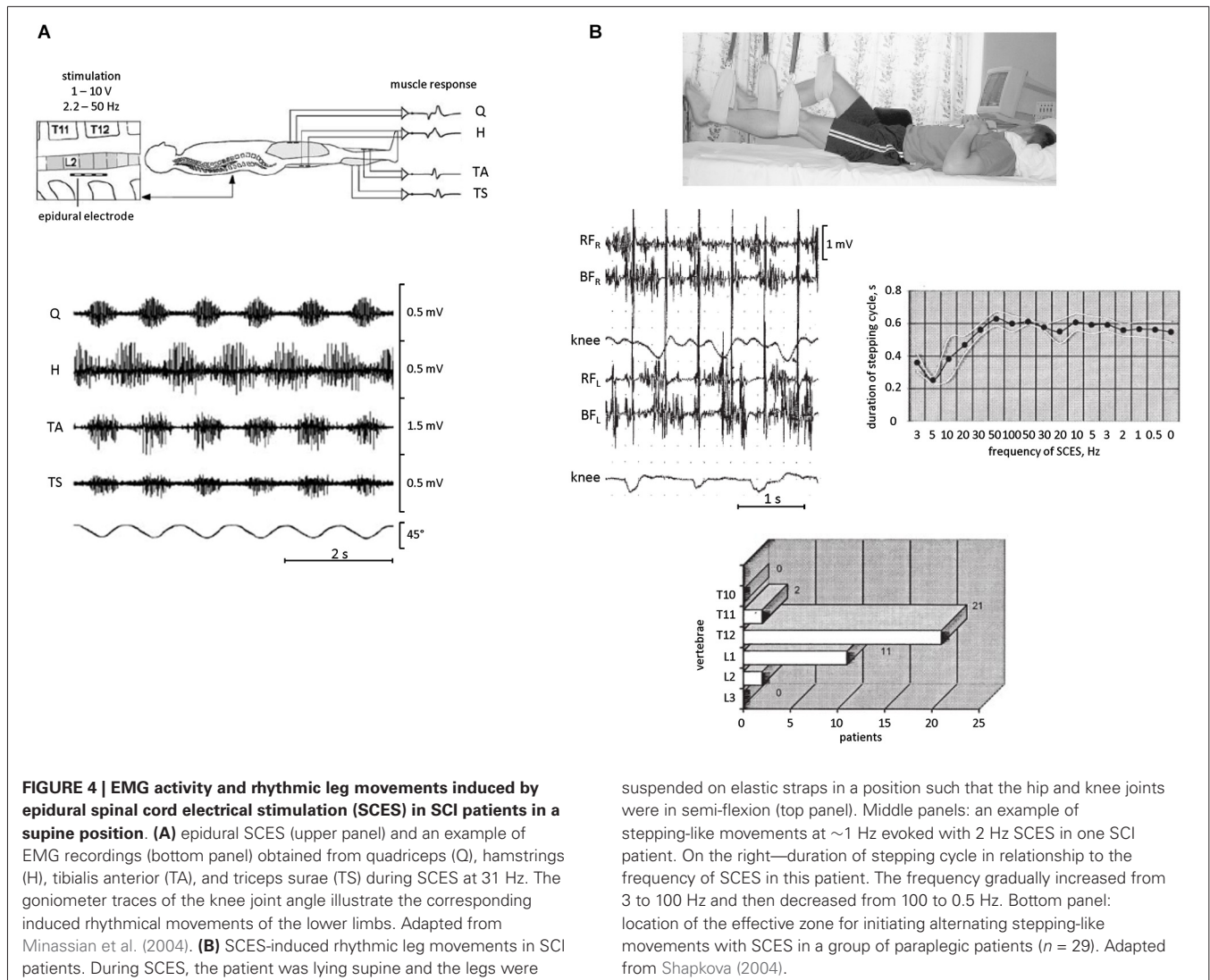
These findings highlight differences between voluntary and non-voluntary activation of the spinal pattern generator circuitry, presumably due to an extra facilitatory effect of voluntary control of stepping on spinal motoneurons and interneurons. It has been argued that the engagement of supraspinal motor areas may be beneficial for gait recovery (van den Brand et al., 2012), and there is a link between facilitation of segmental reflexes and the ability to recover gait (Dietz et al., 2009; Thompson and Wolpaw, 2014). Our results (Figure 3) support this hypothesis, and show an overall facilitatory effect of supraspinally mediated stepping

on reflex responses. Such investigations may contribute to the clinical development of CPG-modulating therapies (Guertin, 2014).

TAPPING INTO RHYTHM GENERATION CIRCUITRY IN NEUROMOTOR DISORDERS

During the last decade, there has been a growing interest in understanding an appropriate state of the spinal circuitry for performing locomotor movements (Hultborn, 2001; Edgerton et al., 2008; van den Brand et al., 2012; Selionov et al., 2013). In particular, to trigger the CPG by neurons with command function (Grillner, 2006), the physiological state of the spinal network needs to be properly prepared (Edgerton et al., 2008) since the same interneurons and motoneurons may contribute to a vast repertoire of motor actions (Hultborn, 2001).

A facilitatory effect of simulated weightlessness can be used for investigating rhythmogenesis of the spinal cord in injured populations and for entraining the spinal locomotor circuitry. Epidural stimulation is a technique that has been used for a number of years to treat individuals with a spinal cord injury, and various experiments emphasized a significant complementary effect of epidural stimulation when combined with pharmacological facilitation, e.g., serotonergic agonists, and step training (Shapkova and Schomburg, 2001; Minassian et al., 2007; Gerasimenko et al., 2008). The existence of a spinal locomotor generator circuitry in humans has been confirmed based on observations in patients with a severe spinal cord injury implanted with an array of electrodes directly placed over the back portion of the lower thoracic-upper lumbar spinal cord (Minassian et al., 2004; Shapkova, 2004). In these experiments, stepping-like movements were produced in patients who were supine with the legs in the resting position (Figure 4A) or suspended in the air (Figure 4B). Epidural stimulation could



even produce rhythmic EMG activities without step-related sensory feedback (stationary legs) or with a rhythm frequency independent of that of passive treadmill stepping (Minassian et al., 2013). Nevertheless, leg suspension significantly facilitates the manifestation of rhythmic motion (Figure 4B) and permits to reveal its characteristics. For instance, depending on the exact location of the stimulating electrodes, the stimulation could produce different patterns of rhythmic leg movements with different involvements of leg joints (Shapkova, 2004), consistent with the idea that there exist individual CPGs for each limb and/or each segment, and are coordinated during natural locomotion to produce a coherent interlimb pattern (Graham Brown, 1912; Grillner, 1981). Epidural stimulation can also transform the CPG circuitry into the active functional state which persists even after a significant decrease of stimulation frequency (Figure 4B, right panel). Interestingly, non-voluntary (evoked by epidural stimulation) air-stepping movements in incomplete spinal cord injury individuals can be sustained for more than 1 h with increasing EMG activity, while voluntarily

initiated rhythmic leg movements in these patients demonstrate progressive fatigue after several minutes (Shapkova, 2004). Thus, even though supraspinally mediated activation of stepping has an overall facilitatory effect on reflex responses (Figure 3) and pattern generation (Solopova et al., 2014), it may also contribute to the development of “central” fatigue (Taylor et al., 2006). Furthermore, daily sessions with epidural stimulation evoking air-stepping rhythmic movements were effective in restoring the locomotor function in some children with a severe spinal cord injury (Shapkova, 2004).

The residual sensory pathways may be critical in regaining voluntary movement. Moreover, the neuromodulation and activation of the “locomotor state” of the spinal circuitry below the lesion may enable completely paralyzed individuals to process conceptual, auditory and visual inputs, and to regain some voluntary control of paralyzed muscles (Angeli et al., 2014). In this study, a stimulation protocol was developed to allow the individuals to stimulate for ~ 1 h while practicing intentional movement in the supine position. Four individuals diagnosed

with clinically motor complete paralysis (classified as AIS-B and AIS-A before implantation) and implanted with a lumbosacral spinal cord stimulator at least 2.2 years post injury were able to generate EMG activity and movement during ankle dorsiflexion in the presence of epidural stimulation following a verbal command. No motor activity was present when attempting to move without epidural stimulation. Furthermore, daily training resulted in the generation of voluntary efforts with higher forces and lower stimulation voltages to reach the thresholds that enabled voluntary motor responses that could be modulated by visual and/or auditory input (Angeli et al., 2014). Hence, it is essential to discern how the spinal pattern generation circuitry is controlled by sensory input and supraspinal networks to design new rehabilitation devices that involve modulation of the physiological state of the spinal cord during training. A degradation of spinal neuronal activity takes place following a spinal cord injury, suggesting that a continuous training approach starting early after injury is necessary to maintain neuronal activity below the level of the lesion (Dietz and Müller, 2004). Future studies may focus on the mechanisms underlying the manifestation of early motor symptoms, muscle tone, impaired sensory feedback and their relation to rhythmogenesis investigated under simulated weightlessness conditions. This may also help facilitating the application of neurophysiological analyses as quantification tools for evaluating new medications useful to assess or augment the rhythmogenesis capacity and gait recovery in neurological disorders.

CONCLUDING REMARKS

Novel pharmacological strategies (Roy et al., 2012; Borton et al., 2014; Guertin, 2014) and electromagnetic stimulation techniques (Shapkova and Schomburg, 2001; Minassian et al., 2007; Gerasimenko et al., 2008; Selionov et al., 2009; Angeli et al., 2014) are being developed aimed at modulating spinal activity and restoring the locomotor function. Even though electrochemical or sensory stimulations do not necessarily induce automated stepping by activating CPG networks, they may transform lumbosacral circuits from non-functional to functional states, enabling the information-processing interface in the spinal cord to utilize multifaceted sensory input as a source of control for locomotion (Courtine et al., 2009). Overall, recent findings highlight the importance of investigating the tonic “state” of the spinal circuits. Since the air-stepping is free from many of the mechanical constraints of normal walking, it may provide an effective model for studying how peripheral inputs influence CPG behavior in human adults (Gurfinkel et al., 1998; Shapkova and Schomburg, 2001; Selionov et al., 2009; Gerasimenko et al., 2010; Solopova et al., 2014; Sylos-Labini et al., 2014a). Thus, the beneficial effect of simulated weightlessness on rhythmogenesis may enhance the utility of spinal cord stimulation techniques for developing CPG-modulating therapies and augmentation of function for disabled people.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research grants #13-04-12076 and #15-04-02825, Italian Health Ministry and Italian Space Agency (COREA Grant).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 05 November 2014; accepted: 27 January 2015; published online: 18 February 2015.

Citation: Solopova IA, Selionov VA, Sylos-Labini F, Gurfinkel VS, Lacquaniti F and Ivanenko YP (2015) Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions. *Front. Syst. Neurosci.* 9:14. doi: 10.3389/fnsys.2015.00014

This article was submitted to the journal *Frontiers in Systems Neuroscience*.

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