Initiation and modulation of locomotor circuitry output with multi-site transcutaneous electrical stimulation of the spinal cord in non-injured humans

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Abstract

The mammalian lumbar spinal cord has the capability to generate locomotor activity in the absence of input from the brain. Previously, we reported that transcutaneous electrical stimulation of the spinal cord at vertebral level T11 can activate the locomotor circuitry in non-injured subjects when their legs are placed in a gravity neutral position (Gorodnichev et al., 2012). In the present study we hypothesized that stimulating multiple spinal sites and therefore unique combinations of networks converging on postural and locomotor lumbosacral networks would be more effective in inducing more robust locomotor behavior and more selective control than stimulation of more restricted networks. We demonstrate that simultaneous stimulation at the cervical, thoracic, and lumbar levels induced coordinated stepping movements with a greater range of motion at multiple joints in five of six non-injured subjects. We show that the addition of stimulation at L1 and/or at C5 to stimulation at T11 immediately resulted in enhancing the kinematics and inter-limb coordination as well as the EMG patterns in proximal and distal leg muscles. Sequential cessation of stimulation at C5 and then at L1 resulted in a progressive degradation of the stepping pattern. The synergistic and interactive effects of transcutaneous stimulation suggest a multi-segmental convergence of descending and ascending, and most likely propriospinal, influences on the spinal neuronal circuitries associated with locomotor activity. The potential impact of using multi-site spinal cord stimulation as a strategy to neuromodulate the spinal circuitry has significant implications in furthering our understanding of the mechanisms controlling posture and locomotion and for regaining significant sensorimotor function even after a severe spinal cord injury.

Key words: multi-site transcutaneous spinal cord stimulation, locomotor neuronal circuitry, stepping movements, non-injured subjects
**Introduction**

The spinal networks controlling stepping movements of the hindlimbs, i.e., the central pattern generator (CPG), are located in the lumbosacral segments of the spinal cord. These networks can generate stepping patterns and adapt to external conditions (Grillner and Zangger 1979; Grillner et al., 2008). We have demonstrated that epidural stimulation applied simultaneously at the L2 and S1 spinal cord segments in the presence of monoamines enables full weight-bearing plantar stepping in rats with a complete mid-thoracic spinal cord transection (Gerasimenko et al., 2007; Courtine et al., 2009; Musienko et al., 2012). Recently we reported that four human patients with a motor complete spinal cord lesion were able to stand independently for prolonged periods as well as facilitate assisted stepping when a broad area of the dorsal surface of the lumbosacral spinal cord involving multiple segmental levels was stimulated via an epidural electrode array (Harkema et al., 2011). Furthermore, over multiple sessions of neuromodulation these patients regained some voluntary control in their legs (Angeli et al., 2014) and reported improvement in some autonomic functions, such as blood pressure, sexual function, and bladder control.

We also have shown that non-invasive electromagnetic spinal cord stimulation (Gerasimenko et al., 2010) and a relatively painless means of transcutaneous electrical stimulation of the spinal cord (painless cutaneous enabling motor control, pcEmc) (Gorodnichev et al., 2012) at vertebral level T11 can activate the locomotor circuitry in non-injured human subjects when their legs are placed in a gravity-neutral position. The purpose of the present study was to determine if this noninvasive strategy of pcEmc could be used to fine-tune the human locomotor circuitry by selectively activating different combinations of motor networks. To
address this issue, we compared the kinematics and EMG patterns enabled by pcEmc at multiple combinations of stimulation sites.

The results show that stimulation at cervical, thoracic, and lumbar spinal levels simultaneously initiates locomotor-like flexion-extension movements more effectively and with larger amplitudes than stimulation at either one or two segments. In addition, the generation of locomotor-like movements was more finely modulated when the spinal circuitry was stimulated at multiple but independent stimulation sources resulting in more coordinated, refined stepping movements. The impact of combining stimulation at cervical with lumbosacral sites provides further evidence of the importance of having the capability to activate those spinal networks that can best mediate the complex behaviors needed to assist in the execution of a specific motor task such as locomotion. The potential impact of using pcEmc as a strategy to neuromodulate spinal circuitries has significant implications in furthering our understanding of the mechanisms that control posture and locomotion and that are involved with regaining motor function after a spinal cord injury and, perhaps, other neuromotor disorders. Preliminary results of this work have been reported elsewhere (Gerasimenko et al., 2012).

Materials and Methods

Six non-injured individuals (males students of Velikie Luky State Academy of Physical Education and Sport, Velikie Luky, Russia) participated in the study. The study was approved by the Human Subject Protection Committee at the Velikie Luky State Academy of Physical Education and Sport, Velikie Luky, and conformed to the principles stated in the Declaration of Helsinki. All subjects signed voluntary written consent forms to participate in these experiments.

Experimental procedures
The experimental setup was similar to that described previously (Gurfinkel et al., 2000; Selionov et al., 2009, Gerasimenko et al., 2010). Briefly, the subjects were tested while lying on their right side with the upper leg supported directly at the shank and the lower leg placed on a rotating brace attached to a horizontal board supported by vertical ropes secured to hooks in the ceiling. By using this supportive device the subject’s legs were placed in a gravity-neutral position. The position of the legs of the subjects in the gravity-neutral apparatus was similar to that published previously (Selionov et al., 2009). The subjects were instructed not to voluntarily intervene with the movements induced by the stimulation.

Based on previous results showing that involuntary stepping movements can be elicited in non-injured subjects by transcutaneous spinal cord stimulation at 5 Hz applied between the T11 and T12 vertebrae (Gorodnichev et al., 2012), we used the same parameters of stimulation. In this study the protocol consisted of stimulation at 5 Hz specifically delivered between the C5 and C6, T11 and T12, and L1 and L2 spinous processes (hereafter referred to as C5, T11, and L1). The intensity of stimulation at each spinal level was adjusted based on the sensations felt by the subject and the motor output generated. Stimulation at any segmental level did not elicit discomfort in the subjects. Individual and multiple combinations of stimulation (5 Hz) at C5, T11, and L1 were tested. In one session the stimulation at each spinal level occurred simultaneously whereas in a second session stimulation occurred sequentially. As a final test, each subject was asked to generate a stepping-like motion voluntarily for 20 sec. Each test session was completed within a 2 h period.

Stimulation procedures

cpEmc stimulation (Patent filed WO 2013071309 A1) was delivered using a 2.5 cm round electrode (Lead-Lok, Sandpoint, USA) placed midline at the C5, T11, and/or L1 spinous
processes as cathodes and two 5.0 × 10.2 cm² rectangular plates made of conductive plastic (Ambu, Ballerup, Germany) placed symmetrically on the skin over the iliac crests as anodes.

Biphasic rectangular 0.3-1.0 msec pulses filled with a carrier frequency of 10 kHz and at an intensity ranging from 30 to 180 mA were used (Fig. 1). The stimulation frequency was 5 Hz and the duration of exposure ranged from 10 to 30 sec. This waveform was used because it has been shown to modulate the spinal cord relatively painlessly and noninvasively (Ward, 2009).

Data recording and analyses

EMG activity was recorded using surface bipolar electrodes from the rectus femoris, biceps femoris, medial gastrocnemius, and tibialis anterior bilaterally as described previously (Gerasimenko et al., 2010). EMG signals were differentially amplified (bandwidth of 10 Hz to 10 kHz) by a ME 6000 16-channel telemetric electroneuromyograph (MegaWin, Finland) and digitized at 2 kHz. The filtered EMG signals (low-pass filtered with a cutoff frequency of 100 Hz) were analyzed off-line.

Kinematics measures of leg movements were recorded using the Qualisy video system (Sweden). Movements of the right (upper) leg were monitored using reflective markers placed at the lateral epicondyle of the humerus, greater trochanter, lateral epicondyle of the femur, lateral malleolus, and hallux. Angular movements of the hip joint were derived from the markers placed at the lateral epicondyle of the humerus, greater trochanter, and lateral epicondyle of the femur. Knee movements were derived from the markers placed at the greater trochanter, lateral epicondyle of the femur, and lateral malleolus. Ankle movements were derived from markers placed at the lateral epicondyle of the femur, lateral malleolus, and hallux. Markers placed at the greater trochanter, lateral epicondyle of the humerus, lateral malleolus, and hallux were used to
reconstruct the kinematics of the stepping movements. Angular movements at the right and left
knee joints also were recorded using goniometers attached laterally to the suspended legs.

Statistical analyses

The data are reported as means ± SD. Overall significant differences among the variables
studied during voluntary stepping and pcEmc with the legs in a gravity-neutral apparatus were
determined using a one-way repeated-measures ANOVA. The level of statistical significance
was set at $P < 0.05$.

Results

Noninvasive stimulation, i.e., pcEmc, was used to gain access to the spinal locomotor
circuits. The stimulation was tolerated easily by the subjects and did not cause any pain even
when the strength of the current was increased to 180 mA, i.e., the maximum current used. The
lack of any pain response can be attributed to the use of biphasic stimuli with a carrier frequency
of 10 kHz, a frequency that suppresses the sensitivity of pain receptors (Ward, 2009). Table 1
lists the mean intensity of pcEmc (mA, based on effect) that was applied at different spinal levels
for each subject.

pcEmc at 5 Hz applied at T11 alone resulted in the initiation of step-like movements (Fig.
2A). The latency for inducing the stepping movements was subject-dependent. The involuntary
stepping movements induced by pcEmc are reflected in the EMG bursting activity in
homologous muscles in the left and right legs (Fig. 2A) and in the kinematics of the left and right
knees (Fig. 2C). Multi-segmental stimulation of the cervical, thoracic, and lumbar spinal cord
segments initiated stepping movements that reached maximal excursions and EMG burst
amplitudes within 2-3 step cycles and were much greater than with stimulation at T11 alone (Fig.
Spinal cord multi-site transcutaneous stimulation

The movements of the right and left knees reflected a distinct alternating stepping pattern (Fig. 2C and D). Reciprocal, alternating patterns were more evident with multi-site compared to single site stimulation.

Figure 3 shows the kinematics (amplitudes of joint movements and cycle periods) of the initial step-like movements induced by pcEmc (5 Hz) applied at one site (T11) vs. simultaneously at multi-sites (T11, L1, and C5) in 4 non-injured subjects. Generally, multi-site stimulation resulted in larger flexion-extension movements and in a more rapid increase in the amplitude of both the hip and knee excursions. The amplitudes of the step-like movements with multi-site stimulation consistently exceeded the amplitudes of the movements during stimulation at T11 alone (Fig. 3). In contrast, the cycle period was somewhat variable and not overtly different between stimulation at one or multiple sites.

We next tested the effects of sequentially adding stimulation at various sites. Stimulation at only T11 induced alternating movements in the right and left legs and corresponding EMG activity in the biceps femoris bilaterally (Fig. 4). The addition of stimulation at L1 and then C5 progressively increased the amplitude of these movements and the EMG activity. Adding stimulation at L1 to T11 immediately resulted in enhancing the kinematics and inter-limb coordination as well as the EMG patterns and involved movement of the ankle joint as reflected by activation of the medial gastrocnemius (Fig. 4A). In general, however, there was little movement at the ankle in most subjects (Fig. 5A). Robust coordinated step-like movements between the left and right legs as well as between joints within a leg were observed in the presence of multi-site stimulation (Fig. 4B and C). Sequential cessation of stimulation at C5 resulted in a progressive decrease in the magnitude of the knee excursions and EMG activity.
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(Fig. 4A-C). Subsequent cessation of stimulation at L1 did not result in a further decrease in these parameters.

The excursions at the hip, knee, and ankle and the cycle periods of the step-like movements under each stimulation condition are shown for 5 subjects in Figure 5. In general the amplitude of the excursions at the hip and knee progressively increased with stimulation at one (T11), two (T11+L1) and then three (T11+L1+C5) sites. Minimal movement was observed at the ankle under any condition. During normal overground locomotion the cycle period as determined by the kinematics of the hip, knee, and ankle are nearly identical. This tight linking of the temporal features at the three joints was not always present in response to pcEmc with the limbs in a gravity-neutral position. This was particularly apparent in three subjects where the mean cycle periods as determined at the knee were shorter than at the hip during stimulation at T11+L1+C5. In addition, in one subject the excursion at the knee was longer than at the hip and in another subject the excursion at the hip was longer than at the knee with stimulation at T11+L1.

Stick diagram decompositions of a single step cycle for three individuals under each stimulation condition are shown in Figure 6. Note that in two subjects (subjects A and B) the effects of stimulation on the step cycle were site specific, whereas in a third subject (subject E) the step cycles were relatively similar across all conditions. For example, minimal movement was observed with stimulation at C5 alone or at L1 alone in subjects A and B and at T11+L1 in subject B. In all three subjects, stimulation at all three sites produced the most robust step cycle. In most cases the movements of the lower limb illustrated by stick figures were qualitatively similar during multi-site stimulation and the trajectory of the hallux reflected less flexion during the swing phase of the step cycle than during voluntary air stepping. The effectiveness of
stimulation at all three sites is highlighted in Figure 7: cessation of stimulation at either C5 or L1 during a bout of stimulation at all three sites resulted in a decrease in the movement excursion at the knees and in the biceps femoris EMG amplitude bilaterally. In addition, the stepping pattern was immediately recovered when all three sites were stimulated again.

Discussion

The results demonstrate that locomotor-like behavior in the legs, as assessed by the excursion of joint movements, muscle EMG activity, and inter-limb coordination, can be initiated involuntarily with pcEmc in non-injured subjects. These step-like patterns can be modulated by stimulation at specific sites along the spinal cord and are subject specific. The most novel finding in the present study is that multi-site stimulation produces a more robust response compared to single site stimulation. We propose that these observations are consistent with the concept of differential modulation of the activation levels of combinations of motor networks projecting to specific combinations of interneurons that coordinate the levels of recruitment of different combinations of motor pools throughout a step cycle.

Transcutaneous stimulation of upper lumbar segments elicits step-like movements similar to other forms of spinal stimulation

Locomotor-like movements can be induced in decerebrated and spinal animals by epidural stimulation (Iwahara et al., 1991; Gerasimenko et al., 2003; Ichiyama et al. 2005; Musienko et al. 2007) and by intraspinal stimulation (Barthelemy et al., 2006; Guevremont et al., 2006; Barthelemy et al., 2007). We have demonstrated that epidural stimulation of the lumbosacral spinal cord is an effective tool for inducing stepping in adult chronic spinal animals (Gerasimenko et al., 2002, 2003; Ichiyama et al., 2005; Lavrov et al., 2006). Functionally, there also is clear evidence that epidural stimulation can be used to facilitate locomotor-like
movements in spinal cord injured human subjects lying in a supine position (Dimitrijevic et al., 1998; Gerasimenko et al., 2001; Minassian et al., 2007; Harkema et al., 2011).

Recently, we described a novel noninvasive method for the activation of spinal locomotor networks in humans, i.e., pcEmc (Gorodnichev et al., 2012). In our early studies using epidural stimulation for mapping of the spinal cord we demonstrated that stimulation at the L2 spinal segment (vertebral level T11-T12) induced locomotor-like movements in the legs of SCI patients more effectively than stimulation at more rostral or caudal segments (Dimitirijevic et al., 1998). Using this region as a trigger of locomotor behavior also is supported by the results of our previous studies using epidural stimulation in complete SCI subjects (Harkema et al., 2011; Angeli et al., 2014). Furthermore, we reported that more effective locomotor movements are induced consistently with electromagnetic spinal cord stimulation at the T11-T12 vertebral level compared to other vertebral levels in non-injured subjects with their legs placed in a gravity-neutral position (Gerasimenko et al., 2010). Studies in animals also have shown that the rostral segments of the lumbar enlargement are critical for the initiation of stepping movements (Langlet et al., 2005; Barthélemy et al., 2007). Based on these observations the area between vertebral levels T11 and T12 was used as the primary site for the transcutaneous spinal cord stimulation. We showed that pcEmc (5-40 Hz) applied at the T11–T12 vertebrae ables involuntary step-like movements in non-injured subjects with their legs suspended in a gravity-neutral position. It also has been shown that transcutaneous spinal cord stimulation (30 Hz) facilitates locomotor-like EMG patterns during assisted treadmill stepping in incomplete (Hofstoetter et al., 2013) and motor complete spinal cord injured subjects (Minassian et al., 2013) when stimulating segments L1-L4 with a 5 cm electrode. It seems rather clear that the upper lumbar segments play a central role in facilitating locomotor movements, regardless of the
different means and methods to stimulate. The novel information in the present study is that there are multiple pathways-networks that can interact with those key segments to achieve unique locomotor movements.

**Rationale for the effectiveness of multi-site stimulation of spinal locomotor-generating networks**

The spinal networks within the lumbosacral region can control stepping movements of the hindlimbs and adapt to external conditions when provided the necessary sensory feedback (Pearson, 2000; Rossignol et al., 2006; Gerasimenko et al., 2007; Courtine et al., 2009; Shah et al., 2012). In mammals it seems that the rhythmogenic capacity of these spinal networks, in the absence of supraspinal and sensory inputs, is distributed throughout the lumbosacral cord along a rostrocaudal gradient (Grillner, 1979; Kiehn, 2006). Experiments performed on spinal cats with a pharmacological block at selected spinal lumbar segments demonstrate that the integrity of the L3-L4 spinal segments is necessary to sustain locomotor activity (Marcoux and Rossignol, 2000). It also has been suggested that the rostral segments of the lumbar cord are critical for the initiation of stepping movements (Langlet et al., 2005). Intraspinal microstimulation of the caudal segments of the lumbosacral enlargement (L7-S1) also can elicit alternating movements in the hindlimbs in spinal cats (Guevremont et al., 2006), although it is unclear whether these movements are a result of direct activation of the caudal networks or are mediated by indirect activation of the rostral networks. Epidural stimulation of the rostral segments (L2) of the lumbar spinal cord has been reported to be a more effective site for inducing rhythmic movements in humans (Dimitrijevic et al., 1998), whereas stimulation of more caudal segments allow for greater postural control (Harkema et al., 2011). Epidural stimulation at the L2 and S1 spinal cord segments in the presence of serotoninergic agonists enabled weight-bearing stepping with plantar
foot placement in complete spinal cord transected rats (Gerasimenko et al., 2007; Courtine et al., 2009; Musienko et al., 2011). All of these observations are suggestive of the possibility that stimulation of multiple spinal sites might be complementary in inducing the most effective stepping-like movements. Depending on how the different combinations of networks interface, however, will determine whether they will be complementary, not additive at all, or even competitive. The present results demonstrate that they can be highly complementary. Herein, we demonstrate that step-like movements were considerably more robust with multi-site pcEmc than with stimulation at one site (Figs. 2 and 5). The effect was especially clear during sequential addition of stimulation sites, i.e., initially at T11, then L1, and then C5 (Fig. 4). Our evidence from spinally evoked potentials at 1 Hz demonstrate that stimulation of rostral and caudal areas of the lumbosacral spinal cord correspond to activation of the motor pools of the more proximal and distal muscles, respectively (unpublished data). Similarly we have reported that stimulation of local regions of the lumbosacral spinal cord via electrodes within an epidural electrode array allow for selective recruitment of motor pools of proximal and distal leg muscles in spinal cord injured human subjects (Sayenko et al., 2014). The different characteristics of motor potentials evoked during transcutaneous stimulation at the lumbosacral enlargement also are consistent with selective topographical recruitment of proximal and distal leg muscles in humans (unpublished data). Combined, these results suggest that activation of proximal and distal interneurons and motoneurons within the lumbosacral spinal cord have complementary effects on locomotor-related neuronal circuits.

**Cervical stimulation facilitates the lumbosacral locomotor-related neuronal circuitry**

There are clear functional interconnections between the locomotor networks for the forelimbs and hindlimbs, providing inter-limb coupling in quadrupedal animals (Shik and
Orlovsky 1965; Cazalets and Bertrand, 2000; Ballion et al., 2001; Juvin et al., 2005, 2012, Gerasimenko et al. 2010) as well as in humans (Zehr and Duysens, 2004; Zehr et al., 2009, Sylos-Labini et al., 2014). Long propriospinal neurons have been suggested to play an important role in the functional coupling of the cervical and lumbosacral locomotor networks, providing forelimb-hindlimb coordination (Yamaguchi, 1986). Recently we reported that hindlimb motor function of rats with a lateral hemisection of the spinal cord could be improved when the forelimbs were engaged simultaneously with the hindlimbs during treadmill step training (Shah et al., 2013). This phenomenon can be attributed, at least in part, to a reorganization and re-engagement of rostrocaudal spinal interneuronal networks involving the propriospinal system. It is known that long propriospinal axons can be activated by stimulation of peripheral nerves in the forelimbs (Lloyd 1942; Shomburg et al., 1978; Menetrey et al., 1985). For example, a strong facilitation of the H-reflex with a delay of ~100 ms was observed in the legs of non-injured humans with a conditioning stimulation of the ulnaris or median nerve. This facilitatory effect is mediated by the long propriospinal system (Meink and Piesiur-Strehlow, 1981; Delwaide and Crenna, 1983; Kagamihara et al., 2003). It has been suggested that stimulation at the cervical level activates the propriospinal system that, in turn, relays the excitatory tonic drive to the lumbosacral locomotor neuronal circuitry (Miller et al, 1998; Cowley et al., 2008). This idea is consistent with the hypothesis that neurons within the propriospinal system are capable of activating spinal interneurons that are involved with stepping (Shik, 1997; Jordan and Schmidt, 2002). Our observations that stimulation at C5 alone induced step-like movements in the legs of one subject (Fig. 6), that the addition of C5 stimulation to T11 and/or L1 stimulation immediately improved stepping performance in another subject (Fig. 5), and that elimination of C5 stimulation during multi-site stimulation impaired the stepping and EMG pattern (Fig. 7) are
all consistent with the long propriospinal system modulating the lumbosacral locomotor circuitry.

Conclusions

Multi-site transcutaneous electrical spinal cord stimulation can be an effective tool for the regulation of locomotor behavior in non-injured subjects. With the lower limbs placed in a gravity-neutral position, pcEmc delivered simultaneously at the C5, T11, and L1 vertebral levels facilitated involuntary stepping movements of a greater magnitude than when stimulating at two sites which tended to be greater when stimulating only one site. When different sequences of stimulation at multiple sites are presented, the withdrawal of C5 in the presence of continuing stimulation at T11 and L1 resulted in a dramatic reduction in limb oscillation amplitude. In spite of this significance effect of C5 withdrawal, stimulation at C5 alone resulted in the smallest oscillatory movement in two of three subjects. Generally the most effective single stimulation site for inducing limb oscillations was T11. These generalizations of relative effectiveness of different combinations of stimulation sites should be tempered given the inter-subject variation in responsiveness. The synergistic and interactive effects of pcEmc suggest a multi-segmental convergence of descending and ascending, and most likely propriospinal, influences on the spinal neuronal circuitries associated with locomotor activity. These data demonstrate the potential of a non-invasive means of stimulating the spinal cord and provide a new tool for modulating spinal locomotor circuits and facilitating locomotion. From a clinical perspective, will be important to determine the effectiveness of this non-invasive, multisite stimulation neuromodulatory technique in facilitating recovery of sensorimotor function after a severe spinal cord injury.
ACKNOWLEDGMENTS

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GRANTS

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AUTHOR CONTRIBUTIONS

YG, RG, and VRE designed the study; YG, RG, AP, TM, AS, and VS performed the experiments; YG, VRE, TM, and AS analyzed the data; YG, TM, RRR, VRE, and DL interpreted the results; YG, VRE, and TM, prepared the figures; YG and VRE drafted the manuscript; YG, RRR, and VRE, edited and revised the manuscript; all authors approved the final version of the manuscript.

DISCLOSURES

D.C.L, V.R.E., R.R.R., and Y.G. have ownership interest in Neuroenabling Technologies Inc., which has been formed to eventually develop spinal cord stimulation devices that have advanced capabilities relative to the devices used in the present studies.

REFERENCE:


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Zehr EP, Hundza SR, Vasudevan EV. The quadrupedal nature of human bipedal locomotion.
Table 1. List of the maximum stimulation amplitudes used for each subject at each stimulation site.

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Figure 1. Diagram of the painless cutaneous stimulation (pcEmc) paradigm. A 10-kHz biphasic stimulation is delivered in 0.3-1.0 ms pulses with these pulses delivered at 5 Hz.

Figure 2. Angular excursions of the right (R) and left (L) knee joints and EMG activity in the right biceps femoris (RBF), right medial gastrocnemius (RMG), left BF (LBF) and left MG (LMG) muscles with pcEmc (5 Hz) at T11 alone (A) and at C5+T11+L1 simultaneously (B). Angle-angle trajectory plots of multiple cycles (50 ms time bins) showing the left (horizontal)-right (vertical) kinematics coupling of the angular movements at the knee with pcEmc at T11 (C) and at C5+T11+L1 (D) as shown in (A) and (B), respectively. Color scheme in (C) and (D) reflects the density of the data points with red being of the highest density.

Figure 3. Peak-to-peak excursion amplitudes at the hip and knee joints and the corresponding cycle period durations are shown for the first five step-like movements in a gravity-neutral apparatus in response to stimulation at T11 only and at T11+L1+C5 for four subjects.

Figure 4. (A) Angular excursions of the right (R) and left (L) knee joints and corresponding EMG activity in the RBF, LBF, and LMG muscles with pcEmc at T11 with and without stimulation at L1 and/or C5 are shown. (B) Stick diagram decompositions (40 ms between
sticks) of the movements of the right leg during one step cycle during stimulation at T11, T11+L1, and T11+L1+C5 are shown. Arrows under the stick diagrams indicate the direction of movement. (C) Angle-angle plots for each segment of the traces shown in (A) and abbreviations, same as described in Figure 2.

Figure 5. Amplitudes of the excursions at the hip, knee, and ankle (A) and the cycle durations (B) during pcEmc (5 Hz) at T11, T11+L1 and C5+T11+L1 are shown. Values are means ± SD (n=10 step cycles for each condition) for five subjects. Mean (n=5 subjects) amplitude (C) and cycle period duration (D) for the hip, knee, and ankle movements during pcEmc at T11, T11+L1 and T11+L1+C5 stimulation are shown.

Figure 6. Stick diagram decompositions (40 ms between sticks) of the movements of the right and left legs during one step cycle in a gravity neutral position for three subjects under each condition are shown.

Figure 7. An example of the on-off effects of multi-site spinal cord stimulation on the excursion at the knee joints and on the EMG activity in the biceps femoris bilaterally is shown. Abbreviations are the same as those used in Figure 2.
Figures
### Spinal cord multi-site transcutaneous stimulation

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Spinal cord multi-site transcutaneous stimulation

![Diagram showing waveforms of RKnee, LKnee, RBF, LBF, C5, L1, T11 with time and voltage scales.]
<table>
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<tr>
<th>Subj</th>
<th>Joint</th>
<th>Amplitude (deg)</th>
<th>Cycle period (ms)</th>
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<td>T11+L1+C5</td>
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