Plantar Cutaneous Afferents Normalize the Reflex Modulation Patterns During Stepping in Chronic Human Spinal Cord Injury

Maria Knikou

1Health Science Doctoral Programs, City University of New York, Staten Island, New York; 2Northwestern University Feinberg School of Medicine; and 3Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, Illinois

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Knikou M. Plantar cutaneous afferents normalize the reflex modulation patterns during stepping in chronic human spinal cord injury. J Neurophysiol 103: 1304–1314, 2010. First published December 30, 2009; doi:10.1152/jn.00880.2009. Plantar cutaneous afferent transmission is critical for recovery of locomotion in spinalized animals, whereas a phase-dependent reflex modulation is apparent during fictive or real locomotion. In nine people with a chronic spinal cord injury (SCI) the effects of foot sole stimulation on the soleus H-reflex and tibialis anterior (TA) flexion reflex modulation patterns during assisted stepping were established on different days. The soleus H-reflex was elicited by posterior tibial nerve stimulation followed by a supramaximal stimulus 100 ms after the test H-reflex to control for movement of recording electrodes. The flexion reflex was evoked by sural nerve stimulation with a 30-ms pulse train, recorded from the ipsilateral TA muscle, and elicited at 1.2- to twofold the reflex threshold. During assisted stepping, spinal reflexes were conditioned by percutaneous stimulation of the ipsilateral metatarsals at threefold perceptual threshold with a 20-ms pulse train delivered at 9- to 11-ms conditioning-test intervals. Stimuli were randomly dispersed across the step cycle, which was divided into 16 equal bins. The conditioned soleus H-reflex was significantly facilitated at midstance and depressed during midswing when compared with the unconditioned soleus H-reflex recorded during stepping. Foot sole stimulation induced a significant facilitation of the long-latency TA flexion reflex before, during, and after stance-to-swing transition when compared with the unconditioned long-latency TA flexion reflex during stepping. This study provides evidence that plantar cutaneous afferents remarkably influence the soleus H-reflex and TA flexion reflex modulation patterns during stepping and support that actions of plantar cutaneous afferents onto spinal interneuronal circuits engaged in locomotion are manifested in a phase-dependent manner in chronic SCI subjects.

INTRODUCTION

Cutaneous afferents and in particular those of the foot contribute profoundly to the reflex regulation of balance and movement in mammals (Rossignol et al. 2006). For instance, in chronic spinal cats cutaneous transmission is modulated in a phase-dependent manner and can reverse muscle responses to promote step progression and correct foot placement in the presence of an obstacle or unexpected perturbation (Abraham et al. 1985; Duysens 1977; Duysens and Pearson 1976; Forssberg et al. 1977; Quevedo et al. 2005). In contrast, lack of sensory feedback from the foot (denervation of cutaneous nerves of the hindpaws in intact cats) changes the locomotor electromyographic (EMG) patterns (Bouyer and Rossignol 2003a). In the same animals, foot placement and locomotor recovery were substantially affected despite prolonged step training after spinalization (Bouyer and Rossignol 2003b). However, preservation of minimal cutaneous feedback in the spinalized animal was sufficient to promote foot placement and weight support (Bouyer and Rossignol 2003b). Further, in spinalized cats it was shown that cutaneous transmission is normalized by locomotor training (Cô tê and Gossard 2004), probably by changes at a premotoneuronal level, leading to recovery of locomotion. Stimulation of low-threshold sural or posterior tibial nerves in freely and spontaneous walking premammillary cats prolonged the ipsilateral flexion and contralateral extension phases when stimuli were delivered before the onset of ankle extensor EMG burst (Duysens and Stein 1978), supporting the notion that signals from cutaneous afferents are integrated by spinal interneuronal circuits involved in locomotion.

In humans, stimulation of cutaneous afferents from the foot evokes complex synaptic actions on leg muscles (Aniss et al. 1992; Delwaide et al. 1981). Cutaneous afferents from the foot provide a neuronal population vector that encodes the direction of the ankle movement and contribute to awareness of our body in space and to upright human posture (Aimonetti et al. 2007; Kavounoudias et al. 2001; Roll et al. 2002). During walking, cutaneomuscular responses are phasically modulated and actions of ankle and thigh muscle reflex responses are reversed (Duysens et al. 1990, 1993; Zehr et al. 1997). The net result of this behavior is step cycle promotion and balance maintenance by shaping of the dynamic postural responses (Meyer et al. 2004). Cutaneomuscular reflexes in patients with hereditary spastic paraparesis or complete/incomplete paraplegia maintain a phasic modulation during walking (Duysens et al. 2004a; Jones and Yang 1994; Knikou et al. 2009b), but are significantly impaired in poststroke patients (Zehr et al. 1998). Disrupted input from cutaneous afferents has been related to an abnormally high variable step cycle duration in patients with sensory polyneuropathy of the feet (Van Wezel et al. 1997). The contribution of these afferents to the reflex regulation of walking may rely on their interactions with spinal interneuronal circuits engaged in locomotion. For example, cutaneous afferents from the foot interact with the interneurons intercalated in the Ib neuronal pathway to α motoneurons, with presynaptic inhibitory interneurons in humans at rest, and in spinalized cats during fictive locomotion (Iles 1996; Ménard et al. 2003; Pierrot-Deseilligny et al. 1981). Cutaneomuscular responses are likely controlled by the spinal locomotor pattern generator, ensuring body stability and ankle extensor loading (Duysens et al. 2004b).

Address for reprint requests and other correspondence: M. Knikou, Health Sciences Doctoral Programs, The Graduate Center, City University of New York, 2800 Victory Boulevard, SN-207, Staten Island, NY 10314 (E-mail: Maria.Knikou@csi.cuny.edu or m-knikou@northwestern.edu).
In most people with a spinal cord injury (SCI) capable of independent or assisted ambulation, the phasic modulation of Ia transmission to α motoneurons during walking is lost or largely impaired (Faist et al. 1999; Knikou et al. 2009a; Yang et al. 1991), but a phase-dependent modulation can be recognized in the short- and long-latency tibialis anterior (TA) flexion reflexes observed following excitation of flexor reflex afferents (FRAs) (Knikou et al. 2009b). Stimulation of the medial plantar region of the foot during early stance and late swing depressed soleus H-reflex excitability in SCI subjects (Fung and Barbeau 1994), whereas in healthy subjects the short-latency soleus H-reflex depression was decreased at the stance phase (Shoji et al. 2005). Thus sensory feedback from the foot may contribute to restoration of a pathological or absent phase-dependent H-reflex modulation in these patients. The presence of a locomotor phase-dependent reflex modulation pattern indicates that spinal (and supraspinal) neural networks for locomotion are engaged in a functional manner and gating of sensory afferent feedback is successful. The research hypothesis was that plantar cutaneous input influences in a functional manner the soleus H-reflex and TA flexion reflex modulation patterns during walking in people with a chronic SCI.

METHODS

The experimental protocol received full Institutional Review Board (IRB) approval from the College of Staten Island and University of Louisville IRB committees. All procedures were conducted in compliance with the 1964 (revised October 2008) Declaration of Helsinki. Each subject signed an informed consent form before participating to compliance with the 1964 (revised October 2008) Declaration of Helsinki. Each subject signed an informed consent form before participating to the study. Nine people (seven male, two female) with chronic SCI (Table 1) participated in the study. Subjects participated in previous studies (Knikou et al. 2009a,b) and are identified here with the same code.

EMG recordings and kinematics

Surface EMGs (Konigsberg Instruments, Pasadena, CA) were recorded from eight muscles bilaterally: SOL, soleus; MG, medial gastrocnemius; TA, tibialis anterior; PL, peroneus longus; MH, medial hamstrings; VL, vastus lateralis; RF, rectus femoris; and GRC, gracilis. Silver/silver chloride bipolar electrode discs of fixed inter-electrode distance were placed following standard skin preparation. All EMG signals were filtered with a cutoff frequency of 10–1,000 Hz and sampled at 2,000 Hz using a data acquisition card (National Instruments, Austin, TX). In the flexion reflex experiments, sagittal plane hip, knee, and ankle joint ranges of motion were acquired continuously and in synchrony with the EMG by the Motion Analysis System (60 Hz, Motion Analysis, Santa Rosa, CA) while using the Helen Hays marker set system. Motion curves for each joint were established with the Eva Real-Time Software (EvART, Motion Analysis).

Assisted stepping

Assisted stepping refers to body weight support (BWS) stepping on a moving treadmill belt (Biodex Medical Systems, Shirley, NY), whereas manual assistance is provided by therapists, as originally proposed by Barbeau and colleagues (1987)—widely used for recovery of walking in SCI patients (see reviews of Dietz 2009; Dietz and Harkema 2004). Each subject during standing wore an upper body harness attached to pulleys that were controlled by a pneumatic closed-loop force-controlled BWS system (Innoventor Engineering, Maryland Heights, MO). During stepping, a therapist was positioned behind the subject and assisted with pelvis and trunk stabilization, as well as with appropriate weight shifting and hip rotation. Therapists positioned at each limb provided manual assistance only when needed. The treadmill speed and BWS (Table 1) were selected for each subject to promote the best stepping pattern.

Elicitation and recording protocol of spinal reflexes

The soleus H-reflex was evoked and recorded similar to the methods used previously during assisted stepping in SCI subjects (Knikou et al. 2009a). With subjects seated, the optimal stimulation site was identified with a stainless steel monopolar electrode used as a probe and was replaced by a permanent electrode under constant pressure throughout the experiment when all criteria were met (Knikou 2008). The indifferent electrode (stainless steel electrode, 5-cm diameter) was placed just above the patella. Each subject was then transferred to the treadmill and while standing at a BWS equivalent to that used during stepping, the soleus H-reflex and M-wave recruitment curves (tibial nerve stimulation was delivered every 5 s) were constructed. During standing, trunk and knee support was provided by the therapists for subjects A33 and B06. Stimulus intensities and peak-to-peak amplitude of the M-wave and H-reflex (measured real time by the customized LabVIEW script; National Instruments, Austin, TX) at each point of the recruitment curve were saved and retrieved as reference values during stepping (Knikou et al. 2009a).

<table>
<thead>
<tr>
<th>ID</th>
<th>Level</th>
<th>ASIA</th>
<th>Age, years</th>
<th>Postinjury, years</th>
<th>Gender</th>
<th>Antispasticity Medication</th>
<th>Ashworth Scale</th>
<th>G</th>
<th>SOL</th>
<th>BWS</th>
<th>Treadmill Speed, m/s</th>
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<tr>
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<td>A</td>
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<tr>
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<td>C5</td>
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<td>F</td>
<td>None</td>
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<td>L: 1, R: 1</td>
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<td>1.07</td>
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<tr>
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<td>39</td>
<td>1.1</td>
<td>M</td>
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<td>Not tested</td>
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</tr>
<tr>
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<td>T10</td>
<td>D</td>
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<td>2.5</td>
<td>F</td>
<td>Baclofen</td>
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<td>0.98</td>
</tr>
<tr>
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<td>C7</td>
<td>D</td>
<td>22</td>
<td>3.2</td>
<td>M</td>
<td>Baclofen</td>
<td>2</td>
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<td>46.0</td>
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<td>D</td>
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<td>6.5</td>
<td>M</td>
<td>Baclofen</td>
<td>3</td>
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<td>0.98</td>
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<tr>
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<td>35</td>
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</table>

The neurologic impairment is indicated in column 3, based on the American Spinal Injury Association (ASIA) Impairment Scale (Maynard et al. 1997). The Ashworth Scale, used to assess the spasticity level at the ankle, is defined as 1 = normal muscle tone; 2 = slight increase in muscle tone; 3 = more marked increase in muscle tone, but limb easily dorsiflexed passively; 4 = considerable increase in muscle tone, passive movement difficult; and 5 = limb rigid in flexion or extension. The ankle clonus in left (L) and right (R) gastrocnemius (G) and soleus (SOL) muscles was graded as 1 = no clonus; 2 = minimal, 1–2 beats; 3 = moderate, 3–9 beats; and 4 = sustained, ≥10 beats. Body weight support (BWS) provided at a level that knee and trunk buckling could be avoided during standing and stepping. F, female; M, male; C, cervical; T, thoracic.

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Because the maximal M-wave (Mmax) varies considerably across the step cycle (Simonsen and Dyhre-Poulsen 1999; see Fig. 4C in the following text) a supramaximal stimulus was delivered to the posterior tibial nerve 100 ms after the test H-reflex during assisted stepping (Fig. 1). This Mmax was used to normalize the associated M-wave and H-reflex, all elicited at the same bin. Conditioning reflex effects are known to rely heavily on the amplitude of the test H-reflex as a percentage of the Mmax (Crone et al. 1990). To meet this criterion, for the control H-reflex during assisted stepping, the customized LabVIEW script adjusted the stimulus intensity at each bin based on the previously constructed H-reflex recruitment curve (M-wave and H-reflex amplitudes) to evoke an H-reflex that was elicited on the ascending part of the recruitment curve and ranged from 20 to 40% of the Mmax. The conditioned H-reflex was elicited on the ascending portion of the M/H recruitment curve, whereas the amplitude of the M-wave was set to a prior range from 4 to 8% of the Mmax evoked at each bin across subjects (Knikou 2008; Knikou et al. 2009a).

For the flexion reflex, a pulse train of 30-ms duration (1-ms pulses at 300 Hz) was triggered from a computer (controlled by a customized LabVIEW script) and delivered to the right (ipsilateral) sural nerve by a constant-current stimulator (DS7A, Digitimer, Hertfordshire, UK) (Conway and Knikou 2008; Knikou 2007; Knikou and Conway 2005). With subjects seated, a bipolar electrode was used to establish the site that elicited a response in the TA muscle at the lowest stimulus intensity. The bipolar electrode was then replaced by two monopolar electrodes (Medicotest, Ølstykkee, Denmark) that were secured throughout the experiment. The stimulus intensity required to elicit an initial response in the ipsilateral TA muscle was identified as reflex threshold. With subjects seated, 20 responses elicited every 10 s were recorded at a stimulus intensity that ranged from 1.2- to twofold the reflex threshold across subjects. This reflex served as the control flexion reflex. During standing with BWS, the stimulus intensity was progressively increased and the response was monitored to observe its sensitivity to the stimulus intensity strength. During stepping, the sural nerve was stimulated between 1.2- and twofold the sensory (or perceptual) threshold was initially established. All conditioning stimuli were delivered at threefold the sensory threshold. At this stimulation intensity, no contraction of the intrinsic foot or ankle muscles was present, responses in TA muscle were absent, and no pain was reported, verifying that the conditioningafferent volley excited mainly large cutaneous afferents of the foot. In the subject without preserved sensation (A33, Table 1), the conditioning stimulus intensity was reduced to half the intensity that induced a response in the TA muscle.

In all subjects, stimulation of posterior tibial and sural nerves and foot sole was triggered based on the signal from the ipsilateral foot switch (Noraxon USA, Scottsdale, AZ), and was delivered randomly every three to ten steps. Only one stimulation sequence was delivered to a step cycle. Stimuli were also randomly dispersed throughout the step cycle, which was divided into 16 equal bins. The step cycle phases of the contralateral leg were also identified by a foot switch. A custom-made script (LabVIEW) was used to identify the 16 bins for the stimulated and nonstimulated steps for both legs. Bin 1 corresponds to heel strike. Bins 8, 9, and 16 correspond approximately to stance-to-swing transition, swing phase initiation, and swing-to-stance transition, respectively. At each bin, at least five reflexes were randomly recorded. Data were sampled at 2,000 Hz, band-pass filtered at 20–1,000 Hz (National Instruments), and stored on a personal computer for off-line analysis.

**Data analysis**

The conditioned and unconditioned H-reflex, M-wave, and Mmax evoked at each bin of the step cycle were measured as peak-to-peak amplitude. All responses recorded at each bin were expressed as a percentage of the associated Mmax. For each subject, the mean amplitude of the conditioned H-reflexes was estimated and compared with the unconditioned H-reflex at each bin with a Wilcoxon rank-sum t-test. Then, the mean conditioned and unconditioned H-reflexes from each subject were grouped based on the bin number and statistical significance was established with a Wilcoxon rank-sum t-test. Subjects were grouped based on the American Spinal Injury Association (ASIA) Impairment Scale level and statistically significant differences were established separately for data from subjects with motor incomplete and complete SCI.

For the flexion reflex, the full-wave rectified area of the short (30-ms poststimulus with 50-ms duration) and long (100-ms poststimulus with 290-ms duration) latency were calculated, grouped for each bin, and averaged. Then, the TA EMG from the nonstimulated steps corresponding to the same time windows was subtracted from the reflex EMGs to remove background activity. The subtracted reflex was then expressed as a percentage of the mean size of the flexion reflex recorded with subjects seated. This was done separately for the conditioned and unconditioned flexion reflex during stepping. For each subject, statistical significance between the conditioned and unconditioned flexion reflex was established at each bin with a Wilcoxon signed rank-sum test. The average conditioned and unconditioned flexion reflexes from each subject were then grouped based on the bin number and statistical significant difference was established with a Wilcoxon rank-sum test.

To establish whether stimulation had an aftereffect, EMG signals of SOL, MG, TA, PL, MH, VL, RF, and GRC muscles from the steps before and after stimulation (H-reflex and flexion reflex experiments)
for the ipsilateral and contralateral legs were full-wave rectified, high-pass filtered at 20 Hz, and low-pass filtered at 500 Hz. After full-wave rectification linear envelopes were obtained at 20-Hz low-pass filter. For each subject and muscle, the mean EMG amplitude for each of the corresponding steps (before and after stimulation) at each bin was determined and normalized to the corresponding maximal locomotor EMG. The overall average (from all bins) normalized EMG of each corresponding muscle was grouped separately for subjects with motor complete and incomplete SCI and a pairwise comparison at 95% confidence interval was conducted separately for the ipsilateral and contralateral limb muscles to establish statistically significant differences on the EMG for the steps before and after stimulation.

The background activity of the ipsilateral TA and SOL muscles for each bin was estimated from the mean value of the rectified and filtered EMG for 60-ms duration (high-pass filtered at 20 Hz, rectified, and low-pass filtered at 400 Hz), beginning 120 ms before sural or posterior tibial nerves stimulation, respectively. The mean amplitudes of the conditioned and control flexion and soleus H reflexes were plotted on the y-axis versus the TA or the SOL background activity (normalized to the maximal EMG) on the x-axis, respectively. A linear least-square regression was then fitted to the data. This analysis was conducted separately for each subject (individual data are not presented) and for the pool data. In all statistical tests, significant differences were established at 95% of confidence level. Results are presented as mean values along with the SE.

RESULTS

Contribution of plantar cutaneous afferents to the soleus H-reflex modulation pattern during stepping

EMG bursts of ankle, knee, and hip flexor and extensor muscles were synchronized to the phase of the step cycle in all motor incomplete SCI subjects during BWS assisted stepping. This is illustrated by representative data from one incomplete SCI subject (D13) in Fig. 2A. In this subject, the MG and SOL muscles from both legs at nonstimulated steps were active during stance, as demonstrated by the corresponding EMG bursts relative to the foot switches. The MG activity occurred in synchrony with that observed in the SOL muscle, whereas the antagonist maximal TA activity occurred at midswing (rectified EMGs; Fig. 2A). However, it is evident from the normalized EMG (Fig. 2A, column 3) that an atypical muscle activation pattern was present, associated with spastic motor activity pronounced in GRC and RF muscles during stance. In the subject with a motor complete SCI (Fig. 2B), based on the EMG bursts and normalized EMG, a synchronized activation of antagonists was absent, the EMG amplitude was of lower amplitude compared with that observed in the incomplete SCI subject, and muscle activation patterns were not modulated in a phase-dependent manner.

The overall EMG amplitude (from all 16 bins) of the ipsilateral and contralateral leg muscles along with the average muscle activation pattern normalized to the maximal corresponding EMG as a function of the step cycle is indicated in Fig. 3A for ASIA C and D subjects and in Fig. 3B for ASIA A and B subjects. EMG averages from all 16 bins are indicated for the steps before and after posterior tibial nerve and foot sole stimulation (left panels). Pairwise comparisons indicated no significant differences in the EMG amplitude for steps before and after stimulation bilaterally for both subject groups \((P > 0.05)\). In both ipsilateral and contralateral muscles, a clonic activity can be seen in both groups, whereas in ASIA A and B subjects a phase-dependent modulation of muscle activity was absent. However, the clonic activity or stimulation did not influence the duration of the step cycle, which had an average of \(1,216 \pm 64.3\) and \(1,250 \pm 52.3\) ms in motor incomplete and complete SCI subjects, respectively.

The average amplitude of the conditioned and unconditioned (or control) soleus H-reflex as a function of the step cycle from ASIA C and D subjects and for subject B06 is indicated in Fig. 4.
and B, respectively. Data from subject A33 were rejected based on the criteria of acceptance outlined in METHODS. Both reflexes during assisted stepping are indicated as a percentage of the Mmax evoked at each bin, with the latter being significantly modulated during stepping (Fig. 4C). In incomplete SCI subjects, the conditioned soleus H-reflex was significantly facilitated at midstance (bins 3, 4, 5, and 6) compared with the control soleus H-reflex (Wilcoxon rank-sum test between conditioned and control H-reflex at each bin, \( P < 0.05 \)). Further, the conditioned soleus H-reflex was significantly depressed during the swing phase (bins 11, 12, and 13; \( P < 0.05 \)), compared with the unconditioned H-reflex. The conditioning reflex effects occurred at SOL background activity levels similar to those observed in the control soleus H-reflex (Fig. 4D, \( P > 0.05 \)). The SOL/TA ratio (Fig. 4E) indicates that SOL muscle activity occurred in a physiological pattern relative to the antagonist TA activity during stepping. A linear relationship between the conditioned soleus H-reflex and SOL background activity (Fig. 4F; \( R^2 = 0.45 \)) was found. A different modulation pattern of the conditioned H-reflex was found in subject B06 (Fig. 4B), whereas facilitation at early stance (bin 1) and depression at late swing (bin 14) were observed.

**Contribution of plantar cutaneous afferents to the TA flexion reflex modulation pattern during stepping**

The muscle activation patterns as a function of the step cycle before and after stimulation were similar in this set of experiments, so EMG data are not reported in this section. The step cycle of the ipsilateral and contralateral legs before sural nerve and foot sole stimulation were identical to that observed after stimulation, with an overall average of 1,235 ± 81.2 ms. The mean sagittal hip, knee, and ankle angles from subjects D11, D13, D15, D16, D17, and D18 as a function of the step cycle are indicated in Fig. 5. Flexion and extension angles are denoted as positive and negative deflections, respectively. Most striking differences for pre- and stimulated steps, compared with the well-established joint kinematics of spinal intact subjects during walking, are the decrement of hip flexion before 20% of the step cycle, lack of knee extension at heel strike, and knee flexion until 30% of the step cycle, absent ankle dorsiflexion at early and midstance (10 and 40% of step cycle), absent plantar flexion at toe off, and absent neutral angle at late swing phase (80–100% of step cycle).

The short-latency TA flexion reflex was observed in four subjects (D11, D15, D17, D18) from whom three were not under antispasticity medication (see Table 1). Because this reflex component was irregularly observed with subjects seated, no comparison between the conditioned and unconditioned TA flexion reflex during stepping was possible. Further, the latency of the long-latency TA flexion reflex in subjects A33 and B06 varied significantly while seated and their data were excluded from the overall analysis. In motor incomplete SCI subjects, the conditioned long-latency TA flexion reflex expressed as a percentage of the control flexion reflex recorded with subjects seated was facilitated just before, during, and after the stance-to-swing transition phase (bins 8, 9, and 10; \( P < 0.05 \), Wilcoxon rank-sum test between the conditioned and the unconditioned long-latency flexion reflex) (Fig. 6A). No significant effects in the remaining swing phase or during stance were observed (\( P > 0.05 \)). The conditioning reflex effects occurred at TA background activity levels similar to those observed in the unconditioned long-latency flexion reflex.
DISCUSSION

The principal findings of this study are that plantar cutaneous afferents selectively facilitated and depressed the soleus H-reflex at midstance and midswing, respectively. Further, excitation of plantar cutaneous afferents during assisted stepping induced a significant facilitation of the long-latency TA flexion reflex before, during, and after the swing-to-stance transition phase. The conditioning reflex effects are consistent with a task-related behavior of the low-threshold plantar cutaneous afferents onto spinal interneuronal circuits engaged in locomotion and appear to be functional in patients with partial spinal cord lesions.

On the effects of foot sole stimulation on the soleus H-reflex modulation during stepping

In most people with SCI capable of independent or assistive ambulation, the phasic modulation of Ia transmission to α motoneurons during walking is lost or largely impaired (Faist et al. 1999; Knikou et al. 2009a; Yang et al. 1991). The most striking abnormalities are the lack of soleus H-reflex depression during the swing phase and an absent progressed and maintained reflex excitation from mid to late stance (Knikou et al. 2009a; Yang et al. 1991).

The phasic soleus H-reflex modulation is the result of complex interactions between different classes of spinal (and supraspinal) interneuronal circuits, but it was restored to some extent with a brief innocuous electrical stimulation of the foot sole during assisted stepping in motor incomplete SCI subjects (Fig. 4A). Specifically, it was facilitated at early-midstance and depressed during midswing, whereas the SOL/TA ratio followed a rather physiological modulation pattern (Fig. 4E). The soleus H-reflex facilitation at midstance (Fig. 4A) is in agreement with the reduced short-latency soleus H-reflex depression by plantar nerve stimulation during the stance phase of walking in healthy subjects (Shoji et al. 2005). Because cutaneous afferents in man interact with Ib inhibitory interneurons (Pierrot-Deseilligny et al. 1981), mechanical or electrical stimulation of the plantar region has inhibitory effects on the soleus...
H-reflex in seated subjects (Knikou and Conway 2001; Shojo et al. 2005) and Ib inhibition is reversed to facilitate weight bearing during the stance phase of walking (Faist et al. 2006), it is likely that afferent volleys from the foot sole interacted with spinal interneuronal circuits mediating information about load and weight bearing.

The soleus H-reflex depression during the swing phase of gait in spinal intact subjects has largely been attributed to reciprocal Ia inhibition (which is under descending control) exerted from the antagonistic TA afferents on soleus motoneurons (Lavoie et al. 1997; Petersen et al. 1999; Schneider et al. 2000). Further, cutaneous afferents inhibit Renshaw cell activity, which is phasically modulated during fictive or real locomotion in animals and humans (Lamy et al. 2008; Pratt and Jordan 1987; Wilson et al. 1964). Renshaw cells are excited from various supraspinal centers, segmental afferents, and the interneurons that mediate reciprocal Ia inhibition (see Knikou 2008 for references). Consequently, excitation of plantar cutaneous afferents may have affected both reciprocal inhibitory interneurons and Renshaw cell activity restoring soleus H-reflex depression during the swing phase in these patients.

The conditioning stimulus to the foot sole was delivered at threefold the sensory threshold, so it is highly likely that large plantar cutaneous afferents were excited. During fictive locomotion in the decerebrate cat, Ménard et al. (2002) showed through intraaxonal recordings in group I afferents of hindlimb muscles that stimulation of different cutaneous nerves evokes a phase-dependent modulation of primary afferent hyperpolarization or depolarization. This means that cutaneous afferents subtract presynaptic inhibition in group I afferents, influencing in this way the effect of proprioceptive feedback on motoneuronal excitability during locomotion. Although muscle afferents can induce phase-dependent presynaptic inhibition of monosynaptic transmission (Gosgnach et al. 2000; Gossard et al. 1991; Ménard et al. 1999; for review see Rudomin 2009), cutaneous input alters this inhibition (decrease of monosynaptic excitatory postsynaptic potential in flexors and extensors) (Ménard et al. 2003), further supporting the interaction of cutaneous afferents with presynaptic inhibitory interneurons and spinal interneuronal circuits of locomotion (see Fig. 9 in Ménard et al. 2003). Excitation of low-threshold receptors of the skin of the distal foot in healthy humans depresses presynaptic inhibition of soleus Ia afferents especially during ankle extension (Iles 1996). Because voluntary muscle contraction fails to change the soleus H-reflex modulation pattern during walking in humans (Yang and Whelan 1993) and conditioning reflex effects were observed at similar levels of SOL background activity compared with those recorded under control conditions during stepping (Fig. 4D), motoneuron pool excitability changes at a postsynaptic level cannot account as the sole mechanism for the observed effects.

To conclude, a possible mechanism account for the phase-dependent conditioning H-reflex effects is phasic modulation of presynaptic inhibition acting on group I afferent terminals by plantar cutaneous afferents. Postsynaptic inhibition, however, cannot readily be excluded. A specific neuronal pathway cannot be identified since cutaneous afferents may have equally interacted either with last order interneurons or with the central pattern generator. Thus more precise methods are needed to further explore the mediating neural structures of the conditioning reflex effects. The demonstration that excitation of plantar cutaneous afferents affected the locomotor modulation pattern of the soleus H-reflex supports the hypothesis that feedback from the foot sole provides afferent-dependent mo-
toneuron excitation gated by the phase of the gait cycle in chronic motor incomplete SCI subjects.

On the effects of foot sole stimulation on the flexion reflex modulation during stepping

Long-latency, long-lasting reflex discharges of ipsilateral flexors and contralateral extensors are released with concomitant depression of the short-latency flexion reflex (absent in decerebrated nonspinalized animals) in spinalized animals after administration of DOPA, whereas short trains of FRA triggered brief bursts of alternating flexor and extensor activity (see Hultborn 2006 and references therein). FRA actions were manifested through excitatory and inhibitory reflex pathways to flexor and extensor motoneurons supporting a reciprocal intralimb innervation pattern with concomitant facilitation of contralateral extensors (Fu et al. 1975; Jankowska et al. 1967; Lundberg 1979; Lundberg et al. 1987). Recent evidence suggests that FRA interneurons interact directly with the central pattern generator for locomotion.1 For example, FRA volleys reset the fictive locomotor rhythm by terminating the ongoing extensor activity and initiating a new flexor burst in L-DOPA–treated decerebrated spinalized animals (Conway et al. 1995; Perreault et al. 1995; Schomburg et al. 1998; Stecina et al. 2005).

A neuronal organization similar to that observed in spinalized animals treated with L-DOPA has been documented for the long-latency (or late) flexion reflex in people with a complete SCI (Roby-Brami and Bussel 1990), whereas FRA volleys modulate spontaneous steplike movements (Bussel et al. 1988, 1989; Calancie et al. 1994). Further, the short (or early) latency flexion reflex is irregularly observed after SCI in humans (Conway and Knikou 2008; Knikou 2007) and resembles the one observed in noninjured humans and spinal cats following nociceptive stimulation (Knikou 2007; Meinck et al. 1985). Regardless of an absent or present early flexion reflex, the long-latency flexion reflex is typically observed after a motor complete or incomplete SCI in humans.

The interneuronal pathways that participate in the generation of the flexion reflex in human SCI may equally participate in the patterned motor activity during locomotion, as described for spinalized animals. In this respect, the long-latency TA flexion reflex is depressed throughout the stance phase and facilitated at the late swing phase in chronic SCI (Knikou et al. 2009b). Foot sole innocuous stimulation changed the long-latency TA flexion modulation pattern by selectively facilitating the reflex before, during, and after the swing-to-stance transition (Fig. 6A). This facilitation may indirectly correct hip and ankle excursions (Fig. 5) when implemented in a locomotor training paradigm. No significant conditioning effects were observed during stance, a phenomenon consistent with lack of facilitatory drive from plantar mechanoreceptors onto flexion reflex pathways in motor complete SCI subjects (Conway and Knikou 2008) and the depressed group II muscle and cutaneous afferents field potentials during fictive locomotion (Perreault et al. 1999).

FIG. 6. Effects of foot sole stimulation on the tibialis anterior (TA) flexion reflex during stepping in SCI. A: the overall amplitude of the conditioned and unconditioned long-latency TA flexion reflex from motor incomplete SCI subjects as a function of the step cycle is indicated as a percentage of the control reflex recorded with subjects seated. Asterisks indicate statistically significant differences between the unconditioned and conditioned TA flexion reflex based on the P value of the Wilcoxon signed rank-sum test at each bin. B: mean normalized TA EMG background activity for the conditioned and unconditioned long-latency TA flexion reflex from motor incomplete SCI subjects as a function of the step cycle. In both graphs, the 16 points correspond to the 16 bins of the step cycle. The step cycle was broken into 16 equal bins and each data point represents the average for a bin from all SCI subjects. Bin 1 corresponds to heel strike. Bins 8, 9, and 16 correspond approximately to stance-to-swing transition, swing phase initiation, and swing-to-stance transition, respectively. C: the relationship between the conditioned long-latency TA flexion reflex amplitude is indicated as a function of normalized TA EMG background activity from all ASIA C and D subjects. Each point corresponds to one bin of the step cycle. Error bars represent the SE.
The observed conditioning reflex effects may be attributed to premotor neuronal mechanisms. In motor complete SCI subjects, excitation of contralateral FRA affects the ongoing presynaptic inhibition of Ia afferents (Roby-Brami and Bussel 1990) similar to the primary afferent depolarization of Ia terminals reported in the acute spinal cat injected with DOPA (Anden et al. 1966). Further, stimulation of the ipsilateral and contralateral sural nerve, cutaneous nerve branches, and light brushing of the dorsal and plantar skin decrease presynaptic inhibition of Ia afferent terminals in humans (Iles 1996). This means that excitation of cutaneous afferents may have potentiated the FRA effects on the interneurons that affect presynaptic inhibition of group I and group II afferents.

Sensory threshold may be shifted after SCI, but subjects with preserved sensation reported no pain following sural nerve or foot sole stimulation. Sural nerve stimulation excited largely cutaneous and muscle afferents, whereas foot sole stimulation excited mainly Aβ fibers originating from slow adapting plantar mechanoreceptors (Merkel or Ruffini disks). The average conduction velocity of Aβ fibers during contraction is 45 ms⁻¹ (Rossi et al. 1996). Thus impulses from Aβ fibers reached the spinal cord about 33 ms after the first pulse of the stimulus train. Because the duration of the conditioning stimulus train was 20 ms and was delivered at a C–T interval of 9–11 ms, impulses from Aβ fibers had reached the spinal cord at the time that the posterior tibial or sural nerves were stimulated. Thus it is likely that polysynaptic neuronal circuits were involved, although a transcortical pathway cannot be excluded (Nielsen et al. 1997).

**Impact of plantar cutaneous afferents on locomotion**

Cutaneous afferents originating from the foot pads are situated just under the base of support. Thus they can provide critical information on foot placement, pressure distribution, and step progression during stepping. During fictive locomotion, cutaneous afferents affect swing initiation by prolonging the stance phase through inhibitory and excitatory connections with the neural networks involved in the generation of flexion and extension (Duyssens 1977; Duyssens and Pearson 1976; Duyssens and Stein 1978; LaBella et al. 1992). Further, plantar cutaneous afferents contribute to body stability following mediolateral perturbations determining the direction of the automatic postural responses (Bolton and Misiaszek 2009; Ting and Macpherson 2004). If we assume that a spinal stepping generator exists in humans (Dietz 2002; Dietz et al. 1994; Rossignol 2006; Rossignol et al. 2009), it is apparent that this modality can be used to promote balance and stepping in people with a chronic SCI.

**Methodological considerations and limitations**

Cutaneous afferents produce postsynaptic potentials in ankle flexor and extensor motoneurons (Aniss et al. 1992; Baldissera et al. 1981; Schieppati and Cenna 1984) through complex spinal pathways. These potentials are modulated in a phase-dependent manner during fictive or real locomotion in animals and humans (Abraham et al. 1985; Andersson et al. 1978; Duyssens and Pearson 1976; Duyssens et al. 1993; Schomburg and Behrends 1978; Zehr et al. 1997) to further assist postural stability and potent presynaptic modulation of group I and group II afferents (part of the FRA system) (Jankowska and Riddell 1995). Therefore the afferents mediating the conditioning volley might have been modulated during assisted stepping themselves beyond their effects on spinal inhibitory/excitatory interneurons.

Cutaneousmuscular reflexes may spread throughout the limb and be a little different when elicited by stimulation compared with a more natural stimulus. In this study, the skin over the foot sole was stimulated because this area is in contact with the support surface more than the heel during walking, whereas plantar cutaneous afferents of this area act as mechanoreceptors mediating pressure. The level of BWS (20–60) among SCI subjects varied significantly and may have affected the population of afferents being excited and thus their effects on spinal interneuronal circuits engaged in locomotion (Bastaanse et al. 2000). However, different level of BWS in incomplete SCI subjects did not affect the modulation pattern of the conditioned reflexes. I thus suggest that a comprehensive investigation of cutaneous afferent transmission as a function of body unloading in patients with spinal lesions at different segmental levels is needed. Manual assistance by therapists during stepping varied among SCI subjects and may have resulted in excitation of different types of afferents, including cutaneous afferents, which modulate and mediate flexion reflexes (Knikou 2007; Knikou et al. 2007). However, understanding the interaction of spinal interneuronal circuits and sensory feedback related to cutaneous input, stretch, and load after neurologic injury with currently used rehabilitation interventions is important for optimizing strategies to improve recovery of walking after SCI.

Last, four of nine patients were under antispasticity medication at the time of the study. It is well known that baclofen, for example, stimulates γ-aminobutyric acid type B receptors that induce a suppression of excitatory neurotransmitter release, decreasing the excitability state of the CNS and clinically resulting in decreased tonic stretch or polysynaptic reflexes (Abbruzzese 2002). The conditioning reflex effects appeared to be related to the type of lesion (complete vs. incomplete) rather than to medication. This is supported by Fig. 4, A and B in which B represents data from a motor complete SCI subject under no medication, whereas A represents data from incomplete SCI subjects of which four of seven were under medication.

**Conclusion**

The phase-dependent reflex modulation during walking is the result of complex interactions among segmental reflex circuits, central pattern generators, descending control, and sensory afferent feedback including vision. The findings of this study suggest that excitation of plantar cutaneous afferents during walking influence the extensor and flexor reflex modulation patterns in a phase-dependent manner in people with a chronic motor incomplete SCI. I thus suggest that this reflex-conditioning protocol could be used along with locomotor training to maximize sensorimotor recovery in these patients. Sensorimotor recovery might be related to reorganization of the sensorimotor cortex (Kaas et al. 2008) and spinal cord due to augmentation of task-related sensory input, although further research is needed to support this.
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