Spinal Cord (2004) 42, 420–424

www.nature.com/sc

Original Article

Potential impact of orthotic gait exercise on natural killer cell activities in thoracic level of spinal cord-injured patients

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Stydy design: Prospective before–after trial.
Objective: To examine the changes of natural killer (NK) cell activity in response to orthotic gait exercise in thoracic level of spinal cord-injured (SCI) patients.
Setting: National Rehabilitation Center for Persons with Disabilities, Japan.
Methods: In all, 10 thoracic level of SCI patients (ranging Th5–Th12), who experienced orthotic gait training, participated in this study. NK cell activity at an effector:target (*E/T*) ratio (20:1) was examined in a sample of peripheral blood taken before and just after orthotic gait exercise for 20 min. On a separate day, to evaluate the physical intensity of the orthotic gait exercise, cardiorespiratory responses at rest and during exercise were measured.
Results: The resting value of the NK cell activity in our SCI patients was remarkably lower than that in normal subjects reported in previous studies. The NK cell activity was significantly increased through a 20 min orthotic gait exercise (pre versus post; 12.7 ± 5.28 versus 17.76 ± 6.71, $P < 0.05$). The steady-state value of oxygen (V_{O_2}) and heart rate (HR) were 18.13 ± 3.92 ml/kg and 142.53 ± 19.84 b/min, respectively. It was noteworthy that a patient who showed decrement of NK cell activity in response to exercise had the highest level of injury (Th5), and showed the higher energy cost of orthotic gait.
Conclusion: These findings suggested that the orthotic gait exercise has the potential to enhance the immune function for SCI persons, although patients with a higher level of SCI may have some difficulties.
Sponsorship: Mitsui Sumitomo Insurance Welfare Foundation
Spinal Cord (2004) **42,** 420–424. doi:10.1038/sj.sc.3101625; Published online 4 May 2004

Keywords: natural killer cell activity; spinal cord injury; orthotic gait; secondary disorder

Introduction

Natural killer (NK) cells have been proposed as a major factor in the first-line defense system against viral infection.[1,2] Previous investigations demonstrated that spinal cord injury (SCI) brings depression of the immune system including decreased NK cell activities, and also reported restoration of the immune function through the rehabilitation therapy.[3]

Orthotic gait training is usually prescribed for paraplegic patients with SCI in the therapeutic phase to promote their general health. On the other hand, there are several obstacles to achieving locomotion for paraplegic patients, in particular the high-energy cost of orthotic gait leads to exhaustion within a few minutes of walking (for a review, see Nene *et al*[4]). Although some positive effects of orthotic gait have been reported,[5] it is still unclear whether the physical intensity of the orthotic gait is suitable for SCI patients to promote their health or not.

We previously examined the physiological characteristics of orthotic gait in thoracic level of SCI patients, and our findings suggested that the physical intensity during walking exercise is suitable to promote the aerobic capacity of SCI patients.[6] In the present study, we designed a direct approach to clarify the effect of orthotic gait exercise on the general health of SCI patients, particularly in terms of exercise-induced changes in NK cell activity. Previous investigations revealed that moderate intensity exercise can enhance NK cell activity.[1,7,8] Therefore, we focused on whether orthotic gait exercise in particular could enhance NK cell activity.

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Methods

Subjects
In all, 10 SCI patients who met the following criteria participated in this study: (i) injured at thoracic level, (ii) complete motor paralysis in the lower limb muscle (ASIA classification; grade A or B), (iii) no history of cardiorespiratory disease. All patients were at least 6 months since time of injury, with time since injury ranging from 8 to 32 months (Table 1). Each subject gave his or her informed consent to the experimental procedure, which was approved by the local biological ethics committee of the National Rehabilitation Center for the Persons with Disabilities (NRCD).

Orthotic gait training
All patients had undergone a standard rehabilitation program, consisting of muscle stretching, balance training, and transfer activity, and participated in orthotic gait training with a weight-bearing control orthosis (WBC) or advanced reciprocating gait orthosis (ARGO). Eight of 10 patients have kept the orthotic gait training for 15 weeks, and the other two patients have kept for 10 (patient E) and 4 weeks (patient G), respectively. Although there is individual variation, in many cases, lower thoracic level of paraplegic patients could walk after 10 weeks of gait training independently, while it needs more practice for higher thoracic level of patients. After the training period, each subject could perform the orthotic gait (patients F and G still required light support to avoid falling) independently, and were able to walk continuously for at least 20 min.

Apparatus
Appearance and sequential picture of walking with WBC and ARGO were shown in Figure 1. The mechanical features of the WBC have been fully described elsewhere.[11,12] This orthosis consists of a rigid frame that supports the user's body weight, a special hip joint device that reciprocally propels each leg forward, a gas-powered foot device that varies the sole thickness of the device for foot/floor clearance, and a control system of the orthosis. As a whole these mechanical features enable a user to ambulate at a faster speed and with less energy expended.[6] The ARGO also has a special hip joint device named 'hip driving cable' which connects both sides of the leg frame. With this device a torque exerted by the right (left) hip joint is mechanically transmitted to the left (right) hip joint, resulting in the torque to the opposite direction exerted by the left (right) hip joint.

Physical intensity during orthotic gait
On a separate day, cardiorespiratory responses at rest and during orthotic gait were measured. Subjects were asked to abstain from alcohol and caffeine for at least 12 h before the experiment. The temperature and humidity on the experiment were 23.5±4.2°C and 68.3±3.3%, respectively. The experimental procedure was as follows: 5 min at rest in the sitting position, 20 min of continuous walking at the most comfortable speed. The cardiorespiratory parameters at rest and during walking were measured continuously with a telemetric device (K4 Cosmed, Italy) and were analyzed in real time. The telemetric device consists of a transmitting unit, a face mask to sample the expired gas, a heart rate chest strip, a battery, and a receiving unit. The following cardiorespiratory parameters were

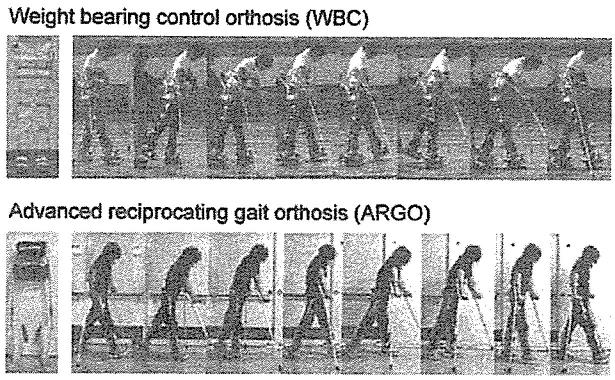

Figure 1 Appearance and sequential picture of walking with weight-bearing control orthosis (WBC; above) and advanced reciprocating gait orthosis (ARGO; below)

Table 1 Characteristics of the patients

Patient	*Sex*	*Age (years)*	*Height (cm)*	*Weight (kg)*	*Lesion level*	*Grade of ASIA*	*Duration of paraplegia (months)*	*Orthosis*
A	M	28	173	63	Th8	A	12	WBC
B	M	27	175	60	Th10	A	10	WBC
C	M	22	175	68	Th12	A	8	WBC
D	M	21	167	46	Th12	B	32	WBC
E	M	36	178	73	Th11	A	20	ARGO
F	M	19	175	53	Th5	B	24	ARGO
G	F	26	156	45	Th10	A	13	ARGO
H	M	30	178	67	Th12	A	13	ARGO
I	M	34	165	54	Th6	A	28	ARGO
J	M	23	168	65	Th8	A	26	ARGO

obtained: oxygen uptake (V_{O_2}) and heart rate (HR). Walking speed in the steady state during walking and rating of perceived exertion (RPE) score were also recorded. After the experiments, the energy consumption and walking energy cost were calculated. The terms adopted were those of Nene and Patrick[9] and calculations performed according to their protocol:

$$\text{Energy consumption (J/kg/s)} = \frac{\text{Ambulatory min } V_{O_2}\text{(ml/min)}}{\text{Weight (kg)} \times 60} \times K$$

$$\text{Energy cost (J/kg/m)} = \frac{\text{Ambulatory min } V_{O_2}\text{(ml/ min)}}{\text{Speed (m/ min)} \times 60} \times K$$

where $K = 20.19$ J/ml, since 1 ml $O_2 = 4.825$ cal and 1 cal = 4.184 J.

NK cell activities

Blood samples were drawn from an antecubital vein with the patient in the seated position before and just after orthotic gait exercise. The exercise consisted of 20 min of continuous walking at the most comfortable speed in the inside of the hospital ward.

NK cell activity was determined by (superscript: 51) Cr-release cytotoxicity assay using the K562 cell-line which derived from cells with chronic myelogenous leukemia as targets, and calculated using the following formula: %NK cell activity = {(experiment mean − spontaneous mean)/(total mean − spontaneous mean)} * 100. In all NK cell activity testing, percent of spontaneous release was less than 5% of total release. Effector:target (*E*:*T*) ratios used were 20:1. Controls included cultures of untreated cells (spontaneous release) and cells treated with 3% sodium dodecyl sulfate (SDS) (total release).

Statistical analysis

Values are given as the mean ± SD. Statistical difference in NK cell activity between pre- and postexercise was tested by paired *t*-test. Significance was accepted at $P<0.05$.

Results

Physical intensity during orthotic gait

The average walking speed during orthotic gait was 18. 01 ± 2.22 m/min. Eight of 10 patients were able to walk continuously, without stumbling, for 20 min. Table 2 shows the cardiorespiratory responses, energy consumption, energy cost, walking speed, and RPE during orthotic gait. During walking, cardiorespiratory parameters clearly showed a significant increase compared with resting rate. The steady-state value of the V_{O_2} ranged from 14.20 to 24.83 ml/kg (average value = 18.13 ± 3.92 ml/kg), and HR was 99.2–166.4 b/min (average value = 142.53 ± 19.84 b/min). The energy consumption and energy cost during walking were 5.94 ± 1.16 J/kg/s and 19.63 ± 5.04 J/kg/m, respectively. The RPE score just after exercise ranged from 13 to 19 (median value: 15).

NK cell activity

The average value of NK cell activity in the SCI patients at rest (12.7 ± 5. 28; ranging from 7.6 to 23.4) was remarkably lower than the standard value in the healthy nondisabled persons (32.9 ± 15.8). Nine of 10 patients showed enhancement of NK cell activity in response to the 20 min of orthotic gait exercise. The total average value of the postexercise NK cell activity was significantly higher than that of the pre-exercise (pre versus post; 12.7 ± 5.28 verses 17.76 ± 6.71, $P<0.05$, Table 3, Figure 2).

Table 2 Physical intensity during orthotic walking

	V_{O_2} (ml/kg)		*HR (beat/min)*		*Energy consmption (J/kg/s)*	*Energy cost (J/kg/m)*	*Walking speed (m/min)*	*RPE score (unit)*
Patient	*Rest*	*Exercise*	*Rest*	*Exercise*				
A	8.84	17.39	84.6	154.0	5.85	17.55	20	15
C	6.76	14.67	104.4	132.9	4.94	16.45	18	15
D	4.57	18.05	96.0	145.1	6.07	16.56	22	13
E	6.71	15.62	78.3	131.5	5.26	17.19	18	13
F	8.29	21.14	104.1	166.4	7.11	29.06	15	19
G	6.78	24.20	62.11	132.5	8.14	26.22	18	17
H	6.70	16.01	40.1	99.2	5.39	16.12	20	13
I	9.75	24.83	81.0	143.6	8.35	29.33	17	17
J	5.80	15.19	107.8	163.0	5.11	19.67	16	17
Mean	7.03	18.13	85.18	142.53	6.10	20.61	18.01	15 (median)
SD	1.53	3.92	21.23	19.84	1.32	5.40	2.22	-

Table 3 NK cell activity in pre- and postexercise

Patient	*Pre*	*Post*	*Δ(%Pre)*
A	10.1	14.4	142.36
B	11.3	14.6	129.19
C	23.4	27.1	115.87
D	18.0	19.4	107.78
E	9.8	17.3	176.53
F	18.3	16.6	90.71
G	9.3	30.8	331.18
H	7.6	11.6	152.63
I	11	17.3	157.27
J	8.3	8.6	103.61
Mean	12.70	17.76	150.71
SD	5.28	6.71	68.87

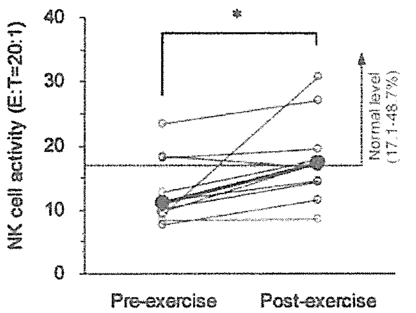

Figure 2 Natural killer (NK) cell activity in response to 20 min of orthotic gait exercise. Thick and thin lines indicate the total averaged ($n = 10$) and each subject's value, respectively. NK cell activity was represented by an E/T ratio (20:1)

Discussion

In the present study, we aimed to examine the effect of orthotic gait exercise on the NK cell activity in SCI patients. The main observations made here were as follows: (i) the NK cell activity in SCI patients was remarkably lower than the standard value in healthy persons; (ii) the NK cell activity was significantly increased through 20 min of orthotic gait exercise, and (iii) the one patient who showed decrement of NK cell activity in response to exercise had an injury of the highest level (Th5) and showed the higher energy cost during orthotic gait exercise.

Previous investigations have reported that SCI was accompanied by depression of immune system including decrement of NK cell activities.[3] These findings, taken together with the fact that paraplegic patients have significantly reduced V_{O_2} peak values as a consequence of the reduction in the daily activity levels,[10] suggest that the decrements of NK cell activity may be the result of limitation of the patient's physical activity.

Because immune resistance is generally regarded as an essential factor for health care, it is conceivable that enhancement of immune function is important for SCI patients to maintain decent physical condition. In this regard, many investigations have reported the possibility of enhancement of immune function through moderate exercise not only in normal persons[1,7,8] but also in SCI patients.[11] Kliesch *et al*[3] demonstrated restoration of immune function through rehabilitation therapy in treated subjects by comparison with those not receiving treatment. The present result of exercise-induced enhancement of NK cell activity is in good agreement with these reports. In the present study, the steady-state value of the V_{O_2} during orthotic gait was 18.13 ± 3.92 ml/kg, and HR was 142.53 ± 19.84 b/min (Table 2). The level of physical intensity implied by these values was considered to be suitable for promoting the general health of SCI patients. Further, all of our subjects, with the exception of patient F, could walk for a considerable time and distance without exhaustion. It is therefore considered that the enhancement of NK cell activities was the result of the suitable aerobic condition during orthotic gait.

Finally, we considered why only patient F showed a decrement in the NK cell activity in response to the orthotic gait exercise. As mentioned above, this patient showed the higher energy cost during orthotic gait and had the highest level of injury in all eight patients. The orthotic gait for SCI patients requires compensatory motion of the residual trunk and upper limbs to swing the paralyzed leg.[12,13] Patient F, who was injured at Th5, could not contract his trunk muscles due to motor paralysis. Consequently, the excess energy expenditure and burden on his upper limbs made it impossible for him to achieve suitable exercise intensity for enhancement of immune function during orthotic gait. His higher RPE score (19: very very hard, Table 2) reflects greater energy consumption than that of the other patients during orthotic gait.

To date, many researchers have reported extremely high-energy requirements of orthotic gait.[4,14,15] Although many devices have been developed to improve this problem to date, it is still unknown whether the orthotic use contribute to facilitate the health care for SCI persons. The present result provides evidence of the effectiveness of the orthotic gait exercise for promotion of the general health of these SCI patients. However, the question remains whether regular exercise training leads to chronically elevated NK cell activity. Further study will be needed to clarify this issue.

Acknowledgements

This work was supported by Research Grants from the Mitsui Sumitomo Insurance Welfare Foundation.

References

1 Shephard RJ, Shek PN. Effects of exercise and training on natural killer cell counts and cytolytic activity. *Sports Med* 1999; **28:** 177–195.
2 Welsh RM. Regulation of virus infections by natural killer cells: A review. *Nat Immun Cell Growth Regul* 1986; **5:** 169–199.

3 Kliesch WF, Cruse JM, Lewis RE, Bishop GR, Brackin B, Lampton JA. Restoration of depressed immune function in spinal cord injury patients receiving rehabilitation therapy. *Paraplegia* 1996; **34:** 82–90.
4 Nene AV, Hermens HJ, Zilvold G. Paraplegic locomotion: a review. *Spinal Cord* 1996; **34:** 507–524.
5 Thoumie P *et al.* Restoration of functional gait in paraplegic patient with the RGO-II hybrid orthosis. A multicenter controlled study. *II: physiological evaluation. Paraplegia* 1995; **33:** 654–659.
6 Kawashima N, Sone Y, Nakazawa K, Akai M, Yano H. Energy expenditure during walking with weight bearing control orthosis (WBC) in thoracic level of paraplegic patients. *Spinal Cord* 2003; **41:** 506–510.
7 Nieman DC *et al.* The effects of moderate exercise training on natural killer cell and acute upper respiratory tract infections. *Int J Sports Med* 1990; **11:** 467–473.
8 Nieman DC *et al.* Moderate exercise training and natural killer cell cytotoxic activity in breast cancer patients. *Int J Sports Med* 1995; **16:** 334–337.
9 Nene AV, Patrick JH. Energy cost of paraplegic locomotion using the parawalker electrical stimulation hybrid orthosis. *Arch Phys Med Rehabil* 1990; **71:** 116–120.
10 Janssen TW, van Oers CA, Rozendaal EP, Willemsen EM, Hollander AP, van der Woude LH. Changes in physical strain and physical capacity in men with spinal cord injuries. *Med Sci Sports Exerc* 1996; **28:** 551–559.
11 Klokker M, Mohr T, Kjaer M, Galbo H, Pedersen BK. The natural killer cell responses to exercise in spinal cord injured individuals. *Eur J Appl Physiol* 1998; **79:** 106–109.
12 Yano H. Weight bearing control orthosis for paraplegics. Organized session. *33rd Annual Scientific Meeting International Medical Society of Paraplegia: Monograph* 1994, pp 1–16.
13 Yano H, Kaneko S, Nakazawa K, Yamamoto S, Bettoh A. A new concept of dynamic orthosis for paraplegia: the weight bearing control (WBC) orthosis. *Prosthetics Ortho Int* 1997; **21:** 222–228.
14 Bernardi M, Canale I, Castellano V, Di Filippo L, Felici F, Marchetti M. The efficiency of walking of paraplegic patients using a reciprocating gait orthosis. *Paraplegia* 1995; **33:** 409–415.
15 Massucci M, Brunetti G, Piperno R, Betti L, Franceschini M. Walking with the advanced reciprocating gait orthosis (ARGO) in thoracic paraplegic patients: energy expenditure and cardiorespiratory performance. *Spinal Cord* 1998; **36:** 223–227.

J Neurophysiol 93: 777–785, 2005.
First published September 22, 2004; doi:10.1152/jn.00817.2004.

Alternate Leg Movement Amplifies Locomotor-Like Muscle Activity in Spinal Cord Injured Persons

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Submitted 11 August 2004; accepted in final form 16 September 2004

Kawashima, Noritaka, Daichi Nozaki, Masaki O. Abe, Masami Akai, and Kimitaka Nakazawa. Alternate leg movement amplifies locomotor-like muscle activity in spinal cord injured persons. *J Neurophysiol* 93: 777–785, 2005. First published September 22, 2004; doi:10.1152/jn.00817.2004. It is now well recognized that muscle activity can be induced even in the paralyzed lower limb muscles of persons with spinal cord injury (SCI) by imposing locomotion-like movements on both of their legs. Although the significant role of the afferent input related to hip joint movement and body load has been emphasized considerably in previous studies, the contribution of the "alternate" leg movement pattern has not been fully investigated. This study was designed to investigate to what extent the alternate leg movement influenced this "locomotor-like" muscle activity. The knee-locked leg swing movement was imposed on 10 complete SCI subjects using a gait training apparatus. The following three different experimental conditions were adopted: *1*) bilateral alternate leg movement, *2*) unilateral leg movement, and *3*) bilateral synchronous (in-phase) leg movement. In all experimental conditions, the passive leg movement induced EMG activity in the soleus and medial head of the gastrocnemius muscles in all SCI subjects and in the biceps femoris muscle in 8 of 10 SCI subjects. On the other hand, the EMG activity was not observed in the tibialis anterior and rectus femoris muscles. The EMG level of these activated muscles, as quantified by integrating the rectified EMG activity recorded from the right leg, was significantly larger for bilateral alternate leg movement than for unilateral and bilateral synchronous movements, although the right hip and ankle joint movements were identical in all experimental conditions. In addition, the difference in the pattern of the load applied to the leg among conditions was unable to explain the enhancement of EMG activity in the bilateral alternate leg movement condition. These results suggest that the sensory information generated by alternate leg movements plays a substantial role in amplifying the induced locomotor-like muscle activity in the lower limbs.

INTRODUCTION

It is now well recognized that the paralyzed lower limb muscles of a person with spinal cord injury (SCI) can be activated by body weight–supported stepping movement on a treadmill (Dietz et al. 1995, 2002; Dobkin et al. 1995; Ferris et al. 2004; Harkema et al. 1997) or by locomotion with the use of a gait orthosis (Kojima et al. 1999; Nakazawa et al. 2004). Since the stepping movement accompanies joint rotation, it is possible that such muscle activity might merely reflect the reflex responses induced by rhythmic muscle-tendon stretches (Dobkin et al. 1995; for review, see Harkema 2001). Nevertheless, this muscle activity is considered to come from the interaction of the central pattern generator (CPG) in the spinal cord (Dimitrijevic et al. 1998) with the sensory input rather than a mere reflex response (Dietz et al. 2002).

This interpretation is justified by several recent findings. Harkema et al. (1997) have shown that the magnitude of EMG activity induced in SCI persons is more closely related with the peak load applied to the leg than with muscle-tendon stretch. Similarly, Dietz et al. (2002) have shown, using a driven gait orthosis, that the ankle muscle activity observed in ordinary locomotion movement was not induced either when the ankle joint alone was moved or when the body weight was completely unloaded. The crucial role of the sensory information of load and hip position in generating and/or shaping the rhythmic output pattern from the CPG has been considerably emphasized by previous studies using reduced animal preparations (Duysens and Pearson 1980; Grillner 1985; Pearson 1995), and these findings also support the view that the muscle activity induced in SCI persons reflects the output from the locomotory CPG.

However, it remains unclear to what extent the induced muscle activity (locomotor-like muscle activity) is actually "locomotory," partly because almost all of the previous studies have not paid attention to a substantial feature of human bipedal locomotion, i.e., alternating leg movements. This is the main point that we focused on in this study. To investigate the significance of alternating leg movements to locomotor-like muscle activity, we compared the magnitude of the EMG activity induced in the complete SCI subjects using a gait-training apparatus in the following different conditions: *1*) ordinary bilateral alternating leg movement, *2*) unilateral leg movement, and *3*) bilateral synchronous (in-phase) leg movement. If the spinal CPG is actually involved in the neural mechanism of the locomotor-like muscle activity, the alternating leg movement pattern should contribute to the generation and/or coordination of the muscle activity.

Part of this study has been presented in abstract form (Kawashima et al. 2003).

METHODS

Participants

Ten male SCI persons (28.8 ± 6.0 yr) participated in this study. All of the subjects had an injury at the thoracic (T) level of the

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spinal cord somewhere between T_5 and T_{12}, complete motor paralysis in their lower limb muscles (American Spinal Injury Association Class: ASIA A or B; Maynard et al. 1997), and moderate degrees of spasticity. At least one-half a year had passed since they were injured. The physical characteristics of the subjects are summarized in Table 1. Each subject gave written informed consent to the experimental procedures, which were conducted in accord with the Helsinki Declaration of 1975 and approved by the ethics committee of the National Rehabilitation Center for Persons with Disabilities, Tokorozawa, Japan.

Passive leg movement apparatus

To impose locomotion-like movements on their legs, we used an apparatus (Fig. 1*A*) originally developed for physical exercise for persons with disabilities (Easy Stand Glider 6000, Altimate Medical). This apparatus enables the SCI persons to stand securely by immobilizing their trunk and pelvis using front and back pads and by preventing hyperextension of the knee joint using the knee pad. It also enables the subjects to swing their legs by moving the handle connected to the foot plate. In this study, the experimenter manually moved the handle back and forth (±17.5 cm from default position) in a sinusoidal manner (Fig. 1*C*) by matching the movement frequency with the sound of a metronome (1 Hz). This handle movement could induce approximately ±14 and ±9° motion in the hip and ankle joints, respectively (these values of range of joint motion depend on the subject's lower limb length). This range of motion of each joint is similar to the data for the normal walking provided by Winter (1990). Although only reciprocal leg movement can be induced at the default setting, synchronous or unilateral leg movement can be induced by removing the bolts that connect bars to both sides.

Experimental protocol

Before the experiment, we checked that the standing posture was stable and that no hypotension was observed. First, bilateral alternate leg movement was imposed for 3 min so that the subjects could experience the standing posture and the imposed leg movement. Then, the experiments were performed under the following three conditions: *1*) bilateral alternate (anti-phase) leg movement; *2*) unilateral leg movement; and *3*) bilateral synchronous (in-phase) leg movement. In the unilateral leg movement, the right leg was moved while the position of the left leg was fixed to be vertical. In the bilateral synchronous leg movement, both legs were passively swung simultaneously in the same direction. Throughout the exercise period, subjects were asked to grasp the bar in front of them and to keep their upper limbs relaxed (Fig. 1*A*). The experimenters had conducted a sufficient number of practices before the testing session so that they could adjust the leg motion to the predetermined pattern (i.e., the range of motion and swing frequency) under all experimental conditions by monitoring the angle data from an electrogoniometer displayed on an oscilloscope. The duration of each session was 1 min, and an interval of ≥1 min was taken for rest between sessions. The order of conditions *1*), *2*), and *3*) was randomized.

TABLE 1. *Characteristics of the SCI subjects*

Subject	Age, yr	Weight, kg	Lesion Level	Grade of ASIA	Duration of Paraplegia, mo
S1	19	53	T_5	B	24
S2	30	74	T_8	A	12
S3	24	75	T_{12}	A	24
S4	32	68	T_{12}	A	32
S5	22	70	T_{10}	A	11
S6	39	67	T_{12}	A	13
S7	35	55	T_6	A	46
S8	27	75	T_{12}	A	22
S9	30	72	T_{11}	B	23
S10	30	60	T_8	A	17

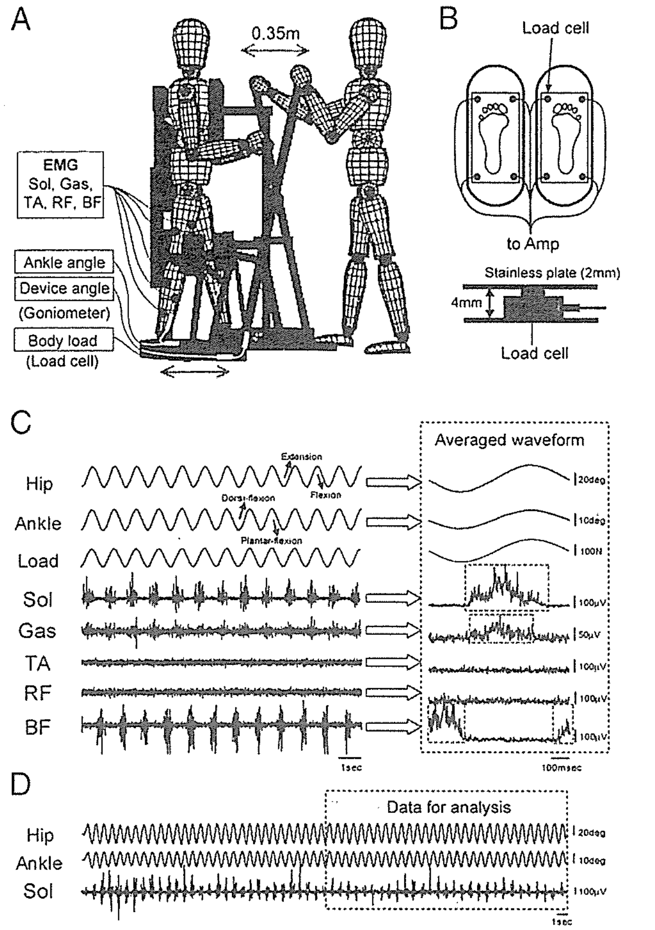

FIG. 1. *A*: overview of the experimental setup. During the experiment, the spinal cord injury (SCI) subject stood securely on a special device (Easy Stand Glider 6000, Altimate Medical). The subject's legs were moved passively by moving the lever back-and-forth in a sinusoidal manner in pace with the tempo of a metronome (1 Hz). *B*: load applied to the foot soles of each leg was measured by 4 load cells placed under the stainless foot plate. *C*: typical example of hip and ankle joint motion and induced EMG activity of an SCI subject (level of lesion, T_{12}) during passive leg movement (Sol; soleus, Gas; medial head of gastrocnemius, TA; tibialis anterior, RF; rectus femoris, BF; biceps femoris). To quantify level of muscle activity, the averaged EMG signal was calculated (*right*). *D*: adaptation-like phenomenon in EMG activity in the Sol. It took ~30 s for the EMG activity to reach steady state. Therefore data from the 1st 30 s were disregarded in the calculation of the averaged EMG signals (*C*).

Data recording

The surface EMG signal was recorded from the soleus (Sol), the medial head of the gastrocnemius (Gas), the tibialis anterior (TA), the rectus femoris (RF), and the long head of the biceps femoris (BF) muscles of both legs with the use of a bipolar electrode. Care was taken to exclude any artifact in the EMG signal (e.g., the skin was washed with a scrub gel and rubbed with sandpaper to reduce the resistance of the skin). The EMG signal was amplified (Bagnoli-8 EMG System, DELSYS) with band-pass filtering between 20 and 450 Hz. Ankle joint motion was recorded with an electrogoniometer (Goniometer System, Biometrics), whose two sensor heads were placed on the lateral part of the shank and foot of the subject (Fig. 1*A*). Hip joint motion was estimated from the data recorded by using another goniometer attached to the lateral aspect of the apparatus (Fig. 1*A*).

In six subjects, the VICON 370 system (Oxford Metrics) was used to analyze the lower limb motion more accurately. Eight markers were attached to the right and left sides of the subject on the skin overlying the following landmarks: the acrominon (SHO), greater trochanter (GTR), lateral malleolus (AKL), and the top of the great toe (TOE). We defined the hip and ankle joint angles as the angles formed by the SHO, GTR, and AKL and by the GTR, AKL, and TOE, respectively. Furthermore, in these subjects, the actual load applied to each foot sole was measured using four load cells (LMA-A-1KN, Kyowa, Tokyo, Japan) placed under the four corners of the stainless foot plate (Fig. 1*B*). During the experiment, all data were continuously monitored by Power Lab software (Chart version 4, AD instruments) and were digitized at 1 kHz for later analysis.

Data analysis

The digitized EMG signal was full-wave rectified after the DC component was subtracted. It was then averaged over the last 30 locomotion cycles (Fig. 1*C*). The data of the first 30 cycles were discarded, because the EMG activity often showed gradual decay, and it took ~30 s (i.e., 30 cycles) to become stationary (Fig. 1*D*). The locomotor-like EMG activity was quantified using the integrated value of the averaged EMG signal and the duration over which the muscle was active (Fig. 1*C*). We regarded the muscle to be active when its averaged EMG signal consistently exceeded the level of resting EMG activity (mean value + 3 × SD). Furthermore, to examine the phase-dependent changes in the EMG activity, the averaged EMG signal was divided into 10 bins, and the mean amplitude in each bin was calculated. The ranges of hip and ankle movements were calculated from the data obtained by electrogoniometers, and those were compared with the VICON data. The load applied on each foot sole was quantified by calculating the summation of the data from four load cells.

Statistics

Values are given as means ± SE. Two-way ANOVA was used to test the difference in the EMG magnitude, duration, and hip and ankle joint range of motion among the three conditions. Tukey's post hoc test was applied to identify differences among the conditions. Significance was accepted at $P < 0.05$.

RESULTS

Pattern of the locomotor-like EMG activity

Figure 2*A* shows the averaged waveform of the joint angle (estimated by electrogoniometers) and the EMG activity obtained from an SCI subject during alternate leg movement. In this subject, EMG bursts modulated with the locomotion cycle were observed in Sol, Gas, and BF. A similar muscle activation pattern was observed in other subjects. Figure 2*B* indicates the number of subjects whose muscle activity was judged to be significant in each of 10 leg movement phases. For all subjects, the EMG activity was observed in Sol and Gas during the backward leg swing phase corresponding to the stance phase in normal locomotion. Similarly, the EMG activity was observed

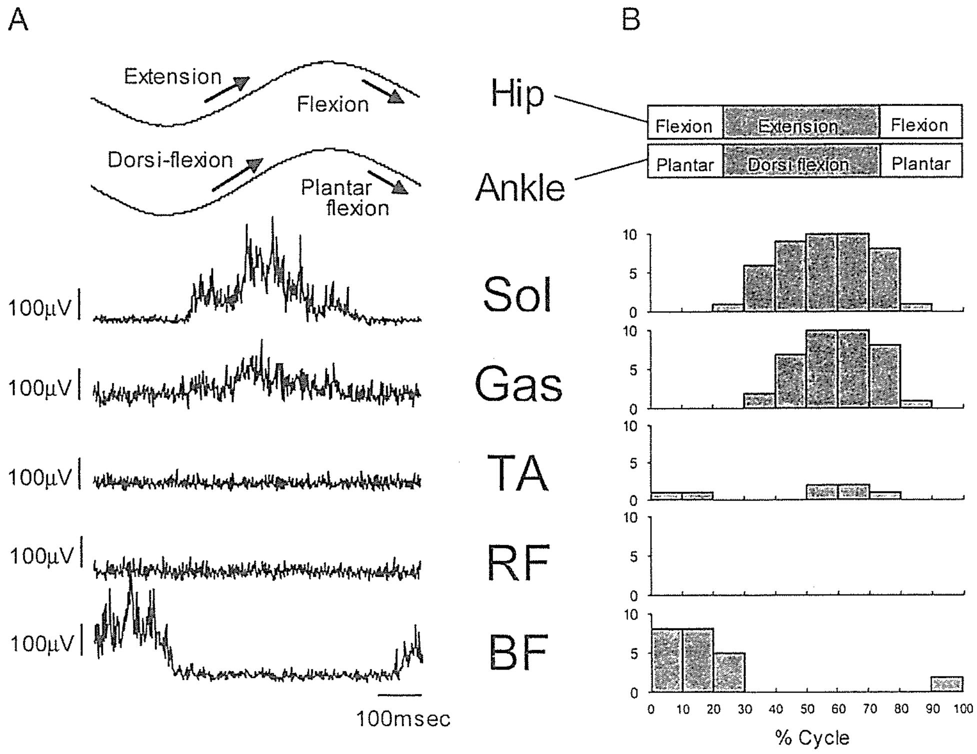

FIG. 2. *A*: ensemble averaged waveform of hip and ankle joint motion and induced EMG activity in each lower limb muscle obtained from an SCI patient. *B*: probability of the occurrence of EMG activity in the lower limb muscles during passive leg movement. Data show number of subjects who showed EMG activity in each leg movement cycle (total number of subjects is 10). Muscle activity was determined to be active when its averaged EMG signal consistently exceeded the level of resting EMG activity (mean value + 3 × SD).

in BF for 8 of 10 subjects during the hip-flexion phase corresponding to the swing phase in normal locomotion. Namely, the active phase of these muscles mainly corresponded with the phase during which they were mechanically stretched. The EMG activity of the TA was observed for two subjects, and no EMG activity was induced in the RF. In the RESULTS and DISCUSSION sections, we will focus only on these activated muscles (Sol, Gas, and BF).

Typical averaged waveforms of the EMG activity for three experimental conditions obtained from two subjects are shown in Fig. 3 (*A* and *D*, bilateral alternate; *B* and *E*, unilateral; *C* and *F*, bilateral synchronous leg movements). As clearly shown in these waveforms, the amount of EMG activity varied from condition to condition. In the unilateral leg movement (Fig. 3, *B* and *E*), no EMG activity was observed in the nonmoving left leg. The magnitude of the EMG activity was smaller for the unilateral leg movement condition (Fig. 3, *B* and *E*) than for the ordinary bilateral alternate leg movement condition (Fig. 3, *A* and *D*). In the bilateral synchronous leg movement condition, the EMG activity was present for both legs (Fig. 3, *C* and *F*); however, its magnitude was smaller than that for the bilateral alternate leg movement condition (Fig. 3, *A* and *C*).

Leg motions and load to foot sole

Figure 4*A* shows a typical example of the hip and ankle joint angle movements obtained using the VICON system. In the right (experimental) leg, both the hip and ankle joint angles moved in a similar manner among three conditions. On the other hand, the left leg movement was completely out of phase between the alternate and synchronous leg movement conditions, and no obvious hip and ankle motion was observed during the unilateral leg movement condition. There was no significant difference in the range of motion of each joint among three conditions for the right leg and between the alternate and synchronous leg movement conditions for the left leg (Fig. 4*B*). In the unilateral leg movement condition, the left leg movement was kept at almost zero (Fig. 4*B*). It should be noted that the data in Fig. 4*B* contain the data measured with electrogoniometers, because the joint angle movement estimated using electrogoniometers was not different from that measured directly using the VICON system.

Figure 5*A* shows a typical example of the load applied to the foot sole in the three conditions. The load was modulated almost sinusoidally with the leg movement cycle. The load was maximal and minimal, respectively, when the hip joint was maximally extended and flexed. Although the load averaged over time was not different from condition to condition (Fig. 5*B*), there was a statistically significant ($P < 0.05$) difference in the peak-to-peak load among the three experimental conditions (Fig. 5*C*). In comparison with the alternate leg movement condition, the load applied to the right leg was 85.5 ± 3.8% in the unilateral leg movement condition and 64.3 ± 12.5% in the synchronous leg movement condition. On the other hand, the peak-to-peak load applied to the left leg was 22.5 ± 4.4% in the unilateral leg movement condition and 69.9 ± 11.9% in the synchronous leg movement condition compared with the alternate leg movement condition.

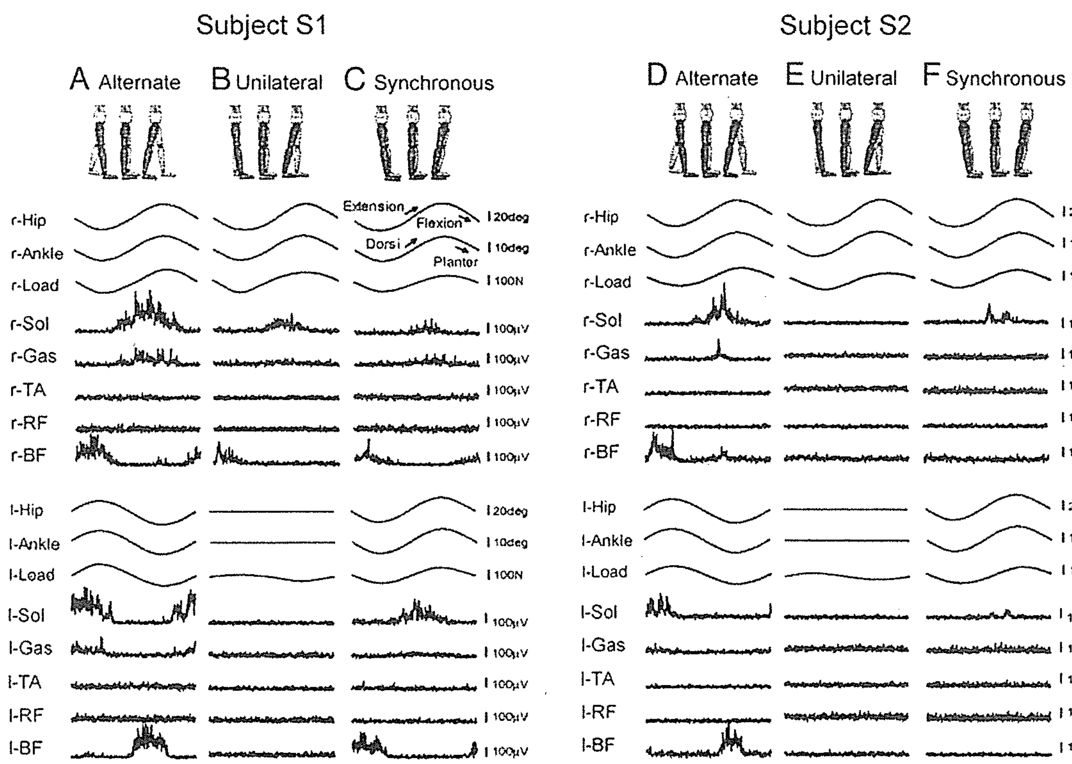

FIG. 3. Averaged waveforms of hip and ankle joint motion, load on the leg, and induced EMG activities of limb muscles recorded from subjects S1 and S2 (*top*, right leg; *bottom*, left leg). *A* and *D*: alternate leg movement condition. *B* and *E*: unilateral leg movement condition. *C* and *F*: synchronous leg movement condition.

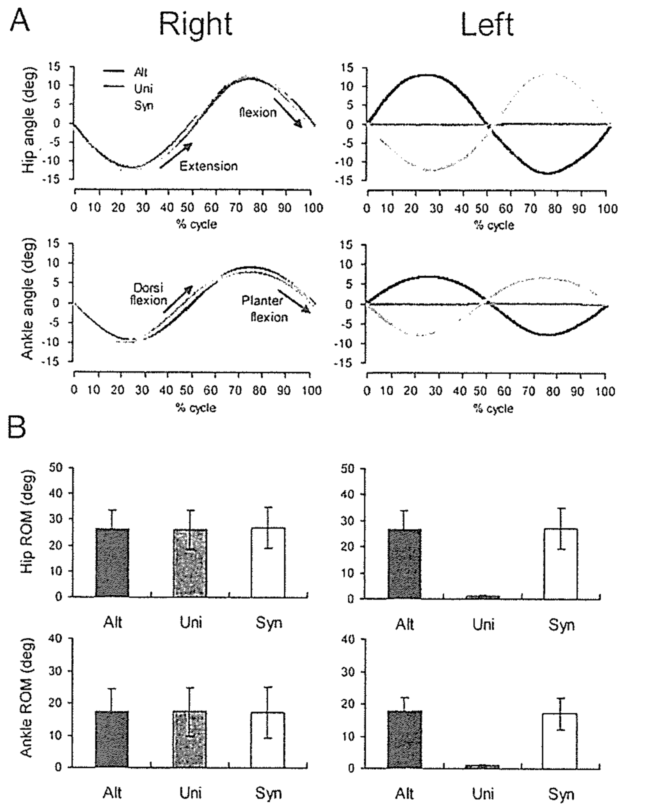

FIG. 4. Kinematical characteristics of imposed leg movement. *A*: changes of hip (*top*) and ankle (*bottom*) joint angular motions in 1 cycle of leg movement recorded from 1 subject using the VICON system. *B*: mean value of range of motion (ROM) of the hip (*top*) and ankle (*bottom*) joint (n = 10). Alt, Uni, and Syn indicate the alternate, unilateral, and synchronous leg movement conditions, respectively. Error bars indicate SE.

Difference in the induced EMG activity among experimental conditions

Figure 6 summarizes the integrated EMG activity of the Sol, Gas, and BF in three experimental conditions. The integrated EMG activity induced by bilateral alternate leg movement was significantly larger (P < 0.05) than that induced during the other conditions. The values of the percentage increase in EMG magnitude induced by alternate leg movement compared with that induced by unilateral leg movement were 291 ± 70, 163 ± 16, and 278 ± 71% for Sol, Gas, and BF, respectively.

Figure 7 shows the mean EMG amplitude in each 10% bin of the locomotion cycle (*top*) and in the period during which the muscle was evaluated to be active (*bottom*). The amplitude of the Sol EMG activity in the bilateral alternate movement was significantly larger (P < 0.05) than that in the unilateral movement from the 30 to 60% cycles, and significantly larger than that in the synchronous movement from the 30 to 70% cycles (Fig. 7*A*). The duration of the EMG activity of the Sol muscle during alternate leg movement was significantly longer (P < 0.05) than that during the other conditions (Fig. 7*A*). Such an amplifying effect of alternate leg movement on the EMG activity was also observed for the Gas and BF muscles (Fig. 7, *B* and *C*).

DISCUSSION

These results show that the locomotor-like EMG activity was significantly larger for alternate leg movement than for unilateral and bilateral synchronous movements. In the DISCUSSION section, the neuronal mechanism underlying these results, mainly in the context of what is known about the spinal locomotor system that was revealed in previous animal and human studies, will be addressed.

Muscle activity induced by passive leg movement

We used the gait-training apparatus (Fig. 1*A*) to impose the locomotory movement. However, the leg movement achieved by this apparatus is different from the ordinary stepping movement in the following two ways. First, the knee joint is locked in an extended position throughout the entire locomotion cycle. Second, the sole always touches the foot plate even during the forward leg swing phase. That is, the sensory information from the foot sole exists even in the swing phase, and there is no clear instant that corresponds to "heel contact." Despite these differences in the movement pattern, the EMG activity was observed in the paralyzed lower limb muscles during the passive leg movement, as was shown during the body weight–supported stepping movement on a treadmill in previous reports (Dietz et al. 1995, 2002; Dobkin et al. 1995; Ferris et al. 2004; Harkema et al. 1997; Ivanenko et al. 2003). This is because several factors that are important to this phenomenon, i.e., hip joint motion (Andersson and Grillner 1983; Grillner and Rossignol 1978) and load information (Dietz and Duysens 2000; Duysens and Pearson 1980), were well preserved, even in our experimental setting. In fact, as for the first difference regarding the knee joint motion, Dietz et al. (2002) have shown

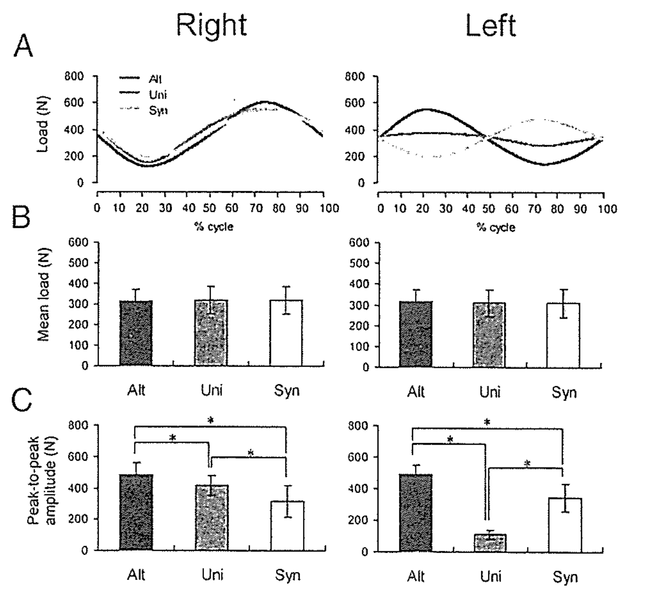

FIG. 5. Characteristics of load change during passive leg movement. *A*: changes of load applied to the foot sole of each leg in 1 cycle of leg movement. *B*: mean value of load averaged over 1 cycle (n = 6). *C*: mean value of peak-to-peak amplitude of load (n = 6). Alt, Uni, and Syn indicate the alternate, unilateral, and synchronous leg movement conditions, respectively. Error bars indicate SE. *Significant difference (P < 0.05).

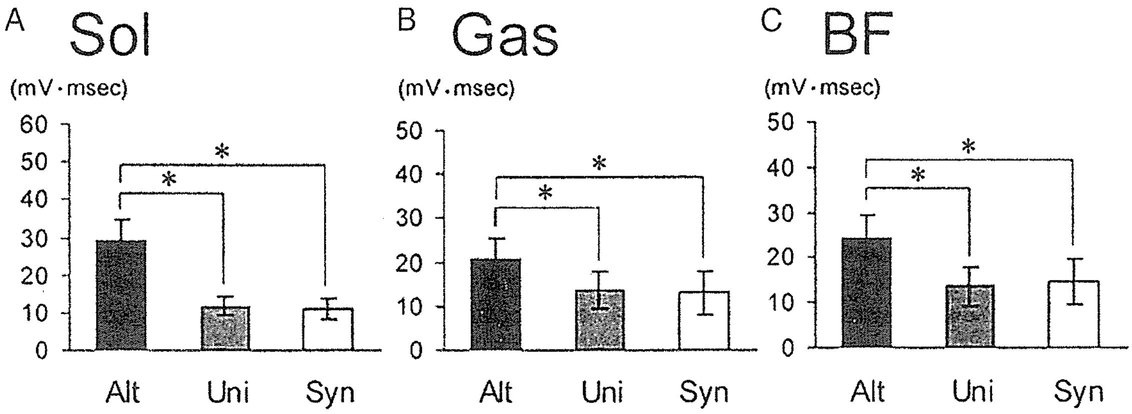

FIG. 6. Effect of leg movement pattern on muscle activity. Mean values of integrated rectified EMG induced by 3 types of leg movement. *A*: soleus (Sol). *B*: gastrocnemius (Gas). *C*: biceps femoris. Induced activity level of all 3 muscles was significantly larger ($P < 0.05$) during the alternate leg movement (Alt) than the unilateral (Uni) and synchronous (Syn) leg movement conditions. Error bars indicate SE. *Significant difference ($P < 0.05$).

that the knee-locked stepping movement (hip walking) does not affect the induced muscle activity. The only difference between normal and hip walking was that RF activity was almost absent in hip walking (see Fig. 4 in their study), a finding that agrees with our result (Figs. 3 and 5). The second difference regarding foot contact might influence the load information associated with the ordinary locomotion cycle; however, as shown in Fig. 5, we ensured that the load applied to the leg was periodically changed with the leg motion cycle in our experimental setting. The load was maximal when the hip joint was nearly maximally extended (Fig. 5), and this loading pattern resembled that observed when a stepping movement was imposed on a treadmill (Ferris et al., 2004). It is therefore likely that a considerable portion of the afferent neural inputs during normal walking could be preserved in our experimental setting.

In all subjects, coordinated EMG bursts can be induced by imposing passive leg movement in the lower limb muscle. As shown in Figs. 2 and 7, the phase in which the muscle activity was observed coincided with the phase in which it was mechanically stretched. That is, Sol and Gas were active while the leg swung toward the backward, and BF was active while the leg swung toward the forward. It is therefore possible that the muscle activity was associated with the stretch reflex response. However, these results show that the muscle activity was observed even in the muscle's shortening phase (Figs. 2 and 7). Concerning this point, Dietz et al. (1998) have also observed that the leg muscle activity is equally distributed during shortening and it seems therefore likely that the locomotor-like muscle activity results from the complex interaction of the afferent inputs and the spinal neural circuits rather than simple stretch reflex.

Contribution of alternate leg movement

One of the most substantial features of human bipedal locomotion is alternating leg movement. Therefore investigating how such an alternate leg movement pattern affects the amount of locomotor-like EMG activity would give us important information, especially regarding the problem of whether the activity is actually "locomotory" or not. A relevant approach has been partly taken by Ferris et al. (2004). They found that muscle activity could be induced for complete SCI patients even in the nonmoving leg when the stepping movement was imposed only on the other leg. Their results have provided evidence that the human spinal cord has a mechanism to efficiently realize alternating leg movement. However, we did not observe any muscle activity in the nonmoving left leg (Fig. 3). This result was similar to the results of the study by Dietz et al. (2002), who ascribed the contradiction with the work of Ferris et al. (2004) to the difference in the speed of stepping and the amount of the load (Dietz and Harkema 2004). Likewise, one of the possible reasons for the contradiction between the results of Ferris et al. (2004) and our results is the difference in the load pattern on the nonmoving leg. In this study, the load was tonically applied and the amount of modulation was small (Fig. 5), while in their study, a load pattern resembling normal stepping was applied.

On the basis of the absence of muscle activity in the nonstepping leg, Dietz et al. (2002) referred the possibility that the interlimb coordination observed in normal subjects requires the supraspinal systems. Concerning this point, a recent study revealed that the interlimb coordination includes the activity of the supplemental motor cortex area (Debaere et al. 2001). However, our data have provided strong evidence that the spinal cord has an ability to coordinate the movement of both legs. Figure 8 shows the relationships between Sol EMG activity and ankle ROM (*left*), hip ROM (*center*), and the peak-to-peak load (*right*) on the right foot sole. The EMG level was significantly larger for locomotion-like alternate leg movement than for unilateral and bilateral synchronous movements, although the hip and ankle joint movements were kept identical in all experimental conditions. This result also indicates that the stretch reflex alone is insufficient to explain the modulation of the EMG activity. If the EMG activity were merely a response to the rhythmic muscle-tendon stretches, the level of muscle activity should have been independent of the contralateral leg movement.

One remaining concern is the difference among the three conditions in the load applied to the right leg (Fig. 8, *right*), because the load-related afferent inputs, such as proprioceptive inputs from the extensor muscle and the sole of the foot, are known to influence the magnitude of the EMG activity

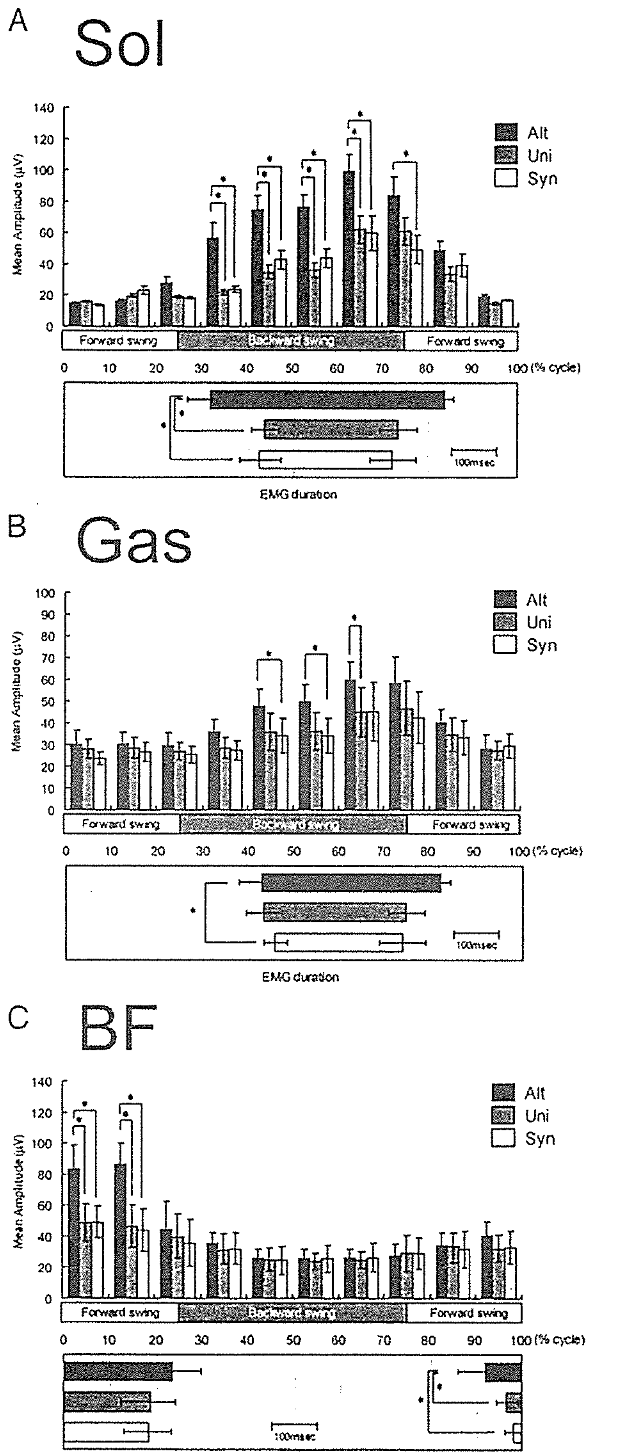

FIG. 7. Comparison of mean EMG amplitude in each 10% cycle bin (*top*) and duration of EMG activity (*bottom*) among the 3 experimental conditions. *A*: soleus (Sol). *B*: gastrocnemius (Gas). *C*: biceps femoris (BF). Alt, Uni, and Syn indicate the alternate, unilateral, and synchronous leg movement conditions, respectively. Error bars indicate SE. *Significant difference ($P < 0.05$).

(Harkema et al. 1997; Kojima et al. 1999). Therefore the larger EMG activity in the alternate leg movement condition could simply result from the load on the right leg having larger peak-to-peak amplitude. However, this is unlikely because the distribution of the Sol EMG activity with respect to the peak-to-peak amplitude of the load is distinctly different from other two conditions (Fig. 8, *right*). Therefore it is difficult to explain such a drastic enhancement of Sol EMG activity based only on the difference in load. In addition, although the peak-to-peak load was larger in the unilateral condition than in the synchronous condition, the Sol activity was almost similar between these two conditions (Fig. 8, *A* and *C*, *right*) and even smaller for the unilateral condition in subject S2 (Fig. 8*B*, *right*), suggesting that the Sol activity does not depend only on the load modulation.

Therefore our results strongly suggest that the afferent input from the contralateral leg plays a substantial role in amplifying the induced locomotor-like muscle activity in the lower limb. In particular, the contralateral leg movement has to be out of phase so that the muscle activity of the ipsilateral leg is well amplified. That is, the alternate leg movement should be added to the recipes for generating locomotor-like muscle activity that have been previously suggested, such as hip joint motion and the load applied to the lower limbs (Pearson 1995).

Interlimb coordination generated within the spinal cord

Previous animal studies, using a variety of preparations, indicate that basic neuronal circuits that generate the locomotive motor output exist in the lumbar level of the spinal cord (Forssberg et al. 1980; Pearson and Rossignol 1991; for a review, see Duysens and Van de Crommert 1998). Such neuronal circuits can operate in the absence of any afferent input (Grillner 1985), whereas the significance of the interaction of such a spinal neuronal circuit with the afferent input has also been pointed out (Duysens and Pearson 1980; Pearson 1995). Recent human studies have shown that the afferent signal from one limb affects the muscle activity of the contralateral limb in locomotory movement in a functional way (Pang and Yang 2001; Ting et al. 2000). However, since these studies were conducted in infants (Pang and Yang 2001) or in healthy subjects (Ting et al. 2000), the supraspinal system's contribution remains unclear. Although the supraspinal system such as the supplementary motor area might contribute to the interlimb coordination (Debaere et al. 2001), these results indicate that some mechanism coordinating the alternate leg movement might exist within the human spinal cord itself. The precise mechanism(s) are unknown at this stage, but it is possible that the neuronal circuits associated with our results have a common origin in the crossed flexor/extensor reflex (Duysens and Loeb 1980; Duysens et al. 1991). Further research is needed to clarify this point.

In summary, this study was designed to investigate to what extent the alternate leg movement influences the locomotor-like EMG activity in the lower limbs of SCI subjects. These results indicated that the alternate leg movements play a substantial role in amplifying the induced muscle activity, and not only suggest the existence of neuronal circuits enabling interlimb coordination within the spinal cord, but might reinforce the interpretation that the muscle activity induced by passive stepping movement is actually locomotory.

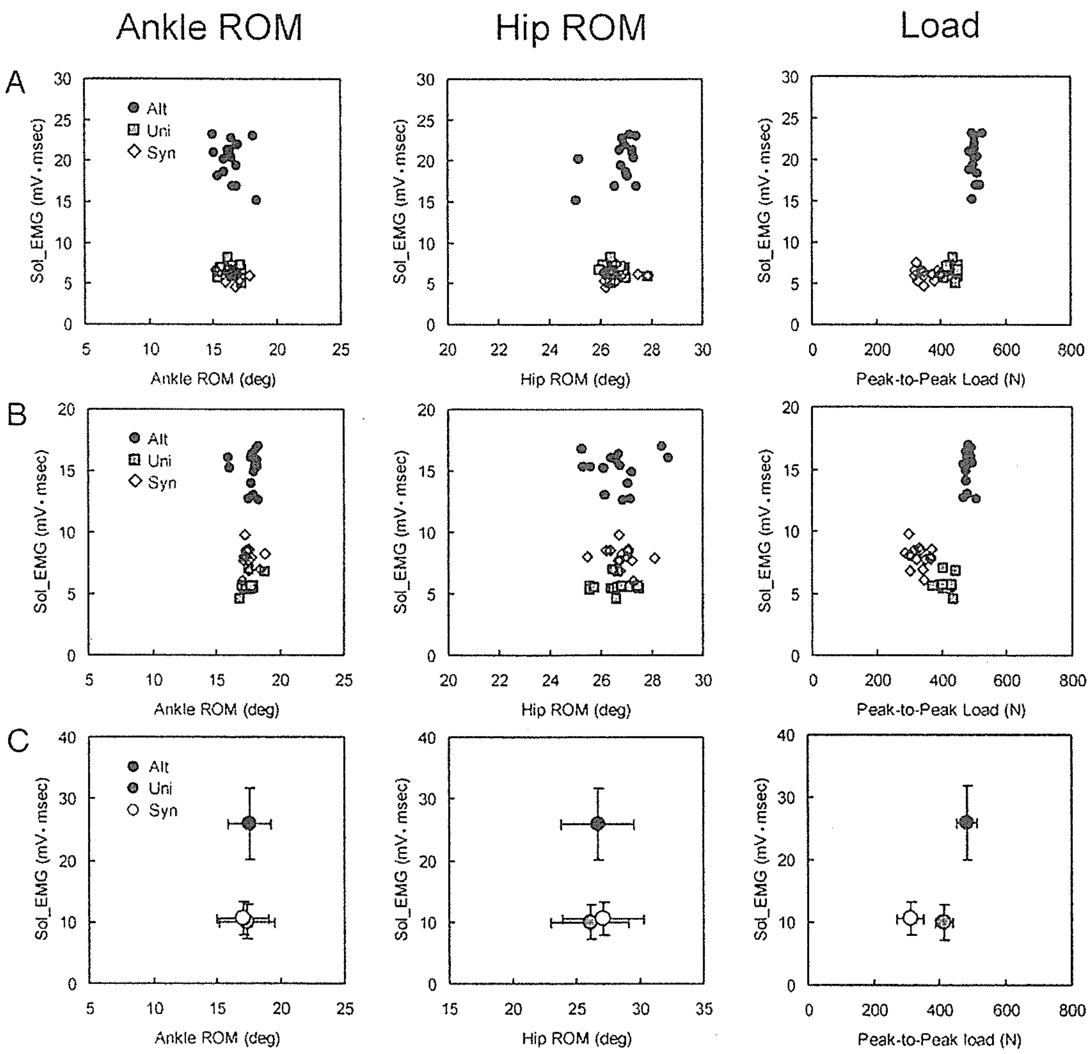

FIG. 8. Relationships between the right soleus EMG magnitude and ROM of the hip joint, ROM of the ankle joint, and peak-to-peak amplitude of load applied to the right leg. *A*: subject S1. *B*: subject S2. *C*: mean data ($n = 6$). In *A* and *B*, each point corresponds to the value obtained in each cycle. Alt, Uni, and Syn indicate the alternate, unilateral, and synchronous leg movement conditions, respectively. In *C*, error bars indicate SE.

REFERENCES

Andersson O and Grillner S. Peripheral control of the cat's step cycle. II. Entrainment of the central pattern generators for locomotion by sinusoidal hip movements during "fictive locomotion." *Acta Physiol Scand* 118: 229–239, 1983.

Debaere F, Swinnen SP, Beatse E, Sunaert S, Van Hecke P, and Duysens J. Brain areas involved in interlimb coordination: a distributed network. *Neuroimage* 14: 947–958, 2001.

Dietz V, Colombo G, Jensen L, and Baumgartner L. Locomotor capacity of spinal cord in paraplegic patients. *Ann Neurol* 37: 574–582, 1995.

Dietz V and Duysens J. Significance of load receptor input during locomotion. *Gait Posture* 11: 102–110, 2000.

Dietz V and Harkema SJ. Locomotor activity in spinal cord-injured persons. *J Appl Physiol* 96: 1954–1960, 2004.

Dietz V, Muller R, and Colombo G. Locomotor activity in spinal man: significance of afferent input from joint and load receptors. *Brain* 125: 2626–2634, 2002.

Dietz V, Wirz M, Colombo G, and Curt A. Locomotor capacity and recovery of spinal cord function in paraplegic patients: a clinical and electrophysiological evaluation. *Electroencepharogr Clin Neurophysiol* 109: 140–153, 1998.

Dimitrijevic MR, Gerasimenko Y, and Pinter MM. Evidence for a spinal central pattern generator in humans. *Ann NY Acad Sci* 860: 360–376, 1998.

Dobkin BH, Harkema S, Requejo P, and Edgerton R. Modulation of locomotor-like EMG activity in subjects with complete and incomplete spinal cord injury. *J Neuro Rehab* 9: 183–190, 1995.

Duysens J and Loeb GE. Modulation of ipsi- and contralateral reflex responses in unrestrained walking cats. *J Neurophysiol* 44: 1024–1037, 1980.

Duysens J and Pearson KG. Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res* 187: 321–323, 1980.

Duysens J, Tax AA, van der Doelen B, Trippel M, and Dietz V. Selective activation of human soleus or gastrocnemius in reflex responses during walking and running. *Exp Brain Res* 87: 193–204, 1991.

Duysens J and Van de Crommert HWAA. Neural control of locomotion: The central pattern generator from cats to humans. *Gait Posture* 7: 131–141, 1998.

Ferris DP, Gordon KE, Beres-Jones JA, and Harkema SJ. Muscle activation during unilateral stepping occurs in the nonstepping limb of humans with clinically complete spinal cord injury. *Spinal Cord* 42: 14–23, 2004.

Forssberg H, Grillner S, and Halbertsma J. The locomotion of the low spinal cat. I. Coordination within a hindlimb. *Acta Physiol Scand* 108: 269–281, 1980.

Grillner S. Neurobiological bases on rhythmic motor acts in vertebrates. *Science* 228: 143–149, 1985.

Grillner S and Rossignol S. On the initiation of the swing phase of locomotion in chronic spinal cats. *Brain Res* 146: 269–277, 1978.

Harkema SJ. Neural plasticity after human spinal cord injury: application of locomotor training to the rehabilitation of walking. *Neuroscientist* 7: 455–468, 2001.

Harkema SJ, Hurley SL, Patel UK, Requejo PS, Dobkin BH, and Edgerton VR. Human lumbosacral spinal cord interprets loading during stepping. *J Neurophysiol* 77: 797–811, 1997.

Ivanenko YP, Grasso R, Zago M, Molinari M, Scivoletto G, Castellano V, Macellari V, and Lacquaniti F. Temporal components of the motor patterns expressed by the human spinal cord reflect foot kinematics. *J Neurophysiol* 90: 3555–3565, 2003.

Kawashima N, Abe M, Nozaki D, Nakazawa K, and Akai M. Alternate leg movements contribute to amplify locomotor-like muscle activity in spinal cord injured patients. Washington, DC: Society for Neuroscience, 2003.

Kojima N, Nakazawa K, and Yano H. Effects of limb loading on the lower-limb electromyographic activity during orthotic locomotion in a paraplegic patient. *Neurosci Lett* 274: 211–213, 1999.

Maynard FM Jr, Bracken MB, Creasey G, Ditunno JF Jr, Donovan WH, Ducker TB, Garber SL, Marino RJ, Stover SL, Tator CH, Waters RL, Wilberger JE, and Young W. International standards for neurological and functional classification of spinal cord injury. *Spinal Cord* 35: 266–274, 1997.

Nakazawa K, Kakihana W, Kawashima N, Akai M, and Yano H. Induction of locomotor-like EMG activity in paraplegic persons by orthotic gait training. *Exp Brain Res* 157: 117–123, 2004.

Pang MY and Yang JF. Interlimb co-ordination in human infant stepping. *J Physiol* 533: 617–622, 2001.

Pearson KG. Proprioceptive regulation of locomotion. *Curr Opin Neurobiol* 5: 786–791, 1995.

Pearson KG and Rossignol S. Fictive motor patterns in chronic spinal cats. *J Neurophysiol* 66: 1874–1887, 1991.

Ting LH, Kautz SA, Brown DA, and Zajac FE. Contralateral movement and extensor force generation alter flexion phase muscle coordination in pedaling. *J Neurophysiol* 83: 3351–3365, 2000.

Van de Crommert HWAA, Mulder T, and Duysens J. Neural control of locomotion: sensory control of the central pattern generator and its relation to treadmill training. *Gait Posture* 7: 131–141, 1998.

Winter DA. *Biomechanics and Motor Control of Human Movement*, 2nd ed. Toronto, Canada: John Wiley, 1990.

Exp Brain Res (2004) 157: 117–123
DOI 10.1007/s00221-003-1826-5

RESEARCH ARTICLES

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Induction of locomotor-like EMG activity in paraplegic persons by orthotic gait training

Received: 2 June 2003 / Accepted: 2 December 2003 / Published online: 17 February 2004

Abstract This is, to our knowledge, the first report demonstrating the effects of orthotic gait training on the activity of the spinal locomotor neural networks. Three subjects with complete spinal cord injury (SCI) performed 1-h training with reciprocating gait orthosis 5 days/week for 12 weeks. The results showed that after 3 (n=1) or 6 weeks (n=2) of training, EMG activities synchronized with locomotor rhythm appeared in the soleus muscle (SOL) in all subjects, although very little EMG activity accompanied the orthotic gait at the early training stage. Our results suggest that the induced modulation in the SOL EMG waveforms might be attributable to changes in the orthotic gait movement pattern, and/or changes in the interneuronal activities of the spinal locomotor neural networks, as a result of orthotic gait training.

Keywords Spinal cord injury · Locomotion · Gait orthosis · Plasticity

Introduction

It has been well established that the human spinal cord has the potential to generate the basic locomotor pattern by interaction of the locomotor neural networks and peripheral sensory information concomitant with limb movements (Harkema et al. 2000). For example, several researchers have demonstrated that in severe spinal cord-injured (SCI) subjects, locomotor-like coordinated electromyographic (EMG) activity can be induced in paralyzed lower limb muscles by passive stepping on a moving treadmill with partial body-unloading (Dietz et al. 1994, 1995; Dobkin et al. 1994; Wernig et al. 1995). However, the nature of the neural networks involved in generation of locomotor EMG activities in SCI persons is not yet fully understood.

During a specific type of upright walking with gait orthosis called weight bearing control orthosis (WBCO) (Yano et al. 1997), modulation of lower leg-muscle activities that synchronize with that particular locomotor cycle can be induced (Kojima et al. 1998). Because the WBCO gait, like other reciprocating gait orthoses, is a "stiff-leg" gait, i.e., a gait with the knee locked in full extension and the ankle in a neutral position, the afferent information thought to primarily contribute to inducing the locomotor-like EMG activity would be associated with hip-joint movement and load on the leg (Dietz et al. 2002). This in turn might mean that use of the orthotic gait would allow us to investigate the contribution of the involvement of hip extension/flexion movement or load on the leg to generation of locomotor-like EMG modulation, specifically in the "lower leg" muscles, which are remote from the hip joint. However, in our experience, little EMG activity appears during the WBCO gait when the user is not well trained, whereas it has been demonstrated in a well-trained SCI subject that locomotor-like EMG is observed (Kojima et al. 1998). These empirical observations might be explained as follows: (1) afferent inputs concomitant with limb movements would not be sufficient to evoke locomotor EMGs during the untrained orthotic gait; and (2) the orthotic gait training induces an alteration in interneuronal activities in spinal neural networks, which would generate the locomotor EMG even when the pattern and amount of afferent inputs are the same. To test these possibilities, we first had to longitudinally evaluate changes in EMG activities in paralyzed muscles, and to relate these EMG changes with gait-motion changes during the time course of training. The purpose of this study, therefore, was to clarify: (1) whether alteration in the EMG activities in the lower leg muscles occurs during the time course of orthotic gait training, and (2) the relation between the EMG activities and kinetic/kinematic alteration of the gait motion due to the training.

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Materials and methods

Subjects and orthotic gait training

Three clinically complete paraplegic men (22–28 years; 60–68 kg; 173–177 cm) with traumatic spinal cord injuries (Th8–Th12) voluntarily participated in this study. The physical characteristics of the subjects are shown in Table 1. The American Spinal Injury Association (ASIA) impairment scale was used for the clinical assessment of subjects, and in each case the sensorimotor functions were classified as A, which means no motor or sensory function below the level of the lesion. The subjects gave their informed consent to the experimental procedures, which were conducted in accord with the Helsinki Declaration of 1975 and approved by the ethics committee of the National Rehabilitation Center for the Disabled, Tokorozawa, Japan. The long brace reciprocating gait orthosis, the WBCO, was used for the training. The subjects performed orthotic gait training with WBCO for 12 weeks, at 1 hour/d for 5 days/week. The mechanical features of the WBCO have been fully described elsewhere (Yano et al. 1997; Kojima et al. 1998). In short it has two specific features that the other existing gait orthoses do not have. The first one is a special gas-powered system to control the foot sole thickness. It can switch the sole thickness depending on the gait phase; when a leg is swinging forward, the sole of this particular leg is held at the thinner position; and just before the heel strikes, the sole gets changed to the thicker position. With this system a user can swing their legs easier without leaning the body sideward to make a clearance between a foot sole and floor. The second one is a special hip joint device. With this device a torque exerted by the right (left) hip joint is mechanically transmitted to the left (right) hip joint, resulting in the torque to the opposite direction exerted by the left (right) hip joint. This system assists each leg to reciprocally propel forward. As a whole these mechanical features enable a user ambulate at faster speed and with less energy expended (Kawashima et al. 2003).

Experiments

To evaluate the kinetic and kinematic changes in the orthotic gait motion during the course of training, the gait motion was measured with a three-dimensional motion-analysis system (VICON370, Oxford Metrics, UK). The motion-analysis system consists of a conventional video-analysis system with seven cameras and Kistler force plates. The force plates, sized 160×450 cm, consisted of two 80×200 cm plates and four 40×250 cm plates. These separate force plates enable us to measure ground reaction forces (GRF) under the feet and canes on both sides, separately. The orthotic gait motion was recorded along with electromyographic (EMG) activities in the right soleus (SOL) and tibialis anterior (TA) muscles. EMGs were recorded by two surface electrodes (Ag/AgCl, 0.8 cm diameter) attached along the muscle fibers over the belly of each muscle and set at an interelectrode distance of 0.5 cm. The EMG signal was detected by a bipolar differential amplifier with upper and lower cutoff frequencies of 50–3000 Hz. Very thin elastic nylon bandages were used to firmly hold both electrodes and lead lines to the body, preventing any small displacement of electrodes and lines that might cause artifacts. These measurements were carried out three times (after 1, 6, and 12 weeks) in subject A; six times (after 1, 2, 3, 4, 10, and 12 weeks of training) in subject B; and twice (after 1 and 6 weeks) in subject C during the training period. For the measurement, subjects ambulated along a 10-m walkway several times at comfortable cadences. They repeated the trials with short-time intermissions, usually a couple of minutes, until the minimum required number of data was obtained. We sampled at least six step cycles for the analysis. Many step cycles, for example more than ten cycles, could not be recorded in the measurements of this study, since high quality VICON data could be obtained only for one or two steps of around five steps in a trial. At the beginning of training, especially, the experimenter had to walk beside the subjects to prevent a fall. This disturbs the motion capture with the VICON system, and makes the space in which the motion capture is possible small. Due to this limitation it would have taken a relatively longer time for the subjects to record many step cycles. To reduce time for the experiment we decided to finish the measurement when at least six step cycles were obtained in good quality.

Changes in the following kinetic and kinematic variables were evaluated from the measured VICON data throughout the training period: kinetic variables, including the impulse and mean vertical GRF (mGRF) under the foot, and kinematic variables, including the stance time and swing time, velocity, cadence and step length, joint range of motion (ROM), and peak velocity during the stance phase of the hip and ankle joints. The digitized EMG signals were full wave-rectified after rejection of the DC component. Then, from the rectified EMG signals, mean values (mEMG) for the stance phase were calculated and normalized by those values at rest.

Stretch reflex test

The reflex EMG responses elicited by mechanical stretches at various velocities were tested to verify whether the stretch reflex mediated the induced EMG activity in the SOL during the orthotic gait. Stretch reflex responses were evoked by imposing a quick dorsiflexion with an amplitude of 10 deg to the SOL muscle, while the subjects were seated comfortably in a chair with the right leg fixed to a foot plate connected to a servo-controlled torque motor (Senoh Inc., Japan). The hip, knee and ankle joints were set at 80 deg, 60 deg flexed and 10 deg plantar-flexed positions (anatomical position is 0 deg), respectively. All of 25 perturbations, each consisting of various angular velocities (50–350 deg/s), were applied to the ankle joint in random order. In the present study, the short latency reflex component, M1, was evaluated, since only M1 component was induced in the three subjects. The onset of the first EMG response was defined as the moment when the rectified EMG activities reached levels higher than the average resting potential plus three times its standard deviation (BGA+3SD), and the response duration was defined as 30 ms from the response onset. The average rectified EMG value above the resting potential level over the response duration was considered as the M1 level, and the relation between the imposed stretch velocity and the M1 level was analyzed for each subject.

Additional experiments

Additional experiments were done for the subject A in order to ascertain whether: (1) the EMG activity was induced with another conventional gait orthosis (advanced reciprocating gait orthosis, ARGO), and (2) in order to compare how different gait velocities affected the induced EMG activity before and after the training. Because this subject continued the training for over half a year,

Table 1 Clinical characteristics of subjects studied

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	Injury level (segment)	ASIA	Time postinjury (months)	Etiology
A	M	27	177	60	T10	A	10	Trauma
B	M	22	174	68	T12	A	8	Trauma
C	M	28	173	63	T8	A	12	Trauma

measurements could be taken at the 1st week (1-W), 4th week (4-W), and 20th week (20-W) of the training. In the measurements, the subject ambulated at various velocities, speeding up his pace on the basis of his comfort.

Statistics

The measured kinetic and kinematic variables during the training period were compared with the first measurement values using the Student's t-test. Data are presented as mean ± SD. Significance was accepted as $p<0.05$.

Results

All subjects could ambulate independently with the aid of canes in the 1st week of training. In subjects A and B, gait velocities were respectively increased from 7.7 to 13.2 m/min, and from 11.8 to 21.2 m/min after the 12-week training; these increases are concomitant with increases in step length, ROM, angular velocity of hip and ankle joints, and the mGRF during the stance phase. In subject C, however, both the hip and ankle-joint angular velocities were decreased; the hip joint's ROM was decreased; and the ankle joint's ROM was increased. These findings were likely due to the markedly faster gait velocity (22.4 m/min) of this subject, as compared to the other two subjects, at the beginning of the training. In none of the subjects was clear modulation in the EMG activities of either muscle observed at the beginning of the training. After three (subj. A) or six (subjs. B and C) weeks of training, however, synchronized EMG bursts with the stance phase commonly appeared in the SOL in all three subjects, whereas no clear modulation was observed in any of the TA EMG waveforms (Fig. 1A). Figure 2 shows changes in the walking velocities, hip and ankle joint angular velocities, mGRFs, and SOL EMGs during the time course of training in the three subjects. As mentioned above, the SOL EMG activities increased during the training period in all subjects, and this increasing time course was qualitatively most similar to the gait velocity and mGRF. The increasing patterns in the SOL EMGs were not necessarily in parallel with those in the hip and ankle joint velocities. In subject C, specifically, the hip joint and ankle joint velocities demonstrated a tendency to decrease, though the SOL EMG increased.

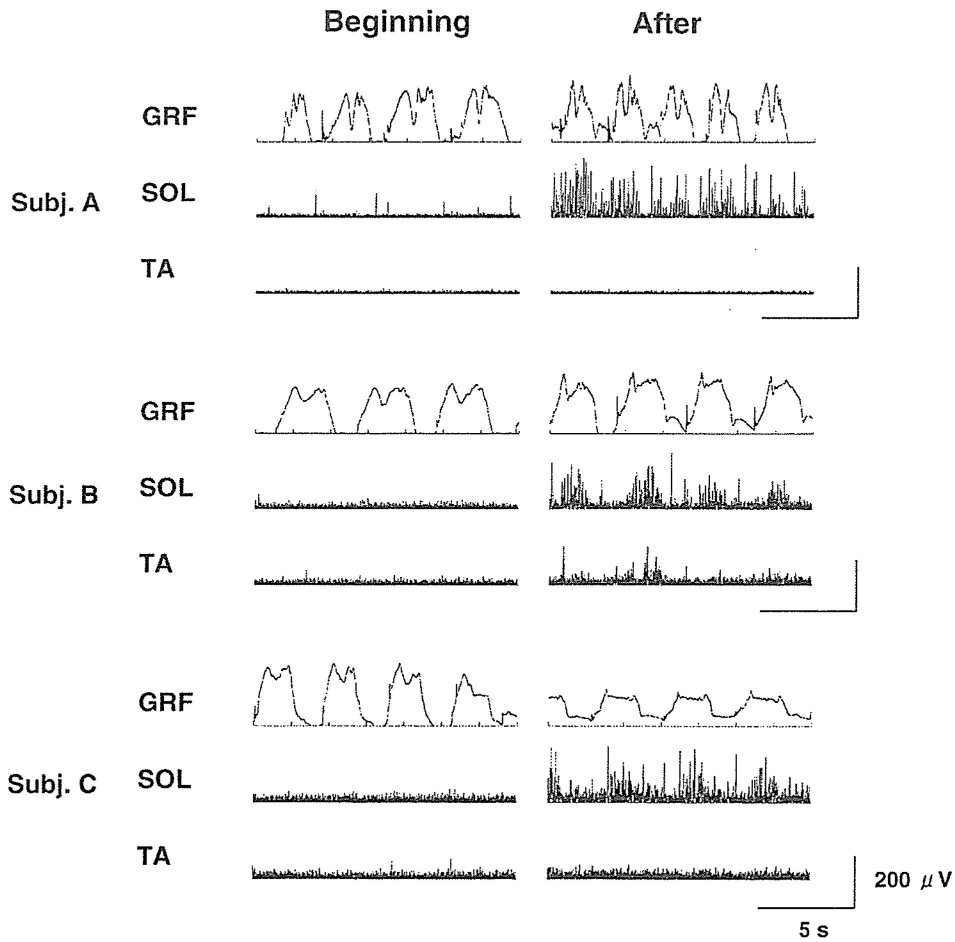

Fig. 1 Changes in EMG activities in the lower leg muscles during orthotic gait before and after the training. Results from the three subjects are shown (*GRF* the vertical ground reaction force, *SOL*, *TA* rectified EMGs from the soleus and tibialis anterior muscles, respectively)

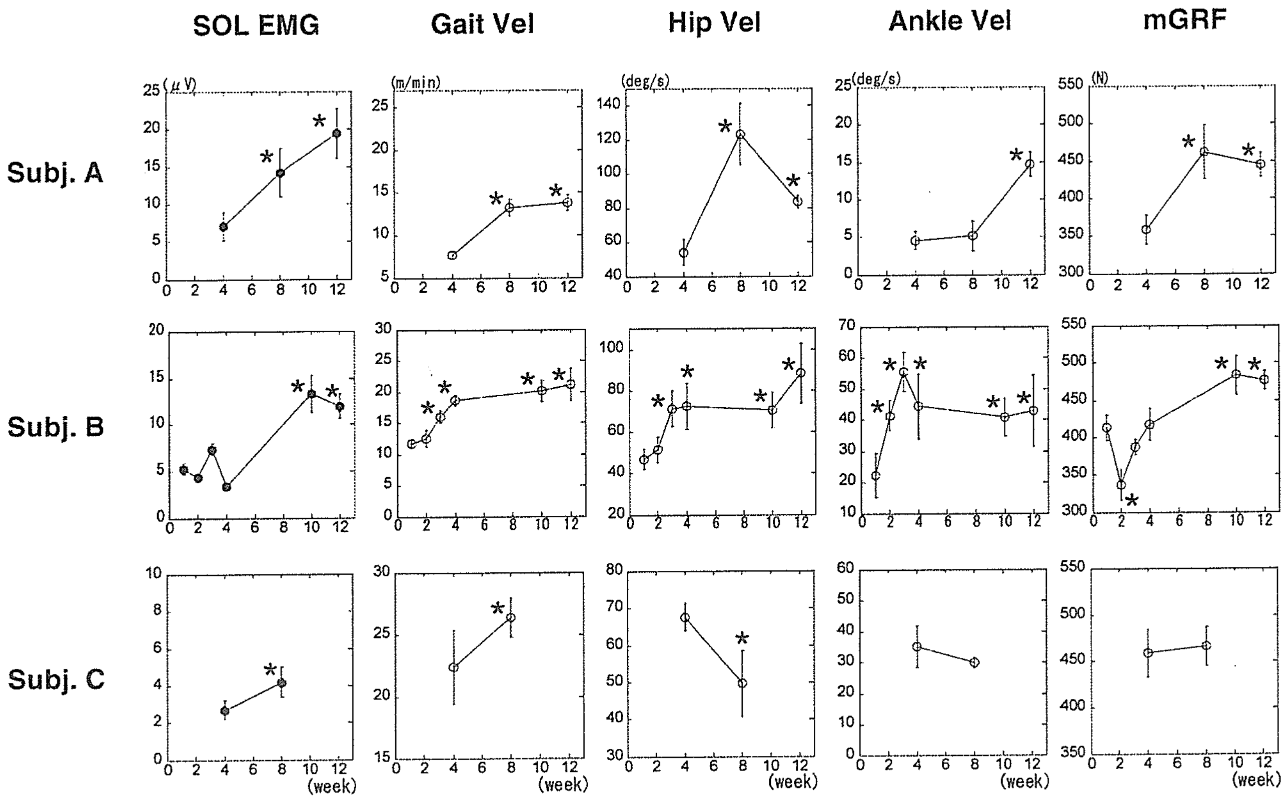

Fig. 2 Changes in the SOL EMGs, the gait velocities, the hip and ankle joint angular velocities, and the ground reaction forces over the training period for the three subjects

Additional experiment

Figure 3 shows the SOL EMG activities during the orthotic gait at three different gait velocities at the 1-W and 4-W measurements. It was demonstrated that the synchronized EMG burst with the stance phase increased drastically with the ambulation velocity after 4 weeks of training; no such clear modulation was observed at the 1st week measurement.

Relationships of the SOL EMGs during the stance phase with the gait velocity, hip velocity, ankle velocity, and ground reaction force, respectively, in each measurement are demonstrated in Fig. 4. In the 1-W measurement, the SOL EMG did not clearly increase with the gait velocity or the other kinetic and kinematic variables; the measurement reflected no qualitatively clear modulation. In the 4-W and 20-W measurements, however, the SOL EMGs covaried with the gait velocity and the other variables. It should be noted that the levels of SOL EMGs in the 4-W and 20-W measurements were greater than those in the 1-W measurement, even though the kinetic and kinematic variables were in similar ranges. This result suggests that the observed increase in the SOL EMG during the time course of training was not merely dependent on the kinetic and kinematic factors; neurological factors were to some degree involved.

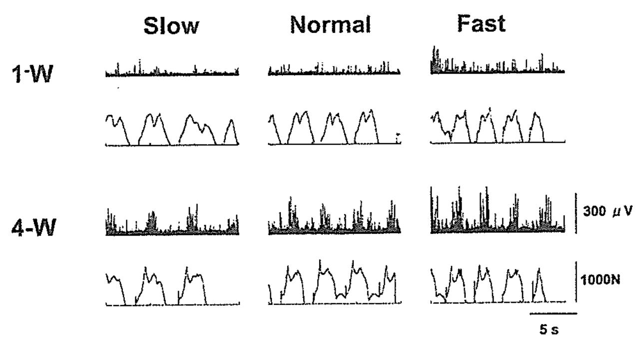

Fig. 3 A comparison of the velocity dependent changes in the SOL EMGs during orthotic gait before and after the 4-week training with the other gait orthosis

Stretch reflex test

Figure 5 demonstrates the relationships between stretch velocity and the reflex EMG responses in the SOL for the three subjects. It was indicated that in all three subjects, the stretch reflex EMG response was induced when the applied stretch velocity was faster than 100 deg/s, meaning the threshold velocity of the reflex was around 100 deg/s. These threshold velocities were well above the peak ankle dorsiflexion velocities observed during the orthotic gait in the three subjects, suggesting that the SOL EMGs

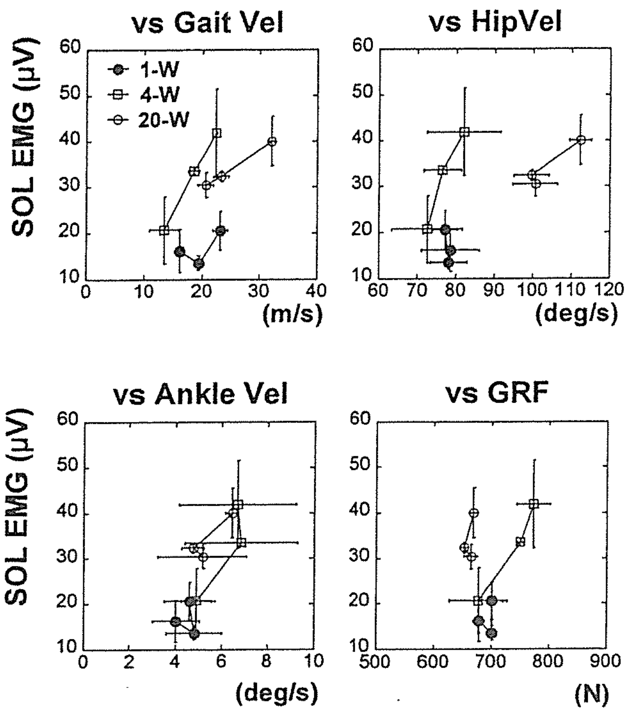

Fig. 4 Comparisons of the relationships of the SOL EMGs to the kinetic and kinematic variables before and after 1 month and 5 months of training. Abbreviations: *vs Gait Vel, vs Hip Vel, vs Ankle Vel,* and *vs GRF* refer to the relationships between the SOL EMG and the gait velocity, hip joint angular velocity, ankle joint angular velocity, and ground reaction force, respectively

observed were not merely mediated by the stretch reflex pathway.

Discussion

The results in the present study demonstrated that intense orthotic gait training induced modulation of EMG activities in the ankle extensor SOL muscle in individuals with clinically complete SCI. These results constitute neurologically significant indirect evidence that knee (and to some degree ankle) movements are far less important than hip movement and loading for the induction of locomotor EMG activity, at least in the SOL. Further, these results have great clinical significance especially in terms of gait rehabilitation of patients with incomplete spinal cord injury. It is worth noting that the results must be carefully interpreted, given that many factors may contribute the observed phenomena. Considering that possibility, we divided the various factors into two different types: (1) kinetic and/or kinematic, related to changes in the orthotic gait movement itself, and (2) neurological factors, namely, supposed changes in the interneuronal activities of the spinal locomotor networks as a result of training. We believe that neither the kinetic/kinematic nor the neurological factors alone can fully explain the observed results

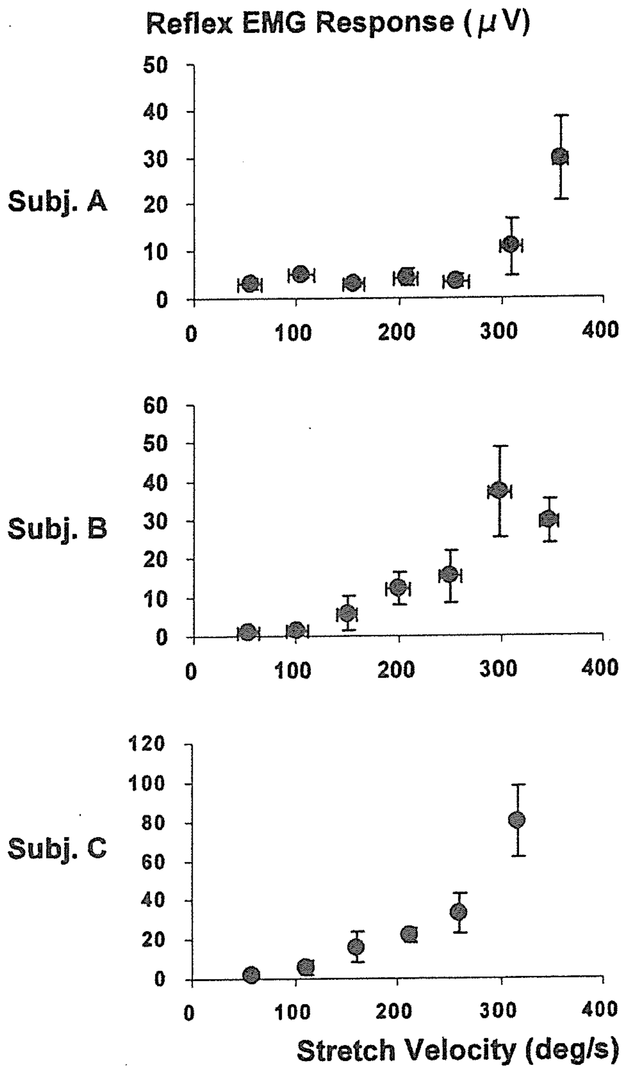

Fig. 5 Relationships between the stretch reflex EMG activities in the SOL and the applied stretch velocity for the three subjects. Note that the estimated threshold velocity to elicit the reflex is over 100 deg/s, which is far faster than the peak ankle joint velocity during the orthotic gait in any of the three subjects

Effects of the orthotic gait motion on modulation of EMG activity

The results of the stretch reflex test in the SOL indicated that the threshold velocities of stretch reflex in the SOL were far higher than the observed ankle dorsiflexion velocity during the orthotic gait. This result strongly suggests that the observed SOL EMGs during the orthotic gait were not induced merely by the spinal stretch reflex. Rather, in the present study, supposed spinal locomotor networks appeared to play a role in the induction of SOL EMGs during orthotic gait.

The observed modulation in the SOL EMG activities synchronized with gait phase confirmed our previous observation that even the knee-locked "stiff-legged" gait with a gait orthosis is effective to induce locomotor-like

EMG activity in lower limb muscles (Kojima et al. 1999). Because the stiff-legged gait is a gait with the knee locked in full extension and the ankle in a neutral position, the afferent information thought to primarily contribute to inducing the observed modulation in the EMG activity would be associated with the hip-joint movement and load on the leg. This result is consistent with recent observations from infant stepping experiments (Pang and Yang 2000) and experiments using a driven gait orthosis for paraplegic subjects (Dietz et al. 2002). Dietz et al. (2002), on the basis of their elegant series of studies on locomotor capacity of human spinal cord and relevant animal and human studies, concluded that "afferent input from hip joints, in combination with that from load receptors, plays a crucial role in the generation of locomotor activity in the isolated human spinal cord". The result in the current study would constitute indirect evidence to support this notion. With respect to afferent input from the hip joint, further, Schimit and Benz (2002) recently demonstrated that imposed hip joint extension/flexion movements in spinal cord-injured subjects induce a unique, stereotypical reflex response in hip, knee, and ankle joints, suggesting that hip movement would activate spinal interneuronal pathways associated with coordinated motor behaviors such as posture and locomotion. Given this notion, the results in the current study suggest that afferent information associated with hip extension during the orthotic gait might activate the spinal neural network responsible, at least in part, for the synchronized EMG activity in the SOL, which may be the common network generating the extensor reflex. With regard to the effect of limb loading on the locomotor activity in the paraplegic human, a growing body of indirect evidence from studies on humans has indicated that load-related afferent inputs play an essential role in the generation of locomotor-like efferent patterns by the human spinal cord (Harkema et al. 1997; Pang and Yang 2000, 2001; Dietz et al. 2002). The observed close relationship between the SOL EMG levels and mGRF during the training period (Fig. 2) and within a single experiment (Fig. 4) in the current study is consistent with that found in previous reports and our previous study (Kojima et al. 1999), in which we demonstrated that the levels of lower limb muscle EMGs during orthotic gait were well correlated to the level of limb loading.

Alteration of the intrinsic property of spinal neural networks due to training

The observed EMG alteration in the SOL might not solely depend on gait-motion changes due to training, but also on alterations in the intrinsic properties of neural networks. This hypothesis is supported by the result of an additional experiment, in which the EMG modulation occurred even under kinematic and kinetic profiles of orthotic gait similar to those of the pre-training gait after 4 weeks of training (Fig. 4). In addition, the effect of changing gait velocity was obviously different before and after several weeks of training, suggesting that the input and output properties in the spinal neural networks during orthotic gait were altered due to the training. Another observation that supports this hypothesis might be the observed EMG changes in subject C, whose orthotic gait motion was at a higher level (i.e., faster velocity and larger ROM, etc.) at the first stage of training and did not largely change during the training period. Nevertheless, modulation of the SOL EMG was induced in this subject, despite rather reduced angular velocities of hip and ankle joints after 8 weeks of training.

Use-dependent plasticity is now a well-known property of spinal neural networks (Hodgson et al. 1994; Muri and Steeves 1997; Raineteau and Schwab 2001). Repeated afferent input accompanying gait training might result in improvement in the transmission efficacy within the neural network responsible for the SOL EMG activity during orthotic gait. This possibility is extremely important with regard to rehabilitation strategy for SCI patients (Field-Fote 2001; Protas et al. 2001). If the act ambulation with an orthosis itself has the potential to improve neuronal activity in the spinal locomotor neuronal networks, a specific gait orthosis one could be designed and developed for locomotor training. Future studies should explore the optimal design for a gait orthosis that can effectively activate the spinal locomotor neural network, using the findings in the current study as the first step in such a series of investigations.

Finally, almost no EMG modulation appeared in the TA in the current study. This might be explained by the following: (1) because the correlation between the load on a limb and EMG activity is less in the TA than in the SOL (Harkema et al. 1997; Kojima et al. 1999), load-related afferent information during the orthotic gait in our study might not have been sufficient to evoke TA activity; and (2) although an imposed hip flexion can induce the ankle flexor response (Schmit and Benz 2002), the flexion amplitude or velocity during the orthotic gait was not sufficient to induce the TA activity. Further, the ankle joint was mechanically immobilized in the orthosis in our study, and the absence of ankle motion might have prevented the elicitation of TA activity. However, further training might induce modulation in TA EMG activity, based on the fact that we observed a reciprocal EMG activity pattern between the ankle extensor and flexor muscles during the orthotic gait in a well-trained SCI subject (Kojima et al. 1998). Further studies are needed to clarify this issue.

References

Dietz V, Colombo G, Jensen L (1994) Locomotor activity in spinal man. Lancet 344:1260–1263

Dietz V, Colombo G, Jensen L, Baumgartner L (1995) Locomotor capacity of spinal cord in paraplegic patients. Ann Neurol 37:574–582

Dietz V, Muller R, Colombo G (2002) Locomotor activity in spinal man: significance of afferent input from joint and load receptors. Brain 125:2626–2634

Dobkin BH, Harkema SJ, Requejo PS, Edgerton R (1995) Modulation of locomotor-like EMG activity in subjects with complete and incomplete spinal cord injury. J Neurol Rehabil 183–190