

# Functional reorganization of soleus H-reflex modulation during stepping after robotic-assisted step training in people with complete and incomplete spinal cord injury

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**Abstract** Body weight–supported (BWS) robotic-assisted step training on a motorized treadmill is utilized with the aim to improve walking ability in people after damage to the spinal cord. However, the potential for reorganization of the injured human spinal neuronal circuitry with this intervention is not known. The objectives of this study were to determine changes in the soleus H-reflex modulation pattern and activation profiles of leg muscles during stepping after BWS robotic-assisted step training in people with chronic spinal cord injury (SCI). Fourteen people who had chronic clinically complete, motor complete, and motor incomplete SCI received an average of 45 training sessions, 5 days per week, 1 h per day. The soleus H-reflex was evoked and recorded via conventional methods at similar BWS levels and treadmill speeds before and after training. After BWS robotic-assisted step training, the soleus

H-reflex was depressed at late stance, stance-to-swing transition, and swing phase initiation, allowing a smooth transition from stance to swing. The soleus H-reflex remained depressed at early and mid-swing phases of the step cycle promoting a reciprocal activation of ankle flexors and extensors. The spinal reflex circuitry reorganization was, however, more complex, with the soleus H-reflex from the right leg being modulated either in a similar or in an opposite manner to that observed in the left leg at a given phase of the step cycle after training. Last, BWS robotic-assisted step training changed the amplitude and onset of muscle activity during stepping, decreased the step duration, and improved the gait speed. BWS robotic-assisted step training reorganized spinal locomotor neuronal networks promoting a functional amplitude modulation of the soleus H-reflex and thus step progression. These findings support that spinal neuronal networks of persons with clinically complete, motor complete, or motor incomplete SCI have the potential to undergo an endogenous-mediated reorganization, and improve spinal reflex function and walking function with BWS robotic-assisted step training.

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## Introduction

Spinal cord injuries (SCI) cause substantial social, economic, and health burdens. Most spinal lesions significantly impair ambulation in young and otherwise healthy individuals, considerably affecting quality of life. In addition to classical rehabilitation strategies, body weight–supported

(BWS) step training with manual facilitation of leg movements and BWS robotic-assisted stepping on a motorized treadmill are utilized to promote recovery of walking ability after damage to the spinal cord (Barbeau et al. 1987; Colombo et al. 2001). These interventions were largely developed based on the theses that the spinal cord of vertebrates contains networks of neurons—which in the absence of inputs from the brain—can produce coordinated efferent activity similar to that observed in actual locomotion (Grillner 1981), and on the capability of the nervous system to utilize reorganizational mechanisms to change following a lesion and after training (Knikou 2010a). In this respect, cats with complete or incomplete spinal transection could learn to step at fast speeds when BWS was provided, while step training improved residual locomotor capacity beyond spontaneous recovery (Lovely et al. 1986; Barbeau and Rossignol 1987; De Leon et al. 1998; Courtine et al. 2009). These changes are likely the result of reorganizational neuronal mechanisms including axonal sprouting, unmasking of previously inactive synapses, and formation of new synapses (Nudo et al. 2001; Maier and Schwab 2006; Goldshmit et al. 2008).

A number of studies have shown that locomotor training with BWS on a motorized treadmill improves step symmetry, limb coordination, walking speed, and walking distance and increases the amplitude of muscle activity in the ankle extensors during the stance phase of walking in people with SCI (Wernig et al. 1995; Dietz et al. 1998; Behrman and Harkema 2000; Field-Fote 2001; Behrman et al. 2005; Wirz et al. 2005; Dobkin et al. 2006, 2007; Field-Fote and Roach 2011). Although reorganization of brain networks following training has already been observed in humans with a SCI (Dobkin 2000; Thomas and Gorassini 2005; Hajela et al. 2013), non-human primates (Aizawa et al. 1991; Nudo et al. 1996, 1997; Xerri et al. 1998), and rats (Donoghue and Sanes 1988; Jones and Schallert 1992), the potential of spinal neuronal networks to reorganize after BWS robotic-assisted step training in people with SCI remains unexplored.

Spinal reflexes have the capacity to affect locomotion by acting directly either on the final common pathway of motor behavior (alpha motoneurons) or on different modules of the spinal pattern generator (Rossignol et al. 2006; Knikou 2010a). In people with a SCI, the soleus H-reflex during stepping varies significantly from a physiological pattern to complete lack of modulation (Yang et al. 1991; Knikou et al. 2009a). The impaired function of spinal reflexes during walking in people with a SCI demonstrates clearly the altered physiological state of the nervous system post-injury (Tansey et al. 2012). In this study, I hypothesized that BWS robotic-assisted step training normalizes the impaired function of the soleus H-reflex during stepping in people with a complete and

incomplete SCI. This hypothesis was tested by establishing the modulation pattern of the soleus H-reflex during assisted stepping before and after BWS robotic-assisted step training in people who had clinically complete and incomplete SCI. People with motor complete SCI participated in the study in order to establish whether functional reorganization of the soleus H-reflex after training could be demonstrated in the absence of voluntary muscle activation.

## Methods

### Participants

Fourteen people with chronic SCI were enrolled in the study. Participant enrollment commenced September 2009, and the final participant completed training in September 2012. Study participation varied depending on the number of training sessions attended (Table 1) and ranged from 1.5 to 3.5 months. One participant had neurological deficit grade A (no sensory or motor function preserved below the lesion) on the American Spinal Injury Association (ASIA) Impairment Scale (AIS) (Marino et al. 2003), one had AIS B (sensory but not motor function preserved below the lesion), four had AIS C (more than half muscles below the lesion had muscle grade <three), and eight had AIS D (at least half key muscles below the lesion had muscle grade  $\geq$ three) (Table 1). The vertebral injury level of SCI ranged from Cervical 5 to Thoracic 10. The motor and sensory (light touch and pin prick) scores for each participant, assessed based on the ASIA guidelines, are indicated in Table 1. All participants signed an informed consent form for neurophysiological tests, clinical evaluation, and locomotor training, which was approved by the Northwestern University (IL, USA) and the City University of New York (NY, USA) institutional review boards. The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

### Electromyography (EMG)

During the experimental sessions on the treadmill, electromyography (EMG), foot switch, and amplitude of body unloading were recorded at 2,000 Hz with custom-written software (National Instruments, Austin, TX). Bilateral EMG activity was recorded via bipolar differential surface electrodes of fixed inter-electrode distance (Motion Lab systems Inc., Baton Rouge, LA, USA) from the vastus lateralis (VL), gracilis (GRC), lateral hamstrings (LH), medial hamstrings (MH), tibialis anterior (TA), peroneus longus (PL), medial gastrocnemius (MG), and soleus (SOL) muscles.

**Table 1** Demographics of participants

ID	Gender	Age (years)	Post-injury (years)	Level	Cause of SCI	AIS scale	Clonus	ASIA (light touch)	ASIA (pin prick)	ASIA (motor)	Medication	# of BWSRST sessions
R06	F	46	1.5	C5-C7	MVA	B	LL 3 RL 3	77	77	LL 11 RL 9	Baclofen 10 mg, not frequent	53
R07	M	31	8	C5-C7	MVA	A	3 3	76	40	23 23	None	53
R08	F	49	4	T5-T7	Fall	D	1 1	75	75	47 39	None during the study	60
R09	M	44	3	C5-C6	Fall	D	0 0	112	112	40 47	Gabapentin 3,600 mg Diazepam 15 mg	30
R10	F	52	11	T7	Fall	D	1 0	78	78	41 49	Neurontin 27 mg Baclofen 60 mg	65
R11	M	39	6	C4	GSW	D	3 3	106	106	42 35	Coumadin	64
R12	M	41	1.5	C5-C6	MVA	D	3 3	54	54	36 27	None during the study	55
R13	F	39	7	T4	Transverse Myelitis	C	3 3	112	74	34 27	Gabapentin 300 mg Baclofen 20 mg	53
R14	M	25	0.5	C5-C6	Diving	D	1 1	112	110	49 29	None during the study	44
R16	M	49	2.5	C5	MVA	C	0 0	64	34	30 24	Baclofen 15 mg	41
R17	M	21	3	T10	GSW	D	3 3	105	105	38 40	Baclofen 60 mg Gabapentin 50,900 mg	48
R18	M	29	2	C7	MVA	D	1 1	86	86	50 45	None	26
R19	M	26	1	C6	Diving	C	3 3	112	97	34 21	Dexapam 10 mg	20
R20	M	55	3	T6-T7	Blood clot during spinal surgery	C	2 3	82	82	33 34	None	21

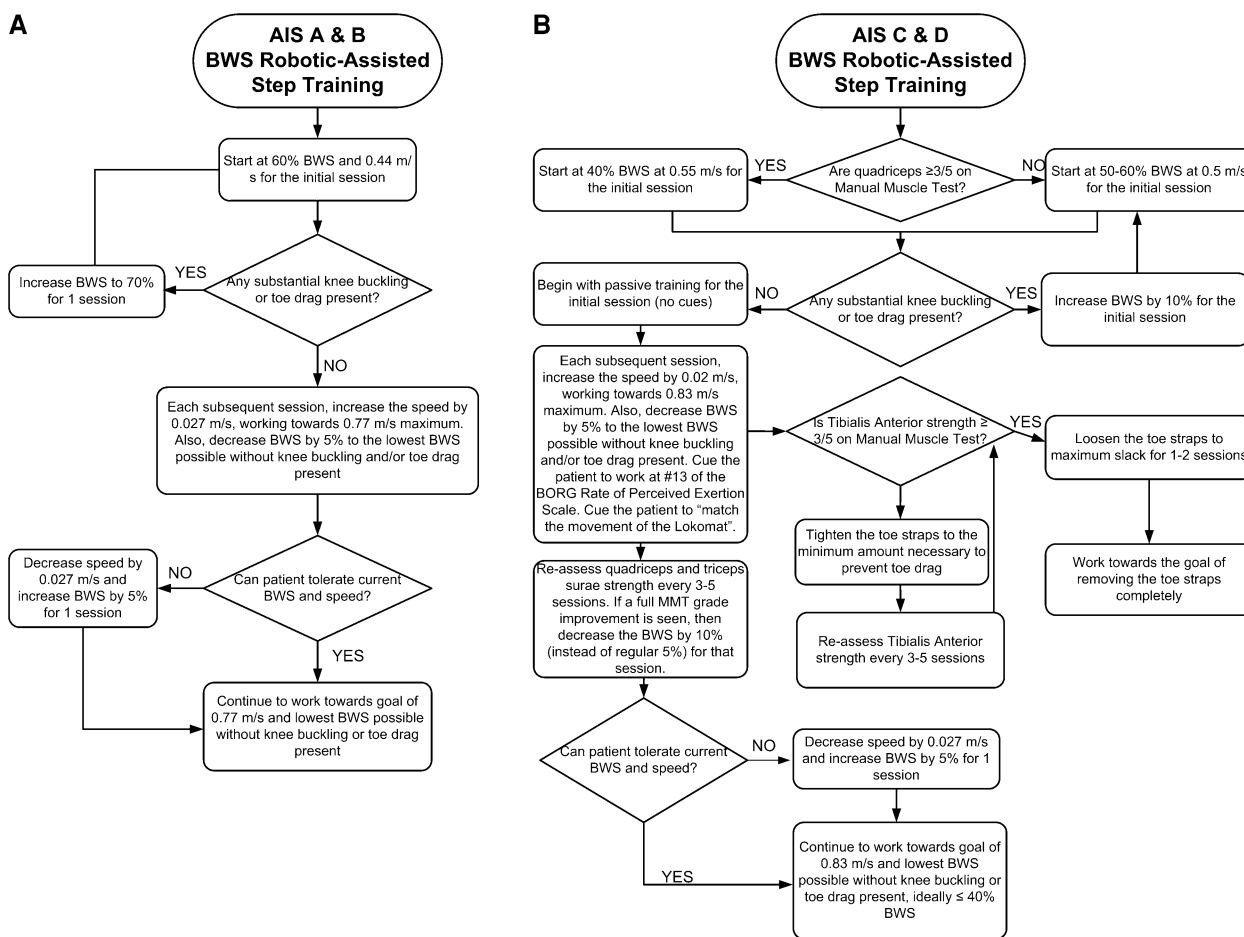
Level of SCI corresponds to vertebral injury level. For each subject, the American Spinal Injury Association (ASIA) standard neurological classification of SCI for sensation (sensory light touch and pin prick; out of 112 maximal points) is shown and evaluated as 0 = absent, 1 = impaired, 2 = normal, *NT* not testable. ASIA motor score (out of 50 maximal points for each leg) is indicated for the left leg (LL) and right leg (RL) based on the manual muscle test of key muscles and evaluated as 0 = No contraction, 1 = Flicker or trace of contraction, 2 = Active movement with gravity eliminated, 3 = Active movement against gravity, 4 = Active movement against resistance, 5 = Normal power. Values for ASIA sensory and motor scores are indicated from clinical evaluation tests conducted before training. After training, these scores did not change and thus are not presented. The ankle clonus for both legs leg is also indicated. Ankle clonus was assessed as follows: 0 = reaction, 1 = mild, clonus was maintained less than 3 s, 2 = moderate, clonus persisted between 3 and 10 s, 3 = severe, clonus persisted for more than 10 s. Medication for each subject is indicated as total mg taken per day

C cervical, T thoracic, MVA motor vehicle accident, GSW gunshot wound, M male, F female, LL left leg, RL right leg, BWSRST body weight-supported robotic-assisted step training

BWS robotic-assisted step training

All participants received BWS robotic-assisted step training with a robotic exoskeleton system (Lokomat Pro®, Hocoma, Switzerland). Each participant was trained 1 h per day, 5 days per week. The mean number of training sessions completed was  $45.21 \pm 15.41$  (mean  $\pm$  SD; range 20–65) (Table 1). In Fig. 1, the protocol employed to train AIS A–B (Fig. 1a) and AIS C–D (Fig. 1b) participants is indicated with respect to the factors that determined decrease in BWS, increase in treadmill speed, and position of toe straps of the ankle braces over the course of training. In AIS A–B subjects, BWS robotic-assisted step training started at 60 % BWS and at 0.44 m/s at the first session. These parameters were adjusted based on the presence of knee buckling or toe drag (Fig. 1a). At each subsequent session, the speed was increased by 0.02 m/s and BWS was decreased by 5 %. In AIS C–D subjects,

if quadriceps manual muscle test score was  $\geq 3/5$ , training started at 40 % BWS at 0.55 m/s. The treadmill speed and BWS were adjusted by 0.02 m/s and 5 % at each subsequent training session, respectively (Fig. 1b). When quadriceps and triceps surae strength was increased by a full grade, then the BWS was decreased by 10 %. Based on the TA muscle strength, the position of the straps of the ankle braces (loosen or tighten) was determined. The TA muscle strength was assessed every 3–5 training sessions. The ultimate training goal in AIS C–D subjects was to reach a treadmill speed of 0.83 m/s at the lowest BWS possible without knee buckling or toe dragging during the stance and swing phases, respectively (Fig. 1b). For each subject, the changes in the BWS and treadmill speed every 5 training sessions are indicated in Table 2. All subjects during the duration of the study did not receive conventional physical therapy and did not participate in any other clinical research study or clinical trial.



**Fig. 1** Diagram indicates the protocol utilized to train AIS A–B (a) and AIS C–D (b) subjects. The training protocol is indicated with respect to the factors that determined decrease in body weight support

(BWS), increase in treadmill speed, and position of toe straps of the ankle braces over the course of BWS robotic-assisted step training

**Table 2** Adjustment of BWS and treadmill speed over the course of training

	R06		R07		R08		R09		R010		R011		R012		R013		R014		R016		R017		R018		R019		R020			
	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed	BWS	Speed		
Day 5	50	0.54	50	0.58	33	0.61	42.5	0.61	25	0.66	25	0.66	27.5	0.65	55	0.44	30	0.69	30	0.69	30	0.61	15	0.55	10	0.80	25	0.52	50	0.61
Day 10	50	0.65	40	0.63	30	0.69	25	0.80	20	0.77	25	0.75	30	0.66	50	0.45	25	0.66	30	0.66	30	0.61	15	0.61	10	0.86	10	0.63	45	0.63
Day 15	50	0.69	40	0.69	30	0.69	22.5	0.88	18	0.83	25	0.80	25	0.69	50	0.50	25	0.69	25	0.69	25	0.61	15	0.61	10	0.88	10	0.69	40	0.69
Day 20	50	0.77	45	0.76	31	0.75	18	0.88	18	0.86	20	0.83	25	0.63	40	0.50	20	0.69	15	0.63	10	0.63	10	0.63	5	0.88	10	0.72	35	0.72
Day 25	54	0.80	45	0.80	25	0.77	17	0.88	18	0.88	19	0.88	20	0.69	40	0.50	20	0.72	10	0.69	10	0.69	10	0.63	5	0.88	5	0.88		
Day 30	50	0.83	40	0.83	25	0.83	15	0.88	15	0.88	19	0.88	17	0.69	40	0.52	18	0.72	10	0.72	10	0.72	10	0.69	10	0.88				
Day 35	45	0.83	43	0.86	35	0.66			15	0.88	20	0.88	17	0.69	40	0.44	10	0.72	10	0.69	10	0.69	10	0.69	10	0.88				
Day 40	35	0.83	35	0.88	27.5	0.75			15	0.88	20	0.88	10	0.69	40	0.47	10	0.72	10	0.72	10	0.72	10	0.72	10	0.88				
Day 45	35	0.88	35	0.88	24	0.80			20	0.88	25	0.88	10	0.69	35	0.47	5	0.72												
Day 50	35	0.88	35	0.88	22	0.83			20	0.88	20	0.88	10	0.69	35	0.47														
Day 55					19	0.86			15	0.88	30	0.88	5	0.69																

For each subject, the BWS (%) and speed (m/s) over the course of body weight–supported (BWS) robotic-assisted step training is indicated. BWS and speed are indicated for every 5 sessions. The Lokomat’s maximal treadmill speed is 0.88 m/s

Neurophysiological recordings before and after training

The modulation pattern of the soleus H-reflex during BWS robotic-assisted stepping was characterized in all subjects for the right leg except in subject R10<sup>1</sup> and in 10 subjects for the left leg before and after training. H-reflexes were recorded in both limbs in order to establish whether spinal reflex function is reorganized differently when the residual muscle strength of the left and right legs is not similar (see ASIA motor scores in Table 1).

Square pulse stimuli of 1-ms duration were delivered by a custom-built constant current stimulator to the posterior tibial nerve. With the subject seated, a stainless steel plate electrode of 4 cm in diameter was placed and secured proximal to the patella. The most optimal stimulation site for the posterior tibial nerve was established via a hand-held monopolar stainless steel head electrode used as a probe (Knikou 2008). Optimal stimulation site corresponded to the site that the M-wave had a similar shape to that of the H-reflex at low and high stimulation intensities, and at the lowest stimulus intensity, an H-reflex could be evoked without an M-wave. When the optimal site was identified, the monopolar electrode was replaced by a pregelled disposable electrode (SuperTrace, Conmed, Utica, NY) that was maintained under constant pressure via an athletic wrap. Then, the subject was transferred to the treadmill and wore an upper body harness that was connected to overhead pulleys. Thigh and shank segments of the robotic device were adjusted based on each subject’s leg length and diameter, and both feet were secured into the foot lifters. The BWS required during stepping for experimental sessions conducted before training was established prior to soleus H-reflex recordings based on the diagram of Fig. 1. With the subject standing at a BWS similar to that required during stepping, the soleus H-reflex and M-wave recruitment curves were constructed. Approximately 80 stimuli in total were delivered at 0.2 Hz to construct the soleus H-reflex and M-wave recruitment curves with the subjects standing (Knikou et al. 2009b).

During walking, the orientation of the recording EMG electrode may vary with respect to the underlying fibers, while the knee joint during the swing phase moves the stimulating electrode away from the tibial nerve, knee extension during the stance phase has the opposite effect. Thus, appropriate adjustments of the stimulation intensity are required across the phases of the step cycle. Last, one of the most important factors in H-reflex studies is the amplitude of the M-wave and H-reflex as a percentage of

<sup>1</sup> In R10 subject, the soleus H-reflex modulation from the right leg was not assessed during stepping due to a pronounced contracture of the ankle joint, which might have masked the soleus H-reflex function.

the maximal M-wave (Knikou 2008). The aforementioned limitations were counteracted by using a custom-built constant current stimulator in which the stimulus intensity was electronically controlled and adjusted, a task that cannot be done with commercially available stimulators when the resolution of the step cycle is large (Knikou personal observations). Further, a supramaximal stimulus to the posterior tibial nerve was delivered 60–80 ms after the test H-reflex (Dyhre-Poulsen and Simonsen 2002; Knikou et al. 2009a, 2011; Knikou and Mummidisetty 2011). The customized Labview software measured the peak-to-peak amplitude of the M-wave and maximal M-wave during stepping and used a self-teaching algorithm to adjust the stimulus intensity at each bin of the step cycle. Adjustment of stimulation intensity was based on the amplitude of the M-wave as a percentage of the maximal M-wave (and with respect to stimulation intensities evoking H-reflexes on the ascending limb of the recruitment curve) which was set to range between 2 and 12 % of the maximal M-wave (Knikou 2008; Knikou et al. 2009a).

Stimulation of the tibial nerve was triggered based on the signal from the ipsilateral foot switch (MA153, Motion Lab Systems Inc., Baton Rouge, LA, USA). In all subjects, stimuli were delivered randomly across the different phases of the step cycle, which was divided into 16 equal bins. Bin 1 corresponds to heel contact. Bins 8, 9, and 16 correspond approximately to stance-to-swing transition, swing phase initiation, and swing-to-stance transition, respectively. Soleus H-reflexes were evoked once every 2–3 steps. For each subject, the soleus H-reflexes during BWS robotic-assisted stepping were recorded 2 days after training was completed. Soleus H-reflex recordings on different days in the same subjects did not affect the results of this study because the soleus H-reflex modulation is reproducible when recorded on different days (Knikou personal unpublished observations; Simonsen and Dyhre-Poulsen 2011). Last, H-reflex recordings post-training were conducted at similar BWS, treadmill speed, and M-wave amplitudes to those utilized before training, while an experimental session was concluded when at least 15 accepted H-reflexes (based on the factors previously described) were recorded at each bin of the step cycle.

#### Clinical evaluation tests conducted before and after training

Before and after training, the walking index for SCI (WISCI II) on a scale of 0–20 with most severe impairment at 0 and least severe impairment at 20 was assessed (Dittuno and Dittuno 2000). Functional walking capacity was assessed with the 6-min walk test (6MWT) (Scivoletto et al. 2011), while the number of sit-to-stand repetitions completed within 30 s, and the time needed (in s) to rise

from a chair, walk for 3 m, and return to the chair (TUGT time up and go test) were also evaluated. The frequency of spasms (0–16) was assessed as the number of spasms the participants reported to have the previous day in all limbs.

#### Data analysis

The soleus H-reflex and M-wave evoked at each bin of the step cycle were measured as peak-to-peak amplitude and were normalized to the associated peak-to-peak amplitude of the maximal M-wave. For each subject, the mean amplitude of the accepted soleus H-reflexes (based on the M-wave amplitude as a percentage of the maximal M-wave) before training was compared to the accepted soleus H-reflexes recorded after training at each bin with a Wilcoxon signed rank sum test. The mean amplitude of the H-reflexes recorded before and after training was grouped based on the bin number and AIS, and statistically significant differences were established with a Kruskal–Wallis rank sum test when data were not normally distributed and with a repeated measures analysis of variance (ANOVA) when data were normally distributed based on the results of individual distribution identification. Bonferroni test for multiple comparisons was used when statistically significant differences were detected.

In order to establish changes in the soleus H-reflex gain with training, the background activity of the ipsilateral SOL muscle for each bin of the step was estimated from the mean value of the filtered and rectified EMG (band-pass filtered 20–400 Hz) for 60 ms beginning 120 ms before posterior tibial nerve stimulation. The mean amplitude of the soleus H-reflex was plotted on the *y*-axis (dependent variable) versus the SOL background EMG activity (independent variable) normalized to the maximal EMG during stepping on the *x*-axis, respectively. A linear least-square regression was then fitted to the data, with its slope reflecting the reflex gain (Ferris et al. 2001). This analysis was conducted separately for each subject (individual data are not presented) and for the pooled data from the left and right soleus H-reflex with the subjects grouped based on the AIS before and after training. Further, in order to establish to what extent functional changes of the soleus H-reflex amplitude during stepping coincided reciprocally with TA muscle activity, a linear relationship between the right and/or left TA background EMG activity and the right and/or left soleus H-reflex during stepping before and after training was also performed.

In order to establish changes in EMG amplitude and activation profiles of muscle activity with training, bilateral EMG from leg muscles was recorded while subjects stepped before and after training on multiple step sessions without stimulation. EMG signals during stepping were full-wave rectified, high-pass filtered at 20 Hz, and

low-pass filtered at 500 Hz. After full-wave rectification, linear EMG envelopes were obtained at 20 Hz low-pass filter. The mean EMG amplitude at each bin across all steps was determined, while the integrated EMG was defined as the area under the linear envelope. For each muscle, the linear EMG envelope at each bin across all steps recorded before training was normalized to the maximal amplitude of the associated linear EMG envelope recorded after training. Then, the normalized EMG envelope from each muscle was grouped across subjects based on the AIS and time of testing (pre-/post-training), and the overall amplitude was estimated.

The BWS, treadmill speed, leg guidance force by the robot, step duration, frequency of spasms, WISCI, 6MWT scores, TUGT scores, and number of sit-to-stand repetitions within 30 s were grouped based on the AIS and were compared before and after training with a paired *t* test, while the percentage of change was also estimated. For all statistical tests, significance was set at  $P < 0.05$ . Results are presented as mean values along with the standard error of the mean (SEM), unless otherwise stated.

## Results

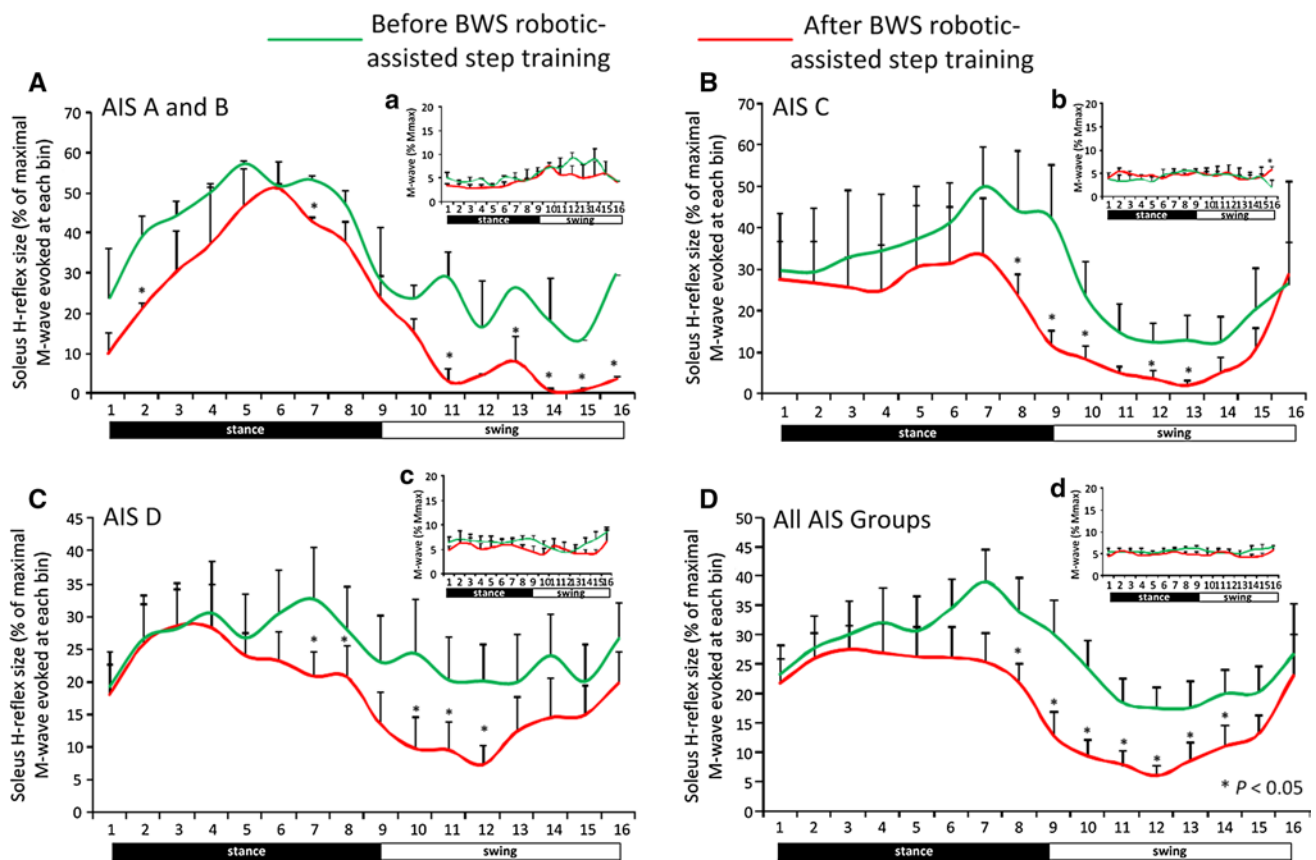
### Reorganization of soleus H-reflex modulation after BWS robotic-assisted step training

Body weight-supported (BWS) robotic-assisted step training reorganized the soleus H-reflex in a functional manner during assisted stepping in people with clinically complete, motor complete and motor incomplete SCI. The soleus H-reflex from the right leg in AIS A and AIS B subjects was decreased just after heel contact (bin 2), at late stance (bin 7), at mid- and late swing phase (bins 13, 14, 15), and at swing-to-stance transition phase (bin 16) after training when compared to the H-reflexes recorded before training ( $P < 0.05$ ; Wilcoxon rank sum test at each bin pre/post) (Fig. 2a). In AIS C subjects, the right soleus H-reflex was decreased at late stance (bin 8), swing phase initiation (bin 9), and during the early and mid-swing phases (bins 10–13) after training when compared to the H-reflexes recorded before training ( $P < 0.05$ ) (Fig. 2b). The soleus H-reflex depression after training in AIS D subjects was noted at similar bins to that observed in AIS C subjects, but the reflex depression occurred earlier, that is from late stance ( $P < 0.05$ ) (Fig. 2c). A repeated measures ANOVA at  $2 \times 16$  levels (2 pre–post-time; 16 bins of step cycle) conducted separately for AIS C and AIS D subjects showed that the difference in mean H-reflex values among the different levels of bins and time were significantly different ( $F_{1,15} = 6.2$ ;  $P < 0.001$ ). The amplitude of the right soleus H-reflex before and after training from all 14 subjects

tested is indicated in Fig. 2d. Overall, the soleus H-reflex was decreased at stance-to-swing transition (bin 8), swing phase initiation (bin 9), and throughout the swing phase (bins 10–14) ( $P < 0.05$ ; Wilcoxon rank sum test at each bin pre/post; Fig. 4d). A three-way repeated measures ANOVA, in which the AIS, number of bins, and time of testing served as sources of variation, showed that the difference in the mean H-reflex sizes among the different levels of bins and time were significantly different ( $P < 0.001$ ), but not across AISs ( $P = 0.14$ ). The changes in H-reflex function were observed at similar amplitudes of M-waves before and after training in all subjects (see a–c in Fig. 2).

In one AIS B subject (R06), two AIS C subjects (R13, R19), and 6 AIS D subjects (R08, R09, R11, R12, R14, and R17), the reorganization of the soleus H-reflex function during stepping after training was recorded also from the left leg. In AIS B subject, the soleus H-reflex after training was decreased at mid-stance (bins 4, 5), late stance (bin 7), just after swing initiation (bin 10), and at late swing (bins 14, 15) when compared to the soleus H-reflexes recorded before training ( $P < 0.05$ ; Wilcoxon rank sum test at each bin pre/post) (Fig. 3a). In AIS C subjects, the soleus H-reflex was decreased at mid-swing phase (bins 10, 11, 12), when compared to the soleus H-reflexes recorded before training ( $P < 0.05$ ; Wilcoxon rank sum test at each bin) (Fig. 3b). In AIS D subjects, the soleus H-reflex was decreased at mid-swing (bin 11) but also during the swing-to-stance transition (bin 16) when compared to the soleus H-reflexes recorded before training ( $P < 0.05$ ; Wilcoxon *t* test at each bin) (Fig. 3c). An ANOVA for repeated measures at  $2 \times 16$  levels (2 pre/post-time; 16 bins of step cycle) on the soleus H-reflex from AIS C subjects showed that the difference in the mean H-reflexes was significantly different before and after training ( $F_{1,15} = 10.34$ ;  $P = 0.003$ ), a result found also for AIS D subjects ( $F_{1,15} = 7.95$ ;  $P < 0.001$ ). The amplitude of the left soleus H-reflex before and after training from all subjects tested is indicated in Fig. 3d. The soleus H-reflex was decreased at mid- and at late swing phases (Fig. 3d). The changes of the soleus H-reflex function were observed at similar amplitudes of M-waves before and after training in all subjects (see a–d in Fig. 3).

In Table 3, the changes observed in the right and left soleus H-reflex modulation after BWS robotic-assisted step training are summarized with respect to the bin number of the step cycle with the subjects grouped based on the AIS. It is apparent that the soleus H-reflex was depressed at stance-to-swing transition and swing phase initiation after training, allowing a smooth transition from stance to swing. Further, the soleus H-reflex was depressed at mid-swing phase, promoting reciprocal activation between ankle flexors and extensors, similar to that observed in healthy control subjects.



**Fig. 2** Changes in soleus H-reflex modulation after BWS robotic-assisted step training (right leg). The mean soleus H-reflex amplitude recorded from the right leg of AIS A and B (a), AIS C (b), and AIS D (c), and all AIS groups (d) before (gray lines) and after (black lines) BWS robotic-assisted step training is shown as a percentage of the associated maximal M-wave elicited after the test H-reflex at each bin of the step cycle during stepping. For each AIS group as well as for all AIS groups, the corresponding M-wave amplitudes recorded

before and after locomotor training are also shown (a, b, c, and d). Asterisks indicate significant differences for H-reflexes recorded before and after training. The step cycle was divided into 16 equal bins. 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. Error bars represent the SEM

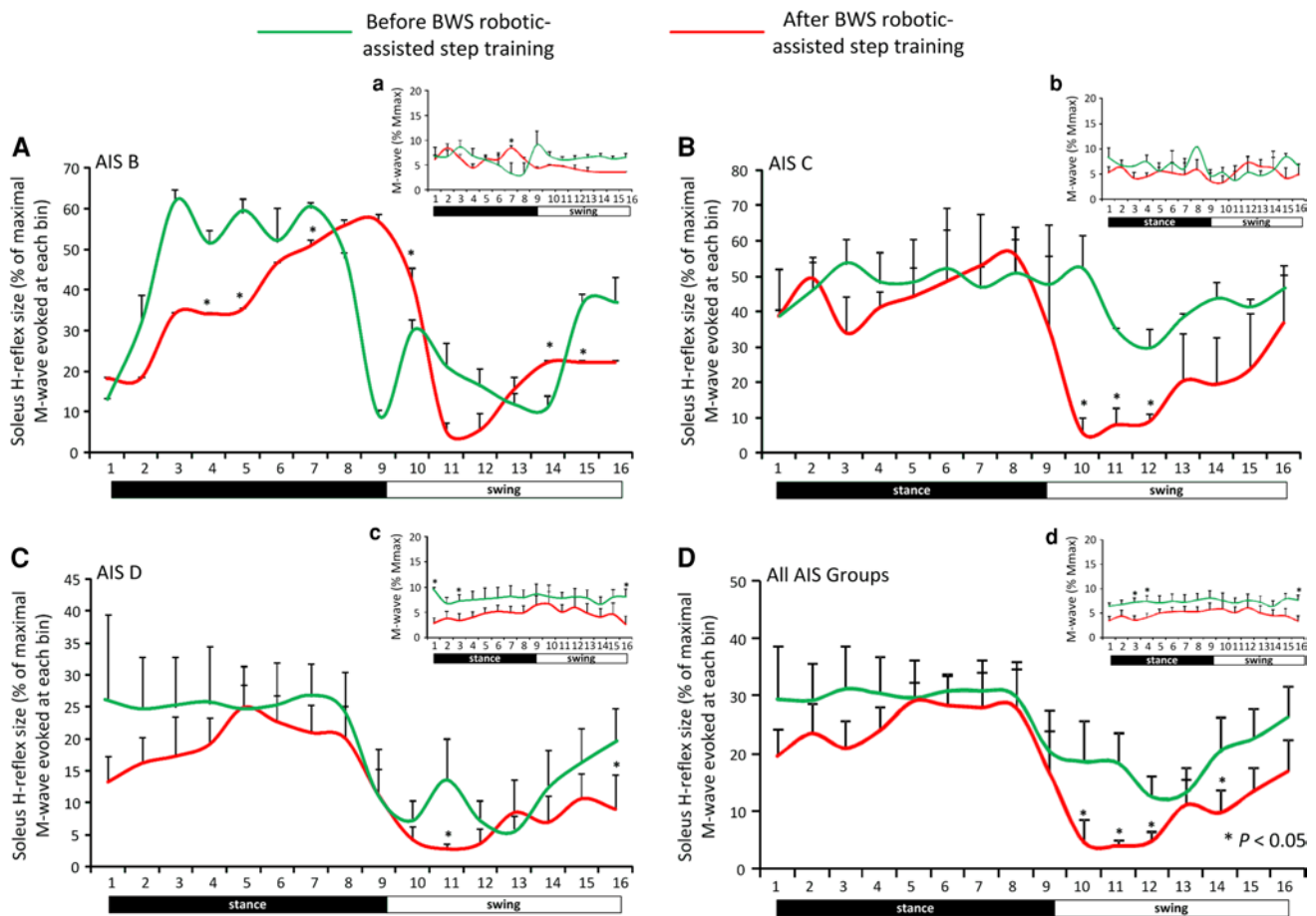
### Reorganization of spinal reflex circuitry

Based on these findings, reorganization of reflex function during stepping emerges as potentiation of soleus H-reflex depression recorded from the right leg before and after training in the same subject. In Table 4, the changes of the left soleus H-reflex are shown against the changes observed on the right soleus H-reflex for each subject. A downward ( $\Downarrow$ ) arrow indicates depression of the H-reflex, while an upward ( $\Uparrow$ ) arrow indicates facilitation. The depression and/or facilitation of the H-reflex is shown for H-reflexes recorded after training when compared to those recorded before training based on the results of Wilcoxon rank sum test conducted at each bin for each subject separately. In summary, the function

of the soleus H-reflex during stepping was reorganized in an homologous pattern (i.e., the soleus H-reflex changed in the same direction in the left and right legs after training) in subjects R06, R11, R12, R13, and R14 (gray rows) and in an heterologous pattern (i.e., the soleus H-reflex changed in the opposite direction in the left and right legs after training) in subjects R08, R17, and R19 (white rows) (Table 4).

Representative examples of the soleus H-reflex recorded from both limbs before and after training are indicated in Fig. 4. After training in subject R13, the soleus H-reflex from the left leg was decreased at swing phase initiation (bin 9), throughout the swing phase, and at swing-to-stance transition (bin 16) ( $P < 0.05$ ; Wilcoxon rank sum test at each bin) (Fig. 4a), while the soleus H-reflex from the right leg was decreased at all bins of the step cycle ( $P < 0.05$ ; Fig. 4b). In subject R17, the soleus H-reflex from the left leg was increased just after heel contact, at mid- and late stance (bins 5–8), early swing, and swing to-stance transition





**Fig. 3** Changes in soleus H-reflex modulation after BWS robotic-assisted step training (left leg). The mean soleus H-reflex amplitude recorded from the left side of AIS B (a), AIS C (b), AIS D (c), and all AIS groups (d) before (*gray lines*) and after (*black lines*) BWS robotic-assisted step training is shown as a percentage of the associated maximal M-wave elicited after the test H-reflex during stepping. For each AIS group as well as for all AIS groups, the corresponding

(bin 16) ( $P < 0.05$ ; Fig. 4c), while the soleus H-reflex from the right leg was decreased in all phases of the step cycle ( $P < 0.05$ ) (Fig. 4d). A heterologous reorganization pattern was also observed in subject R09 (Figs. 4e, f).

#### Relationship between soleus H-reflex and soleus background activity before and after training

BWS robotic-assisted step training re-established a linear relationship between the right SOL background EMG activity and the right soleus H-reflex during stepping in AIS A–B subjects ( $R^2 = 0.66$ ;  $P < 0.001$ ) (Fig. 5a). In AIS C subjects, a linear relationship between the right SOL background EMG activity and the right soleus H-reflex was found for recordings conducted before and after training (Fig. 5a,  $R^2 = 0.64$ ,  $P < 0.001$  and  $R^2 = 0.68$ ;  $P < 0.001$ ), while the slope was significantly decreased after training

M-wave amplitudes recorded before and after locomotor training are also shown (a, b, c, and d). Asterisks indicate statistically significant changes for H-reflexes recorded before and after locomotor training. The step cycle was divided into 16 equal bins. 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. Error bars denote the SEM

compared to that observed before training. In AIS D subjects, although the slope was decreased after training, a linear relationship between the SOL background EMG activity and the soleus H-reflex after training was not found ( $R^2 = 0.02$ ;  $P = 0.56$ ). Similarly, the soleus H-reflex from the left leg was linearly related to the left SOL background EMG activity in AIS C ( $R^2 = 0.65$ ;  $P < 0.001$ ) and AIS D ( $R^2 = 0.61$ ;  $P < 0.001$ ) subjects after training (Fig. 5b). Note that in AIS D subjects, the slope was significantly increased in the left leg after training compared to that observed in the right leg (compare bottom graphs in Fig. 5).

#### Relationship between soleus H-reflex and TA background activity before and after training

In order to establish to what extent the functional changes of the soleus H-reflex during stepping coincided

**Table 3** Summary of neurophysiological changes after BWS robotic-assisted step training

Bins		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AIS A-B or AIS B	Left leg	≈	≈	≈	↓	↓	≈	↓	≈	≈	↑	≈	≈	≈	↑	↓	≈
	Right leg	≈	↓	≈	≈	≈	≈	↓	≈	≈	≈	↓	≈	↓	↓	↓	↓
AIS C	Left leg	≈	≈	≈	≈	≈	≈	≈	≈	≈	↓	↓	↓	≈	≈	≈	≈
	Right leg	≈	≈	≈	≈	≈	≈	≈	↓	↓	↓	≈	↓	↓	≈	≈	≈
AIS D	Left leg	≈	≈	≈	≈	≈	≈	≈	≈	≈	≈	↓	≈	≈	≈	≈	↓
	Right leg	≈	≈	≈	≈	≈	≈	↓	↓	≈	↓	↓	↓	≈	≈	≈	≈

Changes in the left and right soleus H-reflex modulation after BWS robotic-assisted step training are presented with the participants grouped based on the American Spinal Injury Association (ASIA) Impairment Scale (AIS) scale. Symbols (↓), (↑), and (≈) denote depression, facilitation, and no significant changes, respectively. Gray squares denote statistically significant differences ( $P < 0.05$ ) for H-reflexes recorded before and after training

**Table 4** Spinal reflex reorganization after BWS robotic-assisted step training

	Left Side Soleus H-reflex	Right Side Soleus H-reflex
R06	↓ (bin 9)	↓ (bins 2-5, 11, 12, 15, 16)
R08	↓ (bins 1-16)	↑ (bins 2-4, 8, 9, 11-16)
R09	↓ (bin 2, 3, 10, 12); ↑ (bins 5-8, 15)	↓ (bin 8); ↑ (bins)
R11	↓ (bins 13-15)	↓ (bins 8-14)
R12	↓ (bins 1-5, 10, 14-16)	↓ (bins 1-7, 9, 12, 14, 15)
R13	↓ (bins 9-16)	↓ (bins 1-16)
R14	↑ (bins 1-5); ↓ (bins 10-11, 16)	↑ (bins 1-5); ↓ (bins 10-12)
R17	↑ (bins 2, 5-9, 16); ↓ (bins 11-12)	↓ (bins 3, 5-15)
R19	↓ (bins 3-5, 10, 11, 12)	↑ (bins 4-7, 16); ↓ (bins 8-15)

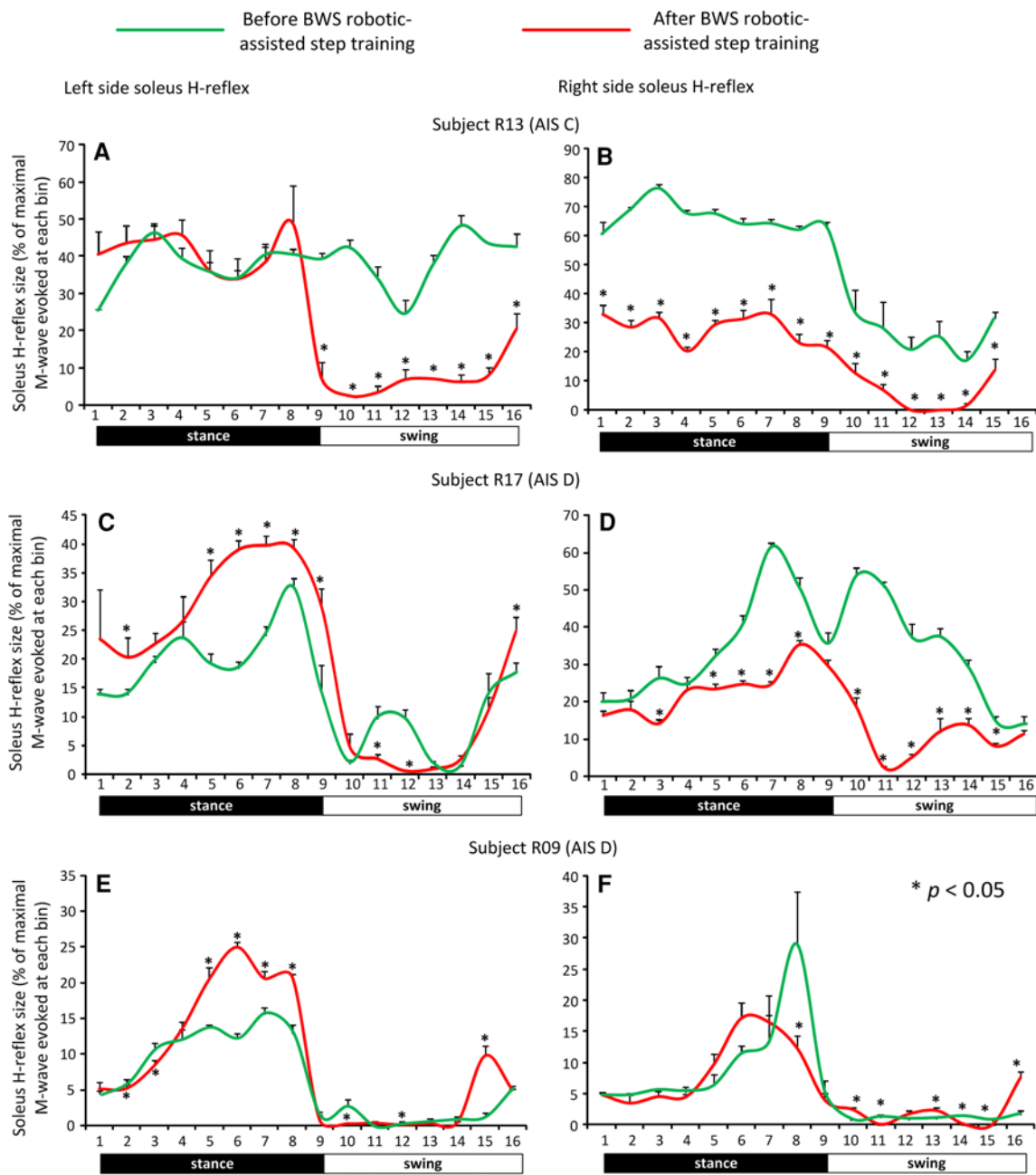
The changes in the left soleus H-reflex are shown against the changes observed on the right soleus H-reflex for each subject in whom reflexes were recorded from both sides before and after training. A downward (↓) arrow indicates depression of the H-reflex, while an upward (↑) arrow indicates facilitation. The depression and/or facilitation of the H-reflex is shown for H-reflexes recorded after training when compared to those recorded before training based on the results of Wilcoxon rank sum test conducted at each bin. The function of the soleus H-reflex during stepping was reorganized in an homologous manner in both limbs in subjects R06, R11, R12, R13, and R14 (gray rows) and in an heterologous manner in subjects R08, R17, and R19 (white rows)

reciprocally with the ongoing antagonistic TA motor neuron pool excitability, a linear relationship between the right and/or left TA background EMG activity and the

right and/or left soleus H-reflex during stepping was performed. In AIS A–B subjects, the right TA background EMG activity was not related negatively to the right soleus H-reflex after training ( $R^2 = 0.001$ ;  $P = 0.88$ ), a phenomenon observed also for the left leg in AIS B subject ( $R^2 = 0.11$ ;  $P = 0.51$ ), and for the left leg in AIS C subjects ( $R^2 = 0.01$ ;  $P = 0.04$ ) (Fig. 6). Potentiation of the negative linear relationship between the soleus H-reflex and TA background activity after BWS robotic-assisted step training was found in AIS D subjects in the left leg ( $R^2 = 0.8$ ;  $P < 0.001$ ). Although the slope for the right leg was decreased after training in AIS D subjects and a negative linear relationship was found ( $R^2 = 0.3$ ;  $P < 0.001$ ) (bottom graph in Fig. 6a), the negative linear relationship was not as strong as that observed in the left leg (compare bottom graphs in Fig. 6).

Changes in EMG activation profiles during stepping after training

The EMG activation profiles and amplitude of EMG activity from both sides grouped based on the AIS during stepping before and after BWS robotic-assisted step training are indicated in Fig. 7. The changes in lumbosacral motor neuron pool activity after training can be summarized as (1) increase in EMG amplitude, (2) decrease in EMG amplitude (left VL and right MH in AIS C), (3) re-establishment of biphasic (when a muscle contracts in more than one phase within a single step cycle) EMG activity (compare left and right MG in AIS A–B and AIS D, respectively), and (4) onset changes of EMG activity (right LH in AIS C and right VL in AIS A–B) (Fig. 7).



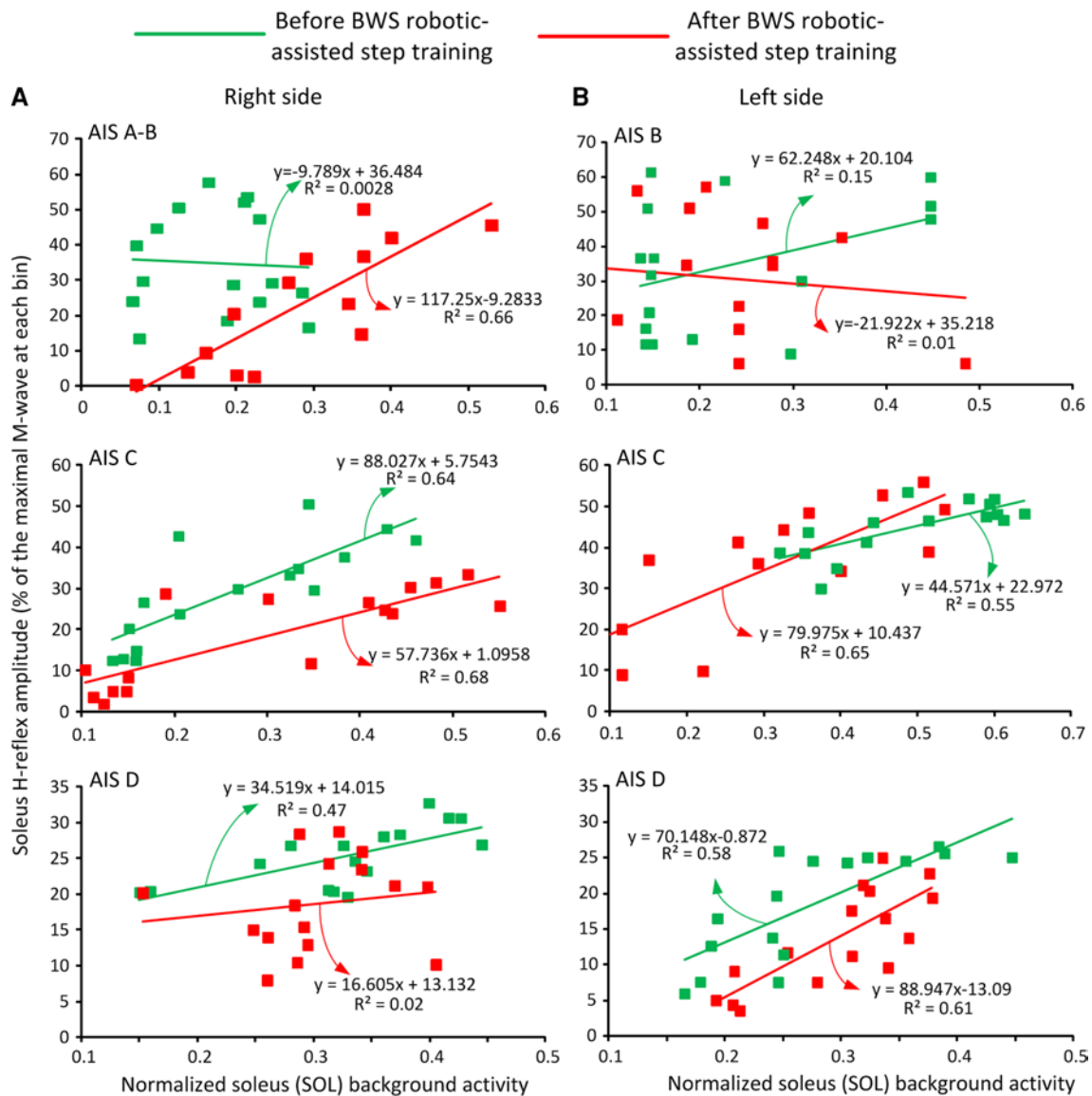
**Fig. 4** Bilateral soleus H-reflex reorganization after BWS robotic-assisted step training. The mean amplitude of the soleus H-reflex recorded from the left and right legs from subject R13 (a, b), R17 (c, d), and R09 (e, f) during assisted stepping before (gray lines) and after (black lines) training. In all graphs, the soleus H-reflexes were normalized to the maximal M-wave elicited after the test H-reflex

during stepping at the same bin of the step cycle. Asterisks indicate statistically significant differences between the H-reflexes recorded before and after training. The duration of the stance and swing phases of the step cycle is identified on the x-axis. Error bars denote the SEM

#### Improvements in walking after BWS robotic-assisted step training

In Table 5, the mean amplitude of gait parameters and the results of clinical tests before and after training are presented with the participants grouped based on the AIS. The

BWS required for stepping without knee buckling decreased significantly after training, with an overall percentage change of 50, 41, and 74.2 % for AIS A–B, AIS C, and AIS D subjects, respectively. The decreased BWS coincided with increased walking speed, decreased leg guidance force by the Lokomat, and decreased step duration of the right



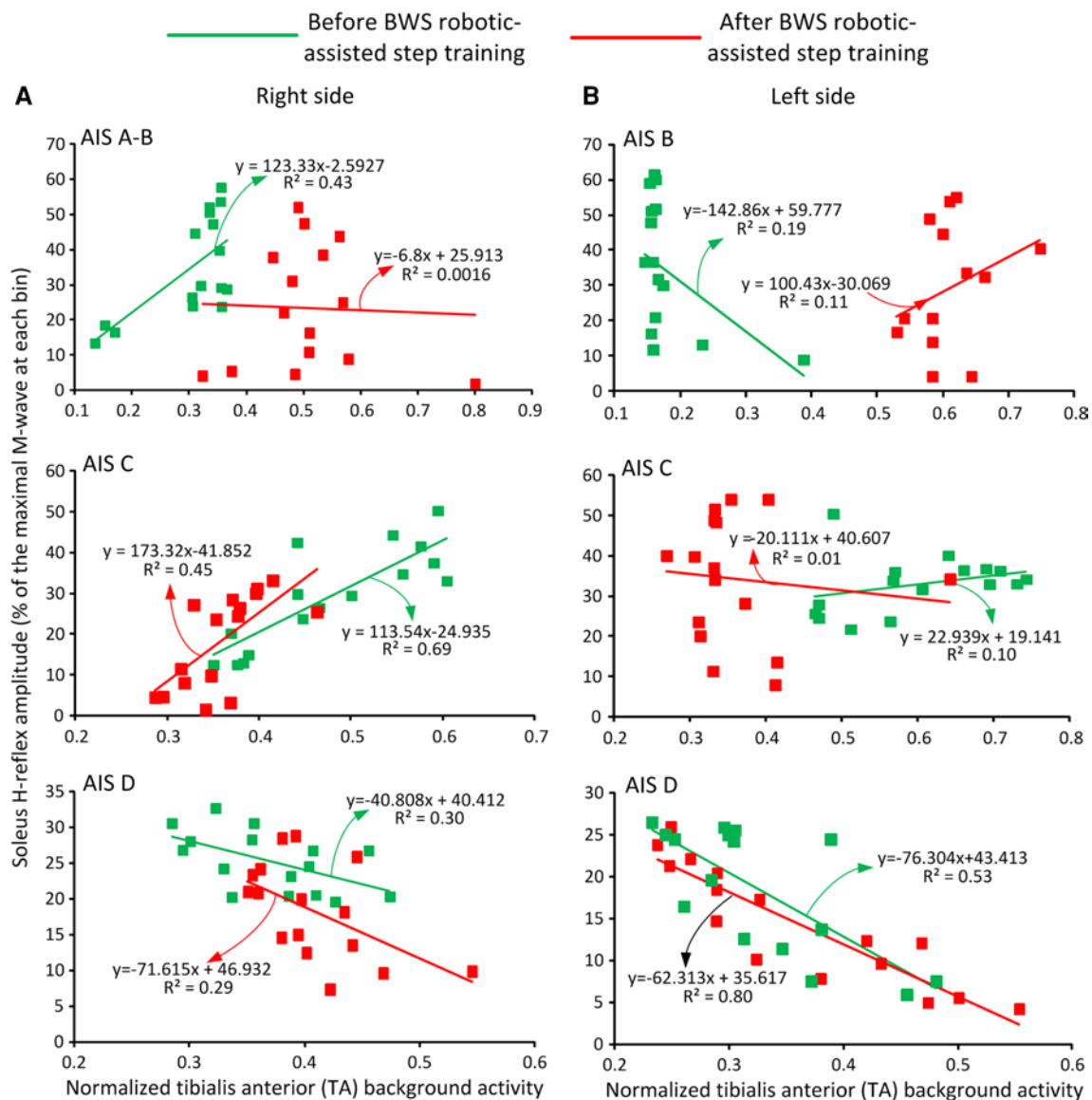
**Fig. 5** Relationship between soleus H-reflex and soleus background EMG activity before and after BWS robotic-assisted step training. The mean amplitude of the soleus H-reflex recorded at each bin before (gray filled squares) and after (black filled squares) training

leg in all subject groups (paired *t* test,  $P < 0.001$ ; Table 5), suggesting improvements in their ability to step. No significant differences were found on the overground walking ability after training, assessed by the 6MWT (paired *t* test,  $P = 0.44$  for AIS C and  $P = 0.35$  for AIS D) (Table 5). A similar result was also found for the TUGT ( $P = 0.39$ ) and sit-to-stand test ( $P = 0.11$ ) in AIS D subjects.

## Discussion

BWS robotic-assisted step training reorganized the modulation of the soleus H-reflex during stepping, in a functional

manner, in people with a clinically chronic motor complete and incomplete SCI. Specifically, after BWS robotic-assisted step training in AIS C and D subjects, the soleus H-reflex was depressed at late stance, stance-to-swing transition, and swing initiation (Table 3), allowing a smooth transition from stance to swing. Further, the soleus H-reflex was depressed at early and mid-swing phases, promoting reciprocal activation of antagonistic ankle flexors and extensors. These neuronal changes coincided with a biphasic activation of the MG muscle for AIS A–B and D groups, increases in EMG amplitude, changes in the onset of LH and VL EMG activity in AIS A–B subjects (Fig. 7), and improvements in gait speed in all subjects (Table 5).



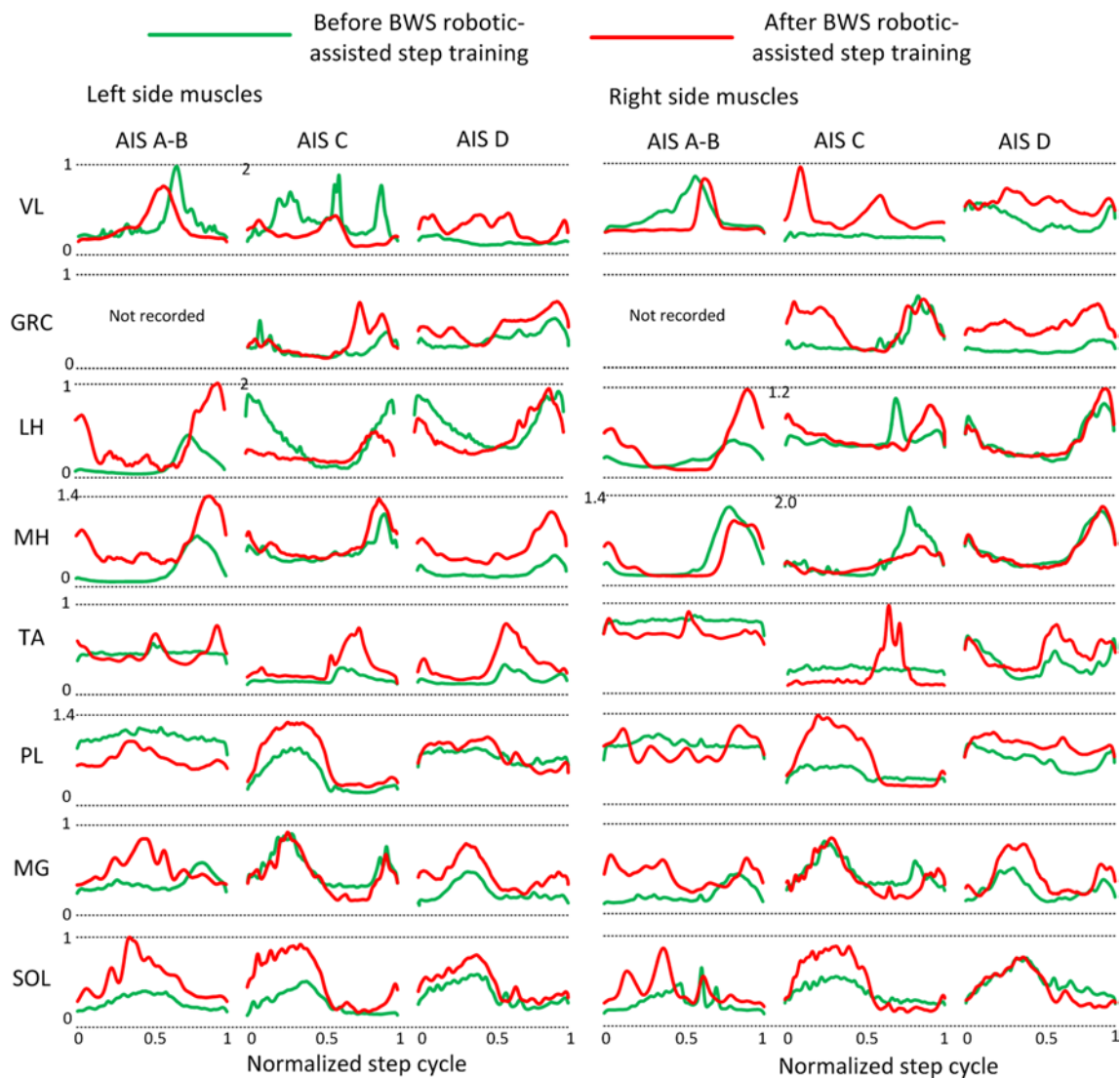
**Fig. 6** Relationship between soleus H-reflex and TA background EMG activity before and after BWS robotic-assisted step training. The mean amplitude of the soleus H-reflex recorded at each bin before (*gray filled squares*) and after (*black filled squares*) training

for AIS A–B, AIS C, and AIS D subjects is plotted against the mean normalized ipsilateral TA EMG background activity for the right leg (**a**) and left leg (**b**), respectively. In all graphs, the 16 points correspond to the 16 bins of the step cycle

The primary source of the neuronal reorganization we observed here is task-specific activity-dependent plasticity of spinal locomotor networks, although the reorganization of supraspinal connections with spinal locomotor networks cannot readily be excluded (Wolpaw and Tennissen 2001; Knikou 2010a). These findings constitute the first neurophysiological evidence on the underlying mechanisms of BWS robotic-assisted step training in people with chronic motor complete and incomplete SCI.

In AIS A and B subjects, the right soleus H-reflex depression at mid- and late swing phases was re-established in the right leg (Fig. 2a; Table 3), during which co-activation of

ankle antagonistic muscles was also present (Fig. 7). The latter is depicted by an absent negative linear relationship between the soleus H-reflex and the TA background EMG activity during stepping (Fig. 6a). Although data were acquired from one subject with AIS A and one subject with AIS B, it should be noted that the soleus H-reflex depression before stance-to-swing transition (Fig. 3a; Table 3) in both legs coincided with an increased activity of ankle extensors during the stance phase (see SOL at 0.5 % of step cycle in Fig. 7). Based on these results, we conclude that in motor complete SCI, the activation of antagonists contributed minimally to the re-establishment of phasic reflex



**Fig. 7** Leg EMG activation profiles during stepping before and after BWS robotic-assisted step training. Mean normalized EMG from the left- and right-side muscles during assisted stepping before (*gray lines*) and after (*black lines*) locomotor training from AIS A–B, AIS C, and AIS D groups. The EMG before locomotor training was nor-

malized to the associated EMG recorded after locomotor training. Y-axis is from zero to one, unless otherwise stated in the plots. Y-axis larger than one indicates that the EMG activity before training was larger than the EMG activity after training

excitability. Thus, based on a syllogistic approach, we can say that BWS robotic-assisted step training reorganized the function of spinal locomotor networks in AIS A and B subjects as a result of repetitive appropriate afferent inputs.

BWS robotic-assisted step training in AIS C and D subjects changed significantly the amplitude of the soleus H-reflex during stepping. In both limbs, the soleus H-reflex was largely depressed at stance-to-swing transition, swing initiation, and early swing phase (Table 3). Absent soleus H-reflex depression at these phases is evident in people with a SCI (Knikou et al. 2009a). Lack of reflex depression at these phases of the step cycle may be driven partly by reduced reciprocal inhibition (Knikou and Mummidisetty

2011), while it produces co-contraction of antagonistic muscles, absent smooth transition from stance-to-swing phase, and poor walking ability. Further, the soleus H-reflex depression after training was re-established at mid- and late swing phases (Figs. 2, 3), supporting for reciprocal activation of ankle flexors and extensors, similar to that described for healthy control subjects. Taken altogether, it is apparent that in all subject groups, BWS robotic-assisted step training resulted in an adaptive process that shifted the timing of soleus H-reflex depression during stepping.

For a phase-dependent H-reflex modulation during human walking to occur, locomotor neuronal networks need to be appropriately engaged at each phase of a step

**Table 5** Walking parameters and clinical evaluation tests before and after locomotor training

		BWS (%)	Speed (m/s)	LGF (%)	RL step duration (s)	Spasm frequency scale (0–16)	6MWT (m)	TUGT (s)	# of sit-to-stand repetitions within 30 s
AIS A-B	Before	50 (0.0)	0.5 (0.0)	100 (0.0)	2.39 (0.12)	10.5 (2.1)	unable	unable	unable
	After	25 (0.0) <b>[-50.0]</b>	0.9 (0.0) <b>[+78.0]</b>	100 (0.0) <b>[+0.0]</b>	1.91 (0.17) <b>[-19.8]</b>	7.5 (6.3) <b>[-28.6]</b>	unable	unable	unable
AIS C	Before	48.7 (10.3)	0.46 (0.06)	77.5 (26.3)	2.41 (0.1)	8.25 (2.6)	36.25 (24.66)	unable	unable
	After	28.75 (23.9) <b>[-41.0]</b>	0.66 (0.13) <b>[+42.1]</b>	31.25 (19.31) <b>[-59.6]</b>	1.93 (0.27) <b>[-19.8]</b>	5.75 (1.71) <b>[-30.3]</b>	39.05 (26.78) <b>[+7.7]</b>	unable	unable
AIS D	Before	43.7 (14)	0.54 (0.1)	93.75 (17.6)	2.07 (0.23)	3.75 (3.28)	252 (138)	16.61 (11)	9.7 (5.88)
	After	11.25 (5.82) <b>[-74.2]</b>	0.82 (0.09) <b>[+53.3]</b>	27.5 (29.8) <b>[-70.6]</b>	1.79 (0.26) <b>[-13.8]</b>	2.0 (2.07) <b>[-46.7]</b>	278.5 (150.8) <b>[+10.5]</b>	15.04 (11.22) <b>[-9.4]</b>	14.43 (9.4) <b>[+48.5]</b>

The mean amplitude of the parameters during stepping and results of clinical tests are presented with the participants grouped based on the American Spinal Injury Association (ASIA) Impairment Scale (AIS) scale. Spasm frequency scale (0–16) represents the number of spasms the participants reported to have the last 24 h in all limbs. Bold numbers in brackets indicate the percentage of change after training compared to that observed before training. Gray squares denote statistically significant differences ( $P < 0.05$ ) for measures taken before and after training. SD is shown in parenthesis

BWS body weight support, LGF leg guidance force provided by the Lokomat, RL right leg, 6MWT 6-min walk test, TUGT time up and go test

cycle. The modulation of the soleus H-reflex during walking in healthy humans has been ascribed to spinal interneuronal circuits, the central pattern generator that regulates the function of spinal reflex circuits which mediate sensory input from the joints and muscles, and descending control of the spinal reflex circuits and the central pattern generator (Knikou 2010a). Thus, the re-appearance of a phase-dependent soleus H-reflex modulation after locomotor training in SCI signifies the presence of functional spinal interneuronal circuits related directly or indirectly to locomotion.

Rhythmic changes in presynaptic inhibition of Ia afferent terminals projecting monosynaptically to soleus motor neuron pool accounts for the phase-dependent soleus H-reflex regulation throughout the step in uninjured humans (Faist et al. 1996). Reciprocal Ia inhibition exerted from ankle flexors onto ankle extensors and recurrent inhibition that affects motor neuron pool excitability through Ia inhibitory interneurons account mostly for the reflex depression during the swing phase in healthy humans (Lavoie et al. 1997;

Petersen et al. 1999; Baret et al. 2003; Lamy et al. 2008). We have recently shown that stepping with a robotic exoskeleton does not alter the phase-dependent modulation pattern of the soleus H-reflex, the reciprocal Ia inhibition, and the presynaptic inhibition of Ia afferents in people with no apparent neurological injury (Knikou et al. 2011; Mummidisetty et al. 2013). Furthermore, step training decreases disynaptic IB inhibition (load pathways) and monosynaptic IA excitation in curarized spinal cats during fictive locomotion, while a stable after-hyperpolarization duration suggests that recovery of locomotion involves plastic changes occurring at a pre-motoneuronal level (Côté et al. 2003), engaging mostly interneurons of the group I pathways to extensors and interneurons of presynaptic inhibition. Plasticity of extensor group I pathways is critical for controlling stance-to-swing transition (Whelan et al. 1995). The source of these changes may be normalization of cutaneous transmission at a pre-motoneuronal level (Côté and Gossard 2004), a sensory-feedback neuronal pathway that normalizes the function of spinal reflexes in untrained people with

a SCI during assisted stepping (Knikou 2010b), and interacts with signals from hip proprioceptors altering soleus H-reflex function (Knikou 2005). Based on these findings, we conclude that the re-establishment of soleus H-reflex depression at late stance, stance-to-swing transition, swing initiation, and mid-swing phases could partly be attributed to changes in these spinal interneuronal circuits that were accelerated by timely and appropriate peripheral and/or descending inputs (Knikou and Conway 2001; Knikou 2005; Wolpaw and Carp 2006).

In this study, we show for the first time that regardless the classification of the neurological injury, people with a chronic SCI regained the lost phase-dependent soleus H-reflex modulation (Yang et al. 1991; Knikou et al. 2009a) through reappearance of spinal inhibition during stepping. However, a more complex spinal reflex reorganization pattern was revealed when we compared the changes between the right and left soleus H-reflex in the same participant (Table 4; Fig. 4). Specifically, the soleus H-reflex was reorganized in an homologous pattern in 5 out of 9 subjects, and in an heterologous pattern in 4 subjects (Table 4). This pattern of adaptation may be related to the level of the residual capacity of the neuromuscular system at the time of the study. For example, based on the ASIA motor scores, it is apparent that the left leg was less impaired compared to the right leg (Table 1). These findings suggest that spinal reflex reorganization pattern likely depends on the motor experiences (good or bad) of remnant neuronal pathways (Wolpaw 2010). It is well established that neuronal interlimb coordination in humans during stepping can be driven by an autonomous spinal pattern generator that interacts with its counterpart of the contralateral limb (Yang et al. 2005), while recent evidence suggests that the spinal pattern generator plays a prominent role in the recovery of locomotion in motor incomplete SCI (Rossignol et al. 2007; Barrière et al. 2008). Further, crossed spinal inhibitory and facilitatory connections are well established in both humans and animals (Roby-Brami and Bussel 1990; Jankowska and Hammar 2002; Frigon and Rossignol 2008). Consequently, the differential spinal reflex reorganization we observed here in the right and left soleus H-reflex may be related to plastic changes in crossed spinal inhibitory and facilitatory connections. Neurophysiological changes over the course of training as a function of the residual motor capacity and whether adaptive reflex plasticity of the right soleus H-reflex affects the left soleus H-reflex and interlimb coordination warrant further investigation.

In addition to these neuronal changes, the BWS required during robotic-assisted stepping, the gait speed, and the step duration changed significantly after training in all AIS subjects (Table 5), suggesting that walking function was improved, consistent with results reported in

other studies (Behrman et al. 2005; Field-Fote and Roach 2011).

### Limitations

A limitation of this study is that data were acquired from one person with AIS A and one person with AIS B, while the sample size for AIS C and D subjects was small. Larger-scale neurophysiological research studies are needed to comprehensively characterize spinal neuronal reorganization of different types and levels of spinal lesions. Further, the lack of improvement of overground walking ability (Table 5) may be related to the variable number of training sessions for each participant, but most importantly to the sensitivity to changes in the clinical tests we used to assess improvements in walking function. For example, the 6-min walk test assesses more walking endurance than improvements in overground walking ability, while the testing conditions affect significantly the outcomes (Scivoletto et al. 2011).

### Conclusions

Locomotor training with a robotic exoskeleton re-established the soleus H-reflex depression at late stance, stance-to-swing transition, swing initiation, and early and mid-swing phases of the step cycle and promoted a physiological activity of leg muscles in people with complete, motor complete, and motor incomplete SCI. While the emergence of soleus H-reflex depression may be due to potentiation or rewiring of spinal inhibitory interneuronal circuits of the injured human spinal cord, further research is needed to comprehensively investigate the source of this neuronal reorganization. Regardless the source of the neuronal reorganization, it is apparent that spinal neuronal networks of persons with clinically complete, motor complete, or motor incomplete SCI have the potential to undergo adaptive reorganization and improve walking function with BWS robotic-assisted step training.

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**Conflict of interest** The author declares that she has no conflict of interest.



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