

# Effects of Stand and Step Training with Epidural Stimulation on Motor Function for Standing in Chronic Complete Paraplegics

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## Abstract

Individuals affected by motor complete spinal cord injury are unable to stand, walk, or move their lower limbs voluntarily; this diagnosis normally implies severe limitations for functional recovery. We have recently shown that the appropriate selection of epidural stimulation parameters was critical to promoting full-body, weight-bearing standing with independent knee extension in four individuals with chronic clinically complete paralysis. In the current study, we examined the effects of stand training and subsequent step training with epidural stimulation on motor function for standing in the same four individuals. After stand training, the ability to stand improved to different extents in the four participants. Step training performed afterwards substantially impaired standing ability in three of the four individuals. Improved standing ability generally coincided with continuous electromyography (EMG) patterns with constant levels of ground reaction forces. Conversely, poorer standing ability was associated with more variable EMG patterns that alternated EMG bursts and longer periods of negligible activity in most of the muscles. Stand and step training also differentially affected the evoked potentials amplitude modulation induced by sitting-to-standing transition. Finally, stand and step training with epidural stimulation were not sufficient to improve motor function for standing without stimulation. These findings show that the spinal circuitry of motor complete paraplegics can generate motor patterns effective for standing in response to task-specific training with optimized stimulation parameters. Conversely, step training can lead to neural adaptations resulting in impaired motor function for standing.

**Keywords:** epidural stimulation; spinal cord injury; spinal motor learning; standing; training

## Introduction

INDIVIDUALS AFFECTED by a motor complete spinal cord injury (SCI) graded A or B by the International Standards for Neurological Classification of Spinal Cord Injury<sup>1</sup> are unable to stand, walk, or move their lower limbs voluntarily, and this diagnosis normally implies severe limitations for functional recovery.<sup>2,3</sup> However, studies in mammals with motor complete paralysis showed that the combined effects of activity-based rehabilitation and lumbosacral spinal cord stimulation can modulate the spinal circuitry and restore, to a relevant extent, motor function of the paralyzed limbs.<sup>4–8</sup> More recently, these two interventions combined together were found effective to progressively reenact voluntary control of lower limbs<sup>9–11</sup> and standing<sup>9</sup> also in humans with motor complete SCI.

Different observations support the view that the mammalian spinal cord is capable of motor learning and that this is conceivably task specific.<sup>12</sup> For example, full weight-bearing stepping ability was about three times greater in complete spinal cats that underwent step

training than in untrained cats that were allowed to recover spontaneously.<sup>13</sup> In this case, difference in stepping ability between step trained and untrained cats coincided with differences in electromyography (EMG) pattern and hindlimb kinematics. Similarly, complete spinal cats trained to stand bilaterally were able to stand bearing full body weight about five times longer than untrained cats.<sup>14</sup> Also, complete spinal cats trained to stand unilaterally selectively improved weight bearing on the trained limb, which showed greater ground reaction forces and extensor muscle EMG activity levels during bilateral standing than the untrained limb. Interestingly, task-specific training can impair the spinal cord ability to perform a different motor task. For example, complete spinal cats trained to stand were not able to use their hindlimbs for locomotion,<sup>15</sup> and complete spinal rats trained to stand showed poorer ability to perform an untrained motor task that involved lower limb flexion as compared with step-trained and non-trained rats.<sup>16</sup>

In humans with clinically complete SCI, locomotor training performed with manual assistance provided by trainers is not sufficient for recovering overground standing or walking.<sup>17–20</sup> However, it

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provides repetitive specific sensory information that can reorganize the spinal circuitry and modulate the locomotor pattern, increasing, to some extent, EMG activity of leg extensors and amount of weight-bearing during assisted standing and stepping. The motor learning ability of the human spinal cord has been also observed in individuals with intact spinal cord by comparing Hoffman reflex responses recorded from highly skilled athletes and unskilled individuals<sup>21,22</sup> as well as by functional imaging<sup>23</sup> detecting the distinct contribution between brain and spinal cord throughout the motor learning process.

Epidural stimulation of the lumbosacral spinal cord after complete SCI has been successfully applied to facilitate standing in mammals.<sup>24–27</sup> These studies demonstrated that, in the absence of supraspinal input, epidural stimulation of the lumbosacral spinal cord can promote motor output effective for standing, and that sensory afferent input as well as a fine tuning of the different stimulation parameters (i.e., stimulation site, frequency, and amplitude) are crucial to control it. More recently, we have demonstrated that four out of four chronic complete paraplegics (two graded as American Spinal Injury Association Impairment Scale [AIS] A and two graded as AIS B) were able to stand overground while epidurally stimulated, bearing full body weight without any external assistance at the knees and using their hands to assist balance.<sup>28</sup> Standing with the least amount of assistance was promoted by individual-specific stimulation parameters that induced little or negligible muscle activation during sitting, while enabling the spinal circuitry to generate overall continuous EMG patterns in response to the sensory information related to the change in body position from sitting to standing.

In this study, we report the effects of activity-based stand training and subsequent activity-based step training with epidural stimulation on motor function for standing in the same four research participants. As standing and stepping differ considerably from each other in neural activation patterns, we hypothesized that stand training would lead to improvements in standing ability, whereas step training performed afterwards would impair standing ability. We also hypothesized that stand and step training with epidural stimulation would not be sufficient for improving motor function for standing in individuals with motor complete SCI when spinal stimulation was not provided.

## Methods

### Participants

Four individuals with chronic paraplegia, who met the following inclusion criteria, were recruited as participants of this study: 1) in stable medical condition without cardiopulmonary disease or dysautonomia that would contraindicate standing or stepping with body weight support training; 2) no painful musculoskeletal dysfunction, unhealed fracture, contracture, pressure sore, or urinary tract infection

that might interfere with stand or step training; 3) no clinically significant depression or ongoing drug abuse; 4) no current antispasticity medication regimen; 5) nonprogressive spinal cord injury above T10; 6) AIS A or B; 7) no motor response present in leg muscles during transmagnetic stimulation; 8) sensory evoked potentials not present or present with bilateral delay; 9) no volitional control during voluntary movement attempts in leg muscles as measured by EMG activity; 10) segmental reflexes remaining functional below the lesion; 11) brain influence on spinal reflexes is not observed as measured by EMG activity; 12) not having received Botox injections in the previous 6 months; 13) unable to stand or step independently; 14) at least 1 year post-injury; and 15)  $\geq 18$  years of age. The research participants signed an informed consent for electrode implantation, stimulation, activity-based training, and physiological monitoring studies approved by the University of Louisville and the University of California, Los Angeles Institutional Review Boards.

Prior to implantation, two clinicians independently performed a physical examination following the International Standards for Neurological Classification of Spinal Cord Injury<sup>29,30</sup> in order to classify the injury using the AIS. Individuals A45 and A53 showed no sensory or motor function below the neurological level of the lesion, and were classified as AIS A. Individuals B07 and B13 showed impaired sensory and no motor function below the lesion, and were classified as AIS B (Table 1). In addition, as previously reported,<sup>10</sup> these four individuals underwent different neurophysiological evaluations (upper and lower extremity somatosensory evoked potentials, transcranial magnetic stimulation [individuals B07, A45, and A53], and assessments of residual motor output). In summary, these evaluations, performed without epidural stimulation, showed that no functional motor connectivity between the supraspinal and spinal centers below the level of injury was detected in any of the four research participants. Moreover, prior to implantation, research participants underwent 80 sessions of locomotor training, which included stand and step training (with stepping comprising the majority of time<sup>31</sup>), with the intent to achieve the positive adaptations induced by activity-based rehabilitation alone before the beginning of training with epidural stimulation.

### Surgical implantation of electrode array and stimulator

The epidural spinal cord stimulation unit (Restore ADVANCED, Medtronic) was used to electrically stimulate the lumbosacral enlargement. As previously described,<sup>9</sup> a 16 electrode array (5-6-5 Specify, Medtronic) was implanted at the T11-L1 vertebral level over spinal cord segments L1-S1 in all individuals. The electrode lead was tunneled to a subcutaneous abdominal pouch where the pulse generator was implanted.

### Experimental procedures

The data reported in the present study were collected during three or four experimental sessions performed by each participant

TABLE 1. CLINICAL CHARACTERISTICS OF INDIVIDUALS

Participant	Age yr	Gender	Post- injury yr	Neuro level	AIS grade	AIS score						Anal sensation
						Sensory (T10-S5, score out of 24)				Motor (lower extremity)		
						L LT	L PP	R LT	R PP	L	R	
B07	24	Male	3.4	T2	B	15	11	18	10	0	0	yes
A45	24	Male	2.2	T4	A	0	0	0	0	0	0	no
B13	33	Male	4.2	C7	B	10	10	10	8	0	0	yes
A53	27	Male	2.3	T4	A	0	0	0	0	0	0	no

Sensory score was designated by light-touch (LT) and pinprick (PP) of the left (L) and right (R) lower limb, below the lesion. Neuro level, neurological level of the lesion; AIS, American Spinal Injury Association (ASIA) Impairment Scale.

after implantation of the stimulation unit, and before any activity-based intervention (pre-training), after 81 ± 1 sessions of stand training (post-stand), and after subsequent 81 ± 2 sessions of step training (post-step) with epidural stimulation (Fig. 1A and B).

A 10 min full weight-bearing standing experimental session was devoted to the assessment of: 1) the ability to stand with the least amount of external assistance for hip and knee extension and 2) the motor pattern generated during such attempt. Standing was performed overground using a custom designed standing frame composed of horizontal bars anterior and lateral to the individual (see Supplementary Video 1) (see online supplementary material at <http://www.liebertpub.com>).

These bars were used for upper extremity support and balance assistance as needed. Stimulation began while the participant was seated. Then, the participant initiated the sit to stand transition by positioning his feet shoulder width apart and shifting his weight forward to begin loading the legs. The participant used the horizontal bars of the standing apparatus during the transition phase to balance and to partially pull himself into a standing position. Trainers positioned at the pelvis and knees manually assisted as needed during the sit to stand transition. When a steady standing position was achieved, the 10 min session began; if the knees or hips flexed beyond the normal standing posture, external assistance at the knees distal to the patella was provided to promote knee extension, and at the hips below the iliac crest to promote hip extension and anterior tilt. Facilitation was provided either manually by a trainer or by elastic cords, which were attached between the two vertical bars of the standing apparatus.

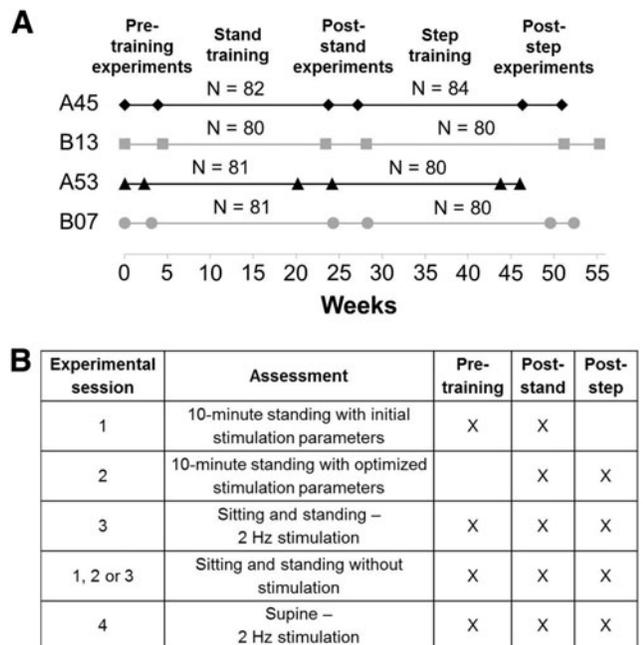
To investigate whether stand and step training induced any neural adaptation sufficient to influence motor function for standing without epidural stimulation, EMG activity of different lower limb muscles was recorded during standing without stimulation and was also compared with that recorded during sitting without stimulation. The neural plasticity induced by stand and step training was also examined by

comparing the evoked potentials to epidural spinal stimulation recorded during standing and sitting while using the same stimulation parameters. In particular, a wide-field electrode configuration with cathodes positioned caudally was selected to deliver at a frequency of 2 Hz the lowest stimulation intensity that activated all the investigated muscles in sitting position during the pre-training assessment. This electrode configuration was selected because it evoked nonlocation-specific responses in both proximal and distal muscles,<sup>32</sup> and because cathodes positioned caudally were shown to possibly promote motor patterns characteristic of standing behavior in subjects with clinically motor complete SCI while lying supine and standing.<sup>28,33</sup> In these two sets of experiment (standing without epidural stimulation and with 2 Hz stimulation), a body weight support system (Innoventor, St. Louis, MO) with a harness was used to provide body weight support (40% of body weight load) during standing in participants A45 and B13, whereas individuals A53 and B07 performed overground standing using the custom designed standing frame. External assistance for hip and knee extension was provided during both standing overground and with body weight support if the knees or hips flexed beyond the normal standing posture.

Finally, in order to investigate whether stand and step training with epidural stimulation induced any neural adaptation in the spinal circuitry that could be detected when no motor tasks were performed, muscle activation threshold and amplitude of evoked potentials were examined in several lower limb muscles with the individuals relaxed in a supine position. Epidural stimulation was delivered at 2 Hz with the wide-field electrode configuration with cathodes positioned caudally; stimulation intensity was increased by 0.1 V until all investigated muscles were active, and then by 0.5 V. Stimulation intensities ranging from 0.1 V to 5 V, which were tolerated from all participants at all three time points, were considered for further analysis.

*Stimulation parameters applied for the assessment of standing ability*

Stimulation parameters applied during the 10 min standing session performed prior to the beginning of stand training were selected during an experimental session following the guidelines reported by Rejc and coworkers.<sup>28</sup> Briefly, the initial stimulation parameters were selected during sitting, and consisted of a wide-field electrode configuration with cathodes positioned caudally, and a stimulation frequency of 25 Hz at near-motor threshold stimulation intensity that did not directly elicit lower limb movements. If the initial stimulation parameters did not promote EMG patterns sufficient to enable the subject to stand bearing full body weight without external assistance for hip and knee extension, electrode configuration adjustments were defined to seek improvements of different aspects of motor output. For example, the electrode field was more focused on the caudal portion of the electrode array to increase the excitability of distal muscles' motoneuron pools, or was extended toward the rostral portion of the array to increase the excitability of proximal muscles' motoneuron pools.<sup>32</sup> Also, active electrodes were unbalanced between the lateral columns of the electrode array with the intent of compensating activation differences between left and right lower limbs, as the lateral placement of the epidural stimulation electrodes with respect to the spinal cord midline was shown to promote motor responses in muscles ipsilateral to the stimulation in rats.<sup>26</sup> In addition, we considered the data recorded from previous experiments performed on the same research participants in the supine position with different bipolar and wide-field electrode configurations (as partially reported by Sayenko and colleagues<sup>32</sup>), which provided individualized maps of motor pools activation, to adjust cathode position to target primarily extensors muscle groups. Also, stimulation frequency and amplitude were modulated synergistically to find the higher stimulation frequency that elicited a continuous (non-rhythmic) EMG pattern effective to bare body weight.



**FIG. 1.** Experimental protocol timeline. (A) Weeks devoted to complete training and experimental sessions (related and unrelated to the present study) performed before any training (pre-training), after stand training (post-stand), and after step training (post-step). Total number of stand and step training sessions performed by each participant is also reported. (B) Assessments performed during different experimental sessions at pre-training, post-stand, and post-step.

Stimulation parameters for standing were also adjusted throughout stand training following the abovementioned guidelines to improve standing. In particular, experimental sessions were performed to monitor standing behavior and EMG from lower limb muscles while using different stimulation parameters to contribute to their selection. Hence, after stand training, the 10 min standing session was performed with the improved stimulation parameters, to achieve standing with the least amount of external assistance. In addition, at the same time point, the 10 min standing session was also performed with the initial stimulation parameters, in some cases at adjusted stimulation intensity. Finally, standing ability after step training was assessed with the optimized stimulation parameters for standing used at the end of stand training.

### Stand training

After the stimulator implantation and the completion of a series of pre-training experiments, research participants underwent on average 81 sessions (range: 80–82 sessions) of full weight-bearing stand training (1 h, five sessions per week). Stand training was always performed with lumbosacral spinal cord epidural stimulation, using the custom designed standing frame described previously. Participants were encouraged to stand for as long as possible throughout the training session, with the goal of standing for 60 min with the least amount of assistance. Seated resting periods occurred when requested by the individuals. If, during standing, the participant's knees or hips flexed beyond the normal standing posture, external assistance to facilitate hip and knee extension was provided either manually by a trainer or by elastic cords, which were attached between the two vertical bars of the standing frame. A research staff member activated a chronometer at the beginning of stand training, and noted the time associated with every onset and offset of the assistance for knee extension.

### Step training

Following the completion of stand training and respective experimental sessions, the research participants performed on average 81 sessions (range: 80 to 84 sessions) of step training with body weight support (Innoventor, St. Louis, MO) on a treadmill (1 h, five sessions per week). Step training was always performed with lumbosacral spinal cord epidural stimulation. Research participants stepped at body weight load and speed adapted to achieve appropriate stepping kinematics.<sup>31</sup> Stepping bout duration was dependent on the participant's endurance and stepping behavior. Following a stepping bout, participants were encouraged to remain standing; body weight support and length of standing break varied. All trainers were careful to provide manual assistance only when needed following standard locomotor training principles.<sup>34</sup>

### Data acquisition

EMG and ground reaction forces data were recorded at 2000 Hz using a custom-written acquisition software (National Instruments, Austin, TX). EMG activity of right (R) and left (L) gluteus maximus (GL), medial hamstring (MH), rectus femoris (RF), vastus lateralis (VL), tibialis anterior (TA), medial gastrocnemius (MG) and soleus (SOL) was recorded by means of bipolar surface electrodes with a fixed inter-electrode distance.<sup>9</sup> Bilateral EMG from the iliopsoas (IL) was recorded with fine-wire electrodes. Two surface electrodes were placed symmetrically lateral to the electrode array incision site over the paraspinal muscles in order to record the stimulation artefacts, which were used as indicators of the stimulation onset (time points when the stimulus pulses were applied). Ground reaction forces were collected using a high-resolution pressure sensing mat (HR mat system, TEKSCAN, Boston, MA).

### Data analysis

The variability of ground reaction forces and EMG activity was assessed by calculating their coefficient of variation (CV) (standard deviation[SD]/mean) over one representative minute of standing. In particular, the CV for EMG activity was calculated from the linear envelope EMG, which was obtained by filtering through a low-pass digital filter at a cutoff frequency of 4 Hz the rectified EMG signal.

The amplitude of EMG activity recorded without epidural stimulation was quantified by root mean square (RMS) and calculated over 10 representative seconds of steady sitting and standing. The amplitude of evoked potentials to epidural stimulation delivered at 2 Hz was quantified by peak to peak amplitude. To investigate the evoked potentials amplitude when subjects were in the supine position, five stimuli were delivered at each stimulation intensity applied, which ranged between 0.1 and 5 V; the average peak to peak amplitude for each stimulation intensity was calculated, and the largest value recorded from each investigated muscle was considered for the comparison across interventions. The average stimulation intensity that induced the largest EMG peak to peak amplitude values corresponded to  $3.0 \pm 1.2$  V. The fact that the largest peak–peak amplitudes were predominantly recorded at submaximal stimulation intensities supports the view that any eventual variability associated with the the relationship of the epidural electrode array with the spinal cord, for example caused by different pressure on the array toward the cord while the subject was lying supine or by the hydration status of the participant,<sup>35</sup> played a negligible role in determining the largest peak to peak amplitude values considered for further analysis.

Muscle activation threshold coincided with the lowest stimulation intensity that induced reproducible evoked potentials with amplitude higher than the mean baseline EMG plus three times its SD.<sup>32</sup> To examine the adaptations brought about by stand and step training, muscle activation thresholds were expressed as percentage of the lowest threshold value at pre-training. If more than one muscle showed the same lowest activation threshold at pre-training, the muscle with the lowest threshold detected at post-stand, and eventually at post-step, was selected. This relative comparison of activation threshold among muscles provides information on muscle recruitment order and also overcomes any eventual variability associated with the the relationship of the epidural electrode array with the spinal cord. In addition, we compared over time the average absolute difference of activation threshold among the investigated muscles, which was calculated as follows:

$$\begin{aligned} & \text{Activation thresholds difference} \\ &= \frac{|Mr - M2| + |Mr - M3| + \dots + |Mr - Mn|}{n - 1} \end{aligned} \quad (1)$$

where *Mr* indicates the muscle with the lowest activation threshold at pre-stand used as the reference (100%), and *M2* to *Mn* indicate all the other analyzed muscles. This parameter was calculated taking into account  $n = 14$  muscles for participants A45, A53, and B13; and  $n = 12$  muscles for participant B07.

### Statistical analysis

Data are reported as means  $\pm$  SD. Statistical analysis was performed using GraphPad Prism (version 5.00 for Windows, GraphPad Software, San Diego, CA). A *p* value  $< 0.05$  was considered statistically significant. The distribution of quantitative variables was tested for normality using the Kolmogorov–Smirnov test. Because the assumption of normality distribution for the investigated variables was not met, the effect of stand and step training on: 1) the ratio between peak–peak EMG amplitude of evoked potentials recorded during standing and sitting, 2) the activation thresholds difference, and 3) the peak–peak EMG amplitude of evoked potentials recorded

with the subject in the supine position were evaluated for each participant by the nonparametric Friedman test, and following multiple comparisons by Dunn's post-hoc test.

## Results

### Time course of stand training

Generally, participants achieved 60 min of full weight-bearing standing every training session, with occasional seated resting periods (Fig. 2A). An exception was shown by participant B07 within the initial 35 training sessions; he often required resting periods and did not achieve the goal of standing for 60 min within a session. At the beginning of stand training, external assistance to facilitate knee extension was required for all participants (Fig. 2A). The ability to stand without knee assistance was shown by each participant for the first time between the 3rd and 27th training session (B13 and B07, respectively). However, the improvement of this ability occurred at different time points and with different time courses among participants. The cumulative amount of standing without knee assistance throughout stand training also varied across participants, ranging from 665 (B07) to 3616 (A45) min (Fig. 2B). The longest bout of standing without knee assistance achieved during training was similar for participants A45 and A53 (63 and 60 min, respectively), whereas B13 and B07 achieved shorter bouts (17 and 4 min, respectively) (Fig. 2C).

### External assistance required for standing before and after stand and step training

Before any training, all participants needed external assistance for hip and knee extension to maintain an upright posture throughout the entire 10 min standing session (Fig. 3). After stand training (post-stand), all four participants showed relevant

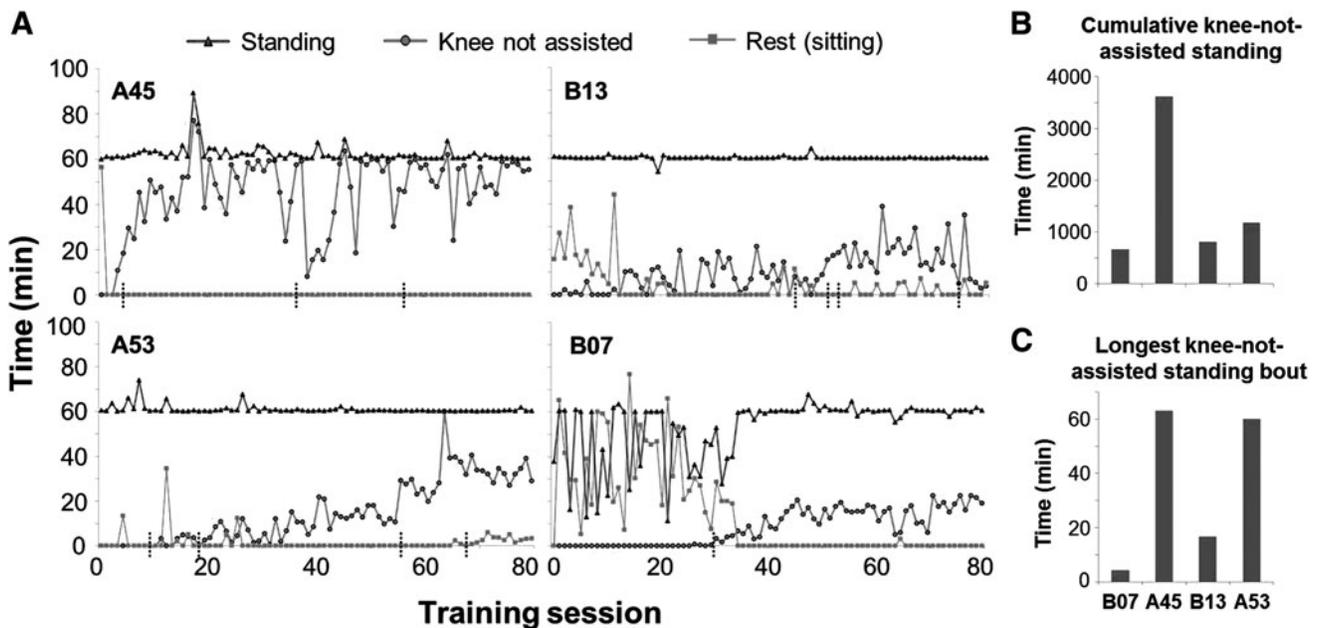
improvements in standing ability when stimulation parameters adjusted throughout stand training were applied. Participants A45 and A53 maintained standing without any external assistance for the entire 10 min session placing their hands on the standing frame to assist balance (see representative standing performed by A45 in Supplementary Video 1). Also, participant B13 (Supplementary Video 2) and B07 achieved standing without knee assistance for 7.0 and 6.8 min, respectively (see online supplementary material at <http://www.liebertpub.com>).

Improvements were less marked when participants used the initial stimulation parameters selected prior to any training (in some cases at adjusted stimulation intensity) (Table 2). In this case, three out of four participants were able to stand without knee assistance for a duration of between 0.6 (B07) and 5.5 (A45) min. Also, participant A45 showed the ability to stand 3.7 min without any external assistance. Conversely, participant A53 needed external assistance at both hip and knee joints during the full attempt.

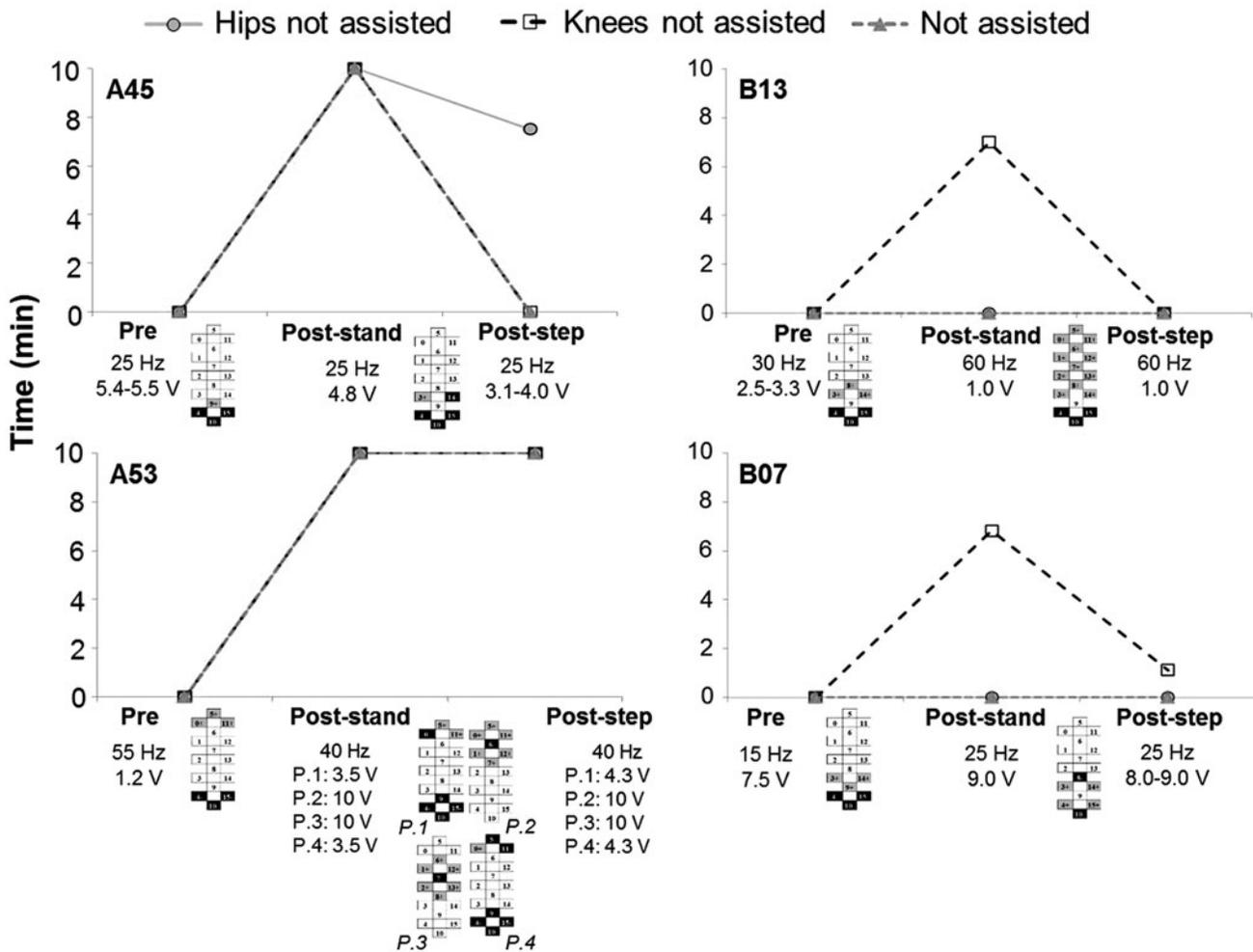
Interestingly, after step training, standing ability was impaired in all participants except A53 (Fig. 3; representative examples shown in Supplementary Videos 1 and 2 for A45 and B13, respectively). In particular, standing time without knee assistance for participants A45, B13, and B07 was on average  $88 \pm 9\%$  lower after step training than at post-stand, even if the same specific stimulation parameters for standing (in some cases at an adjusted stimulation intensity) were used.

### EMG pattern before and after stand and step training

Before training, while trying to achieve steady standing, three out of four participants showed a similar EMG pattern, which resulted in the alternation between EMG bursts and periods of low levels of EMG activity in most of the analyzed muscles (Fig. 4A).



**FIG. 2.** Time course of full weight-bearing standing and resting time throughout stand training. (A) Total standing time, standing time without external assistance for knee extension, and resting (sitting) time are plotted as a function of the number of training sessions performed by the research participants. Vertical black dotted lines indicate that electrode configuration was modified to optimize standing. (B) Cumulative standing time without external assistance for knee extension throughout the 80 stand training sessions. (C) Longest standing bout without external assistance for knee extension performed during stand training.



**FIG. 3.** Full weight-bearing standing time without external assistance for hip and knee extension. The ability to stand without external assistance for hip and knee extension was assessed during a 10 min session before training (pre), after stand training (post-stand), and after step training (post-step). At post-stand and post-step, stimulation parameters adjusted for standing throughout stand training were used. Standing time without external assistance (triangles), without assistance for hip extension (gray circles), and for knee extension (empty squares) is reported for each of the three time points. Stimulation frequency, electrode configuration (cathodes in black, anodes in gray, and inactive in white), and stimulation intensity range are reported for each participant. At post-stand and post-step, participant A53 was stimulated with four programs (P.1 to P.4) delivered sequentially at 10 Hz by the same electrode array, resulting in an ongoing 40 Hz stimulation frequency.

This coincided with unstable levels of ground reaction forces and the need for external knee and hip assistance to maintain upright posture. An exception was shown by participant A53: an overall continuous EMG pattern was recorded from his lower limb muscles before stand training. However, he also needed external assistance for hip and knee extension.

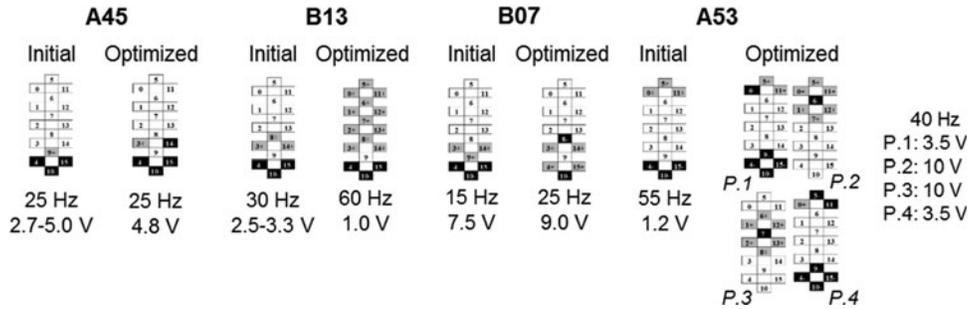
After stand training, applying the stimulation parameters optimized for standing, research participants were able to generate EMG patterns sufficient to support full weight-bearing standing without external assistance at knees (B13 and B07) or without assistance at both hips and knees (A45 and A53) (Fig. 4A). EMG patterns were less variable than before training in all individuals, as quantified by the lower EMG activity CV values ( $-60 \pm 11\%$ , Fig. 4B), and included, for example, the consistent activation of SOL and VL, whereas the EMG activity of other muscles was low or negligible in some cases (i.e., TA in B13 and B07, and IL in A53 and B07). Similarly, the CV of ground reaction forces recorded in this condition was  $84 \pm 7\%$  lower than pre-training. Interestingly,

the CV values recorded in this condition were the lowest compared with the other experimental sessions. At the same time point (post-stand), participants A45 and B13 were able to generate EMG patterns overall continuous and sufficient to support full weight-bearing standing without knee assistance also using the initial stimulation parameters (at adjusted stimulation intensity for participant A45) (Fig. 5A). Participant A53 showed an EMG pattern similar to pre-training, also requiring the same external assistance for standing. The EMG pattern recorded from participant B07 maintained the alternation between EMG bursts and periods of negligible activity in some muscles (i.e., IL and VL) as shown before training; this coincided with the need for external assistance at hips and knees to maintain standing. However, while applying the initial stimulation parameters, the CV of EMG activity was on average  $44 \pm 15\%$  lower than at pre-training (Fig. 5B); this coincided with lower CV of ground reaction forces ( $-74 \pm 6\%$ ) than before training.

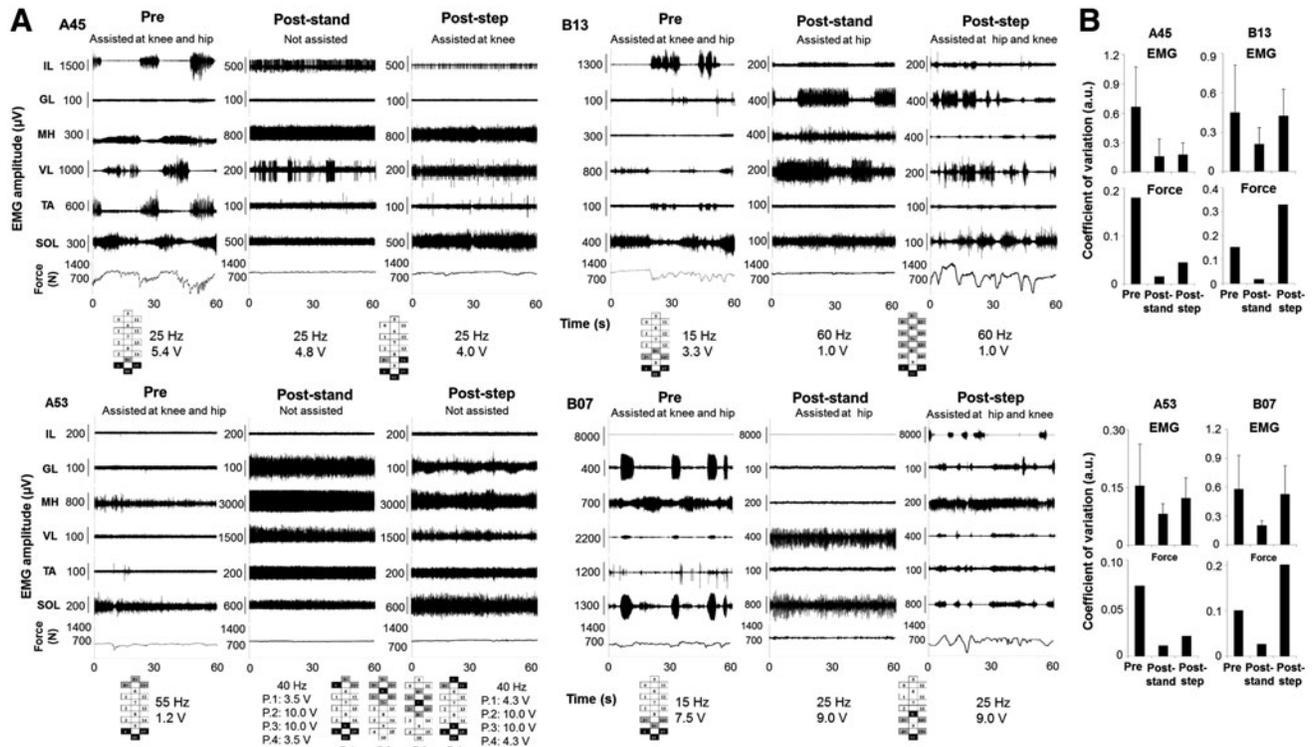
After step training, the EMG pattern recorded from participants A45 and A53 was similar to that observed at post-stand with the

TABLE 2. STANDING TIME WITH DIFFERENT AMOUNT OF ASSISTANCE DURING TEN MINUTES STANDING SESSIONS PERFORMED WITH INITIAL AND OPTIMIZED STIMULATION PARAMETERS AFTER STAND TRAINING

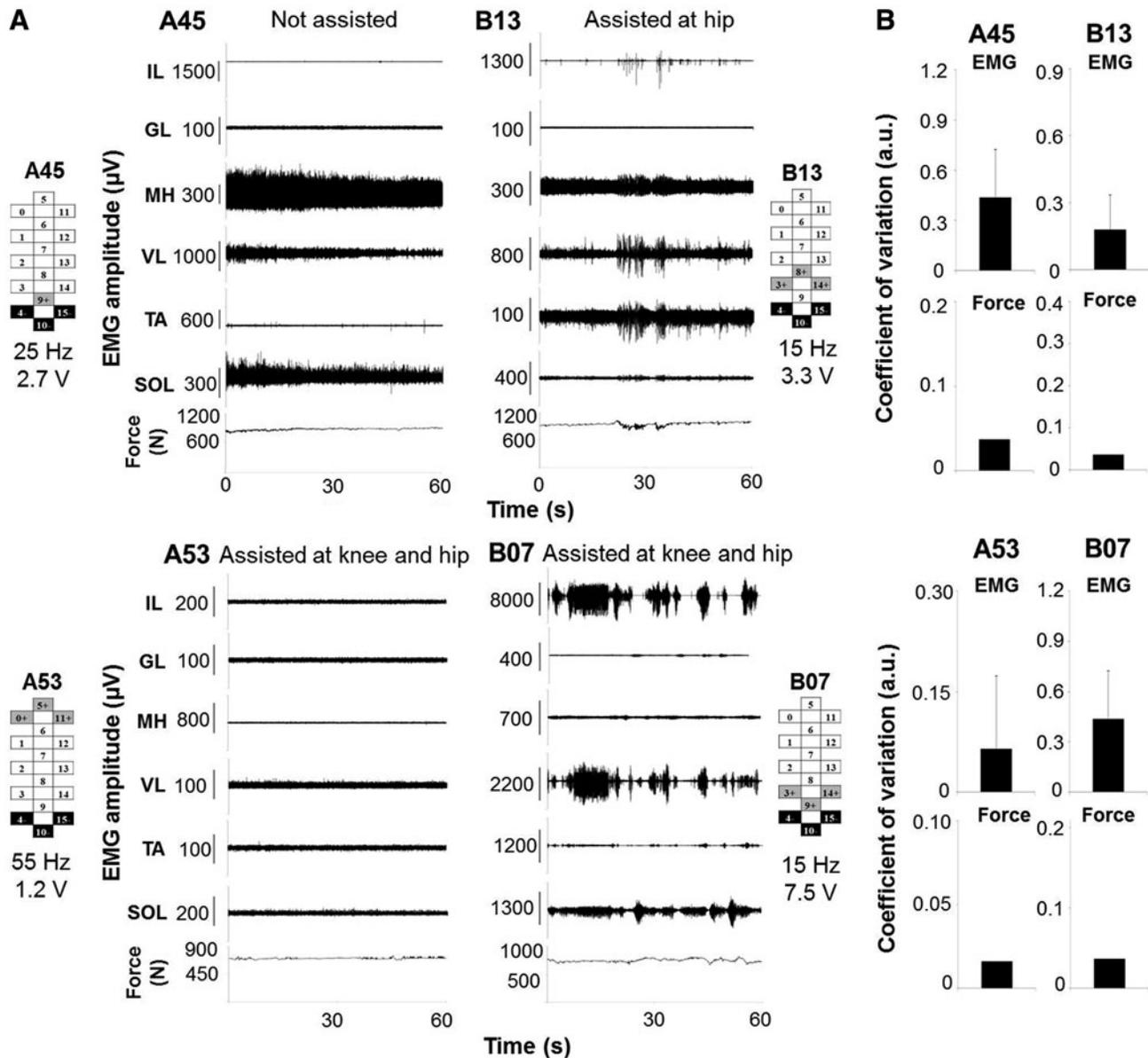
	<i>Not assisted (min)</i>		<i>Knee not assisted (min)</i>		<i>Hip not assisted (min)</i>	
	<i>Initial parameters</i>	<i>Optimized parameters</i>	<i>Initial parameters</i>	<i>Optimized parameters</i>	<i>Initial parameters</i>	<i>Optimized parameters</i>
B07	0.0	0.0	0.6	6.8	0.0	0.0
A45	3.7	10.0	5.5	10.0	8.2	10.0
B13	0.0	0.0	4.2	7.0	0.0	0.0
A53	0.0	10.0	0.0	10.0	0.0	10.0



Electrode configuration (cathodes in black, anodes in gray, and inactive in white), stimulation frequency, and intensity range are reported for each participant. The optimized stimulation parameters applied to participant A53 consisted of four programs (P.1 to P.4) that were delivered sequentially at 10 Hz by the same electrode array, resulting in an ongoing 40 Hz stimulation frequency.



**FIG. 4.** Electromyography (EMG) and ground reaction forces recorded during full weight-bearing standing before any activity-based training, after stand training, and after step training with epidural stimulation. **(A)** Time course of EMG and ground reaction forces recorded during representative full weight-bearing standing performed before stand training (pre), after stand training (post-stand), and after step training (post-step). The amount of external assistance is reported at the top of each panel. At post-stand and post-step, stimulation parameters adjusted for standing throughout stand training were used. Stimulation frequency, amplitude, and electrode configuration (cathodes in black, anodes in gray, and inactive in white) are reported for each participant. At post-stand and post-step, participant A53 was stimulated with four programs (P.1 to P.4) delivered sequentially at 10 Hz by the same electrode array, resulting in an ongoing 40 Hz stimulation frequency. EMG was recorded from the following muscles of the left lower limb: IL, iliopsoas; GL, gluteus maximus; MH, medial hamstring; VL, vastus lateralis; TA, tibialis anterior; SOL, soleus. **(B)** Coefficient of variation calculated for EMG activity (average value among the six investigated muscles) and ground reaction forces is reported in **A**.



**FIG. 5.** Electromyography (EMG) and ground reaction forces recorded during full weight-bearing standing after stand training with initial stimulation parameters. **(A)** Time course of EMG and ground reaction forces recorded during representative full weight-bearing standing performed after stand training with stimulation parameters selected prior to stand training. Stimulation intensity was adjusted for A45 after training. Amount of external assistance is reported at the top of each panel. Stimulation frequency, intensity, and electrode configuration (cathodes in black, anodes in gray, and inactive in white) are reported for each participant. EMG was recorded from the following muscles of the left lower limb: IL, iliopsoas; GL, gluteus maximus; MH, medial hamstring; VL, vastus lateralis; TA, tibialis anterior; SOL, soleus. **(B)** Coefficient of variation calculated for EMG activity (average value among the six investigated muscles) and ground reaction forces are reported in **A**.

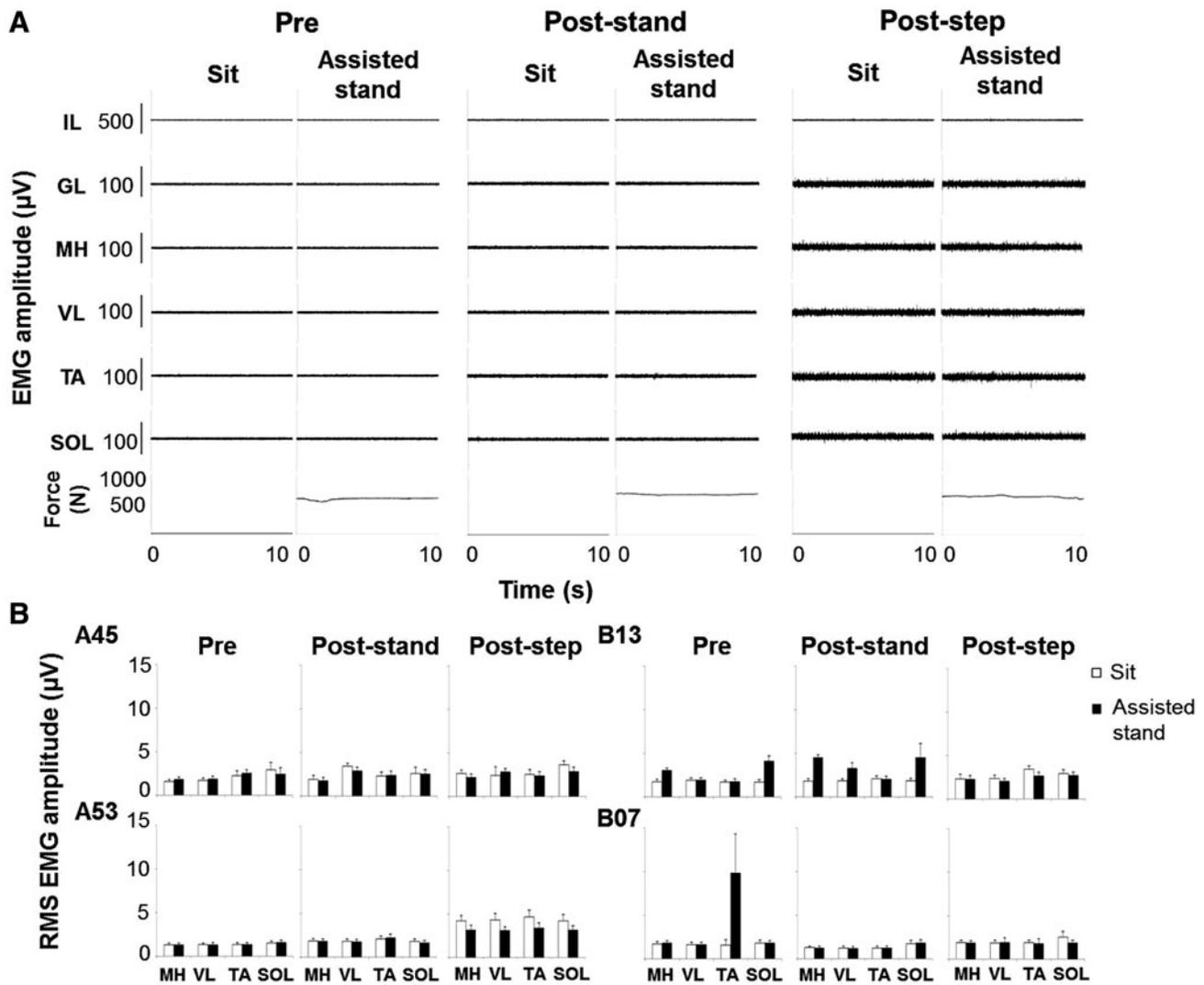
same stimulation parameters optimized for standing (at adjusted stimulation intensity) (Fig. 4A). However, participant A45 consistently required external assistance for maintaining knee extension. These observations were accompanied by an increment in CV values for both EMG activity (+10% and +50%) and ground reaction forces (+181% and +91%) in participants A45 and A53, respectively. The EMG pattern recorded from participants B13 and B07 was substantially different than at post-stand, showing EMG bursts followed by periods of low levels of EMG activity in most of the analyzed muscles, which resulted in greater CV values (+102% and +160%, respectively). These adaptations resulted in the need for external assistance for knee and hip extension and

unstable levels of ground reaction forces, its CV being 644% and 1632% greater than at post-stand in participants B07 and B13, respectively.

On the other hand, without epidural stimulation, all participants showed little or no EMG activity during both steady sitting and assisted standing at all three time points (Fig. 6).

#### *Effects of stand and step training on sitting-to-standing EMG amplitude modulation*

As exemplified in Figure 7A for one muscle (right VL, participant A45), most of the analyzed muscles of all participants showed



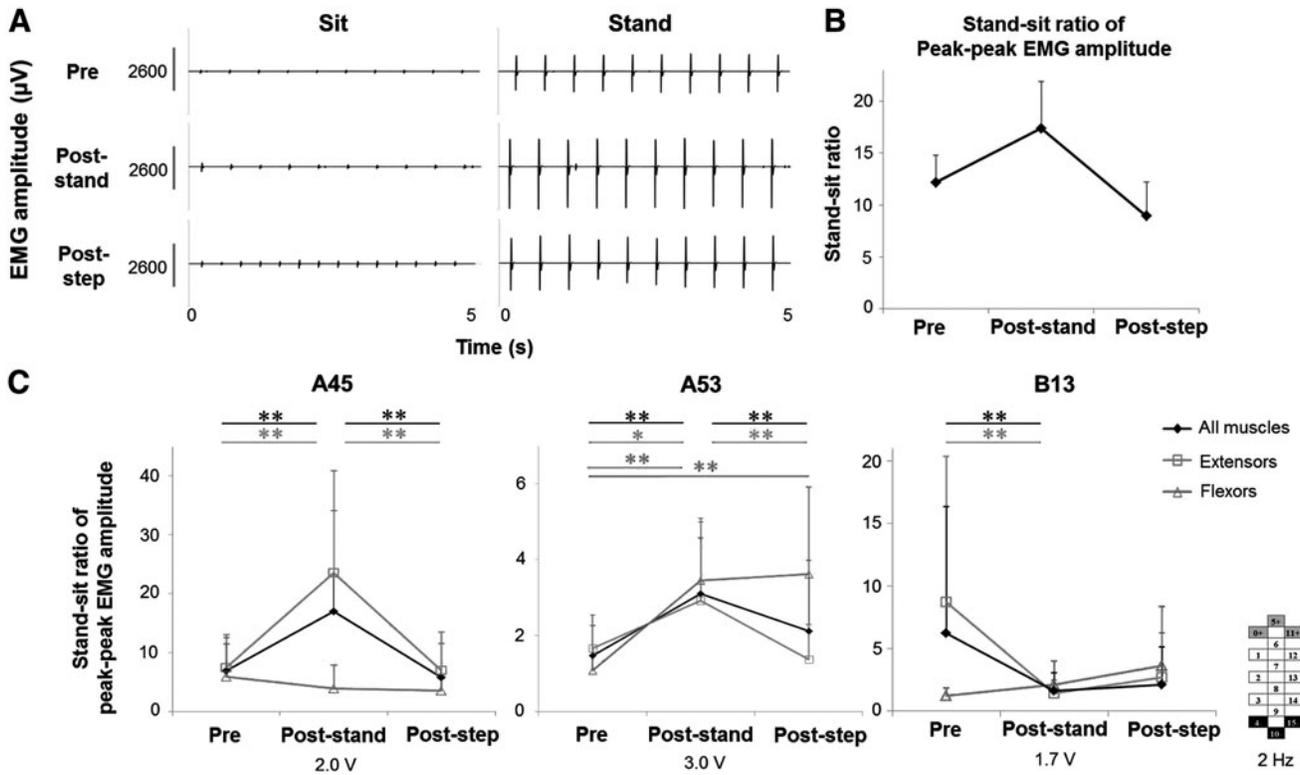
**FIG. 6.** Sitting and assisted standing without epidural stimulation before training, after stand training, and after step training. **(A)** Time course of electromyography (EMG) and ground reaction forces recorded during sitting and assisted standing without epidural stimulation before training (pre), after stand training (post-stand), and after step training (post-step) in participant A53. **(B)** EMG amplitude calculated as the root mean square (RMS) over 10 sec of steady sitting and standing. Standing data were recorded during overground full weight-bearing standing (participants A53 and B07) or during standing with a body weight load of 60% (participants A45 and B13) with external assistance for knee and hip extension provided by trainers. EMG was recorded from the following muscles of the left lower limb: IL, iliopsoas; GL, gluteus maximus; MH, medial hamstring; VL, vastus lateralis; TA, tibialis anterior; SOL, soleus.

greater peak–peak EMG amplitude in standing compared with sitting when the same stimulation parameters were applied. Interestingly, stand and step training affected the ratio between EMG amplitude recorded in standing and sitting, as exemplified in Figure 7B for the same right VL muscle. In particular, stand training induced a substantial increase (+215%,  $p < 0.0001$ ) in EMG amplitude stand–sit ratio for the primarily extensor muscles in participant A45 (Fig. 7C) compared with pre-training. Conversely, after step training, this parameter decreased significantly ( $p < 0.0001$ ) to values similar to pre-training (–7%). On the other hand, training did not significantly affect the EMG amplitude stand–sit ratio of primarily flexor muscles in the same participant ( $p = 0.321$ ), which maintained values comparable with pre-training (–34% at post-stand and –40% at post-step). When all investigated muscles were averaged together, stand training increased EMG amplitude stand–sit ratio by 145%

( $p < 0.0001$ ), whereas step training decreased this parameter to a value similar to pre-training (–16%).

Stand training led to an overall increase of EMG amplitude stand–sit ratio also in participant A53 (+110%,  $p < 0.0001$ ; Fig. 7C). Both extensors and flexors showed a similar positive trend compared with pre-training (+76%,  $p = 0.018$ , and +216%,  $p < 0.0001$ , respectively). As observed in participant A45, the subsequent step training significantly decreased the EMG amplitude stand–sit ratio of extensors and of all muscles pooled together ( $p < 0.0001$ ) to values similar to pre-training (–18% and +44%, respectively). Conversely, flexors maintained EMG amplitude stand–sit ratio value similar to post-stand and still greater than pre-training (+231%,  $p < 0.0001$ ).

As opposed to participants A45 and A53, stand training significantly decreased EMG amplitude stand–sit ratio of extensors (–84%,  $p < 0.0001$ ) and of all muscles averaged together (–74%,



**FIG. 7.** Effects of stand and step training on electromyography (EMG) amplitude modulation induced by the change in body position from sitting to standing. **(A)** Evoked potentials recorded from the right vastus lateralis (R VL) muscle of participant A45 during sitting and assisted standing before training (pre), after stand training (post-stand), and after step training (post-step). **(B)** Ratio between peak-peak EMG amplitude of evoked potentials (mean  $\pm$  SD;  $n=20$ ) recorded during standing and sitting from R VL of participant A45 at the three investigated time points. **(C)** Stand-sit ratio of peak-peak EMG amplitude ( $n=20$  evoked potentials considered for each muscle) expressed as an average among primary extensor muscles (left and right soleus, medial gastrocnemius, vastus lateralis, rectus femoris; empty squares;  $n=160$ ), among primary flexor muscles (left and right tibialis anterior and medial hamstring; empty triangles;  $n=80$ ), and as an average among all investigated muscles (left and right soleus, medial gastrocnemius, vastus lateralis, rectus femoris, tibialis anterior and medial hamstring; black diamonds;  $n=240$ ). Values are mean  $\pm$  SD. Differences were tested by Friedman test, and following multiple comparisons by Dunn's post-hoc test.  $*p \leq 0.05$ ;  $**p < 0.0001$ . Standing data were recorded during overground full weight-bearing standing (participant A53) or during standing with a body weight load of 60% (participants A45 and B13) with external assistance for knee and hip extension provided by trainers. Stimulation frequency, intensity, and electrode configuration (cathodes in black, anodes in gray, and inactive in white) are reported at the bottom of the figure.

**FIG. 8.** Characteristics of evoked potentials recorded with the subject in the supine position. **(A)** Evoked potentials to epidural stimulation recorded from the right vastus lateralis (participant A45) and from the right soleus (participant B13) prior to any training (pre), after stand training (post-stand), and after step training (post-step). The colored traces are the average of five responses represented in gray, which were those with the largest peak to peak amplitude being induced by the stimulation intensity corresponding to the arrows shown in **B** (recruitment curves). Data are shown from the time window between the stimulation onset and 50 ms following the stimulation. **(B)** Representative recruitment curves recorded at pre (diamonds), post-stand (squares), and post-step (triangles) are shown for participants A45 (right vastus lateralis) and B13 (right soleus) at stimulation intensities ranging from 0.1 to 5 V. Each peak-peak electromyography (EMG) amplitude value is reported as mean  $\pm$  SD of  $n=5$  evoked potentials. Arrows indicate the largest value at each time point. **(C)** Peak-peak EMG amplitude ( $n=5$  evoked potentials considered for each muscle) was expressed as percent of pre-training values, and was averaged among primary extensor muscles (left and right soleus, medial gastrocnemius, vastus lateralis, rectus femoris, gluteus maximus; empty squares;  $n=50$ ), among primary flexor muscles (left and right tibialis anterior and medial hamstring; empty triangles;  $n=20$ ), and as an average among all investigated muscles (left and right soleus, medial gastrocnemius, vastus lateralis, rectus femoris, gluteus maximus, tibialis anterior and medial hamstring; black diamonds;  $n=70$ ). Values are mean  $\pm$  SD. Differences were tested by Friedman test, and following multiple comparisons by Dunn's post-hoc test.  $*p \leq 0.05$ ;  $**p < 0.001$ . **(D)** Muscle activation threshold. For each individual, the underlined muscle was that with the lowest activation threshold detected before training (reference muscle). At each time point (pre, diamonds; after stand training, post-stand, squares; after step training, post-step, triangles), the activation threshold of each muscle was expressed as a percent of that observed for the reference muscle. Stimulation intensity range is reported for each participant. L, left; R, right; GL, gluteus maximus; MH, medial hamstring; RF, rectus femoris; VL, vastus lateralis; MG, medial gastrocnemius; SOL, soleus; TA, tibialis anterior. Stimulation frequency and electrode configuration (cathodes in black, anodes in gray, and inactive in white) are reported at the bottom of the figure.

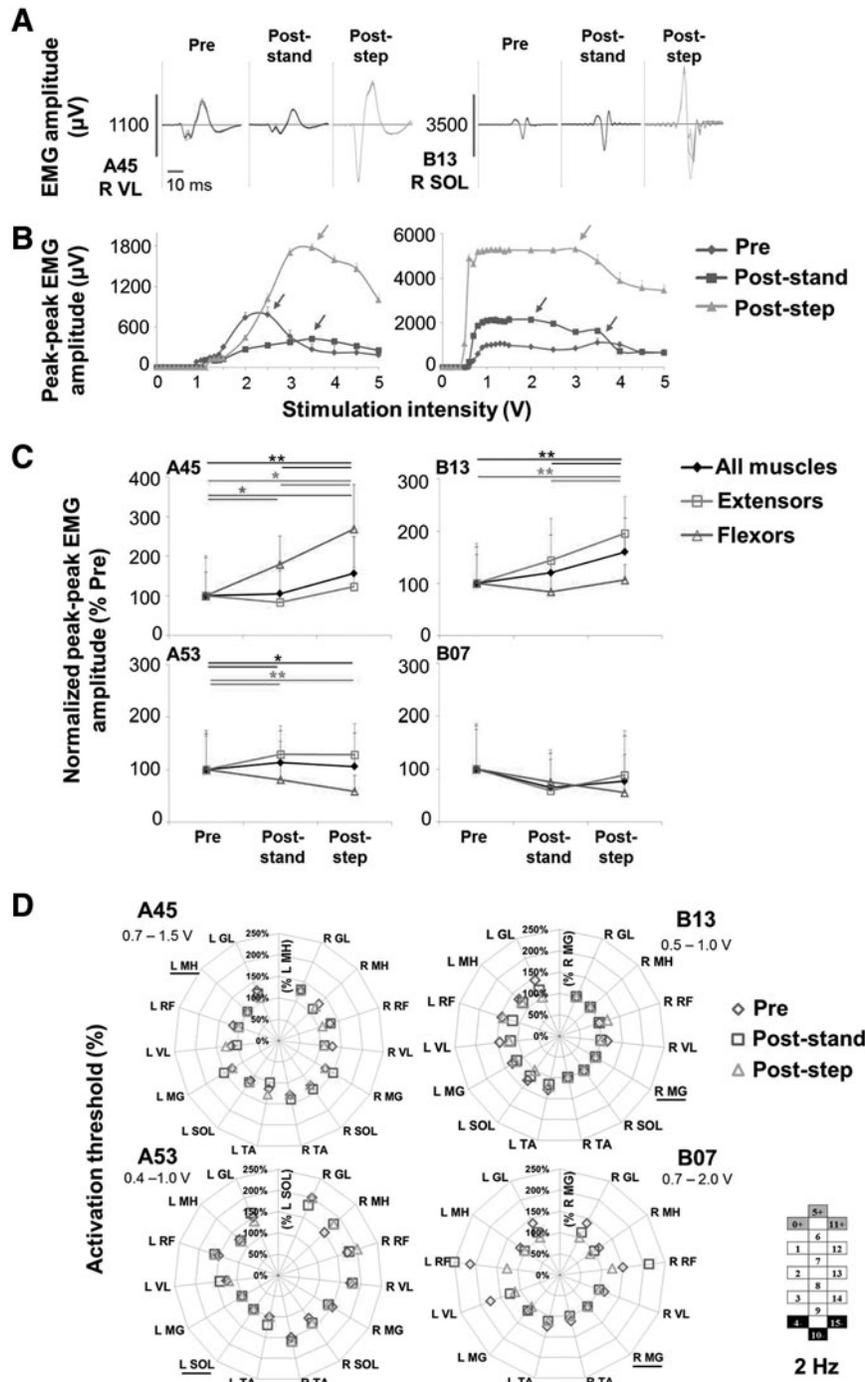
$p < 0.0001$ ) in participant B13 (Fig. 7C). This adaptation was not modified after the subsequent step training. On the other hand, as observed in participant A45, training did not significantly affect EMG amplitude stand–sit ratio of flexor muscles ( $p = 0.987$ ). This set of data was not recorded for participant B07.

*Characteristics of evoked potentials recorded with the subject in supine position*

Representative evoked potentials and recruitment curves during spinal epidural stimulation with the subject in the supine position recorded before training, after stand training, and after step training

are shown in Figure 8A and B. Stand training promoted limited adaptations in the amplitude of evoked potentials. In particular, peak–peak amplitude of flexor muscles increased after stand training in participant A45, whereas peak–peak amplitude of extensors and of all muscles pooled together increased in participant A53 (Fig. 8C). On the other hand, in three out of four individuals (B07 excluded), peak–peak amplitude of evoked potentials recorded from extensor muscles and from all examined muscles pooled together was significantly higher after step training than at pre-training.

Activation threshold of the analyzed muscles was not substantially affected by stand and step training in participants A45, B13, and A53 (Fig. 8D). In particular, the average absolute difference of



activation threshold among the analyzed muscles (activation thresholds difference) assessed for participant A45 at pre-training ( $24 \pm 7\%$ ) was not significantly modified by stand and step training ( $23 \pm 18\%$  and  $20 \pm 9\%$ , respectively;  $p=0.083$ ). In participant B13, stand training tended to decrease activation threshold of the left lower limb muscles (Fig. 8C) and to reduce the activation thresholds difference ( $9 \pm 8\%$ ;  $p=0.069$ ), which was  $19 \pm 16\%$  at pre-training. After step training, this parameter was equal to  $13 \pm 13\%$ , being not significantly different from pre-training and post-stand ( $p=0.172$  and  $0.980$ , respectively). Data recorded from participant A53 showed that a broader range of stimulation intensity was needed to detect evoked potentials in all investigated muscles at pre-training (activation thresholds difference =  $44 \pm 28\%$ ). Also in this participant, activation thresholds difference was not significantly affected by stand and step training ( $51 \pm 26\%$  and  $49 \pm 33\%$ , respectively;  $p=0.232$ ). On the other hand, step training (but not stand training) reduced substantially the activation threshold of RF in participant B07 (Fig. 8C) as well as the activation thresholds difference, which was equal to  $36 \pm 31\%$  at pre-training and  $8 \pm 10\%$  at post-step ( $p=0.017$ ).

## Discussion

We showed that stand training with epidural stimulation improved the ability to stand without assistance for knee extension with different time course and magnitude among four clinically motor complete SCI individuals, with participants A45 and A53 showing the greater improvement. After stand training, standing ability was better than at pre-training in all participants when stimulation parameters optimized for standing were applied; improvements were also observed in participant A45 and, to a lesser extent, in participants B13 and B07, when the initial stimulation parameters were tested. Interestingly, step training performed after stand training substantially impaired standing ability in all participants except A53. Improved standing ability generally coincided with continuous EMG patterns that led to constant levels of ground reaction forces. Conversely, poorer standing ability was associated with more variable EMG patterns that alternated EMG bursts and longer periods of negligible activity in most of the muscles, which resulted in unstable levels of ground reaction forces.

Stand training significantly increased the gain in EMG amplitude induced by the sensory information related to the change in body position from sitting to standing in the two participants with better standing ability (A45 and A53), whereas step training brought this parameter back to pre-training values. On the other hand, training-induced neural adaptations of the spinal circuitry examined by changes in evoked potentials amplitude and activation thresholds during a passive condition (lying supine) were limited after stand training and more pronounced after step training, and were not related to the observed changes in standing ability.

Finally, neither stand nor step training with epidural stimulation was sufficient to improve motor function for standing when spinal stimulation was not provided.

### *Synergistic effects of epidural stimulation and stand training on standing ability and motor pattern*

After stand training, all participants improved their ability to stand while bearing full body weight (Fig. 3). Interestingly, the ability to stand without assistance for knee extension improved with different time courses and magnitude throughout stand training in the four research participants (Fig. 2). The improved selection of stimulation parameters, neural adaptations brought about by

stand training, and individuals' characteristics were conceivably three major factors that influenced these findings.

### *Epidural stimulation*

Experimental studies<sup>32,36–39</sup> and computational models<sup>26,40</sup> provided evidence that lumbosacral spinal cord epidural stimulation engages spinal circuits mainly by recruiting dorsal root fibers carrying somatosensory signals from the limbs at their entry into the spinal cord as well as along the longitudinal portions of the fiber trajectories. To date, the prevailing view is that epidural stimulation impacts many different sensory-motor pathways simultaneously,<sup>32,27</sup> altering the excitability of spinal circuits to a level that can enable sensory information to become a source of motor control.<sup>5,9–11,41,42</sup> In particular, recent studies showed that the motor pattern promoted by epidural stimulation during locomotion is primarily modulated by proprioceptive, muscle spindle feedback circuits.<sup>42,43</sup> However, the stimulation parameters (i.e., site, electrode configuration, frequency, amplitude) are crucial determinants of the extent and proportion of the modulation of these sensory-motor pathways as well as of the motor output generated in rats<sup>26,27,42</sup> and humans.<sup>28,33</sup> For example, when motor complete SCI individuals were epidurally stimulated at a higher intensity in supine position, stimulation frequencies between 5 and 15 Hz were found optimal to elicit a tonic extensor motor pattern of the lower limbs, whereas a rhythmic EMG pattern was induced by higher frequencies (21–31 Hz) without any adjustment in stimulation site or intensity.<sup>33</sup> These results were interpreted as meaning that different stimulation frequencies at the same site of stimulation would access different inhibitory and/or excitatory pathways within the spinal cord networks to elicit different EMG patterns (i.e., rhythmic vs. tonic). Also, we previously showed that higher stimulation frequencies promoted more variable shapes and amplitudes of evoked potentials during standing with epidural stimulation when a relatively low stimulation intensity was applied and an overall continuous EMG pattern was recorded from the lower limb muscles.<sup>28</sup> The view that stimulation parameters are critical determinants of the motor output generated during standing is also supported by the observation that, after stand training, all four research participants of the present study were able to stand with much less external assistance when stimulated with individual-specific parameters that were optimized for standing throughout stand training rather than those selected prior to the beginning of stand training (Table 2). In addition, in some cases, the change in electrode configuration throughout stand training coincided with marked improvements of the ability to stand without knee assistance (i.e., participant A53 at sessions 56 and participant B07 at session 30, Fig. 2A).

### *Stand training*

Previous experiments showed that the mammalian spinal cord is capable of motor learning.<sup>12</sup> Indeed, the repetitive execution of a motor task over a period of weeks can promote the use-dependent strengthening of the targeted sensorimotor pathways; this was shown behaviorally,<sup>44</sup> neurochemically, and physiologically<sup>7,45,46</sup> in complete spinal cats and rats. In particular, observations in spinal cats demonstrated that stand training promoted and substantially increased the duration of full weight-bearing standing, and that this improvement as well as the underlying changes in motor pattern were largely attributable to the repetitive execution of that motor task.<sup>14,47</sup> Our results demonstrated that the combined effects of stand training and the optimization of stimulation parameters promoted improvements in standing ability (Figs. 2 and 3). Further, the facts

that knee-not-assisted time tended to increase throughout portions of stand training without any change in electrode configurations (i.e., participant A45 from session 5 to 36; participant A53 from session 18 to 55; Fig. 2A), and that some improvements in standing ability were shown in three individuals after stand training while using the initial stimulation parameters (Table 2), suggest that stand training *per se* played an important role in the progression of standing ability.

The improved standing behavior shown after stand training coincided with continuous EMG patterns that led to constant ground reaction forces (i.e., data collected at post-stand, Figs. 4 and 5, participants A45 and B13) as opposed to the activation patterns observed before training, which alternated EMG bursts and periods of little or negligible activity in most of the muscles (i.e., data collected at pre-training from participants A45, B13, and B07, Fig. 4). Previous observations based on spinal cord stimulation supported the view that neural networks within the human lumbosacral spinal cord have the capability to promote either continuous extensor motor patterns or rhythmic motor patterns depending on the different inhibitory and/or excitatory pathways accessed and the specific reconfiguration of the interneuronal network.<sup>33,48,49</sup> We hypothesized that the repetitive engagement of cutaneous and propriospinal circuits during the execution of bilateral full body weight-bearing standing strengthened the sensory motor pathways and interneuronal connections responsible for the promotion of continuous extension patterns that are required for standing.

The fact that participants A45 and A53 (AIS A) showed an overall greater standing ability than the two AIS B participants (Figs. 2 and 3) supports the view that clinically detectable sensory input from the lower limbs to the supraspinal structures was not required to achieve full weight-bearing standing without external assistance, and that sensory information projected to the human spinal circuitry plays a primary role in promoting motor patterns sufficient for standing. On the other hand, the lower neurological level of injury in the two individuals graded A compared with the two graded AIS B (Table 1) could have affected the level of trunk control<sup>50</sup> and hence contributed to the different standing ability. This speculation, however, needs further investigation. Also, individual characteristics of the lesion and following reorganization of the spinal circuitry and interneuronal function<sup>51–53</sup> likely contributed to the different standing ability across research participants.

#### *Stand and step training with epidural stimulation differentially affected standing ability and motor pattern*

Our results showed that ~80 sessions of step training performed subsequently to stand training impaired the ability to stand in three out of four individuals (participant A53 excluded, Fig. 3). In participants B13 and B07, poorer standing ability coincided with more variable motor patterns that alternated EMG bursts and periods of little or negligible activity in most of the muscles, resulting in unstable levels of ground reaction forces (Fig. 4). On the other hand, participant A45 lost his ability to stand without knee assistance even if the EMG pattern generated was still continuous. Standing and stepping differ considerably from each other in neural activation patterns and afferent information provided: standing involves a continuous bilateral activation of the lower limb muscles resulting in an antigravity extension pattern, whereas stepping involves a rhythmic, alternated recruitment of flexors and extensors. This fact is relevant considering previous findings that showed that training a specific motor task can impair the spinal cord ability to perform a different motor activity.<sup>12</sup> In particular, complete spinal cats trained to stand were not able to use their hindlimbs for

stepping.<sup>15</sup> Also, complete spinal rats trained to stand showed poorer ability to perform an untrained motor task that involved lower limb flexion compared with step-trained and untrained rats.<sup>16</sup> Interestingly, these adaptations were shown to be reversible, as complete spinal cats trained to stand learned to perform stepping over a 20 month period of step training.<sup>15</sup> On the other hand, the combination of step training with epidural stimulation and serotonergic agonists led to improvements in both stepping pattern and weight-bearing ability in complete spinal rats, enabling locomotion without body weight support.<sup>7</sup> This finding was also accompanied by physiological changes effective for standing, as indicated by the increased amplitude of motor evoked potentials recorded in both extensors and flexors during standing compared with before training. The fact that step training improved weight-bearing stepping as well as motor responses for standing in this experimental model might be partially explained by the concurrent use of quipazine (serotonergic agonist), the primary effect of which was the facilitation of extension pattern, and possibly by intrinsic differences in the spinal circuitry between rats and cats or humans.

However, although task-specific training alone has been shown to be sufficient for recovering weight-bearing standing<sup>14,54</sup> or stepping<sup>13,55</sup> in complete spinal cats, no evidence for a similar plasticity of lumbosacral networks has been found in humans with a clinically complete paralysis.<sup>17–20</sup> Consistently with these findings and with our hypothesis, we observed that months of both stand and step training performed while neuromodulating the physiological state of the lumbosacral spinal cord via epidural stimulation did not promote neural adaptations sufficient to improve motor function for standing when spinal stimulation was not provided (Fig. 6). Indeed, without stimulation, all four research participants achieved and maintained upright posture via external assistance provided by trainers, showing a muscle activation that was negligible and similar to that recorded during sitting.

#### *Training-induced modulation of evoked potentials recorded while subjects are standing, sitting, and supine*

Previous studies highlighted the fact that somatosensory information from the lower limbs play a significant role in modulating the motor output generated during standing and stepping with and without epidural stimulation in mammals.<sup>7,24,56–58</sup> We have also previously shown that the sensory information related to the transition from sitting to standing promoted the generation of EMG patterns sufficient for standing, increasing EMG amplitude in most of the lower limb muscles.<sup>9,28</sup> The results reported in the present study also showed that sensory information related to the change in body position from sitting to standing, without any change in the stimulation parameters, promoted an increased amplitude of the evoked potentials recorded from several lower limb muscles (Fig. 7A–C, stand–sit EMG amplitude ratio >1). Interestingly, stand training increased the stand–sit EMG amplitude ratio in participants A45 and A53, whereas step training brought this parameter back to pre-training values. These results support the view that the spinal circuitry reconfiguration induced by the transition from a passive condition (sitting) to the execution of standing motor task conceivably involved sensory motor pathways that were strengthened during stand training, but not during step training, in these two individuals. On the other hand, stand training decreased the stand–sit EMG amplitude ratio in participant B13, and this parameter was not changed significantly following step training. It is important to note that participants A45 and A53 achieved a

greater amount of standing without knee assistance throughout stand training (Fig. 2B), and an overall better standing ability (Figs. 2C and 3) than participant B13. This fact, as well as the individuals' characteristics (i.e., lesion and following plasticity of the spinal network) are plausibly two of the factors that might explain the different trend observed among individuals. Also, further studies are needed to investigate whether the stand-sit EMG amplitude ratio can be a valuable neurophysiological marker to monitor the training effect on the ability to stand bearing full body weight.

In an effort to understand whether stand and step training induced any adaptation in the spinal circuitry that could be detected in a passive condition (when the circuitry was not reconfigured by the execution of a motor task), the amplitude of evoked potentials and the activation threshold of several lower limb muscles were investigated with the research participants relaxed in supine position. After the first activity-based intervention (stand training), evoked potentials amplitude averaged among all the investigated muscles was larger than at pre-training in participant A53, whereas the same trend was shown for flexor muscles only in participant A45 (Fig. 8C). Conversely, after the subsequent step training, evoked potentials amplitude averaged among all the investigated muscles and among extensors was larger than at pre-training in three out of four individuals. These results suggest that spinal circuits that were not specifically involved in the execution of a motor task were also reinforced after activity-based training with epidural stimulation, and particularly after step training. However, the present data cannot discriminate whether step training intrinsically promoted greater plastic changes in the spinal circuits compared with stand training, or whether the cumulative neurobiological stimuli brought about by ~160 sessions of activity-based training with epidural stimulation, instead of ~80 sessions only, played a major role in promoting the abovementioned adaptations. On the other hand, muscle activation thresholds were generally not affected by activity-based training with epidural stimulation. An exception was shown by participant B07 after step training, which led to a decreased activation threshold, especially in those muscles that showed higher values at the previous time points (i.e., left and right R RF, Fig. 8D). As a consequence, activation thresholds were significantly more similar across the analyzed muscles after step training than at pre-training. Although further investigations are required to better understand the functional meaning of these neurophysiological changes observed with the individuals relaxed in supine position, at this stage they did not provide any information that can be associated to standing ability.

## Conclusion

In conclusion, the synergistic effects of stand training performed with lumbosacral spinal cord epidural stimulation and the optimization of stimulation parameters improved motor function for standing in all four research participants with chronic complete paralysis, allowing them to stand bearing full body weight with minimal self-assistance for balance and without any external assistance for knee extension. The two AIS A individuals were also able to stand without any external facilitation to promote hip extension. On the other hand, the subsequent step training substantially impaired standing ability in three out of four individuals. In addition, the stand and step training protocol performed in this study with spinal cord epidural stimulation did not promote neural adaptations sufficient to improve motor function for standing when spinal stimulation was not provided. These findings highlight the

potential of the human spinal circuitry to learn and optimize the generation of motor patterns effective for standing after months of task-specific training when the lumbosacral spinal cord is neuromodulated with appropriate epidural stimulation parameters. Further efforts should be addressed to the study of different activity-based rehabilitation protocols (i.e., interleaving stand and step training) in order to investigate whether the human spinal cord is capable of concurrently improving the generation of motor patterns appropriate for both standing and stepping.

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## Author Disclosure Statement

No competing financial interests exist.

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