Locomotor training improves premotoneuronal control after chronic spinal cord injury

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Knikou M, Mummidisetty CK. Locomotor training improves premotoneuronal control after chronic spinal cord injury. J Neurophysiol 111: 2264–2275, 2014. First published March 5, 2014; doi:10.1152/jn.00871.2013.—Spinal inhibition is significantly reduced after spinal cord injury (SCI) in humans. In this work, we examined if locomotor training can improve spinal inhibition exerted at a presynaptic level. Sixteen people with chronic SCI received an average of 45 training sessions, 5 days/wk, 1 h/day. The soleus H-reflex depression in response to low-frequency stimulation, presynaptic inhibition of soleus Ia afferents terminals following stimulation of the common peroneal nerve, and bilateral EMG recovery patterns were assessed before and after locomotor training. The soleus H reflexes evoked at 1.0, 0.33, 0.20, 0.14, and 0.11 Hz were normalized to the H reflex evoked at 0.09 Hz. Conditioned H reflexes were normalized to the associated unconditioned H reflex evoked with subjects seated, while during stepping both H reflexes were normalized to the maximal M wave evoked after the test H reflex at each bin of the step cycle. Locomotor training potentiated homosynaptic depression in all participants regardless the type of the SCI. Presynaptic facilitation of soleus Ia afferents remained unaltered in motor complete SCI patients. In motor incomplete SCIs, locomotor training either reduced presynaptic facilitation or replaced presynaptic facilitation with presynaptic inhibition at rest. During stepping, presynaptic inhibition was modulated in a phase-dependent manner. Locomotor training changed the amplitude of locomotor EMG excitability, promoted intralimb and interlimb coordination, and altered cocontraction between knee and ankle antagonistic muscles differently in the more impaired leg compared with the less impaired leg. The results provide strong evidence that locomotor training improves premotoneuronal control after SCI in humans at rest and during walking.

neuromodulation; neuroplasticity; H reflex; interlimb coordination; spinal reflexes and circuits

NEURAL CIRCUITS HAVE THE ABILITY to alter their structure and function in response to motor training (Adkins et al. 2006; Knikou 2010a), while activity-dependent neural plasticity accounts for improvement of motor performance in health and disease (Wolpaw and Tennissen 2001; Wolpaw 2007, 2010). One of the detrimental complications of spinal cord injury (SCI) is reduced locomotor ability. Improvements in locomotor ability and walking are reported after step training with body weight support (BWS) on a motorized treadmill in humans (Wernig et al. 1995; Dietz et al. 1998; Wirz et al. 2005; Dobkin et al. 2007; Field-Fote and Roach 2011) and animals beyond spontaneous recovery (Barbeau and Rossignol 1987; Bélanger et al. 1996; de Leon et al. 1998). However, the neuronal pathways that undergo adaptive changes in humans are poorly understood. These adaptive changes may be related to the return of the lost spinal inhibition after SCI in humans. This conclusion is based on the evidence we recently provided on the return of a physiological soleus H-reflex phase-dependent modulation in people with motor complete and motor incomplete SCI after step training (Knikou 2013). The restored phasic reflex excitability may be the result of changes in the synaptic efficacy of the afferent volley entering the spinal cord (Côté et al. 2003) driven in part by repeated appropriate sensory afferent feedback during walking. Presynaptic inhibition gates sensory afferent feedback before it reaches alpha motoneurons and has been ascribed to modulation of transmitter release at the Ia-motoneuron synapse by means of GABA_A receptors, which consequently increases the efflux of Cl^- ions and produces depolarization of the afferent terminals (Eccles 1964; Rudomin and Schmidt 1999). Presynaptic inhibition is modulated in a phase-dependent manner during fictive and real locomotion in both humans and animals (Ménard et al. 2003; Côté et al. 2003; Mummidisetty et al. 2013) and accounts for the soleus H-reflex phase-dependent modulation in uninjured humans (Faist et al. 1996).

Another form of presynaptic inhibition is that of homosynaptic depression, which is attributed in humans to similar mechanisms to those described in animals (Hultborn et al. 1996; Kohn et al. 1997). Mechanisms account for homosynaptic depression include decrease in the amount of released neurotransmitters (Kuno 1964), depletion of releasable vesicles, failure of action potential conduction at axonal branches (Brody and Yue 2000), decrease of presynaptic quantal size (Chen et al. 2004), and adaptation of exocytosis machinery (Hsu et al. 1996). Impaired function of presynaptic inhibition and homosynaptic depression in SCI (Tanaka 1983; Calancie et al. 1993; Aymard et al. 2000; Field-Fote et al. 2006; Grey et al. 2008) is expected to alter the synaptic efficacy of afferent impulses and contribute to stretch reflex hyperexcitability, clonus, and cocontractions, which characterize the spastic movement disorder after SCI.

We hypothesized that locomotor training can contribute to the return of spinal inhibition after SCI in humans. We demonstrate that locomotor training improves premotoneuronal control based on changes of the soleus H-reflex long-latency depression to heteronymous antagonistic conditioning afferent volleys and soleus H-reflex depression to homonymous affer-
ent discharges at low-stimulation frequencies. Reorganization of spinal networks coincided with improvements in locomotor electromyographic (EMG) activity and intralimb and interlimb coordination.

METHODS

Participants

Sixteen people with chronic SCI participated in the study. Study participation varied depending on the number of locomotor training sessions attended (Table 1) and ranged from 1.5 to 3.5 mo. One participant had neurological deficit grade A (no sensory or motor function preserved below the lesion), six had AIS B (sensory but not motor function preserved below the lesion), six had AIS C (more than half muscles below the lesion had muscle grade <3), and eight had AIS D (at least half key muscles below the lesion had muscle grade ≥3; Table 1). The level of SCI ranged from cervical 3 to thoracic 10. All participants signed an informed consent form before participation to the study for neurophysiological tests, clinical evaluation, and locomotor training, which was approved by Northwestern University (Chicago, IL) and the City University of New York (New York, NY) Institutional Review Boards. Subjects’ consent was obtained according to the Declaration of Helsinki. Subjects formed also a group in a previous study (Knikou 2013) and are identified here with the same code.

Table 1. Characteristics of participants

| ID   | Gender | Age, yr | Postinjury, yr | Level     | Cause of SCI | AIS scale | Clonus | ASIA (light touch) | ASIA (pin prick) | ASIA (motor) | LL          | RL          | Medication                  | No. of Training Sessions |
|------|--------|---------|----------------|-----------|--------------|-----------|--------|-------------------|-----------------|-------------|-------------|-------------|-------------|-----------------------------|--------------------------|
| R04  | F      | 35      | 12             | C3-C4     | MVA          | C         | 1LL, 1RL| 72                | 72              | 21          | 11          | None          | 57           | Not known                  | 57                       |
| R06  | F      | 46      | 1.5            | C5-C7     | MVA          | B         | 3LL, 3RL| 77                | 77              | 11          | 09          | Baclofen: 10 mg not frequent | 53                       |
| R07  | M      | 31      | 8              | C5-C7     | MVA          | A         | 3LL, 3RL| 76                | 40              | 23          | 23          | None          | 53                       |
| R08  | F      | 49      | 4              | T5-T7     | Fall         | D         | 1LL, 1RL| 75                | 75              | 47          | 37          | None during the study       | 60                       |
| R09  | M      | 44      | 3              | C5-C6     | Fall         | D         | 0LL, 0RL| 112               | 112             | 40          | 47          | Gabapentin: 3.6 g           | 30                       |
| R10  | F      | 52      | 11             | T7        | Fall         | D         | 1LL, 0RL| 78                | 78              | 41          | 49          | Diazepam: 15 mg             | 65                       |
| R11  | M      | 39      | 6              | C4        | GSW          | D         | 3LL, 3RL| 106               | 106             | 42          | 35          | Neurontin: 27 mg            | 64                       |
| R12  | M      | 41      | 1.5            | C5-C6     | MVA          | D         | 3LL, 3RL| 54                | 54              | 36          | 27          | Neurontin: 60 mg            | 55                       |
| R13  | F      | 39      | 7              | T4        | Transverse myelitis | C      | 3LL, 3RL| 112               | 74              | 34          | 27          | Gabapentin: 0.3 g           | 53                       |
| R14  | M      | 25      | 0.5            | C5-C6     | Diving       | D         | 1LL, 1RL| 112               | 110             | 49          | 29          | Baclofen: 20 mg             | 44                       |
| R15  | M      | 37      | 10             | C1        | Spinal Tumor | C         | 3LL, 2RL| 64                | 64              | 12          | 05          | None          | 36                       |
| R16  | M      | 49      | 2.5            | C5        | MVA          | C         | 0LL, 0RL| 64                | 64              | 30          | 24          | Baclofen: 15 mg             | 41                       |
| R17  | M      | 21      | 3              | T10       | GSW          | D         | 3LL, 3RL| 105               | 105             | 38          | 40          | Baclofen: 60 mg             | 48                       |
| R18  | M      | 29      | 2              | C7        | MVA          | D         | 1LL, 1RL| 86                | 86              | 50          | 45          | Gabapentin: 50.9 g          | 26                       |
| R19  | M      | 26      | 1              | C6        | Diving       | D         | 3LL, 3RL| 112               | 97              | 34          | 21          | Baclofen: 10 mg             | 20                       |
| R20  | M      | 55      | 3              | T6-T7     | Blood clot during spinal surgery | C | 2LL, 3RL| 82                | 82              | 33          | 34          | None          | 21                       |

Level of spinal cord injury (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; and 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 gravity and resistance; and 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 is also indicated. Ankle clonus was assessed as follows: 0, reaction; 1, mild, clonus was maintained <3 s; 2, moderate, clonus persisted between 3 and 10 s; and 3, severe, clonus persisted for >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.

Locomotor Training

All participants received BWS-assisted locomotor training with a robotic exoskeleton system (Lokomat Pro, Hocoma, Switzerland). Each participant was trained 5 days/wk, 1 h/day. The mean number of training sessions completed was 45.37 ± 14.86 (means ± SD; Table 1). The protocol employed to train persons with motor complete and motor incomplete spinal lesions has been previously published in detail (see Fig. 1 and Table 2 in Knikou 2013). Briefly, adjustments of treadmill speed, BWS, and position of toe straps over the course of training were implemented based on the presence or absence of knee buckling during the stance phase and toe dragging during the swing phase. These were assessed every five sessions along with the strength of quadriceps, triceps, and tibialis anterior (TA) muscles. The ultimate goal in motor incomplete SCIs was to reach a treadmill speed of 0.83 m/s at the lowest BWS without knee buckling or toe dragging. All subjects during the duration of the study did not receive conventional physical therapy and did not participate in other research studies.

Peripheral Nerve Stimulation

Tibial nerve. With the subject seated, a stainless steel plate electrode of 4 cm in diameter was placed and secured proximal to the patella. Rectangular single pulse stimuli of 1-ms duration were delivered by a custom-built constant current stimulator to the tibial nerve at the popliteal fossa. The most optimal stimulation site was established via a hand-held monopolar stainless steel head electrode used as a probe (Knikou 2008). An 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 (SureTrace; Conmed, Utica, NY) that was maintained under constant pressure throughout the experiment with an athletic wrap.

**Common peroneal nerve.** The common peroneal (CP) nerve was stimulated by a bipolar stainless steel electrode placed distal to the head of the fibula. The optimal stimulation site corresponded to the one that at increased levels of intensities the peroneus longus (PL) muscle was silent, the TA motor threshold was always lower than that of the PL muscle, and at increased stimulation intensities selective ankle dorsi flexion without ankle eversion was induced (Knikou 2005, 2008). The CP nerve was stimulated with a single shock of 1 ms in duration, generated by a constant current stimulator (DST7A; Digimer). The stimulus to the CP nerve was delivered at 0.9 to 1.2 TA M-wave threshold across subjects. In subjects in whom presynaptic facilitation was evident at rest when CP nerve stimulation was delivered below or at TA M-wave threshold, the stimulus intensity was adjusted to 1.2 TA M-wave threshold to ensure that the effects were not due to the conditioning stimulus intensity. In subjects in whom the conditioned H reflex did not alter from control reflex values but homosynaptic facilitation was absent, the CP nerve was stimulated at 0.9 TA M-wave threshold. The TA M wave was monitored throughout the experiment to ensure constancy of the conditioning stimulation. For each subject, the conditioning stimulus after training was delivered at the same multiples of TA M-wave threshold to that utilized before training.

**Changes in Premotoneuronal Control**

The neurophysiological tests described below were conducted before and 2 days after step training in the morning on separate days. Recordings posttraining for each subject were conducted at similar BWS levels, treadmill speeds, and M-wave amplitudes to those utilized before training. During stepping, stimulation was triggered based on the signal from the ipsilateral foot switch (MA153; Motion Lab Systems, Baton Rouge, LA). In all subjects, the step cycle was divided into 16 equal bins. Bin 1 corresponds to heel contact. Bins 6, 9, and 16 correspond approximately to stance-to-swing transition, swing phase initiation, and swing-to-stance transition, respectively. EMG and foot switch signals (Motion Lab Systems) were filtered with a cut-off frequency of 10–1000 Hz and sampled at 2,000 Hz using a data acquisition card (NI PCI-6225; National Instruments, Austin, TX).

**Experiment 1: presynaptic inhibition of soleus la afferents.** Having established the most optimal stimulation sites of peripheral nerves, the soleus maximal M wave (Mmax) was evoked and saved for offline analysis. The stimulation intensity was adjusted to evoke control H reflexes that ranged from 20 to 40% of the Mmax. Twenty reflexes elicited at 0.2 Hz were recorded with subjects seated. Then, the effects of CP nerve stimulation on the soleus H reflex at the conditioning-test (C–T) intervals of 20, 60, 80, and 100 ms were established. The soleus H-reflex depression at these C–T intervals has been attributed to presynaptic inhibition of la afferents mediating the afferent volley of the test reflex because the same conditioning afferent volley does not modify the motor evoked potentials at the same long C–T intervals (Capaday et al. 1995) and is long lasting similar to that reported in animals (Eccles and Willis 1962). The C–T interval during which the soleus H reflex was depressed, remained unaltered, or was less facilitated was utilized during assisted stepping. Given the task-dependent modulation of spinal inhibition (Lavoie et al. 1997), a change in body position constitutes a limitation of the study, but this approach was selected because presynaptic inhibition predominates over other spinal inhibitory mechanisms in standing human subjects (Goulart and Valls-Solé 2001).

Each subject was transferred to the treadmill and wore an upper body harness that was connected to overhead pulleys. Thigh and shank segments of the exoskeleton were adjusted based on each subject’s leg length and diameter, and both feet were secured into the foot lifters. With the subject standing at a BWS similar to that utilized during stepping, approximately 80–130 stimuli were delivered at 0.2 Hz to construct the soleus M-wave and H-reflex recruitment curves (Knikou et al. 2009).

The orientation of the recording EMG electrode with respect to the underlying muscle fibers changes during walking. The knee joint during the swing phase moves the stimulating electrode away from the tibial nerve, while knee extension during the stance phase has the opposite effect. To counteract these confounding factors, a supramaximal stimulus to the tibial nerve was delivered 60–80 ms after the test H reflex at each bin of the step cycle (Knikou et al. 2009, 2011; Knikou and Mummendidsetty 2011). The customized Labview software measured the peak-to-peak amplitude of the M wave and Mmax recorded during stepping and used a self-teaching algorithm to adjust the stimulus intensity at each bin of the step cycle. Adjustment of stimulus intensities was based on the amplitude of the M wave as a percentage of the Mmax 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 Mmax (Knikou 2008, 2013; Knikou et al. 2009). These criteria applied to conditioned and unconditioned H reflexes, which were randomly recorded during stepping. The experiment was concluded when at least five accepted conditioned and unconditioned H reflexes were recorded at each bin of the step cycle.

**Experiment 2: homosynaptic depression of the soleus H reflex.** With subjects seated (hip angle 120°, knee angle 160°, and ankle angle 110°) and both feet supported by footrests, the Mmax was evoked and measured as peak-to-peak amplitude on a digital oscilloscope. The stimulus intensity was adjusted to evoke an H reflex and an M wave on the ascending part of the recruitment curve that ranged from 20 to 40% and from 4 to 8% of the Mmax across subjects, respectively. Twenty soleus H reflexes were evoked at this stimulation intensity randomly at 1.0, 0.33, 0.20, 0.14, 0.11, and 0.09 Hz. Soleus H reflexes at these frequencies were recorded from the right leg (AIS A = 1, AIS B = 1, AIS C = 6, and AIS D = 8) and from the left leg (AIS A = 0, AIS B = 1, AIS C = 2, and AIS D = 7) before and after locomotor training with subjects seated.

**Data Analysis**

**Presynaptic inhibition.** All compound muscle action potentials recorded with subjects seated and during stepping were measured as peak-to-peak amplitude of the nonrectified waveforms. The soleus H reflexes (n = 20) conditioned by CP nerve stimulation with subjects seated were expressed as a percentage of the mean amplitude of the unconditioned H reflex. The mean normalized conditioned soleus H reflex from each subject was grouped based on the time of testing, AIS scale, and C–T interval and a repeated-measures ANOVA was conducted to establish statistically significant differences in presynaptic inhibition.

The conditioned and unconditioned soleus H reflexes recorded during stepping were normalized to the associated Mmax evoked at each bin of the step cycle. For each subject, the mean amplitude of the accepted conditioned soleus H reflexes (based on the M-wave amplitude) before training was compared with that recorded after training at each bin with a Wilcoxon rank-sum test. The mean amplitude of the conditioned soleus H reflexes recorded before and after training was grouped based on the bin number and AIS scale, and statistically significant differences were established with a Kruskal-Wallis rank-sum test when data were not normally distributed and with repeated-measures ANOVA when data were normally distributed. Bonferroni test for multiple comparisons was used when statistically significant differences were detected.
To estimate changes in the amount of presynaptic inhibition during stepping, the unconditioned H reflex at each bin of the step cycle was subtracted from the associated conditioned H reflex (M waves for both reflexes ranged from 3 to 9% of the M\text{max}), both normalized to the M\text{max} evoked at each bin (Mummidisetty et al. 2013). This was done to counteract the inherent modulation of the M\text{max} and changes in the soleus H-reflex modulation pattern before and after training during walking. The mean amplitude of the subtracted conditioned H reflex was grouped across subjects based on the bin number and time of testing, and the overall amplitude was estimated. A resultant positive value indicates a condition associated with decreased spinal inhibition, while a negative value indicates increased inhibition at a premotorneuronal level (Mummidisetty et al. 2013).

The soleus H reflexes (n = 20) evoked at 1.0, 0.33, 0.20, 0.14, and 0.11 Hz for each subject were expressed as a percentage of the H reflex evoked at 0.09 Hz before and after training. The mean normalized amplitude of the soleus H reflex from each subject was grouped across subjects based on the bin number and time of testing, and an ANOVA for repeated measures at 2 × 5 levels (2: time; 5: stimulation frequencies) was conducted to establish statistically significant differences in homosynaptic depression across stimulation frequencies before and after training.

The soleus H-reflex gain for conditioned and unconditioned trials during walking was established by estimating the background activity of the ipsilateral soleus muscle (SOL) for each bin of the step from the mean value of the filtered and rectified EMG (band-pass filtered 20–400 Hz) for 60 ms beginning 140 ms before tibial nerve stimulation. The SOL background activity was then normalized to the maximal locomotor EMG background activity. The mean amplitude of the conditioned and unconditioned soleus H reflexes (normalized to the M\text{max} evoked at each bin) was plotted on the y-axis (dependent variable) vs. the associated normalized SOL background activity (independent variable) on the x-axis, respectively. The slope from the linear least-square regression reflects the reflex gain.

Last, the M waves of the unconditioned and conditioned H reflexes during stepping before and after training were normalized to the associated M\text{max} evoked at each bin of the step cycle. The normalized M waves were grouped across subjects based on the bin of the step cycle, time of testing, and type of H reflexes (conditioned vs. unconditioned), and statistically significant differences were established with a three-way ANOVA. This analysis was conducted separately for M waves recorded during stepping in AIS C and AIS D subjects. In all statistical tests, significant differences were tested at 95% of confidence level. Results are presented as mean estimates along with the means ± SE, unless otherwise stated.

**Locomotor EMG.** EMG recovery patterns were established for the SOL, medial gastrocnemius (MG), PL, TA, medial hamstrings (MH), lateral hamstrings (LH), hip adductor gracilis (ADD), vastus lateralis (VL), and vastus medialis (VM) muscles bilaterally. For each subject, EMG signals during stepping before and after locomotor training in absence of peripheral nerve stimulation were full-wave rectified, band-pass filtered (20–1,000 Hz), and plotted as a function of the step cycle. The percentage of change of the maximal EMG after training was also determined. Intralimb and interlimb coordination was determined by locomotor EMG probability distribution plots obtained from the rectified SOL, TA, and MG muscles of the left and right legs before and after training. To elucidate further recovery of EMG, we estimated changes in the cocontraction between the antagonistic muscles. For this, coactivation indexes (CI) between SOL/TA and MH/VM were estimated from the filtered (band-pass filter 40–500 Hz) and rectified EMG for each bin of the step cycle based on Eq. 1 (Kellis et al. 2003). The example shown in Eq. 1 is for the antagonistic pair of SOL and TA muscles at bin 1. Full coactivation between the antagonistic muscles is represented by a CI equal to 100%. CI before and after locomotor training were compared with repeated measures ANOVA at 32 levels.

**RESULTS**

**Presynaptic Inhibition of Soleus Ia Afferents Before and After Locomotor Training**

In motor complete SCI, the soleus H reflex conditioned by excitation of antagonistic group I afferents reached control reflex values when recordings were taken before and after training (Fig. 1A). In contrast, the conditioned H reflexes in motor incomplete SCIs were either facilitated or unaltered before training and were significantly depressed compared with control reflex values after training (Fig. 1A). Presynaptic inhibition of soleus Ia afferents with subjects seated was reorganized in motor incomplete SCI but not in complete SCI (AIS A–B) (Fig. 1B; P > 0.05). In AIS C, the conditioned soleus H reflex was decreased at the C–T interval of 60 and 80 ms after training compared with that recorded before training (P < 0.05; Fig. 1C). The conditioned soleus H reflex varied significantly with respect to the time of testing (F(1,3) = 6.21, P = 0.02) and C–T interval (F(1,3) = 3.55, P = 0.04). Similarly, in AIS D subjects, presynaptic facilitate at the C–T intervals of 60 and 80 ms was replaced by presynaptic inhibition after training (Fig. 1D), while at the C–T interval of 100 ms the presynaptic inhibition was potentiated after training. The conditioned soleus H reflex varied significantly with respect to the time of testing (F(1,3) = 12.42, P = 0.002) but not with respect to the C–T interval tested (F(1,3) = 2.28, P = 0.10).

The overall average amplitudes of the conditioned and unconditioned H reflexes expressed as a percentage of the M\text{max} evoked at each bin during BWS-assisted stepping before and after locomotor training for AIS C subjects are shown in Fig. 2, A and B, respectively. Before training, the conditioned soleus H reflex was significantly decreased at swing-to-stance transition (bin 16) compared with the unconditioned soleus H reflex (P < 0.05; Fig. 2A), while after training the conditioned soleus H reflex was not significantly different from the unconditioned H reflex across all phases of the step cycle (P > 0.05; Fig. 2B). The conditioned H reflex recorded after training was significantly different from the conditioned soleus H reflex recorded before training at early, mid-, and late-swing phases (bins 10–14; P < 0.05; Fig. 2B). These changes were observed at similar amplitudes of M waves before and after training in all subjects (see Fig. 2, Aa and Bb). The M waves were not statistically significant different across bins (F(15) = 0.72, P = 0.76) or time of recording (F(1) = 0.56, P = 0.45). A significant interaction for the M-wave values between bins, time of testing, and type of H reflexes was not found (F(1,15) = 0.12, P = 1.0). In Fig. 2C, the estimated modulation of presynaptic inhibition in AIS C subjects during BWS-assisted stepping is indicated with respect to the time of testing. Presynaptic inhibition was increased at early and mid-stance (bins 3 and 5), during the swing phase (bins 11, 13, and 15), and during the swing-to-stance transition (bin 16) before training (Fig. 2C), while after training presynaptic inhibition decreased at heel contact (bin 1) and was further increased during the swing phase and at the swing-to-stance transition (Fig. 2C).

\[ CI_{SOL1} = \frac{\int_{0}^{bin} EMG_{SOL}(t) dt}{\int_{0}^{bin} (EMG_{SOL} + EMG_{TA})(t) dt} \times 100 \]
The amplitude modulation of the conditioned and unconditioned soleus H reflex in AIS D subjects before and after training is indicated in Fig. 2, D and E, respectively. Before and after training, the conditioned soleus H reflex was significantly decreased at late swing and at swing-to-stance transition (bins 15 and 16) compared with the unconditioned soleus H reflex ($P < 0.05$; Fig. 2, D and E). These changes were observed at similar amplitudes of M waves before and after training in all subjects (see Fig. 2, Dd and Ee). The M waves were not statistically significant different across bins ($F_{15} = 0.83$, $P = 0.64$) or time of recording ($F_1 = 0.53$, $P = 0.35$). A significant interaction for the M-wave values among bins, time of testing, and type of H reflexes was not found ($F_{1,15} = 0.56$, $P = 0.89$). In Fig. 2F, the estimated relative changes of presynaptic inhibition in AIS D subjects during BWS-assisted stepping are indicated with respect to the time of testing. Before training, presynaptic inhibition was increased at late-stance (bin 7; $P < 0.05$), while after training presynaptic inhibition was potentiated at swing-to-stance transition phase (bin 16; $P < 0.05$; Fig. 2F).

The $y$-intercept of the linear regression between the conditioned H reflex and the SOL background activity reached overall amplitudes of $14.1 \pm 3.6$ and $0.36 \pm 0.97$ before and after training (paired $t$-test, $P = 0.09$), while the slope reached overall amplitudes of $113.6 \pm 72.2$ and $32.06 \pm 18.2$ before and after training (paired $t$-test, $P = 0.14$), suggesting that the soleus motoneuron gain was similar before and after training.

**Homosynaptic Depression of Soleus H reflex Before and After Locomotor Training**

Soleus H-reflex nonrectified waveform averages recorded at different stimulation frequencies from one representative subject (R06, AIS B) before and after training for both legs are indicated in Fig. 3. It is apparent that after training the soleus H-reflex amplitude exhibited a stimulation frequency-dependent depression.
The overall amplitude of the soleus H reflex recorded from the left and right legs at 1.0, 0.33, 0.2, 0.14, and 0.11 Hz before and after locomotor training is indicated in Fig. 4. H reflexes are presented as a percentage of the mean amplitude of the H reflex recorded at 0.09 Hz and are grouped together for AIS A–B subjects (Fig. 4, A and B), AIS C subjects (Fig. 4, C and D), and AIS D subjects (Fig. 4, E and F). The soleus H reflex from the right leg in AIS A–B subjects varied significantly at different stimulation frequencies ($F_{8,1} = 5.11, P = 0.04$) and with respect to the time of testing ($F_{8,1} = 10.7, P = 0.007$), with the soleus H reflexes at 1.0 Hz and at 0.33 Hz before training to be significant different from those recorded after training (Fig. 4B). This was also the case for H reflexes recorded at varying stimulation frequencies of the left leg before and after training (Fig. 4A). In AIS C subjects, the soleus H reflex from the right leg varied significantly at different stimulation frequencies ($F_{5,1} = 6.94, P < 0.001$) and with respect to the time of testing only at the stimulation frequency of 1.0 Hz (Fig. 4D). This was the case also for H reflexes recorded at varying stimulation frequencies at the left leg, but H reflexes did not vary before and after training. In AIS D subjects, the soleus H reflex from the right leg varied significantly at different stimulation frequencies ($F_{5,1} = 13.4, P < 0.001$) and with respect to the time of testing ($F_{5,1} = 5.60$, $P = 0.007$).
with the soleus H reflexes at 1.0 Hz and at 0.33 Hz before training to be significant different from those recorded after training (Fig. 4F). This was, however, not the case for the left leg, in which the soleus H reflex varied significantly at different stimulation frequencies ($F_{5,1} = 13.8, P < 0.001$) but not between the time of testing ($F_{5,1} = 0.0068, P = 0.93$).

Recovery of Locomotor EMG, Limb Coordination, and Cocontraction

Bilateral increased EMG activity in soleus muscle coincided with increases in bilateral MG and TA EMG activity after training compared with that observed before training (Fig. 5A). These data suggest that there was greater recruitment of ankle extensor and flexor motoneurons with locomotor training. In contrast, for the knee/thigh flexor muscles and specifically for the medial hamstrings and hip adductor gracilis muscles, a uniform increase in locomotor EMG activity after training was not evident. Specifically, the EMG activity in the medial hamstrings was increased by 60% in the left leg and decreased by 20% in the right leg (Fig. 5B). A similar effect was observed for the lateral hamstrings and hip adductor muscles. The decreased EMG activity of hip flexors, established with locomotor training, most likely is necessary for reducing the amplitude of the spastic gait pattern.

Locomotor training promoted intralimb coordination as reflected by the L-shaped pattern based on the probability distribution plots of the right MG with the right TA, left MG with the left TA, and the left SOL with the left TA locomotor EMG activity obtained after locomotor training (Fig. 5C). However, interlimb coordination was not improved as much as intralimb coordination, being evident in the TA and MG muscles, while SOL EMG coordination between the right and left legs was not improved after training (Fig. 5C). These findings indicate that asynchronous locomotor EMG can become synchronous with respect to the phase of the step cycle, consistent to findings reported in spinalized animals (Martinez et al. 2013). The lack of left and right soleus coordination may be due to 1) neuronal changes occur before changes can be depicted clearly on the locomotor function, 2) SOL interlimb coordination requires more locomotor training sessions, and 3) that an increased locomotor EMG activity with training does not necessarily implicate physiological muscle behaviors since this increased EMG activity may be associated also with increased clonus and cocontraction during walking.

The mean coactivation indices for the SOL/TA and MH/VM pairs from the left and right legs for each bin of the step cycle before and after locomotor training are indicated in Fig. 6. The SOL/TA coactivation index in the left leg was statistically

$P = 0.02$), with the soleus H reflexes at 1.0 Hz and at 0.33 Hz before training to be significant different from those recorded after training (Fig. 4F). This was, however, not the case for the left leg, in which the soleus H reflex varied significantly at different stimulation frequencies ($F_{5,1} = 13.8, P < 0.001$) but not between the time of testing ($F_{5,1} = 0.0068, P = 0.93$).
significant different between the time of testing ($F_{1,15} = 4.6$, $P = 0.03$) but not across the bins of the step cycle ($F_{15} = 0.32$, $P = 0.99$), suggesting that cocontraction between ankle antagonistic muscles decreased after training (Fig. 6A). In contrast, the cocontraction between the knee antagonistic muscles in the left leg increased after training (Fig. 6B; $F_{1,15} = 4.6$, $P = 0.003$). An opposite adaptation in cocontraction of antagonistic muscles was observed in the right leg (Fig. 6, C and D).
SOL/TA coactivation in the right leg increased after training ($F_1 = 41.54, P < 0.001$) but decreased between the knee antagonistic muscles after training ($F_{1,15} = 10.8, P = 0.001$).

**DISCUSSION**

We hypothesized that locomotor training alters the premotoneuronal control after SCI in humans. We demonstrate four novel findings on the reorganization of spinal inhibitory circuitries and muscle function: 1) locomotor training reverses presynaptic facilitation to presynaptic inhibition in motor incomplete but not in motor complete spinal injuries; 2) locomotor training potentiates homosynaptic depression regardless the type of the SCI; 3) locomotor training promotes intralimb and interlimb coordination; and 4) changes the amplitude of cocontraction between knee and ankle antagonistic muscles differently in the more impaired leg compared with the less impaired leg.

In complete SCI, presynaptic facilitation of soleus Ia afferents was evident both before and after training (Fig. 1B), while in motor incomplete SCI locomotor training either reduced presynaptic facilitation or replaced presynaptic facilitation with presynaptic inhibition (Fig. 1, C and D). These findings suggest that for restoration of presynaptic inhibition the descending drive might play a decisive role and that when descending drive is partially present locomotor training can change in a functional manner the premotoneuronal control mediated by heteronymous muscle afferents. The latter is supported by the adaptive changes of presynaptic inhibition during stepping. In motor incomplete spinal injuries, presynaptic inhibition was increased at early and mid-stance, during the swing phase, and during the swing to-stance transition before training, while after training it was decreased at heel contact and was further potentiated during the swing phase and at the swing-to-stance transition (Fig. 2, C and F). This phase-dependent modulation of the presynaptic inhibition is similar to that we have recently reported in healthy control subjects under similar experimental conditions (Mummidisetty et al. 2013) and may account for the return of the soleus H-reflex phase-dependent modulation we recently reported for the same patients after locomotor training (Knikou 2013).

Repetitive activation of Ia afferents at a low frequency reduces substantially monosynaptic excitation, a neuronal phenomenon known as homosynaptic depression and ascribed to presynaptic inhibition related to depletion of neurotransmitters (Hultborn et al. 1996). Limited evidence exists on the reorganization of this spinal inhibitory mechanism with motor training. In uninjured humans, potentiation of homosynaptic depression depends on the type of training since it is increased after cycling (Mazzocchio et al. 2006; Meunier et al. 2007) and not after training with simple unloaded ankle movements.
(Jessop et al. 2013). Homosynaptic depression was increased after just 10 locomotor training sessions in one SCI subject capable of ambulation (Trimble et al. 1998) and after cycling in one person with spastic tetraplegia (Kiser et al. 2005), consistent to the potentiation of homosynaptic depression reported for complete spinal transected rats after passive exercise (Reese et al. 2006). This work revealed that homosynaptic depression was potentiated in the more impaired right leg (see ASIA motor scores in Table 1) regardless the type of the SCI and was potentiated in the left leg only in motor complete SCIs supporting selective neuronal changes at the spinal level.

Mechanisms of Activity-Dependent Plasticity of Presynaptic Inhibition

In complete SCI, locomotor training likely reactivated the spinal locomotor networks through reinforcement of activity-dependent sensory feedback from receptors known to affect locomotor activity (Knikou 2010a; Rossignol 2006). For example, plantar cutaneous afferents can normalize the function of monosynaptic and polysynaptic spinal reflexes during stepping in untrained spinal cord-injured patients (Knikou 2010b), increase limb extension during swimming in spinal hemisected chicks (Muir and Steeves 1997), evoke a phase-dependent modulation of primary afferent depolarization (Ménard et al. 2002), and alter their effects on spinal motoneurons in spinalized cats after step training (Côté and Gossard 2004). Since plantar cutaneous afferents interact with presynaptic inhibitory interneurons in humans at rest and in spinalized cats during fictive locomotion (Iles 1996; Ménard et al. 2003), it is highly likely that the changes in premotoneuronal control we observed here could have been partly mediated by altered transmission in cutaneous pathways. Changes in the strength of the depolarization of muscle afferents constitute also a possible source of neuronal adaptation, since their efficacy of transmission is modulated as a function of the different phases of the step cycle (Dubuc et al. 1985; Gossard et al. 1991; Gossard 1996). It should also be noted that primary afferent depolarization interneurons are under descending control (Lundberg and Vycklympy 1963; Rudomin et al. 1983), and thus the possibility for concomitant spinal and supraspinal reorganization cannot be ruled out.

In incomplete SCIs, the neuronal reorganization is more complex because neuronal structures above the lesion site might adapt the function and behavior of spinal neuronal circuitries known to control locomotor activity through remnant descending pathways. A question that arises is whether reorganization occurs remotely and away from the lesion site or it occurs at the spinal neuronal circuitries. Evidence from animal studies suggests that intrinsic properties of spinal neurons can change with locomotor training. Locomotor training affected the amplitude of the action potential hyperpolarization, stabilized the duration of afterhyperpolarization and the synaptic inputs to motoneurons (Petruska et al. 2007), and decreased the amplitude of monosynaptic and polysynaptic excitation to extensor motoneurons (Côté et al. 2003) in absence of changes in peripheral nerve axons or muscle fibers. Because the duration of afterhyperpolarization was not modified by training, the adaptation of monosynaptic and polysynaptic excitation after training was attributed to changes in the strength of premotoneuronal mechanisms (Côté et al. 2003; Côté and Gossard 2004). Furthermore, locomotor training increased the density of the glycinergic axonal terminals and decreased the size of both glycinergic and GABAergic axon terminals in complete spinal trained transected rats compared with nontrained transected rats (Bras et al. 2013). These findings further support for changes in the intrinsic properties of spinal interneurons with locomotor training. Last, cats subjected to hemisection regained bilateral locomotor ability within 3 wk of training and 1-dy after they were subjected to complete spinal cord section (Gossard et al. 2013), supporting plastic changes that are intrinsic to spinal cord networks. On the other hand, strong evidence support for adaptation of remnant descending pathways with locomotor training in persons with incomplete SCI (Benito-Penalva et al. 2010; Hajela et al. 2013; Thomas and Gorassini 2005; Winchester et al. 2005), which may be mediated through the lumbar propriospi- nal system (Marchand-Pauvert and Nielsen 2002). Based on the discussed evidence, adaptation of premotoneuronal control with locomotor training may be mostly spinal in origin, but we cannot ignore the possibility for an activity-dependent neuromodulation and metaplasticity throughout the central nervous system in incomplete spinal lesions.

Recovery of Motor Function

Reorganization of premotoneuronal control coincided with significant improvements in motor function and locomotor ability. Locomotor EMG activity was increased in distal ankle muscles of both legs but decreased in knee/high flexor (hamstrings and hip adductor) muscles (Fig. 5), supporting that locomotor training improves locomotor function by decreasing the activity of the muscles that contribute primarily to the spastic gait pattern. In addition, the changes in intra- and interlimb coordination (Fig. 5), further support that locomotor training reestablishes a symmetric gait pattern after SCI in humans. Because the soleus muscle after locomotor training was not activated reciprocally between the right and left leg (Fig. 5C, bottom), we theorize that neuronal changes may occur before locomotor EMG function changes can be detected. Further, the increased ankle cocontraction in the right leg (Fig. 6C), which is directly related to presynaptic inhibition (Nielsen and Kagamihara 1993) and partly to recurrent inhibition (Nielsen and Pierrot-Desaulniers 1996), may be necessary to promote step progression of the most impaired leg and be reduced over time with more locomotor training sessions. With respect to gait parameters, the BWS required during stepping was decreased by an average of 55%, the gait speed was increased by 58%, the guidance force by the robotic exoskeleton was decreased by 43%, and improvements in frequency of spasms were also noted (Knikou 2013), supporting improvements in locomotor ability.

Neuromodulation and Rehabilitation

We described here, for the first time reported in the literature, the capability of spinal inhibitory circuitries to reorganize in response to locomotor training in spinal cord-injured persons. While different training interventions are utilized with the solely aim to improve motor function, studies concentrated on the underlying neurophysiological changes are scarce. This reduces the possibility for optimal use of a training intervention and limits significantly a patient-orientated training protocol
approach based on neurophysiological evidence. Our findings provide a strong rationale for larger clinical studies, during which neurophysiological changes over time and clinical outcome measures are utilized to develop prognostic criteria on patients who are likely to benefit the most, and establish appropriate methods for maintaining and/or strengthening metaplasticity by training of the injured nervous system.

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