Changes in Locomotor Muscle Activity After Treadmill Training
in Subjects with Incomplete Spinal Cord Injury

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Abstract

Intensive treadmill training after incomplete spinal cord injury can improve functional walking abilities. To determine the changes in muscle activation patterns that are associated with improvements in walking, we measured the electromyography (EMG) of leg muscles in seventeen individuals with incomplete spinal cord injury during similar walking conditions both before and after training. Specific differences were observed between subjects that eventually gained functional improvements in over-ground walking (responders), compared to subjects where treadmill training was ineffective (non-responders). Although both groups developed a more regular and less clonic EMG pattern on the treadmill, it was only the tibialis anterior and hamstrings muscles in the responders that displayed increases in EMG activation. Likewise, only the responders demonstrated decreases in burst duration and co-contraction of proximal (hamstrings and quadriceps) muscle activity. Surprisingly, the proximal muscle activity in the responders, unlike non-responders, was 3 to 4 times greater than in uninjured control subjects walking at similar speeds and level of body weight support, suggesting that the ability to modify muscle activation patterns after injury may predict the ability of subjects to further compensate in response to motor training. In summary, increases in the amount, and decreases in the duration, of EMG activity of specific muscles are associated with functional recovery of walking skills after treadmill training in subjects that are able to modify muscle activity patterns following incomplete spinal cord injury.
Introduction

Body-weight-supported treadmill training (BWSTT) is an effective treatment method for retraining walking in individuals with motor-incomplete spinal cord injury (iSCI). Intensive training leads to greater ability to walk on the treadmill with less assisted body-weight support, faster walking speeds and better endurance (for review see Harkema 2001; Field-Fote 2000). Further, improvements on the treadmill eventually transfer to increased gains in over-ground walking. Kinematically, training leads to better symmetry of steps (Field-Fote et al. 2005), less stride-to-stride variability (Grasso et al. 2004), more normal excursion in joints of the lower extremity (Barbeau and Blunt 1991; Barbeau et al. 1993) and a toe trajectory that is more similar to the uninjured (Barbeau et al. 1993; Grasso et al. 2004). Clinically, subjects require less assistance with walking, both in terms of personal assistance and walking aids (Wernig et al. 1995; Behrman and Harkema 2000), and they show improved sitting and standing balance (Behrman and Harkema 2000; Dobkin et al. 2006). Improvements in walking are seen whether the training is in the acute (Dobkin et al. 2006; Grasso et al. 2004; Nymark et al. 1998) or chronic phase after injury (Wernig et al. 1995). Moreover, those who regain the ability to walk in the household or community maintain this improvement over several years, and some continued to improve after training (Wernig et al. 1998; Wirz et al. 2001; Hicks et al. 2005).

What are the neural mechanisms that bring about these walking improvements? Is the motor pattern reverting to an uninjured pattern, or is the nervous system finding new solutions? Answers to these questions remain incomplete; however studying the motor pattern through surface EMG has provided some answers. For example, qualitative reports on single subjects suggest better timing of muscle activation (Barbeau and Blunt 1991) and some reduction in co-contraction between
antagonists (Dietz et al. 1994, 1998). Quantitative information exists for the gastrocnemius muscles, which show greater activation in the stance phase after training (Dietz et al. 1994, 1995). However, this change could be explained by the increasing ability of subjects to support more of their own body weight from pre- to post-training (Dietz et al. 1994, 1995, 1998) since extensor EMG is responsive to the amount of weight-bearing, even in individuals with complete spinal cord injury (Harkema et al. 1997). In addition, these subjects were trained acutely after the injury, so spontaneous recovery could also have contributed to the increased EMG activity (Calancie et al 2000). More recently, Grasso and colleagues (2004) reported that subjects show marked improvement in the end-point trajectory of the toe during walking, but the muscle patterns that lead to this improvement are quite different from those seen in uninjured subjects walking under matched conditions (Ivanenko et al. 2003). Their interesting findings suggested that new motor patterns were learned during BWSTT. Perhaps different subjects find their own motor solutions that may not be similar to the uninjured or to other injured individuals. In the Grasso study, only composite EMG scores were reported (i.e., average change in amplitude ratios), so the training-induced changes in EMG activation in individual muscles and between subjects remain unclear.

The purpose of this study was to quantify the changes in EMG activity from several major muscle groups in the lower extremity before and after training in a group of chronically (>0.8 yrs) injured subjects, under walking conditions that were identical (i.e., matched for both walking speed and weight-support). We sought to determine the changes in EMG amplitude, the timing of muscle activity and the amount of co-contraction between antagonists. We also compared the frequency content of the EMG activity both before and after training; a measure that has not been compared in the past, to determine if training has any effect on the degree of clonic activity during walking.
Subjects were separated into two groups: those who gained functional improvements in over-ground walking ability (responders) and those who did not (non-responders). To assess functional over-ground walking ability, we used the Walking Index for Spinal Cord Injury II (WISCI II) (Ditunno and Ditunno 2001) which was developed and validated for the SCI population. The WISCI II contains a larger number of discrete categories compared to other walking scales such as the modified Wernig scale (Hicks et al. 2005; Wernig et al. 1998) or the Hauser scale (Hauser et al. 1983). We also defined improvements in over-ground walking function as any increase in over-ground walking speed of more than 0.06 m/s, as this has recently been shown to be functionally relevant (Musselman 2007). Subjects who eventually showed clinical improvements in over-ground walking showed EMG patterns that were vastly different from uninjured controls and also displayed changes in amplitude and timing in distinct muscles that were not observed in subjects that did not respond to training.
Methods

Subjects

All experiments were carried out with the approval of the Human Research Ethics Board at the University of Alberta and with the written, informed consent of the subjects. Approval from a family physician was also obtained before each subject participated in the training. Our sample comprised of 19 subjects with incomplete spinal cord injury (iSCI) and 6 neurologically intact, control individuals (Table 1). Inclusion criteria for treadmill training were that subjects must have sustained damage to the spinal cord and have the ability to move at least one of the leg joints (ASIA C and D; Ditunno et al. 1994). Exclusion criteria were: 1) orthopaedic problems that could affect walking ability; 2) bone density that was 30% or less of age-matched, non-injured subjects; 3) impaired mental capacity or severe depression; and 4) other medical contraindications to treadmill training (such as cardiovascular problems, pulmonary disorders, history of deep vein thromboses, etc.). In one subject (3M), who had an L1 lesion, data were excluded for the left TA and SOL due to lower motor neuron damage; however, the activity patterns in remaining muscles were similar to other subjects before training with no evidence of denervation activity (i.e., fibrillation potentials) in the raw EMG traces, so 3M was included in the analysis.

Insert Table 1 near here

Training

Training consisted of one hour per day of partial BWSTT, as described elsewhere (Thomas and Gorassini 2005). The target frequency was 5 days per week; on average, subjects trained 3.3±1.3 (SD) days per week for an average of 14 ± 6 weeks. Training continued until subjects reached a plateau in walking function, which was defined as a lack of change in over-ground walking ability.
or speed (see Clinical Assessment below). In subjects in whom no improvements were seen, training lasted for a minimum of 10 weeks. For subject 7M, 2 to 3 treadmill sessions a week were replaced with over-ground training for the last 4 weeks.

Briefly, each training session consisted of body-weight support (BWS) combined with manual assistance of leg movements while the subject walked on a motorized treadmill. Depending on the need, one or two people were positioned at the lower limbs to provide stepping assistance by lifting the foot through swing, flexing the knee at the start of swing and/or stabilizing the knee during stance. A bungee cord tied to the harness and the support frame was sometimes used to help stabilize the subject’s trunk; in other cases, a trainer manually stabilized the trunk. Features of stepping, such as base of support, weight shift between the two legs, step length, postural alignment, hip extension at the end of stance and foot contact during stance (heel to toe), were monitored by the therapist to ensure subjects stepped as efficiently and safely as possible. Subjects were encouraged to swing one or both of their arms when possible. If arm swing resulted in instability of the body, subjects were allowed to grasp horizontal bars positioned at chest level on each side to aid in balance control, but not for weight-bearing. Subjects walked at a slow pace, between 0.2 to 0.6 m/s (0.5 to 1.4 mph), enabling them to concentrate on voluntarily activating their muscles during walking. Rests were taken when needed, but subjects were encouraged to walk/rest at ~10 minute intervals. The amount of body weight support and/or stepping assistance were gradually decreased and the treadmill speed increased when the physical therapist noticed improvements in cardiovascular tolerance, better ability to volitionally control the limbs, smooth weight transition between limbs and better upright trunk alignment. The responders decreased the amount of BWS by 39.1 ± 32.7 lbs on average compared to the non-responders who only decreased the amount of BWS
by 15.0 ± 16.9 lbs (p = 0.07, see Table 1 for individual values). The average increase in walking speed at the end of training compared to the start of training was similar between the two groups (responders 0.12 ± 0.15 m/s vs. non-responders 0.15 ± 0.11 m/s, p = 0.61, Table 1). The average amount of time that a responder walked for during a training session also increased from 25.4±11.2 to 36.2±11.4 minutes with the non-responders increasing from 14.6±11.3 to 29.8±9.2 minutes (average differences not significant, p = 0.4). The amount of assistance given by the physical therapists was not systematically documented in all subjects.

Clinical assessments

Clinical assessments of walking function were performed by experienced physiotherapists. Functional over-ground walking ability was assessed by the WISCI II, a 21-point ordinal scale that incorporates the use of aids required to achieve the walking function (Ditunno and Ditunno 2001). As in our previous study (Norton and Gorassini 2006), we separated subjects who did not show an improvement in WISCI II score (non-responders) from those who did (responders). In addition, subjects that increased their walking speed by 0.06 m/s or more were also considered to be responders as this increment is considered functionally relevant (Musselman 2007). Manual muscle strength (MMS) was assessed by experienced physiotherapists who used the qualitative MRC scale (Medical Research Council 1976) to assess the strength of the major muscle groups in the leg (hip, knee and ankle flexors and extensors, 5 point maximum per muscle group). The maximum score obtainable with this method was 60; (6 muscles per leg x 2 legs x 5 points per muscle = 60). Subjects were also classified using the ASIA classification scale (Ditunno et al. 1994). All participants in this study were classified as either ASIA C or D (Table 1).
**EMG assessment**

Irrespective of the length of the training program, comparisons of EMG activity while walking on the treadmill were made between the start and end of the treadmill training period using the initial (pre-train) BWS and treadmill belt speed values for each subject (Table 1). EMG activity was also measured in 6 uninjured control individuals who walked on the treadmill at the average walking speed (0.3 m/s) and BWS (40%) of the iSCI subjects. In the control subjects, test/retest reliability of the EMG measures was established by using two instances of walking on a treadmill separated by at least a week in which subjects did not alter their exercise routine, to avoid any novel training effects. EMG measurements were also made while controls walked at a more natural speed (0.9 m/s) for comparison. In both iSCI and controls, EMG activity from at least 30 steps was recorded bilaterally from the tibialis anterior (TA), soleus (SOL), hamstrings (HAM) and quadriceps (QUAD) muscles using isolated EMG amplifiers (Octopus, Bortec Technologies, Calgary, AB). Signals were recorded using bipolar surface electrodes (Kendall H59P, Tyco, MA) and were amplified (500 to 1k) and filtered (band-pass 10-1000 Hz) before being stored on a PC using a Digidata1200 AD card and Axoscope software (Axon Instruments, Molecular Devices, Sunnyvale, CA). All data were sampled at a rate of 5 kHz. Changes in knee angle during stepping were recorded with an electrogoniometer (Biometrics Limited, Ladysmith, VA). Data from muscles on the right and left leg in a single subject were considered to be independent because of the asymmetry in lesion location in all subjects.

**Peak and total EMG activity**

Peak and total EMG activity in were analyzed in 17 subjects (1M to 9F, 12M-19M) using custom-written software within the Matlab programming environment (The MathWorks, Natick, MA). The
EMG signals were first rectified and then smoothed using a 150-ms sliding average (Fig. 1A). This data was then re-sampled at 50 Hz. The knee angle was used to estimate the start and end of each step. To obtain the amount of peak EMG activity reached during each step, the maximum and minimum values in the rectified and smoothed EMG were automatically determined. The minimum values were used to characterize the level of background EMG (noise) in each step and were subsequently subtracted from the maximum EMG value to obtain a measure of peak EMG activation in each step. The peak EMG value for each step was then averaged across all steps for a given experiment. The total EMG area was calculated by summing all the points within a step after subtracting the mean level of background EMG (or noise). Likewise, the total EMG area for each step was averaged across all steps.

*Insert Figure 1 near here*

**EMG burst duration and area**

To quantify the percentage of time that a muscle was active during a step cycle (EMG burst duration), the data were first normalized to the step-length (with each step having 100 points, Fig. 1B). The onset and end of the EMG burst were estimated by visual inspection of the normalized recordings (see dashed vertical lines in Fig. 1B) and were expressed as a percentage of the total step cycle (out of 100%). The EMG burst area was calculated by summing all points within the delineated burst after subtracting the background EMG, then averaging across all steps. In some muscles (n = 3), it was not possible to measure the onset or end of the EMG burst due to contact artefact in the EMG signal.
Co-contraction

Co-contraction occurred when antagonistic muscles (TA and SOL or HAM and QUAD) were both active at the same time in the step cycle (see Fig. 1B). Again, the duration of co-contraction between two antagonist pairs of muscles was expressed as a percentage of the total step cycle (100%), and values for each step were averaged for a given experiment.

Variability of muscle on and off times

Because the on or off time of an EMG burst can cross the boundary of a step cycle that is delineated by the knee angle data, we used circular statistics to assess the variability in on and off times for each muscle burst. Using this approach, the on or off time of a particular EMG burst was plotted on the edge of a unit circle at an angle indicating the point in the step cycle that the muscle becomes active or inactive (see Fig. 6A). This process was repeated both before and after training for each muscle on and off time. To measure the degree of spread of the points (i.e., the angular dispersion), the length of the mean vector ($r$) was calculated which is represented as the distance from the origin of the circle to the centre of mass of the points (Zar 1999). In the example shown in Figure 6A, a selection of onset times for a muscle is plotted on a circular measure of the gait cycle along with the associated $r$ value. This normalized value (1 indicating no spread and 0 indicating no clustering) was used to determine if stepping became more regular after training.

Clonus

To determine if treadmill training had any effect on the amount of clonic EMG activity before and after training, custom-written scripts within the Matlab environment were used to rectify the data and apply a fast Fourier transform. The power in the 7.2 to 8.8 Hz band was then calculated per unit
time (Hidler et al. 2002) for the 4 muscles of each leg during a walking trial (data with contact artefact was excluded). For each subject, we averaged the power spectrum of all 8 muscles together as there was not a statistical tendency for any particular muscle group to be any more clonic (contain appreciable power in the ~7 to 9 Hz range) than other muscle groups. Data from two additional subjects (10M and 11M, both responders) were included in the clonic EMG analysis (Table 1). Power in the clonic frequency band was also measured from EMG activity generated during treadmill walking in the 6 uninjured control individuals in the same manner as for the iSCI subjects.

Statistics

Statistical analysis was performed using SigmaPlot 8.0 for Windows (SPSS Inc., Chicago, IL) and Matlab. For normally distributed data, statistical analysis was performed using paired Student’s t-tests to compare parametric data within groups. Wilcoxon tests were used for non-parametric and non-normally distributed data and the unpaired Student’s t-test was used to compare parametric data between responders and non-responders, responders and controls or non-responders and controls. When a Wilcoxon test was used, it is stated in the text; otherwise, Student’s t-tests were used. In all cases a significance level of 95% is considered to be statistically significant and all data are given as means ± SD.
Results:

Changes in walking function

The responder group (subjects 1-9, Table 1) was comprised of four ASIA C and five ASIA D subjects whereas the non-responder group (subjects 12-19) had seven ASIA C and only one ASIA D subject. Likewise, the responders had initial MMS scores for the leg (38±9) that were two times as great as the non-responders (19±11, p < 0.01, Wilcoxon test, see Table 1 for individual values). The average time from the injury date to the start of training was lower in the responders compared to the non-responders (3.1±3 vs. 4.3±7 years, respectively) but the difference was not significant (p > 0.6) and the number of weeks that subjects were trained (14.4±5 responders, 13.5±6 non-responders, p > 0.7) was also similar. The speed of the treadmill belt at which the testing was carried out was higher in the responders (0.33±0.12 m/s) compared to the non-responders (0.24±0.08) but the difference did not reach significance (p = 0.11). However, the non-responders did use a greater amount of percent BWS during testing compared to the non-responders (40±22% responders vs. 59±11% non-responders, p = 0.04). The average improvement in the WISCI II score for the responders was 4.6 points (from 8.3±4 to 12.9±5, p < 0.01, Wilcoxon test) with an average increase in over-ground walking speed of 0.24 m/s (from 0.31±0.3 to 0.55±0.4 m/s, p < 0.05, Wilcoxon test). Non-responders had no increases in WISCI II scores and an average increase in walking speed of less than 0.06 m/s, given that many did not walk over-ground before or after training (from 0.04±0.07 to 0.05±0.08 m/s, p=0.58, Wilcoxon test).

EMG activation in responders, non-responders and controls

Responders showed greater EMG activity during BWS treadmill walking prior to training compared to the non-responders. Typical results are presented in Figure 2 (grey lines, pre training),
which demonstrates that responders (left column) started out with greater EMG activity compared to non-responders (middle column), especially in the TA and HAM muscles for these two subjects. The amount of TA and SOL EMG activation in the responder is comparable to the amount generated in the control subject walking at a similar speed (0.3 m/s) and BWS (40%, right column), but the amount of HAM and QUAD activity is greater in the iSCI subjects, especially in the responder. In addition, the amount of EMG activity generated during treadmill walking increased substantially in the responder after training (Fig. 2 black traces), especially in the TA and HAM muscles, in contrast to the non-responder whose EMG profiles (except for HAM) did not change appreciably in response to training.

Insert Figure 2 near here

Peak EMG Activation

As a group, the amount of peak EMG activation in the responders increased significantly for the TA and HAM muscles (Fig. 3A; p < 0.05; Table 2) but not for the SOL and QUAD muscles in response to training. In fact, the average peak EMG activation in TA and HAM after training was greater than the average values measured in the controls walking at 0.3 m/s as represented by the solid horizontal lines in Fig. 3 (TA p < 0.05; HAM p < 0.01). The amount of SOL peak EMG activation did not change after training and was slightly higher than in controls (p = 0.07), in contrast to the QUAD values which were much higher than in controls both before (p = 0.0001) and after (p = 0.02) training. In the non-responders (Fig. 3B), the initial peak EMG activation for all muscles was about half of that seen in the responders, with SOL and HAM values closer to the controls. Following training, there were small to negligible increases in peak EMG activation in the non-responders
EMG burst duration

In addition to changes in the amplitude of EMG activity with treadmill training, there were also changes in the timing of EMG activity measured in the responders. For example, the percentage of time that a muscle was active in the step cycle (burst duration, see Methods) was reduced in some muscles following training, even though the treadmill speed was kept constant. There was a significant decrease in burst duration for the QUAD muscle after training and a strong trend to decrease in the HAM muscle (Figure 4A, Table 2). After training, the duration of HAM and QUAD burst activity decreased to reach amounts that were closer to values in the controls, whereas the TA and SOL burst durations remained below and above control values, respectively. In contrast, the burst duration times did not change with training in the non-responders, though there was a non-significant decrease in the burst duration of the QUAD muscle after training (p = 0.14; Fig. 4B, Table 2). Note that the large variability in the burst duration for the HAM and QUAD muscles in the control subjects was due to the fact that the burst duration could vary by as much as 50% of the step cycle between subjects. This variability in timing was likely of little functional consequence because the amplitude of HAM and QUAD activation is very low in the non-injured (Figure 3). This is also in agreement with the low levels and high variability of muscle moments about the knee reported by others for walking in non-injured subjects (Winter 1991).
Co-contraction

We also examined the amount of time that there was coincident activity in muscles traditionally defined as antagonists (TA/SOL and HAMS/QUADS), a measure of co-contraction (see Methods). In responders, there was a greater amount of co-contraction in TA/SOL and HAM/QUAD compared to controls ($p < 0.05$, Fig. 5A). In controls, there was a large degree of variability in HAM/QUAD co-contraction because, at the 0.3 m/s walking speed, HAM activity occurred either in the stance or swing phase with QUAD activity occurring mainly in the stance phase. In iSCI subjects, HAM activity mainly occurred in the stance phase alongside the QUAD activity. After training, as a group there were no net changes in co-contraction in the responders given that half of the muscle pairs increased and half of the muscle pairs decreased in co-contraction. Of note, in 6 of the 13 HAM/QUAD muscle pairs examined, a decrease in co-contraction of more than 15% of the total step cycle occurred after training. An example of a decrease in HAM/QUAD co-contraction for a responder subject is shown in Figure 5C whereas an example of a HAM/QUAD increase in co-contraction is shown for the responder subject in Figure 2. The amount of co-contraction for both TA/SOL and HAM/QUAD in the non-responders (Fig. 5B) was lower compared to responders and more similar to control values given the shorter burst durations in the TA and HAM muscles. In only 1 of the 12 HAM/QUAD muscle pairs studied in the non-responders was there a decrease in co-contraction of greater than 15% of the step cycle after training.
Regularity of EMG activation (on and off times of EMG burst)

To determine if training affected the regularity of stepping, the on and off times of an EMG burst for each subject were plotted on a circular representation of the step cycle (Fig 6A) and the angular dispersion (r), a measure of variability, was calculated (see Methods). There was less variability in the on and off times of muscle activity in the responders before training compared to the non-responders as reflected in higher r-values obtained by combining data from all muscles [0.92±0.07 vs. 0.89±0.1, p < 0.05, Figure 6B & C (grey bars)]. After training several muscles in both responders and non-responders showed a statistically significant decrease in variability in either the on and off times, indicated by an increase in the r-value (black bars in Figure 6B&C, p <0.05 in all cases). After training, the amount of variability in the on and off times was similar between the two groups (0.96±0.04 responders vs. 0.94±0.07 non-responders, values combined for all muscles, p = 0.17).

Insert Figure 6 near here

Changes in clonic activity

In the EMG activity of all subjects, including the controls, power in the clonic frequency band (~7 to 9 Hz) was detected. An example of visible clonus in the EMG signal from the HAM muscle generated during the stance phase of walking is shown for a responder in Figure 7A (before training). There were no differences between responders and non-responders in the amount of power in the clonic band before training (4.2±1.1 and 4.0±0.9 dB/s respectively, p > 0.6, Fig. 7C grey bars). After training, the amount of power in the 7 to 9 Hz band decreased as shown for the responder subject in Figure 7B. Similar trends occurred for both groups where the amount of clonic EMG activity decreased to 3.7±0.9 dB/s in the responders and to 3.5±0.9 dB/s in the non-responders.
The iSCI subjects (combined data from responders and non-responders) showed significantly more power in the ~7 to 9 Hz band both before (4.1±1.0 dB/s) and after (3.7±0.9 dB/s) training than the controls (2.5±0.2 dB/s, all p < 0.005).

Reproducibility of EMG measures

Because EMG measurements were made on data acquired on different recording days, it was necessary to ensure that factors such as small differences in positioning and/or impedance of recording electrodes did not appreciably change the measured EMG values. Reproducibility of the measured peak EMG activity was examined by recording muscle activity during treadmill walking in 3 controls (at two intervals separated by at least one week). Muscle activity patterns in the controls should not change since no training was undertaken in the intervening period, allowing us to assess the test/retest reliability of our measures and to ensure that any changes in EMG activity were indeed a result of training. Peak EMG activation for any muscle did not vary by more than 10% (all p > 0.1) and this is in accord with published guidelines on EMG test/retest reliability (Hallet et al. 1994).
Discussion

Although responders and non-responders both developed a more regular and less clonic EMG pattern on the treadmill, only the responders showed increases in EMG activation and shortening of the EMG burst duration in select muscles. Interestingly, the responders also had vastly different EMG profiles in proximal muscles compared to uninjured controls, both before and after training. Therefore, the ability to modify the activation of muscles during walking after injury may predict if a patient can produce further compensations in response to training. Mechanisms responsible for the muscle activation patterns observed during walking after iSCI and the changes induced by training are discussed below.

Training altered EMG activity in certain muscles

Increases in muscle activation have been reported for iSCI subjects undergoing training at an earlier stage of recovery (Grasso et al. 2004: 1-6 months post-injury), but the changes were not reported for individual muscle groups, and subjects who responded positively to the training were averaged with those who did not respond. Moreover, spontaneous recovery could also have contributed to the changes during the relatively acute phase post-injury. The present study controls for spontaneous recovery by examining subjects 8 months or more post-injury who had already plateaued in their walking function. In the responders only, the TA and HAM muscles showed increases in peak, total and burst EMG activity. However, both responders and non-responders showed patterns of stepping that were equally less variable at the end of the training period compared to their initial values; hence, a decrease in variability of walking alone does not account for the changes in EMG activation observed in the responder group. The increase in the regularity of stepping may arise from sources intrinsic to the subject, such as improved modulation of muscle activity associated
with the subject getting more accustomed to the treadmill, or from extrinsic factors, such as improved timing of assistance from the therapists. To avoid the increased regularity of stepping affecting our calculation of EMG amplitudes and timing, we examined each step individually rather than calculating these values from averaged EMG profiles. In addition, we were careful to ensure that the recording conditions before and after training were identical, particularly with regard to treadmill speed and body-weight unloading, which can affect both the amplitude and timing of EMG activation during treadmill walking (Ivanenko et al. 2003, 2006; Harkema et al. 1997).

Changes in EMG activation as a result of changing walking speed are also shown for the control subjects walking at 0.3 and .9 m/s (Table 2).

In the Grasso et al. 2004 study, a correlational analysis of muscle activity before and after treadmill training was presented. In that study, although the kinematics of the movement became more similar to the uninjured, especially the foot trajectory, the muscle activity did not show the same trend, and in some subjects, the activity pattern actually became less like controls. In our recordings, it was surprising to note that the responders, who on average had twice the volitional muscle strength as the non-responders (see MMS scores in Table 1), also displayed EMG activation patterns during walking that were vastly different from uninjured controls. In particular, the proximal HAM and QUAD activity which mainly occurred during stance was 3 to 4 times larger than in controls and likely reflects the greater need to control for postural instabilities resulting from reduced muscle strength and/or control of important muscles for walking. Unlike controls, the responders likely cannot take advantage of the limbs’ biomechanics to help stabilize the body during stance and propel the limbs forwards in swing, but must use additional EMG activation. For example, the knee is often more flexed during stance in individuals with iSCI than in the uninjured,
necessitating more extensor activity to prevent collapse (Winter 1980). Once the responders became more stable in their walking after training, some actually showed a decrease in QUAD muscle activity to reach values closer to the controls, although this was not a consistent trend across all subjects. In contrast, the non-responders were too weak to provide the added muscle activity needed to compensate for the inefficient biomechanics and ironically, had similar amounts of activation in proximal muscles compared to the uninjured controls. As discussed below, a lack of compensation from training in the non-responders may arise from an insufficient amount of residual descending pathways as evidenced by the more pronounced muscle weakness in these subjects compared to the responders.

**Neuronal mechanisms producing changes in EMG activation**

A prolonged period of treadmill training with partial body-weight support leads to an increase in over-ground walking ability that is accompanied by changes in amplitude and timing of select muscles. Observations from animals with anatomically complete spinal cord lesions indicate that the spinal cord contains neural circuits capable of generating a stepping pattern (Lovely et al. 1986). Treadmill training in these adult animals, which presumably occurs by training these neural circuits or central pattern generators (Barbeau and Rossignol 1987), leads to improved treadmill walking, but does not translate to over-ground, self-propulsive walking. Based on the animal findings (Barrière et al. 2008), a possible mechanism to explain our current observations is that we are training spinal circuits or reflexes to generate a better stepping pattern. The observed reduction in clonic activity suggests changes in spinal reflex pathways or circuitry may contribute to the improvements in walking function (Dimitrijevic et al. 1980; Barbeau et al. 1999). However, a
A decrease in the amount of clonic activity was not correlated with an improvement in function as a similar number of responders and non-responders demonstrated decreases in clonic activity.

An alternative and additional mechanism to produce improved walking may be an increase in the activation of descending pathways from the motor cortex and/or brainstem. This may also explain why changes only occurred in the TA and HAM, and not SOL muscles. Estimates of the cortical projections to the leg indicate that the SOL muscle receives fewer cortical inputs than the TA and the more proximal muscles (Brouwer and Ashby 1990; Bawa et al 2002). The SOL is predominantly a postural muscle whose activity is heavily modulated by spinal load-dependent reflexes (Duysens et al. 2000; Harkema et al. 1997) and the similar amounts of SOL EMG activation between the stronger controls and weaker non-responders may reflect the spinally driven element of the SOL activity. Our earlier studies have demonstrated a relationship between functional recovery and descending corticospinal drive. The increase in the size of the maximal motor evoked potential in the TA and QUAD muscles from training was positively correlated with functional improvement in walking (Thomas and Gorassini 2005). The degree of common cortical drive during walking, as measured by coherence of the EMG signal in the 20-40 Hz bandwidth, was also found to be higher for the thigh muscles (HAM & QUAD) of iSCI subjects who responded to treadmill training (Norton and Gorassini 2006). Together with the current data, it suggests that muscles with greater descending drive from the motor cortex are also the ones that change the most with training.

In addition to training-induced increases in the activation of excitatory spinal circuits by spared corticospinal and brainstem inputs, increases in the activation of inhibitory spinal circuits by
descending inputs may also have occurred in response to training. For example, decreases in the burst duration (and co-contraction) of proximal muscles may have been produced by a stronger descending activation of inhibitory interneurons controlling the duration of muscle timing during walking (Shefchyk and Jordan 1985).

Summary and clinical implications

Before training there was a difference in the amplitude of muscle activity during walking between the responders and the non-responders. This initial level of muscle activity produced during walking may be a useful predictor of subjects who will respond to the therapy, especially in the ASIA C class which is very broad and within which there are currently no predictors for who will benefit from treadmill training therapy. This is an issue we will address in a forthcoming publication.

This study has shown distinct changes in the way the damaged nervous system activates the muscles of the leg during walking that parallel functional recovery of walking skills after treadmill training. The altered pattern of EMG activity from training in iSCI subjects likely reflects changes at both spinal and supraspinal levels of the neural axis, although evidence for the former still needs to be established in the human. Finally, this study also highlights that functional gains in over-ground walking ability can be made several years after a spinal cord injury and raises the concern that protocols using both regeneration-based interventions (e.g., olfactory unsheathing cell graphs) and motor training in subjects with chronic injury must take into account the affects of training in isolation.
Acknowledgements

We are grateful to all the subjects in this study for their dedication and enthusiasm. We are also very grateful to Kelly Brunton, Paul Doughty, Scott Jamieson, Jennifer McPhail, Greg Hendricks, Rachelle Lohlun, and all other student volunteers who provided excellent patient care and training. Lisa Guevremont provided advice on the analysis of the gait data.

Grants

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Reference List


Figure Legends

Figure 1. A) For each step cycle (as determined from the knee angle) the minimum value of the rectified and smoothed EMG was subtracted from the maximum value (dashed horizontal lines) to calculate the peak EMG activity for that step. The peak EMG values were then averaged for a given stepping trial. B) To calculate burst duration, the step cycle was first normalized to 100% and the time of onset of the burst was subtracted from the time at the end of the burst (vertical dashed lines) for each step. The burst duration values were then averaged for a given stepping trial. To calculate burst area, the points within the burst were summed. The amount of co-contraction was measured as the percentage of time in the step cycle a pair of antagonist muscles (TA/SOL and HAM/QUAD) were active at the same time.

Figure 2. EMG activity from the TA, SOL, HAM and QUAD muscles recorded during treadmill walking from a responder (left column) and non-responder (middle column) both before (grey lines) and after (black lines) treadmill training. Knee angle is shown in the bottom trace, and the onset of the swing is shown as an upward deflection in the knee angle. Example EMG recording in a control individual is shown in the right column. All EMG signals are displayed on the same y-axis scale.

Figure 3. Summary of the changes in peak EMG activation. A) Responders: changes in peak EMG activity before (grey bars) and after (black bars) training. The numbers in the lower right of each graph represent the number of muscles included in each average. Solid and dashed horizontal lines represent mean and ± standard deviation values, respectively, for the control group. B) Non-responders. Same format as in A. (* p < 0.05).
**Figure 4.** Changes in EMG burst duration with training expressed as a percentage of the total step cycle that is normalized to 100%. Same format as in Figure 3. In some muscles (1 SOL, 1 HAM and 1 QUAD of responders), burst duration values could not be measured because of contact artefact affecting the EMG signal. Solid and dashed horizontal lines represent mean and ± standard deviation values, respectively, for the control group. * p < 0.05, # p = 0.06

**Figure 5.** Changes in co-contraction with training. **A)** Responder group. Amount of co-contraction expressed as a percentage of normalized step cycle both before (grey bars) and after (black bars) treadmill training for the TA/SOL (left graph) and HAM/QUAD (right graph) muscle pairs. Same format as Figures 3 and 4. **B)** Corresponding data for the Non-responder group. **C)** Example data from Responder subject (1M) demonstrating decrease in co-contraction (marked by grey boxes) for the HAM/QUAD muscle pairs after training, which is mainly due to a decrease in burst duration of the HAM muscle.

**Figure 6.** Angular dispersion (r) was used to estimate the variability in onset and offset times for each muscle group. **A)** Each dot on the circle represents the onset time of a muscle expressed as a percentage of the normalized step cycle. The vector points to the centre of gravity of the dots, the length being an indicator of the spread of the dots. **B)** Responders: there was a decrease in the variability of both the onset (left bars) and offset (right bars) times for all muscle groups as indicated by increased r values (pre: grey bars; post: black bars). Increases were significant in 3 cases (paired t-test). **C)** Non-Responders: Increases in r-values were significant in 4 cases. * p<0.05
**Figure 7.** Changes in clonic EMG activation from training. Example HAM EMG during a single step before (A) and after (B) training, respectively. Although some clonic activity in the 7 to 9 Hz range remains after training it is reduced in amplitude. (C) The amount of clonic activity decreased in both the responder and non-responders. Although clonic activity was reduced after training, it remained higher than in controls. * p <0.05, ** p <0.005
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<th>ASIA score</th>
<th>Years Post Injury</th>
<th>Initial MMS (60 max)</th>
<th>Weeks train</th>
<th>Initial BWS (lbs) &amp; (%BWS)</th>
<th>End BWS (lbs) &amp; (%BWS)</th>
<th>Initial Belt Speed (m/s)</th>
<th>End Belt Speed (m/s)</th>
<th>WISCI II Pre-Post (diff)</th>
<th>Over ground Speed (m/s) Pre-Post (diff)</th>
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<td>41</td>
<td>C3-5</td>
<td>C</td>
<td>0.8</td>
<td>35</td>
<td>10</td>
<td>50 (35%)</td>
<td>20 (14%)</td>
<td>0.22</td>
<td>0.36</td>
<td>8-13 (5)</td>
<td>0.05-0.28 (0.23)</td>
</tr>
<tr>
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<td>T7/8</td>
<td>C</td>
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<td>19</td>
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<td>30 (11%)</td>
<td>0.45</td>
<td>0.36</td>
<td>12-16 (4)</td>
<td>0.34-0.53 (0.19)</td>
</tr>
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<td>150 (70%)</td>
<td>60 (28%)</td>
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<td>0.54</td>
<td>1-3 (2)</td>
<td>N/T</td>
</tr>
<tr>
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<td>71</td>
<td>T5-9</td>
<td>D</td>
<td>0.8</td>
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<td>14</td>
<td>150 (73%)</td>
<td>60 (29%)</td>
<td>0.54</td>
<td>0.54</td>
<td>4-8 (4)</td>
<td>N/T</td>
</tr>
<tr>
<td>5M</td>
<td>78</td>
<td>T4/5</td>
<td>D</td>
<td>2</td>
<td>46</td>
<td>10</td>
<td>20 (10%)</td>
<td>20 (10%)</td>
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<td>0.72</td>
<td>13-19 (6)</td>
<td>0.65-0.78 (0.13)</td>
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<td>12</td>
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<td>60 (25%)</td>
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<td>0.36</td>
<td>12-16 (4)</td>
<td>0.57-0.60 (0.03)</td>
</tr>
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<td>D</td>
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<td>44</td>
<td>26</td>
<td>20 (14%)</td>
<td>0 (0%)</td>
<td>0.22</td>
<td>0.59</td>
<td>6-19 (13)</td>
<td>0.33-1.20 (0.87)</td>
</tr>
<tr>
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<td>74</td>
<td>T4-5</td>
<td>D</td>
<td>7.4</td>
<td>45</td>
<td>12</td>
<td>50 (32%)</td>
<td>60 (38%)</td>
<td>0.22</td>
<td>0.27</td>
<td>13-13 (0)</td>
<td>0.10-0.16 (0.06)</td>
</tr>
<tr>
<td>9F</td>
<td>24</td>
<td>T10</td>
<td>C</td>
<td>1</td>
<td>38</td>
<td>17</td>
<td>60 (44%)</td>
<td>30 (22%)</td>
<td>0.22</td>
<td>0.45</td>
<td>6-9 (3)</td>
<td>0.15-0.32 (0.17)</td>
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<td>28.2</td>
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<td>18</td>
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<td>30 (25%)</td>
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<td>0.54</td>
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<td>0.8-1.1 (0.3)</td>
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<td>T11-12</td>
<td>C</td>
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<td>80 (47%)</td>
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<td>0-4 (4)</td>
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</tr>
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<td>C6&amp;T10</td>
<td>C</td>
<td>3.8</td>
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<td>100 (54%)</td>
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<td>T4-5</td>
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<td>2</td>
<td>11</td>
<td>80 (44%)</td>
<td>90 (50%)</td>
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<td>0.72</td>
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<td>23</td>
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<td>50 (30%)</td>
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<td>0.22</td>
<td>9-9</td>
<td>0.11-0.10 (-0.01)</td>
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<td>110 (55%)</td>
<td>0.22</td>
<td>0.27</td>
<td>0-0</td>
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<tr>
<td>16M</td>
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<td>C3&amp;C6</td>
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<td>28</td>
<td>150(75%)</td>
<td>150 (75%)</td>
<td>0.22</td>
<td>0.36</td>
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<td>44</td>
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<td>70 (50%)</td>
<td>50 (36%)</td>
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<td>0.31</td>
<td>9-9</td>
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<td>C7</td>
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<td>10</td>
<td>110 (70%)</td>
<td>90 (57%)</td>
<td>0.22</td>
<td>0.45</td>
<td>9-9</td>
<td>N/T</td>
</tr>
<tr>
<td>19M</td>
<td>42</td>
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<td>C</td>
<td>1</td>
<td>38</td>
<td>11</td>
<td>120 (53%)</td>
<td>100 (44%)</td>
<td>0.13</td>
<td>0.31</td>
<td>0-0</td>
<td>0.0-0.0 (0.0)</td>
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<tr>
<td>20F</td>
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<td>40%</td>
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<td>0.7</td>
<td>N/A</td>
<td>20</td>
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<td>22F</td>
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<td>E</td>
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<td>N/A</td>
<td>40%</td>
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<td>0.7</td>
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<td>20</td>
<td>N/A</td>
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<td>23M</td>
<td>28</td>
<td>N/A</td>
<td>E</td>
<td>60</td>
<td>N/A</td>
<td>40%</td>
<td>N/A</td>
<td>0.7</td>
<td>N/A</td>
<td>20</td>
<td>N/A</td>
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<tr>
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<td>40%</td>
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<td>20</td>
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<tr>
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<td>32</td>
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<td>0.7</td>
<td>N/A</td>
<td>20</td>
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**Table 1.** Demographic, injury, training and experimental details for all iSCI and control subjects. Responders are in white columns, non-responders in light grey (12M-19M), controls in dark grey (20F – 25M). All iSCI subjects sustained trauma to the spinal cord except for subjects 8F and 17F where damage occurred due to transverse myelitis and a surgical bleed, respectively. Subject 9F also sustained a head trauma. Subject 7M performed over-ground training 2-3 times per week in the last 4 months of training. ASIA scores (Ditunno et al. 1994) include global sensory and motor scores from all body segments below the lesion. Years Post Injury is the time between the injury and the onset of training is indicated. MMS = the initial manual muscle strength. BWS = body weight support. The initial BWS and belt speed were used at each EMG testing session. The end
BWS and belt speed were the values used during the final training sessions. N/A = not applicable, N/T = not tested. * subjects included in clonic analysis only.
<table>
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<tr>
<th>Subjects</th>
<th>Peak EMG (µV)</th>
<th>Total Area (µV)</th>
<th>Burst Area (µV)</th>
<th>Burst Duration (% step cycle)</th>
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<td>SOL</td>
<td>38±21</td>
<td>39±22</td>
<td>1731±937</td>
<td>1088±694</td>
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<td><strong>50±43</strong></td>
<td>1419±941</td>
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<tr>
<td>QUAD</td>
<td>54±32</td>
<td>40±40</td>
<td>2234±1364</td>
<td>1919±1299</td>
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<td><strong>Non-Responders</strong></td>
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**Table 2.** Data summarizing EMG analysis for responders, non-responders (before and after training) and control subjects (walking at 0.3 and 0.9 m/s for comparison). Calculation of total area and burst area were done differently (see Methods) and are not directly comparable. Numbers marked in bold represent a statistically significant change post training compared to pre-training (p < 0.05, paired t-test). Differences in EMG values between iSCI subjects and controls are stated in the text. N/A = not applicable. # p = 0.06, † p = 0.10.
Figure 1. A) For each step cycle (as determined from the knee angle) the minimum value of the rectified and smoothed EMG was subtracted from the maximum value (dashed horizontal lines) to calculate the peak EMG activity for that step. The peak EMG values were then averaged for a given stepping trial. B) To calculate burst duration, the step cycle was first normalized to 100% and the time of onset of the burst was subtracted from the time at the end of the burst (vertical dashed lines) for each step. The burst duration values were then averaged for a given stepping trial. To calculate burst area, the points within the burst were summed. The amount of co-contraction was measured as the percentage of time in the step cycle a pair of antagonist muscles (TA/SOL and HAM/QUAD) were active at the same time.
Figure 2. EMG activity from the TA, SOL, HAM and QUAD muscles recorded during treadmill walking from a responder (left column) and non-responder (middle column) both before (grey lines) and after (black lines) treadmill training. Knee angle is shown in the bottom trace, and the onset of the swing is shown as an upward deflection in the knee angle. Example EMG recording in a control individual is shown in the right column. All EMG signals are displayed on the same y-axis scale.
Figure 3. Summary of the changes in peak EMG activation. 

A) Responders: changes in peak EMG activity before (grey bars) and after (black bars) training. The numbers in the lower right of each graph represent the number of muscles included in each average. Solid and dashed horizontal lines represent mean and ± standard deviation values, respectively, for the control group.

B) Non-responders. Same format as in A. (* p < 0.05).
Figure 4. Changes in EMG burst duration with training expressed as a percentage of the total step cycle that is normalized to 100%. Same format as in Figure 3. In some muscles (1 SOL, 1 HAM and 1 QUAD of responders), burst duration values could not be measured because of contact artefact affecting the EMG signal. Solid and dashed horizontal lines represent mean and ± standard deviation values, respectively, for the control group. * p < 0.05, # p = 0.06
Figure 5. Changes in co-contraction with training. A) Responder group. Amount of co-contraction expressed as a percentage of normalized step cycle both before (grey bars) and after (black bars) treadmill training for the TA/SOL (left graph) and HAM/QUAD (right graph) muscle pairs. Same format as Figures 3 and 4. B) Corresponding data for the Non-responder group. C) Example data from Responder subject (1M) demonstrating decrease in co-contraction (marked by grey boxes) for the HAM/QUAD muscle pairs after training, which is mainly due to a decrease in burst duration of the HAM muscle.
Figure 6. Angular dispersion (r) was used to estimate the variability in onset and offset times for each muscle group. A) Each dot on the circle represents the onset time of a muscle expressed as a percentage of the normalized step cycle. The vector points to the centre of gravity of the dots, the length being an indicator of the spread of the dots. B) Responders: there was a decrease in the variability of both the onset (left bars) and offset (right bars) times for all muscle groups as indicated by increased r values (pre: grey bars; post: black bars). Increases were significant in 3 cases (paired t-test). C) Non-Responders: Increases in r-values were significant in 4 cases. * p<0.05
Figure 7. Changes in clonic EMG activation from training. Example HAM EMG during a single step before (A) and after (B) training, respectively. Although some clonic activity in the 7 to 9 Hz range remains after training it is reduced in amplitude. (C) The amount of clonic activity decreased in both the responder and non-responders. Although clonic activity was reduced after training, it remained higher than in controls. * p <0.05, ** p <0.005