Changes in Cortically Related Intermuscular Coherence Accompanying Improvements in Locomotor Skills in Incomplete Spinal Cord Injury

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Norton, Jonathan A., and Monica A. Gorassini. Changes in cortically related intermuscular coherence accompanying improvements in locomotor skills in incomplete spinal cord injury. J Neurophysiol 95: 2580–2589, 2006. First published January 11, 2006; doi:10.1152/jn.01289.2005. In human spinal cord injury, the neuronal mechanisms mediating the improvement of locomotor function in response to intensive treadmill training are not well understood. In this study, we examined if such recovery is mediated, in part, by increases in residual corticospinal drive to muscles of the leg during walking. To do this, we measured the coherence of electromyogram (EMG) activity between two antagonist muscles (intermuscular coherence), specifically at frequencies between 24 and 40 Hz, which is thought to indicate common drive to two muscles from corticospinal inputs. In 12 subjects with incomplete spinal cord injury, intermuscular coherence was measured between hamstrings and vastus lateralis EMG that was activated during walking on a motorized treadmill. Before training, appreciable coherence in the 24–40 Hz frequency band was only present in subjects with moderate voluntary motor strength in their leg muscles (n = 8 subjects) compared with subjects with little or no leg muscle strength (n = 4 subjects), reconfirming that 24–40 Hz frequency coherence is likely mediated by common supraspinal inputs. After training, increases in 24–40 Hz coherence only occurred in the eight subjects with moderate leg muscle strength who also exhibited improvements in locomotor recovery as assessed by the 21 point WISCI II scale (termed responders). In contrast, development of intermuscular coherence in the 24–40 Hz frequency band did not occur in the four subjects with absent or weak muscle strength. These subjects also did not improve in their locomotor ability as reflected in unchanging WISCI II scores (termed nonresponders). Lower-frequency coherence (5–18 Hz), which is thought to contain common drive from spinal inputs, did not change in either group. In a subset of subjects that were previously assessed with transcranial magnetic stimulation (TMS) before and after training (n = 5 responders and 3 nonresponders), there was a significant and positive relationship between increases in 24–40 Hz coherence and increases in evoked muscle responses to TMS of the primary motor cortex. Taken together, increases in higher-frequency EMG coherence in subjects with residual voluntary muscle strength and its parallel relation to changes in TMS-evoked responses provides further evidence that improvements in locomotor function from treadmill training are mediated, in part, by increases in corticospinal drive to muscles of the leg during walking.

INTRODUCTION

In humans, treadmill training therapy involving body weight support and manual assistance of stepping movements can produce improvements in functional walking ability after incomplete spinal cord injury (Dietz and Harkema 2004). Animal studies have shown that modifications in the activation of both inhibitory and locomotor networks intrinsic to the spinal cord are associated with improvements in functional treadmill walking resulting from training, and this occurs even in animals with complete spinal cord lesions (Edgerton et al. 2001; Ribotta et al. 2000; Tillakaratne et al. 2002). In contrast, recovery of functional independent walking in humans is generally only observed in subjects having some preservation of voluntary movement in the legs (Dietz et al. 1994; Wernig and Muller 1992), suggesting that sparing of supraspinal pathways is a necessary prerequisite for locomotor recovery. Recently we have shown in subjects with incomplete spinal cord injury that improvements in locomotor recovery are associated with increases in the strength and excitability of spared corticospinal tracts originating from the primary motor cortex (Thomas and Gorassini 2005). Improvements in locomotor recovery were positively related to increases in evoked muscle responses from transcranial magnetic stimulation (TMS) measured with matched background contractions and consistent, maximal M-wave responses. This suggests that locomotor recovery through training is mediated in part by increasing the function of the spared corticospinal tract to muscles of the leg. Although fairly convincing, these findings are none-the-less correlational, and the TMS responses were not measured during the relevant motor behavior, i.e., during the recovered walking. Thus experimental techniques that address the origin of the improved neural drive to muscles of the leg during walking will help in our understanding of the neurological mechanisms behind training induced locomotor recovery.

Coherence, which measures the similarity between a pair of signals in the frequency domain (Challis and Kitney 1991), such as EMG activity recorded from two muscles, can be used to infer information concerning the neural drive to a given set of muscles (Nielsen 2002). The frequencies at which coherence occurs can be used to infer the source of drive to a pair of muscles as follows. Using electrodes placed on the scalp or directly on the motor cortex, it is possible to record activity associated with movement. Between two or more cortical sources coherence has been found at low frequencies (~10 Hz, α rhythm) and at higher frequencies (~20–35 Hz, β rhythm) (Salenius and Hari 2003). However, only the higher-frequency signal appears to be transmitted to the muscles as electroencephalogram/EMG or magnetoencephalogram/EMG coherence is only found at these higher frequencies (Gross et al. 2000). EMG/EMG coherence between muscle pairs also contains both higher- and lower-frequency bands (Grosse et al. 2002). The higher-frequency coherence in EMG/EMG recordings is
thought to originate from the cortex, whereas the lower-frequency component is thought to arise subcortically (Brown and Marsden 2001; Farmer et al. 1993). For example, in subjects with complete spinal cord lesions, EMG/EMG coherence is only found in the lower-frequency band (peaks in coherence at 10 and 16 Hz in individual subjects) (Norton et al. 2003, 2004), suggesting that the lower band is spinal in origin. In addition, EMG/EMG coherence in the higher-frequency band is reduced after stroke and spinal cord injury, providing evidence that it is supraspinally mediated (Farmer et al. 1993; Hansen et al. 2005).

The first objective of this study was to reconfirm that higher-frequency intermuscular coherence is mediated by supraspinal inputs. To do this, we compared the magnitude of higher-frequency (24–40 Hz) coherence between hamstring and vastus lateralis EMG recorded during treadmill walking in subjects with little to no residual motor strength, i.e., subjects with little to no descending drive, and in subjects with moderate to good residual muscle strength, i.e., in subjects with moderate to good descending drive. We then compared if several months of intensive treadmill training produced increases in common descending drive to the hamstrings and vastus lateralis muscles by measuring changes in 24–40 Hz coherence between these two muscles. Intermuscular coherence below 5 and 18 Hz was also measured to examine if there were changes in common drive having components from spinal sources. Finally, in a subset of subjects, we compared if changes in 24–40 Hz coherence were associated with general increases in evoked muscle responses to TMS to determine if the coherence measurements were related to changes in direct measures of corticospinal tract connectivity. Parts of this paper have been published in abstract form (Norton and Gorassini 2005).

METH ODS

Ethical approval for this study was obtained from the Human Ethics Research Board at the University of Alberta. Three neurologically intact subjects and 12 subjects with incomplete spinal cord injury (iSCI, 11 male) participated in this study. Eight of these iSCI subjects also participated in an earlier study using TMS with data from five of these subjects (1M–5M) included in a previous paper (Thomas and Gorassini 2005). Table 1 shows the demographic data for the subjects in the study including muscle strength and WISCI II scores (Ditunno and Ditunno 2001) before and after training. The iSCI subjects with low muscle-strength scores and the noninjured control group are highlighted in light and dark gray, respectively. None of the subjects with incomplete spinal cord injury had a progressive condition with the average time post injury 5.2 ± 7.6 (SD) yr.

To produce improvements in walking function, treadmill training was administered as described previously (Thomas and Gorassini 2005). Briefly, subjects were trained on five occasions a week for ~4 mo (see Table 1 for individual training durations) for an hour at a time. Training consisted of partial body weight support and manual assistance of leg movements as required while the subjects walked on a motorized treadmill at a slow walking pace (0.8–1.5 km/h). Body-weight support and manual assistance of leg movements were decreased over time as the subject’s ability allowed. Concurrent with the recording of EMG activity on the treadmill the ability of the subjects to walk overground was assessed using the WISCI II score. Muscle strength was assessed by the use of manual muscle testing performed by an experienced physiotherapist before and after the training program using a modified version of the MRC Manual Muscle Strength Scale (Medical Research Council 1976). The muscle strength of the knee flexors and extensors from both legs (maximal score of 5 for each muscle group) were combined to give a maximal total score of 20 (see Table 1). The combined lower extremity muscle score (LEMS) for both legs is indicated in brackets.

Data were collected from the iSCI subjects during walking trials, and so the number of steps taken in each trial varied with their ability. In all trials, subjects walked at a speed of 0.8 km/h because all subjects could walk at this speed both before and after training. Subjects also wore an electromyometer (Biometrics Limited, Ladysmith, VA) around their knee. Electromyometric data were used to segment the data set into individual steps (see following text and Fig. 1). EMG activity was recorded from the vastus lateralis, hamstrings, tibialis anterior, and soleus muscles bilaterally during walking using skin surface electrodes (Kendall Soft EH59P, Kendall - LTP, Huntington Beach, CA). EMG signals were preamplified, band-pass filtered, and digitized at 1000 Hz. The raw EMG signals were low-pass filtered at 100 Hz with a 7th order Butterworth filter. The EEG artifacts were corrected using a point-by-point average reference filter (Brown 1997). The EMG signals were then band-pass filtered at 5–50 Hz with a 7th order Butterworth filter. The signal-to-noise ratio (SNR) was calculated as the ratio of the root mean square amplitude of the EMG signal to the root mean square amplitude of the noise. The SNR was then multiplied by the root mean square amplitude of the filtered EMG signal to obtain the filtered EMG signal. The filtered EMG signal was then used to calculate the intermuscular coherence, which was computed from the power spectral density (PSD) of the filtered EMG signal using the Welch method with a 1024-point fast Fourier transform (FFT) and 512 samples using a Hamming window. The coherence was calculated between the hamstring and vastus lateralis muscles for intermuscular coherence between 5 and 18 Hz and for intermuscular coherence between 5 and 18 Hz.

TABLE 1. Demographic, injury, and experimental details for all spinal cord injured and control subjects

<table>
<thead>
<tr>
<th>Code/ Sex</th>
<th>Age</th>
<th>Cause</th>
<th>Years Postinjury</th>
<th>Injury Level</th>
<th>ASIA Score</th>
<th>Weeks Trained</th>
<th>Pretrain</th>
<th>Posttrain</th>
<th>WISCI II Pretrain</th>
<th>WISCI II Posttrain</th>
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<tr>
<td>1M*</td>
<td>44</td>
<td>Trauma</td>
<td>3</td>
<td>T11/12</td>
<td>C</td>
<td>21</td>
<td>10.7</td>
<td>12</td>
<td>20.7</td>
<td>20</td>
</tr>
<tr>
<td>2M*</td>
<td>52</td>
<td>Trauma</td>
<td>28</td>
<td>C5/6</td>
<td>C</td>
<td>16</td>
<td>11.3</td>
<td>12.3</td>
<td>13.7</td>
<td>13.7</td>
</tr>
<tr>
<td>3M*</td>
<td>60</td>
<td>Trauma</td>
<td>3</td>
<td>L1</td>
<td>C</td>
<td>19</td>
<td>12</td>
<td>13.7</td>
<td>13.7</td>
<td>13.7</td>
</tr>
<tr>
<td>4M*</td>
<td>71</td>
<td>VE</td>
<td>0.8</td>
<td>T5/9</td>
<td>D</td>
<td>13</td>
<td>16.3</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>5M*</td>
<td>78</td>
<td>Tumour</td>
<td>2</td>
<td>T4/5</td>
<td>D</td>
<td>23</td>
<td>20.5</td>
<td>19</td>
<td>20.5</td>
<td>20.5</td>
</tr>
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<td>6M</td>
<td>48</td>
<td>Trauma</td>
<td>10</td>
<td>C4/5</td>
<td>D</td>
<td>11</td>
<td>10.3</td>
<td>9.6</td>
<td>10.3</td>
<td>10.3</td>
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<td>C1-3</td>
<td>D</td>
<td>23</td>
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<td>18</td>
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<td>18</td>
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<td>L1</td>
<td>D</td>
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<td>11.3</td>
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<td>11.7</td>
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<tr>
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<td>Trauma</td>
<td>3.8</td>
<td>C6b/T10</td>
<td>C</td>
<td>10</td>
<td>8</td>
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<td>Trauma</td>
<td>0.92</td>
<td>T4-5</td>
<td>C</td>
<td>11</td>
<td>0</td>
<td>0.27</td>
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<td>C5-6</td>
<td>C</td>
<td>38</td>
<td>2.3</td>
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<td>C5/6</td>
<td>C</td>
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<td>5.7</td>
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<td>N/A</td>
<td>E</td>
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<td>20</td>
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<tr>
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<td>N/A</td>
<td>E</td>
<td>N/A</td>
<td>20</td>
<td>N/A</td>
<td>20</td>
<td>N/A</td>
</tr>
</tbody>
</table>

For subject 4M, VE = viral encephalitis with damage to the spinal cord verified by MRI. Subject 4M became paraplegic after surgical removal of a thoracic epidural abscess and subject 5M became paraplegic after removal of posterior elements of C4–T2. Subject 9M sustained trauma at two levels of the spinal cord as assessed by neurological exam. The weak or nonresponder group is highlighted in light gray and the control group in dark gray. Muscle strength scores for knee flexion and extension only are combined from both legs and the corresponding total Lower Extremity Muscle Score (LEMS) is indicated in parentheses. ASIA scores include global sensory and motor scores from all body segments below the lesion. *Subjects marked with an asterisk also participated in the TMS study. The time between the injury and the onset of training is indicated (years postinjury). N/A, not appropriate.
tered (10–1000 Hz; Octopus, Bortec Technologies, Calgary, Canada) and stored on a PC for off-line analysis using AxoScope hardware and software (DigiData1200 Series and Axoscope respectively, Axon Instruments, Molecular Devices, Sunnyvale CA). Data were sampled at a rate of 5 kHz.

Coherence analysis

The EMG activity for coherence analysis was selected from the period of hamstrings and vastus lateralis co-activation during the stance phase of walking (see Fig. 1A). We measured coherence during stance because the stance phase of walking can be considered quasi-stationary and coherence is known to be higher during the “hold” phase of a task compared with the “movement” phase (Kilner et al. 2000). The period of co-activation, i.e., the EMG window, where coherence was measured was chosen as follows: a threshold crossing was determined for the knee angle (horizontal line in lower graph of Fig. 1A); a delay between the threshold crossing time (vertical line in Fig. 1A) and the onset of the co-activation period was determined (D in Fig. 1A); the length of time of the co-activation (EMG window) was determined (L in Fig. 1A), which on average was 225 ± 15 (SD) ms across all subjects. Placement of the EMG window was performed manually for 20 steps in each walking trial to determine the optimum delay and length values for the trial that were then used automatically for the remaining steps in the trial. On average, there were 70–72 steps analyzed per subject per trial before and after training.

After selection of the EMG window, the data were passed through a Tukey window to reduce the amplitude of the signal at the edges of the window and allow the concatenation of samples without the introduction of high-frequency components at the joints of the segments. The EMG data were then rectified before coherence was calculated. Coherence between the two rectified EMG signals at frequency \( w \) was then calculated using the equation

\[
MSC = |C_{yx}(w)|^2 = \frac{|G_{yx}(w)|^2}{G_{xx}(w)G_{yy}(w)}
\]

(Halliday et al. 1995). MSC is the magnitude squared coherence or coherence, \( G_{xy}(w) \) and \( G_{yx}(w) \) are the power spectra of \( x \) and \( y \), respectively, at frequency \( w \). \( G_{xx}(w) \) is the cross power spectrum of signals \( x \) and \( y \) at frequency \( w \) where \( G_{xx} \) and \( G_{yx} \) correspond to the Fourier transforms of the auto and cross-correlation, respectively (Rosenberg et al. 1989). Coherence was calculated using segments that were 2,048 samples long to give a frequency resolution of the spectra of \( \approx 2.4 \) Hz (5-kHz sample rate/2,048 samples). Calculation of coherence was performed within the Matlab (Mathworks) environment using scripts based on those developed by Neurospec (www.neurospec.org) (Halliday et al. 1995). Coherence is a normalized function bounded between 0 and 1. Zero signifies that the two signals are completely independent at that frequency, and 1 signifies that the two signals are identical at that frequency (Grosse et al. 2002). If two signals show significant coherence (above a confidence limit normally set at 95%, see horizontal line in Fig. 1B), it is assumed that a fraction of the drive to both signals is common based on the coherence estimate.

Coherence analysis should not be performed in isolation and the results must be interpreted in the light of other supporting evidence (Halliday and Rosenberg 1999). Particularly important in this regard is the phase relationship between the muscles. Using the Neurospec software, the phase (Fig. 1B, inset) and cumulant density (Fig. 1C) were also calculated (Halliday and Rosenberg 1999). The cumulant density is a form of the cross-correlogram. Peaks in the cumulant density indicate the relative timing of bursts of activity or periods of inactivity in the muscle pairs. The cumulant density in Fig. 1C peaks at about time 0, indicating that the common neural drive to the two muscles arrived about at the same time as expected for two muscles equidistant from the neural axis. The slope of the phase plot indicates the relative timing of the activity at a given frequency of the two muscles (see Fig. 1B, inset). In regions of significant coherence (above the 95% confidence line), the phase should be constant. However, the phase at different regions of significant coherence may not be consistent, i.e., if there is significant coherence between 5 and 18 Hz and 24 and 40 Hz, the phase relationship in these two different frequency bands need not be the same. The phase spectrum is only valid in the frequency band in which the coherence is statistically significant and hence only those regions are indicated in the phase plots. The x axis is marked in frequency, whereas the y axis is marked in radians. The slope of the line can be used to calculate the lag between the muscles in a frequency band. Both phase and cumulant density (or cross-correlogram) provide timing information; however, the information provided by both is complimentary because the phase splits the information into frequency regions while the cumulant density provides information about the global firing pattern (Halliday and Rosenberg 1999) and thus must be shown together (Grosse et al. 2002; Hamm and McCurdy 1994; Mima and Hallet 1999). We excluded records from subjects demonstrating cross-talk between the EMG records as exhibited by high, narrow peaks in the cumulant density at certain times, a high degree of coherence at all frequencies and a flat phase relationship at all frequencies. For example, cross-talk was evident between soleus and TA EMG in 4 of the 12 iSCI subjects, and thus these two muscles were not analyzed further in this study.

We divided the frequency spectrum into two bands of interest: 5-18 Hz and 24–40 Hz. The lower-frequency band encompasses the range of frequencies that were measured in earlier studies in individuals with motor complete lesions from muscles below the lesion (Norton et al. 2003, 2004) and hence may be mainly spinal in origin. The higher-frequency band encompasses the \( \beta \)-rhythm that is believed to...
originate in the motor cortex and was not seen in individuals with complete lesions. Throughout the study, we used a 95% confidence interval to assess significance of the coherence (Amjad et al. 1997) (see horizontal line in Fig. 1B). In each of the two frequency bands, we measured the area bounded by the coherence estimate and the significance limit in addition to the average amplitude of the significant coherence.

Coherence was also measured during walking and voluntary contractions in the three noninjured control subjects. Walking speeds were set to 0.8 km/h to match the speeds walked by the SCI subjects. A similar number of steps (70) and a similar EMG analysis window (200 ms) were used to match the length of the concatenated data between the two groups. The analysis window was set to maximize the amount of cocontraction between the hamstrings and vastus lateralis EMG during stance, similar to that for the SCI subjects. EMG measurements were repeated on two different days, separated by 1 wk, to test the day-to-day variability of the coherence measures in the noninjured control subjects.

TMS

Responses to TMS in subjects with SCI were assessed as described previously (Thomas and Gorassini 2005). TMS was produced by a Magstim 200 stimulator (Magstim, Wales, UK) and a large double-cone coil with an outer diameter of 125 mm and an inner diameter of 96 mm. Motor evoked potentials (MEPs) were measured from the tibialis anterior or vastus lateralis muscles using skin surface electrodes as subjects maintained a small background contraction (~10% of maximum). For each subject, the location on the scalp where the largest MEP could be evoked (hotspot) was determined using a stimulation intensity that was 1.2 times active motor threshold. A recruitment curve was then produced by measuring MEPS in response to increasing levels of stimulator output in steps of 5% of the maximal stimulator output (% MSO). In addition, the maximum motor response to direct motor nerve stimulation (Mmax) was recorded. The same testing protocol was used before and after training (Thomas and Gorassini 2005) except that matched levels of absolute background EMG were used to match background levels of cortical and motoneuronal excitability pre- and posttraining. The percentage increase in MEPmax from training, which was the largest MEP in the recruitment curve, was determined by (MEPmax after – MEPmax before)/MEPmax before. Percentage increase in MEPmax was plotted against the absolute increase in high-frequency (24–40 Hz) coherence to examine if the two values were related.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 for windows (SPSS). For data that was normally distributed (χ2 for normality), we used the Student’s t-test; for nonnormally distributed data, we used the Mann-Whitney test. The Wilcoxon signed-rank test was used to compare ordinal WISCI II scores before and after training. We tested for correlation between percent increase in MEPmax and change in high-frequency coherence using Pearson’s correlation test. Significance was set to 95%.

RESULTS

Coherence between responders versus nonresponders

To assess the magnitude of higher-frequency (24–40 Hz) EMG coherence in subjects with differing levels of supraspinal input to their musculature, we divided the subjects into two groups based on their volitional muscle strength scores around the knee (see METHODS). Subjects with moderate voluntary activation of their knee muscles were considered to have moderate to good supraspinal input to the hamstrings and vastus lateralis muscles in contrast to the weaker subjects, who were considered to have little or no supraspinal control. Subjects were classed as moderately strong if they had a combined score of >10 (20, 8 subjects) or weak if their score was <10 (4 subjects, see Table 1). The initial muscle strength scores of the two groups were significantly different, (3.7 vs. 13.4, P < 0.05, Mann-Whitney test). The weak subjects also showed no improvement in their functional locomotor scores with training as assessed by the WISCI II scale (see Table 1) and are classified as nonresponders for the remainder of this paper. The strong subjects showed a statistically significant improvement in locomotor function after training as assessed by the WISCI II scale (6.75 ± 4.8 to 12.38 ± 6.4, P < 0.01, Wilcoxon test) and are classified as responders.

Figure 2 compares coherence computed from a nonresponder (A) and a responder (B) before locomotor training. The left panels illustrate the EMG (top 2 traces) from which the coherence was calculated and the knee joint angle (3rd trace). The responder shows better modulation of EMG amplitude over an averaged step-cycle than the nonresponder even in this pretraining state. Although both subjects show a large degree of co-activation of the musculature, the responder is able to reduce the amplitude of EMG to close to zero in both muscles and shows a smooth increase and decrease in EMG amplitude. In the nonresponder (A) there is very little coherence above the 95% confidence line in the lower-frequency band (5–18 Hz) and none in the higher-frequency band (24–40 Hz). A broad peak of ~100 ms was observed in the cumulant density (bottom right graph). In contrast, the responder (B) shows statistically significant coherence in both the lower (5–18 Hz) and higher-frequency (24–40 Hz) bands with a narrow central peak in the cumulant density.

When calculating the total area of coherence above the 95% confidence line (see METHODS), on average, the four nonresponders did not show significant coherence in the higher-frequency band compared with zero (0.015 ± 0.01, right of Fig. 4, P > 0.2, Mann-Whitney test). In contrast, the eight responders showed significant levels of coherence in this band compared with the nonresponders (0.21 ± 0.08, P < 0.01, t-test). This suggests that coherence in the higher-frequency band was mediated by supraspinal pathways because it was present only in subjects with moderate to good residual voluntary muscle strength. The lower-frequency bands (Fig. 4, left), which are thought to contain common drive from spinal (and perhaps supraspinal) sources, were not statistically different between the two groups (0.31 ± 0.1 nonresponders vs. 0.42 ± 0.1 responders, P > 0.4, t-test).

Coherence before and after training

After 3–5 mo of training, a marked increase in higher-frequency (24–40 Hz) coherence occurred in the responders but not in the nonresponders. As shown for the individual case in Fig. 3A, the nonresponders showed some improvement in the modulation of their EMG over the gait cycle compared with their pretraining profiles (compare Figs. 2A and 3A, left) with a smoother modulation of EMG and a decrease in the amount of co-activation and minimum EMG. However, the nonresponders showed only small, nonsignificant changes in coherence in both the lower- and higher-frequency bands (top right graph) with an emergence of a narrower central peak in the
cumulant density in three of the four subjects. A lack of a training effect on the average area of significant coherence for the nonresponders is reflected in the group results of Fig. 4 for both the low- and higher-frequency bands ($P > 0.3$ in both cases, paired $t$-test, see legend for post-training values). In contrast, the responders demonstrated a statistically significant increase in coherence in the higher-frequency band after training compared with before as shown for the individual subject in Fig. 3B and for the group results in Fig. 4 ($0.21 \pm 0.08$ before to $0.47 \pm 0.1$ after, right side, $P = 0.001$, paired $t$-test). Figure 3B also shows the improved EMG and goniometric profile of a responder compared with before training (Fig. 2B, left) where there is greater modulation of EMG and decrease in cocontraction. The responders also showed a small increase in the area of lower-frequency coherence (from $0.42 \pm 0.1$ before to $0.49 \pm 0.1$ after, Fig. 4, left side), but this increase was not significant ($P > 0.2$, paired $t$-test). All eight responders maintained a narrow central peak in the cumulant density (e.g., Fig. 3B, bottom right graph).

Because changes in the area of coherence above the 95% confidence line can reflect changes in both the amplitude and range of significant coherence, we also calculated the latter two variables independently. Similar to the area measurements, the average amplitude of coherence between 24 and 40 Hz that was above the 95% confidence limit did not change after training in the nonresponders ($0.002 \pm 0.003$ before vs. $0.0002 \pm 0.0005$ after, $P > 0.3$) and increased significantly in the responders ($0.007 \pm 0.014$ before vs. $0.031 \pm 0.029$ after, $P < 0.01$, paired $t$-test). In contrast, the average range where the coherence was >95% confidence was similar before and after training (e.g., 24–34 Hz before vs. 24–38 Hz after training in the responders) indicating that the area measurements after
training were mainly influenced by increases in the amplitude of significant coherence. Figure 4, B and C, which represents the differences in significant coherence before and after training averaged across all subjects (see Riddle and Baker 2005), shows a similar trend where the only consistent increase in coherence occurs in the higher-frequency range in the responder group (C) compared with the nonresponder group (B).

Relationship between coherence and functional walking recovery

To assess if increases in coherence in the responder group were related to improvements in walking function as assessed by the WISCI II score, we compared inter-muscular coherence from EMG data obtained on two separate occasions where the functional walking scores stabilized. In three responder subjects (1M, 2M, and 6M), intermuscular coherence was compared between the last 2 mo of treadmill training after the walking score had peaked. There were no appreciable changes in the mean area of high-frequency coherence between the last two recording sessions (0.46 ± 0.1 vs. 0.43 ± 0.2, P > 0.4, paired t-test), indicating a stable level of coherence alongside a stable functional walking score.

Relationship between 24–40 Hz coherence and TMS

Because it is generally considered that 24–40 Hz coherence is an indicator of common cortical drive to muscles, we examined if there was a relationship between changes in the area of coherence and changes in corticospinal tract connectivity as assessed by TMS. In 8 of the 12 subjects, changes in corticospinal tract function were assessed directly by TMS.
before and after training. We plotted the change in 24–40 Hz coherence area against the percentage increase in the maximum MEP size evoked by TMS (MEP\(_{\text{max}}\), Fig. 5). MEP responses were recorded in either the vastus lateralis or tibialis anterior muscle in both nonresponders (open symbols) and responders (closed symbols). There was a positive and significant relationship between the increase in MEP\(_{\text{max}}\) and in the area of coherence in the higher-frequency band (24–40 Hz, \(r = 0.72, P < 0.02\), Fig. 5).

Coherence in noninjured controls

Coherence profiles obtained from neurologically intact subjects (\(n = 3\)) during walking and voluntary cocontractions were compared with the coherence profiles obtained from subjects with incomplete spinal cord injury. Walking speed was set at 0.8 km/h for these trials to match the walking speed to the injured trials. During treadmill walking, there was little coherence, particularly in the 24–40 Hz band where the average area of coherence above the 95% confidence limit was not significantly different from zero (0.04 ± 0.03, \(P > 0.1\), Mann-Whitney test, Fig. 6A). There was some significant coherence near 5 Hz that may have been related to the walking speed (Neilsen 2002). We also asked subjects to perform a strong voluntary cocontraction of the hamstrings and vastus lateralis, a task that would presumably require a greater amount of common corticospinal drive to these muscles (Fig. 6B). When control subjects performed this task, a significant peak in intermuscular coherence emerged around the 30-Hz frequency band that was statistically significant from zero (0.18 ± 0.05, \(P < 0.05\), Mann-Whitney test). Although intermuscular coherence in noninjured controls were low compared with subjects with spinal cord injury, the measured area of significant coherence during walking and cocontractions were very reproducible when re-tested on a separate recording day for both the low- and higher-frequency coherence (inter-day values were not significantly different, \(P = 0.3\) or greater, paired \(t\)-test).

**FIG. 5.** Comparison of the change in the area of coherence between 24–40 Hz and the increase in MEP\(_{\text{max}}\) evoked by TMS in a subset of subjects. Eight subjects participated in both the coherence and TMS studies, and in 2 of these subjects, MEPs were recorded from both sides of the body, giving a total of 10 legs between which to compare changes in MEP\(_{\text{max}}\) and coherence in the 24–40 Hz band. There is a strong, positive correlation between increases in coherence and MEP\(_{\text{max}}\) size (\(r = 0.72, r = \text{correlation coefficient}, *, P < 0.05\). Open symbols, nonresponders; closed symbol, responders.

**DISCUSSION**

High-frequency (24–40 Hz) coherence between hamstrings and vastus lateralis EMG recorded during walking was only
present in responding (moderately strong), but not in nonresponding (weak), subjects with incomplete spinal cord injuries. These findings suggest that the presence of high-frequency coherence during injured walking is mediated by common descending drive to this pair of antagonist muscles. The presence of high-frequency coherence in moderately strong subjects fits with previous studies demonstrating that EEG/EMG coherence, which can be disrupted by TMS to the motor cortex (Hasan and Nielsen 2004), occurs at this higher-frequency band (Brown and Marsden 2001). In contrast, noninjured control subjects exhibited little to no high-frequency coherence between antagonist muscles during walking as reported previously (Halliday et al. 2003; Hansen et al. 2001). The lack of coherence between antagonist muscles in noninjured controls does not necessarily indicate that there is little to no descending drive to muscles of the leg during intact walking. Rather, it is likely that during intact walking there is very little common descending drive to antagonist muscle pairs. Leg muscles appear to be activated rather independently during intact walking because higher-frequency coherence is only present between different motor units of a given muscle or between synergistic muscles (Halliday et al. 2003; Hansen et al. 2001). However, results from this study show that after chronic and incomplete spinal cord injury, the strength of common descending connections to antagonist muscle pairs appears to be increased to generate functional but not necessarily “normal,” walking. Such compensations are likely induced because of the reduced descending drive to individual and synergistic muscles after injury (Hansen et al. 2005).

The high-frequency (24–40 Hz) coherence present in the moderately strong responders increased even further (doubled) after achieving improvements in walking function from intensive treadmill training. We propose that the increased coherence in the 24–40 Hz frequency band represents an increase in
common corticospinal drive to the hamstrings and vastus lateralis muscles because coherence in this band was related to
direct measurements of corticospinal tract strength as assessed
from MEP responses to TMS (Thomas and Gorassini 2005). In
contrast, low-frequency (5–18 Hz) coherence, which is thought
to be mediated mainly by spinal sources, did not change after
training in either the nonresponder or responder group. There is
every possibility that some components of the 5–18 Hz coherence
were also mediated by common drive originating from the
corticospinal tract (see Hansen et al. 2005), but these frequen-
cies appeared not to have any association with functional motor
recovery as they were unchanged in both the nonresponder and
responder group. Likewise, there is every possibility that com-
ponents of the higher-frequency coherence contained drive
from subcortical or spinal sources. Complete identification of
the source of the coherence would require simultaneous re-
cordings from the cortex, brain stem, spinal cord, and muscle
during the movement. Direct recordings from motor cortex and
muscle have been analyzed in the monkey (Jackson et al. 2002)
and in patients (Marsden et al. 2000) and have provided further
evidence for the cortical origin of the higher-frequency coher-
ence at least to hand and upper limb muscles.

Because we found a positive relationship between the in-
crease in the size of the MEP and the increase in coherence in
the 24–40 Hz band, it is likely that some of the changes
underlying the increase in coherence were corticospinal in
origin. The study by Thomas and Gorassini (2005) indicates
that increases in the excitability and strength of the corticospi-
nal tract are associated with improvements in locomotor func-
tion from treadmill training. The coherence results expand this
earlier study by revealing functional connectivity of the corti-
cospinal tract during walking as opposed to just the presence
of a pathway as revealed by TMS (anatomical connectivity) while
seated and making a tonic voluntary contraction. The respond-
ers not only appear, therefore, to have an increased corticospi-
nal capacity but to be able to make use of it during functional
tasks such as walking. This is an important finding and one that
could not have been made using conventional reflex or evoked
response measures given that coherence is measured during the
recording of a normal behavior rather than during an unrelated
motor task (Neilsen 2002).

Increases in common corticospinal drive to the hamstrings
and vastus lateralis muscles from training could arise from
changes occurring at both cortical and spinal levels. One possi-
bility is that training produces a greater recruitment of
cortical cells to activate more descending corticospinal tracts
with common input to hamstring and vastus lateralis motoneu-
ron pools. In the Thomas and Gorassini (2005) study, increases
corticospinal tract function were thought to involve changes
occurring in the primary motor cortex because increases in
MEP responses were also accompanied by increases in the
incidence of subthreshold silent periods and in the duration of
long-lasting (>100 ms) silent periods. Both these phenomenon
are considered to be mediated by cortical, rather than spinal,
circuits (Fuhr et al. 1991; Ikeda et al. 2000). In addition, treadmill training does not produce changes in the size or
modulation of segmental reflexes evoked during walking (J.
Yang and M. Gorassini, personal communication), suggesting
that increases in corticospinal tract function were not largely
influenced by increases in the strength of segmental circuity.

Changes in high-frequency coherence are related to
improvements in walking skill

Evidence that the changes in 24–40 Hz coherence, and
perhaps common corticospinal drive, were related to improve-
ments in walking skill comes from several sources within this
study. First, increases in high-frequency coherence were not
produced simply from exposure to the training and testing
program. Subjects who did not improve in their locomotor or
muscle strength scores (nonresponders) but underwent the
same training and testing program as subjects who did im-
prove, did not show any significant changes in their patterns of
coherence. Second, we analyzed the coherence pattern from
three subjects who had shown improvements in locomotor skill
and then plateaued. The pattern of coherence was not different
between the two plateau sessions, but it was significantly
greater between either of the plateau sessions and that obtained
before the training. This supports our view that in the light of
no changes in walking skill (as assessed by the WISCI II
score), the coherence profile remained consistent. Finally, the
changes in high-frequency coherence in the responder group
were not simply due to postraining increases in locomotor
EMG activity. Coherence is a unitless, normalized measure of
correlation (see METHODS), which means that the absolute am-
plitudes of the EMG signal are not a factor that affects the level
of coherence (Halliday and Rosenberg 1999). For example,
during walking the noninjured control subjects had higher
amplitude EMG than the responders but they had less mea-
sured coherence. As a corollary, the increase in coherence with
training does not necessarily indicate an increase in amplitude
or cocontraction of the EMG signal as responders demon-
strated less cocontraction, but had more high-frequency coher-
ence.

Functional and clinical implications.

We believe that the use of coherence analysis during neu-
rological rehabilitation is a further tool that increases our
ability to map neurophysiological changes during the natural
execution of a task (Nielsen 2002). However, like many cor-
relational tools, coherence can only “look back” one synapse
into a network or system. Thus the level of “common drive”
estimated using coherence is likely an underestimate because if
there is more than one synapse in the pathway, the temporal
variability introduced by the synaptic transmission will mean
that the activity at the recording site may not be completely
coherent, despite having a common origin. However, it re-
mains a powerful tool to estimate common drive for neural
events such as muscle activity or cortical potentials for strong,
direct pathways such as the corticomotoneuronal pathway
(Petersen et al. 2003). Coherence analysis, while compara-
tively complex to perform, requires only simple measures from
the patient, such as EMG during walking, and may be a more
clinically acceptable technique than reflex testing, sensory
evoked potential or TMS mapping. Because only the stronger
subjects and those who had more coherence in the 24–40 Hz
band showed a functional improvement, coherence analysis
may be able to predict those individuals who will benefit from
an intensive locomotor retraining program, especially for pa-
tients in the ASIA C category where some respond to locomo-
tor training, whereas some do not. In summary, the finding that
increases in corticospinal drive are associated with improvements in walking function after spinal cord injury provides the impetus to examine if enhancing cortical excitability via electrical and/or sensory stimulation is a useful technique to enhance spared corticospinal tract function and walking recovery when combined with motor rehabilitation (Hummel et al. 2005).

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