Bilateral Limb Phase Relationship and Its Potential to Alter Muscle Activity Phasing During Locomotion

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1Department of Physical Therapy and Human Movement Sciences, 2Interdepartmental Neuroscience Program (NUIN), and 3Department of Physical Medicine and Rehabilitation, Feinberg School of Medicine, Northwestern University; 4Department of Kinesiology and Nutrition, University Of Illinois, Chicago, Illinois; and 5Department of Physical Therapy, Governors State University, University Park, Illinois

Submitted 11 March 2009; accepted in final form 3 September 2009

Alibiglou L, López-Ortiz C, Walter CB, Brown DA. Bilateral limb phase relationship and its potential to alter muscle activity phasing during locomotion. J Neurophysiol 102: 2856–2865, 2009. First published September 9, 2009; doi:10.1152/jn.00211.2009. It is well established that the sensorimotor state of one limb can influence another limb and therefore bilateral somatosensory inputs make an important contribution to interlimb coordination patterns. However, the relative contribution of interlimb pathways for modifying muscle activation patterns in terms of phasing is less clear. Here we studied adaptation of muscle activity phasing to the relative angular positions of limbs using a split-crank ergometer, where the cranks could be decoupled to allow different spatial angular position relationships. Twenty neurologically healthy individuals performed the specified pedaling tasks at different relative angular positions while surface electromyographic (EMG) signals were recorded bilaterally from eight lower extremity muscles. During each experiment, the relative angular crank positions were altered by increasing or decreasing their difference by randomly ordered increments of 30° over the complete cycle 0° (in phase pedaling); 30, 60, 90, 120, 150, and 180° (standard pedaling); and 210, 240, 270, 300, and 330° out of phase pedaling). We found that manipulating the relative angular positions of limbs in a pedaling task caused muscle activity phasing changes that were either delayed or advanced, dependent on the relative spatial position of the two cranks and this relationship is well-explained by a sine curve. Further, we observed that the magnitude of phasing changes in biarticular muscles (like rectus femoris) was significantly greater than those of uniaxial muscles (like vastus medialis). These results are important because they provide new evidence that muscle phasing can be systematically influenced by interlimb pathways.

INTRODUCTION

The capability to generate complex interlimb coordination during locomotion is one of the most impressive features of the CNS. Interlimb coordination, particularly the maintenance of reciprocal, anti-phase motions of the limbs, is critical for stable human (bipedal) walking (Courtine and Schieppati 2003, 2004). Reciprocal coordination of limbs during locomotion is closely linked to rhythmic activity of circuits that control different muscles and synergies (Bernstein 1967; Bizzi et al. 2000; Dietz 1992, 2003; Dietz et al. 2002; Lundberg et al. 1987; McCrea 1992). Various sensory inputs can reconfigure these circuits’ activity and consequently can control and modulate the onset, intensity, and duration of muscle activity bursts during walking. Indeed interaction of these receptor’s inputs with command signals from higher centers and rhythm-generating circuitry can determine the appropriate coordinated pattern of agonist and antagonist muscles (Duysens et al. 2000).

Muscle timing abnormality is the main reason behind many motor disorders. A better understanding of the contribution of interlimb pathways for generating and modifying muscle activation timing may lead us to novel interventions to enhance functional recovery of the impaired limb after stroke or other neurological impairments.

Split-belt treadmill human walking studies that focused on interlimb coordination have shown that perturbations to one leg invoke a coordinated response of muscle activity with the same latency in both legs during stance and locomotion (Berger et al. 1984; Dietz et al. 1989). These studies suggested that the response measured in the contralateral leg may be mediated by changes in sensory input (such as proprioception, load, and stretch) in the ipsilateral leg. Reisman et al. (2005) tested this relationship by investigating walking using a split-belt treadmill. Their results showed that the locomotor parameters of one limb can adapt to the sensorimotor input from the contralateral limb and also that the adult nervous system can adapt and store new interlimb coordination patterns. This relationship has been supported by pedaling studies as well. Further investigation of this relationship led Ting et al. (1998a) to the conclusion that muscle coordination of the ipsilateral leg during pedaling depends on the sensorimotor state of the contralateral leg, and it can be shaped by interlimb sensorimotor pathways. Moreover they suggested that ipsilateral proprioceptive signals from one leg are insufficient to determine the muscle coordination pattern of that leg (Ting et al. 1998b). In another pedaling study by Ting et al., they proposed that possibly many interlimb coordination patterns exist to coordinate the legs as a functional unit with the specific set of muscles affected and the intensity of each effect depending on the exact bilateral afferent and efferent state (Ting et al. 2000). These findings strengthened the evidence for the potential of interlimb pathways to modify muscle phasing. Consequently, all of these findings are consistent with the idea that interlimb coordination somehow depends on interlimb reflexes and pathways that are regulated by bilateral afferent inputs (Duysens et al. 2000).

Although there is enough evidence to justify the influence of sensorimotor state of one limb on another limb, little is known about how the CNS coordinates muscle phasing during a locomotor task. For example, it isn’t clear whether or not the spatial relationship of the two limbs is primarily important for...
determining relative phasing of muscle activity or whether other factors such as whether one limb is leading the other also can influence phasing of muscle activity. Hypothetically, if muscle coordination in a pedaling task could be generated independently in each leg or if it could just be shaped by sensorimotor signals of the contralateral leg, the electromyographic (EMG) patterns should remain coupled with the spatial phase of the ipsilateral or contralateral leg regardless of relative temporal position of each leg to another. Whereas, if maintaining the same relative angular position of limbs but changing the leading leg can delay or advance EMG activity, it shows that the pattern of muscle activity during a locomotor task mainly depends on the timing of afferents from ipsi- and contralateral limbs during the ongoing cycle. Also with this study, we are the first investigators to systematically test the phasic influence of one leg on the other during an ongoing cyclical task by testing phasic limb positions at regular intervals.

Therefore this study will address these issues by measuring the extent to which ipsilateral muscle activity phasing can be influenced by the relative angular spatial relationship and relative position (i.e., leading or following the ipsilateral limb) of the contralateral limb. Pedaling is ideal for this particular study of locomotion because the task mechanics are controlled and can be manipulated (Kautz and Brown 1998; Kautz et al. 2002; Ting et al. 1998a,b, 1999, 2000). Because the subject is seated, neither balance nor external body weight support is required and the kinetics and kinematics of the legs can be analyzed in isolation of the head, arms, and trunk. It has been shown that biomechanical functions and phasing of the muscles appear to be similar during pedaling and walking despite differences in kinematics (Ting et al. 1998a). Pedaling was therefore an ideal task in this study because it allowed mechanical coupling and decoupling of the lower extremities for testing the importance of interlimb phase relationships in a locomotor activity.

Our study also afforded an opportunity to compare uniaxial muscles (vastus medialis) with biarticular muscles (e.g., rectus femoris) to determine if these two groups respond differently to the phasic limb manipulations. Muscle function during locomotion is thought to be divided into two specific sets of functional muscle groupings (uni- and biarticular) with different roles. Biarticular muscles are thought to be responsible for the regulation of desired direction, whereas the uniaxial muscles seem to mainly work as force generators (Jacobs and van Ingen Schenau 1992). Further, it has been shown that the bifunctional muscles (such as the hamstrings and rectus femoris muscles) responsible for limb transitions between flexion and extension are more strongly influenced by contralateral sensorimotor input than other muscles in neurologically intact individuals (Kautz et al. 2002). This finding raised a new question about whether interlimb pathways are capable of modulating uni- and biarticular muscles timing differently.

The central objective of this study was to determine the extent to which the phasing of the muscle activity of the lower limbs can be influenced by the relative angular relationship of the limbs accomplished by manipulating the relative angular position of the cranks. Our main hypothesis was that the muscle activity phasing in the cycle would systematically adapt to varied leg angular relationships even though the mechanics of the task remained similar. Secondarily, we hypothesized that uniaxial muscles would show little to no substantial phase shifts, whereas biarticular muscles would show substantial phase shifts as a result of the manipulation of relative angular crank position.

**METHODS**

Twenty neurologically intact subjects [13 men, 7 women; age: 53 ± 7 (SD) years] who were naïve to the experimental goals signed consent forms prior to participation in the study. Data were collected from both the dominant and nondominant lower limb of each participant; the dominant lower limb was determined for each subject by asking which lower limb he/she would use to kick a ball. This study was approved by the Institutional Review Board at Northwestern University.

A custom-made, split-crank, bicycle ergometer with instrumented pedals, consisting of a seat with a backrest and a motor driven crank was used for this study (Rogers et al. 2004). Participants were secured to the backrest with nylon straps, and shoulder supports were used to further stabilize the trunk, confining movement to the legs during all tasks. Clipless style pedals with straps allowed the subjects to maintain a rigid connection between the feet and pedal during experiments. The entire ergometer was attached to a hydraulic tilt mechanism that was used to position the backboard at 25° from horizontal to assure seating comfort during all experimental conditions. The ergometer had a split-axe design with a detachable coupling mechanism. The between-axle phase alignment was adjustable, allowing the limbs to be coupled in 15° off-sets from fully anti-phased (standard pedaling) to in-phase relationships while motor driven control was maintained.

Three optical encoders (BEI Model EX116-1024-2), one at each pedal spindle and one coupled to the right crank, provided measurements of the crank and pedal angles with an accuracy of ≥0.3°. Pedaling velocity was controlled by an electric motor (8.1 gear reducer, 10 hp with flux vector drive; model SV3000, Seco Electronics, Lancaster, SC) and was kept constant (40 revolution/min) for all subjects and conditions. This velocity was chosen because a previous study in our lab had shown that for an imposed velocity of 40 rpm, the motor can accurately regulate actual crank speed despite large applied forces (40.5 ± 0.8 rpm) (Rogers et al. 2004).

By controlling crank velocity, the motor provided mechanical isolation of the two limbs even during the bilateral pedaling task such that neither leg influenced the motion of the cranks. This critical feature of the apparatus allowed us to mechanically decouple legs in each pedaling task. Consequently, each leg separately went through the same cycle kinematic trajectory across all conditions (a fundamental characteristic of our experimental design) regardless of effort level or force output, and the only thing that was experimentally manipulated was the relative angular position of the legs. In addition, because the motor-driven crank design does not allow us to control workload directly, feedback of pedal forces was used to assure that effort levels were similar between conditions (see following text).

Bipolar silver surface electrodes (DelSys, 10 mm length, 1 mm width, 1 cm interelectrode distance) were used to record EMGs from eight muscles bilaterally (16 muscles total): vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF), biceps femoris long head (BF), semimembranous (SM), tibialis anterior (TA), medial gastrocnemius (MG), and soleus (Sol). Standard skin preparation was applied prior to the application of bipolar silver surface electrodes. EMG signals were amplified with a gain of 10 at the electrode site before remote differential amplification (common mode rejection ratio: 92 dB, gain range: 100–10,000 times, frequency response: 20–450 Hz) and low-pass filtering (500 Hz, custom-designed filter). The digital optical encoder and force transducer signals were converted to analog with a D/A converter module before sampling. Then all signals were sampled at 1,000 Hz via a 12-bit A/D converter (National Instruments) and Labview software. EMG collection was synchronized with the acquisition position data from the transducers and optical encoders of the cycle ergometer.

**J Neurophysiol • VOL 102 • NOVEMBER 2009 • www.jn.org**
The subjects were asked to pedal with moderate effort, bilaterally, at 12 randomly assigned trials of pedaling at different relative angular relationships with respect to the right leg [0° (in-phase); 30, 60, 90, 120, 150, and 180° (typical standard pedaling); and 210, 240, 270, 300, and 330°] as shown in Fig. 1. During each task, the cycle ergometer motor rotated the legs in the forward direction at the constant velocity of 40 rpm. Subjects were instructed to assist the motor actively by pedaling the crank in a forward direction with a moderate amount of effort using both legs. Pedal forces were monitored throughout the pedaling cycle, using tri-axial force transducers in each pedal (Delta 660, ATI-IA, Garner, NC). Bar graphs with real-time pedal force were displayed as feedback to ensure that subjects used both legs actively. All sessions began with the standard (nominal 180°) pedaling task. While asking subjects to “pedal with moderate effort,” their preferred peak force was determined during the initial standard pedaling task. This force value was used as the preferred effort level. The goal range of force was set at the preferred level ±10%, indicated by error bars on the computer screen. Then for every pedaling task, the pedal forces were monitored and subjects were given feedback to keep approximately the same peak force output that they had produced with the standard pedaling task.

At each angular relationship condition, data were collected for 30 s at a rate of 1,000 Hz to ensure ≥20 complete crank revolutions through mid-down stroke in a steady state. To minimize the possible effects of fatigue, all subjects were provided a minimum of 30 s rest between trials and were given adequate rest periods if further recovery was needed.

Data processing

This study focused on muscle activity phasing, or the relative onset of muscle activation with respect to the crank angle in pedaling at different relative angular relationships between the right leg and left leg. Therefore we used an EMG processing technique that allowed comparison of muscle phasing that was minimally influenced by EMG amplitude. First, to compare muscle activity at the same point in the pedaling cycle for every crank revolution, we rectified the EMG signals and referenced them to the crank position in 1-degree increments. Data that were sampled within any 1° increment were averaged and assigned to the crank position that represented the middle value of the range. Next we integrated the EMG signals (in volts) throughout every pedaling cycle. For each task, the integrated EMGs were averaged across 20 crank cycles. During this process, left and right legs were analyzed independently.

To compare the phasing of EMGs in pedaling at various relative angular positions without regard to EMG amplitude, all EMG profiles were smoothed with a fourth-order, zero-lag, low-pass Butterworth filter with a cutoff frequency of 25 Hz. Then, we cross-correlated each full-wave smoothed EMG signal from different experimental conditions to the EMG signal of the same muscle (at the same side) in the nominal 180° phase relationship using Matlab (R2007b) software. Cross-correlation between pairs of processed EMG curves was performed as follows. Consider two processed EMG profiles of nominal 180° and of another experimental condition. The complete processed EMG signal of each experimental condition was displaced forward and backward in 1° increments through the entire EMG profile of nominal 180°. Correlations were taken and saved at every step. This cross-correlation technique measured the similarity in shape between two curves as a scalar between 0 and 1, analogous to the dot product of two vectors. Two curves with exactly the same shape had a cross-correlation of 1.0. Uniform scaling (changing the amplitude of the curve without changing its shape) did not affect the cross-correlation results. Then the lag at which the highest correlation occurred between EMG profile of nominal 180° and the compared experimental condition was deemed as the phase shift for that specific position. The sign of this phase shift was considered positive or negative relative to EMG profile of nominal 180°. Positive values for the phase shift indicate advanced phasing.

FIG. 1. Schematic view of different angular crank relations in our pedaling tasks. The subjects were asked to pedal at 12 different angular relationships. In all conditions, the right pedal was assumed as reference side and the relative angular relationship of limbs were determined by calculating the angular distance of left pedal from right pedal in clockwise direction. At 0°, both limbs were in-phase. In 5 conditions (30, 60, 90, 120, and 150°) as shown in this figure, the left side led the right side; 180° was typical standard pedaling where there was the greatest angular difference between both limbs. In 5 other conditions (not shown in figure), the right side led the left side (210, 240, 270, 300, and 330°).
whereas negative values indicate delayed phasing. This gave us a value for the number of degrees of phase change that occurred for that muscle within that angular relation condition relative to the nominal pedaling (180°).

The group averages of phasing changes at each relative angular relation were then calculated for each muscle separately. Phase change magnitude versus angular relation were plotted and then fitted with a sine wave by using a program that applied the Levenberg–Marquardt algorithm (OriginPro 8 software). So we were able to compare sine wave parameters (amplitude, phase lag and period) of the fitted sine waves to find the relationships between EMGs’ phase shifts versus limb angular differences for different muscles during our manipulations.

In this study, the sine wave represents the phase shift of muscle activity from the nominal 180° position at each relative angular crank position. Each point along the sine wave (the amplitude of the sine wave at each relative crank position) represents the EMG phase shift that yielded the greatest cross-correlation coefficient for that condition (Fig. 2). The positive values for the amplitude represent an advanced phase shift relative to nominal 180° position, whereas the negative values express the delayed phasing shift relative to nominal 180°. The period of each sine wave was also measured because it helped us to calculate the frequency of each sine wave, which suggests whether the muscles are controlled by oscillators acting at the same or different frequencies. Another parameter of sine function was the phase lag that represents a “shift” of the sine wave from zero phase. For example, in the case of our studies, existence or nonexistence of a phase difference between the sine waves of different muscles during the same experimental task could indicate whether their central oscillators were out of phase or in phase with each other.

These values were analyzed with two-tailed paired sample t-test. We performed this analysis for each of the eight tested muscles and looked for common trends with bi- versus uniarticular muscles, flexors versus extensors, and dominant limb versus nondominant limb. We used a P value of <0.05 to test for significance.

**RESULTS**

Comparison of sine wave parameters representing phase shifts of each muscle

Figure 3 shows a representative set of averaged and smoothed EMG signals recorded from one representative subject during pedaling at different crank angular relationships. During each crank angular relation condition, each muscle exhibited a major burst of EMG activity per cycle. The timing of EMGs shifted earlier and later in the cycle; i.e., the EMG onsets delayed or advanced as a consequence of changing the relative angular relation between the two cranks. Although with each subject changes were evident across all recorded muscles, as shown for the individual in Fig. 3, the magnitudes of these muscle activity phasing changes were different in some manner for each muscle. As described in METHODS, the phasing changes at twelve relative angular crank relations were calculated for each muscle separately. For each subject, calculated phase shifts, with 1° resolution, were plotted and fitted with a sine wave function.

To find out the extent to which dominant and nondominant lower extremities’ phasing responses to relative angular phasing manipulations were similar, we compared the parameters of the sine waves fitted to all recorded muscles, averaged across all subjects, of dominant leg to nondominant leg. All of our participants except one were right dominant. Given that we were interested in comparing the dominant and nondominant legs phasing changes, we discarded the data from the left dominant subject for this dominant/nondominant analysis. Then a two-tailed paired sample t-test was used to determine whether there was any difference between the muscles’ phasing responses of the dominant and nondominant leg to the same angular crank relation condition. Between-group comparisons revealed that both lower extremities’ (i.e., dominant and nondominant) muscles demon-

**Fig. 2.** Illustration of sine wave fitting technique used in our study. A: represents 2 averaged smoothed electromyographic (EMG) signals of a tested muscle during bilateral pedaling task at 180° and 90° relative angular crank positions. In this figure, the “Amp” represents the phase change at which the highest correlation occurred between EMG profile of nominal 180° and 90° angular crank positions. After finding the phase changes for all relative angular crank relations, these values versus angular relation were plotted and then, fitted with a sine wave (see B). B: the sine wave (bold black line) fitted to the “Amps” of the same tested muscle at all relative angular crank positions as an example to illustrate the calculation of each sine wave parameter in our analysis. As described in the preceding text, here in B, the “Amp” represents the amplitude of sine wave at 90° angular crank relation. Indeed each point along the sine wave (amplitude) represents the EMG phase shift that yielded the greatest cross-correlation coefficient for that condition. The positive values for the amplitude represent an advanced phase shift relative to nominal 180° position, whereas the negative values express the delayed phasing shift relative to nominal 180°. “Period” represents the period of each sine wave. “Phase lag” represents the angular shift of a fitted sine wave relative to an ideal zero phase sine wave (gray dashed line).
strated relatively similar behavior at each angular crank relation. Indeed there was no significant difference ($P > 0.05$) among phase lag, period, and amplitude of dominant (right) and nondominant (left) side sine waves for each muscle. We compared dominant to nondominant side because in human locomotion, in which no pathologies are present, different functions of the dominant and nondominant lower extremity have been proposed. For example, Hirokawa (1989) suggested that the dominant limb's primary function is to provide a propulsive force during midstance. Conversely, the nondominant limb's primary function is that of support (Hirokawa 1989; Sadeghi et al. 1997). Contrary to these results, other researchers (Haddad et al. 2005) suggested that both legs contribute to the overall dynamics of gait with no preference. We found no significant difference between dominant and nondominant limb, therefore we only report results from dominant limb muscles from here onward.

We also compared phase changes for VM and VL as two knee uniaxial extensor muscles. By using two-tailed paired sample $t$-test, we identified no significant differences ($P > 0.05$) between phase lag, amplitude and period of fitted sine waves to VM and VL muscle. Therefore we only report results from VM from here onward.

Table 1 represents the group sine wave parameters (phase lag, amplitude, and period) of each measured muscle from

**Table 1. Sine wave parameters fitted to each muscle’s phasing changes relative to the 180° angular crank relation condition**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Phase lag</th>
<th>Period (%)</th>
<th>Amplitude*</th>
<th>Adj. $R^2$</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>182.77 ± 3.7</td>
<td>168.76 ± 5.6</td>
<td>21.85 ± 1.5</td>
<td>0.95</td>
<td>18</td>
</tr>
<tr>
<td>VM</td>
<td>169.88 ± 3.8</td>
<td>164.64 ± 5.5</td>
<td>11.36 ± 0.9</td>
<td>0.93</td>
<td>18</td>
</tr>
<tr>
<td>VL</td>
<td>168.75 ± 3.6</td>
<td>165.14 ± 5.3</td>
<td>13.34 ± 1.0</td>
<td>0.94</td>
<td>18</td>
</tr>
<tr>
<td>BF</td>
<td>122.13 ± 5.1</td>
<td>133.07 ± 6.7</td>
<td>18.94 ± 2.2</td>
<td>0.87</td>
<td>15</td>
</tr>
<tr>
<td>SM</td>
<td>77.38 ± 16.4</td>
<td>138.05 ± 16.8</td>
<td>14.46 ± 3.5</td>
<td>0.57</td>
<td>17</td>
</tr>
<tr>
<td>MG</td>
<td>157.57 ± 7.2</td>
<td>181.69 ± 12.3</td>
<td>13.00 ± 1.6</td>
<td>0.85</td>
<td>17</td>
</tr>
<tr>
<td>Sol</td>
<td>162.78 ± 4.8</td>
<td>155.29 ± 6.4</td>
<td>15.44 ± 1.7</td>
<td>0.88</td>
<td>17</td>
</tr>
<tr>
<td>TA</td>
<td>198.08 ± 6.0</td>
<td>167.41 ± 9.1</td>
<td>24.26 ± 2.5</td>
<td>0.89</td>
<td>18</td>
</tr>
</tbody>
</table>

Values are means ± SE. RF, rectus femoris; VM and VL, vastus medialis and lateralis, respectively; BF, biceps femoris long head; SM, semimembranosus; MG, medial gastrocnemius; Sol, soleus; TA, tibialis anterior. *Amplitude is the degrees shifted relative to 180 degree condition.
the dominant leg. The adjusted $r$-squared values in this table indicate the goodness of fits and therefore confirm that these sine waves were well fitted to the group-averaged data.

Figure 4 shows the fitted sine waves to each muscle’s phasing responses during our experimental tasks in a representative subject and averaged across all subjects. As shown here, the pattern of changes were similar between a representative subject (gray dashed lines) and group mean values (bold black lines). The fitted sine waves showed phase-advanced activity in relative angular crank relations where the ipsilateral leg was leading contralateral leg movement and phase-delayed activity in experimental relations where the ipsilateral leg was following contralateral leg movement. Also this figure shows that the phase lag and magnitude of sine waves fitted to each muscle’s phasing changes varied between muscles, and consequently the pattern of changes were not the same across all muscles even within a subject. We analyzed these differences by comparing uniarticular with biarticular muscle groups as well as between extensor and flexor muscle groups in the following sections. We observed similar results across the group averaged curves (Fig. 4, group curves).

Comparison of uni- and biarticular muscle activity phase changes

Figure 5 shows the comparison of group averaged fitted sine waves for bi- and uniarticular extensor muscles crossing knee and ankle joints. In thigh muscles, the amplitude of the fitted sine wave in the biarticular extensor muscle (RF) was signif-

![Graphs showing phase shifts and relative angular crank relations for different muscles](https://example.com/graphs.png)
We also compared the fitted sine wave parameters of shank bi- and uniarticular extensor muscles (Sol vs. MG), but we found no significant difference for amplitude, phase lag, or period \( (P > 0.05) \). This result reveals that phase shifts of these shank muscles were very similar even though Sol is uniarticular and MG is a biarticular muscle.

**Comparison of extensor and flexor muscle activity phase changes**

We expected to observe a significant difference between extensor and flexor responses to our bilateral angular crank relation manipulation. For purposes of this analysis, we limited our extensor-flexor comparison to the ankle muscles (Sol, extensor; TA, flexor) because we recognize that both of our recorded knee major flexor muscles (BF and SM) and also, one of the knee major extensor muscles, RF, are bifunctional muscles during performance of the pedaling task (Raasch et al. 1997).

We used two-tailed paired sample t-test to compare responses to changes in relative angular crank position, in the form of the derived sine wave parameters, between the two muscles. We observed that the phase lags of the fitted sine waves differed significantly \( (P < 0.05) \) between Sol and TA (Fig. 6). Also the magnitudes of phasing changes (amplitude of sine wave) were significantly greater in TA compared with Sol \( (P < 0.01) \). There were no significant differences between the period of these muscles’ fitted sine wave parameters \( (P > 0.05) \).

**DISCUSSION**

We found that manipulating the angular relation of the cranks in a pedaling task can cause muscle activity phasing changes that are either delayed or advanced, dependent on the relative angular position of the two cranks. Our hypothesis that the muscle phasing in the cycle systematically adapts to varied relative angular relationships in neurologically intact individuals was therefore supported. In addition, these changes were symmetrical about both legs, and uni- and biarticular muscle phasing changes differed from each other as did phase changes compared between ankle extensor and flexor muscles.

Before discussing our results, it is important to re-emphasize that because of the apparatus characteristics and our control of pedaling effort, although we changed the angular relation of the cranks relative to each other, the mechanics of the task remained invariant for each leg across all conditions. Therefore the default neural control strategy for each limb need not necessarily change as a result of manipulations of the relative angular crank relations. Our results suggest that neural constraints related to neural input from task command and/or movement-related afferents from the contralateral leg may be responsible for the observations reported in the results section.

**Changes in muscle activity phasing patterns**

With two- or four-legged locomotion, a high degree of spatiotemporal coordination exists both between (interlimb) and within (intralimb) limb segments where the exact coordinative patterns employed depend on the constraints imposed onto the system (Haddad et al. 2005). It has been suggested that intralimb coordination is typically more stable, making interlimb coordination the primary locus for adaptations during locomotion (Whitall and Caldwell 1992). This has been supported by other studies (e.g., Haddad et al. 2005) that suggested that changes in interlimb coordination were much greater than the changes observed in intralimb coordination during a locomotor task like gait.

While other studies have investigated lower extremity interlimb coordination during split treadmill-based locomotor tasks (Dietz et al. 1994, 2001; Reisman et al. 2005, 2007), the major difference of our novel design with the previous studies of interlimb coordination was that we decoupled both limbs mechanically such that movement of one limb didn’t affect the force exerted in another limb—this type of manipulation could not be accomplished during treadmill studies. Thus we were able to make meaningful comparisons between biomechanically equivalent but neurophysiologically different bilateral tasks that needed to generate an adaptive interlimb coordination strategy.

Appropriate interlimb coordination requires sensorimotor inputs of the contralateral side to contribute to the correct triggering of the spinal networks of the ipsilateral side at a specific phase to produce appropriate and synchronized muscle activity phasing. By manipulating the relative angular crank relations, we manipulated the relative phasing of sensorimotor inputs of one side relative to another side. In other words, although the ipsilateral side still received consistent sensorimotor state signals from the contralateral side (because the intraleg mechanics of the task was the same across all conditions), the relative phasing of these signals arrived at spinal networks of the ipsilateral side differed from condition to condition. Consequently, each limb adapted the muscle activity phasing to maintain the interlimb coordination strategy needed to perform the desired task at different relative angular crank relations. Our findings are consistent with the previous studies described in the preceding text because our results clearly showed that changing relative angular positions of limbs has a significant influence on interlimb coordination patterns.
observed that delayed muscle activity in one leg occurred simultaneously with advanced activity of the same muscle (with the same magnitude) in another leg and vice versa.

The average of the $r$-squared values (Table 1) that we found for fitted sine waves was 0.86, indicating that the degree of fit of the data to the sine wave model was high. According to this finding, we suggest that the CNS uses a simple sine tuning system for controlling the muscle’s phasing to compensate for relative angular changes of limbs. Also applying the sine wave fitting method helped us to identify the pattern of phasing changes. Analysis of EMG parameters related to amplitude and on-off timing could only provide us limited comparisons between each experimental condition’s EMG profile and nominal pedaling (180°). Whereas by using the sine wave model approach, we were able to compare the pattern of these changes through the entire set of relative angular crank manipulations. Indeed comparing the specific sine wave parameters (i.e., amplitude, phase lag, and period) of the fitted curves not only revealed important differences in how muscles respond to these experimental manipulations but also provided novel information about interlimb pattern generation during a locomotor task in terms of bilateral synchronization rather than in terms of individual muscle control. Consequently, we found that sensitivity of the motor output pattern to interlimb relative angular position may appear in different ways rather than just the timing of the EMG signal; i.e., in amplitude changes of fitted sine waves that show muscles’ phasing changes or in period and phase lag changes of the fitted curve that shows relative phasing behavior of the muscle compared with other muscles. Moreover, underlying timing mechanisms of all lower extremity muscles are strongly related as shown by our findings that indicated similar delayed or advanced activity pattern for all muscles, regardless of function, at different relative angular positions.

In general, we found that most of the recorded muscles in our experiments showed phase-advanced activity in relative angular crank relations where the ipsilateral leg was leading the contralateral leg movement and phase-delayed activity in experimental relations where the ipsilateral leg was following contralateral leg movement. This pattern was observed for both the dominant and nondominant lower extremities and sine waves fitted this relationship very strongly with periods of delay and periods of advance. Because the mechanics of the cycling task stayed the same in all conditions, this phenomenon shows to what extent the contralateral leg spatial position is critical to affect ipsilateral muscles’ coordination.

**Uni- versus biarticular muscle phasing changes in pedaling task**

The largest changes in phasing were found in biarticular muscles like RF, BF, and SM. These results were consistent with previous pedaling studies that have shown that biarticular muscles are more susceptible to sensory adaptation than uniarticular muscles (Schindler-Ivens et al. 2004; Ting et al. 1999), and biarticular muscles are more strongly influenced by contralateral sensorimotor input than uniarticular muscles (Kautz et al. 2002).

In addition, Smeets suggested that the accuracy of sensory and motor signals is greatest when associated with movements generated by biarticular muscles (Smeets 1994). He compared the behavior of two sets of muscles (uni- vs. biarticular) in controlling multi-joint arm movements by modeling the response characteristics of the muscle spindle afferents. He showed that both the sensory and the motor accuracy of the set containing biarticular muscles were in general better than those of the set containing only uniarticular muscles. So he stated that one function of biarticular muscles is to ensure good overall accuracy of the sensory and motor information about the speed and direction of a movement. Indeed this argument can explain differences in control strategies of different muscles between situations that do not differ biomechanically from each other like our experimental conditions. Although accuracy considerations put an additional constraint on muscle coordination, it seems to support the larger magnitude of phasing shifts that we have observed with biarticular muscles during our manipulations. Hypothetically, the greater phasing shifts of biarticular muscles compared with uniarticular muscles in response to the same relative angular positions of limbs demonstrate greater sensitivity of the biarticular muscles to this sensorimotor input changes. Because of the higher sensitivity, a small change in the phasing relation of limbs can cause a larger timing change in biarticular muscles relative to uniarticular muscles.

In addition, animal studies have shown that during normal locomotion, biarticular thigh muscles show both complexity and mutability in their activity that is markedly increased compared with that shown by the uniarticular muscles (Pratt et al. 1996). Because the biarticular muscle excitation appears to be closely linked with the intersegmental dynamics (Pratt et al. 1996), central influences are thought to be less important than peripheral influences. Accordingly, it has been suggested that a differential distribution of position and load related afferent information to bi- and uniarticular muscles allows this peripheral input to play a critical role in shaping biarticular muscle excitation (Ingen Schenau et al. 1994).

**Flexor versus extensor muscle phasing changes in pedaling task**

When we compared the Sol ankle extensor and TA ankle flexor muscles’ responses to changes with angular crank relation, we observed a significant difference between phase lags and amplitude of the fitted sine waves. Comparing the TA to the Sol fitted sine wave parameters, we observed the phase lag and the amplitude of TA sine wave were significantly greater than those of Sol sine wave. The latter indicates that the TA muscle was more sensitive to our limb relation manipulations than the Sol.

We expected to see differences between the extensor and flexor muscles’ fitted sine wave parameters because there exists evidence that leg flexor and extensor muscles are controlled differentially in both animals and humans (Cheng et al. 1998; for review, see Dietz 1992). For example, leg flexors have a high responsiveness to visual stimuli but the leg extensors to somatosensory input in the cat (Beloozerova and Sirota 1988) and in human (Dietz 1992). Therefore proprioceptive afferent information continuously modulates the activity of extensors with their antigravity function during gait, whereas the flexor activation is more controlled by central inputs (for a review, see Dietz 1992). Particularly, it had been shown that the corticospinal projections to lower limb motoneurons to the TA muscle are stronger than to the Sol muscle (Brouwer and Asby 1992; Schubert et al. 1997). It also has been suggested that during walking the corticospinal tract is more closely
linked with the segmental neural circuits controlling TA than it is with those controlling Sol (Capaday 1999). In our study, the existence of a significant phase difference between the sine waves of TA and Sol during the same experimental task indicates that their control mechanisms may be centrally controlled oscillators are phase shifted relative to each other. However, the similar period of their sine waves implies that these control mechanisms act at the same frequency.

Furthermore, in some contemporary models of locomotor control (e.g., Hiebert et al. 1996), the flexor half-centers of homologous limbs reciprocally inhibit each other during walking, whereas the extensor half-centers are not directly coupled with each other. Synchronous activation of the leg extensors on both sides can easily be elicited (i.e., stance phase coexisting on both sides), whereas a synchronous activation of flexor is rarely observed. Correspondingly, in human infant stepping, the relationship between the two flexor half-centers is thought to be stronger compared with the extensor half-centers (Pang and Yang 2000). The observations made in these studies would be in line with a more central dominance in the control of leg flexor activity compared with extensor activity. In addition, based on observations in walking thalamic cats, it has been argued that flexor motoneuron activity is more directly controlled by the CPG (Duysens 1977). In this “flexor burst generator” scheme, extensor activity occurs as a consequence of flexor activation.

**Possible underlying neural control schemes**

We observed that timing relations of the bilateral electrical activity (EMG) of leg muscles changed systematically when we altered the spatial relative angular displacement of the legs methodically. These observations provide new evidence for the importance of interlimb pathways and limb position inputs. Our findings raise a question about different control schemes that may generate or control the phasing pattern of locomotor bursts. Due to the important role that muscle coordination plays in the control of locomotion, appropriate intra- and interlimb muscle activity phasing is critical for effective and efficient locomotion (Rossignol et al. 1993). In the following paragraphs, we will discuss some central control theories that are particularly related to the results of our experiment.

One of the central neural mechanisms that had been proposed for generating appropriate interlimb timing is coordination due to spinal cord neural network. It has been suggested that CPGs (i.e., the ensemble of spinal cord neural networks that generate the locomotor bursts) can be the main center of muscle pattern formation. According to theories using the coupled oscillator model (Brown 1914), the appropriate performance of the inhibitory connections cause normal agonist-antagonist muscle activation in interlimb and intralimb levels, whereas inappropriately phased activation of locomotor control network elements can theoretically provoke abnormal movement coordination. The sinusoidal nature of muscle phasing variations that we observed in our study are consistent with this coupled oscillator theory.

The strong correlation that we found between tuning curves of different muscles timing on both sides support the suggestion that there is common rhythm-generating circuitry that receives and processes somatosensory signals of all involved muscles in any locomotor task and modulates their timing accordingly. This can be accomplished with the conceptualization of a unique phasing and pattern control center that can receive inputs from different pathways and networks (upper and lower), while the only output that it generates is timing (phasing) of the locomotor behavior (McCrea and Rybak 2008). Recently, this type of CPG organization model has been proposed in which a two-level CPG has a common rhythm generator that controls the operation of the pattern formation circuitry responsible for motoneuron activation (McCrea and Rybak 2008). This suggests that even locomotor forms requiring different phasing patterns could be coordinated by a common rhythm-generating circuitry and would therefore be subject to cross-modulation.

Even though interlimb coordination is presumably assured through propriospinal pathways, it has been known that supraspinal structures are playing a crucial role as well (Rossignol et al. 1993). A cerebellar contribution via reticulospinal neurons has been suggested in both cats (Ito 1984) and humans (Bonnet et al. 1976), and evidence was presented for a cortical (supplementary motor area) control of interlimb coordination (Debaere et al. 2001) as well. Dietz et al. (2002) showed that the unilateral locomotion in patients with spinal cord injury was associated with a normal pattern of leg muscle EMG activity restricted to the moving side, whereas in the healthy subjects, a bilateral activation occurred. Therefore they suggested that coupling between CPGs is weak when the input from supraspinal structures is reduced and therefore the interlimb coordination observed in neurologically healthy subjects requires the supraspinal inputs. This finding was in line with cat data showing that interlimb coupling in spinal cats was much weaker than in cats with intact supraspinal control (Dietz 2002a,b).

Study of people with cerebellar damage showed that cerebellar damage can disrupt interlimb adaptation and coordination (Morton and Bastian 2006), whereas study of people with stroke demonstrated that cerebral regions were less important for peripherally driven locomotor adaptations (Reisman et al. 2007). A recent neural theory to support the importance of supraspinal structures in interlimb coordination during locomotion is put forward by Drew et al. (2008), who suggested that the subpopulations of motor cortical neurons, active sequentially during the locomotor step cycle, may regulate the activity of small groups of synergistic muscles, likewise active sequentially throughout the step cycle. This group suggested that during locomotor activity, subpopulations of motor cortical neurons may modify the phase of the EMG activity of all muscles contained within a given synergy. However, the relative importance of the different supraspinal or cortical structures in either inter- or intralimb coordination during human locomotion requires further study.

**Conclusions**

Evidence from the amplitudes and phase lags of the sinusoidal variations in muscle timing during our manipulations demonstrated that the muscles’ advanced or delayed activities corresponded to relative angular position of ipsi- and contralateral limbs. Also biarticular muscles responded to a greater extent while participants pedaled bilaterally at all experimental tasks. It is therefore concluded that ipsi- and contralateral limb relative angular position affects muscle phasing during a dynamic pedaling task.

While the novel findings of our study demonstrate the critical importance of bilateral limb phase relationship in modulation of
muscles timing and phasing, they do not identify a neural mechanism for these changes. Now that we have found a way to manipulate muscle activation phasing, the next challenging topic to investigate is the underlying neural mechanism that may generate or modulate the muscle phasing during locomotion.

ACKNOWLEDGMENTS

We gratefully acknowledge L. M. Rogers and C. L. Johnston for invaluable collaborations.

GRANTS

This work was supported by the American Heart Association Grant 0715536Z.

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