1. Title Page

Title: Contribution of afferent feedback to the soleus muscle activity during human locomotion

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2. Abstract

During the stance phase of the human step cycle, the ankle undergoes a natural dorsiflexion that stretches the soleus muscle. The afferent feedback resulting from this stretch enhances the locomotor drive. In this study a robotic actuator was used to slightly enhance or reduce the natural ankle dorsiflexion, in essence, mimicking the small variations in the ankle dorsiflexion movement that take place during the stance phase of the step cycle. The soleus (SOL) and tibialis anterior EMG were analyzed in response to the ankle trajectory modifications. The dorsiflexion enhancements and reductions generated gradual increments and decrements, respectively, in the ongoing SOL EMG. We exercised care to ensure that the imposed ankle movements were too slow to elicit distinct burst-like stretch reflex responses, that have been investigated previously. The increased SOL EMG following the dorsiflexion enhancements was reduced when the group Ia afferents were blocked with peripheral ischemia at the thigh, and during high frequency Achilles tendon vibration. However, neither ischemia nor tendon vibration affected the decrements in the SOL EMG during the dorsiflexion reductions. These findings give evidence of the contribution of afferent feedback to the SOL activity in an on-going basis during the stance phase. The results suggest that mainly feedback from the group Ia pathway is responsible for the increments in the SOL EMG during the dorsiflexion enhancements. However, the decrements in the SOL activity might be mediated by different afferent mechanisms.

Key Words: human walking, sensory feedback.
3. Introduction

In an attempt to investigate the importance of afferent feedback during human walking, several research groups have measured the muscle response to rapid perturbations of the foot and/or leg (Dietz et al. 1985; Dietz et al. 1987; Yang et al. 1991; Sinkjaer et al. 1996). Yang et al. (1991) used a pneumatic device to apply rapid dorsiflexion perturbations in the early stance phase. As a result, burst-like stretch reflex responses, defined as a short latency reflex with a synchronized large amplitude response, were elicited in the SOL muscle. It was concluded that the observed reflex response contribute 30-60% of the SOL EMG during the stance phase of human walking. Sinkjaer et al. (1996) confirmed this conclusion and extended these observations to show that the SOL stretch reflex response is modulated during the gait cycle, being higher at the mid stance phase.

These studies demonstrated the importance of afferent feedback mediating compensatory reflex responses to unexpected external perturbations; however the extrapolation of these results to normal unperturbed walking must be interpreted cautiously. It might be necessary to differentiate the role of afferent feedback during such corrective reactions, and the contribution of afferent feedback to the on-going muscle activation during unperturbed walking. During normal unperturbed walking other neural mechanisms might be implicated (Yang et al. 1991; Nielsen and Sinkjaer 2002). Recent evidence suggests that the afferent feedback that is generated during unperturbed walking and the afferent feedback that signals an unexpected destabilizing perturbation may be centrally processed differently depending on the frequency components of the feedback signal (Morita et al. 1998).
One way to demonstrate the contribution of afferent feedback to the background SOL EMG during walking is to remove the feedback, and observe the effect of this removal on the EMG activity. Sinkjaer et al. (2000) hypothesized that by arresting the ankle dorsiflexion or applying a fast plantarflexion movement during the stance phase, the ankle extensor muscles would be unloaded, and the afferent feedback from these muscles would decrease. Indeed, following the unload movements, a drop of approximately 50% is measured in the SOL EMG, suggesting that afferent feedback from group II muscle spindles and/or tendon receptors contribute to the SOL activity during the stance phase of walking.

The aim of the present study was to investigate the afferent feedback mediated adjustment of SOL activity in the stance phase of normal human walking. We applied small-amplitude, slow-velocity modifications to the ankle trajectory during the stance phase of the gait cycle in order to mimic the variations on the ankle movement that normally occur during human walking. The slow ankle trajectory modifications would therefore simulate the modifications in sensory feedback that might occur during unperturbed walking on flat or slightly uneven surfaces. The protocol involved controlled enhancements and reductions of the natural ankle dorsiflexion during the stance phase of the gait cycle. We hypothesized that if sensory feedback contributes to the enhancement of the plantar flexor muscle activation during walking, the slow dorsiflexion enhancements and reductions would result in coincidental increments or decrements in the ongoing SOL EMG. A semi-portable robotic actuator was used to apply the slow ankle trajectory modifications. We exercised care to ensure that the dorsiflexion enhancements were too slow to produce the distinctive burst-like stretch
reflex responses that are typical to fast perturbations (Dietz et al. 1987; Yang et al. 1991; Sinkjaer et al. 1996). Peripheral ischemia and sustained Achilles tendon vibration were applied together with the ankle trajectory modifications to investigate the contribution of the group Ia to the SOL EMG.
Methods

Twenty-nine volunteers (15 females and 14 males, aged 20-37) with no history of neurological disorders were recruited to participate in this study. The local ethics committee approved the experiments in accordance with the Declaration of Helsinki, and the volunteers gave written informed consent prior to the experiments. The study was divided in 5 experiments, which were performed in different sessions.

Apparatus and Instrumentation

Throughout the experiments the subjects walked on a treadmill (Powerjog EG30, Sport Engineering Ltd, Great Britain) with the left leg attached to a semi-portable robotic actuator capable of rotating the ankle joint in dorsiflexion and plantarflexion. Full details of the device are presented elsewhere (Andersen and Sinkjaer 1995; Andersen and Sinkjaer 2003). Briefly, the device consists of a functional joint aligned with the ankle of the subject and attached to the foot and leg with a polypropylene plaster cast. The actuator is connected to an AC servomotor that applies torque to the functional joint through flexible Bowden cables. The ankle angle was measured with an optical encoder incorporated within the functional joint. The ankle velocity was determined by numerical differentiation of the ankle angular record. The EMG of the SOL and tibialis anterior (TA) muscles of the left leg were recorded with bipolar surface electrodes (Neuroline 720, Medicotest A/S, Denmark) separated by 2 cm, and band pass filtered from 0.5-5000 Hz (DISA, Model 15C01). All signals were sampled at 2 kHz and stored for later analysis (Data Acquisition Card PCL718; 700 MHz IBM
compatible PC). A heel switch based on a force sensitive resistor was placed in the insole of the left shoe to trigger the signal acquisition.

Walking protocol and on-line analysis

At the beginning of the each session the subjects walked on the treadmill for a 5-10 min adaptation period in order to become accustomed to the robotic actuator. During this period they chose a comfortable velocity (3.5 km.h⁻¹), that would be maintained for the rest of the experiment. At the end of this period, data were acquired during normal walking to generate a control profile of the ankle trajectory, SOL and TA EMG. Next, short-latency stretch reflex responses were elicited in the SOL muscle by applying fast dorsiflexion perturbations (500 deg.s⁻¹; 8 deg) during the stance phase. The onset and profile of the short latency stretch reflex response (SLR) were used to examine the presence of such response during the application of slow ankle movements.

Stretch reflex response analysis:
The SOL EMG was rectified, low-pass filtered (40 Hz), and ensemble averaged (approximately 30 trials) to produce a profile of the SLR. The onset of the SLR was defined as the first major deflection in the EMG record following the perturbation as determined by visual inspection of the burst. The magnitude of the SLR was quantified in a 15-20 ms window placed over the SOL EMG record, as the peak-to-peak EMG value within the defined window.
A 200-300 ms time window was defined in mid to late stance phase at the normal ankle trajectory (approximately 200-400 ms after heel contact), for the application of slow ankle trajectory modifications. These ankle trajectory modifications consisted of small-amplitude, slow-velocity enhancements and reductions of the normal ankle dorsiflexion during the stance phase. Perturbations were presented pseudo-randomly every 5-8 steps and a control step was recorded before each perturbation. Data acquisition of every data set was continued until approximately 30 control and perturbed steps were recorded. When a set of records was obtained, the actuator was programmed to present two new perturbation levels and the procedure was repeated. Eight to 12 different levels of dorsiflexion enhancements and reductions were applied in steps of ± 2 deg. The magnitude of the imposed dorsiflexion enhancements was increased until a level was reached such that distinct SOL SLR responses were observed in some of the individual trials. The SLR reflex response was easily recognized in these trials as a sharp large deflection in the EMG within a 20 ms window centered on the onset latency of the SLR latency determined from the muscle response to the fast dorsiflexion perturbations. This level of enhancement was eliminated from the analysis and only the slower-velocity and smaller-amplitude perturbations were analyzed. This general procedure was applied in the experiments described below.

Graded dorsiflexion enhancements and reductions

Graded dorsiflexion enhancements and reductions were applied to investigate the contribution of feedback from muscle and tendon receptors to the ongoing SOL EMG. By slightly enhancing or decreasing the ankle dorsiflexion, the muscle-tendon
complex of ankle extensors would be stretched to a greater or lesser extent than a normal step, and therefore afferent feedback from these ankle extensor muscles would increase or decrease, respectively. In 12 volunteers, 8 to 10 dorsiflexion enhancements and reductions were applied in steps of approximately \( \pm 5 \text{ deg.s}^{-1} \) and \( \pm 2 \text{ deg} \) as described in the previous section.

Effect of the amplitude and velocity of the perturbations

To explore the amplitude and velocity sensitivity of the increments in the SOL EMG following the dorsiflexion enhancements two series of perturbations were applied in 12 volunteers. In one series the dorsiflexions had different velocities and amplitudes, and in the other series, the dorsiflexions had different velocities but the same amplitude. In the first case differences in the SOL EMG increments related to different perturbation levels would be due to changes in the amplitude and velocity of perturbations. In the second series, differences in the SOL activation related to the different perturbation levels would be only caused by the different dorsiflexion velocities, because the amplitude of all the enhancements was the same. In each protocol, 5 to 6 levels of dorsiflexions were applied. Dorsiflexions with the same velocity but different amplitudes were not applied for technical reasons.

Modulation of the SOL response in the mid and late stance phase

To study the SOL response to the dorsiflexion enhancements on different parts of the stance phase, in 12 volunteers the same level of dorsiflexion was applied 200-300 ms after heel contact (mid stance phase), and 150-200 ms later (late stance phase), and
maintained for 150-250 ms. The amplitude and velocity of the perturbations were chosen as the minimum dorsiflexion level that evoked an increment in the SOL EMG (approximately $+5 \text{ deg.s}^{-1}$; $+2 \text{ deg}$).

Ischemic block of the group Ia afferent pathways

In eight subjects peripheral ischemia was combined with dorsiflexion enhancements or reductions to evaluate the contribution of the group Ia afferent pathways to the SOL EMG. In this protocol, the SLR and the medium latency stretch reflex responses (MLR), mediated by the group Ia and II pathways, respectively (Nashner 1976; Allum and Budingen 1979; Diener et al. 1983; Dietz et al. 1987; Toft et al. 1991; Schieppati et al. 1995; Corna et al. 1995), were used to monitor the ischemic block.

Prior to the application of ischemia, fast dorsiflexion perturbations were applied during walking to elicit a stretch reflex response. Next, the actuator was programmed to apply one level of slow dorsiflexion enhancement or reduction during the stance phase.

Ischemia was applied by positioning a pneumatic cuff approximately 10 cm above the left knee and inflating it to a pressure of 220 mmHg while the subjects were seated with their knees flexed at 90 deg. During the first 15 minutes, while the subject remained seated, the progress of the ischemic block was determined by applying 5-10 fast ankle dorsiflexion perturbations every 2 minutes and measuring the amplitude of the SLR. When the amplitude of the SLR decreased to approximately 25% of the initial value, the subjects were asked to walk with the pneumatic cuff inflated and still
in place. During walking, the monitoring was performed by applying fast dorsiflexion perturbations every 6-8 steps, and measuring the SLR response. When the SLR decreased to approximately 15% of the initial value, the group Ia fibers was considered blocked, and the same slow dorsiflexion enhancement or reduction applied before ischemia was repeated. In addition fast dorsiflexion perturbations and control steps were recorded randomly.

The experiment was stopped when the subjects had difficulty walking or when the MLR response started to decrease (Grey et al. 2001). The amplitude of the SLR and MLR responses were restored approximately 10 minutes after the cuff was deflated.

Achilles tendon vibration

High frequency tendon vibration is a powerful stimulus for the group Ia afferents (Burke et al. 1976a; Roll and Vedel 1982; Roll et al. 1989). In this study Achilles tendon vibration was combined with slow dorsiflexion enhancements and reductions to investigate the contribution of the Ia pathways to the SOL EMG during the stance phase.

Achilles tendon vibration during sitting

It has been previously shown that the group-Ia-mediated short latency reflex response is depressed during the application of tendon vibration (Schieppati et al. 2001). Therefore we initially evaluated the effect of constant Achilles vibration on the Ia pathways, by comparing the SOL SLR response before and during tendon vibration,
in the sitting position. A custom designed, high-pressure, hydraulic actuator (MTS-Systems Corporation 215.35, 230 bar) (Voigt et al. 1999) was used to deliver fast dorsiflexion perturbations during sitting, to elicit a stretch reflex response in the SOL muscle. An in-house fabricated portable vibrator was used to deliver the vibratory stimulus. The vibrator consists of a DC motor with an eccentric rotating mass, embedded in a plastic tube (2.5 cm diameter, 5 cm long), with a total weight of 150 g. During the experiment the subjects were sitting with the right knee flexed approximately 20 deg and the right hip flexed approximately 80 deg. The right foot was firmly strapped to a foot adapter on the hydraulic actuator ensuring that the anatomical axes on the ankle of the subject coincided with the center of rotation of the actuator. The subjects were instructed to hold a constant 5% MVC plantar flexion against a footplate; visual feedback was provided through an oscilloscope. Initially, a series of 25 fast dorsiflexion perturbations (600 deg.s\(^{-1}\); 8 deg) were applied to elicit a control profile of the SLR. Next, the vibrator was positioned transversally to the tendon and tightly fixed by means of an elastic ankle support. The Achilles tendon was constantly vibrated at 110 Hz and the fast dorsiflexion perturbations were repeated.

Tendon vibration during walking

In subjects in whom the SOL SLR response was decreased during the application of vibration in the sitting position, the test was repeated during walking. Nine subjects were instrumented with the portable ankle actuator and the portable vibrator on the left leg. The ankle actuator was programmed to impose fast dorsiflexion perturbations, and the SLR responses without and during Achilles tendon vibration
were compared. If the amplitude of the SLR during vibration was reduced to at least 60% of the value without vibration, the slow dorsiflexion enhancements and reductions were applied without and during constant tendon vibration.

Off-line data analysis

Signal processing and analysis were carried out offline. The EMG records were rectified and filtered with a 40 Hz first-order low-pass filter to extract an amplitude envelope. The SOL EMG response to the slow dorsiflexion enhancements and reductions was visually examined for any evidence of SLR response, and all trials that showed such a response were removed from the analysis. In all cases, no more than 5-10 trials were removed from the analysis. The muscle activity was calculated as the area under the EMG signal from the beginning to the end of the perturbations. Changes in SOL EMG due to the dorsiflexion enhancements and reductions were calculated as the difference in activity between perturbed and control steps, and normalized with respect to the background muscle activity. For the protocol with constant-amplitude enhancements, the muscle activity was calculated as the mean EMG value within the dorsiflexion window. In this case the increments were normalized with respect to the same control value. Similarly, when the enhancements were imposed in the mid and the late stance phases, the increments in SOL EMG were normalized with respect to the same control activity. In this case the control activity was measured from the onset of the mid stance perturbation to the end of the late stance perturbation.
The velocity of the ankle movements were expressed as the difference (increment or decrement) with respect to the normal dorsiflexion velocity in (deg.s⁻¹).

Statistical Analysis

Linear regression analyses were used to test the relationship between the changes in the SOL activity and the velocity of the imposed ankle dorsiflexion movements. In the protocol where enhancements with the same and different amplitudes were applied, the slopes of both linear regression analyses were compared. A one-way RM-ANOVA test was used to evaluate the response to the enhancements applied in the mid and late stance. Paired Student t-tests were applied to compare the responses to the slow perturbations without and during ischemia. A two-way RM-ANOVA test was used to analyze the effect of ankle perturbations and Achilles tendon vibration in the SOL activity during control steps, stretch reflexes and slow ankle perturbations. The results of all statistical tests were considered significant at the p<0.05 level. Results are presented as mean ± SD unless otherwise indicated.
Results

The dorsiflexion enhancements and reductions were applied in the mid to late stance phase, approximately 200 - 400 ms after the heel contact when the natural dorsiflexion increases gradually. The reason that the perturbations were not presented earlier in the stance phase is that shortly after heel contact the ankle joint undergoes a rapid transition from plantarflexion to dorsiflexion. Any dorsiflexion enhancement applied in this early phase of the stance would have to be fast, with the consequence of eliciting a burst-like stretch reflex response, that we were taking care to avoid. The ankle trajectory modifications were almost imperceptible to the subjects, possibly because the imposed changes in the dorsiflexion amplitude and velocity were in the range of the normal variability of the ankle movement during the stance phase of walking.

Relationship between the ankle dorsiflexion and the SOL EMG

The normal ankle dorsiflexion was enhanced or reduced during the stance phase, and the influence in the ongoing SOL EMG was tested. Figure 1 is an example of a typical data record for one subject, with each trace representing an ensemble average of 30 trials. In this case, the subject walked at 3 km.h⁻¹ and the natural ankle dorsiflexion was enhanced or reduced for a period of 300 ms, starting approximately 350 ms after heel contact (Figure 1A). The velocity and amplitude of the natural ankle dorsiflexion within the selected time window were 33 deg.s⁻¹ and 10 deg, respectively. While eight different ankle trajectories modifications were applied to this subject, only 4 sets are shown for clarity. The shown dorsiflexion enhancements and reductions have
approximately the following velocities and amplitudes, respectively: ± 6 deg.s\(^{-1}\); ± 2 deg and ± 16 deg.s\(^{-1}\); ± 5 deg. Following the perturbation, the ankle was released and it returned to its normal trajectory after approximately 100 ms. Figure 1B shows the corresponding ensemble average of the rectified and filtered SOL EMG record. Before the perturbations, the SOL EMG of the control and modified steps were similar, but during the perturbations, the EMG increased or decreased when the natural dorsiflexion was enhanced or reduced, respectively. The SOL EMG following the dorsiflexion enhancements and reductions changes smoothly and there are no abrupt changes similar to that observed with rapid perturbations. The TA EMG was unchanged in the perturbed trials compared to the controls (Figure 1C), therefore it was not further analyzed. Figure 1D illustrates the percent changes in SOL EMG in response to the 8 different perturbations applied to this subject.

Figure 1E shows the linear regression analysis for each subject (grey-dotted lines) and across all subjects (black-solid line, n=12), between the percent changes in SOL EMG and the velocity of the imposed dorsiflexions. For every subject, the slope of the linear regression was significantly greater than zero. The regression slope of the group analysis (0.87 % per deg.s\(^{-1}\), \(r = 0.85\)) is significantly different from zero (p<0.0001), indicating that the variation in the SOL EMG is linearly related to the velocity and/or amplitude of the perturbations. The point corresponding to the control step (0 %, 0 deg.s\(^{-1}\)) was not included in the regression analysis but was tested to be within the 95 % confidence interval as would be expected.
The increment in SOL EMG is primarily velocity sensitive

Two different sets of dorsiflexion enhancements were applied in order to explore the amplitude and velocity sensitivity of the observed increment in the SOL EMG. Figure 2 shows an example data for a single subject. In this case the subject walked at 3.4 km.h\(^{-1}\); 12 ankle perturbations were applied, but only 6 are shown for clarity. Figure 2A shows averaged data of the ankle trajectory during control steps and dorsiflexion enhancements. The velocity and amplitude of the normal ankle dorsiflexion within the selected time window were 17 deg.s\(^{-1}\) and 5 deg, respectively. The dorsiflexions enhancements were applied 310 ms after heel contact and maintained for 200-280 ms. In the series shown on the left the velocity and amplitude of the dorsiflexions are +6 deg.s\(^{-1}\), +2 deg; +13 deg.s\(^{-1}\), +4 deg and +26 deg.s\(^{-1}\), +8 deg, respectively. In the series shown on the right, the final amplitude of all dorsiflexions is 6 deg, but the velocities are different: +20 deg.s\(^{-1}\); +26 deg.s\(^{-1}\) and +30 deg.s\(^{-1}\). When the ankle was released it returned to its normal trajectory within approximately 100 ms. Figure 2B shows the corresponding ensemble average of the rectified and filtered SOL EMG. Prior to the dorsiflexions, the SOL EMG of control and modified steps are similar, although the SOL EMG increased when the ankle dorsiflexion was enhanced. After the ankle was released the EMG slowly decreased and returned to the level of the control EMG. In Figure 2C, the linear regression analyses for the same subjects of both protocols are plotted superimposed. The regression lines show the relationship between the percent change in SOL EMG and the velocity of the perturbations. For this subject the dorsiflexions with different amplitudes and velocities had a regression slope of 0.78 % per deg.s\(^{-1}\) (r=0.92), and the protocol of dorsiflexions with the same
amplitude but different velocities had a regression slope of 0.60 \% per deg.s^{-1} (r=0.95).

Across all subjects (n=12), the dorsiflexions with different amplitudes and velocities had a slope 1.2 ± 0.4 \% per deg.s^{-1} (p=0.004), and the dorsiflexions with the same amplitude but different velocities had a slope 0.71 ± 0.2 \% per deg.s^{-1} (p=0.0006). A comparison between the two slopes showed that they were not significantly different from each other (p=0.1).

FIGURE 2 HERE

Equal SOL EMG response in the mid and late stance phases

Slow dorsiflexion enhancements were applied in the mid and late phases of the stance to investigate whether the contribution of afferent feedback to the SOL EMG is modulated during different parts of the stance phase. Figure 3 is a typical data record for a subject walking at 3.2 Km.h^{-1}. In this case the slow enhancements were applied 330 and 500 ms after heel contact (mid and late stance phases), and maintained for 180 ms. Within the corresponding time windows the normal amplitude and velocity of the ankle dorsiflexion were 4 deg and 22 deg.s^{-1}, respectively. The applied dorsiflexion enhancement had amplitude of 3 deg, and a velocity of 16 deg.s^{-1} (see Figure 3A). Figure 3B shows the corresponding ensemble averaged and filtered SOL EMG signals. The SOL EMG during the perturbations increased with respect to the control EMG in both cases, the mid and late stance phases.
Figure 3C shows the percent increments in SOL EMG for mid (23.6 ± 7 %) and late (22.6 ± 7 %) stance phases across all subjects (n=12). In both cases the increments in SOL EMG were greater than zero, but not significantly different from each other (one-way RM-ANOVA: p=0.58).

FIGURE 3 HERE

Contribution of the group Ia to the SOL EMG

Peripheral ischemia was combined with slow dorsiflexion enhancements and reductions to evaluate the contribution of group Ia afferents to the SOL EMG. Figure 4A shows typical data of one subject of the ankle trajectory and SOL EMG during control steps and fast dorsiflexion perturbations, before and during the ischemic block. Note that before the ischemic block, the onset of the SLR was evident and clearly defined, appearing 50 ms after the stretch and lasting approximately 20 ms. Typically the amplitude of the SLR started to decrease approximately 15 minutes after the cuff inflation, and it decreased to less than 15 % of the initial value approximately 18-20 minutes after the cuff inflation. Figure 4B shows the amplitudes of the SLR and MLR responses across all subjects (n=8) before and during ischemia. A paired t-test showed a significant difference (p=0.027) in the amplitude of the SLR before and during the ischemic block (79.5 ± 14 µV and 27.8 ± 7 µV, respectively). However the MLR response was not statistically different before and during the block (93 ± 13 µV and 56.5 ± 10 µV, respectively, p= 0.1). Figures 4C-F show typical data of two subjects of the ankle trajectory and SOL EMG during control steps and dorsiflexion enhancement and reductions, before and during ischemia. Figure 4C shows the ankle
trajectory profile when ischemia was combined with dorsiflexion enhancements. In this example the subject walked at 2.8 km.h⁻¹. The velocity and amplitude of the normal ankle dorsiflexion within perturbation window were 32 deg.s⁻¹ and 8 deg, respectively. The enhancement (+ 20 deg.s⁻¹; + 5 deg) was applied 430 ms after heel contact and maintained for 250 ms. The SOL EMG corresponding to the control and perturbed steps are shown in Figure 4E. The SOL EMG increased following the dorsiflexion before the application of ischemia. However during ischemia there was no increase in the SOL activity.

Figure 4G shows percent increments in the SOL EMG across all subjects (n=8) during the dorsiflexion enhancements. A Student’s Paired t-test showed that the responses to the dorsiflexion enhancements before (30.3 ± 7 %) and during ischemia (3.5 ± 3 %), were significantly different from each other (p = 0.007).

Figure 4D shows a case where ischemia was combined with dorsiflexion reductions. In this example the subject walked at 3 km h⁻¹, and the normal dorsiflexion velocity and amplitude within the perturbation window were 36 deg.s⁻¹ and 9 deg, respectively. The dorsiflexion reduction (- 20 deg.s⁻¹; - 5 deg) was applied 210 ms after heel contact and maintained for 250 ms. Figure 4F shows that the SOL EMG activity decreased during the dorsiflexion reductions in both cases, before and during the ischemic block.

Figure 4H shows percent decrements in the SOL EMG during dorsiflexion reductions across all subjects (n = 8). A Student’s Paired t-test showed that the percent
reductions in EMG before (-16.3 ± 5 %) and during ischemia (- 11.5 ± 4 %) were not statistically different from each other (p = 0.1).

**FIGURE 4 HERE**

Constant Achilles tendon vibration was combined with dorsiflexion enhancements and reductions to evaluate the contribution of group Ia afferents to the SOL EMG. Initially the effect of tendon vibration on the group Ia was evaluated during sitting. Figure 5A shows typical raw data of a subject of the ankle trajectory and SOL SLR during sitting without and during Achilles tendon vibration. In 9 subjects the amplitude of the SLR without and during vibration (0.8 ± 0.2 mV and 0.35 ± 0.2 mV, respectively) were significantly different (p=0.002).

A two-way RM-ANOVA test showed that during walking, both, the ankle perturbations and tendon vibration had a significant effect on the SOL EMG (p<0.01), and there was a significant interaction between both factors. The SOL SLR response without and during vibration (54.3 ± 12 µV and 32.9 ± 8 µV, respectively) were significantly different from each other (p=0.003). However the MLR response without and during vibration (54.6 ± 11 µV and 44.8 ± 6 µV, respectively) were not statistically different (p=0.2) (Figure 5B).

Figures 5C-F show raw data of 2 subjects of the ankle trajectory and SOL EMG during control steps and dorsiflexion enhancements and reductions without and during Achilles tendon vibration. The subject shown in Figure 5C walked at 2.8 km.h⁻¹, and the velocity and amplitude of the normal ankle dorsiflexion within the defined time
window were 32 deg.s\(^{-1}\) and 8 deg, respectively. The dorsiflexion enhancements (+ 7 deg.s\(^{-1}\); + 2 deg) were applied 330 ms after heel contact and maintained for 270 ms. For the case shown in Figure 5D the dorsiflexion reductions (- 18 deg.s\(^{-1}\); -4 deg) were applied 400 ms after heel contact and maintained for 210 ms (see Figure 5D). Figure 5G shows that the SOL EMG during control steps with and without tendon vibration (31.7 ± 7 µV.s and 35 ± 7 µV.s, respectively) were not significantly different from each other (p=0.125). During the dorsiflexion enhancements, the SOL EMG without and during tendon vibration were significantly different from each other (14.4 ± 4 % and 10.5 ± 3 %, respectively, p=0.023) (see Figure 5H). However, the decrement in the SOL EMG during dorsiflexion reductions applied without and during tendon vibration were not statistically different from each other (-12.5 ± 6 % and -12.6 ± 7 %, respectively, p = 0.912) (Figure 5I).

FIGURE 5 HERE
Discussion

The aim of this study was to investigate the afferent feedback mediated adjustments of the SOL muscle activation during the stance phase of human walking. Our experimental paradigm was designed to modify the proprioceptive feedback from ankle extensor muscles by slightly enhancing and decreasing the normal ankle dorsiflexion during the stance phase, thus mimicking the normal variability of the ankle movement during walking on flat or slightly irregular surfaces. The imposed dorsiflexion enhancements and reductions generated gradual increments and decrements respectively, on the ongoing SOL EMG. The SOL EMG increments resulting from the dorsiflexion enhancements were more sensitive to the velocity of the ankle movement than to its amplitude, and were similar in the mid and late stance phases of the step cycle. In addition ischemia and Achilles tendon vibration depressed the increments on the SOL EMG during the dorsiflexion enhancements, but did not affect the decrements on the SOL activity in response to the dorsiflexion reductions. This study provides evidence that afferent feedback contributes to generate adaptive modifications on the SOL activity during walking. Moreover the results from ischemia and Achilles tendon vibration indicate that increments on the SOL activity may be sensitive to feedback from the group Ia pathways, while different afferent mechanisms might mediate the decrements during the dorsiflexion reductions.

Slow dorsiflexion enhancements and reductions

In this study, slow dorsiflexion enhancements and reductions (± 40 deg.s⁻¹) generated gradual increments and decrements on the SOL EMG of ± 45 %. In previous studies
fast dorsiflexion perturbations have been applied in the stance phase of the step cycle, to analyse the contribution of afferent feedback to the SOL activity during walking. For example, Yang et al. (1991) applied dorsiflexion perturbations of up to 100 deg. s\(^{-1}\) in the early stance phase and reported increments of 30-60 % on the SOL EMG. Later, Sinkjaer et al. (1996) applied faster dorsiflexion perturbations (250 deg. s\(^{-1}\)) in different parts of the gait cycle and reported increments of 50-90 % on the SOL EMG during the stance phase. In both studies, the rapid ramp-shaped perturbations elicited distinct burst-like responses in the SOL EMG. In contrast, the slow dorsiflexion enhancements used in the present study generated gradual increments in the SOL activity that extended to the entire range of the perturbation.

It is likely that the afferent feedback that is associated with small-amplitude, slow-velocity variations of the normal ankle trajectory, mediate adaptations in the muscle activity differently than during fast and large unexpected perturbations (Nielsen and Sinkjaer 2002). The slow dorsiflexion enhancements and reductions applied in the present study may modify the sensory feedback in a similar manner to what might happen during normal walking over flat or uneven ground. In contrast, the fast dorsiflexion perturbations that have been used in previous studies might generate an ‘error-like’ signal mediating corrective reflexive responses to these unexpected external events. Morita et al. (1998) proposed that afferent information is processed differently depending on the frequency of the signals. Therefore, the low-frequency sensory information during normal walking might be processed differently by the central nervous system than the high frequency bursts of signal associated with fast and large unexpected perturbations. These two inputs might, therefore, generate different outputs despite the fact that the same pathways might be involved.
Afferent pathways mediating the SOL EMG adaptations

The dorsiflexion enhancements and reductions, respectively, increase and decrease the natural lengthening of the ankle extensor muscles during the stance phase of the step cycle. Therefore, it is very likely that the output from muscle spindle and Golgi tendon organs increased and decreased, respectively, compared with a normal step, contributing to the observed changes in the SOL activity.

The response to the dorsiflexion enhancements was not modulated at least in the mid and late stance phases of the gait cycle. In contrast, it is known that the SOL stretch reflex (Dietz et al. 1985; Yang et al. 1991; Sinkjaer et al. 1996) and the H-reflex (Capaday and Stein 1987) are modulated during the stance phase of the step cycle. For example, Capaday and Stein (1987) reported that the H reflex is relatively low at the time of heel contact, increases progressively during the stance phase, and reaches its maximum amplitude late in the stance phase. Sinkjaer et al. (1996) reported that the stretch reflex reaches its maximum in the mid stance, and decreases at the late stance. Morita et al. (1998) proposed that the stretch reflex is less sensitive to presynaptic inhibition than is the H-reflex and, therefore, the two mechanisms are modulated differently. They suggested that the motoneuronal pool might react differently to a highly-synchronized input, such as the electrical stimuli used to elicit an H-reflex response, compared with a less-synchronized input, such as the mechanical perturbation needed to elicit a stretch reflex response. A similar justification can explain our observation that the response to the slow enhancements was not modulated during the stance phase of the gait cycle. Feedback associated to the slow
trajectory modifications applied in the present study is less synchronized than feedback associated to fast perturbations; therefore these two responses might be modulated differently.

The regression curves for the dorsiflexions with different amplitudes and velocities, and the constant amplitude dorsiflexions were not significantly different. This result suggests that, although the velocity of the imposed dorsiflexion enhancements was slow, the increment in the SOL EMG was more sensitive to the velocity than to the amplitude of the imposed dorsiflexions. While it is well known that the spindle primary endings are more sensitive to velocity than are the spindle secondary endings, both endings respond to amplitude and velocity of the muscle stretch and have the potential to contribute to the observed response (Matthews and Stein 1969).

Peripheral ischemia and Achilles tendon vibration were applied to investigate the contribution of the group Ia pathways to the observed changes on the SOL EMG following the slow perturbations. Surprisingly, both maneuvers affected the response to the dorsiflexion enhancements but not to the reductions, suggesting that these adaptations in the muscle activity may be mediated by different afferent mechanisms.

During the imposed dorsiflexion enhancements the amplitude and velocity of the muscle tendon complex stretch is greater than during a normal step, therefore it is expected that muscle spindle and Golgi tendon organs (GTO) from the stretched muscles increase their firing rate. However, it has been shown that spindle primary afferent pathways (group Ia) are more sensitive to muscle stretch than are spindle secondary and GTO afferent pathways (Burke et al. 1976a; Burke et al. 1976b).
Therefore the observed increments in the SOL EMG may be mostly mediated by feedback from the group Ia pathways. Consequently, when peripheral ischemia and Achilles tendon vibration were applied, this response was largely affected. However, as peripheral ischemia differentiates afferent fibers based on their diameter and in humans there is very likely considerable amount of overlap in the diameter of the groups Ia and Ib fibers (Burke et al. 1983), it is not possible to separate the contribution of these two fiber types with this technique. Moreover, because smaller fibers are blocked with time we cannot exclude the possibility that the largest of the group Ib and II fibers were also blocked at the end of the protocol, and that they also contribute to the observed increment in the SOL EMG.

During the dorsiflexion reductions however, the robotic actuator slowed down the natural ankle joint movement and reduced the stretch of ankle extensor muscles. It has been shown that during small-amplitude, slow-velocity sinusoidal muscle stretch, the Ia pathways are more sensitive than the group II pathways during the stretch phase of the imposed movement, however they become completely silent during the unloading phase (Kakuda 2000). The group II fibres however, follow the sinusoidal load increasing and decreasing its firing rate during all the cycle, even during the unloading phases when the Ia pathways are silent. During the dorsiflexion reductions applied in the present study, although the ankle extensors were still eccentrically stretched, the perturbation prevents the ankle extensors to stretch as it would normally happen. This unloading force might decrease the sensitivity of the group Ia afferent pathways, and therefore feedback from other pathways may become more relevant during this response. As a result, peripheral ischemia and Achilles tendon vibration did not affect the response to the dorsiflexion reductions. This finding is consistent
with the results of Sinkjær et al. (2000) that the drop in the SOL EMG when the ankle
dorsiflexion was arrested during the stance phase of the gait cycle was not affected by
peripheral ischemia.

Achilles tendon vibration did not affect the SOL activation during control steps.
Similar result was reported by Courtaine et al. (2001), by applying bilateral Achilles
tendon vibration during over ground walking. These results suggest that feedback
from the group Ia pathways may not contribute importantly to the background SOL
activation, while feedback from other pathways may play a more significant role.

With the techniques applied in this study, we cannot rule out whether other than the
group Ia afferent pathways are involved in the regulation of the background SOL
EMG during walking. For example, it has been proposed that load feedback from Ib
afferent pathways reinforces the activation of muscles during human walking (Dietz
and Duysens 2000; Duysens et al. 2000) as has been shown on the cat (Conway et al.
1987; Pearson et al. 1992; Gossard et al. 1994; Hiebert and Pearson 1999). It is also
possible that feedback from cutaneous receptors could contribute to the responses
observed in this study. However, cutaneous afferents have not been shown to
contribute to the stretch reflex response produced by a rapid dorsiflexion perturbation
(Grey et al. 2001) or the unload response produced by a rapid plantar flexion
perturbation (Grey et al., unpublished observations). Despite the results suggest that
the observed adaptive modifications in the SOL activity are very likely mediated by
proprioceptive input rather than cutaneous input, the role of feedback from cutaneous
receptors should be assessed in future studies.
Final conclusion

Previous studies have demonstrated that when an unexpected fast dorsiflexion perturbation disrupts the normal ankle trajectory during the stance phase, a burst-like short-latency reflex response is elicited in the SOL muscle (Dietz et al. 1987; Yang et al. 1991; Sinkjaer et al. 1996). We suggest that feedback signaling this kind of extreme fast and large unexpected perturbations is processed differently than the expected proprioceptive information during normal walking, and therefore the latter must be investigated differently. The application of slow and small ankle trajectory modifications, almost imperceptible to the subject, may be a better technique to study the importance of afferent feedback during normal human locomotion. The slow dorsiflexion enhancements and reductions applied in the present study slightly deviate the ankle from its average trajectory generating small variations in the background SOL EMG. The results suggest that continuous contribution of afferent feedback to the muscle activity may automatically adjust the muscle activation to meet external demands of the walking surface.
5. Acknowledgements

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6. Reference List


7. Figure Legends

FIG. 1: Example of an averaged data record (30 trials) for a typical subject during control steps and dorsiflexion enhancements and reductions.
A: Ankle angular position during control steps (thick line) and dorsiflexion enhancements and reductions (thin lines) are shown superimposed. The velocity and amplitude of the ankle dorsiflexion during control steps were: 33 deg.s\(^{-1}\) and 10 deg, respectively. The dorsiflexion enhancements and reductions were applied 350 ms after heel contact and maintained for 300 ms. The imposed perturbations had approximately the following velocities and amplitudes: ± 6 deg.s\(^{-1}\); ± 2 deg and ± 16 deg.s\(^{-1}\); ± 5 deg. B: Rectified and filtered SOL EMG during the corresponding control and perturbed steps. C: TA EMG during the control and perturbed steps. D: Percent changes in SOL EMG for the 8 perturbations applied to this subject. Hatching pattern in panels A-D corresponds with each other. E: Individual (grey-dotted lines) and group linear regression analyses (n=12, black line) between changes in the dorsiflexion velocity and percent changes in the SOL activation.

FIG. 2: Example of an averaged data record for a typical subject during control steps and dorsiflexion enhancements with the different and constant amplitude.
A: Ankle angular position during control steps (thick line) and dorsiflexion enhancements (thin lines). In this subject the velocity and amplitude of the ankle dorsiflexion during the control steps were: 17 deg.s\(^{-1}\) and 5 deg, respectively. The perturbations were applied 310 ms after heel contact. The dorsiflexions shown on the left have different velocities and amplitudes: ± 6 deg.s\(^{-1}\), ± 2 deg; ± 13 deg.s\(^{-1}\), ± 4 deg and ± 26 deg.s\(^{-1}\), ± 8 deg, respectively. In the series shown on the right, the final
amplitude of all dorsiflexions is 6 deg, but the velocities are different: + 20 deg.s\(^{-1}\); + 26 deg.s\(^{-1}\) and + 30 deg.s\(^{-1}\). B: Corresponding rectified and filtered SOL EMG. The hatching pattern in panels A and B corresponds with each other. C: Regression analyses of both protocols superimposed. The symbol (●) corresponds to dorsiflexion enhancements with different amplitudes and velocities, and (○) corresponds to dorsiflexion enhancements with different velocities but same amplitude. The slopes of the regressions were not statistically different from each other (p=0.6).

FIG. 3: Example of an averaged data record for a typical subject during control steps and dorsiflexion enhancements applied in the mid and late stance phase.
A: Ankle angular position during control steps (thick line) and dorsiflexion enhancements applied in the mid and late stance phases (thin lines). The applied dorsiflexion enhancement had a velocity of 16 deg.s\(^{-1}\) and amplitude of 3 deg and was applied 330 and 500 ms after heel contact. B: Corresponding rectified and filtered SOL EMG. C: The percent increments in the SOL EMG in the mid and late stance phases across all subjects (n=12) were not different from each other. The hatching pattern in panels A-C corresponds with each other.

FIG. 4: Example of an averaged data record for a typical subject during fast dorsiflexion perturbations and slow dorsiflexion enhancements and reductions, before and during peripheral ischemia.
A: Ankle trajectory and SOL EMG during control steps and fast dorsiflexion perturbations before (black) and during (grey) ischemia. B: Peak-to-peak increment in the SOL SLR and MLR responses before and during ischemia. The SLR was
decreased during ischemia (p=0.027) however the MLR was not significantly affected (p=0.1). C and D: Ankle trajectory during control steps and dorsiflexion enhancements (+ 20 deg.s⁻¹; + 5 deg) and reductions (- 20 deg.s⁻¹; - 5 deg) before and during ischemia. E and F: SOL EMG during control steps (black thick line) and dorsiflexion enhancements and reductions (thin lines), before and during ischemia. G and H: Percent change in SOL EMG following dorsiflexion enhancements and reductions, before and during ischemia across all the subjects (n=8). Ischemia affected significantly the response to the dorsiflexion enhancements (p=0.007) but not to the dorsiflexion reductions (p=0.1). The hatching pattern in panels C - H corresponds with each other.

FIG. 5: Example of an averaged data record for a typical subject during fast dorsiflexion perturbations applied during sitting, and walking and slow dorsiflexion enhancements and reductions applied during walking, without and during Achilles tendon vibration.

A: Ankle trajectory and SOL EMG during fast dorsiflexion perturbations applied during sitting, without (black) and during (grey) tendon vibration. The bar graph shows the peak-to-peak increment in the SLR without and during tendon vibration across all subjects (n=9). The SLR was significantly decreased during Achilles tendon vibration (p=0.002). B: Ankle trajectory and SOL EMG during control steps (thick line) and fast dorsiflexion perturbations applied during walking, without (thin-black line) and during (thin-grey line) tendon vibration. The bar graph shows the peak-to-peak amplitude of the SLR and MLR responses without and during tendon vibration across all subjects (n=9). The SLR was significantly depressed during Achilles tendon vibration
vibration (p=0.003), however the MLR was not affected (p=0.2). C: Ankle angular trajectory during control steps and dorsiflexion enhancements (+ 7 deg.s\(^{-1}\); + 2 deg) without (thin-black line) and during (thin-grey line) tendon vibration. E: Corresponding SOL EMG. H: Percent increment in the SOL EMG during one level of dorsiflexion enhancement without and during tendon vibration across all the subjects (n=9). The response to the dorsiflexion enhancements was significantly depressed during vibration (p=0.023). D: Ankle angular trajectory during control and dorsiflexion reductions (- 18 deg.s\(^{-1}\); - 4 deg) without (thin-black) and during (thin-grey) tendon vibration. F: Corresponding SOL EMG. I: Percent decrement in the SOL EMG during the imposed dorsiflexion reduction across all subjects (n=9) without and during tendon vibration. The response to the dorsiflexion reductions were not affected by tendon vibration (p=0.912). G: The SOL activity during control steps, without and during tendon vibration were not significantly different (p=0.125).
Heel Contact

Time (s)

A

Ankle Angle (deg)

B

SOL EMG (µV)

C

Perturbation Duration

TA EMG (µV)

D

Group linear regression

Individual linear regressions (n=12)

E

Velocity Changes (deg.s^{-1})

Velocity Changes (deg.s^{-1})

SOL EMG (%)

TA EMG (%)

-40 -20 0 20 40
Enhancements

Heel Contact

Time (s)

Ankle Angle (deg)

SOL EMG (µV)

A

Different Amplitude

Constant Amplitude

B

Enhancements duration

C

SOL EMG Increments (%)

Velocity Increment (deg s⁻¹)
Fast Dorsiflexion Perturbations

A

Ankle Angle (deg)

SOL EMG (µV)

Time (s)

Control

Perturbed without ischemia

Perturbed during ischemia

B

Peak-to-Peak (µV)

Without ischemia

During ischemia

C

Dorsiflexion Enhancements

Ankle Angle (deg)

SOL EMG (µV)

Time (s)

Dorsiflexion Reductions

E

F

G

H

SOL EMG Increment (%)

Without Ischemia

During Ischemia

SOL EMG Decrement (%)

Without Ischemia

During Ischemia

* Significant difference
Dorsiflexion Enhancements

C

Ankle Angle (deg)

SOL EMG (μV)

Time (s)

Dorsiflexion Reductions

D

Ankle Angle (deg)

SOL EMG (μV)

Time (s)

H

Increment in SOL EMG (%) (Without vibration vs. During vibration)

G

SOL EMG (μV/s)

Control

Without vibration

During vibration

I

Dorsiflexion Reductions

Without vibration

During vibration