Lack of On-Going Adaptations in the Soleus Muscle Activity During Walking in Patients Affected by Large-Fiber Neuropathy

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Mazzaro, Nazarena, Michael J. Grey, Thomas Sinkjær, Jacob Buus Andersen, Davide Pareyson, and Marco Schieppati. Lack of on-going adaptations in the soleus muscle activity during walking in patients affected by large-fiber neuropathy. J Neurophysiol 93: 3075–3085, 2005; doi:10.1152/jn.01071.2004. The aim of this study was to investigate the contribution of feedback from large-diameter sensory fibers to the adaptation of soleus muscle activity after small ankle trajectory modifications during human walking. Small-amplitude and slow-velocity ankle dorsiflexion enhancements and reductions were applied during the stance phase of the gait cycle to mimic the normal variability of the ankle trajectory during walking. Patients with demyelination of large sensory fibers (Charcot-Marie-Tooth type 1A and antibodies to myelin-associated glycoprotein neuropathy) and age-matched controls participated in this study. The patients had absent light-touch sense in the toes and feet and absent quadriceps and Achilles tendon reflexes, indicating functional loss of large sensory fibers. Moreover, their soleus stretch reflex response consisted of a single electromyographic (EMG) burst with delayed onset and longer duration (P < 0.01) than the short- and medium-latency reflex responses observed in healthy subjects. In healthy subjects, the soleus EMG gradually increased or decreased when the ankle dorsiflexion was, respectively, enhanced or reduced. In the patients, the soleus EMG increased during the dorsiflexion enhancements; however, the velocity sensitivity of this response was decreased compared with the healthy volunteers. When the dorsiflexion was reduced, the soleus EMG was unchanged. These results indicate that the enhancement of the soleus EMG is mainly sensitive to feedback from primary and secondary muscle spindle afferents and that the reduction may be mediated by feedback from the group Ia pathways. This study provides evidence for the role of sensory feedback in the continuous adaptation of the soleus activity during the stance phase of human walking.

INTRODUCTION

Large-amplitude and fast-velocity dorsiflexion perturbations have been applied to the ankle to investigate the role of sensory feedback to the muscle activation during normal walking (Dietz et al. 1987; Sinkjær et al. 1996; Yang et al. 1991). These unexpected destabilizing perturbations elicited compensatory stretch reflex responses in the plantar flexor muscles, demonstrating that afferent feedback can generate corrective responses to unexpected external perturbations during walking. However, the extrapolation of such results to normal unperturbed walking must be made cautiously. Recently, it has been suggested that sensory feedback during normal unperturbed walking, as part of the locomotor program, might be processed differently than the afferent signal associated to unexpected perturbations (Nielsen 2002; Nielsen and Sinkjær 2002; Sinkjær et al. 2000). Furthermore, Morita et al. (1998) proposed that sensory feedback may be centrally processed differently depending on the frequency components of the feedback signal.

Sinkjær et al. (2000) applied fast plantar flexion perturbations during the stance phase, to unload the ankle extensors muscles, and showed that the soleus (SOL) electromyogram (EMG) decreased markedly after these perturbations. While this study provided evidence that proprioceptive feedback from ankle extensors does indeed contribute to the enhancement of the SOL activation during the stance phase, it did not evaluate whether this feedback can modulate the level of muscle activation on an on-going basis. Such modulation would be important to adapt the plantar flexor activity to the demands of the walking surface. To address this question, Mazzaro et al. (2005) applied small-amplitude, slow-velocity enhancements and reductions to the natural ankle dorsiflexion during the stance phase of walking, thus mimicking variations in the ankle dorsiflexion trajectory during walking. These ankle trajectory modifications were designed to generate changes in sensory feedback in a manner that was similar to what might be expected to occur during normal walking on flat or slightly uneven surfaces. In healthy volunteers, the dorsiflexion enhancements generated gradual increments on the SOL EMG, while the dorsiflexion reductions generated gradual decrements in the SOL EMG. These results suggested that afferent feedback not only contributes to the background SOL EMG, but it also adapts the EMG to variations in the ankle trajectory. Moreover, in the same study it was shown that during Achilles tendon vibration and peripheral ischemia the increments in the SOL activation were depressed; whereas neither of these techniques affected the decrement in the SOL EMG in response to the dorsiflexion reductions. These results suggested that the increments in the SOL EMG were mostly sensitive to feedback from the group Ia pathway, whereas the decrements were probably sensitive to different afferent inputs. However, clear evidence of the pathways involved in the observed responses remained unclear. The contribution of afferent feedback to the

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activation of muscles during normal walking was also investigated by using tendon vibration (Courtine et al. 2001). Contrary to expectations, a tonic Ia input elicited by continuous bilateral triceps surae vibration had only minor effects on lower limb segment coordination and leg muscle activity.

The aim of the present study was to use a new and independent approach to provide further insight into the possible role of the feedback from large-diameter sensory pathways to the modulation of the SOL EMG with changes in ankle angular displacement. Small-amplitude, slow-velocity ankle trajectory modifications were applied to the ankle during the stance phase of walking in patients with demyelination of large-sensory fibers and aged-matched controls. Patients diagnosed with Charcot-Marie-Tooth type 1A (CMT1A) disease and antibodies to myelin-associated glycoprotein (anti-MAG) volunteered to participate in the study. We hypothesized that in the absence of afferent input from the receptors connected to large-sensory fibers, the modulation of the SOL EMG during the stance phase of the gait cycle would be absent or reduced. This would be consistent with the notion that the group I afferent input from the SOL muscle does indeed play a significant role in sustaining and adapting the activity of ankle extensor muscles throughout the stance phase of human walking. In these patients, the smaller-diameter group II spindle afferent fibers are likely preserved and functional (Dyck et al. 1993; Nardone et al. 2000). Therefore this pathological model also allowed us to investigate the possible role of these fibers in the modulation of the SOL activity in response to variations in the ankle dorsiflexion during the stance phase of the step cycle.

METHODS

Twenty-one volunteers participated in this study. Of the 11 patients (9 men and 2 women; mean age: 45 yr, range: 19–76 yr) with demyelination of large-sensory fibers who participated, 7 were affected by CMT1A and 4 were affected by anti-MAG neuropathy. CMT1A is an hereditary demyelinating neuropathy associated with a duplication of the peripheral myelin protein 22 (PMP22) gene. It is characterized by extensive demyelination of motor and sensory nerves.

* TABLE 1. Clinical data of the patients*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, yr</th>
<th>Diagnosis</th>
<th>Weakness Lower Limbs</th>
<th>Achilles Tendon Tap</th>
<th>Pain-Touch Hypoesthesia</th>
<th>Vibration and position hypoesthesia</th>
<th>Motor CV, m/s</th>
<th>Sensory CV, m/s (Sural Nerve)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>54</td>
<td>CMT1A</td>
<td>Mild</td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>29–30</td>
<td>NA</td>
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<tr>
<td>P2</td>
<td>32</td>
<td>CMT1A</td>
<td>Mild</td>
<td>Absent</td>
<td>Moderate</td>
<td>Mild</td>
<td>18 (M)</td>
<td>NR</td>
</tr>
<tr>
<td>P3</td>
<td>46</td>
<td>CMT1A</td>
<td>Mild</td>
<td>Absent</td>
<td>Moderate</td>
<td>Mild</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>P4</td>
<td>19</td>
<td>CMT1A</td>
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<td>Absent</td>
<td>Mild</td>
<td>Mild</td>
<td>NA</td>
<td>NR</td>
</tr>
<tr>
<td>P5</td>
<td>37</td>
<td>CMT1A</td>
<td>Mild</td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>18 (M)</td>
<td>NR</td>
</tr>
<tr>
<td>P6</td>
<td>37</td>
<td>CMT1A</td>
<td>Mild</td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>20–21</td>
<td>16–NR</td>
</tr>
<tr>
<td>P7</td>
<td>31</td>
<td>CMT1A</td>
<td>Mild</td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>22–23</td>
<td>NR</td>
</tr>
<tr>
<td>P8</td>
<td>57</td>
<td>Anti-MAG</td>
<td>No Reduced</td>
<td>Mild</td>
<td>Mild</td>
<td>29–32</td>
<td>36–41</td>
<td>NA</td>
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<tr>
<td>P9*</td>
<td>76</td>
<td>Anti-MAG</td>
<td>No Present</td>
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<td>Mild</td>
<td>45–48</td>
<td>31–32</td>
<td>42–44</td>
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<tr>
<td>P10*</td>
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<td>Anti-MAG</td>
<td>No Right absent</td>
<td>Mild</td>
<td>Mild</td>
<td>49 (M)</td>
<td>26 (P)</td>
<td>43–48</td>
</tr>
<tr>
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<td>Anti-MAG</td>
<td>No Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>56 (M)</td>
<td>17–24</td>
<td>NR</td>
</tr>
</tbody>
</table>

When more than one value is presented in the column of the Sural nerve conduction velocity (CV), they correspond to measurements taken in different sessions. Patients marked with an asterisk were not included in the group of patients for the analysis. For comparison, Kimura (1993) reported the approximate maximal CV in the corresponding nerves in healthy volunteers: Median nerve: 59 ± 2 m/s measured from palm, wrist, and elbow. Ulnar nerve: 62 ± 1 m/s, measured from the wrist, below elbow, and above elbow. Peroneal nerve: 51 ± 1 m/s, measured from ankle and below the knee. Shenefner and Logigian (1994) reported the CV of the sural nerve in healthy volunteers: 55.1 m/s. NA, not assessed; NR, no response.
determined by numerical differentiation of the ankle angular record. A goniometer (Bionomics, Type XM180, Gwent, UK) was placed on the left leg to measure the knee angular displacement. EMG of the SOL, TA, medial gastrocnemius (GM), and rectus femoris (RF) muscles of the left leg were recorded with bipolar surface electrodes (Neuroline 720, Medicotest AS, Denmark, 5 mm diam, 2 cm inter electrode distance). The EMG signals were amplified and band-pass filtered from 20 to 1,000 Hz. 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.

Experimental protocol

Initially, the subjects were instructed to stand with their feet separated by ~10 cm to lean slightly forward to activate the ankle extensor muscles. A series of 20 fast dorsiflexion rotations (6°, 500°/s) were applied during standing to obtain a profile of the SOL stretch reflex response (one perturbation every 4–6 s). Next, the subjects were asked to walk on the treadmill for a 5–to 10-min adaptation period to become accustomed to the robotic actuator. During this period, they choose a comfortable velocity (range: 2.5–3.5 km/h) that was maintained for the rest of the experiment. When the subject felt comfortable with the device, data were acquired to generate a control profile of the ankle, knee trajectory, and the EMG of the different muscles. Data of every step were acquired for 1,500 ms, starting ~200 ms before the heel contact. Next, stretch reflex responses were elicited in the SOL muscle by applying fast dorsiflexion perturbations (6°, 500°/s) in the mid stance phase (~350 ms after heel contact).

Stretch response EMG analysis

The SOL EMG was rectified, low-pass filtered (50 Hz) and ensemble averaged (~20 trials) to produce a stretch-reflex profile. In healthy volunteers, this response is characterized by two distinct large-amplitude bursts of activity, a short- and a medium-latency reflex component (SLR and MLR, respectively) during standing (Allum and Budingen 1979; Corna et al. 1995; Diener et al. 1983; Nashner 1976; Schieppati et al. 1995) and walking (Grey et al. 2001; Sinkjær et al. 1996; Yang et al. 1991). Onset latencies of the SOL EMG responses were determined by visual inspection of the rectified and averaged traces. The onset of the reflex bursts were located and measured using a moveable cursor on the display, by superimposing the averaged SOL EMGs obtained during perturbed and control steps. In the healthy volunteers, the onset latency of the SLR was defined as the first major deflection in the SOL EMG signal after the perturbation. Similarly, the onset of the MLR was defined as the second major deflection in the EMG after the perturbation, appearing immediately after the SLR response. The magnitudes of these bursts were calculated within 30-ms windows positioned on the onsets of the SLR and MLR responses. The magnitudes of the bursts were quantified as the min-max value within the defined 30-ms window. In the patients, the onset and amplitude of the reflex response were measured in the same manner. To compare the amplitudes of the reflexes between the healthy volunteers and the patients, these amplitudes were normalized with respect to the background SOL activity. The normalization consisted in dividing the min-max value by the root mean square (RMS) of the SOL EMG signal during unperturbed trials within the same 30-ms window.

Small-amplitude, slow-velocity dorsiflexion enhancements and reductions

A window was defined on the control ankle trajectory profile for the application of small-amplitude, slow-velocity dorsiflexion enhancements and reductions. Immediately after heel contact the ankle joint undergoes a rapid plantarflexion-dorsiflexion movement. The slow ankle trajectory modifications were applied after this event, on average 200–400 ms after the heel contact, depending on the ankle angular trajectory of the individual subject. The perturbation window was extended to the late stance phase and was terminated before the initiation of the swing phase. On average, the duration of the perturbation window was 200–300 ms depending on the subject’s walking velocity and the duration of the stance phase. Within this window, the velocity of the normal ankle dorsiflexion was approximately constant. A dorsiflexion enhancement or reduction was presented pseudo-randomly every four to six steps. A control step was recorded prior to each perturbed step. Data acquisition was continued until ~25 control and perturbed steps were recorded. Afterwards, two new levels of slow perturbations were presented and the procedure was repeated. Approximately, four to five different levels of ankle trajectory modifications were applied to each subject. The amplitude of the dorsiflexion enhancements and reductions were changed in steps of approximately ±2°. The magnitude of the imposed dorsiflexion enhancements was increased until a level was reached such that distinct SOL short-latency stretch reflex responses were observed in some of the individual trials. The SOL SLR response was easily recognized in these trials as a sharp large deflection in the EMG within a 30-ms window starting at the onset latency of the SLR latency determined from the muscle response to the fast dorsiflexion perturbations. In the present study, the level of dorsiflexion enhancement that elicited such distinct short-latency stretch reflex responses was eliminated from the analysis and only the slower-velocity and smaller-amplitude perturbation levels were analyzed.

Throughout the experiment, the patients walked on the treadmill without assistance, although some patients held the lateral bars of the treadmill for stabilization. All of the patients were able to walk over ground without the assistance of walking aids. The walking session never exceeded 40 min. Some of the patients were encouraged to take short breaks during this period.

Data analysis

The EMG recordings were rectified and filtered with a 40-Hz first-order low-pass filter to extract an amplitude envelope. The muscle activity was quantified as the area under this envelope within the corresponding window of analysis. The SOL EMG of every individual trial during the application of the slow-velocity, small-amplitude dorsiflexion enhancements and reductions was visually examined for any evidence of synchronized SOL stretch reflex responses. If stretch-reflex responses were detected in some of the individual trials corresponding to a specific level of ankle perturbation, the entire set of data records made at that particular trajectory enhancement was eliminated from the analysis, and only slower-velocity, smaller-amplitude ankle-trajectory modifications were further analyzed. In general, this degree of perturbation corresponded to the fastest and largest level of dorsiflexion enhancement applied (generally >40°/s).

For the dorsiflexion enhancements and reductions, the changes in the EMG activity were calculated as the difference in activity between the perturbed and control steps and normalized with respect to the control activity. Moreover, for the dorsiflexion enhancements, the window of analysis was subdivided in two areas of equal duration to evaluate whether the SOL EMG increment was located mainly at the perturbation onset to the middle of the perturbation window and from this point to the end of the perturbation. The velocity of the ankle angular displacements was expressed as the difference with respect to the normal ankle dorsiflexion velocity in degrees per second.

Statistical analysis

A one-way repeated-measures ANOVA was applied to analyze possible differences in the latency, amplitude, or duration of the
stretch reflex responses between patients and control subjects. When a significant difference was detected, a Student-Newman-Keuls multiple comparison post hoc test (SNK) was applied to analyze the differences between the groups. Linear regression analyses were applied to test the relationship between the velocity of the imposed ankle dorsiflexion and the SOL activation throughout the whole range of imposed dorsiflexion perturbation velocities. In a previous study, we found that the responses to the dorsiflexion enhancements and reductions were sensitive to different afferent mechanisms (Mazzaro et al. 2005). Therefore in this study we also applied a separated regression analyses, for the dorsiflexion enhancements and reductions, to test the sensitivity of the SOL EMG to these two types of perturbations in absence of feedback from the group I fibers. The results of the regression analysis for the patients and the healthy volunteers were compared by performing a \( t \)-test for independent samples on the difference of the slopes. The results of all statistical tests were considered significant at the \( P < 0.05 \) level. Results are presented as means \( \pm 1 \) SD unless otherwise indicated.

**RESULTS**

All but two patients presented absent knee- and ankle-jerk responses to tendon percussion when the patient was relaxed and when the responses would be reinforced with a Jendrassik maneuver. The two patients (P9 and P10) who exhibited reflex responses during the tendon tap tests were both diagnosed with anti-MAG. One patient (P9) responded to Achilles and patellar tendon tap in both legs under both, the relaxed condition and Jendrassik maneuver, while the other patient (P10) showed Achilles and patellar tendon tap only on the left leg only during Jendrassik maneuver. Eight patients had absent light-touch sensitivity on the feet, and one patient lacked sensitivity to touch in the foot and leg distal to the knee.

**SOL stretch reflex during quiet stance**

Figure 1 shows typical data of the ankle trajectory and SOL EMG during fast dorsiflexion perturbations applied during standing in a healthy volunteer and a patient. This record is an ensemble average of 25 trials. In the healthy subject (Fig. 1B), the fast ankle dorsiflexion perturbations elicited SLR and MLR responses in the SOL muscle (gray line). For the group of healthy volunteers (\( n = 10 \)), the latencies of the SLR and MLR were 34 \( \pm 10 \) and 69 \( \pm 15 \) ms, and the duration of the bursts were 30 \( \pm 5 \) and 27 \( \pm 2 \) ms, respectively. The min-max amplitudes of the SLR and MLR were 36 \( \pm 11 \) and 45 \( \pm 19 \) \( \mu \)V, respectively.

In 9 patients, the fast dorsiflexion perturbations applied during standing elicited a single burst in the SOL EMG (see gray line in Fig. 1C). Across all patients, the burst had a latency of 110 \( \pm 23 \) ms, duration of 72 \( \pm 16 \) ms, and amplitude of 53 \( \pm 37 \) \( \mu \)V. The perturbations elicited two-burst responses in only two of the patients (P9 and P10), both of whom had palpable ankle-jerk responses to Achilles tendon percussion. In these patients, the latency of the earlier burst was 54 \( \pm 7 \) ms and the latency of the second burst was within the range of the single response observed in the rest of the patients. The fact that patients P9 and P10 present tendon reflexes and SLR response in the SOL suggests that they very likely have preserved feedback from large-sensory fibers. Therefore data of these patients were not included in the analysis as part of the patient group. A summary of the results is shown in Fig. 1D.

A one-way repeated-measures ANOVA test showed that the latencies of the SLR and MLR in healthy subjects (\( n = 10 \)) and the reflex response in patients (\( n = 9 \)) were different from each other (\( P < 0.001 \)). The latency of the SLR in the healthy volunteers was shorter than the latency of the MLR (SNK: \( P = 0.01 \)), and both latencies were shorter than the onset of the single reflex response observed in patients (SNK: \( P < 0.001 \)).
The duration of the SLR and MLR responses in healthy volunteers were shorter than the duration of the single reflex response in patients (one-way repeated-measures ANOVA test: $P = 0.002$). The amplitudes of the reflex bursts were normalized with respect to the background activity and compared between the healthy volunteers and the patients. The analysis showed that the amplitude of the SLR and MLR responses in the healthy volunteers (35 ± 8 and 44 ± 30, respectively) and the patients (52 ± 17) were not significantly different from each other (one-way repeated-measures ANOVA test: $P = 0.7$; Fig. 1D).

**SOL stretch reflex during walking**

Figure 2 shows a set of ensemble averaged data from a healthy volunteer (left) and a patient (right). The figure shows the ankle and knee angular displacements, and the EMG of SOL, TA, GM, and RF during control steps and fast dorsiflexion perturbations. The traces in the figure are an ensemble average of 25 trials. On average the patients walked at $\sim 2.8 \pm 0.4$ km/h, and the control group walked at matched velocity ($3.0 \pm 1.0$ km/h). The amplitude of the background SOL EMG during control steps was measured as the area under the EMG signal from the heel contact to the beginning of the swing.
phase. The background activity of SOL was not significantly different (t-test: $P = 0.14$) between the healthy volunteers ($31 \pm 21 \mu V \cdot s$) and the patients ($20 \pm 8 \mu V \cdot s$).

The fast dorsiflexion perturbations ($6^\circ$, $500^\circ/s$) were applied in the mid stance phase (see Fig. 2A). In the healthy volunteers (left), the perturbations elicited SLR and MLR responses in the SOL EMG. Across all the subjects, the latencies of the SLR and MLR responses were $39 \pm 5$ and $72 \pm 10$ ms, respectively. The durations of the SLR and MLR bursts were $29 \pm 8$ and $25 \pm 4$ ms, respectively, and the min-max amplitudes were $40 \pm 9$ and $37 \pm 5 \mu V$, respectively (Fig. 2G). For all but two patients ($P9$ and $P10$), the perturbations were followed by a single burst in the SOL EMG with a latency of $100 \pm 18$ ms. The duration of the burst was $57 \pm 13$ ms and the min-max amplitude was $38 \pm 12 \mu V$. Patients $P9$ and $P10$ had two bursts of response as it was observed during standing. In these patients, the early burst appeared $~57 \pm 3$ ms after the perturbation, and the second burst appeared $102 \pm 12$ ms after the perturbation. The latency of the second burst in these patients was within the range of the latency of the single burst observed in the other patients. Small burst–like responses were observed in the EMG of the GM and RF muscles in some of the healthy volunteers; these responses were absent in all of the patients (Fig. 2, D and E). In the subject shown in Fig. 2, the TA EMG presented small-amplitude bursts. This is very likely cross-talk with the SOL stretch-reflex response (Fig. 2C) (Toft et al. 1991). In Fig. 2, the knee angular displacement was slightly different between the healthy volunteer and the patient. In the healthy volunteer, the fast ankle perturbation generated a change in the knee kinematics; however, this was not observed in all the volunteers.

A one-way repeated-measures ANOVA test showed that the latencies of the SLR and MLR in the healthy volunteers ($n = 10$), and the latency of the reflex response in patients ($n = 9$) were statistically different from each other ($P < 0.01$). In the healthy volunteers, the latency of the SLR was significantly shorter than that of the MLR (SNK test: $P < 0.02$), and both responses had shorter latencies than the reflex burst observed in the patients (SNK test: $P = 0.002$). The duration of the reflex burst in the patients was longer than the duration of the SLR and MLR bursts in the healthy volunteers ($P < 0.01$). The amplitudes of the reflex bursts were normalized with respect to the background activity and compared between the healthy volunteers and the patients. The analysis showed that the amplitude of the SLR and MLR responses in the healthy volunteers ($43 \pm 26$ and $38 \pm 27$, respectively) and the patients ($33 \pm 15$) were not significantly different from each other (one-way repeated-measures ANOVA: $P = 0.3$). A summary of these results is shown in Fig. 2G.

**Relationship between the slow ankle perturbations and the SOL EMG changes**

Our patient volunteers were more susceptible to fatigue than were our control subjects. Therefore we encouraged some of the patients to take short breaks during the walking session and we limited the duration of their walking. In general, three to four different levels of ankle-trajectory modifications were applied to each patient, whereas we were able to apply four to five different levels with each of the healthy volunteers. Figure 3 shows a typical set of ensemble averaged data for a healthy volunteer (left) and a patient (right) during the application of slow dorsiflexion enhancements and reductions. In the figure, the ankle trajectory and SOL EMG during unperturbed walking and during dorsiflexion enhancements and reductions are superimposed. These records represent an ensemble average of 25 trials.

The small-amplitude, slow-velocity ankle dorsiflexion enhancements and reductions only produced significant changes in the SOL EMG. Therefore in the next sections only results related to this muscle will be reported.

**Dorsiflexion enhancements**

Figure 3A (left) shows a set of ensemble averaged data for a healthy volunteer walking at 3 km/h. In this case, the natural ankle dorsiflexion was enhanced for a period of 280 ms, starting $~330$ ms after heel contact. The velocity and amplitude of the ankle dorsiflexion during control steps within the selected time window were $17^\circ/s$ and $5^\circ$, respectively. The ankle dorsiflexion was enhanced by $3.5^\circ$ at $13^\circ/s$. When the ankle was released, it returned to its normal trajectory after $~60$ ms. Figure 3B (left) shows the corresponding SOL EMG traces during control and the dorsiflexion enhancement. Before the perturbation, the SOL EMG of control and perturbed steps are similar; however, during the dorsiflexion enhancement the SOL EMG gradually increased. Approximately 50 ms after the release of the perturbation the SOL EMG returned to its normal trajectory.

Figure 3A (right) shows a set of ensemble averaged data for a patient walking at 3 km/h. The velocity and amplitude of the ankle dorsiflexion during control steps within the selected time window were $25^\circ/s$ and $7^\circ$, respectively. The dorsiflexion was enhanced by $4^\circ$ at $15^\circ/s$ for a period of 270 ms, starting $~390$ ms after heel contact. Figure 3B (right) shows the corresponding SOL EMG traces during control steps and during the applied dorsiflexion enhancement. Before the perturbation the SOL EMG of control and modified steps were similar. During the dorsiflexion enhancements, the SOL EMG slightly increased with respect to the control step. The bars on the histogram illustrated in Fig. 3C show the percent increments in the SOL EMG for the healthy volunteer and the patient elicited by the level of dorsiflexion enhancement shown on the figure. In the healthy volunteer, the SOL EMG increased 19 $\pm 4\%$ with respect to the background SOL EMG, and in the patient the increment was $7 \pm 5\%$ for approximately the same level of perturbation ($3.5^\circ$, $13^\circ/s$ and $4^\circ$ at $15^\circ/s$, respectively).

**Dorsiflexion reductions**

Figure 3D (left) shows a set of ensemble averaged data for a healthy volunteer during unperturbed steps and the imposed ankle dorsiflexion reduction. In this case, the subject was walking at 3.2 km/h, and the natural ankle dorsiflexion was reduced for a period of 220 ms, starting $~250$ ms after heel contact. The velocity and amplitude of ankle dorsiflexion during control steps within the selected time window were $25^\circ/s$ and $5.5^\circ$, respectively. The ankle dorsiflexion was reduced by $3^\circ$ at $13^\circ/s$. When the ankle was released, it returned to its normal trajectory after $~100$ ms. Figure 3E (left) shows the corresponding SOL EMG during control steps and the dorsiflexion reduction. Before the application of the perturba-
tion, the SOL EMG of control and perturbed steps were similar. However, the SOL EMG gradually decreased during the dorsiflexion reduction. The SOL EMG returned to its normal trajectory 200 ms after the release of the perturbation.

Figure 3D (right) shows a set of ensemble averaged data for a patient during control steps and the imposed dorsiflexion reduction. In this case, the subject was walking at 3 km/h, and the natural ankle dorsiflexion was reduced for a period of 230 ms, starting ~360 ms after heel contact. The velocity and amplitude of the ankle dorsiflexion during control steps within the selected time window were 24°/s and 5.5°, respectively. The dorsiflexion was reduced by 4° at 18°/s. Figure 3E (right) shows the corresponding SOL EMG during control steps and the dorsiflexion reduction. Before the perturbation, the SOL EMG of control and modified steps were similar, but during the perturbation, the SOL EMG slightly decreased with respect to the background EMG level. Figure 3F depicts the percent decrement on the SOL EMG for the healthy volunteer and the patient, elicited by the level of dorsiflexion reduction shown on the figure. In the healthy volunteer, the SOL EMG decreased by 14 ± 4% with respect to the background SOL EMG, whereas the decrement was only 2.6 ± 5.0% in the patient with approximately the same level of perturbation (~3° at 13°/s, and ~4° at 18°/s, respectively).

Figure 3A shows data corresponding to all the subjects, of all the levels of ankle perturbations applied and their associated SOL EMG change (3–4 perturbations levels for each subject). Linear regression analyses were performed on the data corresponding to the group of healthy volunteers and the group of patients. Figure 4A shows the superimposition of the regression lines calculated for the healthy volunteers (n = 10) and for the patients (n = 9). The regression line corresponding to the healthy volunteers had a significantly positive slope (P < 0.001) and an intercept that was not significantly different from zero (y = 0.69 × +1.7). For the patients, the slope of the regression line was not significantly different from zero (P = 0.08) and had a significantly positive offset (P = 0.02) (y = 0.3 × +7). A comparison between the regression lines showed that the slope of the regression corresponding to the healthy volunteers was higher (P < 0.05) than the slope corresponding to the patients, and the offset values were significantly different from each other (P = 0.01).

The regression analysis was also applied for the dorsiflexion enhancements and the reductions separately. For the healthy volunteers, the slopes of both regression lines were positive (enhancements: 0.48% per °/s and reductions: 0.81% per °/s; P = 0.04). However, for the patients, the slopes for the enhancements and reductions (~0.14% per °/s and 0.035% per °/s, respectively) were not significantly different from zero. Two t-tests were used to compare the slopes corresponding to the dorsiflexion enhancement and reductions between the group of healthy volunteers and the patients. The t-test showed
that the regression slopes for the dorsiflexion enhancement and reductions, between healthy volunteers and patients, were significantly different from each other ($P < 0.001$).

The histogram bars of Fig. 4B show the change in the SOL EMG across all the healthy volunteers and the patients, grouping all the levels of enhancements and reductions applied to each individual subject. The mean increment in the SOL EMG across all the healthy volunteers (21.1 ± 16.0%, $t$-test $P < 0.001$) and the patients (18.9 ± 14.0%, $t$-test $P < 0.001$) were both significantly greater than zero. To evaluate whether the increment on the SOL EMG was equally distributed in the 1st (full-black) and 2nd (striped-black) sub-window of analysis. In the patients, the SOL EMG increment was mainly located in the last period of the perturbation (striped-gray). The increment in the SOL EMG within the 1st window of analysis was significantly different between healthy volunteers and patients. The mean decrement in the SOL EMG during the dorsiflexion reductions was significantly different between the healthy volunteers and the patients.

DISCUSSION

The aim of this study was to investigate the contribution of sensory feedback from large-diameter afferents to the adaptive modifications of the SOL EMG during the stance phase of human walking. Healthy volunteers and patients with peripheral neuropathy affecting the large-diameter sensory afferent nerves participated in this study. The patients exhibited loss of light-touch sense in the toes and feet and lack of deep quadriceps and Achilles tendon reflexes during the clinical examination. In agreement with previous studies (Krajewski et al. 2000; Nardone et al. 2000), in the present study, we observed that the patients suffering from CMT1A had absent knee and ankle jerks to tendon percussion. In Nardone et al. (2000), it was also demonstrated that these patients have absent H-reflex responses; this gives evidence for the complete functional loss of the group Ia pathways. The loss or decreased light-touch sense suggests that large cutaneous afferents ($A\alpha\beta$) were also affected in these patients. The decrement in light-touch sense was more severe to the periphery as was previously reported in other studies (Krajewski et al. 2000). Patients with two different neuropathies (CMT1A and anti-MAG) were incorporated in the study and analyzed together. The reason for grouping them was that both peripheral neuropathies fulfill the inclusion criteria of this study, damage of large-diameter sensory fibers (see in Table 1 the decrease CV compared with normal subjects), while myelinated fibers of smaller diameter are relatively well preserved (Dyck et al. 1993). The patients of both groups showed similarly altered SOL stretch-reflex responses to fast toe-up perturbations during standing and walking with respect to the healthy volunteers.

The slow ankle-trajectory modifications generated correspondingly small but consistent and gradual changes in the SOL EMG. Therefore these trajectory modifications appear to constitute an appropriate protocol to study the role of sensory feedback during normal walking, because they may modify sensory feedback as it might occur during walking, in contrast to the large and fast ankle perturbations applied in previous studies.

The results showed that in the healthy volunteers the SOL EMG gradually increased or decreased when the ankle dorsiflexion was, respectively, enhanced or reduced during the stance phase of the gait cycle. In the patients, the sensitivity of the SOL muscle activity to the same imposed ankle-trajectory modifications was reduced compared with the healthy volunteers.
Methodological considerations

Two of the patients investigated in this study held the lateral bars of the treadmill for stabilization during the experimental session. It may be argued that holding the lateral bars introduced a source of variance in the response to the fast and slow ankle perturbations. Postural stabilization, as obtained by holding a bar located in front of the subject, can affect the amplitude but not the latency of the medium-latency response (MLR) to muscle stretch produced by fast ankle rotations (Nardone et al. 1990; Schieppati and Nardone 1991). However, in the present study, the responses to fast perturbations of these two patients did not appear to be significantly depressed compared with those of the control subjects. The lack of MLR depression in these two patients suggests that holding the bar may have not cause the depression in the response to the slow and small ankle dorsiflexion enhancement. To strengthen this inference, we checked that the average increment and decrement on the SOL EMG in the patient group was not brought down by the average enhancement and reduction of the SOL EMG corresponding to these patients.

Stretch reflex response to fast toe-up perturbations

In the healthy volunteers, the fast toe-up perturbations elicited two synchronized burst-like responses in the SOL EMG; a SLR and a MLR response. However, in the neuropathic patients, the fast dorsiflexion perturbations elicited a single delayed burst as previously reported (Nardone et al. 2000). This single burst response had longer latency and duration than the SLR and MLR responses observed in healthy subjects. Based on the estimation of the conduction velocity of the fibers responsible of the reflex burst, Nardone et al. (2000) suggested that the response observed in the patients corresponds to a delayed MLR and not to a SLR. In the same study, it was reported that biopsies of the sural nerve of several CMT1A patients showed loss of large myelinated fibers. Further, these patients had absent H-reflex, quadriceps reflex, and Achilles tendon tap reflex responses, as would be expected for functional loss of Ia spindle pathways. Morphometric studies in CMT1A patients indicate that only large sensory fibers (groups I and Aaβ) are affected by the neuropathy. Fibers of smaller diameter and slower conduction velocity, such as the group II fibers, that mediate the MLR response (Corna et al. 1995; Dietz et al. 1985; Grey et al. 2001; Nardone and Schieppati 1998; Schieppati and Nardone 1997, 1999) are less affected (Dyck et al. 1993). These observations suggest that in patients suffering from CMT1A, the single burst observed in the SOL EMG in response to the fast toe-up perturbations is very likely not mediated by the group Ia afferent fibers. The present study provides additional evidence that this response might be mediated by the spindle group II afferent fibers. Two of the anti-MAG patients had tendon tap reflexes, albeit decreased, and showed two bursts of response after the fast toe-up perturbations. In these patients, the first burst was slightly delayed with respect to the SLR in healthy volunteers, and the latency of the second burst was similar to the latency of the single burst response observed in the rest of the patients. This might suggest that in the rest of the patients the single SOL burst response was very likely a delayed MLR, and that the SLR response was completely absent. Nardone et al. (2000) suggested that the delay in the MLR response may be caused by a decreased conduction velocity (CV) of the motor fibers specifically rather than the sensory fibers. They estimated, although indirectly, that the motor CV of the tibial nerve in patients (20.3 ± 4.0 m/s) was significantly slower than the motor CV of the same nerve calculated for healthy volunteers (45.2 ± 2.2 m/s). From this result, the afferent time of the MLR response (calculated as the difference between the total latency and the efferent time) was not different between patients and healthy volunteers. However, it is possible that at least the largest of the group II fibers are also affected by the disease. Therefore the degeneration of some of these fibers may also contribute to the delay in the stretch-reflex response observed in patients. In turn, the increased duration of the reflex burst in the patients may be due to the fact that the neuropathy likely affects individual motor and sensory axons in different grades; therefore the fast muscle stretch produces a desynchronized afferent response that combined with a desynchronized efferent response results in a long duration burst.

Results from the present study also show that during walking the stretch reflex response of the control subjects and the patients showed similar characteristics than during standing. In the healthy volunteers, the fast dorsiflexion perturbations applied during the stance phase elicited SLR and MLR responses. However, in the patients, a single delayed reflex burst was observed after the perturbations. This result was expected due to the known loss of feedback from large-sensory fibers.

Slow ankle trajectory modifications

Small-amplitude, slow-velocity ankle trajectory modifications were also applied in the present study to test the hypothesis that feedback from large-sensory fibers contribute to the on-line adaptive modifications of the SOL activity to small deviations in the ankle angular displacement. As shown in Mazzaro et al. (2005), in the healthy volunteers the dorsiflexion enhancements and reductions generated gradual increments and decrements, respectively, on the SOL EMG. The positive linear relationship between the velocity of the imposed ankle angular displacements and percent changes in the SOL EMG suggests that the SOL activity changes in an on-going basis in demand of the ankle trajectory, and that these EMG adaptations are very likely mediated by feedback from ankle extensor proprioceptors.

In the healthy volunteers, the slope of the regression line between the velocity of the ankle dorsiflexion and the changes in SOL EMG was bigger than for the patients (Fig. 4A). This suggests that in patients with loss or decreased feedback from large-sensory fibers the adaptability of the SOL activity to variations in the amplitude and velocity of the ankle movement during walking was decreased. When the regression analysis was separated for the enhancements and the reductions, the slopes for both regressions were positive in the case of healthy volunteers. However, the slopes of both regression analyses were not significantly different from zero in the group of neuropathic patients, and the regression line for enhancements had a significantly positive offset. This may indicate that in the patients, although there was an increase in the SOL activation during the dorsiflexion enhancements; the response was hardly modulated with respect to the velocity of the imposed movement. This means that increasing levels of dorsiflexion en-
hancements elicited similar levels of increments on the SOL EMG. In the case of the dorsiflexion reductions, this perturbation did not generate significant responses in the SOL EMG in the neuropathic patients (Figs. 3F and 4B).

We have previously suggested that in healthy volunteers, the increments in the SOL EMG during dorsiflexion enhancement are partially mediated by feedback from the velocity-sensitive Ia pathways (Mazzaro et al. 2005). The results from the present study are consistent with this hypothesis, as the decreased sensitivity of the SOL EMG to different levels of dorsiflexion enhancements in the neuropathic patients can be explained by a decreased feedback from the no longer viable velocity-sensitive group Ia terminations. However, the fact that in the patients there was still an increase on the SOL EMG during dorsiflexion enhancements (Fig. 3A) suggests that this response is also sensitive to feedback from other sensory pathways. One possible candidate is the group II afferent pathways because it is likely preserved in these neuropathies. Moreover, these pathways originate from spindle receptors that transduce primarily muscle length and have a facilitatory effect on the SOL motoneurons (Schieppati and Nardone 1999; Simonetta-Moreau et al. 1999).

Regarding the ankle dorsiflexion reductions, the present study suggests that feedback from the group I pathways may contribute to the decrements on the SOL EMG in healthy volunteers because this decrement was not significant in the neuropathic patients. However, in Mazzaro et al. (2005), the contribution of the group Ia fibers to this response was excluded based on results from peripheral ischemia and Achilles tendon vibration. It is therefore possible that other large-diameter afferent pathways, which may also be affected by the neuropathy, contribute to this response. One possible candidate is the group Ib afferent pathways, as their diameter is likely close to the diameter of Ia spindle afferent fibers (Burke et al. 1983), they provide force feedback, and have been suggested to contribute to enhance the muscle activity during human walking (Dietz 1998; Dietz and Duysens 2000; Duysens et al. 2000; Grey et al. 2004). Other possible contributors are large cutaneous afferents (Aαβ), as all the patients reported decreased or absent light-touch sense in their toes, indicating a loss of feedback related to these pathways. Inhibitory reflexes from sural skin afferents (activated at two times the perception threshold) onto leg extensor muscles have been shown to be depressed by load receptor input during locomotion (Bastiaanse et al. 2000), opening the possibility that these inhibitory effects might be unmasked by large fiber loss (including Ib afferents). However, we do not know whether these smaller-diameter skin afferents were still present in the patients and, if so, whether or not they were activated during the stance phase of gait. Recently, cutaneous input from the foot sole has been shown to have a substantial effect on the ankle torque during perturbed standing (Meyer et al. 2004). However, in recent experiments on healthy subjects (Grey et al. 2004), we have observed that large cutaneous afferents may not contribute to the ankle extensor activity during the stance phase of walking.

Adaptability of the nervous system

Although the patients had decreased or absent feedback from large-myelinated sensory fibers, they walked without assistance both in and out of the laboratory environment. The patients’ normal gait pattern can be explained in terms of the flexibility and adaptability of the human motor control. Mulder et al. (2001) showed that patients suffering from CMT1A were able to perform fine movements as long as the movements were well known and learned. However, the performance deteriorated when they had to execute a new motor pattern. Mulder et al. (2001) proposed that even when parts of the sensory-motor system are damaged, the output remains optimal by means of shifting between input sources. The CNS adapts to changes in the peripheral input to keep the output optimal. Nardone et al. (2000) showed that the body sway of CMT1A patients during standing was almost normal. However, in patients with diabetic neuropathy, in which large- and small-diameter sensory fibers are affected, the body sway was larger compared with normal subjects and CMT1A patients (Nardone and Schieppati 2004). It was then suggested that when sensory feedback from only one kind of afferent fiber is affected, the performance of movements remains almost normal. However, when the neuropathy affects a broader range of sensory fibers, the control of movements is impaired. This idea is supported by studies in which selective block of specific afferent pathways was applied during standing, showing that the elimination of single sensory input hardly affects balance; however, unsteadiness appears when a second sensory input is blocked (Diener et al. 1984; Dietz et al. 1980; Magnusson et al. 1990a,b).

The findings of the present study suggest that the afferent feedback in healthy subjects contributes to adaptation of the SOL EMG with changes in the ankle movement occurring during the stance phase of walking. Feedback from primary and secondary spindle receptors may contribute to the enhancement of the background locomotor activity, whereas feedback from other large-sensory pathways (e.g., group Ib) may contribute to the reduction of this activation in an on-going basis to compensate for variations on the ankle trajectory during walking.

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