Fast Responses to Stepping on an Unexpected Surface Height Depend on Intact Large-Diameter Nerve Fibers: A Study on Charcot–Marie–Tooth Type 1A Disease

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1Department of Rehabilitation and 2Department of Neurology, Radboud University Nijmegen Medical Centre; 3Sint Maartenskliniek, Research Development and Education, Nijmegen, The Netherlands; and 4Motor Control Laboratory, Department of Biomedical Kinesiology, Katholieke Universiteit Leuven, Leuven, Belgium

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van der Linden MH, de Kam D, van Engelen BGM, Hendricks HT, Duysens J. Fast responses to stepping on an unexpected surface height depend on intact large-diameter nerve fibers: a study on Charcot–Marie–Tooth type 1A disease. J Neurophysiol 102: 1684–1698, 2009. First published July 22, 2009; doi:10.1152/jn.91142.2008. The contribution of reflexes from the large myelinated afferents in the control of normal and perturbed gait in humans is a highly debated issue. One way to investigate this topic is by studying normal and perturbed gait in patients lacking large myelinated fibers in the distal limb (Charcot–Marie–Tooth [CMT] type 1A disease). Such patients should have delayed and decreased reflexes if the latter depend on these large myelinated fibers. To elicit the reflexes, both patients and controls had to step on a platform that was either at the same level or lowered by 5 cm. In control subjects, landing on a level surface induced short-latency responses in the biceps femoris and tibialis anterior muscles, whereas such responses were largely absent in the patients. Similarly, stepping down unexpectedly induced a very fast muscle synergy, leading to a brake of the forward propulsion in the controls, which was significantly reduced and delayed (on average 32 ms) in the patients. The observed changes correlated with both sensory and motor deficits. Nevertheless, it is concluded that the results are primarily related to the sensory deficits, since the delayed or absent responses appeared in both upper and lower leg muscles, whereas only the latter showed motor deficits. The data are taken as evidence that large-diameter afferents from the distal leg are essential for fast reflex activations induced by stepping on a level or lowered surface unexpectedly.

INTRODUCTION

A disturbance related to the interaction with the ground surface is sensed primarily by cutaneous afferents from the foot sole or by the fast-conducting group I proprioceptive afferents (Duysens et al. 2000; Rossignol 1996). It has been proposed that such afferent input is used to elicit reflexes that can assist posture and gait (Dietz and Noth 1981; Duysens and Pearson 1980; Duysens et al. 2000; Fitzpatrick et al. 1992; Horak et al. 1990; Nielsen and Sinkjær 2002; Pearson 1995, 2004; Stein et al. 2000; Thoumie and Do 1996; Verschueren et al. 2003). If these propositions are correct one would expect that these reflexes are reduced in patients who lack the large myelinated fibers in the lower limb. In patients with Charcot–Marie–Tooth (CMT) 1A disease, the afferents with the largest diameter (group I of the proprioceptors, group II of the cutaneous afferents) are primarily affected, along with a variable amount of efferents to muscles in the distal lower leg. In agreement with expectations, studies on CMT 1A patients during gait and stance showed an absence of the cutaneous P2 responses (at ~80 ms; Van Wezel et al. 2000) and of the early stretch responses (short-latency response [SLR], at ~36 ms; Mazzaro et al. 2005b; Nardone et al. 2000), thereby convincingly illustrating that the largest-diameter afferents are essential for these responses.

The previous studies investigated the role of the large-diameter afferents during gait, using methods such as nerve stimulation and imposed muscle stretch. Yet, an important remaining question is how CMT patients react to perturbations that mimic daily-life situations. Under normal conditions, such as good lighting and an even surface, patients perform relatively well, indicating that they are well able to compensate for their motor and sensory impairments. However, when encountering a challenging environment under poor lighting, falls frequently occur. In a group of elderly suffering from polynuropathy, for example, most of the falls (79%) were indeed caused by stepping on an irregular surface (DeMott et al. 2007). This suggests that such patients are not able to execute the appropriate response strategies when faced with an unexpected change in support surface conditions. One commonly observed situation leading to a fall is that one steps on a surface that is lower than expected, such as when missing a curb or the last staircase step.

This type of perturbation has been introduced in the laboratory both for cats (“foot in hole”; Gorassini et al. 1994) and for humans (“unexpected step-down”; Shinya et al. 2009; Van der Linden et al. 2007; Van Dieën et al. 2007). In the cat experiments, the steps in a hole caused a pausing of the ongoing ankle extensor activity (Gorassini et al. 1994). This was taken as evidence that group I afferent input provides reinforcing feedback in extensor muscles, as proposed earlier by Duysens and Pearson (1980). Further support for this interpretation came from experiments in which the pausing could be eliminated by providing extra activation of group I afferents through electrical stimulation (Hiebert et al. 1995). Inversely, when such fast-conducting afferents were blocked by administering pyridoxine, the deafferented cats failed to show appropriately
timed and scaled responses to perturbations of gait (Allum et al. 1998).

In humans, the unexpected step-down was characterized by an increased forward momentum of the body followed by a rapid step of the nonperturbed leg (Van Dieën et al. 2007). Regarding the muscular responses, the reactions to the “foot-in-hole” experiments in humans differ from those of cats because humans have no ongoing extensor activity at the time of expected foot contact. Instead it was found that stepping down unexpectedly triggered a fast bilateral braking reaction (Shinya et al. 2009; Van der Linden et al. 2007). The braking reaction consisted of increased activity of the medial gastrocnemius (MG) and rectus femoris (RF) muscles on the perturbed side and increased tibialis anterior (TA) and biceps femoris (BF) activity on the nonperturbed side, 47–69 ms after the moment of expected foot contact (Van der Linden et al. 2007). This complex synergy that flexed the nonperturbed leg and extended the perturbed leg is reminiscent of a braking reaction that is seen when humans are asked to quickly stop walking (Hase and Stein 1998). In addition, there was increased electromyographic (EMG) activity in all muscles immediately after the delayed impact to stabilize the ankle and knee, resembling the increased postlanding reflex activity found in false-floor and unexpected-landing experiments (Kamibayashi and Muro 2006; McDonagh and Duncan 2002) and in experiments in which subjects stepped on a surface that suddenly collapsed (Marigold and Patla 2005; Nakazawa et al. 2004).

When stepping in a hole, the expected feedback, both cutaneous and proprioceptive, from the foot and lower leg at landing is temporarily absent. One can therefore assume that the absence of expected sensory input is sensed as a discrepancy, which then triggers the fast braking reaction. Based on the impaired function of the large-diameter afferents, CMT 1A patients are presumably less able to detect this. The present hypothesis is that the “braking synergy” is absent or delayed in these patients. Furthermore, due to the distal muscle atrophy of the CMT 1A patients, a further decrement in the amplitude of the response can be expected. In addition, secondary biomechanical constraints of the polynuropathy, such as a reduced ankle range of motion, may play an important role in the ability to respond to these perturbations as well. Thus both motor and sensory deficits might contribute to a less effective braking reaction, ultimately leading to loss of balance. The contribution of the motor and sensory impairments regarding the maintenance of postural stability after stepping on a surface of unexpected height in polynuropathy patients has not yet been extensively studied. It has been shown that falls in elderly patients with an acquired form of polynuropathy are associated with both motor (peroneus, dorsiflexion strength) and sensory deficits (vibration sense, neuropathy score; Richardson 2002), although similar studies in CMT 1A patients are currently lacking.

In summary, little is known about the role of the large-diameter afferents in response to an unexpected step-down, as occurs regularly in daily life. Therefore the purpose of this study was to investigate the muscular and kinematic responses of CMT 1A patients to stepping on a surface that is unexpectedly lowered by 5 cm. In addition, the contribution of the sensory and motor impairments to the responses of the CMT 1A patients will be examined. With regard to the unexpected step-down, two important features will be addressed: 1) the early braking reaction between the moment of expected and real foot contact and 2) the postlanding reflex activity following the delayed impact. To determine the exact onset latency and muscle synergy of the early braking reaction, the unexpected step-downs need to be compared with a condition in which the preprogrammed anticipatory muscle activity prior to the moment of the expected foot contact is the same, to ensure that the subjects approached the surface similarly. Such a condition is level walking, while being unaware of the support surface height. Second, to calculate the increase in the amplitude of the postlanding activity, the unexpected step-downs need to be compared with a condition in which the physical condition of the task is similar, whereas the level of expectancy differs: an expected step-down.

METHODS

Subjects

Eleven CMT 1A patients (mean ± SD: age, 40.6 ± 10.3 yr; height, 1.77 ± 0.1 m; weight, 82.8 ± 10.3 kg; 8 men and 3 women) were compared with 11 matched control subjects, without any known orthopedic or neurological deficits (age, 45 ± 9.8 yr; height, 1.75 ± 0.14 m; weight, 81.5 ± 13.4 kg; 6 men and 5 women). None of the subjects had undergone ankle–foot surgery or suffered from comorbidities. All patients had a duplication of the p11.2–12 region on the short arm of chromosome 17, as diagnosed through DNA analysis. The patient population consisted of relatively good walkers, who were able to walk independently without orthopedic aids for ≥1 h. Most of the patients had mild pes cavus. None of the patients had structural pes equinovarus, since patients who were unable to achieve bilateral heel loading with fully extended knees were not included in the study. To reduce the risk for ankle sprains, the strength of the peroneus muscle of all patients had to be ≥3 on the Medical Research Council scale (MRC 1981), indicating the ability to evert the foot against gravity. Patients were recruited from the Department of Neurology at the University Medical Hospital in Nijmegen and through the Dutch Society for Neuromuscular Disorders. Written informed consent was obtained from all subjects. The study was approved by the regional ethics committee and performed in accordance with the Declaration of Helsinki.

Clinical examination

All patients underwent a standardized clinical evaluation of their motor and sensory impairments. To grade the motor impairments, muscle strengths of the distal lower leg muscles were scored bilaterally on the modified MRC scale, consistent with previously published methods for impairment profiles in neuromuscular disorders (Carter et al. 1995; Fowler Jr et al. 1995). In the current patient population, MRC scores could range between 3 and 5, which corresponds to: 3, full range of motion (ROM) against gravity without resistance; 3.25, full ROM against slight resistance; 3.75, movement against moderate resistance; 4, movement against strong resistance, but some weakness; 4.25, movement against strong resistance with slight weakness; and 5, full strength. Distal muscle strength was determined for the following muscles: tibialis anterior, tibialis posterior, gastrocnemius, peroneus longus, extensor digitorum longus, and hallucis longus. The strength of these muscles was averaged into a mean distal MRC score. In addition, proximal muscle strength was determined for the quadriceps, hamstrings, hip abductor, and adductor muscles. The passive ROM of the ankle was addressed as well, by measuring the maximal passive dorsi- and plantar flexion angles with a handheld goniometer. While the patient sat, the upper arm of the goniometer was positioned parallel to the peroneus longus muscle and the lower arm parallel to
the lateral border of the foot, just inferior to the lateral malleolus. A goniometer value of 90° was considered neutral (0° dorsiflexion).

To grade the sensory impairments, the superficial tactile perception threshold of the sole and dorsum of the foot was examined with a monofilament (buckling force, 10 g). The sole of the foot was divided in three regions: the heel, middle, and ball of the foot. The monofilament was pushed against each area at least three times. Patients lay with their eyes closed and gave a verbal sign when they felt the monofilament. The monofilament scores ranged from 0 to 2: 0, the patient did not feel the monofilament; 1, the patient felt the monofilament, but not consistently; 2, the patient consistently detected the monofilament. The monofilament scores of both feet were averaged into single scores for further analysis.

Vibration detection threshold (VDT) was assessed bilaterally by means of a 128-Hz Rydel–Seiffer tuning fork (US Neurologics). The tuning fork was vibrated maximally and then applied at three locations: the medial malleolus and lateral malleolus of the ankle and the head of the first metatarsal bone. Subjects were instructed to respond verbally when they no longer perceived vibration, at which point the corresponding number on the tuning fork [ranging from 0 (no vibration sense) to 8 (optimal vibration sense)] was scored by the examiner. The distal VDT score was calculated by averaging all VDT scores. Finally, patellar and Achilles tendon reflexes were tested and scored.

Electrophysiological data of the patients were collected from their medical records whenever possible. The motor nerve conduction was absent or present. The distal VDT score was calculated by averaging all VDT scores. Finally, patellar and Achilles tendon reflexes were tested and scored absent or present.

Electrophysiological data of the patients were collected from their medical records whenever possible. The motor nerve conduction velocity (NCV) of the peroneal nerve and the compound motor action potential (CMAP) of the tibialis anterior (TA) were collected.

**Experimental setup and instrumentation**

The experimental setup was identical to that used in previous research (Fig. 1; Van der Linden et al. 2007). The setup consisted of a 2.6-m wooden walkway, ending on a gravity-driven platform of 1 m² (see Fig. 1A), supported at each corner by two electromagnets (Commissaris et al. 2002). Release of the electromagnets resulted in a 5-cm lowering of the platform. Subjects were instructed to walk four steps, starting with their right foot, and then come to a full stop with their feet placed next to each other. The second step, with the left (contralateral) foot, was the step prior to the step on the platform and triggered the start of the data collection. The third step, with the right (ipsilateral) foot, was placed on the gravity-driven platform that was sometimes unexpectedly lowered. In the series of unexpected trials, subjects were asked to step in place for 20 s with their eyes closed in between the trials, while falling platforms were played on earphones, preventing subjects from hearing, seeing, or feeling the movement of the platform. All subjects wore a safety harness that was attached to a rail system on the ceiling. During the whole experiment, a physical assistant was present to assist in case of loss of balance. In addition, subjects could grasp a lateral sidebar, when they experienced severe loss of balance. Step length and cadence were standardized by foot placement instructions and the use of a metronome. Subjects performed several practice trials to become familiar with the correct foot placement and cadence prior to data collection. During all trials, subjects were instructed to look at a marker on a wall straight ahead, to control for head position. In addition, subjects wore glasses that blocked the lower part of the visual field, to prevent them from visual feedback of the platform position.

Surface electromyographic (EMG) measurements were performed bilaterally on the tibialis anterior (TA), medial gastrocnemius (MG), rectus femoris (RF), and biceps femoris (BF) muscles. Skin impedance was reduced to a value <10 kΩ by shaving and scrubbing the skin with scrub gel, before placing the electrodes. Two electrodes (Kendall ECG, 2.0-cm diameter) were placed adjacent to each other (interelectrode distance of 2.0 cm) on the center of the muscle belly, in parallel with the direction of the muscle fibers. EMG signals were preamplified and low- (300 Hz) and high-pass (3 Hz) filtered (electrical analogue band-pass filter) before they were stored for further off-line analysis. Electrogoniometers (Penny and Giles, UK Biometrics, Gwent, UK) were placed on the lateral side of both knees and

A: methods of experimental setup. A 2.6-m walkway was built that embedded a platform that could be lowered 5 cm before foot contact. The step on the platform is referred to as ipsilateral (i), the preplatform step as contralateral (c). B: illustrated is the moment of expected foot contact (EFC) as measured by an interruption in the infrared (IR) signal. The moment of actual foot contact (AFC) was measured by footswitch signals, placed on the insoles of the subjects’ shoes. Simultaneously, force transducers under the platform measured vertical ground reaction force. C: schematic overview of the experimental definitions and protocol.
ankles to measure the joint trajectories. Heel and toe contacts were measured by footswitches (designed in collaboration with Algra Fotometaal, Wormerveer, The Netherlands) that were placed on the soles. All subjects were identical, appropriately sized gymnastic shoes. The moment of expected foot contact (EFC) in the unexpected step-down trials was measured by an infrared system, consisting of two horizontally placed transmitters and receivers at level height (see Fig. 1B, dashed arrow). The moment at which the infrared beam was interrupted by the right foot was defined as the moment of EFC. Force plates (Dtaxe Weighing system, type 104A) were placed underneath the four corners of the platform. A steep increase in the force signal closest to the right foot followed the footswitch signal by on average 7.4 ms, presumably due to damping (Fig. 1B). When the footswitch data were missing, the force signal was used for determining the moment of actual foot contact (AFC), taking into account the 7.4-ms delay. All data were sampled with a frequency of 1 kHz, from 1 s prior to 2 s after heel contact of the pre-perturbed step. Data were A/D converted and stored for further off-line analysis. An RsScan pressure plate that was placed on the platform detected the area of first foot contact (RsScan International, Olen, Belgium; 0.4 × 0.5 m, two sensors/cm², 500 Hz).

Protocol

The experimental definitions and protocol, similar to those used in a previous study (Van der Linden et al. 2007), are schematically illustrated in Fig. 1C. The series of trials were divided in two categories: expected (E) and unexpected (U). The U trials were preceded by the warning that the platform might be lowered 5 cm during each of the following trials. Strictly speaking, the lowered platform trials were not totally unexpected, since the subjects were aware of the possibility of a lowered platform. This terminology was used to distinguish these from the second group, the expected trials (E). In the latter trials, subjects were told beforehand whether the platform was lowered. Furthermore, the trials received a second label, based on the physical condition of the landing surface. The level trials were termed level (L) and the 5-cm-lowered platform trials were termed down (D).

Subjects started the protocol with 10 trials of expected level walking (EL, expected level). Next, the platform was unexpectedly lowered 9 times (UD, unexpected down; UD 1, UD 2, etc.). Each UD was followed by 8–10 trials of level walking (UL, unexpected level; UL1, UL2, etc.). The exact number of UD trials varied to prevent the subjects from being able to predict the next UD. In the last part of the experiment, the subjects performed 10 expected step-down trials (ED, expected down).

All UL trials were intrinsically unexpected, since the subjects were always in anticipation of a possible step-down trial. However, the level of anticipation gradually adapted during the subsequent UD trials (see Van der Linden et al. 2007). During the UL ≥7 trials, the walking pattern of the subjects had stabilized and was no longer strongly biased towards expecting a step-down. However, this walking pattern was still slightly different from expected level walking. Because of these reasons, the trials UL ≥7 were selected as control trials to compare with the UD condition (UD–UL).

Data analysis

All signals were rectified off-line. For each subject, the EMG data were normalized with respect to the maximum EMG that was measured during a UL control trial. The maximum EMG was determined for each muscle, by calculating a moving average with a window of 30 ms, over a total step cycle. To further quantify the reflex activity, the experimental condition (UD) was compared with two control conditions: unexpected level walking (UL ≥7) and expected down (ED). In the UD condition, the subjects were unaware of the support surface condition. Therefore the surface was approached similarly as in the UD condition. As a consequence, the preprogrammed anticipatory muscle activity prior to the moment of the expected foot contact (EFC) was the same. Thus this comparison allowed us to determine the exact onset latency and muscle synergy triggered by the absence of expected foot contact. In the second comparison (UD vs. ED), the physical conditions of the tasks were the same (step-down), whereas the level of expectancy differed. This allowed us to answer to what extent postlanding muscle activity was enhanced as a consequence of the unexpectedness of the step-down. For the first comparison, UD–UL, the data were aligned such that the EFC of the UD trials coincided with the actual heel contact (AHC) of the UL trials. As a consequence, the conditions in this comparison were equal until the moment of expected heel contact. For the second comparison, UD–ED, the data of both conditions were aligned at the moment of actual foot contact (AFC).

To determine the amplitude of the EMG response, the control conditions UL ≥7 and ED were averaged and subtracted from the single UD trials. After subtraction, the EMG data were averaged in 10-ms bins starting at EFC. The bin data were averaged per subject, muscle, and finally per group. The onset latency of the EMG response was determined by visual inspection. For each subject and muscle, the UD trials were superimposed on the mean ±2SD of the UL ≥7 trials, in the period of EFC until 200 ms after AFC. For graphic use only, the EMG trials were filtered (zero-lag, second-order, bidirectional Butterworth filter, low-pass 50 Hz). Using a custom-made MATLAB program, onset latencies were determined by placing a cursor at the moment where the UD curve exceeded the mean ±2SD of the UL curve for a period of ±30 ms. Afterward, group averages of the onset latencies were calculated for all muscles, based on three consecutive onset latencies per person. One patient and two control subjects failed to show three consecutive onset latencies in the predefined window, for the TA (patient), MGI, and TAi (controls), respectively. Therefore these trials were not included for further analysis. The onset latencies of the kinematic responses were similarly identified, although over a longer time window (until 400 ms after EFC).

The subjects used different strategies for landing from the step-down: heel, combined (heel–forefoot), and forefoot landings. The control subjects landed mostly on the heel (52%), followed by combined (“flatfooted,” 35%) and forefoot landings (12%). The patients used slightly different landing strategies: heel (35%), combined (62%), and forefoot (2%). To ensure that the EMG responses were not affected by the way the foot hit the platform, the EMG responses of the different landing strategies were compared using an ANOVA for all subjects together and for the patient and control group separately. The onset latencies of the EMG responses showed no significant differences between the different landing strategies. Significant differences in response amplitude in the period of 50–100 ms after EFC, based on the UD–UL comparison, were not found either, except for the forefoot landings. The forefoot landings were associated with significantly increased activity compared with the other landing strategies, 50–70 ms after expected landing in the RFc of the control subjects \( F_{2,21} = 4.27; P = 0.029 \) and 70–90 ms after expected landing in the TAi of the patients \( F_{2,17} = 6.42; P = 0.01 \). Thus the effects of landing strategy were present but quite limited (only 2% of the patient trials were forefoot landings) and did not largely affect the main braking synergy. Therefore all foot-landing strategies were grouped for further analysis.

Statistical analysis

The results are presented as means ± SD, unless otherwise specified. Group differences were tested with an independent samples \( t \)-test. To verify whether the average subtracted EMG amplitudes of the 10-ms bins were significantly different from zero, multiple one-sample \( t \)-tests were performed. When three or more consecutive bins were significantly different from zero, it was considered a reflexive
burst. A two-way ANOVA with Bonferroni post hoc comparisons was used to test whether the onset latencies of the EMG response differed across muscles and groups. The associations between the EMG responses and the clinical measures were tested with two-tailed nonparametric Spearman’s rho correlation coefficients. The level of significance was set at a P value of 0.05.

RESULTS

Subject characteristics

The clinical characteristics of the patients are depicted in Table 1. The average distal muscle strength was moderately impaired in the patients (CMT 1A, MRC 4.7 ± 0.4 vs. control, MRC 5). The strength of the peroneus muscle was most severely affected (4.4 ± 0.5; see also Table 1), followed by the extensor digitorum longus (4.6 ± 0.6) and hallucis longus (4.7 ± 0.6). The strength of the calf muscles was always intact (MRC 5). Proximal muscle strength was always intact as well, except for the hamstrings in two patients (MRC 4). Only the most severely affected muscle (peroneus) was included for further analysis.

The impairments of the mean vibration detection threshold (VDT; range 0–8) had a distal to proximal distribution in the CMT 1A patients. The VDT of the hallux was most severely affected (2.5 ± 2.7), followed by the medial (4.8 ± 2.0) and lateral malleolus (5.4 ± 2.1). These VDT values were significantly lower compared with the normal VDT values, as determined for a group of healthy controls in the age range of 40 to 49 yr (hallux 6.3 ± 1.0, P < 0.001; medial malleolus 6.0 ± 0.9, P = 0.08; adapted from Martina et al. 1998). With regard to the superficial tactile sensation, five patients showed impairments at the ball of the foot and three at the dorsum of the foot. The electrophysiological data of the patients showed slowed nerve conduction velocities (22.1 ± 3.6 m/s) and reduced CMAPs (3 ± 2.5 mV), consistent with CMT type 1A disease. For four patients, electrophysiological data could not be collected from their medical records. The average maximum passive dorsiflexion of the ankle tended to be slightly impaired (−6 ± 10°), whereas the average passive plantar flexion (76 ± 12°) was generally intact. Yet, all patients were able to achieve bilateral heel loading, sometimes by hyperextending the knees. Achilles and patella tendon jerks were absent in all but one patient. Joint position sense at the ankle and hallux were all intact, except for the eversion/inversion of the ankle in one patient.

Experimental conditions

All patients and controls successfully completed the experimental protocol. Since the patients were more susceptible to exhaustion, they were encouraged to take a break and sit down in case of fatigue. The unexpected step-down trials generated a forward propulsion of the body, which triggered a fast braking reaction (see also Van der Linden et al. 2007). The forward propulsion frequently induced a loss of balance in the patients, in which case a physical assistant provided extra support. Furthermore, the patients regularly reached for the lateral sidebar. In contrast, loss of balance was never observed in the healthy controls. Thus the braking reaction appeared to be less effective in the patients than that in the controls. When the braking was insufficient the patients made a few short corrective steps. Therefore a shortening of the stance phase of the perturbed step was considered a good indicator of loss of stability. As illustrated in Fig. 2, the stance-phase duration of the ipsilateral (perturbed) step was significantly shorter in the patients than that in the controls in the trials with an unexpected step down (594 vs. 725 ms, P < 0.001). In contrast, the duration of the stance phase of the preperturbed (contralateral) step did not differ between the patients and controls, indicating that both groups approached the platform with similar walking speed.

The EMG data of the three main experimental conditions (UL, ED, and UD) were further analyzed by averaging the normalized responses of all patients and controls, as illustrated in Fig. 3. The UL condition contains only UL ≥7 trials, that is the trials after there had been six consecutive trials of level landing in a series of trials in which subjects did not know beforehand whether they would land on a level or a lowered surface (see also Protocol in METHODS). Significant group differences, as calculated over 10-ms bins of EMG data, are illustrated by the black bars underneath the plots. Only those muscles are illustrated that had significant group differences in

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<th>MRC P</th>
<th>VDT</th>
<th>MON D</th>
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Illustrated are the muscle strength of the peroneus muscle (MRC P; range, 0–5), the average distal vibration detection threshold [VDT; range, 0 (absent) to 8 (optimal)], and the monofilament scores of the dorsum [MON D; range, 0 (absent) to 2 (optimal)] and ball (MON B) of the foot. Electrophysiological findings are represented by the nerve conduction velocity of the peroneal nerve (NCV in m/s) and the compound motor action potential of the tibialis anterior (CMAP in mV). The passive range of motion of the ankle is represented by the DF (maximal dorsiflexion angle) and PF (maximal plantarflexion angle). Note that a neutral position of the ankle (90°) was scored as 0.
Prior to foot landing (AFC) the activation patterns in UL and ED were quite similar for the patients and the controls, except for some small extra activity in the RFi of the patients in the UL condition. After touchdown, however, substantial differences were seen. The most striking feature was the distinct burst in the BFi in the controls, starting some 50 ms after AFC in UL. This burst was absent in the patients. Similarly, there was increased activity in the TAi of the controls, some 30–70 ms after AFC in UL and ED. This burst was reduced (UL, nonsignificant) and lacking (ED) in the patients. For the MGi in the ED condition, a small burst was observed shortly after AFC in the controls, which was delayed in the patients. Due to the delay, the patients showed increased MGi activity some 70–100 ms after AFC. For the contralateral TAc, the patients showed significantly increased activity in UL and ED, starting at about 60 ms after ipsilateral foot contact. In the UD condition, there were strong bursts of activity in all illustrated muscles in the period just before and/or after AFC. These bursts were significantly smaller in the patients than those in the controls. To further analyze the data, it was essential to compare the various experimental conditions, with respect to both the actual movement made (down vs. level, UD–UL; see following section) and the level of expectation (unexpected vs. expected, UD–ED; see Association between response characteristics and clinical symptoms).

**FIG. 2.** Average stance phase durations for the contralateral (preperturbed) and ipsilateral (perturbed) step in the unexpected step-down condition. The black bar represents the Charcot–Marie–Tooth (CMT) 1A patients (n = 11); the gray bar represents the healthy controls (n = 11). Error bars represent SD. *P < 0.05.

EMG amplitude in the period of 100 ms before to 150 ms after actual foot contact (AFC): MGi, TAi, RFi, BFi, and TAc.

**FIG. 3.** Average normalized electromyographic amplitudes for the unexpected level (UL ≥7), expected step-down (ED), and unexpected step-down (UD) conditions. The black trace represents the average ±2SD of the healthy controls (n = 11); the gray trace represents the average amplitude of the CMT 1A patients (n = 11). Significant group differences are illustrated by the black bars underneath the plots. Illustrated are the muscles that showed significant group differences in the window of 100 ms before to 150 ms after foot contact. Note that these were predominantly found on the ipsilateral side (i). The vertical line indicates the moment of AFC. MG, medial gastrocnemius; TA, tibialis anterior; RF, rectus femoris; BF, biceps femoris muscles. i, ipsilateral; c, contralateral.
Unexpected step-down compared with unexpected level (UD–UL)

To study the exact onset and amplitude of the reflex activity that occurred as a consequence of stepping down unexpectedly, the UD condition was compared with the UL condition. In the UL condition, the subjects were unaware of the support surface condition. Therefore the surface was approached similarly as in the UD condition. As a consequence, the preprogrammed anticipatory muscle activity until the moment of expected foot contact (EFC) was similar. Illustrated in Fig. 4 (top left) is the average activity of the ipsilateral MGi of a healthy subject for the UD and UL conditions, aligned at the moment of EFC. For the UD condition, EFC occurred 82 ms before actual foot contact (AFC), whereas for the UL condition the two coincided. As can be seen, the absence of expected foot contact at EFC triggered a large burst of reflex activity in the MGi, well before AFC.

To further quantify the reflex amplitude, the average activity of the UL condition was subtracted from the UD condition (UD/H1102/UL), yielding the plots as shown in Fig. 4 for the CMT 1A patients (gray) and the healthy controls (black). The left side represents the response amplitude of the ipsilateral, perturbed leg. The right side represents the contralateral, unperturbed leg. Reflex activity that is significantly different from zero is indicated by the arrows beneath each plot. The difference in activity appears either as positive (UD > UL) or as negative (UD < UL) and will be referred to as positive and negative responses. Significant group differences are illustrated by the black bars.

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**Fig. 4.** Unexpected down (UD) vs. unexpected level (UL) walking. Top: average MGi amplitude of the UD and UL ≥7 condition of a healthy control (n = 1). The other panels show the subtracted response amplitudes: UD – UL ≥7. Each bin represents the group average (n = 11) of the response amplitude for a period of 10 ms: the black squares for the controls, the gray diamonds for the CMT 1A patients. The solid vertical line represents the moment of EFC, the dotted vertical line the moment of AFC in the UD condition. The moment of EFC in UD was aligned with the AFC in UL (AFC UL). A positive deflection implies increased UD activity; a negative deflection implies decreased UD activity compared with the UL activity. Bin amplitudes that are significantly different from zero (P < 0.05) are marked by the black (control) and gray (patient) arrows beneath the x-axis. Significant group differences are illustrated by the black bars beneath the plots. Error bars represent the SE.
Bilateral changes in muscle activity were found well before AFC in both the patient and control group. The positive response in the ipsilateral leg was characterized by increased activity of the MGi and RFi, consistent with our previous study (Van der Linden et al. 2007). On the contralateral side, the positive response was characterized by a clear burst in the TAc and BFc well before AFC, followed by increased RFc activity. Negative-response amplitudes were seen in the ipsilateral TAi and BFi in the period before AFC and originated from activity that was present after foot contact in UL, but not in UD (see also Fig. 3). The negative TAi and BFi responses were observed only in controls and not in patients because the latter lacked the activity bursts after foot contact in the UL condition.

As appears from Fig. 4, the muscle synergies of the patients and the controls were largely similar. However, there was a significant reduction in the amplitude of the reflex responses of the patients. Well before AFC, the MGi and TAc activity of the patients was strongly reduced, followed by significant reductions in TAi, RFi, and RFc activity immediately after AFC. To determine the exact onset of the response synergy, the onset latencies of the positive responses were determined quantitatively for each trial (see Data analysis in METHODS).

The average onset latencies of the various illustrated muscles ranged from 38 to 72 ms in the controls and from 64 to 88 ms in the CMT 1A patients (see Fig. 5), indicating that the responses of the CMT 1A patients were generally delayed. More specifically, significant group differences were found for the MGi, TAc, and BFc, which were respectively 48, 27, and 21 ms delayed in the patients. To test whether the responses in the various muscles belonged to a single synergy the onset latencies of these deviations were measured for both patients and controls. Before going into detail about these differences, it was necessary to ensure that the basic gait patterns of the patients and the controls were comparable. Therefore the average ankle and knee trajectories of the UL condition were analyzed for both patient and control groups, based on 10 consecutive trials per person (see Fig. 6). Illustrated is the period of 800 ms before, until 1,600 ms after the step on the platform (ipsilateral foot contact). One of the subjects with reduced passive ankle dorsiflexion was excluded from this analysis as a result of technical difficulties during the recording. The average joint trajectories were largely comparable. No significant differences in the amplitude of the ankle or knee could be observed, as calculated over 100-ms bins. Somewhat remarkably, the impaired passive dorsiflexion observed in four patients (see Table 1) did not result in significantly altered average ankle patterns during gait. It must be mentioned, however, that the dorsiflexion impairments during passive dorsiflexion testing were generally larger than those during gait.

Since the termination of the ongoing movement in the UD condition originated from a flexor response in the contralateral leg, further analysis was focused on the ankle and knee trajectory of the contralateral leg. For this analysis, a comparison was made between the UD and the UL conditions, since expectations about the support surface condition were similar until the moment of EFC. Figure 7 illustrates the average ankle and knee trajectory of a typical healthy control.

How did these muscle responses translate into behavior? As described in a previous article (Van der Linden et al. 2007), the activated muscle synergy resembled a braking reaction. The latter was characterized by a flexor response in the contralateral leg, trying to stop the ongoing movement by decreasing the push-off force, and an extensor response in the ipsilateral leg, preparing for the delayed impact. Based on the less-effective braking reaction of the patients, we hypothesized that there would be differences between the joint angle trajectories of the patients and controls. Before going into detail about these differences, it was necessary to ensure that the basic gait patterns of the patients and the controls were comparable.

Kinematic responses

Both patients and controls showed an exaggerated knee flexion at the end of the stance phase. The group data are illustrated in Fig. 8. It can be seen that the plantar flexion at the end of the stance phase was terminated prematurely in the UD condition and that the knee flexion was exaggerated. To quantify this, the onset latency of these deviations were measured for both patients and controls (see arrows in Fig. 7 and Data analysis in METHODS). The group data are illustrated in Fig. 8.

For the contralateral ankle, the onset latency of the reaction could be reliably identified in seven patients. For these seven
patients, the onset of the deviation was significantly delayed compared with the healthy controls \((315 \pm 146 \text{ ms} (n = 7) \text{ vs. } 200 \pm 53 \text{ ms}, P < 0.04; \text{ see Fig. } 8A, \text{ left})\). The onset of the ankle deviation in the patients correlated significantly with the activity of the TAc at AFC (UD–UL comparison; \(r = -0.74, P < 0.01\)) and with the postlanding TAc activity (30–60 ms after AFC; UD–ED comparison; \(r = -0.79, P < 0.01; \text{ scatterplots not shown}\)). Thus patients with reduced TAc activity (an ankle dorsiflexor) had a delayed termination of the contralateral plantar flexion.

For the contralateral knee, the onset could be reliably detected in 10 patients. The mean onset of the enhanced knee flexion was slightly delayed in the patients compared with that in the controls (168 ± 53 vs. 132 ± 33 ms), but this was not significantly different \((P = 0.08; \text{ see Fig. } 8A)\). For the patients, the onset of the contralateral knee flexion correlated significantly with the onset of the BFc burst, as determined from the UD–UL comparison \((r = 0.62, P < 0.05)\), the amplitude of the BFc activity at AFC (UD–UL comparison; \(r = -0.69, P < 0.05\)), and the postlanding BFc activity (30–60 ms after AFC; UD–ED comparison; \(r = -0.72, P < 0.05\); scatterplots not shown). Thus patients with slowed and reduced BFc activity (a knee flexor) had a delayed onset of knee flexion in the UD condition.

To further establish the effectiveness of the braking reaction, the maximum (plantar) flexion of the ankle and knee was studied [see Figs. 7 (max) and 8B]. It was hypothesized that the patients would not be able to reduce the plantar flexion as much as could the controls—this was confirmed by the data. The healthy controls showed 12° plantar flexion in the UL condition, compared with 0.2° in the UD condition. In the patients, this reduction in plantar flexion (UL–UD; 9 – 4°) was significantly smaller \([F_{(1,18)} = 6.7, P = 0.02; \text{ see Fig. } 8B, \text{ top}]\).

Similarly for the knee, it was predicted that patients would show less extra knee flexion in the UD condition. It was found that healthy subjects increased their knee flexion with 17° in the UD condition. The increase in knee flexion was indeed smaller in the CMT 1A patients (12°), but not significantly different.

**Association between response characteristics and clinical symptoms**

One of the aims of the study was to identify associations between the reflex response of the patients and their sensory or motor deficits. It was hypothesized that strong correlations could indicate whether the changes in reflex activity were primarily due either to the sensory or to the motor deficits of these patients. For this purpose, a distinction was made between tests aimed at measuring the sensory deficits (distal vibration detection threshold, monofilament sensibility at the dorsum and ball of the foot), the motor loss (peroneus strength—most severely affected muscle), and secondary biomechanical constraints (maximal passive plantar- and dorsiflexion of the ankle; see Table 2). The clinical outcome measures were correlated with both the onset latency and the amplitude of the muscles involved in the braking synergy.

Significant negative correlations were found between the onset latency of the MGi, TAc, and BFc and both peroneus strength and vibration detection threshold (see also Fig. 9 for scatterplots). This indicates that patients with reduced strength and vibration sense had slowed responses. Recall that the MGi, TAc, and BFc represent the “fast onset” muscles in the controls (40, 38, and 41 ms, respectively). In addition, a longer TAc onset corresponded with this reduction in plantar flexion (UL–UD; 9 – 4°) was significantly smaller \([P = 0.01]\) and with the postlanding TAc activity (30–60 ms after AFC; UD–ED comparison; \(r = -0.79, P < 0.01; \text{ scatterplots not shown}\)). Thus patients with reduced TAc activity (an ankle dorsiflexor) had a delayed termination of the contralateral plantar flexion.

**FIG. 6.** Joint angle curves of the ipsi- and contralateral ankle and knee in the UL = 7 condition. The black trace represents the average trajectory of the healthy controls \((n = 11)\); the gray trace represents the average amplitude of the CMT 1A patients \((n = 9)\). The vertical dotted line represents the moment of ipsilateral foot contact (iFC). PF, plantar flexion; F, flexion. There were no significant group differences in the average ankle or knee angles, based on 100-ms bins.
decreased monofilament sensitivity. For the RFi and RFc, which were activated slightly later, significant negative correlations were found with the peroneal NCV, indicating that patients with a reduced NCV had slower responses. Additionally, the onset of the RFc showed a significant negative correlation with the maximal passive dorsiflexion of the ankle, indicating that the more the dorsiflexion of the ankle was impaired, the slower was the RFc response.

For the amplitude analysis the muscle activity at the moment of actual foot contact was selected (AFC, 90–100 ms after EFC, UD–UL comparison). Significant correlations were found with both sensory and motor tests. The amplitude of the MGi at AFC correlated significantly with peroneus strength and vibration detection threshold, indicating that patients with decreased strength and vibration sense had less MGi activity at AFC; moreover, the BFc amplitude correlated significantly with peroneus strength. The RFc amplitude correlated significantly with monofilament sensitivity, indicating that patients with decreased superficial sensitivity had less RFc activity at AFC. It follows that both the onset latency and the amplitude of the braking synergy were related to measures of both sensory and motor functions.

Unexpected step-down compared with expected down (UD–ED)

To study the extent to which the postlanding muscle activity was influenced as a consequence of the unexpectedness of stepping down, the UD trials were compared with the expected down (ED) trials, in which the subjects made the same step-down, while being aware that the surface was down (Fig. 10). Thus the physical conditions of the tasks were the same (5-cm step-down), whereas the level of expectancy differed (U vs. E). To compare the two conditions, the data were lined up with respect to AFC.

At the top of Fig. 10, the average normalized MGi activity for the UD and the ED conditions is shown for a single control subject. The traces illustrate that the burst of activity that is normally generated in MGi at foot contact in ED starts earlier and is exaggerated in UD. The resulting difference shows up as extra activity in the plot below, which illustrates the average subtracted muscle responses (UD–ED) for the total group of patients (gray, n = 11) and controls (black, n = 11). The muscles that were previously identified to play a role in the early braking reaction (MGi, RFi, TAc, BFc) again showed extra activity prior to AFC.

**FIG. 7.** Joint angle curves of the contralateral ankle (cAN) and knee (cKN) of a healthy control (n = 1). The panels show the mean ± SD of the UL ≥7 condition (black). Superimposed are the average joint angles of the UD condition (gray). The onset of the deviation between the UD and UL conditions is illustrated by the arrow. The maximum (plantar) flexion following the expected moment of foot contact (EFC) is illustrated by the dotted lines (max). PF, plantarflexion; F, flexion.

**FIG. 8.** A: onset latency of the kinematic responses in the contralateral ankle (cAN) and knee (cKN), based on the UD–UL comparison. The black bars represent the CMT 1A patients (n = 11); the gray bars represent the healthy controls (n = 11). Error bars represent SD. B: maximum (plantar) flexion angle of the ankle [cAN, max. (°)] and knee [cKN, max. (°)] for the UL ≥7 (white) and UD (gray) conditions. The left side represents the controls, the right side the CMT 1A patients. *P < 0.05.
After AFC, other muscles showed increased activity as well (TAi, BFi, RFc). All reflex amplitudes were positive except for the TAi, which showed a brief negative amplitude, originating from activity that was present prior to foot contact in ED, but not in UD (see Fig. 10). This brief negative amplitude was observed in the controls but not in the patients.

To further study the amplitude of the postlanding reflexes, the period of 30–60 ms after AFC was selected, since reflex

### Table 2. Associations between braking reaction and clinical characteristics

<table>
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<th>Channel</th>
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<th>VDT (A.U.)</th>
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<th>MON B</th>
<th>NCV</th>
<th>CMAP</th>
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<td>-0.21</td>
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Two-tailed Spearman’s rho correlation coefficients between the clinical outcome measures of the CMT 1A patients \( (n = 11) \) and the following EMG characteristics of the braking reaction: A, the onset latency; B, the response amplitude at actual foot contact (AFC; UD–UL comparison); and C, the postlanding response amplitude, 30–60 ms after the moment of AFC (UD–ED comparison). The average peroneus strength is represented by MRC P. The sensory outcomes are represented by the VDT (average distal vibration detection threshold), MON D (monofilament score at the dorsum of foot), and MON B (monofilament score at the ball of foot). Electrophysiological findings are illustrated by the NVC (nerve conduction velocity) of the peroneal nerve \( (n = 7) \) and CMAP (compound motor action potential) of the tibialis anterior muscle \( (n = 7) \). The passive range of motion of the ankle is illustrated by the DF (maximal dorsiflexion) and PF (maximal plantarflexion). MG, medial gastrocnemius; TA, tibialis anterior; RF, rectus femoris; BF, biceps femoris. i, the ipsilateral, step-down leg; c, the contralateral, unperturbed leg. * \( P < 0.05 \); ** \( P < 0.01 \).

After AFC, other muscles showed increased activity as well (TAi, BFi, RFc). All reflex amplitudes were positive except for the TAi, which showed a brief negative amplitude, originating from activity that was present prior to foot contact in ED, but not in UD (see Fig. 10). This brief negative amplitude was observed in the controls but not in the patients.

To further study the amplitude of the postlanding reflexes, the period of 30–60 ms after AFC was selected, since reflex

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**FIG. 9.** Scatterplots of the clinical outcome measures of the CMT 1A patients and the onset latency of the MGi and TAi muscles (UD–UL comparison). MRC P, peroneus strength; VDT, vibration detection threshold; A.U., arbitrary units.
activity that is elicited by foot landing is expected to occur in this period. In the ipsilateral leg, significantly increased postlanding activity in this period was found in all muscles in both the patient and the control groups (Fig. 10, left; see horizontal arrows). This indicates that there was cocontraction of the muscles around the knee and ankle, 30–60 ms after AFC. In the contralateral leg, both groups showed increased postlanding activity in the TAc and BFc, followed by increased activity in the RFc, indicating cocontraction around the knee and flexion of the ankle in the postlanding period (Fig. 10, right).

The amplitude of the postlanding activity was generally smaller for the patients than that for the healthy controls. Significant group differences were found for the MGi, TAi, RFi, and TAc (see Fig. 10, black bars), whereas a nonsignificant decrease was found for the BFi and BFc. To identify possible associations between the postlanding amplitude and the motor and sensory deficits of the patients, correlation coefficients were calculated (see Table 2, bottom).

The amplitude of the postlanding MGi and TAc activity correlated significantly with the peroneal NCV. Furthermore, the postlanding BFi amplitude correlated significantly with peroneus strength, whereas the contralateral BFc correlated significantly with monofilament sensibility. For the RFi, there was a trend that reduced postlanding activity was associated with impaired vibration sense ($r = 0.56, P = 0.07$). Furthermore, some associations were found with the passive range of motion of the ankle. Reduced passive dorsiflexion was associated with reduced postlanding TAi activity, whereas increased
passive plantar flexion was associated with reduced BF activity.

DISCUSSION

The main result of the present study in mildly affected CMT 1A patients was the reduction and absence of various muscular reactions when stepping on a level and lowered surface, either expectedly or unexpectedly. Consequently, the CMT 1A patients had considerable problems recovering from the experimental perturbations, whereas controls showed no loss of balance at all. Both motor and sensory pathways could have played a role in the disturbed balance reactions of the patients. However, the results are highly indicative of a primary role of the sensory afferents, as will be argued in the next sections.

Short-latency reflexes after touchdown are altered in CMT 1A patients

A first major finding was the absence, in patients, of short-latency reflex (SLR) activity in various muscles after touchdown. Within the 30- to 60-ms window after landing, there was a distinct BFi burst in controls during level walking (UL) that was absent in the patients. As a consequence, controls showed a negative BFi burst in the UD–UL comparison that was absent in the patients. These results are in line with previous studies on reactions to postural perturbations during standing and gait in CMT 1A patients (Mazzaro et al. 2005b; Nardone et al. 2000). In these latter studies it was shown that the SLRs (~35 ms) were absent, whereas the medium-latency reflexes (MLRs, ~85 ms) were present but delayed. These results were obtained in lower leg muscles, either in the flexor digitorum brevis (FDB; Nardone et al. 2000) or in soleus (SOL; Mazzaro et al. 2005b). The present results extend these findings to the upper leg BF muscle.

The observation that thigh muscles participate in group I-mediated reflex activations at onset stance during walking confirms previous suggestions. It was argued that group I reflexes could contribute to normally occurring BF activity during gait (Dietz and Noth 1981; Duyssens et al. 1998; Prochazka et al. 1979, 2002). The claim was partly based on the finding that SLRs to BF tendon jerks during both reduced (stiff leg) and normal gait were largest in the swing-to-stance transition (Faist et al. 1999; Van de Crommert et al. 1996). Further evidence came from a study in which tendon vibration, a powerful source of Ia afferent stimulation, was applied to various muscles during gait (Verschueren et al. 2003). It was found that BF vibration induced extra activity in the BF at onset stance.

In the lower leg, distinct short-latency bursts after foot contact were seen in the TAi during normal walking (UL) and when stepping down (ED). These TA bursts were present in the controls, but less pronounced (UL) and even absent (ED) in the patients. As a consequence, the controls showed significant negative TA bursts in both the UD–UL and UD–ED comparisons, which were absent in the patients. Thus postlanding TA activity might be Ia mediated, especially when stepping down.

For the MGi, no evidence was seen for short-latency bursts at onset stance during normal gait, which is consistent with previous findings that tendon vibration does not induce extra EMG activation in that period (Verschueren et al. 2003). It should be pointed out that for some muscles such as MGi, a group I reflex contribution during later phases of stance during gait is more likely than that immediately after touchdown (Duyssens and Pearson 1980; Grey et al. 2004, 2007; Mazzaro et al. 2005a,b; Yang et al. 1991). Previous work on CMT 1A patients is in accordance with this suggestion (Mazzaro et al. 2005b). Although no SLRs were seen during level walking, such reflexes possibly occur under conditions of more abrupt landing, such as when stepping on a collapsing surface (Nieuwenhuijzen and Duyssens 2007; Nieuwenhuijzen et al. 2002) and when landing from jumps (Grüneberg et al. 2003; McDonagh and Duncan 2002). Consistent with these latter findings, it was found that landing after an expected step-down elicited a small MG burst in the controls, which was delayed in the patients. Since the 30- to 60-ms burst was absent in the patients, it is suggested that it normally relies on the presence of large myelinated fibers.

Prelanding activity: braking reaction is delayed and reduced in CMT 1A patients

Encountering an unexpected step-down of 5 cm during walking triggered a rapid bilateral braking reaction, enabling the subject to swiftly put the perturbed foot on the floor and keep the body behind the forward leg. Results of the healthy control group (mean age, 45 yr) in the current study were consistent with those observed in the younger controls (mean age, 24 yr) as described in a previous study, thereby illustrating that there were no major aging effects within these age groups (Van der Linden et al. 2007). The results were also consistent with those of Shinya et al. (2009) who showed a similar braking synergy and postlanding cocontraction in the lower leg, triggered by a “completely” unexpected step-down without a previous warning, after eight steps of walking.

The rapid braking reaction to stepping down unexpectedly was observed both in healthy subjects and CMT 1A patients, but the latter group had slower and smaller responses compared with those of the controls. They had significantly increased onset latencies in the MGi, TAi, and BFc muscles, with an average delay of 26–48 ms. The reduced response amplitudes were predominantly found in the distal MGi, TAi, and TA and, to a lesser extent, in the more proximal RFi and RFc. The delayed and reduced activity in several of the muscles participating in the braking synergy occurred in parallel with a less-effective braking reaction after touchdown. This was illustrated by a shortening of the perturbed ipsilateral stance phase, indicative of a greater forward propulsion of the body. The shortened ipsilateral step was often followed by some rapid corrective steps in the patients. When the ability to make such rapid steps fails, falls may occur (Van Dieën et al. 2007). Although falls were not observed in the current study, the patients frequently grabbed for the lateral sidebar and some experienced a loss of balance to the extent that a physical assistant had to provide support. The fact that the braking reaction was less effective in the patients was further illustrated by the delayed and reduced flexion responses of the contralateral ankle and knee. For both the ankle and the knee, these delayed onsets were associated with the altered EMG characteristics of the CMT 1A patients.
Postlanding activity: cocontraction around the ankle and knees is reduced in CMT 1A patients

To investigate to what extent the postlanding responses to an unexpected step-down depended on the “surprise element,” a comparison was made with the responses observed after a step-down on a known lowered surface. The subjects performed the same step-down in both conditions, whereas they were fully expecting it only in the “expected” step-down condition. It was found that the unexpected step-down elicited a wide cocontraction in various muscles after impact, consistent with our previous study (Van der Linden et al. 2007). This extra activity was severely reduced and delayed in the CMT 1A patients, especially in the distal MGi, TAi, and TAc muscles, which may have resulted in reduced ankle stiffness, resembling findings in patients with diabetic polyneuropathy (Nielsen et al. 2004). The reduced amplitude of the postlanding activity was associated with both sensory (tactile sensation) and motor deficits (peroneal NCV and strength), as well as biomechanical constraints (reduced ankle dorsiflexion and excessive plantar flexion).

Delayed responses due to afferent or efferent impairments?

One of the most prominent features of the braking synergy consisted of the early activations of the MGi and RFi on the step-down side and the TAc and BFc on the contralateral side. These activations were present well before touchdown and thus were not induced by afferent feedback due to contact with the floor, given that they are in other paradigms (i.e., when stepping on a compliant surface: Marigold and Patla 2005; or a collapsing surface: Nakazawa et al. 2004). Previously, it was argued that these prelanding activations are triggered by the absence of expected afferent feedback when passing the level of expected foot contact (Van der Linden et al. 2007). On the other hand, these responses require fast efferents as well. Based on the identified correlations with vibration sense and tactile sensation in the current study, the group I afferents are likely to play an important role in the onset of the braking reaction. Yet, the same holds true for the identified correlations with peroneal NCV and strength. In the neurophysiology literature on CMT 1A there is no consensus about the contribution of sensory and motor symptoms to disability in patients with polyneuropathy. Some attribute all the observed changes in reflexes to the sensory loss (Inglis et al. 1994; Mazzaro et al. 2005b), whereas others argue that the slowed and impaired efferent system is the principal cause of change (Nardone et al. 2000; Vinci and Perelli 2002). Strong evidence for a dominant role of the loss of sensory afferents as the source of reduced corrective reflexes was obtained in a study in cats treated with pyridoxine (Stapley et al. 2002). In these cats the efferent system is intact but there is selective destruction of large sensory afferents (mostly group Ia) in conjunction with delayed postural responses. Overall, this is in line with the present study. Although both sensory and motor symptoms were related to the delayed responses, there is evidence for a dominant involvement of the sensory system. A major argument is that the delay was similar for upper and lower leg muscles, yet proximal muscle strength was always intact in these patients. This rules out the possibility that a lack of muscle strength, due to a loss of efferent fibers, contributed to the delay in the response in these muscles.

Limitations

The kinematic patterns of both groups were largely comparable. Yet, a subgroup of the patients showed a substantial reduction in the passive dorsiflexion of the ankle (< −6°; n = 4). Theoretically, this group might have affected their EMG patterns. Therefore subgroup analysis was done for the onset of the EMG responses, the early braking reaction (UD–UL bins), and the postlanding activity (UD–ED bins). Occasionally, there were small differences between the subgroups, but these never extended beyond a duration of 10 ms. This was presumably caused by the fact that the responses triggered by the perturbation were much larger compared with the muscle activations during normal gait (see Fig. 3, UL vs. UD). Therefore it is unlikely that the reduced passive dorsiflexion strongly influenced the results.

Theoretically, the identified correlations in the CMT 1A group could have been caused by a general “disease severity,” including both sensory and motor losses—however, this was not the case. The current study included a group of patients that varied in the extent of their motor and sensory deficits. Accordingly, no associations were found between their sensory (tactile sensation, vibration sense) and motor impairments (peroneus strength: r = −0.05 and r = 0.48, ns), nor between their tactile sensation and vibration sense (r = 0.48, ns). A significant association between peroneus strength and dorsiflexion impairments was present (r = 0.87; P < 0.01), however, indicating that restrictions in the passive ankle range of motion often coincided with reduced distal muscle strength.

Implications for rehabilitation

This is the first study to prove that the responses to an unexpectedly lowered support surface are impaired in relatively mildly impaired CMT 1A patients. It is important to recall that the patients were able to walk unaided for ≥1 h and did not suffer from severe foot deformities (structural pes equinovarus). However, their response to a small change in surface height (5 cm) led to large balance disturbances. This finding is consistent with the results of DeMott et al. (2007) who showed that an uneven support surface is the most common cause of falls in polyneuropathy patients (79%). Slowed and reduced responses may be one of the causes of falls in these patients because they may not be able to respond adequately to such perturbations in daily life. From the present results it cannot be concluded whether the delay or the decreased responses contributed most to the deficient balance reaction in the CMT 1A patients. However, extrapolating from studies on elderly, the amplitude reduction presumably plays an important role (Pijnappels et al. 2008; Schillings et al. 2005). In conclusion, the present findings indicate that even mildly affected CMT 1A patients might experience substantial balance problems in daily life when faced with terrain that is lower than expected. Delayed and reduced corrective responses may be at the basis of these balance deficits.

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REFERENCES


