`

Differential effects of plantar cutaneous afferents excitation on soleus stretch and H-reflex

Authors:

Dimitry G. Sayenko[1], M.D., Ph.D., Albert H. Vette[2], Dipl.-Ing., Hiroki Obata[3], M.Sc., Maria I. Alekhina[3], M.Sc., Masami Akai[3], M.D., and Kimitaka Nakazawa[3], Ph.D.

Affiliations:

[1] Rehabilitation Engineering Laboratory, Toronto Rehabilitation Institute, Lyndhurst Centre, 520 Sutherland Drive, Toronto, Ontario M4G 3V9, Canada

[2] Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College Street, Toronto, Ontario M5S 3G9, Canada

[3] Department of Rehabilitation for Movement Functions, Research Institute of National Rehabilitation Center for Persons with Disabilities, 4-1 Namiki, Tokorozawa 359-8555, Japan

Corresponding author: Dimitry G. Sayenko

Phone: +1-416-597-3422 ext. 6213

Fax: +1-416-425-9923

E-mail: dimitry.sayenko@utoronto.ca

Rehabilitation Engineering Laboratory, Toronto Rehabilitation Institute, Lyndhurst Centre, 520 Sutherland Drive, Toronto, Ontario M4G 3V9, Canada

Running title:

Differential modulation of spinal reflexes

Abstract

Previous studies have demonstrated that plantar cutaneous afferents can adjust motoneuron excitability, which may contribute significantly to the control of human posture and locomotion. However, the role of plantar cutaneous afferents in modulating the excitability of stretch and H-reflex with respect to the location of their excitation remains unclear. In the present study, it was hypothesized that electrical stimulation delivered to the sole of the foot might be followed by a modulation of spinal excitability that depends on both 1) the stimulation location and 2) the reflex studied. In these experiments, conditioned and unconditioned stretch and H-reflexes were evoked in sixteen healthy subjects in a seated position. Both reflexes were conditioned by non-noxious electrical plantar cutaneous afferent stimulation at two different sites, i.e., the heel and metatarsal regions, and four different conditioning test (CT) intervals. The conditioning stimulation delivered to the heel caused a significant facilitation of the soleus stretch reflex for all CT intervals, whereas the soleus H-reflex experienced a significant facilitation solely at the CT interval of 50 ms and a significant inhibition at longer CT intervals. Stimulation delivered to the metatarsal region on the other hand resulted mainly in reduced stretch and H-reflex sizes. This study extends the reported findings on the contribution of plantar cutaneous afferents within spinal interneuron reflex circuits as a function of their location and the reflex studied.

Keywords

Motoneuron excitability, plantar cutaneous afferents, sensorimotor integration, soleus H-reflex, soleus stretch reflex.

Introduction

Considerable evidence has been brought forward that shows cutaneous afferents of the foot sole can adjust excitability of spinal motoneurons that innervate the muscles that act about the ankle joint[1,9,11]. Additionally, it has been revealed that cutaneous afferents play a significant role in the control of locomotion[45,46] and posture[24,27,28,34].

During locomotion, the particular contribution of plantar cutaneous afferents has become evident in multiple ways: for example, studies with spinalized and decerebrate cats indicated that cutaneous afferents can establish inhibitory and excitatory connections with spinal coordinating centers involved in the generation of flexion during walking[5]. As a result, cutaneous afferent activity was able to modify the limb trajectory during the swing phase of locomotion[10]. In humans, excitation of cutaneous afferents due to stimulation of nerves innervating the foot has been shown to result in cutaneous reflexes during locomotion that are 1) task-dependent (revealed by stimulation of sural nerve)[7,45,46], 2) phase-dependent (revealed by stimulation of tibial[31] or sural[8] nerves)[45,46], 3) context-dependent (revealed by stimulation of tibial or superficial peroneal nerves)[15,46], and 4) intensity-dependent (revealed by stimulation of tibial or sural nerves)[6,45]. During standing, disrupted plantar pressure sensation resulted in balance deficits[24,27,28], implying that cutaneous afferents might not only contribute to the control of locomotion, but also posture. This notion is supported by the fact that plantar cutaneous afferents have specific direction- and phase-dependent tasks in the control of compensatory stepping reactions after postural perturbations[28,34].

Besides this apparent contribution of cutaneous afferents, however, it has also been shown that the stretch reflex plays an important role in locomotion and posture control by increasing ankle stiffness during balance perturbations while walking[33] or standing[32]. Due to this proposed common involvement in postural control, we have recently investigated how electrical stimulation of plantar cutaneous afferents located at the heel affect the soleus stretch reflex[40]. This study revealed pronounced facilitation of the soleus stretch reflex for conditioning/test (CT) intervals from 30 to 70 ms. These results, however, seem to contradict numerous quantitative studies that indicate predominantly inhibitory effects of plantar cutaneous afferent stimulation on the soleus H-reflex[22,35]. One explanation for these opposite stimulation effects on the modulation of the soleus stretch[40] and H-reflex[22,35] could be that electrical stimulation delivered to cutaneous afferents may affect the motoneurons innervating the lower limb muscles dependent on the site of stimulation. For example, a study with a voluntary pre-contraction of the lower leg muscles has shown that, in soleus, a facilitated response was observed following stimulation of the heel region, but it turned into a decreased response following stimulation of the metatarsal region[30]. As such, this study might shed light on the previously mentioned findings, which imply that stimulation of plantar cutaneous afferents located at the *metatarsal region* of the foot[22,35] might cause inhibition of the soleus H-reflex, whereas that delivered to the *heel*[40] might result in a facilitation of the soleus stretch reflex.

Additionally, one should consider organizational differences between the stretch and H-reflex. On the one hand, the stretch reflex is elicited through activation of muscle spindle primary endings, whose sensitivity is controlled by γ-efferents. The H-reflex, on the other hand, results from electrical stimulation of Ia afferents and is characterized by a better synchronization and shorter duration of the afferent Ia volleys in comparison with the stretch reflex[2,36]. Accordingly, Morita *et al.*[29] proposed a different sensitivity of the stretch and H-reflexes to presynaptic inhibition following a conditioning stimulation. Such explanation would also agree with the findings that 1) the soleus H-reflex was *reduced* in standing compared to seated or prone subjects[16,19,23] and 2) the stretch reflex was *facilitated* during standing[32] and the stance phase of walking[3,41].

As a consequence, the present study was undertaken to clarify the role of plantar cutaneous afferents in modulating the soleus stretch and H-reflexes when using the same characteristics for the conditioning stimulation. Taking advantage of the aforementioned findings on the topographic organization of cutaneous reflexes, it was hypothesized that electrical stimulation delivered to the sole of the foot might be followed by a modulation of spinal excitability that is dependent on the stimulation location and the reflex studied. In particular, the purpose of the present study was to investigate and compare the effect of electrical stimulation of plantar cutaneous afferents located at the heel and the metatarsal region on the excitability of the two reflexes. Such a study identifying the effects of inputs from cutaneous afferents of the foot sole on spinal interneuron circuits might be fundamental for understanding the contribution of each reflex mechanism to the control of gait and posture, especially during unexpected perturbations.

Methods

Subjects

Experiments were conducted in sixteen healthy subjects (nine male, seven female) between the ages of 21 and 31 years (Mean ± SD: 25.8 ± 3.1) and the heights of 154 and 182 cm (170.3 ± 8.2). None of the subjects had any known history of neurological disorders. Each subject gave written informed consent to the experimental procedure, which was approved by the local ethics committee in accordance with the declaration of Helsinki on the use of human subjects in experiments.

During the experiments, the participants were seated in an adjustable chair with the right foot firmly strapped to a foot plate. The positions of the hip and knee joints were set to 120º and 160º of flexion, respectively, and that of the ankle joints to neutral position (0º dorsi-/plantar-flexion).

Elicitation of soleus stretch reflex

The axis of rotation of the ankle joint was aligned with the axis of rotation of the foot plate. Soleus stretch reflexes of the right leg were evoked by rotating the foot plate in the dorsiflexion direction by a custom-made servo-controlled torque motor (Senoh Inc., Tokyo, Japan). The perturbations were applied to the ankle joint at an angular velocity of approximately 200º s^{-1}, which resulted in joint rotations of 10º dorsiflexion[13,20]. The time between two successive perturbations was randomized between five and eight seconds[37,47]. For each test condition, i.e., for the control reflexes and those conditioned at different CT intervals, ten soleus stretch reflex responses were evoked. Note that the reflexes were elicited randomly across the different CT intervals and controls.

Elicitation of soleus H-reflex

The tibial nerve was stimulated by a monopolar stainless-steel electrode with a diameter of 1 cm (cathode), placed on the skin above the posterior tibial nerve at the right popliteal fossa. The anode, a 35-cm^2 copper plate electrode, was placed above the patella. The optimal location for the stimulating electrode was identified as the one for which the lowest stimulus intensity was needed to evoke a threshold soleus H-reflex without a preceding M-wave. Having established this site, a neoprene knee brace was used to hold the two electrodes in place under constant pressure.

The H-reflex was evoked by electrical stimulation (1 ms pulse width) using a constant voltage stimulator (DPS-1300D, Dia Medical System Co., Tokyo, Japan). For normalization of the H-reflex, the amplitude of the soleus maximal M-wave (M_{max}) after supramaximal stimulation of the posterior tibial nerve was calculated by averaging the five highest peak-to-peak M-waves. Two separate intensities were applied for H-reflex elicitation: the first one was chosen such that the peak-to-peak amplitude of the control H-reflex of each subject was similar in magnitude to the control stretch reflex obtained during the stretch reflex session of that particular subject (*stretch reflex calibrated*). Note that, due to the generally small magnitude of the stretch reflex (below 10 % of M_{max}), the H-reflex obtained under these conditions was not preceded by an M-wave in five of the sixteen subjects. In the other eleven subjects, the M-wave was continuously monitored over this part of the experiment to ensure the stimulus constancy. The second stimulation intensity for the H-reflex was chosen to generate a control reflex with an amplitude equivalent to 20 to 30 % of M_{max} (*M-wave calibrated*). With this stimulus intensity, the M-wave occurred in all trials of all subjects and was continuously monitored to ensure that the same Ia afferents were excited during stimulation. The time between two successive H-reflex elicitations was randomized between five and eight seconds[37,47]. For each test condition, i.e., for the control reflexes and those conditioned at different CT intervals, twenty soleus H-reflex responses were evoked (ten for each H-reflex elicitation intensity). Note that the reflexes were elicited randomly across the different CT intervals and controls.

Conditioning stimulation of plantar cutaneous afferents

The soleus stretch and H-reflexes were conditioned by non-noxious plantar skin stimulation. Two pairs of self-adhesive, disposable surface electrodes (2.5 × 4.5 cm, Vitrode W, Nihon Kohden) were placed over two different stimulation sites, i.e., the surface of the right heel and of the metatarsal region. Specifically, during heel stimulation the anode was located over the medial side-surface of the heel, and the cathode was placed laterally to the anode over the stance-surface of the heel. During metatarsal stimulation, the electrodes were placed transversely across the first and third metatarsals with the anode located proximally and the cathode distally (Fig. 1).

Using a constant voltage stimulator (DPS-1300D, Dia Medical System Co., Tokyo, Japan), the perceptual threshold (PT) that corresponded to the stimulus intensity first perceived by the subject was established. All conditioning stimuli were equivalent to three times PT[21,22]. For pulse trains at this stimulation intensity, no movement of the foot muscles was elicited, and no pain was reported. The subjects experienced a short strong parasthesia at the site of stimulation that did not radiate to surrounding sites. In addition, it was confirmed that the non-noxious stimulation had no influence on the background electromyogram (EMG) activity of the soleus and tibialis anterior muscles. The conditioning pulse train, which consisted of five 1 ms-pulses and four inter-stimuli intervals of 3 ms each, had a duration of 17 ms and preceded the test reflexes (soleus stretch and H-reflexes) at different CT intervals. The CT intervals were measured as the time between the end of the conditioning pulse train and the onset of the ankle rotation (in case of stretch reflex) or the single pulse delivered to the posterior tibial nerve (in case of H-reflex). The different latencies of the stretch and H-reflexes were taken into account such that all reflexes had the same latency in relation to the conditioning stimulation. In the case of the soleus H-reflex, the CT intervals ranged from 25 to 100 ms (25, 50, 75, and 100 ms), and in the case of the stretch reflex, the CT intervals were delayed by 10 ms in relation to the H-reflex conditioning stimulation (15, 40, 65, 90 ms, respectively). The CT intervals were delivered randomly, and the experimental part with heel stimulation was executed prior to the part with stimulation of the metatarsal region.

EMG recording and data collection

Surface EMG signals were recorded via bipolar surface electrodes (Ag-AgCl, diameter of 7 mm, Vitrode F, Nihon Kohden) that were placed longitudinally on the soleus muscle on one half of the distance between the mid-popliteal crease and the medial malleolus[47], with an inter-electrode distance of 20 mm after cleansing and light mechanical exfoliation of the skin. For the reference electrode, a belt-type surface electrode with a width of 20 mm was used (Ag-AgCl, Shimizu Electronic Ind., Niigata, Japan), which was wrapped around the right shin at the tibial tuberosity level. The EMG signals were amplified by 1k and band-pass filtered (15 Hz to 3 kHz) with a conventional bioamplifier (AB-651J, Nihon

Kohden, Tokyo, Japan). Finally, the EMG data of the right soleus as well as the change in angle of the right ankle joint during the stretch reflex session were digitized at a sampling rate of 10 kHz.

Data processing

The digitized EMG time series were full-wave rectified after subtraction of the mean background EMG. In accordance with previous studies on the quiescent soleus[29,44], only the short-latency component of the stretch reflex, i.e., the M1 response was identified. Both M1 and the H-reflex responses were analyzed by calculating the area under the curve of the full-wave rectified time series. The onset of the M1 response was defined as the moment when the EMG activity reached levels higher than the mean background EMG plus two times its standard deviation. For the M1 duration of each subject, a constant interval of 35 ms was used. H-reflex responses were processed for the time interval of 25 to 60 ms after tibial nerve stimulation.

Statistical analysis

The areas of the conditioned reflex responses (M1 and H-reflex) recorded for each stimulation site and CT interval were expressed as percentages of the mean area of respective control reflexes. Then, a one-way ANOVA with repeated measures ($\alpha = 0.05$ and $\alpha = 0.01$) along with a subsequent Bonferroni test was applied to the individual and pool data to identify significant differences in the magnitude of the conditioned reflexes across the CT intervals investigated. The results for the pool data are presented as mean values and standard errors of the means (SEM).

Results

Stretch reflex

In the left panels of Fig. 2, the effects of plantar cutaneous afferent stimulation delivered to the heel at the two shortest CT intervals on the soleus stretch reflex are illustrated for one subject. The plots exemplify the average stretch reflex (*n* = 10) evoked at an angular velocity of 200º s^{-1} under control conditions (bold black line) and during reflex conditioning (bold gray line) for CT intervals of 40 ms (Fig. 2a) and 15 ms (Fig. 2b). It can be seen that the conditioning stimulation resulted in significant facilitation of the soleus stretch reflex for the presented CT intervals. The most pronounced facilitation of the conditioned stretch reflex occurred at the CT interval of 40 ms. It exceeded the magnitude of the subject's control reflex by more than three times (Fig. 2a). The waveform of the conditioned reflexes was characterized by an earlier onset as well as a steep reflex augmentation in comparison to the control reflex. In Fig. 2c, all control and conditioned stretch reflexes from Fig. 2a and Fig. 2b are shown in relationship to the stimulation artifacts recorded in the soleus background EMG (total of 30 trials). It can be clearly seen that the conditioning stimulation was delivered consistently for both CT intervals.

The right panels of Fig. 2 depict the effects of plantar cutaneous afferent stimulation delivered to the metatarsal region on the soleus stretch reflex for the same subject and the same CT intervals. At the CT intervals of 40 ms (Fig. 2d) and 15 ms (Fig. 2e), the magnitudes of the conditioned stretch reflex were significantly reduced in comparison to the respective control reflex, with the most pronounced inhibition occurring at the CT interval of 15 ms (Fig. 2e). In Fig. 2f, all control and conditioned stretch reflexes from Fig. 2d and Fig. 2e are shown in relationship to the stimulation artifacts recorded in the soleus background EMG (total of 30 trials). The plots indicate that the conditioning stimulation was delivered consistently for both CT intervals.

In Fig. 3, the pool data of the stretch reflex size is illustrated for all subjects after plantar cutaneous afferents excitation delivered to the heel (Fig. 3a) and the metatarsal region (Fig. 3b). Note that the reflexes are presented for all CT intervals and as percentages of the average control reflexes. Asterisks indicate statistically significant differences between the control and the conditioned reflex sizes as identified with the post-hoc Bonferroni test (* P<0.05; ** P<0.01). The CT dependency of the stretch reflex facilitation during cutaneous stimulation of the heel was observed consistently across all subjects and can easily be recognized in Fig. 3a. The changes in magnitude of the soleus stretch reflex were quite prominent for all CT intervals tested (15 to 90 ms): The significant reflex increases for the CT intervals of 15, 40, 65, and 90 ms reached 149 ± 12 %, 159 ± 14 %, 139 ± 11 %, and 121 ± 9 % of the control value, respectively.

During stimulation of plantar cutaneous afferents delivered to the metatarsal region at the shorter CT intervals (15 and 40 ms), the stretch reflex was significantly decreased. It reached a maximal inhibition of 79 ± 5 % (CT = 15 ms). At longer CT intervals of 65 and 90 ms, the stretch reflexes were not statistically different and had sizes of 105 ± 4 % and 110 ± 6 % of the control value, respectively, (Fig. 3b).

H-reflex

Somewhat different effects of plantar cutaneous afferent excitation delivered to the heel on the stretch reflex calibrated H-reflex can be seen in the left panels of Fig. 4 (same subject as in Fig. 2). The changes in the conditioned H-reflex magnitude in all trials occurred without significant changes in the M-wave (see Fig. 5, *lower panels*), indicating stable stimulation and recording conditions. Stimulation at a CT interval of 50 ms provoked a strongly pronounced rise in the H-reflex, reaching more than twice the magnitude of the subject's control reflex (Fig. 4a). At the shortest CT interval of 25 ms, excitation of plantar cutaneous afferents of the heel did not significantly affect the soleus H-reflex (Fig. 4b). In Fig. 4c, all control and conditioned H-reflexes from Fig. 4a and Fig. 4b are shown in relation to the stimulation artifacts recorded in the soleus background EMG (total of 30 trials). It can be seen clearly that the conditioning stimulation was delivered consistently for both CT intervals.

The right panels of Fig. 4 depict the effects of plantar cutaneous afferent stimulation delivered to the metatarsal region on the soleus H-reflex for the same subject as in Fig. 2. The reflex modulation observed for the soleus H-reflex was similar to the one obtained for the soleus stretch reflex after cutaneous afferent stimulation of the metatarsal region (see Fig. 2, *right panels*). Accordingly, the magnitude of the conditioned H-reflex was significantly reduced at the CT interval of 25 ms (Fig. 4e). In Fig. 4f, all control and conditioned H-reflexes from Fig. 4d and Fig. 4e are shown in relation to the stimulation artifacts recorded in the soleus background EMG (total of 30 trials). The plots indicate that the conditioning stimulation was delivered consistently for both CT intervals.

In the upper panels of Fig. 5, the pool data of the size of the stretch reflex calibrated H-reflex is shown for all subjects after plantar cutaneous afferent excitation delivered to the heel (Fig. 5a, white bars) and the metatarsal region (Fig. 5b, white bars).

Note that the reflexes are presented for all CT intervals and as percentages of the average control reflexes. Asterisks indicate statistically significant differences between the control and the conditioned reflex sizes as identified with the post-hoc Bonferroni test (* $P<0.05$; ** $P<0.01$). The changes in magnitude of the conditioned H-reflex following cutaneous stimulation of the heel area were characterized by an inconsistent modulation pattern (Fig. 5a, white bars). At the longer CT intervals of 75 and 100 ms, the H-reflex was significantly lower, reaching 85 ± 4 %, and 80 ± 6 % of the control value, respectively. However, at a CT interval of 50 ms, the conditioned H-reflex was characterized by significant facilitation, reaching 154 ± 13 % of the control value (P = 0.002). There was no observable change in reflex magnitude during the conditioning stimulation delivered at the shortest CT interval of 25 ms. During the stimulation delivered to the metatarsal region, the H-reflex was significantly decreased at all but the longest CT interval, reaching a maximal inhibition of 65 ± 4 % at the CT interval of 25 ms (Fig. 5b, white bars). The changes in the conditioned H-reflex magnitude in all trials occurred without significant change in the M-wave (Fig. 5, *lower panels*, white circles).

H-reflex conditioning with alternative elicitation level

In the case where the stimulus intensity for the H-reflex elicitation was set to induce control reflexes of 20 to 30 % of M_{max} (M-wave calibrated), very similar H-reflex modulation patterns were observed as for the stretch reflex calibrated H-reflex conditioning (Fig. 5a and 5b, gray bars). For the CT interval of 50 ms, cutaneous stimulation of the heel caused a pronounced facilitation of the H-reflex, reaching 141 ± 8 % of the control value ($P = 0.004$), whereas the stimulus delivery at longer CT intervals resulted in a decrease of the H-reflex magnitude (Fig. 5a, gray bars). Cutaneous stimulation delivered to the metatarsal region at CT intervals of 25 and 50 ms caused significant inhibition of the H-reflex, reaching 79 ± 4 % and 92 ± 2 % of the control value, respectively (Fig. 5b, gray bars). The changes in the conditioned H-reflex magnitude in all trials occurred without significant change in the M-wave (Fig. 5, *lower panels*, gray boxes). It has to be emphasized that the observed consistency in results for the two different H-reflex elicitation methods provides strong evidence for the validity of the applied H-reflex conditioning procedure.

Discussion

This study was performed to investigate the effects of cutaneous stimulation delivered to the heel and metatarsal regions of the foot on the soleus stretch and H-reflexes. In summary, two main results were found: First, electrical stimulation of different sites of the sole of the foot was followed by facilitation or inhibition of the reflexes dependent on the site of stimulation and conditioning test interval, thus demonstrating a location- and conditioning-dependent effect of cutaneous stimulation on spinal reflex pathways. Second, the conditioning cutaneous stimulation delivered to the heel resulted in different modulation patterns of the soleus stretch and H-reflexes. In what follows, these outcomes as well as the mechanisms that might be responsible for the observed phenomena will be discussed in more detail.

Location-dependent effect of cutaneous stimulation on spinal reflex pathways

Facilitation of the soleus motor output following excitation of plantar cutaneous afferents around the heel seems plausible, as plantar flexion is needed to raise the heel off a painful stimulus. Although the stimulus intensity used in our study was not high enough to result in either a withdrawal reflex or even a noticeable change of the background EMG activity in the soleus, we can assume that electrical excitation of cutaneous afferents around the heel could result in facilitation of the soleus reflexes through connections with afferent withdrawal reflex pathways. Note that such a mechanism agrees with the notion that cutaneous mechanoreceptors can adjust excitability of both flexor and extensor motoneurons through 'alternative reflex pathways'[25,43]. Our assumption is supported by the most pronounced facilitation of the soleus stretch and H-reflexes occurring at CT intervals of 40 and 50 ms, respectively. This implies that the time between the end of the conditioning pulse train delivered to the sole of the foot and the onset of the soleus stretch or H-reflex (CT + reflex latency ≈ 80 ms) agrees with the delay of the middle-latency cutaneous withdrawal reflex (≈ 70-110 ms). As such, an action potential generated due to muscle stretch (in the case of the stretch reflex) or electrical stimulation of the posterior tibial nerve (in the case of the H-reflex) arrives at the soleus motoneurons at a time when the motoneuron pool is being activated by the conditioning stimulation. Consequently, this superposition might result in recruitment of additional, larger (high-threshold) motoneurons, causing augmentation of the overall response[4,17]. Note that this explanation agrees with previous studies performed in cats that showing that low-intensity electrical stimulation activates large spindle afferents of motor nerves, thereby generating contraction of the muscle through the monosynaptic stretch reflex pathway[12,14].

The opposite effect occurred in the soleus stretch and H-reflexes following the excitation of cutaneous afferents located at the metatarsal region. Again, in the view of the location specificity of plantar cutaneous excitation, this outcome seems logical, as an inhibition of the ankle extensor activity supports the dorsiflexors' goal of lifting the forefoot off a painful stimulus. Furthermore, this effect agrees with previous findings that demonstrated inhibitory effects of metatarsal region stimulation on the soleus motor output[22,35].

Besides the assumed connections with withdrawal reflex pathways, additional potential mechanisms that could contribute to modulation of the soleus stretch and H-reflexes following excitation of plantar cutaneous afferents include changes in the amount of both pre- and postsynaptic inhibition[18,22,35,38,39]. It has been shown, for example, that 1) cutaneous afferents modulate α-motoneuron excitability by affecting the amount of presynaptic inhibition acting on soleus Ia afferents[18], 2) flexion reflex afferents induce presynaptic inhibition of Ia afferent terminals[38], and 3) ipsilateral cutaneous stimulation increases reciprocal inhibition of soleus α-motoneurons[39]. Nonetheless, in light of the current experimental protocol, it is difficult to fully ascribe particular roles to pre- and postsynaptic mechanisms.

Our results on the location-dependent effect of cutaneous stimulation on spinal reflex pathways are, in fact, in agreement with previous studies that suggested a location-specific organization of cutaneous reflexes of the plantar foot[30,42]. At the same time, although the CT dependency during the stimulation of the metatarsal region was very similar for the stretch and H-reflexes (Fig. 3b and 5b), a comparable pattern of stretch and H-reflex modulation during stimulation of the heel occurred only for one scenario (Fig. 3a and 5a): the facilitation of the stretch and H-reflexes reached its maximum at CT intervals of 40 and 50 ms, respectively. The reflex modulation patterns due to the conditioning stimulation delivered at the other CT intervals, however, were somewhat different.

Difference in soleus stretch and H-reflex modulation during cutaneous heel stimulation

While facilitation of the soleus stretch reflex was quite prominent across CT intervals from 15 to 90 ms (Fig. 3a), the soleus H-reflex did not change significantly at the CT interval of 25 ms and was even reduced at the CT intervals of 75 and 100 ms (Fig. 5a). In the context of the topographic reflex mapping, this finding confirms the reported facilitative effect of the plantar cutaneous afferent excitation at the heel onto the soleus stretch reflex[40] and the reported inhibitory effect of the same conditioning stimulation on the soleus H-reflex[26]. Note that this remarkable difference, which suggests that different mechanisms are responsible for modulation of the soleus stretch and H-reflexes following a conditioning stimulation, might be explained by different temporal dispersion of the afferent volleys evoked by mechanical and electrical stimuli[29].

Following muscle stretch, the Ia afferent volleys are considerably dispersed in time with respect to their arrival at the spinal cord[2]. As such, the conditioning stimulation delivered at all CT intervals would continually pre-activate a certain number of additional motoneurons that are recruited in the conditioned response. This also explains the observed earlier onset of the stretch reflex conditioned at the CT interval of 40 ms (Fig. 2a). On the contrary, electrical nerve stimulation activates the Ia afferents almost simultaneously and therefore elicits a volley that is only slightly dispersed in time with respect to its arrival at the spinal cord[2]. Consequently, significant facilitation of the soleus H-reflex at a CT interval of 50 ms could be explained by a particular time-matching of the afferent volley that *initiates* the H-reflex itself and the arrival of the afferent inputs that *condition* the H-reflex. Depression of the H-reflex for conditioning stimulation delivered at longer CT intervals (namely, of 75 and 100 ms) might on the other hand result from depression of transmission in the Ia afferent synapses caused by the conditioning stimuli[29].

Conclusions

The present work extends reported findings on the contribution of plantar cutaneous afferents within the spinal interneuron reflex circuits. Although the present results agree with most of the previous findings, we demonstrated that electrical stimulation delivered to the sole of the foot might be followed by modulation of spinal excitability dependent not only on the stimulation location, but also on the reflex studied. Using corresponding protocols for the conditioning stimulation, the present study revealed a significant difference in modulation of the soleus stretch and H-reflexes following the conditioning cutaneous stimulation delivered to the heel. Note that this difference can be explained by different temporal dispersion of the afferent volleys evoked by mechanical and electrical stimuli when they are conditioned by electrical stimulation of the heel region. Further research with different postural conditions is needed to explicate the role of plantar cutaneous afferents in the modulation of spinal excitability during gait and posture.

Acknowledgements

The authors thank Dr. Hirofumi Sekiguchi for assistance in data processing and Dr. Daichi Nozaki, José Zariffa, and Egor Sanin for reviewing the manuscript. The first author would like to thank Prof. I. Kozlovskaya for her academic guidance in the past. This study was supported by the grant and fellowship program of the Japan Society for the Promotion of Science.

Abbreviations

ANOVA	analysis of variance
CT intervals	conditioning test intervals
EMG	electromyogram
M_{max}	maximal M-wave
SD	standard deviation
SEM	standard errors of the means

References

1. Aniss AM, Gandevia SC, Burke D. Reflex responses in active muscles elicited by stimulation of low-threshold afferents from the human foot. J Neurophysiol 1992; 67:1375-1384.
2. Burke D, Gandevia SC, McKeon B. The afferent volleys responsible for spinal proprioceptive reflexes in man. J Physiol 1983; 339:535-552.
3. Christensen LO, Petersen N, Andersen JB, Sinkjaer T, Nielsen JB. Evidence for transcortical reflex pathways in the lower limb of man. Prog Neurobiol 2000; 62:251-272.
4. Crone C, Hultborn H, Mazieres L, Morin C, Nielsen J, Pierrot-Deseilligny E. Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a study in man and the cat. Exp Brain Res 1990; 81:35-45.
5. Duysens J. Reflex control of locomotion as revealed by stimulation of cutaneous afferents in spontaneously walking premammillary cats. J Neurophysiol 1977; 40:737-751.
6. Duysens J, Trippel M, Horstmann GA, Dietz V. Gating and reversal of reflexes in ankle muscles during human walking. Exp Brain Res 1990; 82:351-8.
7. Duysens J, Tax AA, Trippel M, Dietz V. Increased amplitude of cutaneous reflexes during human running as compared to standing Brain Res. 1993; 613:230-8.
8. Duysens J, Bastiaanse CM, Smits-Engelsman BC, Dietz V. Gait acts as a gate for reflexes from the foot. Can J Physiol Pharmacol 2004; 82:715-722.

9. Fallon JB, Bent LR, McNulty PA, Macefield VG. Evidence for strong synaptic coupling between single tactile afferents from the sole of the foot and motoneurons supplying leg muscles. J Neurophysiol 2005; 94:3795-3804.

10. Forssberg H. Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. J Neurophysiol 1979; 42:936-953.

11. Gibbs J, Harrison LM, Stephens JA. Cutaneomuscular reflexes recorded from the lower limb in man during different tasks. J Physiol 1995; 487:237-242.

12. Gordon J. Spinal mechanisms of motor coordination. In: Kandel ER, Schwartz JH, and Jessell TM, editors. Principles of neural science, 3rd edition Norwalk. CT: Appleton & Lange; 1991. p 5-17.

13. Grey MJ, Klinge K, Crone C, Lorentzen J, Biering-Sørensen F, Ravnborg M, Nielsen JB. Post-activation depression of Soleus stretch reflexes in healthy and spastic humans. Exp Brain Res 2008;185(2):189-197.

14. Hagbarth KE. Excitatory and inhibitory skin areas for flexor and extensor motoneurons. Acta Physiol Scand Suppl 1952; 26:1-58.

15. Haridas C, Zehr EP, Misiaszek JE. Postural uncertainty leads to dynamic control of cutaneous reflexes from the foot during human walking. Brain Res 2005; 1062:48-62.

16. Hayashi R, Tako K, Tokuda T, Yanagisawa N. Comparison of amplitude of human soleus H-reflex during sitting and standing. Neurosci Res 1992; 13:227-233.

17. Hennemann E, Mendell LM. Functional organization of motoneuron pool and its inputs. In: Brooks VB, editor. Handbook of physiology, Section 1. The nervous system. Bethesda: American Physiological Society; 1981. Vol. 2, p 423-507.

18. Iles JF. Evidence for cutaneous and corticospinal modulation of presynaptic inhibition of Ia afferents from the human lower limb. J Physiol 1996; 491:197-207.

19. Katz R, Meunier S, Pierrot-Deseilligny E. Changes in presynaptic inhibition of Ia fibers in man while standing. Brain 1988; 111:417-437.

20. Kawashima N, Nakazawa K, Yamamoto SI, Nozaki D, Akai M, Yano H. Stretch reflex excitability of the anti-gravity ankle extensor muscle in elderly humans. Acta Physiol Scand 2004;180:99-105.

21. Knikou M, Rymer Z. Effects of changes in hip joint angle on H-reflex excitability in humans. Exp Brain Res 2002;143:149-59.

22. Knikou M. Plantar cutaneous input modulates differently spinal reflexes in subjects with intact and injured spinal cord. Spinal Cord 2007; 45:69-77.

23. Koceja DM, Trimble MH, Earles DR. Inhibition of the soleus H-reflex in standing man. Brain Res 1993; 629:155-158.

24. Kozlovskaya I, Dmitrieva I, Grigorieva L, Kirenskaya A, Kreidich Y. Gravitational mechanisms in the motor system. Studies in real and simulated weightlessness. In: Gurfinkel VS, Ioffe ME, Massion J, Roll JP, editors. Stance and motion: facts and concepts. Plenum Press, New York; 1988. p 37-48.

25. Lundberg A, Malmgren K, Schomburg ED. Reflex pathways from group II muscle afferents. 3. Secondary spindle afferents and the FRA: a new hypothesis. Exp Brain Res 1987; 65:294-306.

26. Marque P, Nicolas G, Marchand-Pauvert V, Gautier J, Simonetta-Moreau M, Pierrot-Deseilligny E. Group I projections from intrinsic foot muscles to motoneurones of leg and thigh muscles in humans. J Physiol 2001; 536:313-327.

27. Meyer PF, Oddsson LIE, De Luca CJ. The role of plantar cutaneous sensation in unperturbed stance. Exp Brain Res 2004; 156:505-512.

28. Meyer PF, Oddsson LIE, De Luca CJ. Reduced plantar sensitivity alters postural responses to lateral perturbations of balance. Exp Brain Res 2004; 157:526-536.

29. Morita H, Petersen N, Christensen LO, Sinkjaer T, Nielsen J. Sensitivity of H-reflexes and stretch reflexes to presynaptic inhibition in humans. J Neurophysiol 1998; 80:610-620.

30. Nakajima T, Sakamoto M, Tazoe T, Endoh T, Komiyama T. Location specificity of plantar cutaneous reflexes involving lower limb muscles in humans. Exp Brain Res 2006; 175:514-525.

31. Nakajima T, Kamibayashi K, Takahashi M, Komiyama T, Nakazawa K. Phase-dependent modulation of cutaneous reflexes in tibialis anterior muscle during passive stepping. Neurol Res 2008; 30:46-51.

32. Nakazawa K, Kawashima N, Obata H, Yamanaka K, Nozaki D, Akai M. Facilitation of both stretch reflex and corticospinal pathways of the tibialis anterior muscle during standing in humans. Neurosci Lett 2003; 338:53-56.

33. Nakazawa K, Kawashima N, Akai M, Yano H. On the reflex coactivation of ankle flexor and extensor muscles induced by a sudden drop of support surface during walking in humans. J Appl Physiol 2004; 96:604-611.

34. Perry SD, McIlroy WE, Maki BE. The role of plantar cutaneous mechanoreceptors in the control of compensatory stepping reactions evoked by unpredictable, multi-directional perturbation. Brain Res 2000; 877:401-406.

35. Pierrot-Deseilligny E, Bussel B, Sideri G, Cathala HP, Castaigne P. Effect of voluntary contraction on H reflex changes induced by cutaneous stimulation in normal man. Electroencephalogr Clin Neurophysiol 1973; 34:185-92.

36. Pierrot-Deseilligny E, Mazevet D. The monosynaptic reflex: a tool to investigate motor control in humans. Interest and limits. Neurophysiol Clin 2000; 30:67-80.

37. Pierrot-Deseilligny E, Burke D. General methodology. In: The Circuitry of the Human Spinal Cord: Its Role in Motor Control and Movement Disorders. Cambridge University Press; 2005. p 1-62.

38. Roby-Brami A, Bussel B. Effects of flexor reflex afferent stimulation on the soleus H-reflex in patients with a complete spinal cord lesion: evidence for presynaptic inhibition of Ia transmission. Exp Brain Res 1990; 81:593-601.

39. Rossi A, Mazzocchio R. Cutaneous control of group I pathways from ankle flexors to extensors in man. Exp Brain Res 1988; 73:8-14.

40. Sayenko DG, Vette AH, Kamibayashi K, Nakajima T, Akai M, Nakazawa K. Facilitation of the soleus stretch reflex induced by electrical excitation of plantar cutaneous afferents located around the heel. Neurosci Lett 2007; 415:294-298.

41. Sinkjaer T, Andersen JB, Nielsen JF, Hansen HJ. Soleus long-latency stretch reflexes during walking in healthy and spastic humans. Clin Neurophysiol 1999; 110:951-959.

42. Sonnenborg FA, Andersen OK, Arendt-Nielsen L. Modular organization of excitatory and inhibitory reflex receptive fields elicited by electrical stimulation of the foot sole in man. Clin Neurophysiol 2000; 111:2160-2169.

43. Steffens H, Schomburg ED. Convergence in segmental reflex pathways from nociceptive and non-nociceptive afferents to alpha-motoneurones in the cat. J Physiol 1993; 466:191-211.

44. Toft E, Sinkjaer T, Andreassen S, Larsen K. Mechanical and electromyographic responses to stretch of the human ankle extensors. J Neurophysiol 1991; 65:1402-1410.

45. Zehr EP, Komiyama T, Stein RB. Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. J Neurophysiol. 1997; 77:3311-25.

46. Zehr EP, Stein RB. What functions do reflexes serve during human locomotion? Prog Neurobiol 1999; 58:185-205.

47. Zehr PE. Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 2002; 86:455-68.

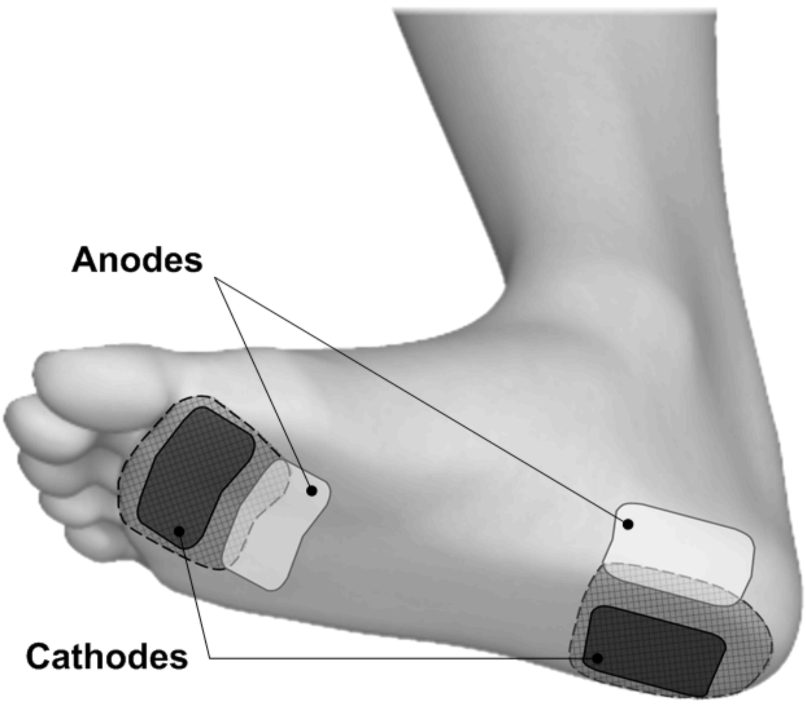

Figure 1. Location of cathodes (black) and anodes (white) for conditioning stimulation of plantar cutaneous afferents. Shaded areas at the heel and the metatarsal region indicate zones of prevalent sensation during the stimulation.

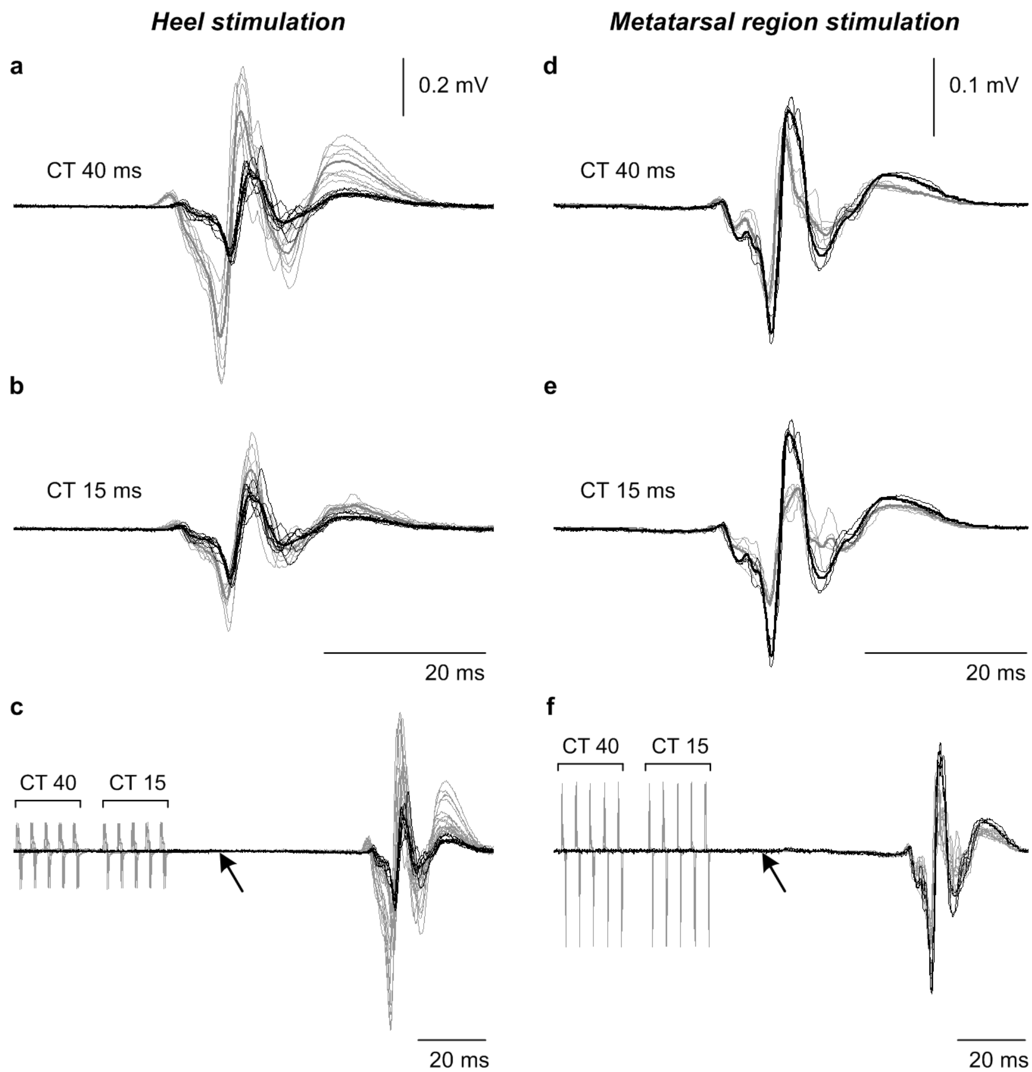

Figure 2. Raw soleus stretch reflexes obtained for one subject under control conditions (black lines) and during reflex conditioning (gray lines) via plantar cutaneous afferent excitation delivered to the heel (*left panels*) and the metatarsal region (*right panels*). The thin lines indicate individual trials, whereas the bold lines indicate the average of 10 trials under control conditions (black) and during reflex conditioning (gray). Shown are different CT intervals of 40 ms (a,d) and 15 ms (b,e). Fig. 2c and Fig. 2f show the evoked reflexes in relationship to the stimulation artifacts recorded in the background EMG, with the arrow indicating the onset of the ankle rotation.

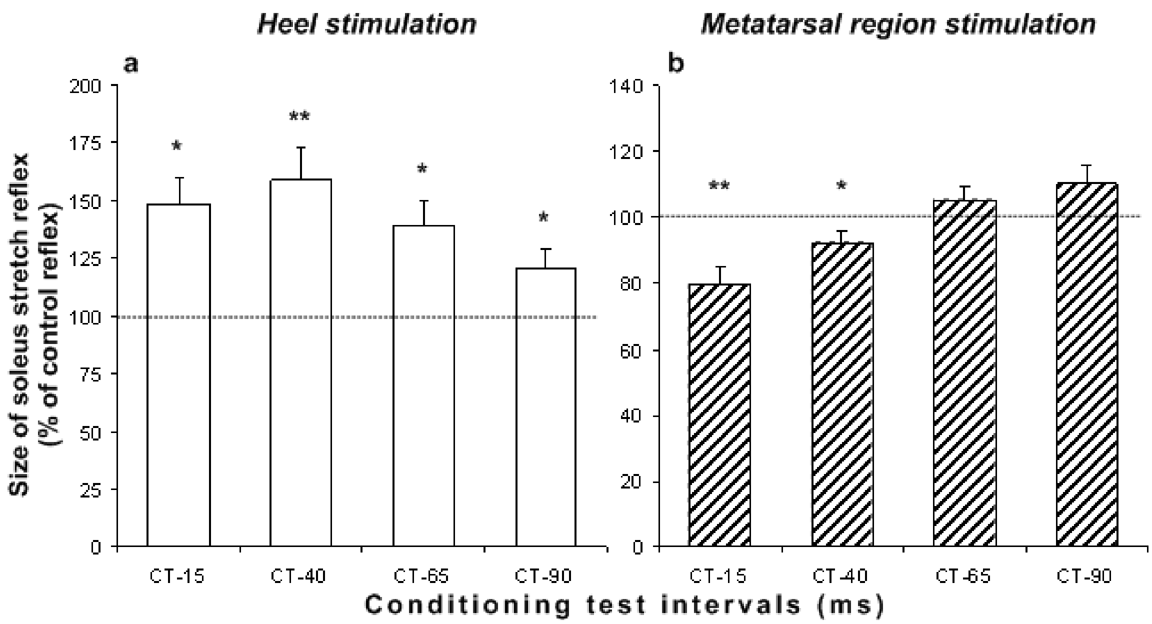

Figure 3. Pool data showing the effect of plantar cutaneous afferent excitation delivered to (a) the heel and (b) the metatarsal region at different CT intervals on the soleus stretch reflex. For each CT interval tested, the overall average size of the conditioned soleus stretch reflex is presented as a percentage of the overall average control size (mean ± SEM). Asterisks indicate statistically significant differences between the control and the conditioned reflex sizes (* $P < 0.05$; ** $P < 0.01$).

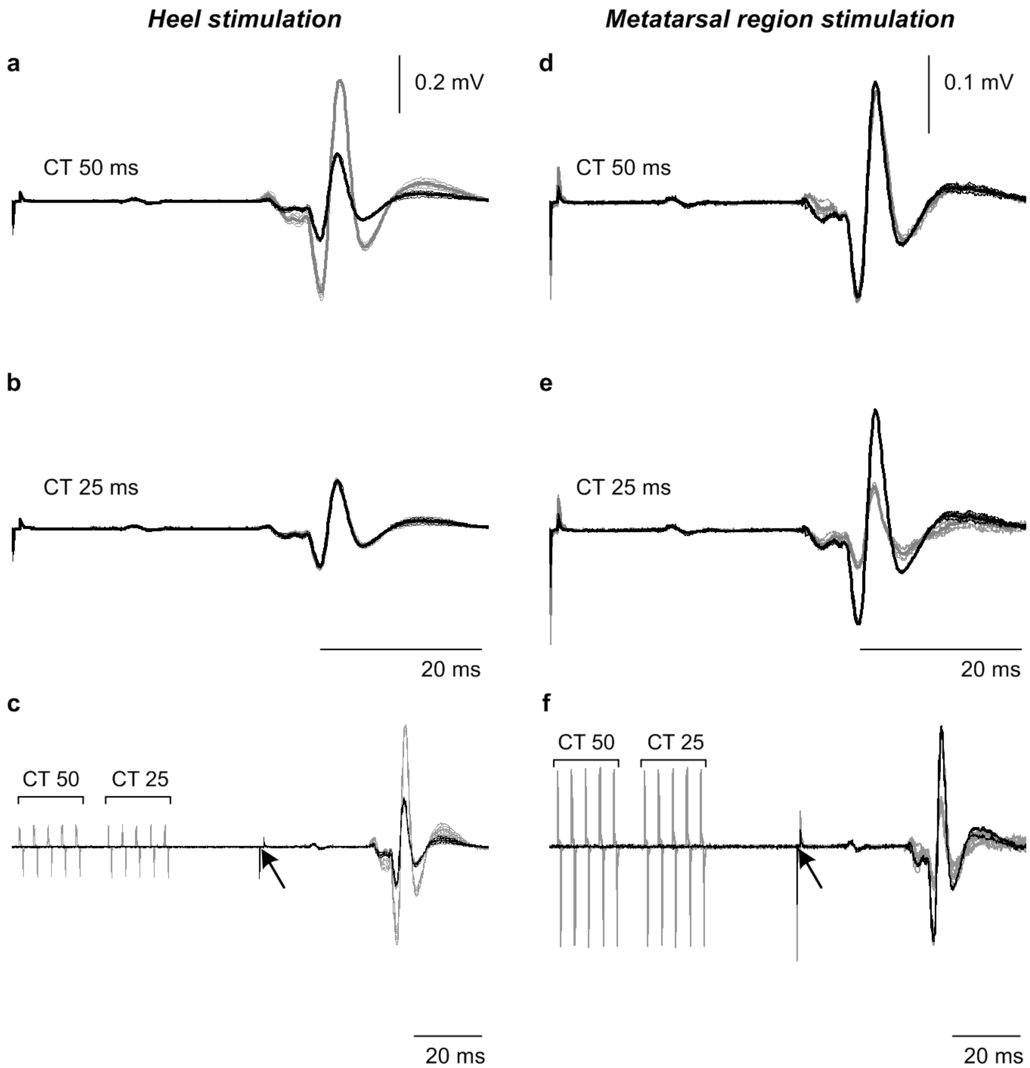

Figure 4. Raw soleus H-reflexes obtained for one subject under control conditions (black lines) and during reflex conditioning (gray lines) via plantar cutaneous afferent excitation delivered to the heel (*left panels*) and the metatarsal region (*right panels*). The thin lines indicate individual trials, whereas the bold lines indicate the average of 10 trials under control conditions (black) and during reflex conditioning (gray). Different CT intervals of 50 ms (a,d) and 25 ms (b,e) are shown. Fig. 4c and 4f show the evoked reflexes in relationship to the stimulation artifacts recorded in the background EMG, with the arrow indicating the moment of posterior tibial nerve stimulation. Note that the peak-to-peak amplitude of the control H-reflex was matched to that of the control stretch reflex.

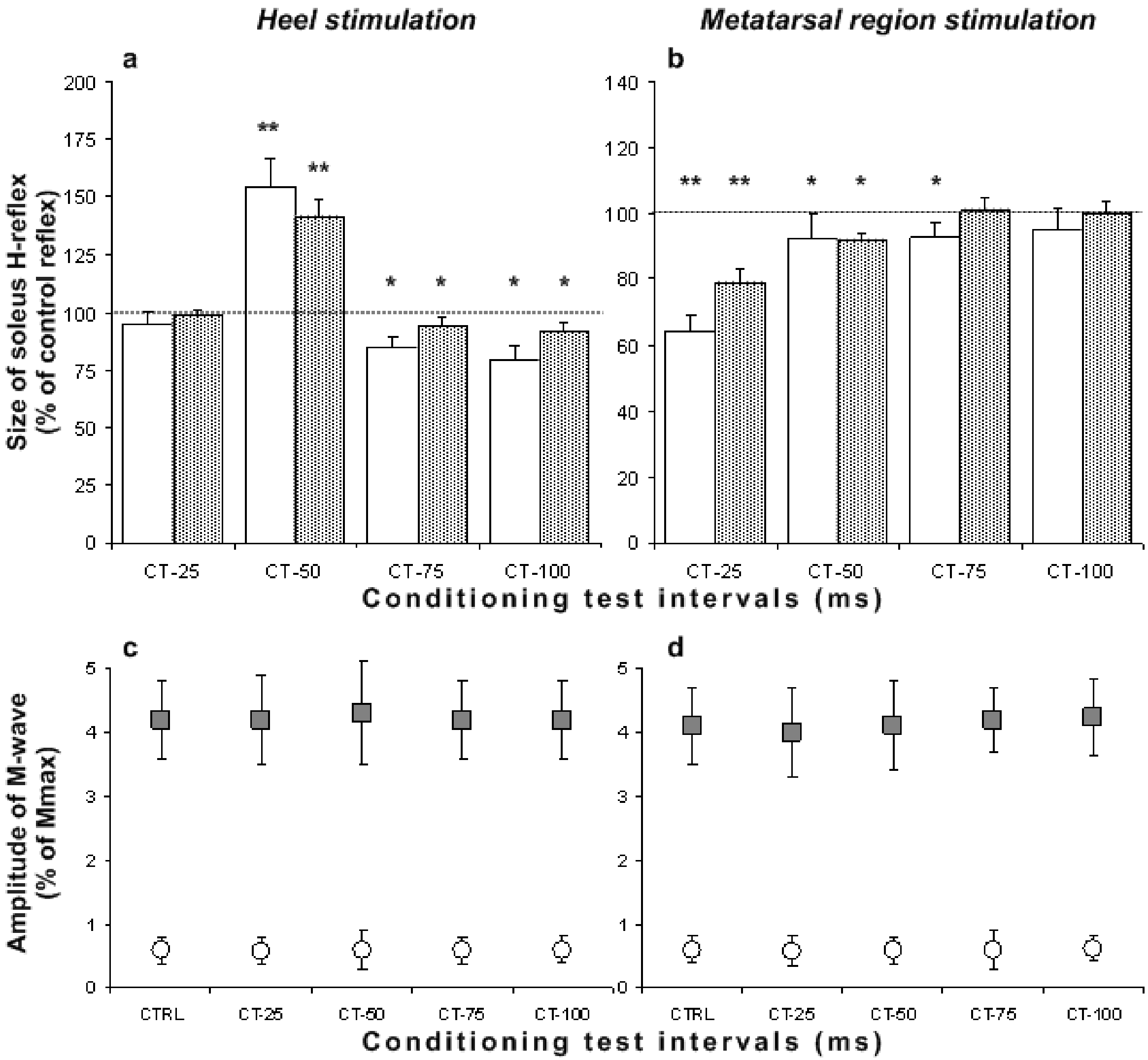

Figure 5. *Upper panel*: Pool data showing the effect of plantar cutaneous afferent excitation delivered to (a) the heel and (b) the metatarsal region at different CT intervals on the soleus H-reflex. White bars represent the trials when the peak-to-peak amplitude of the control H-reflex was matched to that of the control stretch reflex; gray bars represent the trials when the peak-to-peak amplitude of the control H-reflex was equivalent to 20 to 30 % of the maximal M-wave. For each CT interval tested, the overall average size of the conditioned soleus H-reflex is presented as a percentage of the overall average control size (mean ± SEM). *Lower panel*: Pool data showing the average amplitude of the M-waves for the control and conditioned reflexes following the conditioning stimulation delivered to (c) the heel and (d) the metatarsal region. Empty white circles represent the trials when the peak-to-peak amplitude of the control H-reflex was matched to that of the control stretch reflex (11 subjects); filled gray boxes represent the trials when the peak-to-peak amplitude of the control H-reflex was equivalent to 20 to 30 % of the maximal M-wave (16 subjects). Asterisks indicate statistically significant differences between the control and the conditioned reflex sizes (* $P < 0.05$; ** $P < 0.01$).