Crossed reflex reversal during human locomotion

Sabata Gervasio¹, Dario Farina², Thomas Sinkjær¹,³ and Natalie Mrachacz-Kersting¹

¹Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Aalborg University, Fredrik Bajers Vej 7 D-3, Aalborg - DK 9220, Denmark.
²Department of Neurorehabilitation Engineering, Bernstein Focus Neurotechnology Göttingen, Bernstein Center for Computational Neuroscience, University Medical Center Göttingen, Georg-August University, Von-Siebold-Str. 4, 37075 Göttingen, Germany
³Danish National Research Foundation, Holbergsgade 14, 1., Copenhagen K-DK-1057, Denmark

Running title: Crossed reflex reversal

Key words: interlimb reflexes, locomotion, human, reflex reversal

Corresponding author:
Natalie Mrachacz-Kersting
Center for Sensory-Motor Interaction (SMI)
Department of Health Science and Technology
Aalborg University
Fredrik Bajers Vej 7 D-3
Aalborg - DK 9220 (Denmark)
Email: nm@hst.aau.dk
ABSTRACT

During human walking, precise coordination between the two legs is required in order to react promptly to any sudden hazard that could threaten stability. The networks involved in this coordination are not yet completely known, but a direct spinal connection between soleus (SOL) muscles has recently been revealed. For this response to be functional, as previously suggested, we hypothesize that it will be accompanied by a reaction in synergistic muscles, such as gastrocnemius lateralis (GL), and that a reversal of the response would occur when an opposite reaction is required. In the current study, surface EMG of contralateral SOL and GL were analyzed following tibial nerve (TN), sural nerve (SuN) and medial plantar nerve (MpN) stimulation during two tasks in which opposite reactions are functionally expected: normal walking (NW), just before ipsilateral heel strike, and hybrid walking (HW) (legs walking in opposite directions), at ipsilateral push off and contralateral touch down. Early crossed facilitations were observed in the contralateral GL following TN stimulation during NW and a reversal of such responses occurred during HW. These results underline the functional significance of short-latency crossed responses and represent the first evidence for short-latency reflex reversal in the contralateral limb for humans. Muscle afferents seem to mediate the response during NW while during HW cutaneous afferents are likely involved. It is thus possible that different afferents mediate the crossed response during different tasks.

Abbreviations

cGL, contralateral gastrocnemius lateralis; cSOL, contralateral soleus; GL, gastrocnemius lateralis; GM, gastrocnemius medialis; HW, hybrid walking; iGL, ipsilateral gastrocnemius lateralis; iMpN, ipsilateral medial plantar nerve; iSOL, ipsilateral soleus; iSuN, ipsilateral sural nerve; iTN, ipsilateral tibial nerve; MpN, medial planter nerve; NW, normal walking; PT, perception threshold; RMS, root mean square; SOL, soleus; SuN, sural nerve; TN, tibial nerve.
INTRODUCTION

In human walking, a fine coordination between the two legs is required, in particular when an unexpected change in the over ground surface or the encountering of an obstacle threaten our balance. The topology of the networks involved in this coordination are not yet completely known, however studies involving locomotion on a split belt treadmill suggest a coupling of the neuronal circuits controlling each leg (Reisman et al., 2005) and the involvement in this connection of spinocerebellar pathways (Reisman et al., 2007).

In animals, direct commissural interneurons connecting opposing limbs have been identified within the spinal cord (Jankowska & Noga, 1990; Edgley et al., 2003; Jankowska, 2008). Recent evidence suggests that similar interneurons may exist in healthy humans. For example, a short-latency inhibition of the contralateral soleus muscle (cSOL) has been quantified following ipsilateral tibial nerve stimulation (iTN) (Stubbs & Mrachacz-Kersting, 2009; Stubbs et al., 2011b, 2011a). This response showed a phase dependent modulation during locomotion, with the most prominent inhibition occurring before and during the swing to stance transition of the stimulated leg (Stubbs et al., 2011b).

Evidence of phase-dependent reflex modulation in the ipsilateral limb has been provided both for cats (Forssberg et al., 1975; Duysens & Pearson, 1976) and humans (Duysens et al., 1990; Yang & Stein, 1990; Sinkjaer et al., 1996; Zehr et al., 1997; Andersen & Sinkjaer, 1999; Baken et al., 2005). In its most extreme form, this modulation exhibits responses with opposite signs and is termed “phase-dependent reflex reversal” when it occurs with the same latency for an identical stimulus (Duysens et al., 1992, 2004; De Serres et al., 1995). A functional implication has been hypothesized for this phenomenon (Yang & Stein, 1990; Marchand-Pauvert & Nielsen, 2002).

Even though it is known to exist for ipsilateral reflexes, in the contralateral limb a reflex reversal has been observed only in animal studies (Magnus, 1909, 1910; Grillner & Rossignol, 1978; Rossignol & Gauthier, 1980; Duysens et al., 1980). In chronic spinal dogs, the contralateral response to a knee tap is an extension or a flexion depending on whether the contralateral limb was initially flexed or extended (Magnus, 1909, 1910). Similarly, spinal cats treated with clonidine showed a reversal from crossed flexion to crossed extension when the position of the contralateral hind limb was altered (Grillner & Rossignol, 1978; Rossignol &
A reversal from flexor to extensor responses has also been observed in intact walking cats when stimuli were delivered at the end of the contralateral stance phase (Duysens et al., 1980).

Since the inhibition in the human cSOL observed by Stubbs et al. (2011) may have the functional purpose of preventing the push off of the contralateral foot and the progress of the perturbed step, a facilitation of the cSOL would be expected when it is required to stiffen the ankle joint and increase the stability of the ankle. Furthermore, a complementary facilitation in the gastrocnemii would enhance the degree of mechanical coupling between ankle and knee and ensure the stability of the limb (Duysens et al., 1991).

The current study investigates the functional significance of the short-latency crossed response in the human soleus muscle by quantifying the observed responses in the synergist gastrocnemius lateralis (GL) and comparing the behavior of muscles in two walking tasks in which an opposite functional response would be expected. Secondly, since the reflex reversals reported for ipsilateral pathways have been shown to partly arise from cutaneous afferents (Forssberg et al., 1975; Duysens et al., 1980; Yang & Stein, 1990; De Serres et al., 1995), the contribution of these fibers to the modulation of the cSOL and contralateral gastrocnemius lateralis (cGL) were investigated.
MATERIAL AND METHODS

**Ethical approval.** Fourteen healthy subjects (11 males and 3 women, mean age 31 ± 5 (SD)) with no known central or peripheral disorders took part in the experiments. In accordance with the standards of the Declaration of Helsinki, the participants provided written informed consent to the protocol approved by the Scientific Ethics Committee of Nord-Jutland (approval number: N-20110040).

**General experimental protocol.** The subjects were asked to walk on a split belt treadmill (Woodway) that allowed independent control of the two belts. Tibial Nerve (TN) (Experiment 1), Sural Nerve (SuN) and Medial Plantar Nerve (MpN, also referred to as posterior tibial nerve) electrical stimuli (Experiment 2) were applied to the ipsilateral leg, and short-latency responses (appearing in a time window between 40 -80 ms after stimulation) were investigated in the SOL and GL of the contralateral leg. In order to investigate the functional meaning of such direct connections, responses elicited during normal forward walking were compared with responses elicited during hybrid walking, in which the two legs walked at the same speed but in opposite directions, thus disturbing the natural coupling of the legs during normal locomotion. Hybrid walking thus creates a biomechanical condition in which an opposite reaction to the one observed during normal walking would be expected. Subjects started randomly with normal or hybrid walking. Subjects wore a safety harness that did not alter their natural body weight support (figure 1.A). In order to reduce stride time variability due to the adaptation process, subjects were exposed to familiarization to the hybrid walking task at least 24 hours prior to the experimental sessions, to minimize the possible occurrence of muscle fatigue during the recording session. The familiarization lasted 15 minutes, since in previous studies it was observed that 10 minutes were sufficient to adapt to a split belt task (Choi & Bastian, 2007).

The experimental session started with 2-3 minutes of familiarization with the treadmill during which subjects were asked to select their preferred walking speed. It has been observed that at preferred speeds, stability and adaptability of the gait cycle are enhanced (Jordan *et al.*, 2007). The selected speed was then maintained for the duration of the experiment both during the normal and the hybrid walking task. During the hybrid walking task, the direction of the belt under the contralateral leg was inverted, causing the subject to walk forward with the ipsilateral leg and backward with the contralateral leg. The mean duration of the gait cycle...
was estimated as the average of the stride time of 30 steps. Stride time was defined as the time between two
consecutive touchdowns of the leg ipsilateral to the stimulation. The same procedure was repeated for the
hybrid walking task. During normal walking, the stimulation time was identified as 80 or 90% of the
ipsilateral gait cycle. At these times, short latency crossed responses have previously been observed in the
cSOL (Stubbs et al., 2011b). Since functional significance of these responses was hypothesized in the form
of preventing the contralateral leg to progress in the pushing off phase (Stubbs et al., 2011b), the selected
stimulation time for the hybrid walking task was 40 or 50% of the gait cycle (figure 1.B). At these times,
with the ipsilateral foot pushing off and the contralateral foot touching down a facilitation in the cSOL,
rather than an inhibition would be expected to allow adequate control of the positioning of the foot on the
ground. In addition, cSOL and cGL activation levels and ankle joint angles are comparable between the two
tasks at the chosen timings (figure 2), allowing either a facilitation or an inhibition to be observed in the
sEMG signals. The gait cycle percentage was defined so that the ipsilateral touch down corresponds to 0% of
the gait cycle and the next ipsilateral touch down corresponded to 100%.

To reduce inter-individual differences, all participants wore the same model of shoes (Biltema, Super Star).
The subjects were asked to maintain the same pace throughout the testing period in order to limit the inter-
step variability. For the same reason, the stride time was monitored during the experiment and when this
differed from the mean gait cycle duration by more than 10% of the mean gait cycle duration, the subject
was instructed to increase or decrease the stride time.

Data for an entire gait cycle were recorded every 2-4 steps, and, to create a control case, gait cycles in which
a stimulation was delivered were alternated randomly with gait cycles with no stimulation. Between the
normal walking and hybrid walking task, a resting period of approximately five minutes was given to the
subjects. However, they were also able to stop at any time and rest in case of fatigue or for any other reasons.
Data from 30 to 40 gait cycles were recorded during the normal walking task per stimulation intensity and
for the control condition of “no stimulation”. For the hybrid walking condition, due to the larger step
variability observed visually, the number of gait cycles recorded per stimulation intensity and for the control
condition was between 40 and 60.
Stimulation and data acquisition. Surface EMG was recorded using a bipolar differential configuration from the ipsilateral and contralateral SOL muscles (iSOL and cSOL) and from the contralateral gastrocnemius lateralis (cGL). Single use surface electrodes (Neuroline 720 silver/silver-chloride, AMBU A/S, Denmark) were placed in accordance with the recommendations of Cram et al. (1998) following appropriate skin preparation. Ground electrodes were placed over the tibial bone. Foot switch sensors were attached over the sole of the foot at the level of the heel of the ipsilateral leg in order to determine the stride times. Surface EMGs were pre-amplified, all signals were sampled at 2 kHz using scientific software Mr. Kick II 2.3 (Knud Larsen, Center for Sensory-Motor Interaction, Aalborg University, Denmark) and stored for off-line analysis.

Experiment 1. Fourteen subjects (11 males and 3 women, mean age 31 ±5 (SD)) took part in this experiment. TN stimulation at an intensity of 85% of the maximum direct motor response (M-max) (Pierrot-Deseilligny & Burke, 2005) of the iSOL muscle was elicited during normal and hybrid walking using an isolated stimulator (Noxitest IES 230). This stimulation intensity was chosen as it has been shown to evoke the most prominent short-latency response in the cSOL (Stubbs et al., 2011b). The stimulating electrodes were placed while standing; the cathode (PALs Platinum round electrode, Model No. 879100, 3.2 cm diam., Axelgaard Man) was placed over the popliteal fossa and the anode (PALs Platinum rectangular electrode, Model No. 895240, 5 x 9 cm, Axelgaard Man) at the anterior aspect of the knee joint just above the patella. In order to find the optimal location of the electrodes, monopolar stimuli, 1 ms duration, were delivered every 4 to 7 ms and the cathode’s position was adjusted until an M-wave was observed. The subject was then asked to walk on the treadmill and the recording session started. The stimulation time was established as described in General experimental protocol and the intensity was selected by observing the M-wave peak-to-peak amplitude starting at 0 mA and increasing with increments of 5 mA every 3 stimuli. When no more increases in the M-wave amplitude were observed the previous stimulation intensity was noted and the corresponding M-wave peak-to-peak amplitude was labeled as the M-max. The stimulation intensity that elicited 85% M-max was then identified using increments of 1 mA. This intensity was used to record a total of 40 or 60 gait-cycles (40 for normal walking and 60 for hybrid walking condition) randomly alternated with the same number of “no stimulation” control gait cycles every 2 to 4 steps. The recording session was repeated both during normal and hybrid walking conditions.
Experiment 2. Five subjects (2 males and 3 women, mean age 29 ± 3 (SD)) took part in this experiment. The
SuN and MpN are primarily cutaneous nerves that adjoin to the TN. These two nerves were stimulated to
investigate the contribution of cutaneous afferents to the short-latency crossed response. Subjects started
randomly with the SuN or with the MpN stimulation. Surface stimulating electrodes PALs Platinum round
electrodes (Model No. 879100, 3.2 cm diam., Axelgaard Man) were placed on the subjects while standing.
For MpN stimulation, the electrodes were located posterior and inferior to the medial malleolus. For SuN
stimulation, they were located between the lateral malleolus and calcaneal tendon, posterior and inferior to
the lateral malleolus. To determine the optimal location of the electrodes, a train of 3 pulses, 1ms duration
for each pulse with an inter-pulse interval of 3 ms, were delivered at an intensity above the subject’s
perception threshold (PT) (between 2 and 5 mA), that allowed the subjects to perceive an irradiating
sensation. Subjects were then asked to describe the sensation. The expected sensation for MpN stimulation
was a spreading on the plantar side of the foot to the first and second metatarsal. For the SuN the expected
sensation was a spreading on the lateral side of the foot toward the fifth metatarsal. The location of the
electrodes was moved until the subject described the desired sensation. The subjects were then asked to
report when they were feeling the stimulation while the stimulation intensity was increased from 0 with 0,5
mA increments to find the PT. Increments of 0,1 mA were then used to find the exact PT. When, increasing
and decreasing the intensity, if the subject reported the same threshold three times, the PT was identified. In
case the reported threshold while increasing the stimulation intensity was higher than the reported threshold
while decreasing the intensity, the highest threshold was taken. The recording session was then started and
the subject was asked to walk on the treadmill (either normal or hybrid walking). Once the mean gait cycle
duration was found (see General experimental protocol) three consecutive stimuli, 1 ms duration, at an
interval of 3 ms (Nielsen et al., 1997), were applied randomly at the defined stimulation time (see General
experimental protocol) every 3 to 5 steps at an intensity of 1x, 2x, and 3x PT. Non stimulated gait cycles
were also recorded randomly in order to create a control condition. A total of 30 gait cycles per stimulation
intensity and for the control “no stimulation” condition were collected for the normal walking. During hybrid
walking a total of 40 gait cycles per stimulation intensity and for the control “no stimulation” condition for
the hybrid walking were recorded.
Data analysis. All data were processed off line. Data were first inspected using the acquisition software Mr. Kick II 2.3 (Center for Sensory-Motor Interaction, Aalborg University, Denmark) and gait cycles with a stride time shorter or longer than the main gait cycle duration ± 10% of the main gait cycle duration itself were excluded. Data were then exported and processed with the computing environment MATLAB R2010b (MathWorks). Surface EMG signals of the cSOL and cGL were band pass filtered between 25 and 400 Hz. Stimulated and control gait cycles were then rectified, low pass filtered with a cuff-off frequency of 40Hz and averaged. To evaluate the magnitude of the response, the root mean square value (RMS) in a defined time window was computed from the stimulated gait cycle and expressed as a percentage of the RMS in the same window of the control gait cycle. For the cSOL, a time window of 40 to 55 ms after the stimulation or after the onset of the stimulus train, was used in accordance with Stubbs et al. (2011). For the cGL a later facilitation during normal walking was observed by visual inspection. The occurrence of the peak of this facilitation was computed in a time window of 68 to 88 ms after the stimulation. A time window of 20 ms centered on this peak was used to evaluate the magnitude of this response. The onset of the response was evaluated as the time in which the averaged stimulated gait cycle exceeded the value of the averaged control gait cycle for an amount of 2 times the standard deviation of the averaged control gait cycle computed in a time window of 25 to 120 ms after the stimulation. The duration of the response was evaluated as the time at which the averaged stimulation gait cycle remained above this threshold. If the duration was shorter than 10 ms, the response was discarded. In order to compare the responses elicited with iTN and cutaneous stimulation, in Experiment 2, the amplitude of the possible responses was quantified within the same time window used in Experiment 1 for each subject.

Statistical analysis. For experiment 1, two tailed paired sample t-test was used to compare the RMS of the averaged stimulation gait cycle in the defined time window and RMS of the averaged control gait cycle in the same time window. This was done to establish the significance of the response for both muscles in the two tasks. The same test was performed to compare the magnitude of the responses for both cSOL and cGL during normal and hybrid walking. To ensure the reliability of the stimulation during the two walking conditions the reproducibility of the M-wave in the iSOL sEMG signal was tested using a paired sample t-test. The M-wave peak-to-peak value was computed from the non-rectified averaged signal in a time window
From 5 to 25 ms after the stimulation, normalized to the M-max recorded during the respective walking task and compared between normal and hybrid walking conditions.

To ensure that the muscle activation during the control steps was reliable, a paired sample $t$-test was performed between RMS values of the control signal during normal and hybrid walking at the time in which the stimulation was delivered. In the former a time window of 20 ms (centered on the stimulation onset) was used. In the latter the RMS was computed in the same time window used for quantifying the response.

In order to verify whether bigger responses could have been explained by increased muscle activation, linear regression analysis was performed between the magnitude of the responses and the background activation level computed as the RMS of the control signal in the same time window of the responses. Spearman correlation coefficients were computed to assess this relation.

For experiment 2, one-way ANOVA was performed with the magnitudes of the responses of TN, SuN and MpN stimulation for cSOL and cGL, during normal and hybrid walking. Responses elicited by TN and each singular intensity of SuN and MpN stimulations ($1\times$, $2\times$, and $3\times$ PT) were also compared-way ANOVA. When significant differences were identified, the post hoc Fishers LSD multiple comparisons test was performed to establish the nature of the difference. The results of the statistical tests were considered significant when $P$ was less than 0.05.
RESULTS

On average, the subjects selected a preferred walking speed of 3.3 (mean) ± 0.3 (SD) km/h. The selected speeds ranged within limits that ensured a comparison between subjects. As in previous studies (Choi & Bastian, 2007), the subjects were able to produce a walking pattern in both legs during hybrid walking. All subjects reported being familiar with the hybrid walking task, after being exposed to that task prior to the recording session.

Responses in the cSOL and cGL during normal forward walking

As in previous studies (Stubbs et al., 2011b) we observed a significant (P = 0.009) short-latency inhibition in the cSOL when stimulating the iTN at the end of the ipsilateral swing phase of normal forward walking. Contralateral cGL responses after iTN stimulation were investigated in order to uphold the hypothesis of a functional significance of the cSOL short-latency responses (Stubbs & Mrachacz-Kersting, 2009; Stubbs et al., 2011b, 2011a). Figure 3 displays the averaged iSOL and cGL sEMG traces for 30 trials for one subject. The onset of the iTN stimulation is indicated by the dashed vertical gray line. The cGL shows a facilitation (indicated by the vertical dotted line; Fig.2.B) with an onset of 65 ms after the stimulation. The magnitude of the response is 165.0 % of the control signal.

Significant facilitation in the cGL (P = 0.01) was observed in 12 out of 14 subjects following iTN stimulation. Some of these subjects (n = 4) had more prominent responses when stimulation occurred at 80% of the gait cycle while the others (n = 8) had greater responses when the stimulation was delivered at 90% of the gait cycle, likely due to different walking patterns between subjects. Only the condition in which subjects showed the biggest response was used for further analysis.

The responses showed an average magnitude of 138.1 (mean) ± 24.5 (SD) as a percentage of the control signal, with onset and peak occurring at 69.6 (mean) ± 9.3 (SD) ms and 81.1 (mean) ± 6.6 (SD) ms respectively after the stimulation. In two cases the response appeared within the selected time window (68 to 88 ms after the stimulation) however later larger facilitation was seen which appeared to mask the first peak and which was out of the time window. In these two cases the magnitude of the response was computed from
78 to 98 ms after the stimulation, including only part of the response. When computing the mean peak occurrence, however, the time of actual peak was used.

Comparison between normal and hybrid walking

Of 12 subjects that showed a facilitation during normal walking, 11 revealed a reduction in the cGL sEMG when performing hybrid walking. The response was significantly different (P = 0.009) from the control signal (magnitude = 84.0 (mean) ± 13.9 (SD) % of control). In addition, when comparing the same time window, the magnitudes of the responses were significantly different between normal and hybrid walking (P = 0.0003). An example of the inhibition elicited in the cGL when stimulating during hybrid walking is shown in figure 3.C. The onset of the iTN stimulation is indicated by the dashed vertical grey line and the magnitude of the response is 83.1 % of the control signal. Figure 4.A shows the magnitudes of responses (mean ± SD) for cGL during normal and hybrid walking; the no response situation is indicated with a horizontal dotted line.

For cSOL, 10 subjects out of 14 showed a significant increase (P =0.02) in the response during hybrid walking compared to the one observed during the normal forward walking task. However, in the hybrid walking task, the response was not different from the control condition (P = 0.73). Differences in magnitudes of responses (mean ± SD) during normal and hybrid walking for cSOL are shown in figure 4.B where the no response situation is indicated in the figure with a horizontal dotted line.

The M-wave peak to peak amplitude normalized to the respective M-max was similar between the normal and hybrid conditions (P = 0.127), indicating that the same stimulation intensity was applied to the nerve regardless of the position of the knee joint. Similarly, the background activation of the cSOL and cGL, did not significantly differ between normal and hybrid walking at the time of the stimulation (P = 0.206; P = 0.378). There was no correlation between the magnitude of the responses and the background activity.

During normal walking the Spearman correlation coefficients for the cSOL and cGL were r = -0.137 and r = 0.195 respectively while for the hybrid walking task the values were r = -0.1064 and r = 0.831. Cutaneous nerve stimulation
The MpN and SuN were stimulated in five subjects and the effects on the cSOL and cGL quantified. However, one subject did not show a facilitation in cGL and thus a time window for the analysis could not be defined. As a consequence data from five subjects were used for the cSOL’s analysis while for cGL, data from four subjects were used. Figure 5 shows an example of cSOL (A to D) and cGL (E to H) for a single subject after iSuN (A, B, E, F) and iMpN (C, D, G, H) stimulation. In both figures, signals recorded during normal walking are shown on the left while the signals in the right column were recorded during hybrid walking. The grey and black traces indicate control steps and stimulated steps (SuN or MpN stimulation) respectively. The vertical dotted lines represent the time window in which the response occurred following TN stimulation. For this subject the RMS of the signal within the time windows was 95 and 163% of the control for cSOL and cGL respectively. Across all subjects, the cSOL responded differently following iTN, iSuN and iMpN stimulations (1-way Anova test, P < 0.01, F(2, 41) = 6.81) during normal walking. Fisher LSD post hoc test showed a significant difference in the responses after iTN stimulation compared to the SuN and MpN stimulation (P < 0.01) indicating an unlikely contribution of the cutaneous afferents to the observed response during normal walking.

Significant differences in the magnitude of the responses in the cSOL after iTN stimulation were found when compared to those responses elicited by iSuN at 2 × PT (Fisher LSD test, P < 0.01) and iSuN at 3 × PT (Fisher LSD test, P < 0.05) but not with iSuN at 1 × PT (Fisher LSD test, P =0.09). For the iMpN, the TN stimulation differed significantly from iMpN at 1 × PT (Fisher LSD test, P < 0.01) and iMpN at 2 × PT (Fisher LSD test, P < 0.05) but not from iMpN at 3 × PT (Fisher LSD test, P =0.141). No differences were observed between the responses elicited by iSuN and iMpN stimulation (Fisher LSD test, P = 0.808).

The cGL also had different responses during normal walking depending on the stimulated nerve (1-way-Anova test, P < 0.01, F(2,33) = 16.163). Fisher LSD post hoc tests revealed a significant difference between the responses evoked by iTN and iSuN or iMpN stimulation (P < 0.01) suggesting, as for the response in the cSOL, that cutaneous afferents are unlikely to contribute to the cGL response during normal walking. A significant difference was also revealed between responses provoked by iSuN and iMpN stimulation (Fisher LSD test, P = 0.033). If the SuN and MpN stimuli were separated according to intensity, then significant
differences were found between iTN and iSuN at all the stimulation intensities (Fisher LSD test, P < 0.01) and with iMpN stimulation at 1 × PT and 2 × PT (Fisher LSD post hoc test, P < 0.01) but not with iMpN at 3 × PT (Fisher LSD test, P =0.346).

During hybrid walking, no significant difference in cSOL and cGL responses were found between TN, SuN or MpN stimuli (1-way-Anova test, P = 0.387, F = 0.973, df = 2 for cSOL and P =0.867, F=0.143, df = 2 for cGL), suggesting that, during this walking task, cutaneous fibers may have a role in the mediation of the response.
DISCUSSION

The current study further suggests the functional significance of the short-latency crossed responses in the SOL muscle. Indeed, early crossed facilitations are also elicited in the cGL at the end of the swing phase, validating our hypothesis that, if functional, cSOL responses should have been accompanied by response in synergistic muscles. In addition, when an opposite reaction is required, a reversal of these responses can occur and were observed in the cGL. This represents the first evidence for reflex reversal in the contralateral limb for humans. Muscle afferents likely mediate the response during normal walking, as stimulation of cutaneous afferents did not produce similar responses. However, during hybrid walking, when the reversal occurred, responses provoked by iTN, iSuN and iMpN stimuli did not differ. It is thus possible that cutaneous afferents play a more relevant role during hybrid walking, for example due to the uncertainty of the new task.

Short-latency crossed responses in cGL

In the current study, a facilitation of the cGL occurred 69.6 ± 9.3 ms following iTN stimulation. These latencies accord with previous studies in which crossed responses with latencies ranging from 65 to 112 ms after ipsilateral nerve stimulation or mechanical perturbation have been shown in the contralateral gastrocnemius during walking (Berger et al., 1984; Dietz et al., 1986, 1989; Duysens et al., 1991).

The facilitation in the cGL occurred around 30 ms after the suppression of the cSOL. Different behavior of SOL and gastrocnemius muscles has previously been observed in intact walking cats, where stimulation of the GM elicited facilitation in the cGM with a latency of 20 ms during the stance phase, but failed to elicit responses in the cSOL (Duysens & Loeb, 1980). This different behavior has also been shown in human walking by Duysens et al. (1991), where a separate control of SOL and gastrocnemius medialis (GM) was proposed. Stimulation of cutaneous afferents during the contralateral early stance phase when both cGM and cSOL are active produced a facilitatory response in the cSOL but a suppression in the cGL (Duysens et al., 1991). However, the responses were observed in a time window of 100 to 120 ms after the stimulation, substantially later compared to the responses described in the current study, and might thus be mediated by different pathways. Nevertheless, both studies confirm the hypothesis of a separate control for SOL and
gastrocnemius muscles. Even if SOL, GL and GM all act on the ankle joint, these muscles differ in terms of anatomy, action and motor units’ property (Nardone et al., 1990). For instance, the SOL is monoarticular and purely plantarflexor, while the gastrocnemius muscles are biarticular and contribute to knee flexion (Gravel et al., 1987). Hence, in the current study, the inhibition of cSOL and the subsequent facilitation of cGL at the end of the ipsilateral swing phase, could have the function of increasing the degree of mechanical coupling of ankle and knee joints, reinforcing the stability of the limb in a “critical” situation such as walking (Nichols, 1989).

The difference in latencies between cSOL and cGL responses is again in accordance with previous studies where iTN and iSuN stimulation during stance produced earlier responses in the cSOL than cGM (Duysens et al., 1991). It is possible that the different onsets are due to different pathways; this will be further discussed in the following sections.

Reflex reversal and functional significance

The current study compared crossed responses in the SOL and GL during normal walking at 80-90% of the gait cycle to the responses elicited during hybrid walking at 40-50% of the gait cycle. The latter timings were chosen since the instability of this phase (ipsilateral leg in push off and contralateral leg in touch down) would require appropriate interlimb coordination in order to react rapidly to a disturbance of balance. As previously mentioned, a different number of gait cycles was recorded during the two tasks, to take into account the larger step variability induced by hybrid walking. However, when repeating the analysis using the same number of gait cycles for both tasks, the same results were obtained. For this reason, results using all the available gait cycles are shown.

Most of the subjects (n = 10) showed an increase in the cSOL response during hybrid walking compared to the normal walking task. This increase was significantly different between the two tasks, but the response, during the hybrid walking task did not differ from the control, indicating that a reduction, but not a reversal, of the response occurred. On the contrary, all subjects except one showed a significant reversal in the cGL from facilitation during normal walking to inhibition during hybrid walking. This behavior provides the first
evidence for reflex reversal in the contralateral limb in humans and, in addition, confirms the task
dependence of short latency crossed responses. While during normal walking the inhibition of cSOL and the
facilitation of the knee flexor cGL might prevent the push off of the contralateral foot, maintain the body
weight on the contralateral leg and reinforce the stability of the ankle and knee joints, during hybrid walking,
a crossed inhibition in the gastrocnemii when occurring after a facilitation in the cSOL, could have the
function of preventing the knee flexion from contaminating the induced reduction in ankle dorsiflexion in the
contralateral leg (Duysens et al., 1991). In addition unlike during normal walking, at the time the stimulation
was delivered during hybrid walking the contralateral leg was not yet completely on the ground (see figure
1B) and it might not be prepared to support the possible body weight shift provoked by the ipsilateral
perturbation. The observed cGL inhibition may, in this case, be directed to resolve the functional need of
preparing the contralateral leg to support the body weight, accelerating the knee extension and thus forcing
the heel to the ground. The facilitation we expected in the cSOL that may function to aid in stiffening the
ankle joint was not seen. This may be due to the fact that during hybrid walking the toes touch the ground
first and the heel is lowered only in the mid-stance phase. In this sense the stiffening of the ankle joint may
be controlled by other mechanisms. However there is an alternative possibility, as the cSOL inhibition during
normal walking is relatively smaller than the facilitation in the cGL, it is possible that changes in the
response were masked by the variability of the walking pattern induced during the hybrid walking. Stubbs et
al. (2011) observed that not all the participants displayed short latency inhibitory responses in the cSOL and
further, that the percentage of the gait cycle in which the inhibition commenced varied between subjects.
This is unlike the response in the cGL which always appeared at 80% of the gait cycle in the current study.
Stubbs et al. (2011) suggested that different walking strategies adopted by the subjects may produce different
feedback, resulting in a diverse expression of the crossed SOL response. Furthermore, chronic recordings in
cat have shown that activation patterns and reflexes during locomotion are quite variable from one animal to
another but very consistent in the same animal (Loeb, 1993). Thus, even if the locomotor program has a very
strong genetic component (Yang et al., 2004), during development, inter-individual differences within the
locomotor circuitry could arise due to activity dependent mechanisms and interactions with the environment
(Frigon, 2011). The fact that conditioning of the soleus H-reflex can induce persistent changes in the spinal
circuitry of adult rats confirms this hypothesis (reviewed in Wolpaw and Tennissen, 2001; Wolpaw, 2007).

Therefore, we suggest that this response could be influenced by inter-individual variability and not being present in all subjects.

Possible pathways and neural mechanisms

Stubbs and Mrachacz-Kersting (2009) suggested that the observed crossed responses in the cSOL could be spinally mediated due to the short latency of the onset, as supported by invasive recordings on cats (Baxendale & Rosenberg, 1976; Arya et al., 1991; Jankowska et al., 2005). Group I muscle afferents of the ipsilateral leg might be involved since application of ischemia to the ipsilateral thigh delayed the response during sitting (Stubbs & Mrachacz-Kersting, 2009). However group II afferents, may also be involved since the ischemic block did not abolish the response (Stubbs & Mrachacz-Kersting, 2009). The contribution of cutaneous fibers has been excluded since iSuN and iMpN stimulations did not produce the same response (Stubbs et al., 2011, confirmed by the present study). Even if occurring slightly later, the crossed response, observed in this study in the cGL, could be spinally mediated as well. In fact, Petersen et al. (1998) reported a minimum latency for a transcortical pathway’s contribution to ipsilateral reflexes in the TA of 79 ms. TA and GL are located at similar distance from the cortex, therefore even the latest responses we observed for cGL started too early to be mediated by a transcortical pathway. However, further investigation will be necessary to confirm this hypothesis. As for the cSOL (Stubbs & Mrachacz-Kersting, 2009; Stubbs et al., 2011b), iSuN and iMpN stimulations did not produce in the cGL a response similar to the iTN stimulation. An exception is the iMpN stimulation at 3 x PT where the response elicited in both muscles does not differ from the one observed after iTN stimulation. The MpN is not a pure cutaneous nerve and it is possible that, at this intensity, not only cutaneous but also muscle fibers were activated (Gracies et al., 1994; Nielsen et al., 1997). Hence, muscle afferents remain the likeliest source for short-latency responses in both cSOL and cGL. During hybrid walking, however, there were no statistically significant differences between responses provoked by iTN, iSuN and iMpN. Experiments on the cat have however shown that after cutaneous nerve transection, the kinematics can be completely recovered for normal walking but not for tasks such as walking on a ladder or inclines (Bouyer & Rossignol, 2003). This evidence supports the conclusion that, although
cutaneous inputs are not essential in locomotion (Sherrington, 1910; Grillner & Zangger, 1979), the
information provided by these afferents might be crucial in more demanding tasks (Bouyer & Rossignol,
2003). If this is the case for the human as well, a possible explanation for the results of the current study is
that cutaneous afferents might contribute in challenging tasks, such as hybrid walking.

The difference in responses and their latencies between the cSOL and cGL could thus arise from different
afferent contributions or different fiber composition. The response in SOL can be due to the excitability of
slow motoneurones in response to Ia afferent fibers (Nadeau & Vanden-Abeele, 1988). The subsequent
response in GL could be due to a predominant input of group II afferent fibers from both spindles and joint
receptors (Lundberg et al., 1987). Moreover, natural or electrical stimulation of the SuN in decerebrate cats
excited preferentially large motor units, of which the gastrocnemius is mainly composed, and inhibited
preferentially small motor units, such as those of which the SOL is comprised (Kanda et al., 1977).

In addition animal research suggests that, even if in animal preparations (Holmqvist, 1961), stimulus
intensities (Megirian, 1962) and size of the stimulated cutaneous fiber (Perl, 1957) could produce a reversal
in the response, the main factor determining whether the crossed response results in an extension or a flexion
is the position of the contralateral limb (Magnus, 1909, 1910; Grillner & Rossignol, 1978; Rossignol &
Gauthier, 1980). It has been suggested that responses are directed toward the muscle that is more stretched
(Uexküll, 1904; Magnus, 1909, 1910). However, since flexion or extension responses are still present after
tenotomy of both antagonist muscle, it has been hypothesized that the response pattern is due to other intact
pairs of muscles acting on the same joint (Grillner & Rossignol, 1978; Rossignol & Gauthier, 1980). After
complete deafferentation, only crossed extensor responses are observed, (Rossignol & Gauthier, 1980),
leading to the conclusion that crossed extension would be evoked unless flexor muscles are stretched. In the
functional view point though the most observed crossed response is an extension as a result of an ipsilateral
nociceptive stimulus, unless such a response would be inappropriate as observed in the walking intact cat
(Duysens et al., 1980). This condition, such as at the end of the ipsilateral swing phase when the contralateral
limb is fully extended, would be signaled by the stretch of the flexor muscles (Rossignol & Gauthier, 1980).
The results of the current study allow to extend such a theory to the human. During normal walking at the
time when the stimulus was delivered the contralateral knee was extended (figures 1.B and 2.D), leading the
gastrocnemius, as knee flexor, in a state of stretch, and eliciting a facilitation of this muscle. During hybrid
walking, the knee joint was flexed (figures 1.B and 2.H) at the time in which the stimulus was delivered, thus
a flexion response would not been expected.

A characterization of the neural mechanism at the base of the observed crossed reflex reversal would provide
relevant information about the networks controlling the lower limbs during locomotion. Unfortunately, this
mechanism is difficult to address in the intact human. We can exclude that a diverse stimulation during the
normal and hybrid task was the cause of the difference in the responses during normal and hybrid walking.
The iSOL M-wave peak-to-peak amplitude, namely the magnitude of the direct motor response produced by
the stimulation, was indeed comparable in the two conditions. This indicates that the same efferent fiber
population was stimulated, although it cannot confirm that the afferent volley remained constant during the
two tasks. Moreover, the size of the response was not influenced by the muscle’s background activation
since the level of muscle activity was similar in the two conditions for both cSOL and cGL. The reversal
mechanism is likely controlled at a premotoneuronal level since there was no correlation between the
magnitude of the response and the background activity level. Through motor unit recording in the tibialis
anterior, De Serres et al. (1995) observed that the same motor unit was excited during swing and inhibited
during the transition from swing to stance during locomotion. They suggested that parallel excitatory and
inhibitory pathways from cutaneous afferents to single motoneurons are involved in the reversal and that a
shift between the two pathways occurs during the gait cycle. We sustain this hypothesis; however, since the
current study shows that cutaneous afferents may contribute to the response during hybrid walking but not
during normal walking, and referring to previous studies on cat, where it has been observed that crossed
deflexor responses had a lower threshold than crossed extensor responses (Duysens et al., 1980), we argue that
different afferent populations from the stimulated leg could mediate the reflex reversal. Further studies will
be necessary to investigate whether the state of enhanced excitability provoked by the stretch of antagonist
muscles acting on the same joint in the contralateral leg contributes to the reversal of the response.
REFERENCES


Acknowledgement:

This study was supported by Det Obelske Familiefond, the Spar Nord Fonden, and “Mathilde and Jeppe Juhl’s Mindelegat”
FIGURE LEGENDS

Figure 1: **Experimental set up.** A: Wearing a safety harness, the subjects walked on a split belt treadmill that allowed to control each belt separately. TN, SuN and MpN stimulations were delivered to the ipsilateral leg during separate sessions and EMG signals from iSOL, cSOL and cGL were recorded. B: Legs’ position at the time the gait was perturbed. The ipsilateral and contralateral legs are shown in white and gray respectively. During normal walking, electrical stimulation was delivered at 90 or 80% of the gait cycle when the ipsilateral leg is about to touch down. During hybrid walking the stimulation was delivered at 50 or 40% of the gait cycle, when the ipsilateral leg is about to push off and the contralateral leg is about to touch down. The gait cycle percentage was defined so that the ipsilateral touch down corresponds to 0% of the gait cycle and the next ipsilateral touch down corresponds to 100%.

Figure 2: **Muscle activation patterns and joint kinematics during normal and hybrid walking.** The graphs on the left represent signals recorded during a gait cycle of normal forward walking, in order SOL (A) and GL (B) EMG, and ankle (C) and knee (D) kinematics. Same signals but recorded during hybrid walking are shown on the right (E, F, G, H). Gray and black traces represent the ipsilateral and contralater leg respectively. For the GL, only the contralateral sEMG was recorded, consequently only this signal is displayed. The arrows indicate the timings when the stimulations were delivered (80 and 90% of the ipsilateral gait cycle during normal walking and 40 and 50% of the ipsilateral gait cycle during hybrid walking). The black bars in the bottom indicate the contralateral stance phase, from heel strike to toe off for normal walking and from toe strike to heel off for hybrid walking. Ipsilateral stance phase is not shown since ipsilateral toe off was not recorded. While muscle patterns and joint kinematics of the ipsilateral leg do not change between normal and hybrid walking, the figure shows that cSOL and cGL activation levels and ankle joint angles are comparable between the two tasks at the timings the stimulation is delivered. Contrarily, the knee joint angle differs at these timings between the two tasks.

Figure 3: **Short-latency responses in the cGL after iTN stimulation.** The traces are the averages of 40 stimuli for one subject. The time axis in the figures has been shifted so that onset of the stimulation corresponds to 0 ms and is indicated by the dashed vertical lines. A and C show the iSOL sEMG from 20 ms
before to 60 ms after the stimulation for normal and hybrid walking respectively. The M-wave occurs between 5 and 20 ms after the stimulation. B and D show the rectified sEMG for the cGL from 20 ms before to 200 ms after the stimulation during normal (B) and hybrid walking (D). The black traces represent the averaged test signal while the gray lines are the average of the control gait cycles. B: a facilitation occurs in the cGL after iTN. The onset of the facilitation is signaled by the vertical dotted gray line and occurs 65 ms after the stimulation. D: An inhibition is instead elicited in the cGL during hybrid walking.

Figure 4: **Comparison of cGL and cSOL responses during NW and HW after iTN stimulation.** Magnitude of the responses (mean ± SD) for the cGL (A) (11 subjects) and for cSOL (10 subjects). The responses are expressed as percentage of the control signal during normal (left) and hybrid walking (right). The dotted horizontal line represents 100% of the control, indication “no response”. cGL responses (A) changes significantly from a facilitation during normal walking to an inhibition during hybrid walking, while for the cSOL (B) the responses are significantly different between normal and hybrid walking but during the latter task the magnitude of the response does not differ from the control.

Figure 5: **Responses to cutaneous stimulation.** Signals recorded from the cSOL (A-D) and cGL (E-H) of a single subject after iSuN (A, B, E, F) and iMpN (C, D, G, H) stimuli, during normal (right column) and hybrid walking (left column). The gray line represents the control signal (no stimulation) and the black lines represent the sEMG signal when the stimulation was performed at 1x, 2x and 3x PT. All traces are the rectified averages of 30 trials for normal walking and 40 trials for hybrid walking. The dashed vertical line indicates the time at which the stimulation was delivered while the two vertical dotted lines indicate the time window in which the responses were observed following iTN stimulation. No responses are observed in the time window.
Comparison of knee kinematics and cGL-sEMG activity during normal walking and hybrid walking.

A) Normal walking: cGL-sEMG activity shows distinct patterns for the ipsilateral and contralateral legs.

B) Hybrid walking: cGL-sEMG activity is compared for the ipsilateral and contralateral legs.

C) Ankle kinematics: The graphs illustrate the movement of the ankle during both normal and hybrid walking.

D) Knee kinematics: The graphs illustrate the movement of the knee during both normal and hybrid walking.

E) Normal walking: The graph shows the knee kinematics for the ipsilateral leg in black and the contralateral leg in gray.

F) Hybrid walking: The graph shows the knee kinematics for the ipsilateral leg in black and the contralateral leg in gray.

G) Normal walking: The graph shows the ankle kinematics for the ipsilateral leg in black and the contralateral leg in gray.

H) Hybrid walking: The graph shows the ankle kinematics for the ipsilateral leg in black and the contralateral leg in gray.

Arrows indicate key points of comparison between the two types of walking.
A

Walking task

B

Walking task