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Interlimb communication following unexpected changes in treadmill velocity during human walking

Andrew J. T. Stevenson[1], Svend S. Geertsen[2,3], Thomas Sinkjær[1,4], Jens B. Nielsen[2,3], and Natalie Mrachacz-Kersting[1]

[1]*Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Aalborg University, Fredrik Bajers Vej 7 D-3, Aalborg - DK 9220, Denmark*
[2]*Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen – DK 2200, Denmark*
[3]*Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen – DK 2200, Denmark*
[4]*The Danish National Research Foundation, Copenhagen – DK 1057, Denmark*

Running head: Interlimb reflexes following treadmill velocity changes

Corresponding author:
Natalie Mrachacz-Kersting
Address: Fredrik Bajers Vej 7 D3, 9220 Aalborg, Denmark.
Email: nm@hst.aau.dk
Telephone: +45 9635 7571
FAX: +45 9815 4008

Author contributions:
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Abstract

Interlimb reflexes play an important role in human walking, particularly when dynamic stability is threatened by external perturbations or changes in the walking surface. Interlimb reflexes have recently been demonstrated in the contralateral biceps femoris (cBF) following knee joint rotations applied to the ipsilateral leg (iKnee) during the late stance phase of human gait (Stevenson et al. 2013). This interlimb reflex likely acts to slow the forward progression of the body in order to maintain dynamic stability following the perturbations. We examined this hypothesis by unexpectedly increasing or decreasing the velocity of the treadmill before (-100 ms and -50 ms), at the same time, or following (+50 ms) the onset of iKnee perturbations in twelve healthy volunteers. We quantified the cBF reflex amplitude when the iKnee perturbation was delivered alone, the treadmill velocity change was delivered alone, or when the two perturbations were combined. When the treadmill velocity was suddenly increased (or decreased) 100 or 50 ms prior to the iKnee perturbations, the combined cBF reflex was significantly larger (or smaller) than the algebraic sum of the two perturbations delivered separately. Furthermore, unexpected changes in treadmill velocity increased the incidence of reflexes in other contralateral leg muscles when the iKnee perturbations were elicited alone. These results suggest a context dependency for interlimb reflexes. They also show that the cBF reflex changed in a predictable manner to slow the forward progression of the body and maintaining dynamic stability during walking, thus signifying a functional role for interlimb reflexes.

Keywords: interlimb, reflex, gait, human

Introduction

Human walking requires precise coordination between the two legs, particularly when encountering unexpected perturbations or changes in the walking surface. Spinal and supraspinal interlimb reflexes have been proposed to play an important role in compensating for threats to dynamic stability during locomotion (Haridas et al. 2006; Zehr et al. 2001).

To quantify interlimb reflexes, different methodologies have been applied such as halting the leg during the swing phase of the gait cycle (Dietz et al. 1986), applying body loading or unloading (Bachmann et al. 2008), and treadmill accelerations during normal stance and walking (Berger et al. 1984; Dietz et al. 1989; Dietz et al. 1984; Dietz et al. 1987). Results indicate that muscle afferent feedback has a dominant role in mediating interlimb reflexes. Whole body perturbations are likely more expressive of what occurs in real life situations when stumbling than single-joint perturbations. However, they may induce converging input from many afferent sources onto the motoneuron pool, thus not allowing the determination of which specific input instigates the largest contribution. Furthermore, the precise role of specific interlimb reflexes cannot be elucidated due to the different sensory inputs.

We have recently applied unilateral knee joint rotations using a custom-made actuator system (Andersen and Sinkjær 2003) and quantified interlimb reflexes during the late ipsilateral stance phase of human gait (Stevenson et al. 2013). An unexpected ipsilateral knee (iKnee) extension joint rotation elicited a facilitation in the contralateral biceps femoris (cBF) muscle at a latency of 76 ms. A transcortical pathway contributes to this response, potentially allowing for integration with other sensory information and therefore more appropriate adaptations to varying situations than purely spinally mediated reflexes (Christensen et al. 2001; Christensen et al. 2000; Stevenson et al. 2013; Zuur et al. 2009). The cBF reflex may be involved in slowing the forward progression of the body in order to maintain dynamic stability during walking. This is consistent with the reflexive braking reaction observed in the cBF muscle, among others, when the walking surface of the ipsilateral foot was unexpectedly lowered at heel contact (van der Linden et al. 2007).

The aim of this study was to investigate if the cBF reflex is involved in slowing the forward progression of the body following iKnee extension joint rotations during the late stance phase of human walking. Therefore, the treadmill velocity was unexpectedly increased or decreased before

(-100 ms and -50 ms), at the same time, or after (+50 ms) the onset of iKnee extension joint rotations. We hypothesized that by decreasing the treadmill velocity, the requirement of the cBF reflex to slow the body's forward progression would be decreased, resulting in a diminished cBF reflex. In contrast, by increasing the treadmill velocity, the requirement of the cBF reflex to slow the forward progression of the body would be increased, resulting in a facilitated cBF reflex. Furthermore, we hypothesized that if the treadmill velocity was altered too close to the onset of the cBF reflex (e.g., +50 ms), the cBF reflex would be unaltered. A preliminary account of the work has been published in abstract form (Stevenson et al. 2014).

Methods

Participants

Twelve participants (six female) aged 19-55 years (26.4 ± 9.9, mean ± S.D.) provided written informed consent to take part in this study. At the time of the study, all participants were free of any known physical or neurological disorders. Approval for the study was given by the scientific ethics committee for Nordjylland (Reference Number: N-20110076). The study was performed in accordance with the declaration of Helsinki.

Apparatus and instrumentation

The knee perturbator used in this study was a semi-portable device that could induce unexpected knee joint rotations (Figure 1; see Andersen and Sinkjær 2003; Stevenson et al. 2013 for further details). The functional joint consisted of a two-link joint connected to a powerful actuator by Bowden wires. The actuator was positioned next to the treadmill that the participant walked on. The motor was regulated by position feedback from the joint in such a way that it followed the movement of the knee joint without influencing the gait pattern. The perturbator was programmed to randomly apply knee extension joint rotations at 50% of the gait cycle, which has previously been shown to elicit facilitatory interlimb reflexes in the cBF muscle (Stevenson et al. 2013). The gait cycle percentage was defined as one ipsilateral leg heel contact (corresponding to 0% of the gait cycle) to the next ipsilateral leg heel contact (corresponding to 100% of the gait cycle). The perturbations had a mean ramp and hold of 193.3 ± 41.4 ms, a mean velocity of 293.5 ± 28.8°/s, and a mean amplitude of 7.6 ± 0.6° (see Mrachacz-Kersting et al. 2004; Stevenson et al. 2013). During walking, the weight of the portable stretching apparatus added an extra load of approximately 2 kg to the left leg. This has previously been shown not to change the normal walking pattern compared to when participants walked without the device (Mrachacz-Kersting et al. 2004).

[Insert **Figure 1** about here]

During this study, participants walked on a split-belt treadmill (Split 70/157/ASK, Woodway GmbH, Weil am Rhein, Germany) wearing a safety harness that did not alter their natural body weight support (Figure 1). The treadmill was used to influence the participants' gait by rapid changes in

velocities (of both belts) at different points in the gait cycle; relative to knee perturbation onset at 50% of the gait cycle (-100 ms, -50 ms, 0 ms, and +50 ms), and at ipsilateral heel strike (0% of the gait cycle). The main purpose of this study was to look at the effects of the initial velocity change, which was to either speed up (+velocity trials) or slow down (–velocity trials) the treadmill. During +velocity trials, the treadmill velocity increased from the initial velocity by 0.56 m/s, then decreased by 1.12 m/s, then increased by 1.12 m/s. During –velocity trials, the treadmill velocity decreased from its initial velocity by 0.56 m/s, then increased by 1.12 m/s, then decreased by 1.12 m/s. Each of the velocity changes lasted for 500 ms, thus the whole treadmill perturbation lasted for 1.5 seconds. The values above were chosen such that the resulting velocities were never below 0 m/s or above 1.81 m/s (i.e., fast walking; Sousa and Tavares 2012). The velocity changes occurred with an acceleration of ± 5 m/s^2.

Bipolar surface electrodes (Medicotest 720-01-K, AMBU A/S, Ballerup, Denmark) were used to record electromyographic (EMG) activity of the ipsilateral (left) rectus femoris (iRF) and biceps femoris (iBF) muscles, along with the rectus femoris (cRF), cBF, vastus lateralis (cVL), soleus (cSOL) and tibialis anterior (cTA) muscles of the contralateral (right) leg. The EMG signals were amplified and band-pass filtered at 10 Hz – 1 kHz and rectified. A flexible electrogoniometer (XM180 series, Biometrics Ltd., Cwmfelinfach, Newport, UK) was used on two participants to trace the right knee joint angle. A force sensitive resistor was placed under the heel of the participants' left shoe and used to trigger the sampling to the computer and the onset of experimental events. A custom-made PC system controlled the acquisition of the signals from the position-feedback channels and the surface EMGs. All data were collected at a sampling frequency of 2 kHz.

Experimental procedures

During the experiment, participants walked at a self-selected velocity between 0.83 and 1.11 m/s (mean velocity: 1.07 ± 0.05 m/s). Prior to data collection, participants walked on the treadmill for five minutes to become accustomed to the selected walking velocity and the semi-portable device attached to the left leg. Following this, 20 steps were recorded to establish the non-perturbed walking profile of each participant. From the walking profile, 50% of the gait cycle of the ipsilateral leg was calculated. If the participants began to vary from their initial stride time (±100 ms), they

were verbally asked to increase or decrease their stride time (necessary in three experimental sessions). All data were analyzed off-line.

Following gait profile assessment, one of the 11 conditions presented in Table 1 were administered randomly every four to six steps. There were a total of 720 trials. Participants were allowed to rest every 100 recorded steps in order to prevent fatigue. Each experimental recording session lasted between 1.5 to 2.5 hours.

[Insert **Table 1** about here]

Data analysis

Data for each individual participant were averaged within conditions and the quantification of responses was performed on these averaged trials. 2.8% of all trials were discarded due to an incorrect registration of heel strike or a gait cycle time greater than ± 10% of the mean gait cycle time. The onset of the reflex responses following iKnee perturbation only trials was determined for each contralateral muscle in each participant by using an algorithm in MATLAB, and was defined as the first deviation of the mean rectified EMG data above or below two standard deviations of the mean rectified EMG in the normal gait condition that lasted for at least 10 ms (Gervasio et al. 2013a; Stevenson et al. 2013). The offset of the reflex responses was defined as the point where the mean rectified EMG in the iKnee perturbation only trials returned to within two standard deviations of the control gait trials for at least 10 ms. The reflex onsets and offsets were manually verified for accuracy. The onsets and offsets of responses in the cBF following all treadmill only conditions were determined in the same way.

To quantify the amplitude of the contralateral reflex responses following the iKnee perturbation only condition (for each contralateral muscle), and the amplitude of the cBF responses following the treadmill only conditions, the root mean square (RMS) of the mean rectified EMG was calculated for the duration of the response. To obtain the absolute mean amplitude of the responses, the ongoing background activity from the normal gait condition, where no iKnee or treadmill perturbations were imposed, was subtracted from trials when perturbations were applied. These corrected values were subsequently averaged across all participants to obtain the mean response across the group.

In order to compare the effects of combining the iKnee perturbations with the sudden changes in treadmill velocity, a window commencing from the onset of the cBF reflex until 120 ms following iKnee onset was individually specified for each participant to determine the initial reflex amplitude of the cBF response. The RMS of the cBF EMG in the specific time window for each participant was extracted for each condition, and for conditions 3-10 the RMS amplitude was extracted separately for each treadmill velocity change direction. To obtain the expected level of convergence from pairing ipsilateral knee extension joint rotations with abrupt changes in treadmill velocity, the algebraic sum of the iKnee only and treadmill only conditions was calculated from the cBF data for each participant for each corresponding treadmill perturbation timing (-100 ms -50 ms, 0 ms, +50 ms) and direction (+velocity, -velocity). The cBF RMS value for the algebraic sum of the iKnee only and treadmill only conditions was then compared to the combined iKnee & Treadmill condition. Background activity (normal gait cBF RMS value) was subtracted from these trials before comparison.

Statistical analysis

For the iKnee perturbation only condition, the means ± SD were reported for response onsets, durations, and amplitudes of ipsilateral and contralateral muscles when responses were observed in at least five out of 12 participants (see Table 1). When contralateral reflex responses were observed in all 12 participants, single-sample Student's *t* tests were conducted on the response amplitudes (normalized to normal gait).

For the treadmill only conditions, the means ± SD were reported for response onsets, durations, and amplitudes of the cBF muscle for each treadmill timing and velocity change direction (see Table 3). To determine whether the cBF response was modulated by the timing (-100 ms, -50 ms, 0 ms, +50 ms) or direction (+velocity, –velocity) of the sudden treadmill velocity changes, four (timing) by two (direction) within-subjects analyses of variance (ANOVAs) were performed on the cBF response parameters (onset, duration, and amplitude).

In order to assess the effect of combining the treadmill perturbations and the iKnee extension joint rotations, the algebraic sum of the treadmill perturbation only condition at each of the four timings and the iKnee joint rotation only condition was compared to the combined treadmill perturbation and iKnee extension joint rotation condition in a four (timing: -100 ms, -50 ms, 0 ms,

+50 ms) by two (condition: algebraic sum of the treadmill perturbation only condition and the iKnee joint rotation only condition, combined treadmill perturbation & iKnee extension joint rotation condition) within-subjects ANOVA. Separate ANOVAs were completed for the two different treadmill perturbation directions (+velocity and –velocity).

Greenhouse-Geisser corrected degrees of freedom were used to correct for violations of the assumption of sphericity. Differences with a probability of <0.05 were considered significant. Tukey's honestly significant difference post hoc tests were administered to determine the locus of the differences.

Results

Reflex responses following iKnee perturbation only trials

A summary of the mean ipsilateral and contralateral reflex response data across all participants following iKnee extension joint rotations at 50% of the gait cycle, including means and standard deviations, are provided in Table 2. Across all participants, the stretch reflex response in the iBF had a mean onset latency of 25 ± 3 ms. The cBF reflex was observed in all participants and had a mean onset latency of 80 ± 11 ms. The mean amplitude of the cBF reflex (250 ± 172% above normal gait) was significantly greater than the EMG activity during normal gait, $t_{(11)} = 4.61$, $p < 0.001$. Facilitatory contralateral reflex responses were also observed in the cSOL (11 out of 12 participants, mean onset latency 91 ± 20 ms) and in the cTA (six out of 12 participants, mean onset latency 96 ± 16 ms), while inhibitory contralateral reflex responses were observed in the cVL (eight out of 12 participants, mean onset latency 80 ± 11 ms) and cRF (six out of 12 participants, mean onset latency 80 ± 23 ms). No detectable responses were observed in the iRF. Mean data from one representative participant following iKnee extension joint rotations at 50% of the gait cycle are shown in Figure 2 (60 control and 60 iKnee perturbation steps). Mean reflex response onsets for this participant were: iBF = 22 ms; cBF = 73 ms; cRF = no response; cVL = 65 ms; cTA = 83 ms; cSOL = 83 ms.

[Insert **Table 2** and **Figure 2** about here]

cBF responses following treadmill only trials

Facilitation responses were also observed in the cBF muscle following trials with only sudden increases and decreases in treadmill velocity at all timings examined relative to 50% of the ipsilateral gait cycle (-100 ms, -50 ms, 0 ms, +50 ms). A summary of the mean cBF response onset latencies, durations, and amplitudes across all participants following treadmill only perturbation trials, including means and standard deviations, are presented in Table 3. Across all participants, the mean (*M*) onset latency of responses in the cBF following sudden increases or decreases in treadmill velocity ranged from 195 to 235 ms across the four timings. There were no significant main effects of either the timing or the direction of the treadmill velocity changes on the cBF response onset latencies, nor was there a significant interaction effect (all p's > 0.20). There was a

significant main effect of timing on the duration of the cBF responses following treadmill only trials, $F_{(3,33)} = 4.03$, $p = 0.015$. Post-hoc analyses revealed that sudden changes in treadmill velocity delivered 100 ms prior to 50% of the gait cycle (M = 315 ms) resulted in significantly longer responses in the cBF than when sudden changes in treadmill velocity occurred at 0 ms timing (M = 259 ms; $p = 0.035$) or 50 ms after 50% of the gait cycle (M = 264 ms; $p = 0.037$). There was also a significant main effect of timing on the amplitude of the cBF responses following treadmill only trials, $F_{(3,33)} = 5.31$, $p = 0.004$. Post-hoc analyses revealed that sudden changes in treadmill velocity delivered 50 ms after 50% of the gait cycle (M = 143% above normal gait) resulted in significantly smaller cBF response amplitudes than when sudden changes in treadmill velocity were applied 100 ms prior to (M = 214% above normal gait; $p = 0.034$), 50 ms prior to (M = 200% above normal gait; $p = 0.006$), or at 50% of the gait cycle (M = 170% above normal gait; $p = 0.048$). Additionally, cBF response amplitudes were significantly smaller following treadmill only perturbations at 50% of the gait cycle than when they were delivered 50 ms after 50% of the gait cycle ($p = 0.037$). For both cBF response duration and amplitude there were no effects of treadmill velocity change direction, nor were there any significant interaction effects (all p's > 0.05).

[Insert **Table 3** about here]

Effects of sudden changes in treadmill velocity on the cBF reflex

Results depicting the combined iKnee perturbation and treadmill velocity change conditions together with the algebraic sum of the treadmill perturbation only condition and the iKnee extension joint rotation only condition across all treadmill timings are shown in Figure 3. The same individual participant's data are also shown in Figure 2. The reflex analysis window for this participant was between 73 and 120 ms following iKnee perturbation onset. When the treadmill velocity was suddenly increased 100 ms and 50 ms prior to the onset of the iKnee perturbation, the combined iKnee & Treadmill condition resulted in a larger cBF reflex than the algebraic sum of the iKnee only and treadmill only conditions (Figure 3A and 3B, left panels). Conversely, when the treadmill velocity was suddenly decreased 100 ms and 50 ms prior to the onset of the iKnee perturbation, the combined iKnee perturbation & Treadmill condition resulted in a smaller cBF reflex (Figure 3A and 3B, right panels). When the sudden changes in treadmill velocity (+velocity or –velocity) occurred at the same time or 50 ms after the onset of the iKnee perturbation, the cBF

reflex of the combined iKnee & Treadmill condition was the same size as the algebraic sum of the iKnee only and treadmill only trials (Figure 3C and 3D).

[Insert **Figure 3** about here]

The results shown in Figure 3 were quantitatively similar across all participants, and confirmed by significant interaction effects between treadmill perturbation timing (-100 ms vs. -50 ms vs. 0 ms vs. +50 ms) and condition (algebraic sum of the treadmill perturbation only condition and the iKnee extension joint rotation only condition vs. combined treadmill perturbation & iKnee extension joint rotation condition), $F_{(3,33)} = 7.57$, $p = 0.001$ and $F_{(3,33)} = 13.69$, $p < 0.001$, for both sudden increases and sudden decreases in treadmill velocity, respectively. Figure 4 shows the mean data across all participants for the combined conditions when the sudden treadmill velocity changes occurred 100 ms, 50 ms, at (0 ms), or 50 ms after the onset of the iKnee perturbation. Post-hoc analyses revealed that, following sudden increases in treadmill velocity either 100 ms or 50 ms prior to iKnee onset, the initial component of the cBF reflex for the combined treadmill perturbation & iKnee extension joint rotation condition (-100 ms *M* = 331 % above normal gait; -50 ms *M* = 137% above normal gait) was significantly facilitated compared to the algebraic sum of the treadmill perturbation only condition and the iKnee joint rotation only condition (-100 ms *M* = 216 % above normal gait; -50 ms *M* = 106% above normal gait; both *p*'s ≤ 0.001). Conversely, following sudden decreases in treadmill velocity either 100 ms or 50 ms prior to iKnee onset, the initial component of the cBF reflex for the combined treadmill perturbation & iKnee extension joint rotation condition (-100 ms *M* = 127 % above normal gait; -50 ms *M* = 73% above normal gait) was significantly inhibited compared to the algebraic sum of the treadmill perturbation only condition and the iKnee joint rotation only condition (-100 ms *M* = 207 % above normal gait; -50 ms *M* = 112% above normal gait; both *p*'s ≤ 0.002). No such effects were observed when the sudden treadmill velocity changes occurred at the same time or 50 ms after iKnee perturbation onset (all *p*'s >0.15).

[Insert **Figure 4** about here]

Discussion

In this study, we examined the role of the cBF reflex in slowing the forward progression of the body following iKnee extension joint rotations applied during the late stance phase of the gait cycle. A significant decrease in the cBF reflex amplitude was observed when the treadmill velocity was decreased 100 ms and 50 ms prior to the onset of the iKnee perturbation compared to the algebraic sum of the cBF reflex when the two perturbations were elicited in isolation. Conversely, a significant increase in the cBF reflex amplitude was observed when the treadmill velocity was increased 100 ms and 50 ms prior to the onset of the iKnee perturbation. These results indicate for the first time in intact humans that contralateral reflexes are driven by sensory feedback arising from muscles located in the ipsilateral leg and that their expression is context dependent. This has important implications for the development of rehabilitation strategies for stroke survivors with gait asymmetries.

Previous studies have implicated the biceps femoris muscle in the reflexive braking or abrupt termination of human walking (Hase and Stein 1998; van der Linden et al. 2007). Rapid bilateral muscle responses occurred at 47-69 ms in the ipsilateral medial gastrocnemius, iRF, cTA and cBF when the support surface of the ipsilateral leg was unexpectedly lowered (van der Linden et al. 2007). The authors proposed that the muscle synergy triggered by the absence of expected heel contact was released in order to arrest the forward propulsion of the body. A similar muscle synergy was observed when participants were required to stop walking after detecting an electrical stimulus applied to the superficial peroneal nerve. However, the response onset latencies reported by Hase and Stein (1998) occurred later at 150-200 ms after the electrical stimulus and can be comparable to simple reaction time tasks in the lower limb. In the current study, the cBF reflex responses following iKnee extension joint rotations were observed at a mean onset latency of 80 ms, while sudden treadmill velocity changes alone resulted in responses in the cBF with mean onset latencies ranging between 195-207 ms. Given that voluntary influences may be included in the EMG signal after 120 ms following an unexpected muscle stretch (Lee and Tatton 1975), the responses in the cBF to the treadmill perturbations alone are unlikely to be considered involuntary reflexes, but voluntary responses arising from supraspinal structures similar to the responses reported by Hase and Stein (1998). Following expected or unexpected treadmill accelerations occurring during the initial stance phase of the right leg, responses in the

iBF muscle were observed ranging from 100-170 ms (Dietz et al. 1987). Methodological differences in relation to onset measures (from onset of the sudden treadmill velocity changes in the current study, vs from the onset of ankle joint displacement in the study by Dietz et al. (1987)) likely accounts for the discrepancy in response onset. However, differences in the amplitude or acceleration of the treadmill velocity change, are also not comparable between the two studies since Dietz et al. (1987) reported these with regards to the angle and angular acceleration of the right ankle joint, which we did not quantify in the present study.

An important consideration is that hip or ankle displacements may have contributed to the observed cBF responses. We believe this to be unlikely for several reasons. First, we previously found no changes in hip joint angles following iKnee extension joint rotations (Stevenson et al. 2013). Second, due to the late responses recorded in the cBF in the present study following treadmill perturbations alone (195-207 ms), it is likely that any movement at the hip would occur after this time and therefore not influence the amplitude of the cBF reflex response following combined iKnee & treadmill perturbations. Indeed, the current study and others investigating treadmill perturbations alone (e.g., Berger et al. 1984; Dietz et al. 1989; Dietz et al. 1984; Dietz et al. 1987), did not measure hip joint kinematics, thus it is difficult to speculate on the nature of any specific movement about the hip joints following the treadmill perturbations alone. Finally, regarding the ipsilateral ankle joint following the iKnee perturbations, pilot data (including ankle kinematics) collected prior to our previous study (Stevenson et al. 2013) revealed no changes in movement at the ankle joints within the first 120 ms following the iKnee perturbation (unpublished data).

Because a transcortical pathway contributes to the cBF reflex (Stevenson et al. 2013), it likely allows for more adaptable responses than purely spinally mediated reflexes due to integration with other sensory input at a cortical level, such as afferent information arising from the contralateral leg, in addition to visual and motivational factors (Christensen et al. 2001; Gervasio et al. 2013b; Zuur et al. 2009). Given the inherent instability in bipedal human walking compared to quadrupedal walking, cortical integration of various sources of sensory information may be advantageous in terms of maintaining dynamic stability following external perturbations (Christensen et al. 2001). However, there must be sufficient time for this sensory integration to occur. When the treadmill velocity was altered at the same time as, or 50 ms after, the iKnee

perturbation onset, the initial reflex component of the cBF reflex was unchanged. For example, the mean onset latency of the cBF reflex in the present study was 80 ms, so when the treadmill velocity was altered at the same time as the iKnee perturbation (i.e. 80 ms prior to the cBF reflex onset), the cBF reflex amplitude was unchanged. However, when the treadmill velocity was altered 50 ms prior to iKnee perturbation onset (i.e. 130 ms prior to cBF reflex onset), there was sufficient time for the cBF reflex to be significantly altered. Given that a transcortical pathway contributes to the cBF reflex, it is possible that the integration of sensory input arising from the iKnee extension joint rotation and the sudden treadmill velocity change occurs at a cortical level. However, this would need to be imperially verified using a combination of transcranial magnetic and electrical stimulation protocols.

In addition to demonstrating that the cBF reflex is modulated in a predictable manner following treadmill velocity changes, we also observed that the incidence of interlimb reflexes in other contralateral muscles increased following iKnee extension joint rotations delivered without treadmill perturbations (see Table 2). Increasing the level of postural threat during walking increases the amplitude of both intra- and interlimb reflexes in response to cutaneous stimulation (Haridas et al. 2006; 2005). Haridas et al. (2006; 2005) manipulated dynamic stability during walking by either applying unpredictable anterior-posterior (AP) perturbations to the trunk, having participants walk with their arms crossed, or a combination of unpredictable AP perturbations and arms crossed, and tested cutaneous reflexes during control strides where no external AP perturbations were applied. In the present experiment, participants were exposed to an unpredictable environment by perturbing walking with unexpected treadmill velocity changes, increasing postural threat. iKnee extension joint rotations were elicited in isolation as a control condition interspersed with these unexpected treadmill perturbations. In this way, the contralateral reflexes were elicited during a condition where the participants were made to believe that the situation was more unstable or unpredictable without any mechanical changes.

When the iKnee extension joint rotations were elicited in isolation interspersed with these unexpected treadmill perturbations, contralateral reflexes were observed in the cVL (eight out of 12 participants), cRF (six out of 12 participants), cSOL (11 out of 12 participants), and cTA (nine out of 12 participants) muscles (see Table 2), in addition to the cBF reflex. In comparison to previous studies, additional contralateral reflexes were observed in only three, two, four and seven out of

10 participants for each muscle, respectively, using the same iKnee perturbation parameters without any treadmill velocity perturbations throughout the experiment (Stevenson et al. 2013). This may suggest that the afferent input from the iBF muscle caused by the iKnee extension joint rotation converges onto motoneurons of these other contralateral muscles in such a way that the weighting is increased by the uncertain environment.

The uncertain environment created by the unexpected treadmill velocity perturbations allows us to examine the coordination of reflex responses in the contralateral leg following iKnee extension joint rotations (see Figure 2). For example, in eight out of 12 participants, the cBF reflex was accompanied by an inhibition in the cVL, and in six out of 12 participants an inhibition was also observed in the cRF. If the cBF reflex signifies a preparation of the contralateral leg for early load bearing (Stevenson et al. 2013), it might be expected that knee extensor EMG would also be augmented. However, the BF is biarticular, so it is possible that responses were also present in other muscles spanning the hip joint which were not measured. In support of a stabilizing response, facilitatory interlimb reflexes were observed in the cSOL (11 out of 12 participants) and cTA (nine out of 12 participants), indicating a level of co-contraction around the contralateral ankle joint. The mean onset latencies of the contralateral reflexes ranged between 80 and 96 ms, indicating the possibility that a transcortical pathway also contributes to these reflexes. The increased incidence of reflexes observed in the other contralateral leg muscles provides further evidence that specific interlimb reflex pathways during walking may be regulated appropriately to the environmental context (Haridas et al. 2006).

While eleven out of twelve participants were aged between 19-34 years (23.8 ± 4.3, mean ± S.D.), one participant was 55 years of age and may have been more affected by the side-effects of ageing than the others, which is particularly relevant in the context of dynamic balance (Krasovsky et al. 2012). However, the 55 year old participant reported no previous lower limb injuries or neurological conditions, and the participant's results were quantitatively similar to the other participants. For example, the onset of the cBF reflex was 72 ms with a duration of 454 ms and amplitude of 299% above normal walking (compare with means in Table 2). Furthermore, for the -100 ms, -50 ms, 0 ms, and +50 ms timings, the cBF reflex amplitudes following the combined iKnee & treadmill condition were 145%, 167%, 106%, and 96% of the algebraic sum of the iKnee and treadmill perturbations delivered alone in the +velocity trials, respectively (compare these values

414 with Figure 4). The corresponding cBF amplitudes following the combined –velocity trials were
415 48%, 61%, 104%, and 112%, respectively. Despite 55 years of age being at the lower end (or
416 outside) of the age range in studies investigating older adults (e.g., Krasovsky et al. 2012; Maver et
417 al. 2011; Stubbs et al. 2012), it is important to consider the possible differences as gait stability
418 and interlimb coordination are different in older adults (Krasovsky et al. 2012).

419 In conclusion, we observed a significant reduction of the cBF reflex elicited by iKnee extension
420 joint rotations when the treadmill velocity was suddenly decreased, and a significant facilitation of
421 the cBF reflex when the treadmill velocity was suddenly increased 50 or 100 ms prior to the iKnee
422 perturbation. The results suggest a functional role for the cBF reflex in slowing the forward
423 progression of the body and maintaining dynamic stability during walking. An increased
424 uncertainty in the walking surface created by the abrupt changes in treadmill velocity also
425 increased the incidence of responses in the other contralateral muscles, indicating a context
426 dependency for interlimb reflexes. A greater knowledge of the functionality of such interlimb
427 reflexes is important in understanding the neural control of human walking, particularly from a
428 rehabilitation perspective.

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Figure Captions

Figure 1: Experimental setup. (*A*) Wearing a safety harness, participants walked on a treadmill that allowed for the velocity of the belt to be changed rapidly. EMG signals were recorded from the ipsilateral (left) rectus femoris (iRF) and biceps femoris (iBF) muscles, along with the contralateral (right) rectus femoris (cRF), biceps femoris (cBF), vastus lateralis (cVL), soleus (cSOL) and tibialis anterior (cTA) muscles. (*B*) The gait cycle was defined so that the ipsilateral heel strike corresponded to 0% of the gait cycle and the next ipsilateral heel strike corresponded to 100%, represented by the black horizontal bar. Ipsilateral knee (iKnee) extension joint rotations were applied to the left knee joint during the late stance phase (50%) of the gait cycle when the ipsilateral leg was about to push off, and the contralateral leg had just touched down (black arrow). On certain steps, the treadmill velocity was unexpectedly increased (+velocity trials) or decreased (–velocity trials) before (-100 ms and -50 ms), at the same time (0 ms), following (+50 ms) the onset of iKnee perturbations, or at 0% of the gait cycle (gray arrows).

Figure 2: Ipsilateral and contralateral reflex responses following ipsilateral knee extension joint rotation (iKnee) only trials. Mean data from one participant for either normal gait (60 trials; black lines) or following ipsilateral knee extension joint rotations (60 trials; gray lines) during the late stance phase (50%) of the gait cycle (0-100%). (*A*) Ipsilateral knee angle; (*B*) mean rectified iBF electromyography (EMG); (*C*) contralateral knee angle; (*D*) mean rectified cBF EMG; (*E*) mean rectified cRF EMG; (*F*) mean rectified cVL EMG; (*G*) mean rectified cTA EMG; and (*H*) mean rectified cSOL EMG. Perturbation onset is represented by the vertical long-dashed lines. Reflex response onset is represented by the vertical short-dashed lines. Ipsilateral and contralateral stance phases are represented by the black bars below H. Note the period of facilitation in the cBF EMG following iKnee only trials, beginning about 73 ms after perturbation onset. Note also that this participant did not display any reflex responses in the cRF.

Figure 3: Evidence for extra-facilitation (+velocity trials) and inhibition (–velocity trials) of the cBF reflex following the combined iKnee perturbation and treadmill condition when the sudden changes in treadmill velocity were applied either 100 ms or 50 ms prior to iKnee perturbation onset. Mean rectified cBF EMG for one participant depicting either the treadmill only condition

(30 trials; dark gray lines), the combined iKnee perturbation and treadmill velocity change condition (30 trials; light gray lines), or the summation of the iKnee perturbation only (60 trials) and treadmill velocity change only conditions (black lines) following sudden increases (left panels) or decreases (right panels) in treadmill velocity at -100 ms (*A*), -50 ms (*B*), 0 ms (*C*) and +50 ms (*D*) relative to iKnee perturbation onset (vertical dashed lines). The onset of the treadmill velocity change is represented by the black arrows. The participant is the same as in Figure 1. Note the extra facilitation of the cBF reflex in the left panels of (*A*) and (*B*), and the inhibition of the cBF reflex in the right panels of (*A*) and (*B*), following the combined iKnee perturbation and treadmill velocity change condition.

Figure 4: Effect of altering timing of sudden treadmill velocity changes on the cBF reflex amplitude. Mean amplitude of the initial reflex component of the cBF reflex following a combination of iKnee perturbations delivered at 50% of the gait cycle, and sudden changes in treadmill velocity at the four different timings relative to iKnee perturbation onset investigated. Data are expressed as a percentage of the algebraic sum of the iKnee perturbation only and sudden treadmill velocity change conditions. The horizontal dashed line represents 100%. Filled circles represent sudden increases in treadmill velocity, while open circles represent sudden decreases in treadmill velocity. The asterisks indicate that the cBF reflex following combined iKnee and treadmill perturbations were significantly different than the algebraic sum of the iKnee only and treadmill only conditions for the -100 ms and -50 ms timings (all *P*'s < .01). Error bars represent standard error of the mean.

Table Legends

Table 1: Description of experimental conditions.

Table 2: Mean reflex response data for all participants following iKnee perturbation only trials. Data are included for all muscles collected, with standard deviations given in brackets.

Table 3: Mean cBF response characteristics for all participants following Treadmill only conditions. Data are included for the four different timings investigated, with standard deviations given in brackets.

560 **Table 1**

	Condition	Description	Number of Trials
1.	*Normal gait*	No ipsilateral knee extension joint rotations and no treadmill perturbations	60 trials
2.	*iKnee perturbation only*	Ipsilateral knee extension joint rotations delivered at 50% of the gait cycle	60 trials
3.	*Treadmill only –100 ms*	Treadmill perturbations delivered 100 ms prior to 50% of the gait cycle	30 +velocity trials and 30 –velocity trials
4.	*iKnee & Treadmill –100 ms*	A combination of conditions 2 and 3	30 +velocity trials and 30 –velocity trials
5.	*Treadmill only –50 ms*	Treadmill perturbations delivered 50 ms prior to 50% of the gait cycle	30 +velocity trials and 30 –velocity trials
6.	*iKnee & Treadmill –50 ms*	A combination of conditions 2 and 5	30 +velocity trials and 30 –velocity trials
7.	*Treadmill only 0 ms*	Treadmill perturbations delivered at 50% of the gait cycle	30 +velocity trials and 30 –velocity trials
8.	*iKnee & Treadmill 0 ms*	A combination of conditions 2 and 7	30 +velocity trials and 30 –velocity trials
9.	*Treadmill only +50 ms*	Treadmill perturbations delivered 50 ms following 50% of the gait cycle	30 +velocity trials and 30 –velocity trials
10.	*iKnee & Treadmill +50 ms*	A combination of conditions 2 and 9	30 +velocity trials and 30 –velocity trials
11.	*Control trials*	Treadmill perturbations at 0% of the gait cycle in order to decrease adaptation to the above treadmill perturbation timings	60 +velocity trials and 60 –velocity trials

561

562 **Table 2**

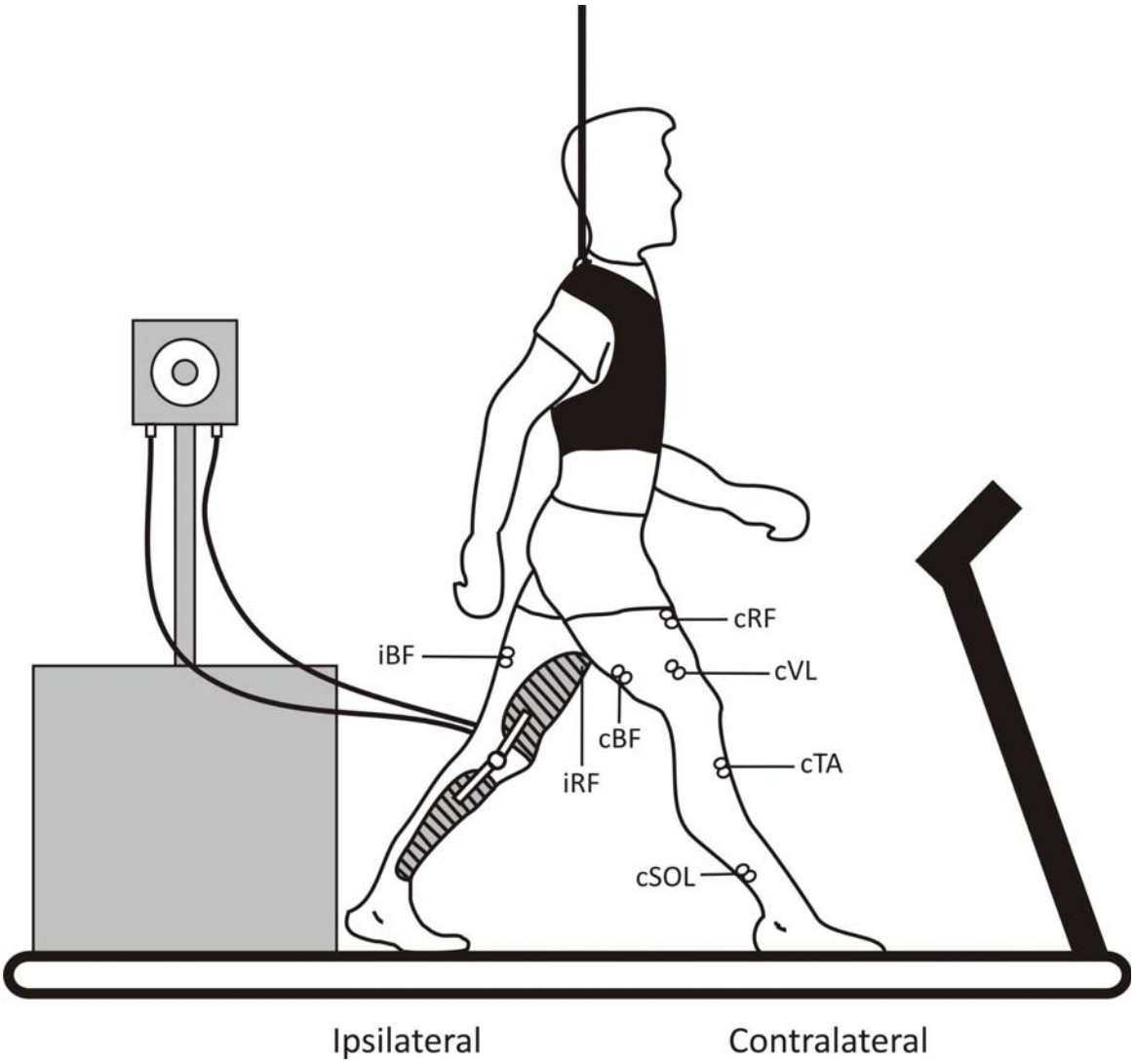
	iRF	iBF	cVL	cRF	cBF	cSOL	cTA
Count (no. of participants)	--	12/12	8/12	6/12	12/12	11/12	9/12
Onset (ms)		25.3 (3.1)	79.8 (11.0)	79.7 (23.2)	80.1 (10.8)	90.7 (19.5)	96.0 (16.3)
Duration (ms)		97.0 (84.9)	59.8 (14.4)	54.3 (30.6)	382.7 (124.0)	168.4 (92.7)	175.3 (103.0)
Amplitude (% above normal gait)		281.1 (214.2)	-26.7 (5.5)	-22.2 (7.8)	241.9 (181.9)	91.8 (30.9)	90.1 (50.5)

563 **Table 3**

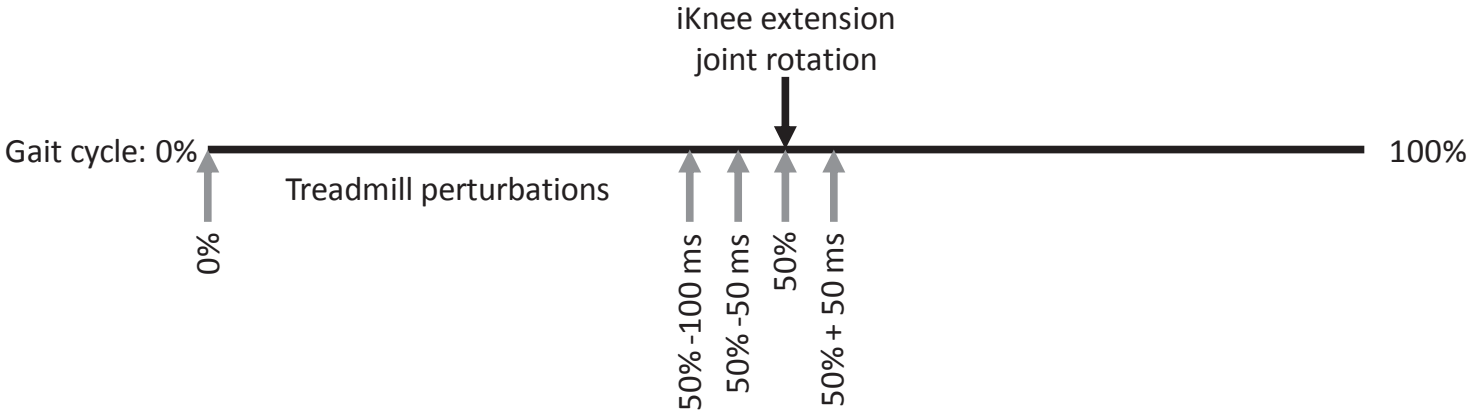
	+Velocity Trials		
Timing	*Response Latency (ms)*	*Response Duration (ms)*	*Response Amplitude (% above normal gait)*
-100 ms	207.4 (92.5)	232.9 (82.7)	277.0 (170.0)
-50 ms	199.1 (86.8)	259.5 (102.9)	247.3 (165.0)
0 ms	202.0 (94.7)	244.8 (104.7)	205.1 (120.9)
+50 ms	195.3 (82.1)	241.1 (99.3)	164.0 (135.1)
	–Velocity Trials		
Timing	*Response Latency (ms)*	*Response Duration (ms)*	*Response Amplitude (% above normal gait)*
-100 ms	235.3 (81.0)	396.3 (212.9)	151.0 (73.9)
-50 ms	207.1 (64.8)	330.0 (244.0)	152.2 (114.4)
0 ms	218.8 (87.1)	273.7 (204.3)	152.2 (114.4)
+50 ms	210.9 (54.0)	288.8 (194.2)	122.1 (118.5)

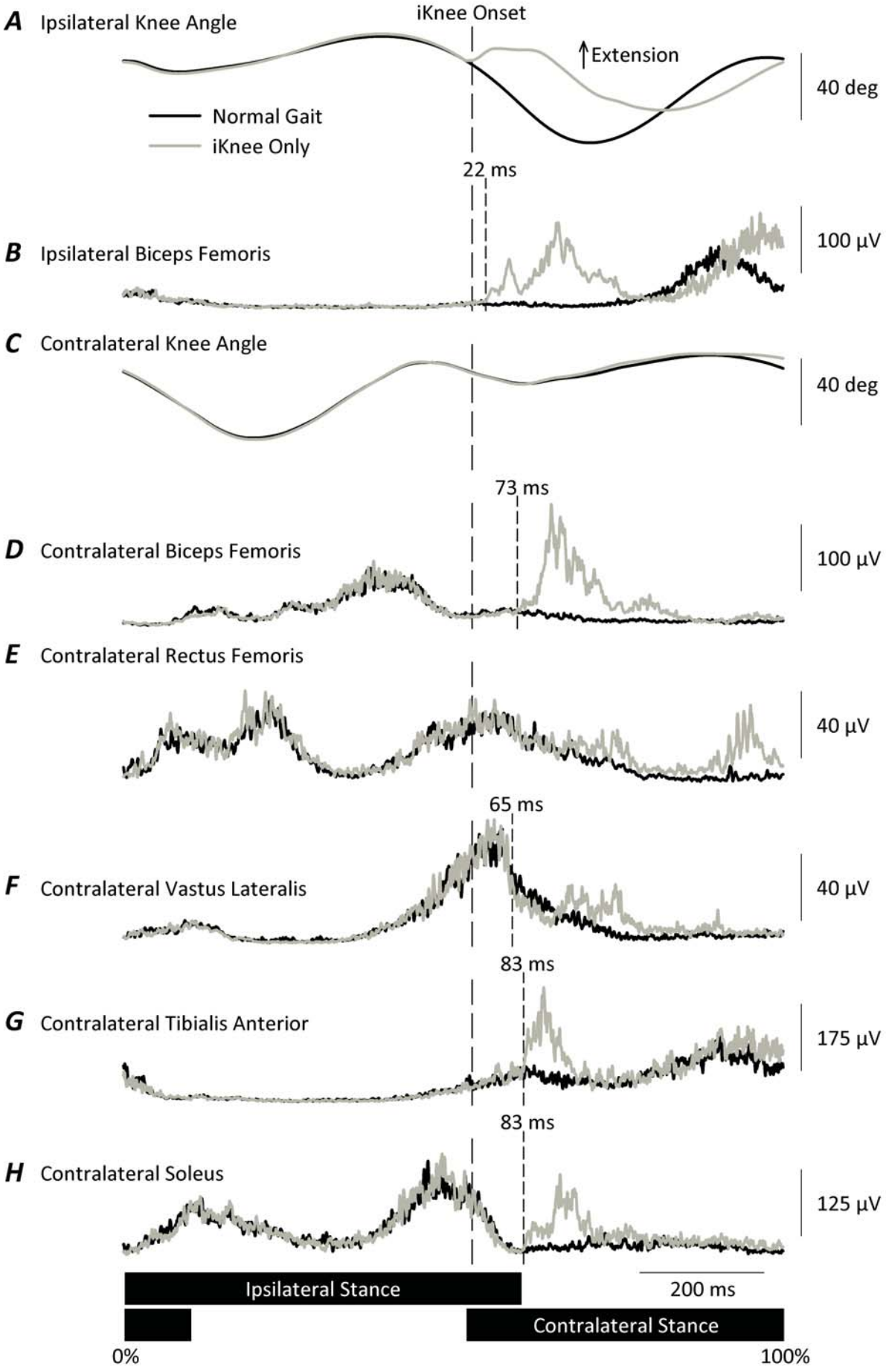
564

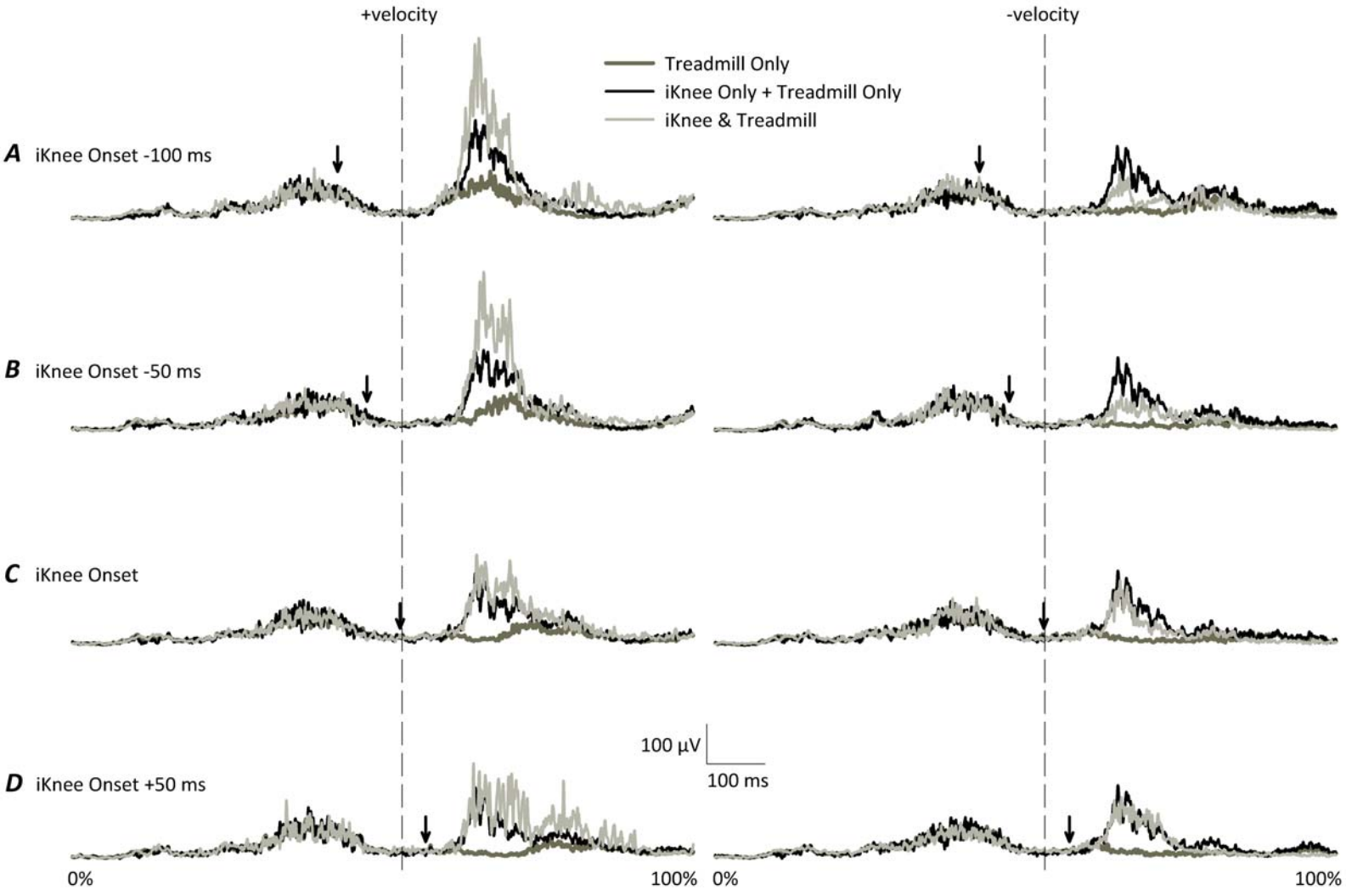
A

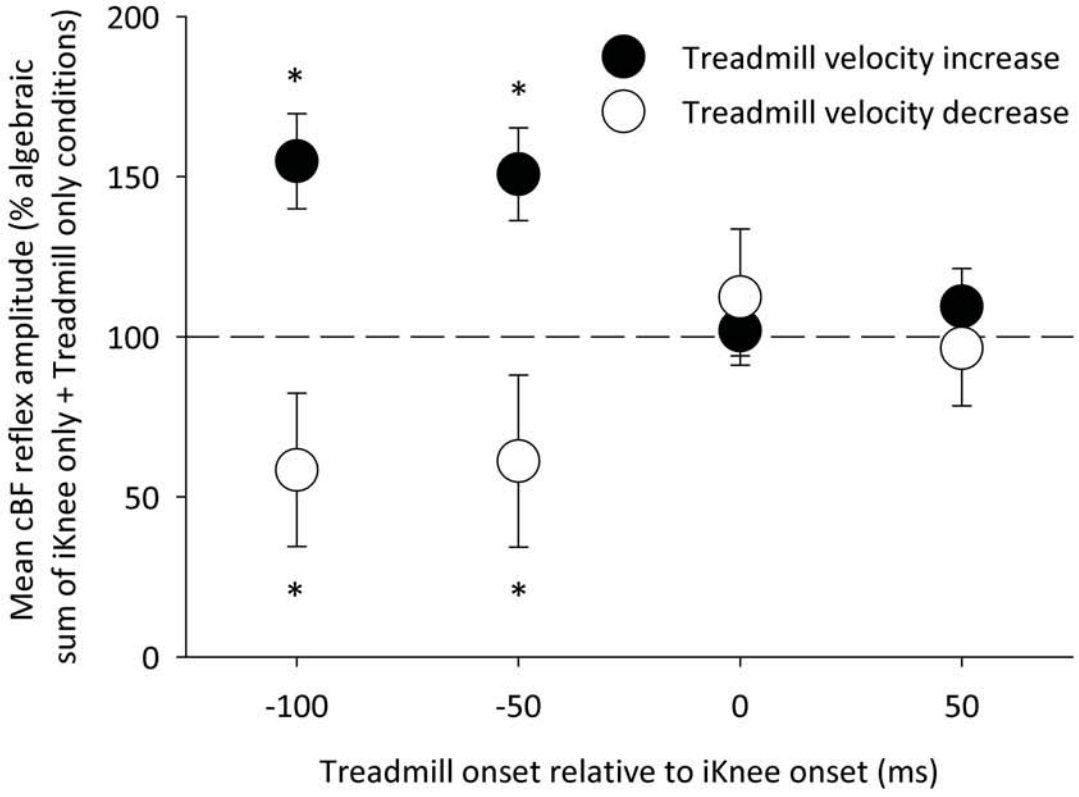

cRF
iBF
cVL
cBF
cTA
iRF
cSOL
Ipsilateral
Contralateral

B


iKnee extension
joint rotation
Gait cycle: 0%
100%
Treadmill perturbations
0%
50% -100 ms
50% -50 ms
50%
50% + 50 ms


A Ipsilateral Knee Angle
iKnee Onset
Extension
40 deg
Normal Gait
iKnee Only
22 ms
B Ipsilateral Biceps Femoris
100 µV
C Contralateral Knee Angle
40 deg
73 ms
D Contralateral Biceps Femoris
100 µV
E Contralateral Rectus Femoris
40 µV
65 ms
F Contralateral Vastus Lateralis
40 µV
83 ms
G Contralateral Tibialis Anterior
175 µV
83 ms
H Contralateral Soleus
125 µV
Ipsilateral Stance
200 ms
Contralateral Stance
0%
100%


+velocity
-velocity
Treadmill Only
iKnee Only + Treadmill Only
iKnee & Treadmill
A iKnee Onset -100 ms
B iKnee Onset -50 ms
C iKnee Onset
D iKnee Onset +50 ms
100 μV
100 ms
0%
100%
0%
100%


Mean cBF reflex amplitude (% algebraic sum of iKnee only + Treadmill only conditions)
200
150
100
50
0
*
*
*
*
Treadmill velocity increase
Treadmill velocity decrease
-100
-50
0
50
Treadmill onset relative to iKnee onset (ms)