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## **Interlimb communication**

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**INTERLIMB COMMUNICATION:  
CROSSED RESPONSES IN THE  
HUMAN BICEPS FEMORIS MUSCLE**

**BY  
ANDREW J. T. STEVENSON**

DISSERTATION SUBMITTED 2015



**AALBORG UNIVERSITY**  
DENMARK



# **Interlimb communication: Crossed responses in the human biceps femoris muscle**

A Ph.D. Thesis

By

**Andrew J. T. Stevenson, B.Sc., M.Sc.**

Department of Health Science and Technology



**AALBORG UNIVERSITY**  
DENMARK

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Ph.D. supervisor: Associate Professor Natalie Mrachacz-Kersting  
Aalborg University

Ph.D. co-supervisors: Professor Thomas Sinkjær  
Danish National Research Foundation  
Aalborg University

Professor Jens Bo Nielsen  
University of Copenhagen

Dr. Svend Sparre Geertsen  
University of Copenhagen

Ph.D. committee: Professor T. Richard Nichols  
Georgia Institute of Technology

Professor Albert Gollhofer  
University of Freiburg

Associate Professor Mark de Zee (chairman)  
Aalborg University

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## ABOUT THE AUTHOR



Andrew J. T. Stevenson was born in Nelson, New Zealand on 21 August 1985. He graduated with a Bachelor of Science degree in psychology and sport and exercise science from the University of Auckland, New Zealand in 2008. In 2007, Andrew was awarded the senior prize in the Department of Sport and Exercise Science at the University of Auckland. From November 2007 to June 2008, he completed a research internship in the Movement Neuroscience Laboratory at the University of Auckland under the supervision of Professor Winston D. Byblow and Dr. Cathy Stinear. Andrew completed a Master of Science degree in kinesiology at the University of British Columbia, Vancouver, Canada in 2011 under the primary supervision of Professor Ian M. Franks and committee members Professor Romeo Chua, Associate Professor Jean-Sébastien Blouin, and Dr. Dana Maslovat. The title of his Master's thesis was "*Examining cortical involvement in the StartReact effect using transcranial magnetic stimulation*". From 2011 to 2015, he was enrolled as a Ph.D. student at the Center for Sensory-Motor Interaction at Aalborg University, Denmark. His main research interests include movement neuroscience, neurophysiology, motor control and learning, electrophysiology, cortical stimulation, and gait rehabilitation.





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## PREFACE

The work presented in this Ph.D. thesis is the result of research carried out at the Center for Sensory-Motor Interaction (SMI) at Aalborg University, Denmark, and the Helene Elsass Center, Copenhagen, Denmark in the period from September 2011 to October 2014. The research was supported by SparNord Fonden, Det Obelske Familiefond, Oticon Fonden, and Otto Mønstedts Fond.

This thesis investigates the neural pathways that might underlie interlimb coordination in healthy humans. The overall objective of this thesis was to further investigate and elucidate neural pathways underlying interlimb communication in humans, focusing primarily on the likely interlimb connections to the biceps femoris muscle. Furthermore, this thesis aimed to provide evidence for the functional role of the observed interlimb reflexes during walking. In order to fulfill these aims, a number of different neurophysiological and behavioral techniques were applied to elucidate the pathways mediating the contralateral reflexes and their functional role.

Throughout the thesis, the studies are referred to by the Roman numerals I-IV. This thesis is based on four experimental studies; three studies have been published in the *Journal of Physiology* (Study I and III) and the *Journal of Neurophysiology* (Study IV), and the final manuscript is unpublished (Study II).

This thesis contains five chapters. The Introduction chapter presents the reader to the background and motivation for this project and provides a general overview of the Ph.D. thesis. The Methods chapter introduces the adopted methods and elaborates on the background for choosing these. The Results chapter presents the main findings in this thesis; the reported findings are further elaborated in the original papers. The Discussion chapter aims at interpreting the significance and implications of these findings. Finally, the Conclusions chapter sums up the main findings and future perspectives of this work. The appendix includes complete versions of the papers.

Andrew J. T. Stevenson,

Aalborg, 2015



## LIST OF ARTICLES

The Ph.D. thesis is based on four original studies that produced three peer-reviewed journal articles and one unpublished manuscript:

- I. **Stevenson, A. J. T.**, Kamavuako, E. N., Geertsen, S. S., Farina, D. & Mrachacz-Kersting, N. Short-latency crossed responses in the human biceps femoris muscle. *Journal of Physiology*, doi: 10.1113/JP270422.
- II. **Stevenson, A. J. T.** & Mrachacz-Kersting, N. *Influence of ipsilateral knee joint rotation velocity and amplitude on short-latency crossed responses in the human biceps femoris muscle*. Unpublished.
- III. **Stevenson, A. J. T.**, Geertsen, S. S., Andersen, J. B., Sinkjær, T., Nielsen, J. B., & Mrachacz-Kersting, N. (2013). Interlimb communication to the knee flexors during walking in humans. *Journal of Physiology*, 591(19), 4921-4935, doi: 10.1113/jphysiol.2013.257949.
- IV. **Stevenson, A. J. T.**, Geertsen, S. S., Sinkjær, T., Nielsen, J. B., & Mrachacz-Kersting, N. (2015). Interlimb communication following unexpected changes in treadmill velocity during human walking. *Journal of Neurophysiology*, 113(9), 3151-3158, doi: 10.1152/jn.00794.2014.

The original papers may be found in the appendix. In addition, several conference abstracts were based on the research conducted in this Ph.D. thesis.



## OTHER PUBLICATIONS RELATED TO THIS THESIS

- Mrachacz-Kersting, N., Geertsen, S. S., **Stevenson, A. J. T.**, & Nielsen, J. B. *Convergence of ipsi- and contralateral Ia afferents on common interneurons mediating reciprocal inhibition of ankle plantarflexors in humans.* In preparation.
- Stevenson, A. J. T.**, Kamavuako, E. N., Geertsen, S. S., Sinkjær, T., Farina, D. & Mrachacz-Kersting, N. (2015). *Short-latency crossed responses in the human biceps femoris muscle.* Paper presented at the annual conference of the Society for the Neural Control of Movement, Charleston, SC, United States.
- Stevenson, A. J. T.**, Geertsen, S. S., Sinkjær, T., Nielsen, J. B., & Mrachacz-Kersting, N. (2014). *Functionality of the contralateral biceps femoris reflex response during human walking.* In *Replace, Repair, Restore, Relieve – Bridging Clinical and Engineering Solutions in Neurorehabilitation: Proceedings of the 2nd International Conference on NeuroRehabilitation (ICNR2014), Aalborg, 24-26 June, 2014*, ed. Jensen W., Andersen O. K., & Akay M., pp. 765-773, Springer International Publishing, doi: [http://dx.doi.org/10.1007/978-3-319-08072-7\\_106](http://dx.doi.org/10.1007/978-3-319-08072-7_106).
- Stevenson, A. J. T.**, Geertsen, S. S., Sinkjær, T., Nielsen, J. B., & Mrachacz-Kersting, N. (2014). *Interlimb communication: Short-latency crossed spinal reflexes in the biceps femoris following ipsilateral knee joint rotations in seated humans.* Paper presented at the annual conference of the Society for Neuroscience, Washington, DC, United States.
- Stevenson, A. J. T.**, Geertsen, S. S., Sinkjær, T., Nielsen, J. B., & Mrachacz-Kersting, N. (2013). *Interlimb communication: Functionality of the contralateral biceps femoris reflex response during human walking.* Paper presented at the annual conference of the Society for Neuroscience, San Diego, CA, United States.
- Stevenson, A. J. T.**, Sinkjær, T., & Mrachacz-Kersting, N. (2012). *Interlimb communication between knee flexors during a sitting task.* Paper presented
-

at the annual conference of the Society for Neuroscience, New Orleans, LA, United States.

**Stevenson, A. J. T.**, Andersen, J. B., & Mrachacz-Kersting, N. (2012). *Interlimb communication between knee flexors in the late stance phase of human walking*. Paper presented at the annual conference of the International Society of Electromyography and Kinesiology, Brisbane, Australia.

Geertsen, S. S., **Stevenson, A. J. T.**, Nielsen, J. B., & Mrachacz-Kersting, N. (2012). *Spatial facilitation of reciprocal inhibition and crossed inhibitory responses to soleus motoneurons*. Paper presented at the biannual conference of the Federation of European Neuroscience Societies, Barcelona, Spain.

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inspiration, encouragement, and motivation. Thank you for being with me every step of the way – I could not have done it without you.



## ENGLISH SUMMARY

A continual coordination between the two legs is necessary for maintaining a symmetric walking pattern and adapting to changes in the external environment. Recent evidence in animals and humans suggests that spinal interneuronal circuits under supraspinal control may mediate communication between the lower limbs. The overall objective of the present thesis was to further investigate and elucidate neural pathways underlying interlimb communication in humans, focusing primarily on the possible interlimb connections to the biceps femoris muscle. The major aims were 1) to investigate whether interlimb reflexes are present in sitting and walking following ipsilateral knee (iKnee) joint rotations (Studies I and III), 2) to elucidate the neural pathways involved in mediating the interlimb reflexes (Studies I, II and III), and 3) to investigate the functional role of the observed interlimb reflexes during walking (Study IV).

Study I demonstrated that short-latency (44 ms) crossed-spinal reflexes are present in the contralateral biceps femoris (cBF) muscle during sitting. The cBF reflexes were inhibitory following iKnee extension joint rotations, facilitatory following iKnee flexions, and intramuscular recordings revealed that the same population of cBF motor units were involved in the reversal in sign of the reflex. Study II indicated that velocity sensitive muscle spindle afferents likely contribute to the short-latency cBF reflexes.

Study III showed that, while short-latency interlimb reflexes were not observed during walking, strong cBF reflex responses were evoked from iKnee extension joint rotations in the late stance phase with an onset latency of 76 ms. An increase in evoked responses in the cBF from transcranial magnetic stimulation (TMS) and not transcranial electrical stimulation (TES) following iKnee extensions provided evidence for a transcortical pathway contributing to this interlimb reflex. Study IV demonstrated that the functional role of the cBF reflex is likely a preparation for early load bearing, slowing the forward progression of the body to maintain dynamic equilibrium during walking. Therefore, the transcortical cBF reflex may be integrated with other sensory input, allowing for responses that are more adaptable to the environmental demands.

These results provide new insights into the neural mechanisms underlying human interlimb communication, as well as their functional relevance to human locomotion. Although it is difficult to propose the exact neural pathways mediating interlimb reflexes to the cBF muscle, this thesis provides the basis for future studies.

## DANISH SUMMARY / DANSK RESUMÉ

En kontinuerlig koordination mellem de to ben er nødvendig for at opretholde et symmetrisk gangmønster og for at kunne adaptere til ændringer i det eksterne miljø. Nylige studier i dyr og mennesker tyder på, at interneuronale kredsløb i rygmarven, under supraspinal kontrol, kan mediere kommunikationen mellem underekstremiteterne (såkaldt interlimb kommunikation). Det overordnede formål med denne afhandling var, at undersøge og belyse nervebanerne bag interlimb kommunikation hos mennesker, primært med fokus på de mulige interlimb forbindelser til m. biceps femoris. Mere præcist var målet 1) at undersøge, om interlimb reflekser er til stede i siddende position og under gang efter ipsilaterale knæledsrotationer (iKnæ) (Studie I og III), 2) at belyse hvilke nervebaner der er involveret i at mediere interlimb reflekser (Studie I, II og III), og 3) at undersøge den funktionelle betydning af de observerede interlimb reflekser under gang (Studie IV).

Studie I viste, at krydsende, rygmarvsreflekser med kort latenstid (44 ms) er til stede i den kontralaterale biceps femoris (cBF) muskel i siddende mennesker. cBF-reflekserne var inhibitoriske efter iKnæ-ekstension og ledrotationer og excitatoriske efter iKnæ-fleksioner, og intramuskulære målinger viste, at den samme population af cBF-motoriske enheder var involveret i ændringen af refleksens fortegn. Studie II viste, at hastighedsfølsomme muskeltene afferenter sandsynligvis bidrager til de kort-latente cBF-reflekser.

Studie III viste, at mens kort-latente interlimb-reflekser ikke blev observeret under gang, så fremkaldte iKnæ-ekstensioner i den sene standfase stærke cBF-refleksvar med en latenstid på 76 ms. Data fra forsøg med transkranial magnetstimulation (TMS) og transkranial elektrisk stimulation (TES) indikerer at en transkortikal forbindelse bidrager til denne interlimb-refleks. Studie IV viste, at cBF-refleksens funktionelle rolle sandsynligvis er, at forberede det kontralaterale ben til at bære kroppens vægt tidligere, og bremse den fremadgående bevægelse af kroppen til at kunne opretholde dynamisk balance under gang. Derfor kan den transkortikale cBF-refleks integreres med andre sanseindtryk, der giver mulighed for reaktioner, som kan tilpasses til eksterne krav.

Disse resultater giver ny viden om de neurale mekanismer bag interlimb kommunikation hos mennesker samt deres funktionelle betydning for menneskets gang. Selv om det er vanskeligt at fastslå de nøjagtige nervebaner bag interlimb reflekser til cBF-musklen, danner denne afhandling grundlag for fremtidige undersøgelser.

## LIST OF ABBREVIATIONS

ANOVA,	analysis of variance
cAnkle	contralateral ankle
cBF,	contralateral biceps femoris
cGL,	contralateral gastrocnemius lateralis
cHip	contralateral hip
cKnee	contralateral knee
comINs,	commissural interneurons
cRF,	contralateral rectus femoris
cSOL,	contralateral soleus
cTA,	contralateral tibialis anterior
cVL,	contralateral vastus lateralis
EMG,	electromyography
EPSP,	excitatory postsynaptic potential
iBF,	ipsilateral biceps femoris
iEMG	integrated electromyography
iKnee,	ipsilateral knee
IPSP,	inhibitory postsynaptic potential
iRF,	ipsilateral rectus femoris
<i>M</i> ,	mean
MEP,	motor evoked potential
MU,	motor unit
MVC,	maximum voluntary contraction
pps,	pulses per second
PSTH,	peristimulus time histogram
PTN,	posterior tibial nerve
RMS,	root mean square
rTMS,	repetitive transcranial magnetic stimulation
SD,	standard deviation
SEM,	standard error of the mean
SLR,	short-latency reflex
TA,	tibialis anterior
TES,	transcranial electrical stimulation
TMS,	transcranial magnetic stimulation
+velocity,	trials with an initial sudden increase in treadmill velocity
-velocity,	trials with an initial sudden decrease in treadmill velocity



## THESIS AT A GLANCE

	Question	Methods	Answer
I	<p>a. Are there short-latency crossed-spinal reflexes in the cBF following iKnee joint rotations during pre-sitting?</p> <p>b. Do the cBF reflexes follow the automatic gain principle?</p> <p>c. Can the cBF reflexes be quantified at the motor unit level?</p>	<p>25 healthy volunteers, sitting, iKnee joint rotations, different pre-contraction levels, surface EMG, intramuscular EMG.</p>	<p>a. Yes, short-latency (44 ms) inhibitory and facilitatory crossed-spinal reflexes were observed in the cBF following iKnee extension and flexion joint rotations, respectively.</p> <p>b. Yes, the short-latency cBF reflexes increased in magnitude with increases in pre-contraction levels in the cBF.</p> <p>c. Yes, the short-latency cBF inhibition and facilitation were observed in the same population of motor units.</p>
II	<p>Do velocity- and amplitude-sensitive muscle spindle afferents contribute to the short-latency cBF reflexes?</p>	<p>13 healthy volunteers, sitting, iKnee joint rotations, manipulation of perturbation velocity and amplitude, surface EMG.</p>	<p>Velocity sensitive muscle spindle afferents contribute to both the short-latency cBF inhibition and facilitation.</p>
III	<p>a. Do afferent pathways from the iKnee joint muscles contribute to interlimb reflexes in the contralateral leg during human walking?</p> <p>b. Does a transcortical pathway contribute to the cBF reflex?</p>	<p>17 healthy volunteers, walking, iKnee joint rotations, surface EMG, TMS, TES.</p>	<p>a. Yes, consistent reflexes were observed in the cBF muscle (onset 76 ms) following iKnee extension joint rotations during the late stance phase of the gait cycle.</p> <p>b. A, transcortical pathway likely contributes to the cBF reflex.</p>
IV	<p>What is the functional role of the cBF reflex in maintaining dynamic stability following iKnee extension joint rotations during walking?</p>	<p>12 healthy volunteers, walking, iKnee extension joint rotations, surface EMG, sudden treadmill velocity changes.</p>	<p>The cBF reflex likely acts to slow the forward progression of the body in order to maintain dynamic stability following the iKnee extension joint rotations.</p>

cBF, contralateral biceps femoris; EMG, electromyography; iKnee, ipsilateral knee; TES, transcranial electrical stimulation; TMS, transcranial magnetic stimulation.





# 1

## INTRODUCTION

Human walking involves the complex coordination of many different muscles, joints, and limbs. The human locomotor system contains a large amount of flexibility and adaptability, facilitating the impressive ability to adapt to a variety of different external perturbations or changes to the walking surface. The integrated activities of descending supraspinal motor commands, ascending sensory feedback signals and spinal neuronal circuitries allows for this flexibility (Duysens & Van de Crommert, 1998; Duysens *et al.*, 2002; Nielsen, 2003). Of particular importance to maintaining dynamic stability and balance during walking when faced with adapting to changes in the external environment is the precise adjustment of coordinated muscle activation between the limbs, known as interlimb coordination. The aim of this chapter is to review the current knowledge about interlimb coordination as it relates to locomotion, beginning with a brief overview of the motor control of human walking.

### 1.1 Motor control of human walking

During human walking, the central nervous system coordinates a large number of muscles in both legs to contract and relax with precise timing and force. The general consensus is that the complex control of human walking is executed by an interaction of descending motor commands from supraspinal structures, the activation of spinal neuronal networks that generate rhythmic movements (i.e., central pattern generators, CPGs), and sensory feedback (Duysens & Van de Crommert, 1998; Duysens *et al.*, 2002; Nielsen, 2003). While a thorough review of the motor control of human walking is beyond the scope of this thesis, I will briefly introduce the above-mentioned terms below in order to place human interlimb coordination into context.

Due to the limitations in performing invasive experiments in humans, and the similarities that exist between animals and humans, much of the existing literature on human walking results from knowledge gained from animal

models, particularly from cats (Nielsen, 2003). However, it is important to consider the differences in the control of locomotion between cats and humans. The most obvious difference is the fact that locomotion in cats is quadrupedal, while it is bipedal in humans due in part to the upper limbs in humans becoming specialized to perform skilled hand movements. Additional structural differences in the bipedal human compared to the quadrupedal cat include an increase in the angle between the spine and pelvis to ensure an upright position of the upper body, the spine is more curved to keep the upper body centered above the pelvis, the foramen magnum is repositioned such that the head is balanced on the spine, the lower limbs are elongated, and the femurs are angled more inwardly so that the legs are positioned beneath the pelvis (Nielsen, 2003). Bipedal walking provides unique challenges for the motor control of human walking, such as maintaining a tall body above an unstable support of two long legs (Nielsen *et al.*, 1993; Dietz, 2002).

### 1.1.1 Central pattern generators (CPGs)

While there is a large amount of evidence in animals on the existence of CPGs, evidence for their existence in humans remains indirect (see Duysens & Van de Crommert, 1998; McCrea & Rybak, 2007; Ivanenko *et al.*, 2009; Molinari, 2009 for reviews). CPGs refer to functional neuronal networks located in the spinal cord with the ability to generate rhythmical activity, typically between flexor and extensor muscle groups (Duysens & Van de Crommert, 1998). Experiments have shown that cats still have the ability to generate rhythmic alternating contractions in flexor and extensor muscles of the ankle despite a completely transected spinal cord and the removal of essentially all sensory input by severing the dorsal root afferents to the spinal cord (Brown, 1911, 1912). These experiments confirm the intrinsic ability of the spinal cord to generate rhythmic activity. While invasive experiments, such as severing neural pathways, are not possible in humans, indirect evidence for CPGs in humans comes from patients with spinal cord injuries displaying stepping-like movements (Calancie *et al.*, 1994; Dobkin *et al.*, 1995) and infant stepping (Lam & Yang, 2000). For example, alternating rhythmical activity in leg muscles has been elicited in rare cases in patients with complete spinal cord lesions (Bussel *et al.*, 1996a, b; Dimitrijevic *et al.*, 1998), and involuntary stepping-like movements were observed in incomplete spinal cord injured patients unable to produce voluntary movements (Calancie *et al.*, 1994;

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Dobkin *et al.*, 1995). However, supraspinal input cannot be completely ruled out in these human studies due to the spinal cord injuries being incomplete.

It has been recently suggested that human bipeds use quadrupedal coordination (Dietz *et al.*, 2002). Experiments in humans have indicated that there is a neural coupling between upper and lower limb muscles during various locomotor activities, such as walking, crawling and swimming (Wannier *et al.*, 2001). In these activities, movements of the arms and legs are locked with a fixed frequency relationship. For example, even if the leg movements during swimming are slowed by flippers, or if the mechanical resistance of the movements is minimized, the coordination between legs and arms is preserved (Wannier *et al.*, 2001). Additional evidence for neural coupling between upper and lower limbs comes from experiments where mechanical perturbations of one leg evoked reflexes in the upper limbs during walking, the amplitude of which are modulated throughout the gait cycle (Dietz *et al.*, 2001). Cutaneous nerve stimulation in the legs also elicited phase-dependent reflexes in the arms during walking (Dietz *et al.*, 2001). These arm muscle responses were absent while participants were standing while voluntarily swinging their arms, or sitting while writing, demonstrating the task dependency relating to rhythmical activity (Dietz *et al.*, 2001).

### 1.1.2 Supraspinal control

While Section 1.1.1 describes how rhythmical movements are possible without descending supraspinal input (at least in animals), evidence suggests that drive from descending pathways contributes importantly to human walking (Capaday, 2002; Nielsen, 2002; Nielsen, 2003; Yang & Gorassini, 2006; Barthelemy *et al.*, 2011). The primary descending pathways can be divided into three groups, including the corticospinal tract and two groups descending from the brainstem; the first comprising the tectospinal, vestibulospinal, interstitiospinal, and part of the reticulospinal tract, and the second comprising the rubrospinal pathway and the crossed reticulospinal tract from the pontine lateral tegmental regions (Rothwell, 1986). The vestibulospinal and reticulospinal tracts of the first brainstem group make abundant collateral connections and are involved in the synergistic activation of many of muscles. Transections of these pathways in cats results in significant locomotor and postural deficits (Brustein & Rossignol, 1998), while

these deficits are not observed in humans when the descending pathways of the second brainstem group are impaired (Nathan, 1994).

The corticospinal tract, arising from the primary motor cortex, is more important in humans than in cats (Nielsen, 2003). While the primary motor cortex is essential to human walking, motor commands generated in the primary motor cortex do not necessarily require an intact corticospinal tract to be transmitted to the spinal cord as other descending pathways, such as the reticulospinal tract, may be able to compensate (Nathan, 1994; Nielsen, 2003; Barthelemy *et al.*, 2011). In patients who have experienced a lesion to the sensorimotor cortex, hemiparesis can cause abnormal control of the paretic lower limb, resulting in an asymmetrical gait pattern and gait impairments (Wade *et al.*, 1987; Jorgensen *et al.*, 1995). For example, only 37% of stroke survivors are able to walk within the first week (Jorgensen *et al.*, 1995).

Non-invasive neurophysiological techniques have also demonstrated the importance of the primary motor cortex during walking. Suprathreshold transcranial magnetic stimulation applied to the primary motor cortex has demonstrated that an overall decrease in corticospinal excitability occurs in walking compared to standing, and that corticospinal excitability is modulated throughout the gait cycle (Petersen *et al.*, 1998b; Capaday *et al.*, 1999). However, since modulations in corticospinal excitability during walking may be caused by changes at the cortical or subcortical level, it was not possible to determine the extent of involvement of the primary motor cortex in walking from this study (Capaday *et al.*, 1999). Applying subthreshold TMS to the primary motor cortex, on the other hand, only activates inhibitory intracortical networks, not the descending corticospinal tract, and has been shown to suppress the ongoing muscle activity by reducing corticospinal drive (Davey *et al.*, 1994). Applied during walking, subthreshold TMS has been demonstrated to temporarily suppress muscle activity in the soleus and tibialis anterior (TA) muscles, thereby providing evidence that the primary motor cortex plays an important role during human walking (Petersen *et al.*, 2001).

Functional imaging studies have provided further evidence for the involvement of the primary motor cortex during walking and cycling (Fukuyama *et al.*, 1997; Christensen *et al.*, 2000a). A study using single photon emission tomography demonstrated a significant increase in blood flow to the leg area of the sensory-motor cortex, the cerebellum, and the frontal cortex

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during walking in healthy humans (Fukuyama *et al.*, 1997). However, it was unclear how much of the increased blood flow was related to the afferent feedback and how much was related to the generation of motor output. Using positron emission tomography, Christensen *et al.* (2000a) demonstrated that the only area with a larger cerebral blood flow when comparing passive cycling (i.e., only afferent feedback) to voluntary cycling was in a restricted area corresponding to the leg representation in the primary motor cortex. Therefore, most of the cerebral activity during cyclical movements appears to be generated by sensory afferent feedback (Nielsen, 2003).

Besides the primary motor cortex, the cerebellum is also an important structure in the supraspinal control of human walking, posture and balance (Morton & Bastian, 2004a, 2007). Therefore, one of the most common impairments in patients with cerebellar damage is walking ataxia, or walking incoordination (Earhart & Bastian, 2001; Morton & Bastian, 2004a, 2007). Cerebellar patients are often described as having a 'drunken gait'. The cerebellum plays a role in the generation of appropriate patterns of limb movements, the dynamic regulation of balance, and the adaptation of posture and locomotion through practice (Morton & Bastian, 2004a). Importantly, patients with cerebellar damage show consistent impairments in adapting to perturbations during standing and walking (Rand *et al.*, 1998), and adapting to novel walking environments (Morton & Bastian, 2004b; Morton & Bastian, 2006).

### 1.1.3 Sensory feedback

Sensory information is proposed to play at least three major roles in the motor control of human walking: 1) providing an error signal informing the brain of differences between the intended movement and the movement that was actually executed, 2) driving active motoneurons, and 3) contributing to corrective reflexes following sudden perturbations (Nielsen & Sinkjær, 2002; Nielsen, 2003). With respect to the first of these roles, the error signals informed by sensory afferent information may be used to adapt and update the locomotor pattern in the future (Prokop *et al.*, 1995; Erni & Dietz, 2001).

In support of the second role, strong evidence for sensory feedback driving active motoneurons during human walking was provided by suddenly altering the angle of the ankle joint during walking using a portable mechanical actuator device (Sinkjær *et al.*, 2000). When the actuator imposed

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a sudden plantar flexion movement of the ankle during the stance phase, the plantarflexors were shortened and the active muscles unloaded. Unloading the active plantarflexor muscles during the stance phase caused a significant decrease ongoing plantarflexor muscle activity. Control experiments showed that the drop in activity of the plantar flexors was not simply due to reciprocal inhibition from the antagonistic dorsiflexor muscles, indicating that the cause was the cessation of sensory afferent activity, leading to a decreased motoneuronal drive to the plantarflexor motoneurons from these afferents (Sinkjær *et al.*, 2000).

Many experiments have examined the third role sensory feedback plays in human walking by examining corrective reflexes following sudden perturbations at different phases of the gait cycle (Berger *et al.*, 1984; Duysens *et al.*, 1990; Zehr *et al.*, 2001; e.g., Mrachacz-Kersting *et al.*, 2004; Stubbs & Mrachacz-Kersting, 2009). Fast, functional changes in the muscle activation of both legs occur in response to perturbations during both stance and swing phases, and these changes depend greatly on the particular phase of the gait cycle in which these perturbations occur (Berger *et al.*, 1984; Dietz *et al.*, 1986; Haridas *et al.*, 2006; Bachmann *et al.*, 2008). Intralimb and interlimb reflexes have been proposed to play an important role in the central neural coordination of different limbs in both animals and humans (Miller & van der Meché, 1976; Nichols, 1989; Zehr *et al.*, 2001; Haridas *et al.*, 2006; Honeycutt *et al.*, 2009; Stubbs *et al.*, 2011b). For example, a sudden forward or backward acceleration of one leg during standing or applying a holding impulse to the leg during the swing phase of the gait cycle evoked reflex responses in synergistic muscles of both legs (Dietz *et al.*, 1986; Dietz *et al.*, 1989). The role of interlimb reflexes in the control of interlimb coordination during walking, including the neural mechanisms and pathways mediating interlimb reflexes, is the primary focus of the current Ph.D. thesis. The next section will provide an overview of the background on interlimb reflexes in both animal models and humans as they relate to locomotion.

## 1.2 The role of interlimb reflexes in interlimb coordination

### 1.2.1 Interlimb reflexes

Since Sherrington (1910) first discovered the crossed extensor reflex in the cat, numerous studies have been conducted attempting to characterize and investigate the importance of interlimb reflexes to the coordination between limbs in both animal models and humans. Spinal and supraspinal interlimb reflexes have been proposed to play an important role in adjusting to external perturbations and compensating for threats to dynamic stability during locomotion (Zehr *et al.*, 2001; Haridas *et al.*, 2006). Different techniques are used to highlight these interlimb reflex pathways in animals and humans, and it may be speculated that if they are present during particular points in the gait cycle, they may have a role in actively contributing to the ongoing locomotor EMG. Gaining a deeper understanding of the underlying neural mechanisms of interlimb reflexes in humans is important for the development of improved rehabilitation strategies in patients with gait impairments.

Interlimb reflexes can arise following a stimulus (e.g., electrical), mechanical perturbation or disturbance to one limb, resulting in changes to muscle activity (e.g., facilitation, inhibition) and/or joint angles in a different limb. The reflexes can be caused by activation of muscle spindles, golgi tendon organs, cutaneous (skin) receptors, and/or nociceptive (pain) receptors. Afferent nerves from these sensory receptors carry information to the spinal cord and brain where it is integrated with descending motor commands. Motor (efferent) nerves arising from the spinal cord transmit the integrated information to the muscles of other limbs, causing them to contract or relax. While interlimb reflexes can be elicited from and recorded in, the upper limbs in humans (Zehr *et al.*, 2001; Dietz, 2002), the primary focus of the current thesis is on interlimb reflexes between the lower limbs. Much of the knowledge about interlimb reflexes in humans is based on the results of more invasive experiments in animal models. The next section therefore provides a brief overview of the literature in animals.

### 1.2.2 Interlimb reflexes in animals

Commissural interneurons (comINs), characterized in animal studies, are spinal interneurons connecting afferent nerves from one side of the spinal

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cord to interneurons and motoneurons on the contralateral side (Jankowska, 2008). They are proposed to play a pivotal role in interlimb coordination in animals and possibly humans, in part through the mediation of interlimb reflexes (Jankowska, 2008). ComINs have been identified within the spinal cord of cats in lamina IV, V (Matsushita, 1970; Bras *et al.*, 1989; Bannatyne *et al.*, 2006), VI, VII (Jankowska *et al.*, 2009) and VIII (Matsushita, 1970; Jankowska & Noga, 1990; Edgley *et al.*, 2003; Hammar *et al.*, 2004) of the dorsal horn. ComINs project to a number of areas in the contralateral spinal cord, including contralateral motoneurons and interneurons (Bannatyne *et al.*, 2006; Jankowska *et al.*, 2009).

The effects of stimulating various types ipsilateral afferents on the responses observed in the contralateral motoneurons have been investigated in animal preparations (Arya *et al.*, 1991; Jankowska, 2008). Following stimulation of group Ia and Ib ipsilateral muscle afferents, both inhibitory and excitatory responses were observed in contralateral motoneurons with latencies consistent with di-, tri-, and polysynaptic pathways (Jankowska, 2008). Following stimulation of group II muscle afferents, the majority of responses observed in the contralateral motoneurons were inhibitory (Arya *et al.*, 1991). Additionally, stimulation of contralateral group Ia, group Ib and group II afferents has shown that comINs project to group Ia, group Ib and group II interneurons on the contralateral side of the spinal cord (Jankowska *et al.*, 2005; Jankowska, 2008; Jankowska *et al.*, 2009).

Descending supraspinal input has been shown to have a strong influence on the behavior of comINs in cats, which has been highlighted by animal studies where the spinal cord is completely transected (Arya *et al.*, 1991). In cats with an intact spinal cord, electrical stimulation of ipsilateral group II afferents elicited predominantly inhibitory postsynaptic potentials (IPSPs) in contralateral motoneurons. However, following spinal cord transection, a reversal in sign of responses from IPSPs to excitatory postsynaptic potentials (EPSPs) was observed in contralateral extensor muscle motoneurons, while the effects on contralateral flexor muscle motoneurons remained inhibitory (Arya *et al.*, 1991). Furthermore, the administration of serotonin restored IPSPs to the contralateral extensor muscle motoneurons in the spinalized cats, while the administration of a serotonin agonist reversed the effects back to EPSPs (Aggelopoulos *et al.*, 1996). Taken together, these results indicate that a tonic descending drive involving serotonin neurotransmitters is likely necessary to



generate IPSPs in contralateral motoneurons following group II afferent input via comINs (Arya *et al.*, 1991; Aggelopoulos *et al.*, 1996).

Many other descending pathways have been implicated in the control of interlimb coordination via comINs in cats. Brainstem networks including the rubrospinal tract (Stecina *et al.*, 2008), the reticulospinal tract (Matsuyama & Jankowska, 2004), the vestibulospinal tract (Krutki *et al.*, 2003), and the mesencephalic locomotor region (Le Ray *et al.*, 2011), along with corticospinal tract neurons (Jankowska *et al.*, 2006; Stecina & Jankowska, 2007) all provide inputs to comINs. The mesencephalic locomotor region may be particularly important in the control of interlimb coordination; stimulation of this area following a transection superior to the mesencephalon resulted in walking, running and trotting (Shik & Orlovsky, 1976). While much is known about interlimb reflexes in animals, an understanding of the prevalence and importance of such reflexes are limited in humans, due in part to the inability to perform invasive studies in humans as can be done with animals.

### 1.2.3 Interlimb reflexes in humans

To quantify interlimb reflexes in humans, different non-invasive 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.*, 1984; Dietz *et al.*, 1987; Dietz *et al.*, 1989). A sudden forward or backward acceleration of one leg during standing or applying a holding impulse to the leg during the swing phase of the gait cycle evoked reflex responses in synergistic muscles of both legs, likely in order to increase stability and compensate for the perturbation (Dietz *et al.*, 1986; Dietz *et al.*, 1989). Similarly, a full body loading or unloading during the stance phase of walking altered the activation of muscles in both limbs (Bachmann *et al.*, 2008).

Different perturbations of the walking surface elicited bilateral reflex responses specific to the mode of perturbation, and were dependent on the phase of the gait cycle (Berger *et al.*, 1984). For example, sudden decreases in treadmill velocity evoked a bilateral TA muscle activation, while sudden increases in treadmill velocity evoked an ipsilateral gastrocnemius activation and contralateral TA activation (Berger *et al.*, 1984). In the same study, tibial nerve stimulation at the beginning of stance phase was followed by an

ipsilateral TA activation, while during the swing phase it was followed by an ipsilateral TA activation and a contralateral gastrocnemius activation (Berger *et al.*, 1984). Despite the different muscle activation patterns following these different perturbations during gait, it was hypothesised that the same basic functional mechanism was involved: the early ipsilateral response achieved a repositioning of the displaced foot and leg, while the early contralateral and later ipsilateral responses provided compensation for body displacement (Berger *et al.*, 1984).

One of the major roles of interlimb reflexes during walking is to maintain dynamic equilibrium (Dietz, 2002). While both intra- and interlimb reflexes are phase-modulated throughout the gait cycle, bilateral responses are relatively stronger when perturbations are delivered during phase transitions between stance to swing or vice versa (Berger *et al.*, 1984; Dietz *et al.*, 2004). Furthermore, when external perturbations act against the physiological movement trajectory of specific lower limb joints, responses are much larger, indicating that these response patterns are intended to restore the physiological movement trajectory (Dietz *et al.*, 2004). This suggests that there may be an increased need for momentary interlimb coordination at these times in order to compensate for external perturbations.

Results of the studies mentioned above indicate that muscle afferent feedback has a dominant role in mediating these interlimb reflexes, possibly at a spinal level. This is somewhat surprising as the onset of the responses recorded ranged between 65 to 112 ms. Given that ipsilateral reflexes in the lower limb with latencies greater than 54 ms (rectus femoris muscle) and 79 ms (TA muscle) can be cortically mediated (Petersen *et al.*, 1998a; Mrachacz-Kersting *et al.*, 2006), it is possible that some of these contralateral reflexes could be mediated by a transcortical pathway. 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.

Although comINs have not been directly identified in humans due to the inability to perform similar invasive studies as in animals, recent studies have provided evidence for short-latency crossed spinal reflex pathways in humans (Stubbs & Mrachacz-Kersting, 2009; Stubbs *et al.*, 2011a, b; Stubbs *et*

*al.*, 2012; Gervasio *et al.*, 2013a). Stubbs and Mrachacz-Kersting (2009) found a short-latency inhibition in the contralateral soleus (cSOL) following electrical stimulation applied to the posterior tibial nerve (PTN) of the ipsilateral leg during isometric plantar flexion. Since the latency of the depression was very short (onset 37-41 ms), the response must be mediated by spinal pathways. Indeed, the minimum latency for a transcortical pathway to mediate ipsilateral responses in the TA muscle, which is located at a similar distance from the cortex as the triceps surae, is 79 ms (Petersen *et al.*, 1998a). Taken together, these results support the hypothesis that comINs exist in humans and contribute to interlimb coordination (Stubbs & Mrachacz-Kersting, 2009; Stubbs *et al.*, 2011a, b).

Ischemia of the lower leg delayed, but did not abolish, the crossed-spinal inhibition, suggesting that both larger-diameter group I and smaller-diameter group II muscle afferents mediate the response (Stubbs & Mrachacz-Kersting, 2009). Further support for the crossed-spinal inhibition being mediated by input from ipsilateral muscle or tendon afferents from the homologous muscle was provided by the fact that no similar crossed responses were elicited by stimulation of the ipsilateral sural or medial plantar nerves, which are primarily cutaneous nerves (Stubbs & Mrachacz-Kersting, 2009; Stubbs *et al.*, 2011b).

The short-latency crossed-spinal inhibitory response in the cSOL is modulated throughout different phases of the gait cycle, suggesting that the response has a functional role and has a contribution of input from higher centers (Stubbs *et al.*, 2011b). The cSOL inhibition was largest immediately prior to the swing to stance transition of the ipsilateral leg (90% of the gait cycle), which is when the threat to balance is greatest. The PTN stimulation evokes a plantar flexion in the ipsilateral ankle joint, while at 90% of the gait cycle the ankle is moving into dorsiflexion. The effect of the short-latency inhibition in the cSOL likely acts to slow the forward progression of the body and maintain dynamic stability by decreasing the amount of push off in the contralateral leg following the unexpected perturbation (Stubbs *et al.*, 2011b).

In addition to an inhibition in the cSOL inhibition following ipsilateral PTN stimulation during the late swing phase of the gait cycle, a short-latency crossed facilitation was observed in the synergistic contralateral gastrocnemius lateralis (cGL) muscle (Gervasio *et al.*, 2013a). Further underlining the functional importance of short-latency crossed responses, a

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crossed reflex reversal occurred in the cGL muscle during hybrid walking (legs walking in opposite directions) when the contralateral leg was touching down (Gervasio *et al.*, 2013a). The cGL response also produces an anterior-medial displacement in the center of pressure, likely preserving dynamic stability by accelerating the propulsion phase of the contralateral leg thus preparing for a faster step in the event that the stimulated leg is not able to sustain body weight (Gervasio, 2014).

Short-latency crossed-spinal reflexes arising from ankle joint muscle afferents have also recently been identified in human upper leg muscles following isolated ankle joint rotations that seem to be functionally relevant (Mrachacz-Kersting *et al.*, 2011). An excitation response in the contralateral biceps femoris (cBF) muscle (onset 62 ms) was quantified following unexpected ipsilateral ankle joint rotations during gait, unloading the ipsilateral soleus during the mid-stance phase (Mrachacz-Kersting *et al.*, 2011). The reflex in the cBF muscle may indicate a preparation of the contralateral leg for an earlier touch down, because an unloading of the ipsilateral soleus during mid-stance signals an earlier take-off of the ipsilateral leg (Mrachacz-Kersting *et al.*, 2011). Ischemia diminished the response, suggesting that larger-diameter afferents arising from the ipsilateral ankle muscles mediated this contralateral reflex (Mrachacz-Kersting *et al.*, 2011).

#### **1.2.4 Supraspinal input to lower limb reflex pathways in humans**

Aside from descending input from higher centers, afferent feedback from muscle receptors contributes to the activation pattern of agonist and antagonist muscles of the same leg, and is known to play an important role in modifying their activation to attain stability and to adapt to varying situations (Dietz, 1992; Duysens *et al.*, 1998; Sinkjær *et al.*, 2000; Nielsen, 2002; Nielsen, 2004). Ipsilateral muscle generated afferent feedback may be mediated by either purely spinal pathways, or via a transcortical loop (Christensen *et al.*, 2000b; Christensen *et al.*, 2001; Zuur *et al.*, 2009). It has been suggested that transcortically mediated reflexes may have the ability to be integrated with other sensory information at the level of the cortex, and therefore be better adapted to varying situations than reflexes mediated via the spinal cord (Christensen *et al.*, 2001; Zuur *et al.*, 2009).

Christensen *et al.* (2001) provided the first evidence for a contribution of the primary motor cortex to the late component of stretch reflexes in leg muscles during walking. A stretch reflex was evoked in the TA muscle after a plantar flexion perturbation in the mid-swing phase of walking. TMS delivered to the primary motor cortex 60-120 ms after plantar flexion perturbations resulted in a facilitation of the MEPs measured in the TA. However, MEPs evoked by transcranial electrical stimulation (TES), which are not influenced by motor cortical excitability, were not facilitated, indicating therefore that the late component of the stretch reflex in this situation was likely to have been mediated by the primary motor cortex. In addition, Zuur *et al.* (2009) provided evidence that the primary motor cortex contributes to the TA stretch reflex elicited during the mid-stance phase of the gait cycle by applying 1Hz repetitive TMS (rTMS) over the primary motor cortex, which temporarily reduces the excitability of the cortex. Furthermore, the second component of the short-latency crossed-spinal reflex in the cGL following ipsilateral PTN stimulation also appears to involve the contribution of a transcortical pathway (Mrachacz-Kersting *et al.*, 2014). While the first component of the cGL reflex appears to be spinally mediated and was unaffected by subthreshold TMS, the second, later component was inhibited by subthreshold TMS (Mrachacz-Kersting *et al.*, 2014). Taken together, these results provide strong evidence that the primary motor cortex plays an important role in reacting to external perturbations in both ipsilateral and contralateral legs during walking.

In addition to phase modulation throughout the gait cycle, further evidence that the short-latency crossed-spinal inhibition in the cSOL is under cortical control arises from the finding that the response is altered in stroke patients during both sitting (Stubbs *et al.*, 2012) and walking (Stubbs *et al.*, in preparation). Responses were significantly more variable in sub-acute than chronic patients, likely due to the continuing reorganization of cortical networks that become more stable from three to six months post-stroke (Stubbs *et al.*, 2012). Impaired interlimb reflexes in stroke patients potentially indicates an inability to coordinate the two legs following external perturbations, which may contribute to an increased risk of falls (Stubbs *et al.*, 2012).

## 1.3 Methodological considerations

### 1.3.1 Ankle vs. knee joints

The majority of recent studies in humans have examined interlimb reflexes mostly by electrically stimulating afferent nerves arising from muscles crossing the ankle joint (Stubbs & Mrachacz-Kersting, 2009; Mrachacz-Kersting *et al.*, 2011; Stubbs *et al.*, 2011b; Stubbs *et al.*, 2012). However, animal models have shown that afferent nerves arising from the quadriceps and hamstring muscle groups have stronger contralateral reflex connections compared to ankle muscle afferent nerves, especially from group II afferents (Arya *et al.*, 1991). Afferent input from upper leg muscles also plays an important role in aiding transition between phases of the gait cycle, specifically from flexion to extension. Furthermore, muscles acting about the knee joint have particularly strong ipsilateral connections in humans (Pierrot-Deseilligny *et al.*, 1981; Marchand-Pauvert & Nielsen, 2002). Based on these findings, the studies in the present Ph.D. thesis were designed to assess contralateral responses following isolated knee joint rotations during sitting and walking in humans.

### 1.3.2 Mechanical perturbations

There are considerable differences in how mechanical perturbations and electrical stimulation activate the sensory-motor system in humans. While both electrical stimulation and mechanical perturbations activate sensory nerve pathways, peripheral nerve stimulation bypasses the fusimotor system and the mechanical stimulus of the muscle spindles due to direct stimulation of the afferent nerve fibres. In addition, mechanical perturbations are not as specific as electrical stimulation. While some muscles are stretched by a perturbation, antagonistic muscles are unloaded, and single-joint perturbations can influence multi-joint muscles. Furthermore, in contrast to the stretch reflex, peripheral nerve stimulation causes a very short, temporally summated, synchronized firing of nerve afferents. Mechanical perturbations eliciting stretch reflexes may therefore provide a more natural input to the nervous system than electrical nerve stimulation.

A semi-portable actuator system with a functional joint has been developed at Aalborg University's Center for Sensory-Motor Interaction, which can

induce unexpected ankle (Andersen & Sinkjær, 1995) or knee (Andersen & Sinkjær, 2003) joint rotations throughout the gait cycle. Sinkjær et al. (1996) used the device about the ankle to demonstrate that the stretch reflex of the soleus muscle is significantly modulated during the gait cycle, while Mrachacz-Kersting et al. (2004) found a modulation in the quadriceps stretch reflex during the transition from swing to stance phase when using the device about the knee. The semi-portable actuator system provides a useful methodology to further investigate interlimb coordination during the gait cycle in humans.

## 1.4 Aims and hypotheses

The overall objective of the present thesis was to further investigate and elucidate neural pathways underlying interlimb communication in humans, focusing primarily on the likely interlimb connections to the biceps femoris muscle. There were three major aims of this Ph.D. project:

1. To investigate whether interlimb reflexes are present in sitting and walking following ipsilateral knee (iKnee) joint rotations.
2. To elucidate the neural pathways involved in mediating interlimb reflexes.
3. To investigate the functional role of the observed interlimb reflexes during walking.

Four studies were performed to fulfill these aims:

**Study I:** Short-latency crossed responses in the human biceps femoris muscle.

The primary aim of Study I was to investigate whether short-latency crossed reflexes are present in the cBF muscle following iKnee joint rotations during a sitting task, where participants maintained a slight pre-contraction in the target muscle. It was hypothesized that short-latency crossed-spinal reflexes would be observed in the cBF muscle following iKnee joint rotations, and that these interlimb reflexes would depend on the direction of the rotation applied to the iKnee joint.

**Study II:** Influence of ipsilateral knee joint rotation velocity and amplitude on short-latency crossed responses in the human biceps femoris muscle.

The primary aim of Study II was to determine whether ipsilateral velocity- and/or amplitude-sensitive muscle spindle afferents are involved in mediating the short-latency crossed reflexes in the cBF observed in Study I by independently manipulating the velocity and amplitude of the iKnee joint rotations. It was hypothesized that changes in both perturbation velocity and amplitude will influence the size of the cBF reflex, independent of perturbation direction.

**Study III:** Interlimb communication to the knee flexors during human walking.

The primary aim of Study III was to assess interlimb reflexes following isolated iKnee joint rotations during human gait. Unilateral flexion perturbations were applied to the left knee joint at 0%, 10%, 30%, 50% and 90% of the gait cycle, as these are points where transitions occur from periods of single support to periods of double support and vice versa and a stronger requirement for interlimb coordination is expected during phase transitions in the gait cycle (Berger *et al.*, 1984; Dietz *et al.*, 2004). It was hypothesized that extension perturbations would elicit short-latency reflex (SLR) responses in the contralateral hamstrings muscles, and flexion perturbations would elicit SLR responses in the contralateral quadriceps muscles, and that these responses would be modulated throughout the gait cycle. Contrary to our initial hypothesis, consistent reflex responses were only observed in the cBF muscle following knee extension joint rotations. Based on the latency of the observed interlimb reflex response, it was investigated whether a transcortical pathway could contribute to the response.

**Study IV:** Interlimb communication following unexpected changes in treadmill velocity during human walking.

The aim of Study IV was to investigate whether the transcortical 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. It was 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, it was hypothesized that if the treadmill velocity was changed too close to the onset of the cBF reflex (e.g., +50 ms), the cBF reflex would be unaltered.



# 2

## METHODS

This chapter will describe the methods applied in this Ph.D. thesis. After a description of the general methodology common to all the studies, a description of the techniques applied in the individual studies will follow.

### 2.1 Participants

Healthy participants with no physical or neurological disorders took part in the experiments. In accordance with the standards of the Declaration of Helsinki, all participants provided their written informed consent to the protocol approved by the Scientific Ethics Committee of Nordjylland (approval number: 20110076) and the Capital Region of Denmark (Experiments 2 and 3 in Study III; approval number: H-A-2008-029). Participant numbers, age ranges and mean ages are presented in Table 2.1.

Table 2.1: Participant details for all studies.

Study	Number of participants	Number of males and females	Age range (years)	Mean age (years; $\pm$ SD)
I	32	20 males, 12 females	18-39	25.5 $\pm$ 4.2
II	13	9 males, 4 females	19-30	23.7 $\pm$ 4.1
III	17	10 males, 7 females	22-41	30.3 $\pm$ 6.3
IV	12	6 males, 6 females	19-55	26.4 $\pm$ 4.4

For all experiments included in this Ph.D. thesis, interlimb reflex responses were investigated by an externally applied unexpected knee joint rotation, TMS, TES, unexpected treadmill velocity changes, or by a combination of these. The unexpected knee joint rotations were applied with either a stationary hydraulic joint actuator system or a portable mechanical joint actuator device (Andersen & Sinkjær, 1995; Voigt *et al.*, 1999; Andersen & Sinkjær, 2003).

## 2.2 Apparatus and instrumentation

Bipolar surface electromyography (EMG) quantified muscle activity throughout the Ph.D. project. For Studies I and II, surface EMG electrodes were placed over the belly of the ipsilateral (right) rectus femoris (iRF) and biceps femoris (iBF), along with the contralateral (left) RF (cRF) and BF (cBF) muscles (see Figure 2.1). In Experiment 3 of Study I, surface EMG activity was only collected from the iRF and cBF muscles. For Studies III and IV, the EMG activity was recorded by bipolar surface electrodes (Medicotest 720-01-K) placed over the belly of 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 TA (cTA) muscles (see Figure 2.2). In Experiments 2 and 3 of Study III, EMG activity was only collected from the iBF, cBF, and cSOL muscles. The electrodes were placed in accordance with the recommendations of Cram *et al.* (1998). The surface EMG signals were amplified and band-pass filtered at 10 Hz to 1 kHz and sampled at 2 kHz. They were also rectified and low pass filtered at 20 Hz offline (Butterworth 1st order digital filter).

Intramuscular EMG was recorded from the cBF muscle using two bipolar wire electrodes in Experiment 3 of Study I. Intramuscular recordings were adopted primarily to verify whether the same cBF motoneurons could have different behaviors depending on the inhibitory or facilitatory reflexes, and to confirm that changes in the surface EMG are not interference produced by the mechanical perturbation. Intramuscular wire electrodes were made of Teflon-coated stainless steel (A-M Systems, Carlsborg WA, diameter 50  $\mu\text{m}$ ) and were inserted with a sterile 25-gauge hypodermic needle. The insulated wires were cut to expose 3 mm of the wire. The needle was inserted into the muscle and then removed to leave the wire electrodes inside the muscle. Intramuscular signals were analog bandpass filtered between 0.1 and 4.4 kHz and sampled at 10 kHz.

Flexible electrogoniometers (XM180 series, Biometrics Ltd, UK) were used in Study I (Experiment 1b) to measure the left (contralateral) hip (cHip), knee (cKnee) and ankle (cAnkle) joint angles, and in Study III (Experiment 1) to trace the left and right hip joint angles of all participants, as well as the right knee joint angle. One electrogoniometer was used in Study IV to trace the right knee joint angle of two participants.

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In Studies III and IV, 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 position-feedback data were collected at a sampling frequency of 2 kHz.

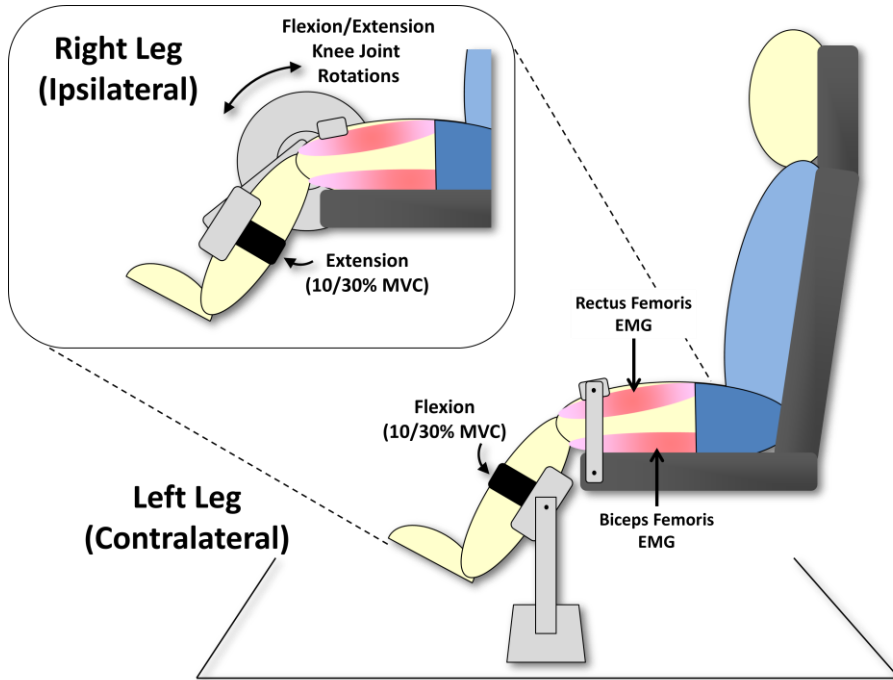
## 2.3 Stationary hydraulic joint actuator system (Studies I and II)

In Studies I and II, a stationary device was implemented to rotate the iKnee joint (see Figure 2.1). Participants were seated in a chair that was fixed to the floor, with their hip and knee joints flexed at 90° and 45°, respectively. The right (ipsilateral) leg was affixed to a servo-controlled hydraulic actuator (MTS-systems Corporation 215.35; Voigt *et al.*, 1999), such that the anatomical knee axis of rotation was closely aligned with the fulcrum of the actuator. The lower segment of the right leg of the participant was firmly strapped to a custom-made plate that extended from the actuator, thus producing a tight interface between the arm of the motor and the leg of the participant. The lower segment of the left (contralateral) leg was firmly strapped to a custom-made plate that extended from the floor such that the left leg was in the same start position as the right leg. In addition, the knees of both legs were fixed with a custom-made adjustable bar fixed to the chair and placed over the upper thigh slightly proximal to the knee joint. This position minimized both the hip and ankle movement, ensuring that the movement of the actuator was transmitted solely to the knee joint. The angular position of the actuator was monitored with an angular displacement transducer (DC ADT series 500, Trans-Tek Inc., USA).

### 2.3.1 General setup for sitting experiments

For Studies I and II, visual feedback of the rectified surface EMG activity from the iRF and cBF was provided on a computer monitor placed in front of the participants with a custom-made computer program. Initially, the participants were asked to perform a maximal isometric voluntary contraction (MVC) of the iKnee extensors and contralateral knee flexors, simultaneously. This was repeated twice and the best effort for each muscle was deemed the MVC. In all subsequent trials, the participants were asked to

tonically pre-activate the iRF and cBF to a level that produced EMG activity corresponding to 10%, or 30% of MVC. This was chosen to reflex the opposite actions of the legs during the gait cycle.



**Figure 2.1: General experimental setup of the stationary hydraulic joint actuator system used in Studies I and II.** Participants sat comfortably in a chair with their hip and knee joints flexed at 90° and 45°, respectively. The right (ipsilateral) leg was affixed to a servo-controlled hydraulic actuator such that the anatomical knee axis of rotation was closely aligned with the fulcrum of the actuator. The lower segment of the right leg of the participant was firmly strapped to a custom-made plate that extended from the actuator. The lower segment of the left (contralateral) leg was firmly strapped to a custom-made plate that extended from the floor such that the left leg was in the same start position as the right leg. The knees of both legs were fixed with a custom-made adjustable bar fixed to the chair and placed over the thigh slightly proximal to the knee joint. Surface EMG activity was recorded from the iRF and iBF muscles, along with the cRF and cBF muscles. Intramuscular EMG activity was also recorded from the cBF in Experiment 3 of Study I. Participants were asked to isometrically pre-activate the iRF and cBF to a level that produced EMG activity corresponding to 10% or 30% MVC. Knee flexion and extension joint rotations (4-8° and 75-200°/s) were imposed in separate blocks of 30-60 trials every 3-5 s.

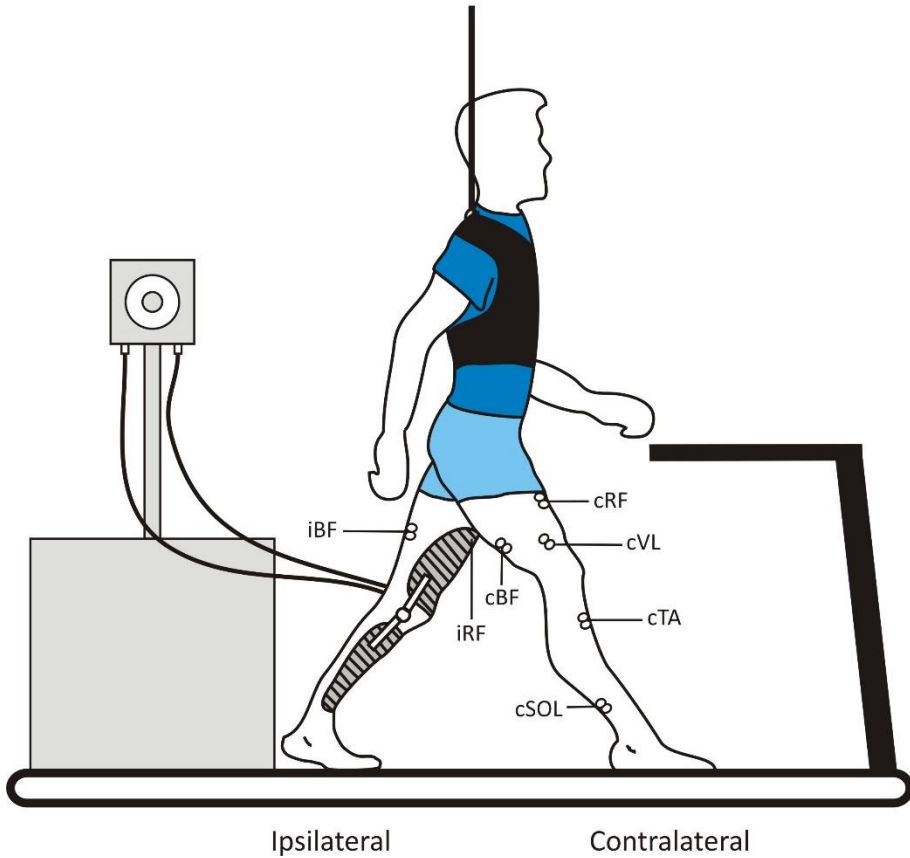
## 2.4 Portable mechanical joint actuator device (Studies III and IV)

The portable mechanical joint actuator device used in Studies III and IV consisted of a functional joint that could reliably induce unexpected knee joint rotations (see Figure 2.2) (Andersen & Sinkjær, 2003). To align the axis of rotation of the functional joint with the anatomical knee axis of rotation, an individual brace was constructed for each participant. First, a positive plaster cast of the left leg of each participant was created. The polyurethane brace was then constructed by molding it onto the positive plaster cast. The functional joint and a supporting joint were mounted on the brace, leveled and aligned with the knee joint axis of rotation. A thin layer of foam rubber was applied to the inside of the brace to make it comfortable to wear. For the testing session, the brace was attached to the left leg of the participant by Velcro straps and duct tape.

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 rotations in either the flexion or extension direction during the gait cycle (Andersen & Sinkjær, 2003). The mean perturbation characteristics for all conditions in Studies III and IV are presented in Table 2.2 (see also Mrachacz-Kersting *et al.*, 2004). During walking, the weight of the portable stretching apparatus added an extra load of approximately 2 kg to the left leg. This has been previously shown not to change the normal walking pattern compared to when participants walked without the device (Mrachacz-Kersting *et al.*, 2004).

Table 2.2: Mean ( $\pm$  SD) perturbation characteristics of the portable mechanical joint actuator device for all conditions.

	Mean ramp and hold time (ms)	Mean angular velocity ( $^{\circ}$ /s)	Mean angular displacement ( $^{\circ}$ )
Study III	203 $\pm$ 8	302 $\pm$ 35	9.0 $\pm$ 0.9
Study IV	193 $\pm$ 41	294 $\pm$ 29	7.6 $\pm$ 0.6



**Figure 2.2: General experimental setup of the portable mechanical joint actuator device used in Studies III and IV.** Ipsilateral knee (iKnee) extension and flexion joint rotations were applied to the left knee joint during various points during the gait cycle. 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. Participants in Study IV walked on a treadmill that allowed for the velocity of the belt to be changed rapidly wearing a safety harness. 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%.

### 2.4.1 General setup for walking experiments

For Studies III and IV, participants walked at a self-selected velocity between  $0.83$  and  $1.11 \text{ ms}^{-1}$  (mean velocity:  $0.97 \pm 0.12 \text{ ms}^{-1}$ ). 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. 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). If the participants began to vary from their initial stride time ( $\pm 100$  ms), they were verbally asked to increase or decrease their stride time (necessary in three experimental sessions).

## **2.5 Study I: Ipsilateral knee joint rotations during sitting**

### **2.5.1 Experiment 1a: Ipsilateral knee flexion and extension joint rotations**

Nine participants (mean age:  $27.8 \pm 4.2$  years) took part in this experiment. Following the setup described in Section 2.3.1, participants were asked to tonically pre-activate the iRF and cBF to a level that produced EMG activity corresponding to 10% of MVC. Knee flexion and extension joint rotations ( $\pm 6^\circ$  amplitude with a peak angular velocity of  $\pm 150^\circ/\text{s}$ ) were imposed in separate blocks every three to five seconds. The new joint position was held for 200 ms and then released, giving a total duration of the perturbation of 300 ms. Thirty trials were collected for each perturbation direction for each participant, for a total of 60 trials. Data collection for each trial began 100 ms prior to the perturbation and lasted for 500 ms. The order of the iKnee joint rotation direction was randomized. The instructions to the participants were to maintain the level of contraction level as displayed on the computer monitor and not to intervene when the knee joint rotation was applied.

### **2.5.2 Experiment 1b: Measurement of contralateral joint kinematics**

To verify that the short-latency reflexes observed in the cBF in Experiment 1a were not due to movement of the contralateral leg caused by the mechanical linkage between the legs, cHip, cKnee and cAnkle joint angles were measured following iKnee joint rotations. Eight participants (mean age:  $26.82 \pm 1.5$  years)

took part in this experiment. The experimental procedures were identical to the procedures described in Section 2.5.1.

### **2.5.3 Experiment 2: Manipulation of background muscle contraction level**

To further verify that the SLRs observed in the cBF in Experiment 1a were not simply due to vibrations caused by the mechanical actuator, the automatic gain control principle (Matthews, 1986) was investigated by varying the level of background contraction in the iRF and cBF. Thirteen participants (mean age:  $23.7 \pm 4.1$  years) took part in this experiment. Participants were asked to tonically pre-activate the iRF and cBF to a level that produced EMG activity corresponding to either 10% or 30% of MVC. Knee flexion or knee extension joint rotations ( $6^\circ$  and  $150^\circ/s$ ) were imposed in separate blocks of 30 trials every three to five seconds, with the iRF and cBF pre-contracting at different levels. The combinations of pre-contraction included: iRF 10% MVC and cBF 10% MVC; iRF 10% MVC and cBF 30% MVC; iRF 30% MVC and cBF 10% MVC; and iRF 30% MVC and cBF 30% MVC. Participants therefore received a total of 240 knee joint rotations separated into eight blocks of 30 trials. The new joint position for all conditions was held for 200 ms and then released, giving a total duration of the perturbation of 300 ms. Data collection for each trial began 100 ms prior to the perturbation and lasted for 500 ms. The order of the blocks varied across participants, and participants were allowed to rest between blocks as required in order to prevent fatigue. The instructions to the participants were to maintain the level of contraction level as displayed on the computer monitor and not to intervene when the knee joint rotation was applied.

### **2.5.4 Experiment 3: Intramuscular EMG recordings in the cBF**

In this experiment, intramuscular EMG was recorded in the cBF to determine whether the reversal in the sign of the short-latency cBF reflexes measured by surface EMG following either iKnee extension or flexion joint rotations can be quantified at the motor unit (MU) level and involved the same population of MUs (De Serres *et al.*, 1995). Eleven participants (mean age:  $25.5 \pm 4.8$  years) took part in this experiment. Participants were asked to maintain a comfortable level of activation in the iRF and cBF to a level that produced

EMG activity constrained between 5-10% of MVC. Knee flexion or knee extension joint rotations ( $\pm 8^\circ$  amplitude with a peak angular velocity of  $\pm 150^\circ/\text{s}$ ) were imposed in separate blocks of 60 trials every three to five seconds. This condition was performed since MU activity is more sensitive than surface EMG. Participants therefore received a grand total of 120 knee joint rotations separated into two blocks of 60 trials. The new joint position for all conditions was held for 200 ms and then released, giving a total duration of the perturbation of 300 ms. Data collection for each trial began 1 s prior to the perturbation and lasted for 3 s. The order of the conditions was randomized. The instructions to the participants were to maintain the level of contraction level as displayed on the computer monitor and not to intervene when the knee joint rotation was applied.

## **2.6 Study II: Manipulation of ipsilateral knee joint rotation velocity and amplitude during sitting**

Following the setup described in Section 2.3.1, participants were asked to tonically pre-activate the iRF and cBF to a level that produced EMG activity corresponding to 10% of MVC. Knee flexion or knee extension joint rotations of different amplitudes and velocities were imposed in separate blocks of 30 trials every three to five seconds. The velocities applied were 75, 100, 150, 175 or  $200^\circ/\text{s}$  at an amplitude of six degrees, and the amplitudes were four, six or eight degrees at  $150^\circ/\text{s}$ . Participants therefore received a total of 420 knee joint rotations separated into 14 blocks of 30 trials. The new joint position for all conditions was held for 200 ms and then released, giving a total duration of the perturbation of 300 ms. Data collection for each trial began 100 ms prior to the perturbation and lasted for 500 ms. The order of the blocks varied across participants, and participants were allowed to rest between blocks as required in order to prevent fatigue. The instructions to the participants were to maintain the level of contraction level as displayed on the computer monitor and not to intervene when the knee joint rotation was applied.

## **2.7 Study III: Ipsilateral knee joint rotations during walking**

Following gait profile assessment (see Section 2.4.1), 0, 10, 30, 50, and 90% of the gait cycle of the ipsilateral leg were calculated. These percentages of the gait cycle were chosen to explore transitions in the gait cycle from periods of

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double support to periods of single support, and vice-versa. For all experiments, predefined iKnee joint rotations were administered every six to ten steps. For every perturbation step collected, one step was also collected every six to ten steps without any perturbations in order to serve as a control condition. The participants were allowed to rest between each gait cycle phase and perturbation direction (Experiment 1) and between experiments (Experiments 2 and 3), and were also able to pause the experiment at any stage if they reported fatigue. If the participants began to vary from their initial stride time ( $\pm 50$  ms), they were verbally asked to increase or decrease their stride time (necessary in three experimental sessions).

### **2.7.1 Experiment 1: Ipsilateral knee flexion and extension joint rotations**

Ten participants (mean age:  $28.2 \pm 6.5$  years) partook in this experiment. Following the setup described above and the calculation of the perturbation timings to be used in the experiment, a total of 30 knee flexion joint rotations were applied in each of the five percentages in the gait cycle for each participant in separate blocks. Additionally, 30 knee extension joint rotations were applied to each participant at 50% of the gait cycle. Participants therefore received a total of 180 iKnee joint rotations (150 flexion perturbations and 30 extension perturbations) during the experimental session. The order of the perturbation timings and directions was randomized.

### **2.7.2 Experiment 2: Transcranial magnetic stimulation and ipsilateral knee extension joint rotations**

Nine participants (mean age:  $31.4 \pm 5.5$  years) partook in this experiment, two of which also participated Experiment 1. The most consistent contralateral reflex response observed in Experiment 1 was in the cBF following iKnee extension joint rotations applied at 50% of the gait cycle. TMS was applied to the left primary motor cortex in order to investigate whether a transcortical pathway contributed to the cBF response observed in Experiment 1. Prior to the application of TMS in Experiment 2, the mean onset latency of the cBF response was calculated by applying 20 knee extension joint rotations to the ipsilateral leg at 50% of the gait cycle, as described above.

Motor evoked potentials (MEPs) in the cBF were elicited by magnetic stimulation applied to the left primary motor cortex. In Experiments 2 and 3, TMS was delivered using a magnetic stimulator (Magstim Company Ltd., Dyfed, UK) and a prototype figure-of-eight (bended bat) coil (loop diameter 9 cm). At the beginning of TMS application, the coil was adjusted to find the best location for eliciting MEPs in the right BF, usually near the vertex on the left side of the scalp. The coil was fixed in place by using a specially designed harness (Balgrist Tec, Zurich, Switzerland) as described previously by Schubert *et al.* (1997) and Petersen *et al.* (1998b). The coil was maneuvered by the experimenter until the lowest threshold point for evoking MEPs was found. The threshold was established by visually detecting MEPs clearly distinguishable from the background activity during walking (i.e., greater than 50  $\mu$ V) in four out of eight trials. TMS was delivered such that the onset of the MEP would coincide with the onset of the cBF response. For example, if one participant had a mean cBF response onset of 75 ms following the perturbation, and their mean MEP onset in the cBF was 25 ms, the TMS would be delivered at 50 ms following the onset of perturbation. Intensities between 1.1 and 1.5 times MEP threshold were used as the conditioning stimulus.

Once the TMS coil placement was finalized, the position marked on the scalp, and the stimulation timing and intensity set, participants again walked on the treadmill at their preferred velocity. Four different conditions were applied every three to five steps: no iKnee joint rotations and no TMS (control), iKnee extension joint rotations only (perturbation only), TMS only, and a combination of iKnee extension joint rotations (perturbation & TMS). Twenty trials were collected per condition, and the order of presentation was randomized. TMS coil position was monitored by the experimenters throughout the experimental session.

In order to obtain a time course of changes in MEP size both prior to and after the cBF response onset, the timing of magnetic stimulation relative to cBF response onset was varied in six out of nine participants (in the same experimental session as above). The TMS was timed such that MEPs would occur at -30, -15, 0, +15, +30, and +250 ms with respect to cBF response onset. For example, if one participant had a mean cBF response onset of 75 ms following the perturbation, and their mean MEP onset in the cBF was 25 ms, the TMS would be delivered at 20, 35, 50, 65, 80, and 300 ms following the onset of perturbation. Given that the onset of sensory evoked potentials

following mechanical stretching of upper leg muscles occurs at about 24 ms (Mrachacz-Kersting *et al.*, 2006) and allowing 10 ms for central processing delays (34 ms total; Deuschl *et al.*, 1989; Petersen *et al.*, 1998a), convergence between the afferent input from the iKnee joint rotation and TMS to the left primary motor cortex was expected when the MEP was timed to arrive either at the same time (0 ms) or immediately after (+15 and +30 ms) the cBF response onset. However, no convergence was expected when the MEP was timed to arrive before the onset of the cBF response (-30 and -15 ms). The +250 ms timing was chosen as a control condition, where no convergence between the afferent input from the perturbation and TMS to the left primary motor cortex was expected.

Twenty trials were collected for each of the six timings both with (10 trials) and without (10 trials) iKnee extension joint rotations (120 trials total). In addition, 10 control trials (no perturbation or TMS) and 10 perturbation only trials were collected, for a grand total of 140 trials per participant. The order in which the conditions were presented was randomized, with one combination of TMS timing (none, -30, -15, 0, +15, +30, or +250 ms) and perturbation (present or absent) occurring every three to five steps.

### **2.7.3 Experiment 3: Transcranial electrical stimulation and ipsilateral knee extension joint rotations**

Three participants (mean age:  $37.0 \pm 5.3$  years) partook in this experiment (all three also participated in Experiment 2). In order to further investigate the possibility of a transcortical pathway contributing to the cBF response following iKnee extension joint rotations at 50% of the gait cycle, both electrical and magnetic stimulation were applied to the left primary motor cortex. Initially, Experiment 3 was the same as Experiment 2 with the exception of investigating the time course changes in MEP size. Once the four conditions (control, perturbation only, TMS only, perturbation & TMS) had been collected with magnetic stimulation applied to the primary motor cortex, the same procedure was conducted with electrical stimulation applied to the primary motor cortex.

MEPs in the cBF were evoked by TES using a high voltage constant current stimulator (Digitimer D180A, Digitimer Ltd., UK), with a maximal output of 1500 V. A high-voltage electrical pulse (100  $\mu$ s duration) was passed between

cup electrodes, with the cathode placed 4 cm anterior to the vertex and the anode placed 2-3 cm to the left of the vertex (Nielsen *et al.*, 1995). Identical to the TMS condition above, TES was delivered such that the onset of the MEP would coincide with the onset of the cBF response. To account for the shorter MEP onset latencies following TES than following TMS (Nielsen *et al.*, 1995), TES was delivered 2 ms closer to the time of cBF onset relative to when the TMS was delivered. For example, if one participant had a mean cBF response onset of 75 ms following the perturbation, and their mean TMS-evoked MEP onset in the cBF was 25 ms, the TMS would be delivered at 50 ms following the onset of perturbation, and the TES would be delivered at 52 ms following onset of perturbation. TES intensity was set such that it elicited MEPs of similar size to the MEPs elicited by the TMS only condition.

Once the TES intensity was set, the same four conditions were applied every three to five steps with TES: no iKnee extension joint rotations and no TES (control), iKnee extension joint rotations only (perturbation only), TES only, and a combination of iKnee extension joint rotations (perturbation & TES). Five trials were collected per condition for a total of 20 trials per participant. One condition was presented every three to five steps, and the order of presentation was randomized.

## **2.8 Study IV: Influence of treadmill velocity changes on interlimb reflexes during walking**

For Study IV, 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 2.2). 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

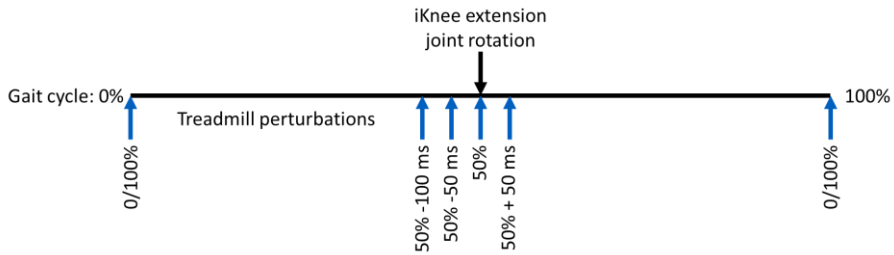
lasted for 1.5 seconds. The values above were chosen such that the resulting velocities were never below 0 m/s or above 1.81 m/s (i.e., fast walking; Sousa & Tavares, 2012). The velocity changes occurred with an acceleration of  $\pm 5$  m/s<sup>2</sup>.

Following gait profile assessment (see Section 2.4.1), 50% of the gait cycle of the ipsilateral leg was calculated. One of the 11 conditions presented in were administered randomly every four to six steps. Figure 2.3 displays the relative timings of treadmill and iKnee perturbations. 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.



**Table 2.3: Description of experimental conditions for Study IV.**

	<b>Condition</b>	<b>Description</b>	<b>Number of Trials</b>
1.	Normal gait	No iKnee extension joint rotations and no treadmill perturbations	60 trials
2.	iKnee perturbation only	iKnee 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



**Figure 2.3: Relative timing of treadmill and iKnee perturbations.** 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 0/100% of the gait cycle (blue arrows).

## 2.9 Data analysis

### 2.9.1 Studies I and II

Data were analyzed off-line for all studies. For the surface EMG data in Studies I and II, the amplitude of the responses in each muscle for each condition was calculated as the area under the mean, filtered, full-wave rectified surface EMG signal. For each condition, the amplitude of the responses was expressed as a percentage of the baseline surface EMG, recorded in the 100 ms preceding the perturbation to the iKnee joint. The onset of the reflex responses was determined for each 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 (SDs) of the mean rectified baseline EMG that lasted for at least 10 ms. The offset of the reflex responses was defined as the point where the mean rectified EMG in the perturbation trials returned to within two SDs of the baseline EMG for at least 10 ms. The reflex onsets and offsets were manually verified for accuracy. When either a depression or facilitation was observed in the EMG, the onset and duration of the response were recorded. To quantify the amplitude of the response to the imposed knee joint rotations for each muscle, the mean integrated EMG (iEMG) activity was calculated from the onset for the

duration of the response. Due to the lack of background EMG in the iBF and cRF, only responses from the iRF and cBF were analyzed.

For Experiment 1b of Study I, the onsets of cHip, cKnee and cAnkle joint movement were defined as the first deviation of the mean joint angles above or below two standard deviations of the mean baseline joint angle that lasted for at least 10 ms. When deflections in joint angles were observed, the onset, direction and peak amplitude were recorded.

Intramuscular EMG provides means to decode the neural drive as the ensemble of the timings of activations of the motoneurons innervating the muscle (Study I, Experiment 3). Thus, most of the information lies in the number of MU action potentials discharged per unit time. Intramuscular EMG data were decomposed (EMGLAB, McGill *et al.*, 2005) into constituent MU action potentials and analyzed in two ways: (1) the total number of action potentials (over all 60 trials) was quantified using a 10 ms window to create a peristimulus time histogram (PSTH) expressed in number of counts as a percentage of the number of perturbations. 10 ms was used to ensure a reliable resolution in determining the onset and duration of the response. (2) The instantaneous discharge frequencies (discharge rate) for each MU were quantified 100 ms pre and post perturbation to investigate how they were affected by the perturbation. This analysis was carried out for all detected MUs; however, signals from the two conditions were concatenated prior to MU decomposition to enable tracking and estimation of the number of common units across conditions using EMGLAB software.

### 2.9.2 Studies III and IV

For Studies III (Experiment 1) and IV, 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  $\pm 10\%$  of the mean gait cycle time. The onsets of the reflex responses following the knee joint rotation only trials were determined for each muscle in each participant by using an algorithm in MATLAB, and were defined as the first deviation of the mean rectified EMG data above or below two SDs of the mean rectified EMG in the control trials that lasted for at least 10 ms (Gervasio *et al.*, 2013a). The offsets of the reflex responses were defined as the point where the mean rectified EMG in the perturbation trials returned to within two SDs of the

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control 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 (Study IV). In some participants, the perturbation introduced a brief artifact in the recorded EMG within the first 5 ms following the perturbation. However, the artifact occurred prior to any possible reflex activity via even the shortest monosynaptic pathways (Mrachacz-Kersting *et al.*, 2004).

To quantify the amplitude of the reflex responses following iKnee joint rotations, and the amplitude of the cBF responses following the treadmill only conditions (Study IV), the iEMG (Study III) or the root mean square (RMS) of the mean rectified EMG (Study IV) were calculated for the duration of the response. To obtain the absolute mean amplitude of the responses, the ongoing background activity from the normal walking trials, where no iKnee or treadmill perturbations were imposed, was subtracted from trials when perturbations were applied. To enable inter-participant comparisons of these responses, the resulting reflex amplitude data were normalized to the maximum reflex response for each particular time point in the gait cycle. These corrected values were subsequently averaged across all participants to obtain the mean response across the group.

For Experiments 2 and 3 in Study III, the mean iEMG was calculated in the cBF from MEP onset for 20 ms (or the same corresponding time period in conditions without either TMS or TES) for each condition. To obtain the expected level of convergence from pairing iKnee extension joint rotations with TMS or TES, the algebraic sum of the perturbation only and TMS or TES only conditions was calculated from the cBF data for each participant. To allow for inter-participant comparisons, the data were then normalized to the algebraic sum of the perturbation only condition and the TMS only (Experiment 2) or stimulation only (TMS or TES; Experiment 3) conditions. For the time course data in Experiment 2, the cBF intramuscular EMG data from the combined perturbation & TMS condition were also normalized to the expected level of convergence for each of the six different timings (Petersen *et al.*, 1998a). Additionally, because the optimal TMS coil location for eliciting MEPs in the cBF also elicited MEPs in the iBF due to the close proximity of their respective representations in the primary motor cortex, the iBF time course data in Experiment 2 were analyzed in the same way as the cBF data above. It could therefore be considered whether any increase in MEP

size in the cBF was specific to the cBF, or due to a global increase in cortical excitability (in which case an increase in iBF MEP size would also be expected).

In order to compare the effects of combining the iKnee perturbations with the sudden changes in treadmill velocity in Study IV, 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 iKnee 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.

## 2.10 Statistical analysis

For Experiment 1a in Study I, the amplitude of the responses observed in the cBF for each perturbation direction was tested for significance with respect to baseline levels using single-sample Student's *t*-tests. For Experiment 1b, the onset of the joint angle movements were compared with the cBF reflex onset using a repeated measures analysis of variance (ANOVA) for each perturbation direction, with mean onset (cBF reflex, cHip, cKnee, cAnkle) as the within-subjects factor. For Experiment 2 in Study I, the effects of altering the cBF pre-contraction level on the cBF response magnitude, correlation analyses were performed between cBF pre-contraction level and cBF response amplitude (with background activity subtracted). Separate analyses were performed for each level of pre-contraction in the iRF (10% and 30% MVC) and for each perturbation direction (flexion and extension). For each perturbation direction, the cBF reflex amplitudes for the two different iRF contraction level (10% and 30% MVC) were tested for significance using paired-samples *t*-tests.

For Experiment 3 in Study I, mean PSTHs for both extension and flexion perturbations were calculated across all participants. The mean background MU firing counts and firing frequencies were calculated during the 100 ms period immediately prior to perturbation onset. Periods of facilitation and inhibition were determined if the firing counts in three or more adjacent bins were above (or below) the mean background firing plus (or minus) three SDs (Mao *et al.*, 1984). The background firing frequencies in the cBF for each perturbation direction were tested for significance using a paired-samples *t*-test. The effects of perturbation direction on intramuscular EMG firing frequency in the cBF were assessed via a two-way repeated measures ANOVA with perturbation direction (extension, flexion) and time (background, response window) as within-subject factors.

The effects of perturbation amplitude on cBF response onset, magnitude and duration in Study II were assessed by performing separate two-way repeated measures ANOVAs with perturbation amplitude (four, six, eight degrees) and perturbation direction (flexion, extension) as within-subjects factors. The effects of perturbation velocity were assessed by performing two-way repeated measures ANOVAs with perturbation velocity (75, 100, 150, 175, 200°/s) and perturbation direction (flexion, extension) as within-subjects factors.

For Studies III (Experiment 1) and IV (for the iKnee perturbation only condition), the means  $\pm$  SD were reported for reflex response onsets, durations, and amplitudes for ipsilateral and contralateral muscles for each perturbation timing and direction when responses were observed in at least four participants (see Table 3.5 and Table 3.6). When contralateral reflex responses were observed in all ten participants, single sample Student's *t*-tests were conducted on the response magnitudes (normalized to normal walking). In order to determine whether the iRF reflex response was modulated across the gait cycle in Study III (0, 10, 30, 50, and 90%), one-way repeated measures analyses of variance (ANOVAs) were performed on the mean iRF reflex response parameters (onset, duration, and amplitude).

For Experiment 2 in Study III, a one-way (condition; control, perturbation only, TMS only, perturbation & TMS) ANOVA was performed on the normalized cBF EMG data. Additionally, the expected level of convergence in the cBF EMG data was compared to the observed convergence (normalized

perturbation & TMS condition) using Student's one-sample *t*-test. For the time course data, one-tailed single sample Student's *t*-tests were used to test the a-priori hypothesis that the combined perturbation & TMS condition evoked a larger response than the algebraic sum of the responses to separate perturbation only and TMS only conditions for both the cBF and iBF muscles (Petersen *et al.*, 1998a). The Holm-Bonferroni method was used to control for the Familywise error rate (Holm, 1979). Due to the relatively small number of participants (three), statistical analyses were not performed comparing the TMS and TES data from Experiment 3 (Study III).

For the treadmill only conditions in Study IV, the means  $\pm$  SD were reported for response onsets, durations, and amplitudes of the cBF muscle for each treadmill timing and velocity change direction (see Table 3.7). 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 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 in Study IV, 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).

For all studies, 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.





# 3

## RESULTS

### 3.1 Study I: Ipsilateral knee joint rotations during sitting

A summary of the mean ipsilateral and contralateral reflex response data across all participants following iKnee flexion and extension joint rotations in Experiment 1a of Study I, including means and SDs, are provided in Table 3.1. The main result of Experiment 1a was that, following iKnee extension joint rotations an inhibition response was observed in the cBF muscle, while following iKnee flexion joint rotations a facilitation response was observed in the cBF muscle. The mean onset latencies of the inhibition and facilitation responses were 44.3 ms and 43.7 ms, respectively.

**Table 3.1: Mean reflex response data for all participants following ipsilateral knee extension and flexion joint rotations during sitting.** Data are included for each perturbation direction for iRF and cBF muscles, with SDs given in brackets.

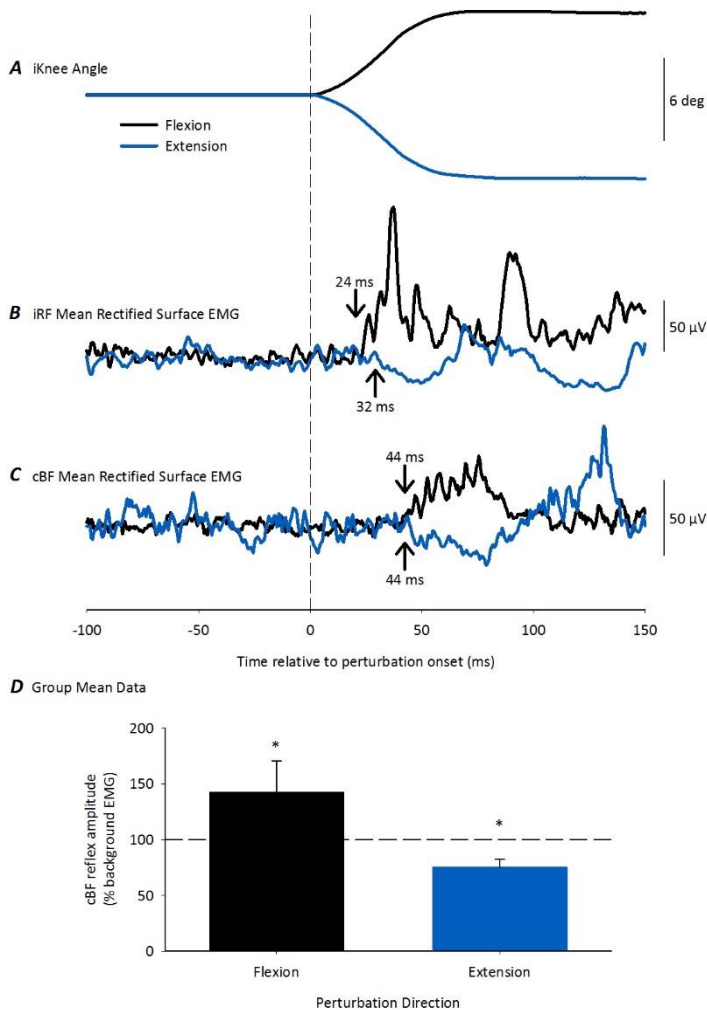
Variable	Perturbation Direction			
	Extension		Flexion	
	iRF	cBF	iRF	cBF
Onset (ms)	31.5 (6.3)	44.3 (10.7)	24.2 (3.2)	43.7 (7.2)
Duration (ms)	25.9 (14.0)	45.3 (20.9)	35.2 (19.7)	45.8 (18.9)
Amplitude (% baseline)	82.0 (16.1)	75.2 (7.1)	411.0 (402.9)	142.3 (28.1)

#### 3.1.1 Ipsilateral knee flexion and extension joint rotations during sitting

Mean data from one representative participant following both iKnee flexion and extension joint rotations in Experiment 1a of Study I are shown in Figure

3.1 (30 trials). Across all participants, the stretch reflex response in the iRF following iKnee flexion joint rotations had a mean latency of  $24 \pm 3$  ms (Figure 3.1B). The mean amplitude of the iRF stretch reflex response ( $411 \pm 403\%$  of baseline) was significantly greater than mean background EMG activity prior to the perturbation,  $t_8 = 2.32$ ,  $P = 0.049$ . Additionally, a facilitatory response occurred in the cBF in all participants with a mean onset latency of  $44 \pm 7$  ms (Figure 3.1). The mean amplitude of the cBF response ( $142 \pm 28\%$  of baseline) was significantly greater than mean background EMG activity prior to the perturbation,  $t_8 = 4.52$ ,  $P = 0.002$  (Figure 3.1D).

An inhibition response was observed in all participants in the iRF following iKnee extension joint rotations with a mean onset latency of  $32 \pm 6$  ms (see Figure 3.1B). The mean amplitude of the iRF inhibition response ( $82 \pm 16\%$  of baseline) was significantly less than mean background EMG activity prior to the perturbation,  $t_8 = 3.35$ ,  $P = 0.01$ . Additionally, an inhibition response occurred in the cBF in all participants with a mean onset latency of  $44 \pm 11$  ms (Figure 3.1C). The mean amplitude of the cBF inhibition response ( $75 \pm 7\%$  of baseline) was significantly less than mean background EMG activity prior to the perturbation,  $t_8 = 10.5$ ,  $P < 0.001$  (Figure 3.1D).



**Figure 3.1: Mean data from one participant (panels A-C) and group mean data (panel D) following extension (blue lines and blue bar) and flexion (black lines and black bar) joint rotations (six degrees, 150°/s) to the right (ipsilateral) knee joint during a sitting task.** Participants isometrically contracted their iRF and cBF muscles to 10% MVC prior to the perturbation. Panel A shows the ipsilateral knee angle, panel B shows mean rectified iRF EMG, and panel C shows mean rectified cBF EMG (30 trials). The vertical dashed line represents perturbation onset. Mean response onsets are denoted by the arrows. Note the period of inhibition in the cBF EMG, beginning at 44 ms following extension perturbation onset, and the period of facilitation beginning 44 ms following flexion perturbation onset. Panel D shows group mean data for cBF response amplitudes following ipsilateral knee extension and flexion perturbations. The horizontal dashed line represents cBF background EMG levels. Error bars represent SD. The asterisks denote significant differences from background EMG.

### 3.1.2 Contralateral joint kinematics

To verify that the short-latency reflexes observed in the cBF in Experiment 1a of Study 1 were not due to movement of the contralateral leg caused by the mechanical linkage between the legs, hip, knee and ankle joint angles were measured in the contralateral leg. A summary of the mean cBF reflex and contralateral leg joint movement onset latencies across all participants in Experiment 1b, including means and standard deviations, are provided in Table 3.2. Overall, the cBF reflex onsets were shorter than the contralateral joint movement onset latencies. Following iKnee extension perturbations, a repeated measures ANOVA revealed significant differences of mean onset latency between the cBF reflex and contralateral joint movement,  $F_{3,21} = 5.57$ ,  $P = .006$ . Post-hoc analysis revealed that the cBF reflex onset ( $M = 44.5$  ms) was significantly shorter than the movement onset of the cKnee ( $M = 83.7$  ms) and cAnkle ( $M = 99.9$  ms; both  $P$ 's  $< .02$ ). The cHip movement onset ( $M = 82.3$  ms) was not significantly different that the cBF reflex onset ( $P = 0.12$ ). Following iKnee flexion perturbations, a repeated measures ANOVA revealed significant differences of mean onset latency between the cBF reflex and contralateral joint movement,  $F_{3,21} = 3.81$ ,  $P = .025$ . Post-hoc analysis revealed that the cBF reflex onset ( $M = 43.1$  ms) was significantly shorter than the movement onset of the cAnkle ( $M = 59.2$  ms;  $P = .025$ ). The cHip ( $M = 67.9$  ms) and cKnee ( $M = 50.5$  ms) movement onsets were not significantly different that the cBF reflex onset (both  $P$ 's  $> 0.2$ ).

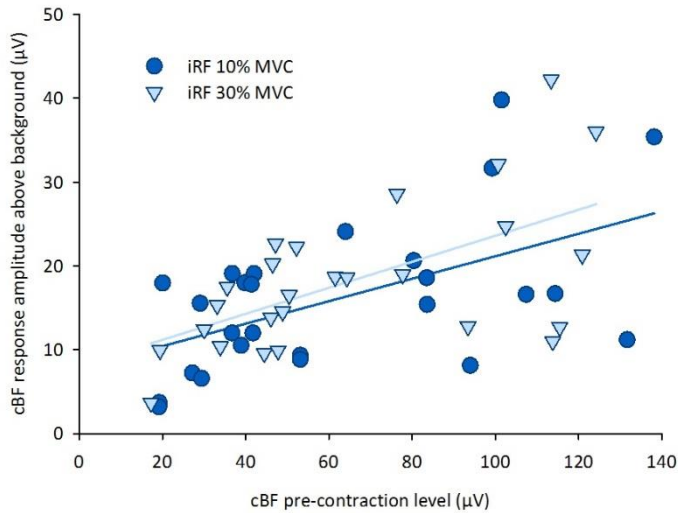
**Table 3.2: Mean cBF reflex onsets and contralateral joint movement onset, direction and amplitude data for all participants following iKnee extension and flexion joint rotations.** Data are included for each perturbation direction, with standard deviations given in brackets.

Variable	Perturbation Direction							
	Extension				Flexion			
	cBF	cHip	cKnee	cAnkle	cBF	cHip	cKnee	cAnkle
Mean onset (ms)	44.7 (4.9)	83.3 (4.1)	83.7 (23.7)	99.9 (21.8)	43.1 (5.2)	67.9 (27.4)	50.5 (10.5)	59.2 (9.2)
Joint displacement direction		Extension	Flexion	Plantar		Flexion	Extension	Plantar
Amplitude of joint displacement (degrees)		0.17 (0.28)	-0.12 (0.28)	0.18 (0.43)		-0.22 (0.33)	0.50 (0.33)	0.34 (0.54)

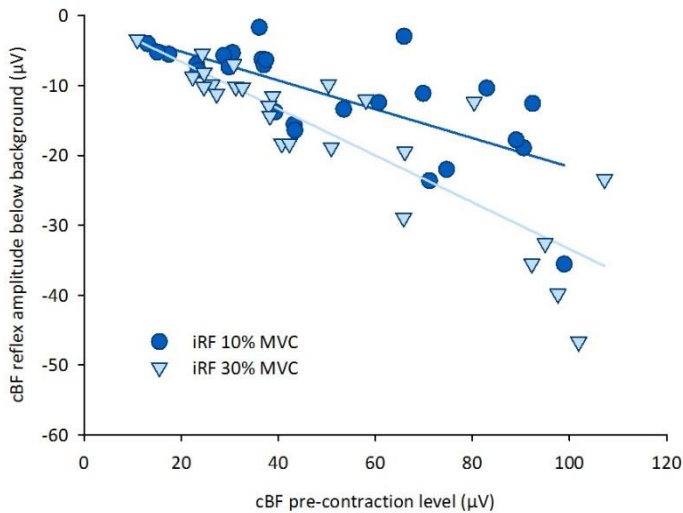
### 3.1.3 Effect of altering background muscle contraction level on cBF reflexes

To further verify that the SLRs observed in the cBF in Experiment 1a of Study I were not simply due to vibrations caused by the mechanical actuator, we tested the automatic gain control principle (Matthews, 1986) by varying the level of background contraction in the iRF and cBF. Figure 3.2A displays the relationship between cBF pre-contraction level and the short-latency cBF facilitation amplitude above background EMG following iKnee flexion joint rotations, while Figure 3.2B displays the relationship between cBF pre-contraction level and the short-latency cBF inhibition amplitude below background EMG following iKnee extension joint rotations. Data are plotted separately for the two different levels of iRF pre-contraction (10% and 30% MVC). Following iKnee flexion joint rotations, there was a significantly strong positive correlation between cBF background activity and the amplitude of the short-latency cBF facilitation response when the iRF was pre-contracted to 10% MVC,  $r_{24} = 0.54$ ,  $P = 0.002$ , and when the iRF was pre-contracted to 30% MVC,  $r_{24} = 0.59$ ,  $P = 0.001$ . Thus, as the cBF pre-contraction level increased, the amplitude of the short-latency cBF facilitation also increased. Following iKnee extension joint rotations, there was a significantly strong negative correlation between cBF background activity and the amplitude of the short-latency cBF inhibition response when the iRF was pre-contracted to 10% MVC,  $r_{24} = 0.71$ ,  $P < 0.001$ , and when the iRF was pre-contracted to 30% MVC,  $r_{24} = 0.86$ ,  $P < 0.001$ . Thus, as the cBF pre-contraction level increased, the amplitude of the short-latency cBF inhibition also increased. For both perturbation directions, the cBF reflex amplitude was not significantly different when the iRF was contracted at 10% or 30% MVC, both  $P$ 's  $> .12$ .

### A Flexion Perturbations



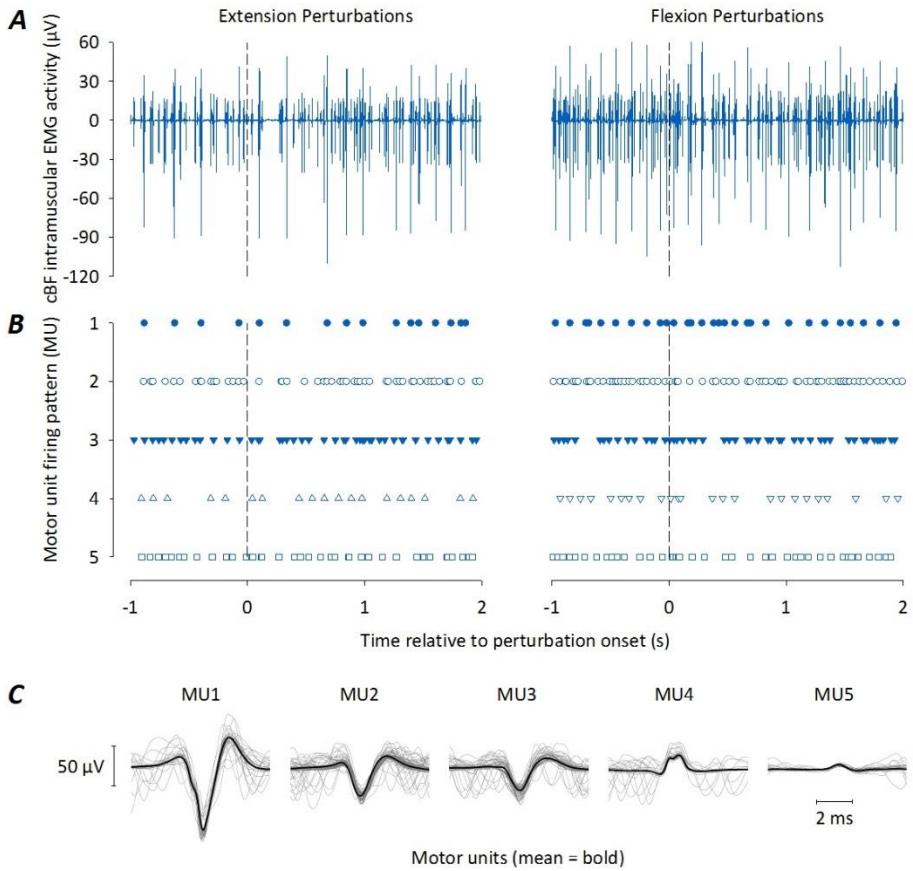
### B Extension Perturbations



**Figure 3.2: Relationship between cBF pre-contraction level and cBF response amplitude following ipsilateral knee flexion (panel A) and extension (panel B) joint rotations (six degrees, 150°/s) in all participants.** Dark blue circles and dark blue regression lines represent conditions when the pre-contraction level of the iRF muscle was 10% MVC, while the light blue triangles and light blue regression lines represent conditions when the pre-contraction level of the iRF muscle was 30% MVC. Note that the amplitude of the cBF facilitation response and the cBF inhibition response increases as the background cBF activity increases following ipsilateral knee flexion and extension joint rotations, respectively.

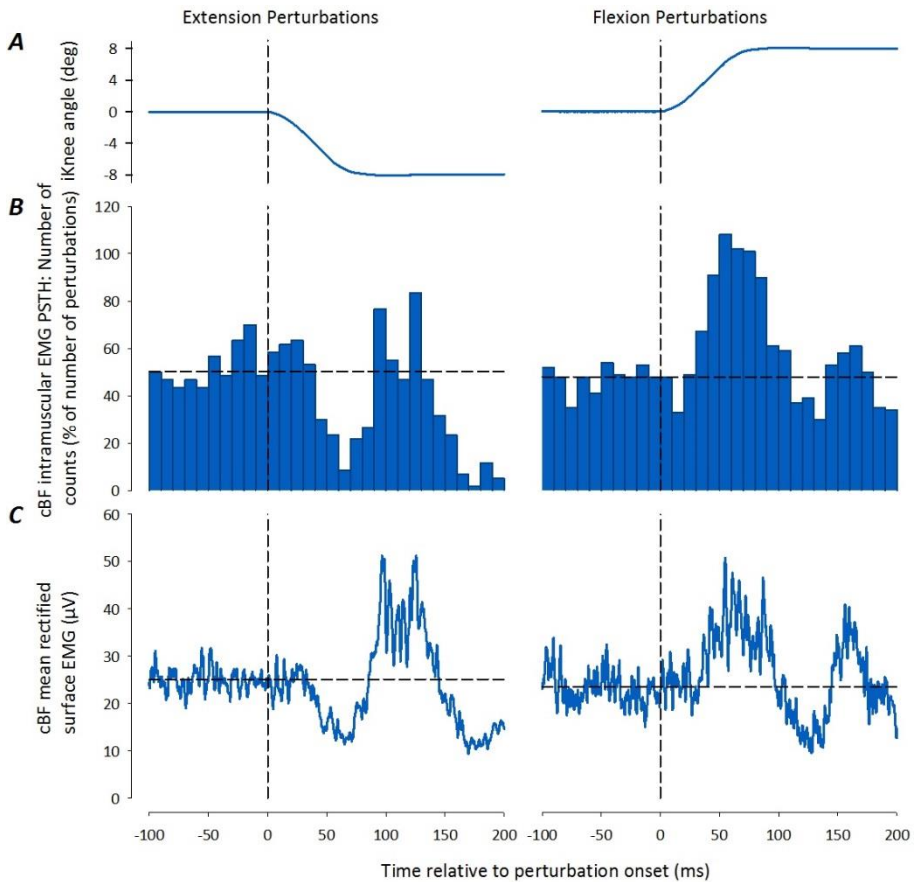
### 3.1.4 Intramuscular EMG recordings in the cBF

Intramuscular EMG was recorded in the cBF to determine whether the reversal in the sign of the short-latency cBF reflexes following either iKnee extension or flexion joint rotations can be quantified at the MU level and involved the same population of MUs (De Serres *et al.*, 1995). Figure 3.3 shows raw intramuscular EMG data following one flexion and one extension iKnee joint rotation trial from one representative participant, along with the firing pattern and templates of the five MUs that were identified during decomposition for the corresponding raw data. For all participants, the median number of active MUs during both iKnee flexion and extension conditions was eight and the median number of active MUs in only one or the other condition was two. Mean data from the same representative participant following both iKnee flexion and extension joint rotations in Experiment 3 of Study I are shown in Figure 3.4 (60 trials). The probability of firing of single MUs before and after perturbation onset is represented in the PSTHs of Figure 3.4C, while the mean surface EMG is shown in Figure 3.4B. For this participant, the period of inhibition in the cBF surface EMG began at 41 ms following extension perturbation onset, and the period of facilitation in the cBF surface EMG began at 41 ms following flexion perturbation onset. The total number of MUs making up the PSTH for this participant was five, and the mean background firing frequency in the cBF was 10.5 pulses per second (pps) and 9.6 pps during extension and flexion perturbations, respectively. Periods of inhibition and facilitation can also be observed in the PSTHs for this participant beginning at 40 ms following iKnee extension and flexion joint rotations, respectively.



**Figure 3.3: Motor unit decomposition.** Panel A shows the raw intramuscular EMG data recorded from the cBF following one flexion (left) and one extension (right) iKnee joint rotation trial from one participant. Panel B shows the firing pattern of the five different motor units that were identified during decomposition for the corresponding raw data in A. The vertical dashed lines indicate iKnee flexion or extension perturbation onset. Panel C shows the mean templates of the five different motor units that were identified during decomposition (thick black lines), together with each of the separate instances or each motor unit firing (thin gray lines). Note that the same five motor units were identified during decomposition following both iKnee flexion and extension joint rotations.



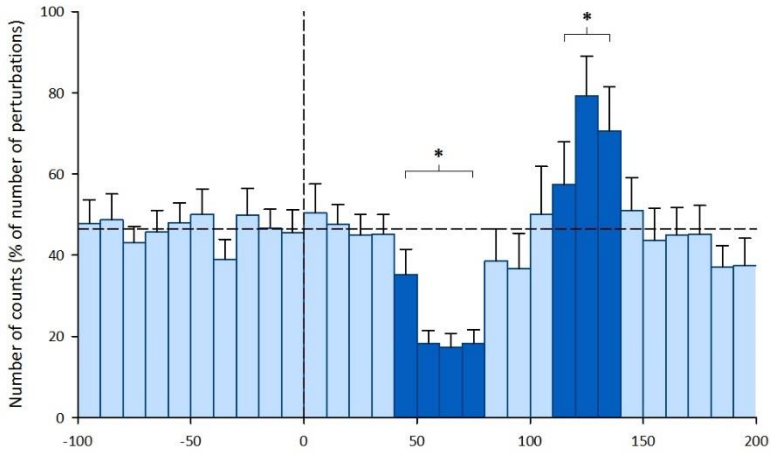


**Figure 3.4: Mean cBF intramuscular and surface EMG data from one participant following extension (left panels) and flexion (right panels) joint rotations (eight degrees, 150°/s) to the right (ipsilateral) knee joint during a sitting task.** Participants isometrically contracted their iRF and cBF muscles to 10% MVC prior to the perturbation. The top panels (A) show the ipsilateral knee angle, the middle panels (B) show the peristimulus time histogram of motor unit firing counts in the cBF normalized to the number of perturbations in bins of 10 ms, and the bottom panels (C) show the mean rectified cBF surface EMG (60 trials). The vertical dashed line represents perturbation onset and the horizontal dashed line represents mean cBF background levels. Note the period of inhibition in the cBF surface EMG beginning at 41 ms following extension perturbation onset, and the period of facilitation in the cBF surface EMG beginning 41 ms following flexion perturbation onset.

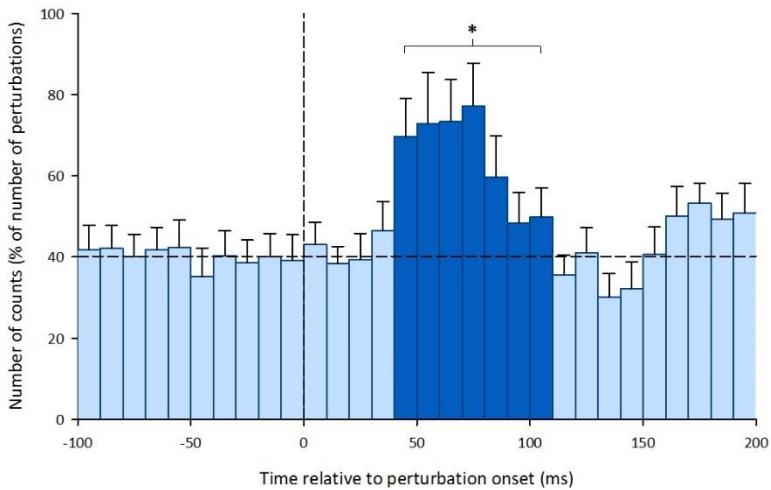
The overall mean responses of the cBF MU activity following iKnee extension and flexion joint rotations for all participants are shown in Figure 3.5. Responses in the mean cBF PSTHs were considered significant if differences greater than three SDs from the mean cBF background levels occurred that lasted for at least three consecutive bins (Mao *et al.*, 1984). The horizontal dashed line represents the mean cBF background level, and was calculated during the 100 ms period immediately prior to perturbation onset. Across all participants, an inhibitory reflex in the cBF was observed at a latency of 40-50 ms after the onset of iKnee extension joint rotations, lasting for 30-40 ms, while a facilitatory response was also observed at an onset latency of 110-120 ms (Figure 3.5A). Following iKnee flexion joint rotations, a facilitatory reflex was observed in the cBF with an onset latency of 40-50 ms, lasting for 60-70 ms.

The total number of MUs making up the PSTHs across all participants was 93, and their background firing frequencies in the cBF were  $12.4 \pm 3.2$  pps and  $13.5 \pm 4.3$  pps during flexion and extension perturbations, respectively. The mean background instantaneous firing frequency was not significantly different between perturbation directions,  $t_{10} = 1.36$ ,  $P = 0.2$ . Participants were able to reproduce approximately the same discharge rate across trials due to the visual feedback provided. A two-way repeated measures ANOVA revealed a significant interaction between perturbation direction and time window (background, response)  $F_{1,10} = 48.8$ ,  $P < 0.001$ . Post hoc analyses revealed that, following iKnee extension joint rotations, the cBF instantaneous firing frequency during the response window (mean,  $M = 11.4 \pm 2.5$  pps) was significantly lower than during background activity ( $P = 0.017$ ). Conversely, following iKnee flexion joint rotations, the cBF instantaneous firing frequency during the response window ( $M = 16.2 \pm 4.6$  pps) was significantly higher than during background activity ( $P < 0.001$ ). All participants showed the same general trend.

### A Extension Perturbations



### B Flexion Perturbations



**Figure 3.5: Group mean cBF intramuscular EMG data showing peristimulus time histograms following extension (panel A) and flexion (panel B) joint rotations (eight degrees, 150°/s) to the right (ipsilateral) knee joint during a sitting task.** Peristimulus time histogram data show group mean motor unit firing counts in the cBF normalized to the number of perturbations in bins of 10 ms. The vertical dashed line represents perturbation onset and the horizontal dashed line represents the mean cBF background level. Error bars represent standard error of the mean. The asterisks and dark blue bars denote differences greater than three SDs from the mean cBF background levels lasting for at least three consecutive bins. Note the period of inhibition following extension perturbations lasting from 40-80 ms, and the period of facilitation following flexion perturbations lasting from 40-110 ms.

### 3.2 Study II: Manipulation of ipsilateral knee joint rotation velocity and amplitude during sitting

A summary of the mean cBF response data across all participants following iKnee flexion and extension joint rotations in Study II, including means and SDs, are provided in Table 3.2 and Table 3.4. The main results of Study II were that manipulations in perturbation velocity, but not perturbation amplitude, increased the size of the short-latency cBF facilitation following iKnee flexion joint rotations and increased the size of the short-latency cBF inhibition following iKnee extension joint rotations.

**Table 3.3: Mean cBF reflex response characteristics for all participants following manipulations in ipsilateral knee extension and flexion joint rotation velocity with a constant amplitude of 6° during a sitting task.** Data is included for each perturbation direction and velocity, with SDs given in brackets.

Variable	Perturbation Direction/Velocity				
	Extension				
	75°/s	100°/s	150°/s	175°/s	200°/s
Onset (ms)	47.2 (8.5)	47.5 (6.4)	45.0 (8.1)	46.9 (6.3)	42.8 (5.1)
Duration (ms)	52.4 (14.0)	50.9 (13.1)	50.1 (13.4)	43.2 (13.0)	42.0 (14.3)
Amplitude (% baseline)	77.8 (8.0)	74.7 (6.7)	70.6 (6.5)	66.3 (8.6)	65.2 (9.3)
Variable	Flexion				
	75°/s	100°/s	150°/s	175°/s	200°/s
	75°/s	100°/s	150°/s	175°/s	200°/s
Onset (ms)	45.1 (6.6)	45.4 (7.7)	44.9 (9.5)	45.6 (9.9)	43.1 (3.9)
Duration (ms)	39.9 (21.2)	51.0 (33.4)	45.6 (21.3)	48.8 (38.3)	38.7 (35.1)
Amplitude (% baseline)	128.3 (20.2)	132.7 (18.0)	142.3 (21.9)	152.7 (30.2)	163.7 (35.2)

**Table 3.4: Mean cBF reflex response characteristics for all participants following manipulations in ipsilateral knee extension and flexion joint rotation amplitude with a constant velocity of 150°/s during a sitting task.** Data is included for each perturbation direction and amplitude, with SDs given in brackets.

Variable	Perturbation Direction/Amplitude					
	Extension			Flexion		
	4°	6°	8°	4°	6°	8°
Onset (ms)	43.2 (3.2)	45.0 (8.1)	47.2 (6.9)	42.5 (2.4)	44.9 (9.5)	45.9 (11.2)
Duration (ms)	43.6 (10.1)	50.1 (13.4)	51.7 (12.5)	49.3 (10.8)	45.6 (21.3)	54.0 (37.1)
Amplitude (% baseline)	72.9 (9.6)	70.6 (6.5)	66.7 (6.0)	148.7 (24.7)	142.3 (21.9)	150.9 (36.4)

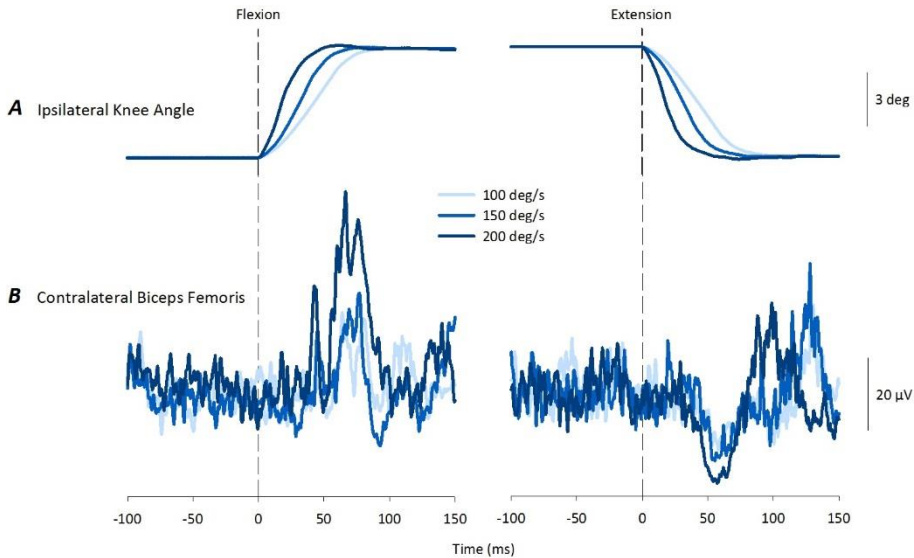
### 3.2.1 Effect of altering perturbation velocity on cBF reflex characteristics

The effect of altering the velocity of the iKnee flexion and extension joint rotations, while the amplitude remained unchanged at six degrees, was investigated using five different velocities; 75, 100, 150, 175 and 200°/s. For these conditions, cBF reflex onset latencies ranged between 42.8 and 47.5 ms, while the duration of the reflexes ranged between 38.7 to 52.4 ms (see

Table 3.3). Two-way repeated measures ANOVAs revealed no significant main effects or interaction effects of perturbation velocity and direction for cBF reflex onset latency or duration (all  $P$ 's > 0.15).

Figure 3.6 shows the mean (30 trials) rectified cBF EMG and iKnee angle data for one participant at three different flexion and extension perturbation velocities (100, 150 and 200°/s). The black, blue and red lines represent a perturbation velocity of 100°/s, 150°/s and 200°/s, respectively. In all trials, participants were asked to pre-contract the iRF and cBF muscles to approximately 10% MVC. Across all participants, the mean amplitude of the short-latency cBF facilitation following ipsilateral flexion perturbations increased with increasing perturbation velocities, while the mean amplitude of the short-latency cBF inhibition following ipsilateral extension perturbations also increased. Figure 3.7A displays the mean short-latency cBF response magnitudes for all participants ( $n = 13$ ) at all velocities investigated as a percentage of background EMG. A two-way repeated measures ANOVA

revealed a significant interaction between perturbation velocity and perturbation direction,  $F_{4,48} = 73.7$ ,  $P < 0.001$ . Post hoc analyses revealed that, following iKnee flexion joint rotations, the short-latency cBF facilitation was significantly larger when the perturbation velocity was  $200^\circ/\text{s}$  ( $M = 164\%$  background EMG) than when the perturbation velocity was  $75^\circ/\text{s}$  ( $M = 128\%$  background EMG),  $100^\circ/\text{s}$  ( $M = 133\%$  background EMG) or  $150^\circ/\text{s}$  ( $M = 142\%$  background EMG; all  $P$ 's  $< 0.005$ ). In addition, the cBF facilitation was significantly larger when the flexion perturbation velocity was  $175^\circ/\text{s}$  ( $M = 153\%$  background EMG) than when the velocity was  $75^\circ/\text{s}$  or  $100^\circ/\text{s}$  (all  $P$ 's  $< 0.02$ ), and the cBF facilitation was also significantly larger when the velocity was  $150^\circ/\text{s}$  than when the velocity was  $75^\circ/\text{s}$  ( $P = 0.002$ ). Following iKnee extension joint rotations, the short-latency cBF inhibition was significantly larger when the perturbation velocity was  $175^\circ/\text{s}$  ( $M = 66\%$  background EMG) or  $200^\circ/\text{s}$  ( $M = 65\%$  background EMG) than when the perturbation velocity was  $75^\circ/\text{s}$  ( $M = 78\%$  background EMG) or  $100^\circ/\text{s}$  ( $M = 75\%$  background EMG; all  $P$ 's  $< .02$ ). In addition, the cBF inhibition was significantly larger when the extension perturbation velocity was  $150^\circ/\text{s}$  ( $M = 71\%$  background EMG) than when the velocity was  $75^\circ/\text{s}$  ( $P = 0.002$ ). In addition, there was a significant main effect of perturbation direction,  $F_{1,12} = 87.2$ ,  $P < 0.001$ , revealing that the cBF reflex following iKnee flexion joint rotations ( $M = 144\%$  background EMG) was significantly larger than the cBF response following iKnee extension joint rotations ( $M = 71\%$  background EMG).



**Figure 3.6: Increases in ipsilateral knee flexion and extension joint rotation enhance short-latency cBF facilitation and inhibition responses, respectively, during a sitting task.** Mean data from one participant following ipsilateral knee flexion (left panels) and extension (right panels) joint rotations. Panel A shows the ipsilateral knee angle and panel B shows mean rectified cBF EMG activity (30 trials). Three different perturbation velocities are shown; 100°/s (light blue lines), 150°/s (blue lines), and 200°/s (dark blue lines), each with an amplitude of six degrees. Participants isometrically contracted their iRF and cBF muscles to 10% MVC prior to the perturbation. The vertical dashed lines represent perturbation onset. Note the increase in magnitude of the short-latency cBF facilitation following flexion perturbations and cBF inhibition following extension perturbations as perturbation velocity increases.

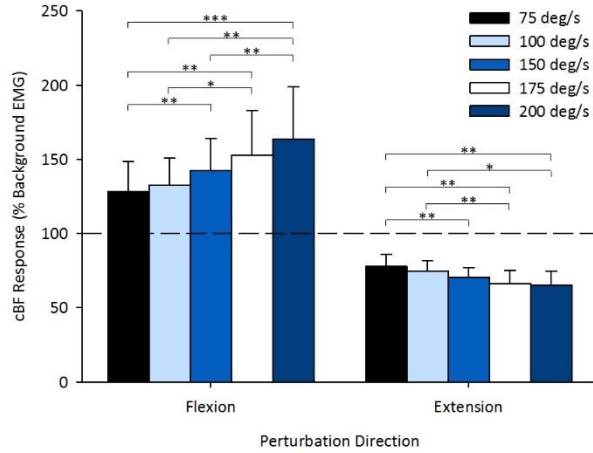
### 3.2.2 Effect of altering perturbation amplitude on cBF reflex characteristics

The effect of altering the amplitude of the iKnee flexion and extension joint rotations, while the velocity remained unchanged at 150°/s, was investigated using three different amplitudes; four, six and eight degrees. For these conditions, cBF reflex onset latencies ranged between 43.2 and 47.2 ms, while the duration of the reflexes ranged between 43.6 to 54.0 ms (see Table 3.4). Two-way repeated measures ANOVAs revealed no significant main effects or interaction effects of perturbation amplitude and direction for cBF reflex onset latency or duration (all  $P$ 's > 0.15).

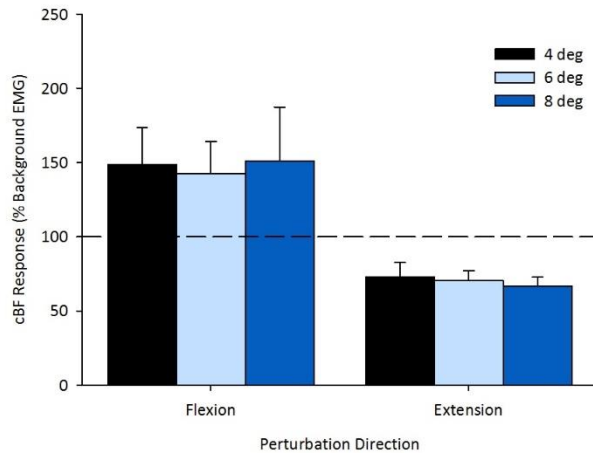
Figure 3.7B displays the mean short-latency cBF response magnitudes for all participants ( $n = 13$ ) at all amplitudes investigated as a percentage of background EMG. Across all participants, there was no significant difference in the mean amplitude of the short-latency cBF facilitation following ipsilateral flexion perturbations or cBF inhibition following ipsilateral extension perturbations. This was confirmed by the lack of a significant main effect of perturbation amplitude and the lack of a significant interaction between perturbation amplitude and direction (both  $P$ 's  $> .10$ ). There was a significant main effect of perturbation direction,  $F_{1,12} = 94.8$ ,  $P < 0.001$ , revealing that the cBF reflex following iKnee flexion joint rotations ( $M = 147\%$  background EMG) was significantly larger than the cBF response following iKnee extension joint rotations ( $M = 70\%$  background EMG).



### A Perturbation Velocity



### B Perturbation Amplitude



**Figure 3.7: Increases in ipsilateral knee flexion and extension joint rotation enhance short-latency cBF facilitation and inhibition responses during a sitting task, respectively, while increases in perturbation amplitude do not.** Group mean data of cBF response amplitude following manipulations in the velocity (panel A) or amplitude (panel B) of ipsilateral knee flexion and extension joint rotations. There were five different perturbation velocities (75, 100, 150, 175 and 200°/s) with an amplitude six degrees, and three different amplitudes (four, six and eight degrees) with a velocity of 150°/s. Participants isometrically contracted their iRF and cBF muscles to 10% MVC prior to the perturbation. The horizontal dashed line represents cBF background EMG levels. Error bars represent SD. \*, \*\* and \*\*\* represent significant differences to  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ , respectively. Increases in cBF facilitation and inhibition magnitudes were found following increases in the velocity of flexion and extension perturbations, respectively.

### 3.3 Study III: Ipsilateral knee joint rotations during walking

A summary of the mean ipsilateral and contralateral reflex response data across all participants for Experiment 1 in Study III following iKnee flexion and extension joint rotations, including means and SDs, are provided in Table 3.5. Group means were not calculated for contralateral muscles with reflex responses in less than four out of ten participants. No detectable responses were observed in the iBF following iKnee flexion joint rotations, or in the iRF following iKnee extension joint rotations. Therefore, these muscles were not analyzed for the relevant perturbation direction. The main result of Experiment 1 was that, following iKnee extension joint rotations at 50% of the gait cycle, a reflex response was observed in the cBF in all participants with a mean onset latency of 76 ms.

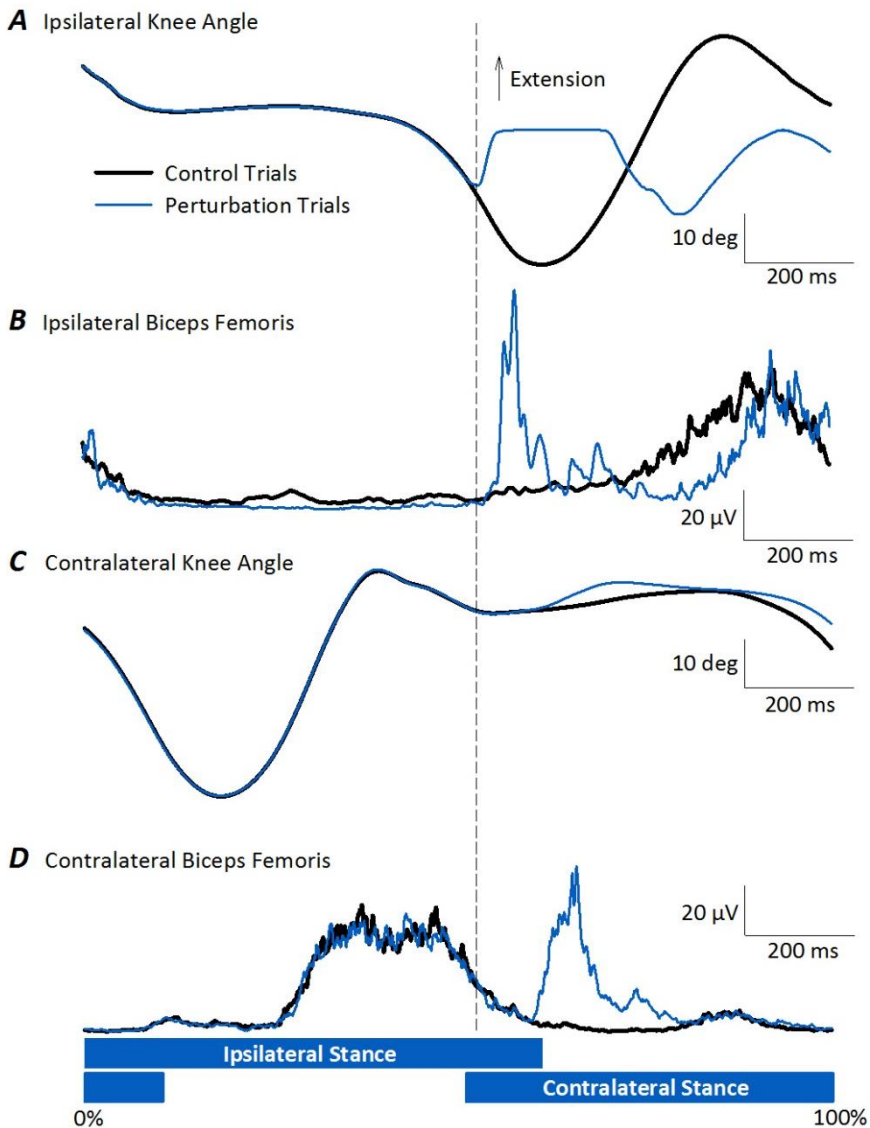
**Table 3.5: Mean reflex response data for all participants following iKnee extension and flexion joint rotations during walking.** Data is included for each time during the gait cycle investigated and each perturbation direction for all muscles collected, with SDs given in brackets.

Perturbation (timing and direction)		iRF	iBF	cVL	cRF	cBF	cSOL	cTA
<b>50% Extension</b>	Count (no. of participants)	--	10/10	3/10	2/10	10/10	4/10	7/10
	Onset (ms)		28.9 (5.6)			76.3 (6.2)	79.9 (13.4)	77.4 (11.2)
	Duration (ms)		144.4 (101.7)			210.4 (83.2)	135.5 (62.7)	159.2 (62.6)
	Amplitude (% above control)		179.6 (196.9)			460.6 (444.2)	103.9 (53.5)	147.8 (85.4)
<b>0% Flexion</b>	Count (no. of participants)	10/10	--	2/10	4/10	0/10	1/10	1/10
	Onset (ms)	29.9 (13.3)			70.3 (42.0)			
	Duration (ms)	117.2 (35.2)			61.1 (16.4)			
	Amplitude (% above control)	110.6 (159.2)			57.9 (8.2)			
<b>10% Flexion</b>	Count (no. of participants)	10/10	--	0/10	0/10	0/10	1/10	1/10
	Onset (ms)	29.5 (10.9)						
	Duration (ms)	188.9 (58.8)						
	Amplitude (% above control)	207.5 (154.8)						
<b>30% Flexion</b>	Count (no. of participants)	10/10	--	3/10	1/10	0/10	1/10	3/10
	Onset (ms)	32.4 (20.0)						
	Duration (ms)	170.9 (44.5)						
	Amplitude (% above control)	356.1 (159.0)						
<b>50% Flexion</b>	Count (no. of participants)	10/10	--	0/10	0/10	1/10	1/10	2/10
	Onset (ms)	22.2 (8.9)						
	Duration (ms)	120.8 (98.8)						
	Amplitude (% above control)	146.3 (102.9)						
<b>90% Flexion</b>	Count (no. of participants)	10/10	--	3/10	3/10	3/10	0/10	5/10
	Onset (ms)	26.1 (10.8)						79.0 (29.4)
	Duration (ms)	148.2 (70.0)						81.0 (23.2)
	Amplitude (% above control)	286.8 (399.3)						129.6 (76.1)

Mean data from one representative participant following iKnee extension joint rotations at 50% of the gait cycle are shown in Figure 3.8 (30 control steps and 30 perturbation steps). Across all participants, the stretch reflex response in the iBF had a mean onset latency of  $29 \pm 6$  ms. Additionally, a facilitatory response occurred in the cBF in all participants with a mean onset latency of  $76 \pm 6$  ms. The mean amplitude of the cBF response ( $461 \pm 444\%$  above control steps) was significantly greater than mean EMG activity during control steps,  $t_9 = 3.28$ ,  $P = 0.01$ . Contralateral reflex responses were also observed in the cTA

(7/10 participants, mean onset latency  $77 \pm 11$  ms) and in the cSOL (4/10 participants, mean onset latency  $80 \pm 13$  ms). The mean sizes of the responses were  $148 \pm 85\%$  and  $104 \pm 54\%$  above background activity during control steps, respectively. Infrequent reflex responses were observed in the cVL (3/10 participants) and the cRF (2/10 participants). The extension perturbation did not change the joint angles of the contralateral knee (see Figure 3.8) or hip angles.

The most consistent contralateral reflex observed in Study III was in the contralateral hamstrings following a stretch of the ipsilateral hamstrings muscles. iKnee extension joint rotations applied during the late stance phase (50%) of the gait cycle induced a strong reflex response in the cBF muscle at a mean latency of 76 ms in all participants (see Figure 3.8). In contrast to the contralateral responses observed following iKnee extension joint rotations, no consistent contralateral reflex responses were observed across all participants following iKnee flexion joint rotations at any of the five timings examined during the gait cycle. At the time of perturbations it is possible that flexion of the knee joint did not result in enough neural summation arising from knee afferents to activate any contralateral reflex pathways because the perturbation was not strong enough to disrupt the stability of normal gait. For example, at 50% of the gait cycle, where iKnee extension joint rotations elicited a strong facilitation in the cBF, the iKnee is flexing prior to toe-off and the contralateral leg has just touched down. Applying additional flexion to the knee joint at this time may not have provided the necessary input required to activate interlimb reflex pathways, while iKnee extension joint rotations at this time while the knee was flexing provided more of a threat to postural stability and the progression of the gait cycle. Many factors contribute to interlimb communication, including limb position, load, and temporal features of the cyclical activity (Duysens *et al.*, 2000), thus it is also likely that the position of the contralateral leg greatly influences the necessity for compensatory interlimb reflexes. Consequently, when iKnee flexion joint rotations were applied immediately prior to or during the contralateral swing phase (0%, 10%, 30%, and 90% of the gait cycle), the requirement for contralateral reflexes to maintain postural equilibrium and balance may have been decreased.

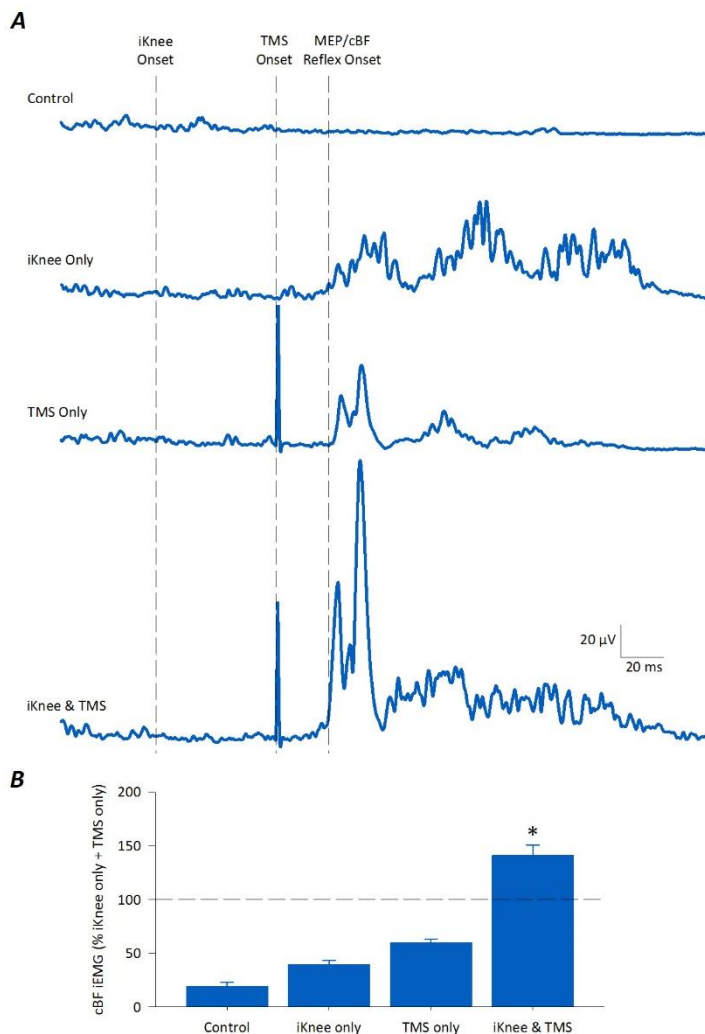


**Figure 3.8: Facilitation of cBF following ipsilateral knee extension joint rotations during walking.** Mean data from one participant for either control steps (black line) or following ipsilateral knee extension joint rotations (blue line) during the late stance phase (50%) of the gait cycle (0-100%). Panel A shows ipsilateral knee angle, panel B shows mean rectified iBF EMG, panel C shows contralateral knee angle, and panel D shows mean rectified cBF EMG. Perturbation onset is represented by the vertical dashed line. Ipsilateral and contralateral stance phases are represented by the blue bars below the panel D. Note the period of facilitation in the cBF EMG following perturbation trials, beginning around 75 ms after perturbation onset.

### 3.3.1 Conditioning MEPs elicited in the cBF by magnetic stimulation with ipsilateral knee extension joint rotations

Given that the minimum latency for a transcortical pathway in the ipsilateral RF following a mechanical flexion of the knee was found to be 54 ms (Mrachacz-Kersting *et al.*, 2006), it was examined whether spinal or supraspinal pathways contributed to the cBF response in Experiments 2 and 3 of Study III. In the first part of Experiment 2, TMS was applied such that MEPs were evoked in the cBF at the same time as the expected onset latency of the cBF reflex response following iKnee extension joint rotations at 50% of the gait cycle. The mean stimulation intensity used was  $69.9 \pm 7.1\%$  of maximum stimulator output, and the mean onset latency of the MEPs was  $23.5 \pm 1.8$  ms.

Figure 3.9A shows mean cBF EMG data from one representative participant following each of the four conditions (20 trials per condition): control steps with no iKnee extension joint rotations or TMS, iKnee extension joint rotations applied alone, TMS applied alone, or a combination of iKnee extension joint rotation and TMS. Note that when MEPs elicited by TMS were combined with perturbations to the iKnee joint, the MEPs were greatly facilitated compared to control MEPs elicited when TMS was delivered in isolation. This effect was observed in all nine participants (Figure 3.9B), and was confirmed by a significant repeated measures ANOVA,  $F_{3,24} = 78.83$ ,  $P < 0.001$ . Post-hoc analyses revealed that MEPs following a combination of iKnee extension joint rotations and TMS ( $141 \pm 29\%$ ) were significantly larger than control MEPs elicited by TMS alone ( $60 \pm 10\%$ ), and cBF EMG activity during the corresponding time windows following iKnee extension joint rotations alone ( $40 \pm 10\%$ ) or control steps ( $19 \pm 19\%$ ). Additionally, cBF EMG activity was significantly greater following TMS alone than in control steps. In addition, Figure 3.9B shows that the combination of iKnee extension joint rotations and the MEP was significantly larger than the algebraic sum of the two potentials when evoked separately,  $t_8 = 4.24$ ,  $P = 0.003$ .

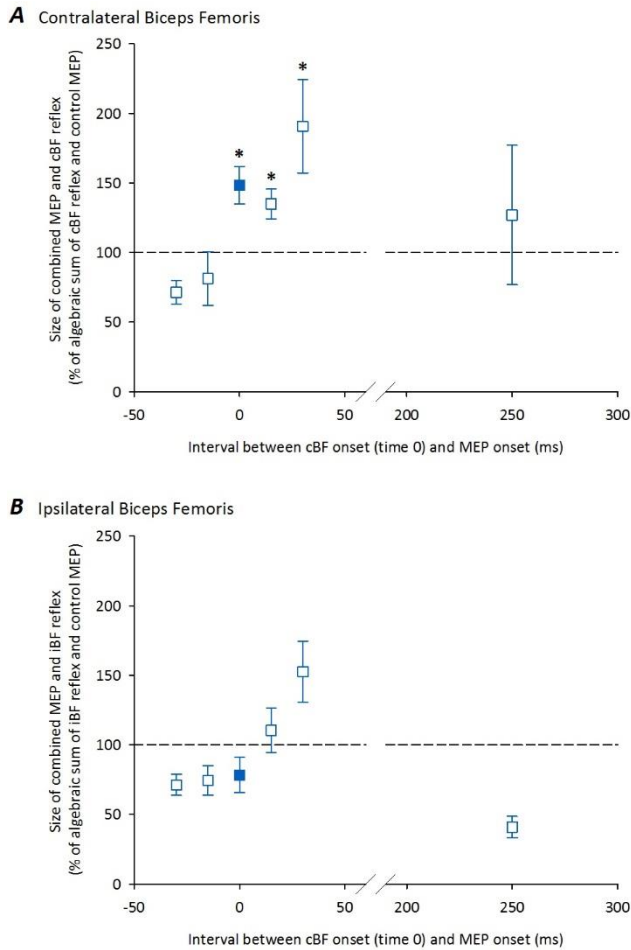


**Figure 3.9: Evidence of extra-facilitation of magnetically induced MEPs in the cBF following ipsilateral knee extension joint rotations during walking.** Panel A shows mean rectified cBF EMG for one participant following either control steps, iKnee perturbation only trials, TMS only trials, or iKnee perturbation & TMS trials. MEPs evoked by TMS were timed to coincide with the onset of the cBF reflex response. Vertical dashed lines represent perturbation onset, TMS onset, and MEP/cBF reflex onset, respectively. Panel B shows group mean cBF EMG data for the 20 ms following MEP/cBF reflex onset for the four conditions for nine participants. All data were normalized to the algebraic sum of the iKnee perturbation only and TMS only conditions (horizontal dashed line in panel B). The asterisk denotes a significant difference of the iKnee perturbation & TMS condition compared with the algebraic sum of the iKnee perturbation only and TMS only conditions. Error bars in panel B represent SEM.

In the second part of Experiment 2, a time course of changes in MEP size prior to, at, or after the cBF reflex response onset was investigated. MEPs elicited by TMS were timed such that they occurred before (-30 and -15 ms), at (0 ms), or after (+15, +30, and +250 ms) the cBF reflex response onset (Figure 3.10A). For each timing, the cBF MEP size following the combined perturbation & TMS condition was normalized to the algebraic sum of the cBF reflex response (perturbation only condition) and the control MEP (TMS only condition). When the MEP arrived 30 ms prior to the onset of the cBF reflex response (-30 ms), the conditioned MEP was significantly less than the algebraic sum of the cBF reflex response and the control MEP,  $t_5 = 3.47$ ,  $P = .018$ , and when the MEP arrived 15 ms prior (-15 ms) or 250 ms after (+250 ms) the cBF onset there were no significant differences ( $P$ 's > .35). However, when MEPs were evoked either at (0 ms,  $t_5 = 3.66$ ,  $P = 0.015$ ) or up to 30 ms following the cBF reflex response onset (+15 ms,  $t_5 = 3.28$ ,  $P = 0.022$ ; +30 ms,  $t_5 = 2.71$ ,  $P = 0.042$ ), the conditioned MEPs were significantly greater than the algebraic sum of the cBF reflex response and the control MEPs.

The optimal TMS coil location for eliciting MEPs in the cBF also elicited MEPs in the iBF due to the close proximity of their respective representations in the primary motor cortex. The iBF iEMG data were analyzed for the same respective time windows described above for the cBF (see Figure 3.10B). When the MEPs were evoked in the iBF either 30 ms prior (-30 ms,  $t_5 = 3.78$ ,  $P = 0.013$ ) or 250 ms after (+250 ms,  $t_5 = 7.65$ ,  $P < 0.001$ ) the cBF onset, the conditioned MEPs were significantly less than the algebraic sum of the iBF reflex response and the control MEP. When the MEP arrived at the same time (0 ms), 15 ms prior (-15 ms), or up to 30 ms following the cBF reflex response onset (+15 ms, +30 ms) there were no significant differences ( $P$ 's > 0.05). This indicates that the significant increases in MEPs observed in the cBF were not due to a global increase in cortical excitability following the iKnee extension joint rotations.



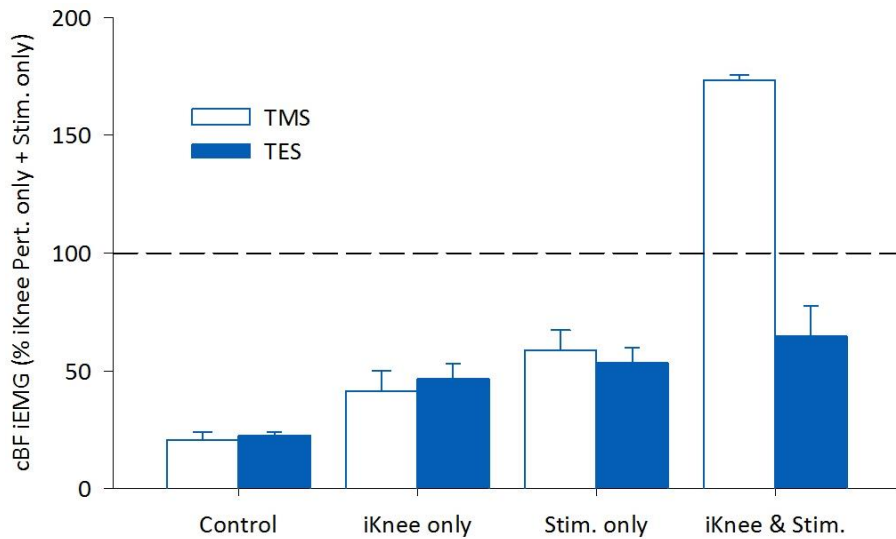


**Figure 3.10: Time course of extra-facilitation of magnetically induced MEPs in the cBF (A) and iBF (B) following ipsilateral knee extension joint rotations during walking.** MEPs were evoked by TMS at different timings (-30, -15, 0, +15, +30, and +250 ms) relative to the cBF reflex response onset (time 0, filled square). Panel A shows the size of the conditioned MEPs (iKnee perturbation & TMS condition) expressed as a percentage of the algebraic sum of perturbation only and TMS only conditions for each time interval in the cBF. Panel B shows the size of the conditioned MEPs (iKnee perturbation & TMS condition) expressed as a percentage of the algebraic sum of perturbation only and TMS only conditions for each time interval in the iBF. The horizontal dashed lines represents 100%. Error bars represent SEM. The asterisks indicate that the combined iKnee perturbation & TMS condition resulted in MEPs significantly greater ( $P < 0.05$ ) than the sum of the cBF reflex response and the control MEP in the cBF only. Note that there was only a significant increase in the cBF MEPs when the MEPs were times to arrive at or immediately after the onset of the cBF response, and that there was no such increase in the MEPs evoked in the iBF at these timings.

### 3.3.2 Comparison of magnetic and electrical stimulation to the primary motor cortex

In Experiment 3 of Study III, MEPs elicited by either magnetic or electrical stimulation to the primary motor cortex were compared when they were timed to coincide with the onset of the cBF reflex response following iKnee extension joint rotations at 50% of the gait cycle. Initially, MEPs were elicited by TMS under the same four conditions as the first part of Experiment 2: control steps with no iKnee extension joint rotations or TMS, iKnee extension joint rotations applied alone, TMS applied alone, or a combination of iKnee extension joint rotation and TMS. Following this, the same four conditions were repeated with MEPs being elicited by TES. The mean TMS intensity used was  $60.3 \pm 2.5\%$  maximum stimulator output, and the mean onset latency of the MEPs following TMS was  $24.8 \pm 0.7$  ms. The mean TES intensity used was  $24.2 \pm 4.3\%$  maximum stimulator output, and the mean onset latency of the MEPs following TES was  $23.1 \pm 0.7$  ms.

The mean cBF iEMG data for the 20 ms following MEP/cBF reflex onset for the three participants are shown in Figure 3.11. Note that, as for Experiment 2, when MEPs elicited by TMS were combined with perturbations to the iKnee joint ( $173 \pm 4\%$ ), they were greatly facilitated compared to the cBF iEMG following the other conditions (control trials,  $21 \pm 6\%$ ; perturbation only,  $41 \pm 15\%$ ; stimulation only,  $59 \pm 15\%$ ). However, MEPs elicited by TES were not facilitated when combined with perturbations to the iKnee joint ( $65 \pm 22\%$ ) compared to the other conditions (control trials,  $23 \pm 3\%$ ; perturbation only,  $47 \pm 11\%$ ; stimulation only,  $53 \pm 11\%$ ). This effect was observed in all three participants. Due to the relatively small sample size (three participants), only descriptive statistics are reported without statistical analyses.



**Figure 3.11: Comparison of TMS or TES induced MEPs in the cBF following iKnee extension joint rotations during walking.** Mean cBF iEMG data for the 20 ms following MEP/cBF reflex onset in three participants following either: control steps, iKnee perturbation only trials, stimulation (TMS or TES) only trials, or combined iKnee perturbation & stimulation (TMS or TES) trials. MEPs evoked by either TMS or TES were timed to coincide with the onset of the cBF reflex response. All data were normalized to the algebraic sum of the perturbation only and the stimulation (TMS or TES) only conditions (horizontal dashed line). Note that there is a clear facilitation above the algebraic sum of the iKnee perturbation only and the stimulation only conditions when the primary motor cortex was magnetically stimulated, but not electrically stimulated, when either stimulation was combined with ipsilateral knee extension joint rotations (iKnee perturbation & stimulation trials). Error bars represent SEM.

### 3.4 Study IV: Influence of treadmill velocity changes on interlimb reflexes during walking

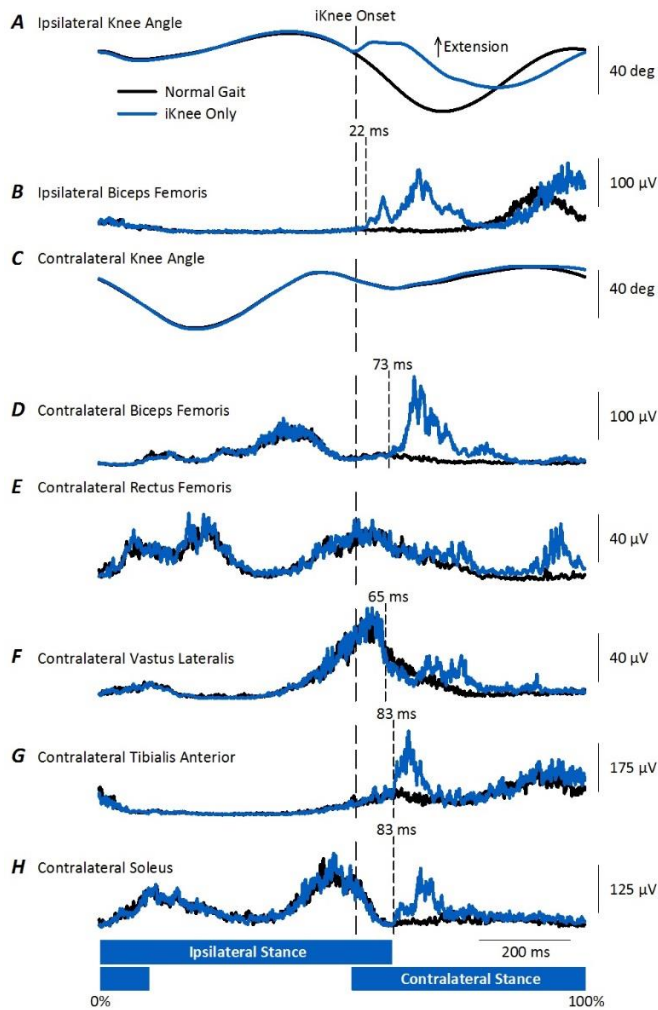
#### 3.4.1 Reflex responses following iKnee perturbation only trials

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 in Study IV, including means and SDs, are provided in Table 3.6. Across all participants, the stretch reflex response in the iBF had a mean onset latency of  $25 \pm 3$  ms. The cBF reflex was observed in all participants and had a mean onset latency of  $80 \pm 11$  ms. The mean amplitude of the cBF reflex (250

$\pm 172\%$  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  $91 \pm 20$  ms) and in the cTA (six out of 12 participants, mean onset latency  $96 \pm 16$  ms), while inhibitory contralateral reflex responses were observed in the cVL (eight out of 12 participants, mean onset latency  $80 \pm 11$  ms) and cRF (six out of 12 participants, mean onset latency  $80 \pm 23$  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 3.12 (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.

**Table 3.6: Mean reflex response data for all participants following iKnee perturbation only trials during walking.** Data are included for all muscles collected, with SDs given in brackets.

Perturbation (timing and direction)		iRF	iBF	cVL	cRF	cBF	cSOL	cTA
50% Extension	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 control)		281.1 (214.2)	-26.7 (5.5)	-22.2 (7.8)	241.9 (181.9)	91.8 (30.9)	90.1 (50.5)



**Figure 3.12: Ipsilateral and contralateral reflex responses following ipsilateral knee extension joint rotation (iKnee) only trials during walking.** Mean data from one participant for either normal gait (60 trials; black lines) or following iKnee extension joint rotations (60 trials; blue 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 blue 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.

### 3.4.2 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 SDs, are presented in Table 3.7. Across all participants, the mean 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).

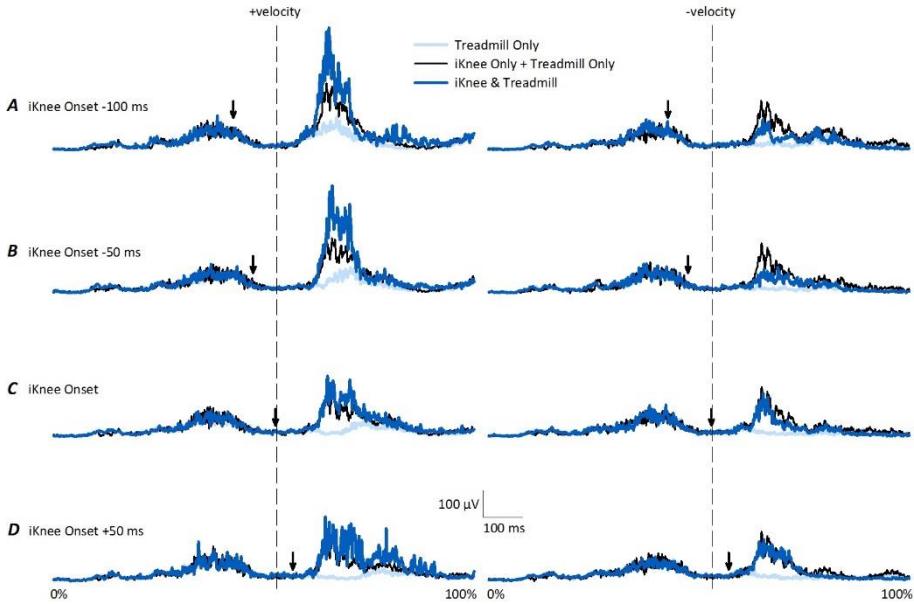
**Table 3.7: Mean cBF response characteristics for all participants following Treadmill only conditions.** Data are included for the four different timings investigated, with SDs given in brackets.

<b>+Velocity Trials</b>			
<b>Timing</b>	<b>Response Latency (ms)</b>	<b>Response Duration (ms)</b>	<b>Response Amplitude (% above normal gait)</b>
-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)
<b>-Velocity Trials</b>			
<b>Timing</b>	<b>Response Latency (ms)</b>	<b>Response Duration (ms)</b>	<b>Response Amplitude (% above normal gait)</b>
-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)

### 3.4.3 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 3.13. The same individual participant’s data are also shown in Figure 3.12. 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 3.13A and B, 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 3.13A and B, 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 3.13C and D).

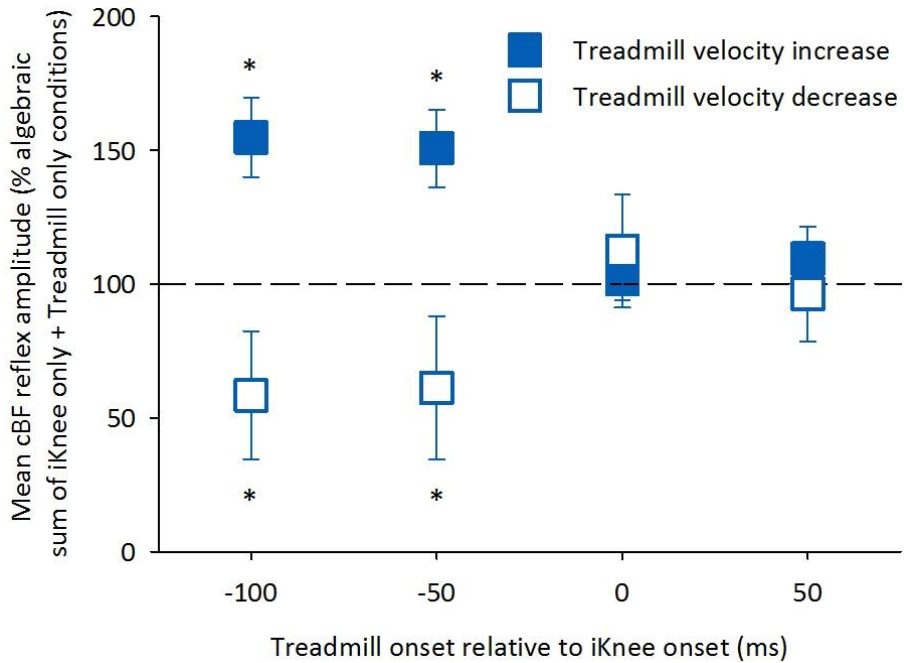


**Figure 3.13: Evidence for extra-facilitation (+velocity trials) and inhibition (-velocity trials) of the cBF reflex following the combined iKnee perturbation and treadmill condition when the sudden changes in treadmill velocity were applied either 100 ms or 50 ms prior to iKnee perturbation onset.** Mean rectified cBF EMG for one participant depicting either the treadmill only condition (30 trials; light blue lines), the combined iKnee perturbation and treadmill velocity change condition (30 trials; dark blue 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 3.12. 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.

The results shown in Figure 3.13 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 3.14 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  $\leq 0.001$ ). 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  $\leq 0.002$ ). 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  $> 0.15$ ).

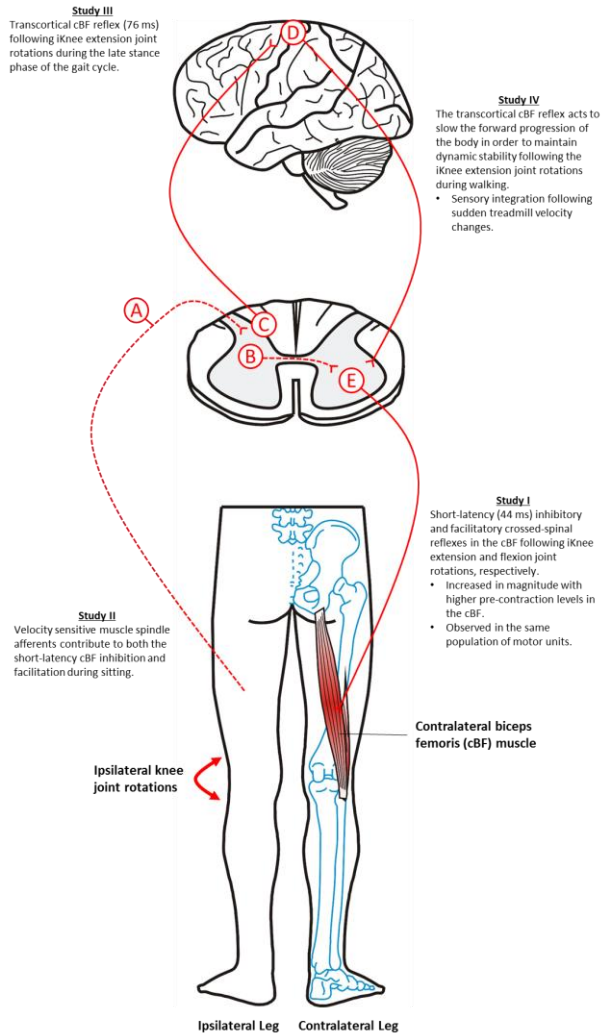


**Figure 3.14: Effect of altering timing of sudden treadmill velocity changes on the cBF reflex amplitude.** Mean amplitude of the initial reflex component of the cBF reflex following a combination of iKnee perturbations delivered at 50% of the gait cycle, and sudden changes in treadmill velocity at 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 squares represent sudden increases in treadmill velocity, while open squares 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 < 0.01). Error bars represent standard error of the mean.

# 4

## DISCUSSION

The current thesis investigated human interlimb neural pathways mediating interlimb reflexes to the cBF muscle following iKnee joint rotations during sitting (Studies I and II) and walking (Studies III and IV). This chapter will present a discussion of the main findings of the thesis. Figure 4.1 summarizes the main findings. Briefly, iKnee extension and flexion joint rotations resulted in spinally mediated (possibly via comINs) short-latency inhibitory and facilitatory reflexes in the cBF muscle, respectively, (Studies I and II). The short latency cBF reflexes were observed in the same population of cBF MUs (Study I), and velocity sensitive group Ia afferents likely contribute (Study II). During walking, a transcortically-mediated interlimb reflex was observed in the cBF following iKnee extension joint rotations applied during the late stance phase of the gait cycle (Study III). The transcortical cBF reflex likely has a functional role in slowing the forward progression of the body in order to maintain dynamic equilibrium during walking (Study IV).



**Figure 4.1: Summary of the main results from the current Ph.D. thesis.** Briefly, iKnee extension and flexion joint rotations resulted in spinally mediated (possibly via comINs, B) short-latency inhibitory and facilitatory reflexes in the cBF muscle, respectively, (Studies I and II). The short latency cBF reflexes were observed in the same population of cBF MUs (E, Study I), and velocity sensitive group Ia afferents (A) likely contribute (Study II). During walking, a transcortically-mediated (C and D) interlimb reflex was observed in the cBF following iKnee extension joint rotations applied during the late stance phase of the gait cycle (Study III). The transcortical cBF reflex likely has a functional role in slowing the forward progression of the body in order to maintain dynamic equilibrium during walking (Study IV). Note that this figure is for summary purposes and does not necessarily reflect the exact neural pathways involved in these interlimb reflexes.

## 4.1 Short-latency crossed responses in the human biceps femoris muscle during sitting

Studies I and II are, to our best knowledge, the first to demonstrate short-latency crossed-spinal responses in human upper leg muscles following mechanical rotations to the iKnee joint. An iKnee extension joint rotations, induced an inhibitory response in the cBF muscle with a mean onset latency of 44 ms. An iKnee flexion joint rotations on the other hand, induced a facilitatory response with a mean onset latency of 44 ms. Both the short-latency cBF inhibition and facilitation responses followed the automatic gain control principle (Matthews, 1986), with the size of the response increasing as the level of background pre-contraction in the cBF muscle increased. Furthermore, both short-latency inhibitory and facilitatory responses in the cBF were observed at the MU level using intramuscular EMG recordings. The same population of cBF MUs that were inhibited following iKnee extension joint rotations were facilitated following iKnee flexion joint rotations, suggesting that the perturbation direction-dependent reversal of the sign of the short-latency cBF reflex can be explained by parallel interneuronal pathways from ipsilateral afferents to single motoneurons in the contralateral leg (De Serres *et al.*, 1995).

### 4.1.1 Short-latency crossed responses in the cBF

The latencies of both the facilitation and inhibition are much shorter than previously reported latencies ranging from 62 to 80 ms after ipsilateral nerve stimulation or mechanical perturbations during walking (Dietz *et al.*, 1986; Nielsen *et al.*, 2008; Mrachacz-Kersting *et al.*, 2011). However, the latencies are similar to recent experiments showing short-latency crossed responses in the cSOL muscle following ipsilateral PTN stimulation with onsets of 37-41 ms during sitting and walking (Stubbs & Mrachacz-Kersting, 2009; Stubbs *et al.*, 2011a, b; Stubbs *et al.*, 2012; Gervasio *et al.*, 2013a; Hanna-Boutros *et al.*, 2014). Despite the distance of the neural pathway to the cSOL being further than to the cBF, the slightly longer cBF reflex latency is likely due to the asynchronous activation of afferents following the iKnee joint rotations compared with the synchronous activation of electrical stimulation to the ipsilateral PTN.

Stubbs and Mrachacz-Kersting (2009) suggested that the crossed responses in the cSOL is spinally mediated because of the short latency of the onset. The

crossed response in the cBF is also likely purely spinally mediated since previous studies have suggested that the minimum latency for a transcortical pathway in the iRF following mechanical flexion of the knee was 54 ms. As the cBF muscle under investigation is also an upper leg muscle, the minimum time for a transcortical pathway to the cBF in Study I is likely similar. Although crossed spinal interneurons have not been directly identified in humans, the results of the Study I contribute to the growing evidence that comINs may contribute to interlimb coordination in humans as well as in cats (Arya *et al.*, 1991; Jankowska *et al.*, 2009; Stubbs & Mrachacz-Kersting, 2009).

#### 4.1.2 Methodological considerations during sitting experiments

Two possible explanations for the short-latency cBF reflexes are that vibrations transferred from the mechanical actuator to the seat of the participants caused the reflexes, or that movement of the contralateral leg due to a mechanical linkage between the legs caused the cBF reflexes. Four pieces of evidence from the Study I argue against these possibilities. First, the sign of the cBF responses depended on the direction of the iKnee perturbation. Secondly, any movement observed in the contralateral leg occurred after the onset of the cBF reflexes (Experiment 1b). Therefore, the cBF reflexes were not caused by movement of the contralateral leg. Thirdly, the onset of the cBF reflexes occur much earlier than the termination of the movement of the mechanical actuator (see Figure 3.1 and Figure 3.4). Finally, if mechanical vibrations transferred from the mechanical actuator caused the cBF reflexes, increasing the cBF contraction level (and stiffness around the cKnee joint) would result in no changes to the amplitude of the reflexes. However, the amplitude of the cBF reflexes increase in magnitude with increasing levels of background pre-contraction in the cBF, following the automatic gain control principle (Matthews, 1986). Based on experiments on animals or humans at rest, this principle states that the amplitude of reflex responses increases with increments in background contraction of the target muscle due to an increase in excitability within the motoneuron pool, for example, because of an increased central drive (Duysens & Tax, 1994). In Study I, both the short-latency cBF facilitation elicited by iKnee flexion joint rotations and the cBF inhibition following iKnee extension joint rotations increased in magnitude with increases in cBF background pre-contraction level (see Figure 3.2). Furthermore, increasing the stiffness of the cKnee joint would also dampen

the oscillations provided by the perturbation, and Experiment 1b showed that any movement of the contralateral joints occurred after the onset of the cBF reflexes.

### 4.1.3 Possible afferent contribution to the short-latency cBF reflexes

In order to examine the afferent pathways mediating the short-latency cBF reflexes discovered in Study I, iKnee perturbation velocity and amplitude were independently manipulated in Study II. Group Ia afferent fibres arising from primary endings in muscle spindles are sensitive to both the static and dynamic components of muscle stretch. Group II afferent fibres arising from secondary endings in muscle spindles, on the other hand, are predominantly sensitive to the static component of muscle stretch and much less sensitive to the dynamic component (see Pierrot-Deseilligny & Burke, 2005). Based on the slower onset latency of the cBF reflexes (44 ms in the surface EMG) relative to the shortest responses in the iRF following iKnee flexion perturbations, which are predominantly mediated by fast conducting group Ia afferents (24 ms, see Table 3.1), it was expected that slower conducting group II afferents likely mediate the cBF reflexes. This is also supported by interlimb reflex data in animals models, where it has been shown that group II afferents play a major role in mediating interlimb reflexes and have direct projections onto commissural interneurons (Arya *et al.*, 1991; Jankowska *et al.*, 2005; Jankowska, 2008; Jankowska *et al.*, 2009). On the other hand, stimulation of group Ia and Ib ipsilateral muscle afferents in cats resulted in both inhibitory and excitatory responses in contralateral motoneurons (Jankowska, 2008).

Increasing the velocity of the iKnee extension and flexion joint rotations resulted in a concomitant increase in the amplitude of the short-latency cBF inhibition and facilitation responses, respectively, while maintaining the same perturbation velocity and increasing the perturbation amplitude resulted in no change to the cBF reflexes. This is in agreement with the velocity sensitivity of the group Ia afferents arising from muscle spindles. Group Ia afferents were also shown to contribute to the SLR component of the stretch reflex in the quadriceps muscles following mechanical knee flexion perturbations, with increases in perturbation velocity, but not amplitude, increasing the size of the SLR (Mrachacz-Kersting, 2014). Furthermore, the short-latency crossed cSOL inhibition is also likely partially mediated by group Ia afferents. Because

ischemia to the ipsilateral thigh delayed, but did not abolish, the cSOL response, group Ia, Ib and II muscle afferents likely contribute (Stubbs & Mrachacz-Kersting, 2009). Indeed, conditioning of the cSOL H-reflex by IPTN stimulation revealed that the early and late phases of the cSOL inhibition were produced at intensities of 0.7 and 1.0 X motor threshold (Hanna-Boutros *et al.*, 2014). These intensities are compatible with the activation of group I and group II afferents, respectively (Marchand-Pauvert *et al.*, 2005; Pierrot-Deseilligny & Burke, 2005). Cutaneous afferents do not contribute to the cSOL reflex since stimulation of the ipsilateral sural and medial plantar nerves did not produce the same response (Stubbs & Mrachacz-Kersting, 2009; Gervasio *et al.*, 2013a). Further evidence of interlimb connections to the cBF arising from ipsilateral muscle afferents in humans was provided by Mrachacz-Kersting *et al.* (2011). Following an unloading of the ipsilateral soleus muscle during the mid to late stance phase of the gait cycle, signaling an early push off, the cBF responded with a significant facilitation after 62 ms, likely signifying the contralateral leg for an earlier touch down. Ischemia applied to the ipsilateral lower leg almost abolished the cBF reflex, indicating that large diameter muscle afferent feedback arising from the ipsilateral ankle muscles provide an important contribution to the activation of the contralateral knee flexors (Mrachacz-Kersting *et al.*, 2011).

From the data in Studies I and II, it is not possible to ascertain whether the cBF reflexes are mediated primarily by afferents from the ipsilateral knee flexors, or the knee extensors, or both. It is possible that the cBF inhibition following iKnee extensions arises from a stretching of the ipsilateral hamstrings muscles, or an unloading of the ipsilateral quadriceps muscles, and vice versa for the cBF facilitation following iKnee flexions. For example, during a knee extension perturbation the hamstrings muscle group is stretched and the pre-activated quadriceps muscles are unloaded (see Figure 3.1B), and following a knee flexion perturbation, the quadriceps are stretched and the hamstrings muscles are shortened. However, because the iRF is pre-contracted prior to the iKnee perturbations, it is likely that afferents from the iRF play a more dominant role than afferents from the iBF. If the iBF muscle afferents were primarily involved in mediating the cBF inhibition following iKnee extensions, the onset latency would likely be delayed compared to the cBF facilitation following iKnee flexions due to the iBF not being active at the time of the perturbations, requiring up to 5 ms for the slackness of the muscle fibers to be taken up (Fellows & Thilmann, 1989).



Based on the results of Study II, it is speculated that velocity sensitive group Ia afferents predominantly mediate the short-latency cBF reflexes. However, to rule out contributions from other afferents, or indeed other muscles, one would have to apply traditional methods for investigating the afferent pathways involved in spinal and supraspinal reflexes arising from perturbations involving the ankle joint, including nerve cooling, lidocaine nerve blocks (Grey *et al.*, 2001), and ischemia (Stubbs & Mrachacz-Kersting, 2009; Mrachacz-Kersting *et al.*, 2011). Due to the anatomical location of the quadriceps and hamstrings muscle groups, however, these techniques are not feasible and further studies are required to determine the source of afferent input mediating the short-latency crossed reflexes in the cBF.

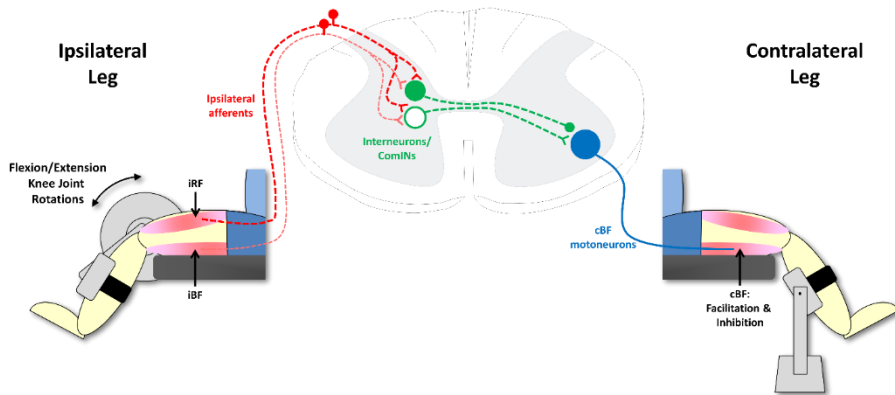
#### **4.1.4 Possible pathways underlying the perturbation direction-dependent reversal in sign of the short-latency cBF reflex**

In Study I, it was observed that the perturbation direction-dependent reversal in sign of the short-latency cBF reflex during sitting was present in the same population of MUs recorded with intramuscular EMG. De Serres *et al.* (1995) observed that the same MUs in the ipsilateral TA muscle were facilitated during swing and inhibited during the transition from swing to stance during human walking following electrical stimuli to the ipsilateral PTN. They suggested that there are parallel excitatory and inhibitory pathways from cutaneous afferents to single motoneurons of the TA muscle, and that a shift in balance between the two pathways throughout the gait cycle likely generated the reflex reversal. It is possible that a similar parallel pathway mediates the observed reversal in sign of the cBF reflex given that the same population of MUs were implicated in both inhibitory and facilitatory reflexes. While the reflex reversal observed in the TA is mediated by the same cutaneous afferents (De Serres *et al.*, 1995), it is possible that different afferent populations from the perturbed ipsilateral leg mediate the cBF reflexes (see Section 4.1.3).

According to the parallel pathways hypothesis by De Serres *et al.* (1995), the reflex reversal could result from an alternate gating or weighting of two parallel interneuronal pathways from afferents innervating the same motoneurons (see Figure 1C in De Serres *et al.*, 1995). Applied to the cBF reflex in Study I, one of these pathways is excitatory and activated following iKnee

flexion joint rotations, while the other is inhibitory and is activated following iKnee extension joint rotations. It is unknown how many interneurons (likely including comINs) exist between the afferent fibres and the motoneurons. However, evidence from animal work suggests that interneurons are a likely site for this type of reversal (De Serres *et al.*, 1995). Figure 4.2 depicts a simplified schematic representation of the proposed parallel interneuronal pathways mediating the perturbation direction-dependent reflex reversal in the cBF muscle in Study I.

An alternative explanation is a single pathway where the specific ipsilateral afferents resulting in the cBF facilitation following iKnee flexion perturbations also result in an inhibition following iKnee extensions due to an increase or decrease in their firing rate. Therefore, the same pathway may be responsible for the perturbation direction-dependent reversal in sign of the cBF reflexes through either more or less afferent input that thus acts differently on the MU pool.



**Figure 4.2: Schematic diagram depicting the proposed parallel interneuronal pathway mediating the cBF reflex reversal following ipsilateral knee extension and flexion joint rotations during sitting.** Given that the same population of motoneurons to the cBF is inhibited following ipsilateral knee extension joint rotations and facilitated following ipsilateral knee flexion joint rotations, they all belong to one functional group. There are inhibitory (filled interneuron) and excitatory (open interneuron) pathways converging onto each motoneuron from the ipsilateral afferents. There are an unknown number of interneurons/comINs between the ipsilateral afferents and the cBF motoneurons (represented by the broken lines). It is proposed that the reflex reversal results from a gating or weighting of one of the pathways depending on the direction of the ipsilateral knee perturbation. The ipsilateral afferents mediating the cBF reflex reversal are unknown and may be of muscular and/or cutaneous origin (represented by the broken lines). Because the iRF is pre-contracted prior to perturbation onset, the afferents arising from the iRF likely play a dominant role (represented by the thicker afferents arising from the iRF than the iBF).

The functional relevance of reflex reversals has been highlighted by their phase dependence during walking in both animals and humans (Duysens *et al.*, 1980; Yang & Stein, 1990; Marchand-Pauvert & Nielsen, 2002). By stimulating cutaneous and muscle afferents in humans, several studies have demonstrated functional, phase-dependent reflex reversals in the ipsilateral limb during walking in humans (Duysens *et al.*, 1990; Yang & Stein, 1990; Duysens *et al.*, 1992; De Serres *et al.*, 1995; Marchand-Pauvert & Nielsen, 2002). Phase dependent reflex reversals have also been identified in the contralateral limb in animals (Duysens *et al.*, 1980; Rossignol & Gauthier, 1980), and more recently in humans (Gervasio *et al.*, 2013a). An interlimb reflex reversal was demonstrated for the first time in humans in the cGL muscle following electrical stimulation to the PTN during normal walking or hybrid walking (Gervasio *et al.*, 2013a). A facilitation occurred in the cGL in response to PTN stimulation during normal walking at ipsilateral push off and contralateral

touchdown, while a reversal in the cGL response was observed at a similar phase of hybrid walking, when both legs are walking in opposite directions. While this finding underlies the functional significance and task dependence of short-latency interlimb reflexes, Studies I and II are the first to show a perturbation direction-dependent reflex reversal in the contralateral limb in humans.

While interlimb reflexes to the cBF following ipsilateral ankle joint rotations appear to have a functional role in maintaining dynamic stability during locomotion (Mrachacz-Kersting *et al.*, 2011), the role of the short-latency perturbation direction-dependent reversal in sign of the cBF reflexes during sitting remains unclear. However, the short-latency cBF reflexes provide further evidence to the notion that comINs play a role in mediating interlimb reflexes in humans. Future in depth studies are required to further elucidate the source and pathways of these responses, including the functional implication in humans. Study III aimed to investigate the functional role of the short-latency interlimb reflex reversal in the cBF to interlimb coordination during walking in humans.

## **4.2 Interlimb reflexes in the human biceps femoris muscle during walking**

### **4.2.1 Ipsilateral reflex responses to mechanical knee joint rotations**

The primary focus of Studies III and IV was to investigate interlimb reflexes following iKnee joint rotations. However, ipsilateral responses to the iKnee perturbations are also of importance. Similar to previous findings, the iBF and iRF responded with a SLR response to the iKnee extension and flexion joint rotations, respectively (Bayoumi & Ashby, 1989; Mrachacz-Kersting *et al.*, 2004). iKnee flexion joint rotations resulted in SLRs in the iRF, with mean onset latencies varying non-significantly throughout the phases of the gait cycle tested between 22 and 30 ms. These times were comparable to what has been reported previously for the knee extensors during human gait following knee joint flexion rotations (Mrachacz-Kersting *et al.*, 2004). The amplitude of the iRF reflex response was modulated throughout the gait cycle, with perturbations at 30% (ipsilateral leg mid-stance) eliciting significantly larger responses than at 0% (ipsilateral heel contact). Mrachacz-Kersting *et al.* (2004)

found a similar modulation in the ipsilateral quadriceps throughout the gait cycle. In their study, the medium and late components of the stretch reflex responses in the ipsilateral quadriceps muscles increased significantly as the gait cycle progressed from late swing phase to the early and mid-stance phases. Following iKnee extension joint rotations applied at 50% of the gait cycle, a stretch reflex was observed in the iBF at a latency of 29 ms (see Figure 3.8). This onset latency is in agreement with studies investigating SLRs in the iBF following mechanical tendon taps during both reduced and normal gait (Van de Crommert *et al.*, 1996; Faist *et al.*, 1999). Given the conduction velocities of the afferent fibres as well as the distance between the thigh muscles and the spinal cord, the SLRs in the iRF and iBF are likely mediated by the velocity sensitive group Ia muscle spindle afferents (Matthews, 1986; Mrachacz-Kersting *et al.*, 2004). Based on the results discussed above, the portable mechanical actuator used in these studies can elicit stretch reflexes in the ipsilateral leg. The following sections will discuss the effects of these perturbations on interlimb reflexes measured in the contralateral leg.

#### **4.2.2 Contralateral reflex responses following ipsilateral knee joint rotations**

The most consistent contralateral reflex observed in Study III was in the contralateral hamstrings following a stretch of the ipsilateral hamstrings muscles. iKnee extension joint rotations applied during the late stance phase (50%) of the gait cycle induced a strong reflex response in the cBF muscle at a mean latency of 76 ms in all participants (see Figure 3.8). In contrast to the contralateral responses observed following iKnee extension joint rotations, no consistent contralateral reflex responses were observed across all participants following iKnee flexion joint rotations at any of the five timings examined during the gait cycle. At the time of perturbations it is possible that flexion of the knee joint did not result in enough neural summation arising from knee afferents to activate any contralateral reflex pathways because the perturbation was not strong enough to disrupt the stability of normal gait. For example, at 50% of the gait cycle, where iKnee extension joint rotations elicited a strong facilitation in the cBF, the iKnee is flexing prior to toe-off and the contralateral leg has just touched down. Applying additional flexion to the knee joint at this time may not have provided the necessary input required to activate interlimb reflex pathways, while iKnee extension joint rotations at this time while the knee was flexing provided more of a threat to postural

stability and the progression of the gait cycle. Many factors contribute to interlimb communication, including limb position, load, and temporal features of the cyclical activity (Duysens *et al.*, 2000), thus it is also likely that the position of the contralateral leg greatly influences the necessity for compensatory interlimb reflexes. Indeed, contralateral afferents have been shown to contribute to interlimb reflexes in the cGM following iTN stimulation (Gervasio *et al.*, 2013b). Consequently, when iKnee flexion joint rotations were applied immediately prior to or during the contralateral swing phase (0%, 10%, 30%, and 90% of the gait cycle), the requirement for contralateral reflexes to maintain postural equilibrium and balance may have been decreased (Berger *et al.*, 1984; Dietz *et al.*, 2004).

The longer-latency (76 ms) facilitatory responses observed during the late stance phase of the gait cycle in Study III are in contrast to the short-latency (44 ms) of the cBF responses observed during sitting in Studies I and II. Many ipsilateral and contralateral reflexes are diminished or absent during walking, likely due to an increase in descending input modulating presynaptic inhibition in order to maintain stability and balance (Morin *et al.*, 1982; Capaday & Stein, 1986; Hayashi *et al.*, 1992; Hanna-Boutros *et al.*, 2014), which may account for the lack of SLRs in the cBF during walking. For example, TMS diminished the short-latency crossed cSOL inhibition elicited by ipsilateral PTN stimulation, while the late phase of the cSOL inhibition was particularly depressed during the stance phase of walking compared with sitting, highlighting the importance of descending input on regulating short-latency crossed-spinal reflexes (Hanna-Boutros *et al.*, 2014). The larger reflexes observed during standing, for example, are consistent with the control of position required to maintain a stable static posture in this task (Capaday & Stein, 1986). Further studies are required to probe the functional significance of the short-latency cBF reflexes during sitting, including whether the same cBF MUs are implicated following iKnee perturbations during sitting and walking.

### **4.2.3 Evidence that a transcortical pathway contributes to the cBF reflex response during walking**

Mrachacz-Kersting *et al.* (2006) reported that the minimum latency for a transcortical pathway to the ipsilateral RF following a mechanical flexion of the knee was 54 ms by summing the mean onset of sensory evoked potentials

(24 ms) together with mean MEP onset latencies (20 ms), in addition to a 10 ms central processing delay. Since the BF muscle under investigation is also an upper leg muscle, the minimum time for a transcortical pathway to the cBF in Study III is likely similar. Indeed, the mean MEP onset latencies following TMS in Experiments 2 and 3 were 23.5 and 24.8 ms, respectively. In Experiments 2 and 3 of Study III, when MEPs in the cBF elicited by magnetic stimulation to the primary motor cortex were timed to coincide with the cBF reflex onset evoked by iKnee extension joint rotations at 50% of the gait cycle they were significantly larger than the algebraic sum of either potential evoked alone (Figure 3.9B). However, when MEPs in the cBF were elicited by electrical stimulation to the primary motor cortex were timed to coincide with the cBF reflex response onset, there was no extra-facilitation of the MEPs compared to the algebraic sum of the potentials evoked alone (Figure 3.11). Petersen *et al.* (1998a) found a similar effect when investigating the long-latency stretch reflex response in the ipsilateral TA muscle and concluded that the results were best explained by an increase in cortical excitability following a stretch to the TA muscle, supporting the hypothesis that the M3 response in the TA is at least in part mediated by a transcortical pathway.

This result provides strong evidence for the involvement of a transcortical pathway in the cBF response because magnetic and electrical stimulation to the cortex activate the same corticospinal tract fibres, but at different levels (Petersen *et al.*, 1998a; Petersen *et al.*, 2003). Electrical stimulation, even at low intensities, penetrates deep into the white matter of the brain and is believed to directly activates the axons of corticospinal cells, while magnetic stimulation activates the corticospinal cells indirectly (via intracortical interneurons) or close to the cell soma (Day *et al.*, 1989; Nielsen *et al.*, 1995; Edgley *et al.*, 1997; Petersen *et al.*, 1998a; Petersen *et al.*, 2003). MEPs elicited by TMS are therefore influenced by the excitability of cortical cells, while MEPs elicited by TES are not or less so.

Further evidence suggesting that a transcortical pathway contributes to the cBF reflex response comes from the result that, when MEPs in the cBF elicited by TMS were timed to arrive 30 or 15 ms before the cBF reflex response onset there was no extra facilitation of the MEPs significantly above the algebraic sum of either potential evoked alone (Figure 3.10A). The mean cBF response onset following iKnee extension joint rotations in Study III was 76 ms, and the mean TMS-evoked MEP onset in Experiment 2 was 24 ms, thus TMS was

delivered to the area of the primary motor cortex controlling the cBF muscle on average 52 ms following perturbation onset. As described above, the mean onset latency of sensory evoked potentials following mechanical stretching of pre-contracted upper leg muscles is 24 ms (Mrachacz-Keresting *et al.*, 2006). An additional 5 ms should be added because the iBF is not active at the time of the perturbation to allow for the slackness in the muscle fibers to be taken up (Fellows & Thilmann, 1989). Adding an extra 10 ms for central processing time (Deuschl *et al.*, 1989; Petersen *et al.*, 1998a) means that the minimum time for afferent information arising from the iKnee extension joint rotation to influence the excitability of the cortical cells in the primary motor cortex controlling the cBF is 39 ms. Convergence at the cortical level between the afferent input from the perturbation and TMS was indeed observed in Study III when TMS was delivered on average 52, 67, and 82 ms (timings 0, +15, and +30 ms) following perturbation onset, but not when TMS was delivered on average 22, 37, or 303 ms (timings -30, -15, and +250 ms) following perturbation onset (see Figure 3.10A).

Furthermore, due to the close proximity of the cortical representation of the iBF and cBF muscles in the primary motor cortex, TMS in Experiment 2 also elicited MEPs in the iBF. There was no increase in the iBF MEP size corresponding to the facilitation observed in the cBF (Figure 3.10B), indicating that the cBF facilitation was not due to a global increase in cortical excitability. Therefore, the most parsimonious explanation is that the iKnee extension rotations applied at 50% of the gait cycle increased the excitability of cortical cells, causing a facilitation of the MEPs elicited in the cBF by TMS, but not TES, when the cortical stimulation was applied to coincide with this increase in cortical excitability.

#### **4.2.4 Functionality of a transcortical cBF reflex at 50% of the gait cycle**

One of the major roles of interlimb reflexes during human walking is believed to be to maintain postural equilibrium (Dietz, 2002). Berger *et al.* (1984) and Dietz *et al.* (2004) noted that perturbations delivered during phase transitions between swing and stance (and vice-versa) and in the opposite direction to the normal joint progression elicited relatively stronger ipsilateral and contralateral responses, suggesting that there may be an increased need for momentary interlimb coordination in order to compensate for external



perturbations at these times. The reflex response in the cBF in Study III was evoked by iKnee extension joint rotations delivered during the late stance phase (50%) of the gait cycle. At this time, the ipsilateral leg is flexing at the knee joint and is about to transit from stance to swing, while the contralateral leg has just touched down and is preparing to take the full weight of the body during single support when the ipsilateral leg pushes off. Our findings further support the idea of a stabilizing response. It is hypothesized that the cBF response likely signifies a preparation of the contralateral leg for early load bearing and slowing the forward progression of the body, thereby helping to maintain postural stability when transitioning from double support to single support and allowing for the continuation of locomotion without a loss of balance. This assumption is supported by the lack of any compensatory movement at the contralateral hip and knee joints following the perturbation (see Figure 3.8C). If the cBF response signifies a preparation of the contralateral leg for early load bearing, it might be expected that knee extensor EMG would also be augmented. This was not found consistently in Study III; three out of ten participants showed responses in the cVL, and two participants showed responses in the cRF. However, the BF is a biarticular muscle, so it is possible that responses were also present in other muscles spanning the hip joint which were not measured.

Study IV 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 that the expression of interlimb reflexes arising from sensory afferents located in the ipsilateral leg 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 & 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 Study IV, 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 & 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 Study IV, 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 was not quantified in Study IV.

To our knowledge, the finding of a transcortical contribution to the cBF response in Study III is the first showing that a transcortical pathway contributes to an interlimb reflex in the upper leg muscles. During walking, human ipsilateral (e.g., Capaday & Stein, 1987; Faist *et al.*, 1996; Sinkjær *et al.*, 1996; Mrachacz-Kersting *et al.*, 2004) and interlimb (e.g., Stubbs *et al.*, 2011b; Mrachacz-Kersting *et al.*, 2014) reflexes in various lower limb muscles are modulated by supraspinal areas throughout the different phases of the gait cycle. Therefore, it makes sense that transcortical pathways contribute to the neural communication between limbs following unexpected perturbations during walking. Such transcortical interlimb reflexes likely allows for more adaptable responses than purely spinally mediated reflexes due to integration

with other sensory information at a cortical level, such as afferent information arising from the contralateral leg, in addition to visual and motivational factors (Christensen *et al.*, 2001; Zuur *et al.*, 2009; Gervasio *et al.*, 2013b). 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 Study IV 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.

#### **4.2.5 Methodological considerations during walking experiments**

In Study III, it would also have been desirable to determine whether the cBF reflex response following knee extension perturbations was modulated throughout the gait cycle in order to further probe the functionality of the response. However, given that the knee joint is close to full extension for much of the gait cycle (mid swing to late stance), this was not possible with the mechanical actuator system that was used to implement the perturbations. The mechanical actuator was set up with strict end stops built in to both the hardware and software to ensure the safety of the participants, so the perturbations could not hyperextend the participants' knee joint.

Given the location of the muscles acting about the knee joint in the human and the nerves innervating these muscles, it is difficult to determine the exact afferent nerves involved in mediating the cBF response observed during

walking in Studies III and IV. Traditional methods for investigating the afferent pathways involved in spinal and supraspinal reflexes arising from perturbations involving the ankle joint, such as nerve cooling, lidocaine nerve blocks (Grey *et al.*, 2001), and ischemia (Stubbs & Mrachacz-Kersting, 2009; Mrachacz-Kersting *et al.*, 2011) are often not possible for the knee joint. It is hypothesized that muscle generated afferent feedback at least partly mediates the cBF response given that SLRs were observed in the iBF following iKnee extension joint rotations (see Figure 3.8B). However, contributions from knee joint and cutaneous afferents cannot be excluded. Furthermore, it is possible that the cBF response following iKnee extension joint rotations arises from a stretching of the ipsilateral hamstrings muscles, or an unloading of the ipsilateral quadriceps muscles. Finally, it cannot be ruled out that vestibular inputs also contribute to the cBF reflex during walking.

An important consideration is that hip or ankle displacements may have contributed to the observed cBF responses in Study IV. We believe this to be unlikely for several reasons. First, no changes in hip joint angles following iKnee extension joint rotations (Study III). Second, due to the late responses recorded in the cBF in Study IV 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 Study IV and others investigating treadmill perturbations alone (e.g., Berger *et al.*, 1984; Dietz *et al.*, 1984; Dietz *et al.*, 1987; Dietz *et al.*, 1989), 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 Study III revealed no changes in movement at the ankle joints within the first 120 ms following the iKnee perturbation (unpublished data).

While eleven out of twelve participants in Study IV were aged between 19-34 years ( $23.8 \pm 4.3$ , mean  $\pm$  S.D.), one participant was 55 years of age and may have been more affected 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 with Figure 4). The corresponding cBF amplitudes following the combined -velocity trials were 48%, 61%, 104%, and 112%, respectively. Despite 55 years of age being at the lower end (or outside) of the age range in studies investigating older adults (e.g., Maver *et al.*, 2011; Krasovsky *et al.*, 2012; Stubbs *et al.*, 2012), it is important to consider the possible differences as gait stability and interlimb coordination are different in older adults (Krasovsky *et al.*, 2012).

#### 4.2.6 Walking in an unstable environment

In addition to demonstrating that the cBF reflex is modulated in a predictable manner following treadmill velocity changes in Study IV, it was also observed that the incidence of interlimb reflexes in other contralateral muscles increased following iKnee extension joint rotations delivered without treadmill perturbations (see Table 3.6). 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.*, 2005, 2006). Haridas *et al.* (2005, 2006) manipulated dynamic stability during walking by either applying unpredictable anterior-posterior perturbations to the trunk, having participants walk with their arms crossed, or a combination of unpredictable anterior-posterior perturbations and arms crossed, and tested cutaneous reflexes during control strides where no external anterior-posterior perturbations were applied. In Study IV, 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 3.6), in addition to the cBF reflex. In comparison to Study III, 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 (see Table 3.5). 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 3.12). 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, 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).

In conclusion for the transcortical cBF reflex during walking, a significant reduction of the cBF reflex elicited by iKnee extension joint rotations was observed when the treadmill velocity was suddenly decreased, and a significant facilitation of the cBF reflex when the treadmill velocity was suddenly increased 50 or 100 ms prior to the iKnee perturbation. The results suggest a functional role for the cBF reflex in slowing the forward progression of the body and maintaining dynamic stability during walking. An increased uncertainty in the walking surface created by the abrupt changes in treadmill velocity in Study IV also increased the incidence of responses in the other

contralateral muscles, indicating a context dependency for interlimb reflexes. A greater knowledge of the functionality of such interlimb reflexes is important in understanding the neural control of human walking, particularly from a rehabilitation perspective.





# 5

## CONCLUSION

This Ph.D. thesis explored interlimb neural pathways to the human biceps femoris muscle by applying iKnee joint rotations during both sitting and walking.

Study I demonstrated that short-latency (44 ms) crossed-spinal reflexes are present in the cBF muscle during sitting, providing further evidence for the presence of comINs in humans. The cBF reflexes were inhibitory following iKnee extension joint rotations, and facilitatory following iKnee flexion joint rotations. Additionally, both short-latency inhibitory and facilitatory cBF reflexes were observable at the MU level by intramuscular EMG, and the same population of cBF MUs that were inhibited following iKnee extension joint rotations were facilitated following iKnee flexion joint rotations. Therefore, parallel interneuronal pathways (likely involving comINs) from ipsilateral afferents to common motoneurons in the contralateral leg can likely explain the perturbation direction-dependent reversal in the sign of the short-latency cBF reflexes. The results of Study II indicated that velocity sensitive muscle spindle afferents likely contribute to the short-latency cBF reflexes.

Study III showed that while short-latency interlimb reflexes following iKnee joint rotations were not observed during walking, strong cBF reflex responses evoked only from iKnee extension joint rotations in the late stance phase with an onset latency of 76 ms. TMS and TES provided evidence for a transcortical pathway contributing to this interlimb reflex. Study IV demonstrated that the function of the cBF reflex is likely a preparation for early load bearing, slowing the forward progression of the body to maintain dynamic equilibrium during walking. Therefore, the transcortical cBF reflex may be integrated with other sensory input, allowing for responses that are more flexible.

These results provide new insights into the neural mechanisms underlying human interlimb coordination, as well as their functional relevance to human locomotion. Although it is difficult to propose the exact neural pathways

mediating interlimb reflexes to the cBF muscle, this thesis provides the basis for future studies.

## 5.1 Future perspectives

The studies in this thesis contribute to the further understanding of interlimb coordination and the neural control of walking, and in turn open up a broad range of unknowns, which will hopefully lead to future research.

While short-latency crossed responses in the lower leg in humans have a functional role during walking (Stubbs *et al.*, 2011b; Gervasio *et al.*, 2013a), the short-latency cBF reflexes observed during sitting were not observed in the upper leg muscles during walking. During walking, longer-latency transcortical interlimb reflexes appear to have a central role. It will be of interest to examine the functional significance of the perturbation-direction dependent reversal in sign of the short-latency cBF reflexes during sitting, and to examine their lack of significance during walking.

One methodological development that may aid in the answering of these questions is the development of a reliable sciatic nerve stimulation technique for eliciting H-reflexes in the hamstrings muscles (Palmieri *et al.*, 2004; Dueholm *et al.*, 2013). A reliable technique for stimulating the sciatic nerve will also allow for the modulation of interlimb reflexes arising from hamstrings muscle afferents to be assessed throughout the gait cycle, which was not possible due to methodological constraints in Study III. Furthermore, it will provide another tool for elucidating the afferent pathways mediating interlimb reflexes in the cBF muscle during both sitting and walking.

While many studies investigating interlimb reflexes involve walking in a stable environment (i.e., on a treadmill with a constant velocity), the reality is that humans seldom walk in such predictable conditions. In Study IV, it was observed that the incidence of interlimb reflexes in other contralateral leg muscles increased while walking in a more unstable environment compared to walking in a more stable environment in Study III. The more unstable environment was created in Study IV by the addition of sudden changes in velocity of the treadmill, which was stable in Study III. It will be important to compare these two situations in one single study in order to directly investigate the integration of interlimb reflexes with other sensory

information and to elucidate how they may be regulated depending on the environmental demands.

Finally, because the results of the current thesis suggest a functional role of the transcortical cBF reflex in maintaining dynamic stability following an external perturbation during walking, future research should consider how such reflexes are impaired in patients with neurological damage, such as stroke or spinal cord injury. Short-latency crossed reflexes in the cSOL are impaired in stroke patients, which may indicate an inability to appropriately coordinate the two legs following external perturbations during walking, potentially leading to an increased risk of falls (Stubbs *et al.*, 2012). Gaining a better understanding of the underlying neural mechanisms regulating the integration of specific interlimb reflexes with other sensory information in healthy participants and beginning to understand how they are impaired in patients will allow for the development of improved rehabilitation strategies in patients with gait impairments.



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## SUMMARY

A continual coordination between the two legs is necessary for maintaining a symmetric walking pattern and adapting to changes in the external environment. Recent evidence in animals and humans suggests that spinal interneuronal circuits under supraspinal control may mediate communication between the lower limbs. The overall objective of the present thesis was to further investigate and elucidate neural pathways underlying interlimb communication in humans, focusing primarily on the possible interlimb connections to the biceps femoris muscle. The major aims were 1) to investigate whether interlimb reflexes are present in sitting and walking following ipsilateral knee (iKnee) joint rotations (Studies I and III), 2) to elucidate the neural pathways involved in mediating the interlimb reflexes (Studies I, II and III), and 3) to investigate the functional role of the observed interlimb reflexes during walking (Study IV).

The results of the this thesis provide new insights into the neural mechanisms underlying human interlimb communication, as well as their functional relevance to human locomotion. Although it is difficult to propose the exact neural pathways mediating interlimb reflexes to the contralateral biceps femoris muscle, this thesis provides the basis for future studies.