Center for Sensory-Motor Interaction

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PhD Thesis by
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"Many of life's failures are people who did not realize how close they were to success when they gave up."

Thomas A. Edison

"It always seems impossible until it's done."

Nelson Mandela

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Preface

The work presented in this PhD thesis is the result of research carried out at the Center for Sensory-Motor interaction (SMI) at Aalborg University (Denmark) in the period from October 2010 to January 2014. The research was supported by Det Obelske Familiefond, Oticon Fonden, Otto Mønsteds Fond and Familien Hede Nielsens Fond.

This thesis investigates spinal pathways that might underlie interlimb coordination in healthy humans. The aim of this thesis was to provide insight to the neural pathway mediating short-latency crossed responses (SLCR) in the gastrocnemius muscle, investigating the possibility of a cortical contribution to this response and how contralateral sensory information is integrated to generate the response. Moreover this thesis aimed at providing evidence supporting its functionality. In order to fulfill these aims electrophysiological experiments, advanced mathematical modeling and movement analysis techniques were applied to elucidate the pathways mediating the crossed responses and their functionality.

This thesis contains five chapters. The Introduction presents the reader to the background and motivation for this project and gives a general overview of the PhD thesis. The Methods chapter introduces the adopted methods and elaborates 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 significance and implications of these findings. Finally, the Conclusions chapter sums up the main findings and future perspective of this work.

List of articles

The thesis is based on four original studies that produced four peer-reviewed journal papers and a peer-reviewed conference paper:

I. Gervasio S., Farina D., Sinkjær T. and Mrachacz-Kersting N. **Crossed reflex reversal during human locomotion.** J Neurophysiol 109:2335-2344, 2013.

II. Gervasio S., Kersting U., Farina D., and Mrachacz-Kersting N. **The effect of crossed responses on dynamic stability.** (Submitted).

III. Gervasio S., Voigt M., Kersting U., Farina D., Sinkjær T., and Mrachacz-Kersting N. **Sensory feedback in interlimb coordination: contralateral afferent contribution to crossed responses during human locomotion.** (Submitted).

IV. Mrachacz-Kersting N., Gervasio S. and Farina D. **Cortical contribution to crossed responses during human locomotion.** (In preparation)

V. Mrachacz-Kersting N., Gervasio S. and Farina D. **Cortical contribution to crossed reflexes in walking humans.** (Submitted)

In addition, several conference abstracts were based on the research conducted in this project.

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Last but not least, I wish to thank my boyfriend, house mate and best friend Steffen. He shared with me the best and worst moments of these last few years and he never left my side. His priceless help, love and at some times immense patience have accompanied me in this trip.

English summary

Walking requires a precise and continuous adjustment of the coordination between movements of the two legs in order to promptly react to any sudden obstacles in the path or any changes in the ground's surface. Research findings suggest that lower interlimb coordination may be mediated by spinal interneuronal circuits under supra-spinal control. While such circuits are well documented in animals, evidence suggesting that direct connections between muscles on either side exist in humans has only recently emerged; afferent feedback arising from the soleus muscle on the ipsilateral side have been shown to affect the activation of the soleus on the contralateral side. This pathway is significantly affected in patients following stroke and may thus also contribute to abnormal symmetry between the legs' movement. It is predictable that the connection between soleus muscles is only one of the pathways underling interlimb coordination. The aim of this thesis was to provide further insight into interlimb coordination, investigating the interlimb neural pathways connecting gastrocnemii muscles during human locomotion.

Short-latency crossed responses (SLCRs) were elicited in the two heads of the contralateral gastrocnemius by ipsilateral tibial nerve stimulation. The functional significance of the responses was investigated by comparing the responses elicited during normal and hybrid walking (in which one leg moves forwards while the other moves backwards) since opposite responses were expected in the two conditions (study I). Furthermore, pressure sensitive insoles were used to assess whether the response produced any changes in pressure distribution under the contralateral foot (study II). To investigate how contralateral sensory information is integrated to generate the SLCR, a mathematical model based on inverse dynamics was adopted to estimate gastrocnemius afferent activity; the estimated afferent activity was then compared with the response elicited in gastrocnemius medialis (study III). Finally, transcranial magnetic stimulation (TMS) was applied to evaluate the possibility of a cortical contribution to the response (study IV).

The results of this thesis confirm the functionality of the SLCR in gastrocnemius since the response is modulated during the gait cycle and a reverse in the response occurs when an opposite reaction is expected. Moreover, the response produces a displacement in center of pressure (CoP) location toward the medial and anterior direction; this displacement might be a method to preserve dynamic stability, preparing the contralateral leg to a faster step in case the perturbed ipsilateral leg is not able to sustain the body weight. Moreover, the results of this thesis suggest that sensory feedback from the contralateral limb, and not only from the one that has been perturbed, is essential to generate a suitable reaction when the natural progression of one limb is threatened. Specifically, autogenic feedback from the same muscle and heterogenic length related feedback from gastrocnemius lateralis likely contributes to the generation of the SLCR in the gastrocnemius medialis muscle. Two components of the response can be commonly distinguished. While the first components seem to be spinally mediated, the second component might be mediated by a supraspinal pathway, since only motor evoked potentials (MEP) elicited by TMS at the time of the second component of the SLCR were facilitated to a greater extent than the algebraic sum of the SLCR and MEP elicited separately.

These results provide new insight into the neural mechanism behind interlimb coordination during human locomotion. Due to the functionality of the gastrocnemius SLCR, future research should investigate the perspective of using the knowledge about these pathways to design innovative rehabilitation approaches to increase dynamical stability in patients with impaired locomotion.

Danish summary / Dansk sammenfating

For at opretholde gang kræves en præcis og løbende tilpasning af koordineringen mellem bevægelser af de to ben for hurtigt at kunne reagere pludselige forhindringer eller eventuelle ændringer i jordens overflade. Forskningsresultater tyder på, at koordination imellem benene kan medieres af spinale interneuronale kredsløb under supra-spinal kontrol. Selv om sådanne kredsløb er veldokumenterede i dyr, er tilsvarende beviser på direkte forbindelser mellem muskler på hver side findes hos mennesker først for nylig dukket op. Afferent information fra soleus musklen på den ipsilaterale side, påvirker aktivering af soleus på den kontralaterale side. Denne kommunikation er væsentligt påvirket i patienter efter slagtilfælde og kan således også bidrage til unormal symmetri mellem benenes bevægelse. Det forventes, at forbindelsen mellem soleus musklerne kun er en af de underliggende mekanismer, der styrer koordineringen imellem benene. Formålet med denne afhandling var at give yderligere indsigt i koordineringen imellem benene og undersøge hvilke nervebaner der forbinder gastrocnemius musklerne under menneskelig bevægelse.

Kort-latens kryds-reaktioner (short-latency crossed responses, SLCRs) blev fremkaldt i hver del af den kontralaterale gastrocnemiusmuskel ved ipsilateral tibial nerve stimulation. Den funktionelle betydning af svarene blev undersøgt ved at sammenligne reaktioner fremkaldt under normal og hybrid gang (hvor det ene ben bevæger sig fremad, mens den anden bevæger sig baglæns) (studie I). Desuden blev trykfølsomme indlægssåler brugt til at vurdere, om en evt. reaktion gav ændringer i trykfordeling under den kontralaterale fod (studie II). For at undersøge hvordan kontralateral sensorisk information er integreret til at generere SLCR, blev der implementeret en matematisk model baseret på invers dynamik for at estimere gastrocnemius afferente aktivitet; den estimerede afferente aktivitet blev derefter sammenlignet med den respons, der kunne måles i gastrocnemius medialis (studie III). Endelig blev transkraniel magnetisk stimulation (TMS)

anvendt til at vurdere muligheden for evt. kortikale bidrag til koordinationen imellem benene (studie IV).

Resultaterne af denne afhandling bekræfter funktionaliteten af SLCR i gastrocnemius, da responset i disse musklerer moduleret under gangcyklusen, og der opstår et omvendt respons, når der forventes en modsat reaktion. Foruden SLCR, fandtes der en forskydning i midten af tryk (center of pressure: COP) i den mediale og anterior retning; denne forskydning kan være en metode til at bevare den dynamiske stabilitet, som er med til at forberede det kontralaterale ben til et hurtigere skridt i tilfælde af det perturberede ipsilaterale ben ikke er i stand at opretholde kropsvægten. Desuden viser resultaterne af denne afhandling, at sensorisk feedback fra den kontralaterale ekstremitet, og ikke kun fra den der er blevet forstyrret, er afgørende for at generere en egnet reaktion, når den naturlige progression af et lem er truet. Specifikt feedback fra gastrocnemius medialis og længde-relateret-feedback fra gastrocnemius lateralis bidrager sandsynligvis til frembringelsen af SLCR i gastrocnemius medialis muskel. Normalt kan der skelnes imellem to komponenter i SLCR. De første komponenter lader til at være spinalt medieret, mens den anden komponent lader til at være supraspinal medieret, da kun motor evokerede potentialer (MEP) fremkaldt af TMS på tidspunktet for den anden komponent af SLCR var større end den algebraiske sum af SLCR og MEP fremkaldt separat.

Disse resultater giver ny indsigt i den neurale mekanisme bag koordinationen imellem benene under gang. På grund af funktionaliteten af gastrocnemius' SLCR bør fremtidig forskning undersøge hvorvidt det er mulgit atbruge denne viden om disse nervebaner kan være med til at designe innovative rehabilitering fremgangsmåder til at øge dynamiske stabilitet hos patienter med nedsat bevægelsesevne

List of abbreviations

5-HT,	5-hydroxytryptamine;
c5met,	contralateral fifth metatarsal joint;
cCond,	contralateral lateral epicondyle of the femur;
cGL,	contralateral gastrocnemius lateralis;
cGM,	contralateral gastrocnemius medialis;
cMall,	contralateral lateral malleolus;
CoM,	center of mass;
comINs,	commissural interneurons;
CoP,	center of pressure;
CPG,	central pattern generator;
cSOL,	contralateral soleus;
cTroc,	contralateral great trochanter;
DSCT,	dorsal spinocerebellar tract;
EPSPs,	excitatory postsynaptic potentials;
GL,	gastrocnemius lateralis;
GM,	gastrocnemius medialis;
GRF,	ground reaction force;
GTO,	Golgi tendon organ;
iCalc,	ipsilateral calcaneus;
iGL,	ipsilateral gastrocnemius lateralis;
iMpN,	ipsilateral medial plantar nerve;
IPSPs,	inhibitory postsynaptic potentials;
ISI,	inter stimulus interval;
iSOL,	ipsilateral soleus;

iSuN,	ipsilateral sural nerve;
iTN,	ipsilateral tibial nerve;
MEP,	motor evoked potential;
MpN,	medial planter nerve;
PCSA,	physiological cross-sectional area;
RMS,	root mean square;
sacr,	sacral bone;
SD,	standard deviation;
sEMG,	surface EMG;
SLCR,	short-latency crossed response;
SOL,	soleus;
SuN,	sural nerve;
TES,	transcranial electrical stimulation;
TMS,	transcranial magnetic stimulation;
TN,	tibial nerve;
VSCT,	ventral spinocerebellar tract;

CHAPTER 1

1. Introduction

In order to maintain stability while walking, locomotor patterns must be flexible enough to accommodate changes in speed or in the ground's surface or to promptly react to any sudden obstacles that can be encountered in the path. Maintaining stability requires a continuous adjustment of coordination of muscle activation between the limbs, referred to as interlimb coordination.

In this chapter, the current knowledge about the control of interlimb coordination during locomotion will be reviewed. Since the use of invasive procedures is limited in humans and some similarities exists between the human and other animals (Nielsen, 2003), much of the knowledge about interlimb coordination results from animal models and especially from cats. However, differences in the control of locomotion, and therefore in interlimb coordination, between humans and cats cannot be ignored; upper limbs in the humans have become specialized to perform skilled hand movements and some other features, such as the lengthening contraction of the ankle plantarflexors during most of the stance phase, make human walking unique (Nielsen, 2003). Therefore, the main findings about interlimb coordination in cats will be presented first, followed by the principal outcomes of research studies investigating lower limb coordination in humans.

Since the focus of this thesis is interlimb communication, this chapter will not deal with the general control of locomotion, on which extensive literature exists (Duysens and Van de Crommert, 1998; Nielsen, 2003), but will focus on how afferent feedback arising from receptors within one limb can affect the activity of the other. This communication is more evident when the balance is threatened; in this case, interlimb reflexes have been shown to occur, and these appear to have the functional purpose of maintaining postural stability after an unilateral perturbation (Berger et al.,

1984; Dietz et al., 1989, 2004). Therefore, studies suggesting functional significance of these interlimb reflex pathways will be briefly reviewed. Subsequently, the knowledge about spinal structures that might underlie interlimb coordination will be presented both for cats and humans. These structures, and in particular the existence of a possible interlimb spinal connection between gastrocnemii muscles will be the focus of the following chapters.

Interlimb coordination during walking

1.1.1. Interlimb coordination in the cat

It is commonly accepted that rhythmic activities in animals are generated by special neural networks referred to as Central Pattern Generators (CPG) (Grillner, 1985). It is assumed that each limb is controlled by its own CPG, located in the spinal cord and that the interconnection of the CPGs in the Central Nervous System (CNS) generates the different interlimb coordination patterns used for various forms of locomotion, as for example walking and running.

Bilateral muscle activation persists even in the absence of sensory input from the periphery, as shown from fictive locomotion; in this experimental preparation the locomotor output is present in a motionless animal, where afferent feedback is removed and muscles are either paralyzed or removed. In decerebrate cats, the locomotor pattern remains after bilateral deafferentation of hind limbs (Miller et al., 1975).

However, sensory inputs from one side affect the rhythmic activity of the other side; in spinal cats the rhythm in one limb can be altered by manipulation of another limb (Rossignol et al., 1993). Moreover, in decerebrate cat, the coordination between the four limbs has been shown to adapt to changes in the environment (Yanagihara et al., 1993); when the cats were exposed to locomotion on a split-belt treadmill, where one hind limb is forced to go faster than the others, the duration of the double support phase adjusted asymmetrically in the two sides and the gait cycle duration is

significantly shortened (Forssberg et al., 1980). It is unclear how much of this adjustment is due to brain stem or cerebellar activity. These areas have been suggested to play a relevant role in interlimb coordination (Kato, 1992; Swinnen and Duysens, 2004). Neurons in the ventral spinocerebellar tract (VSCT) carry information about the timing of the step cycle from more than one limb (Arshavsky et al., 1984). Neurons in the dorsal spinocerebellar tract (DSCT) receive bilateral sensory input from the limbs. The reticulospinal tract provides output signals related to the activity of groups of flexor and extensor muscles in single or multiple limbs (Matsuyama and Drew, 2000). However, spinal cats are able to adapt interlimb coordination when the walking speed of left and right side is unequal, like in the split belt condition (Forssberg et al., 1980; Frigon et al., 2013). Therefore, although for a full expression of this coordination supraspinal and afferent inputs are essential, the basic wiring for interlimb coordination in cats seems to be present in the spinal cord (Swinnen and Duysens, 2004; Frigon et al., 2013).

1.1.2. Interlimb coordination in humans

Unlike animal studies, demonstrating the existence of CPGs in the intact human is not an easy task; however some evidence supporting the existence of CPGs in humans has been provided by cases where supraspinal control is limited, e.g. in patients with spinal cord injuries (Harkema et al., 1997) and infants (Yang et al., 1998). Although there is a lack of functional motor recovery in patients with a complete spinal cord lesion (Nielsen, 2003), alternating rhythmical activity in leg muscles has been elicited in rare cases in patients with complete spinal cord lesions (Bussel et al., 1996; Dimitrijevic et al., 1998). Moreover, involuntary stepping-like movements were observed in incomplete spinal cord injured patients unable to produce voluntary movements (Calancie et al., 1994; Dobkin et al., 1995). Besides, studies on infants, between 2 and 11 months, which have immature supraspinal pathways, showed well organized phase-dependent and location specific reflex responses to mechanical disturbances during walking in various directions requiring very

different interlimb coordination (Pang and Yang, 2000, 2002). Walking in infants is possible with weight support and is largely controlled by the spinal and brainstem circuitry. Dominici and colleagues (Dominici et al., 2011) recorded EMG activity form trunk and legs muscles of stepping neonates, toddlers, preschoolers, and adults, showing that the locomotor output of neonates can be described by two basic activation patterns that are retained and augmented through development by two new patterns. Similar patterns were observed also in several animal species. The combined evidence from these studies suggests that CPGs may exist in humans as in other vertebrates, (likely arising from a common ancestral network), and that the interconnection between spinal CPGs may be important for human interlimb coordination. Nevertheless, a partial descending control is present in both patients with incomplete spinal cord lesions and infants and therefore these cases cannot provide conclusive evidence for the role CPG in humans. The experiments of Pang and Yang (2000) showed, however, that in human infants, as in decerebrate and spinal cats, the stepping patterns is regulated by afferent input, such as limb loading and hip position.

The role of afferent feedback in human interlimb coordination has been investigated using various experimental paradigms. For example, data recorded during cycling or during "reduced gait", where the subject is limping with one leg on a moving split belt while the other leg is held stiff on a stationary belt to reduce sensory feedback, underline that the activity in one leg influences reflex modulation or muscle activity in the other (Van de Crommert et al., 1996; Ting et al., 1998, 2000; Faist et al., 1999). The importance of afferent feedback in interlimb coordination in humans has also been investigated using split belt locomotion. This walking pattern mimics the unloading of one limb that occurs for example when making a turn where the body's center of mass (CoM) moves toward the inner foot. In this situation the inner foot (on the slower belt) is loaded, while the external foot (on the faster belt) is unloaded (Vallis et al., 2001) and this change in afferent feedback is accompanied by a change in the electromyographic (EMG) signal (Stephens and Yang,

1999) and an increase in stride length on the fast side (outer) compared to the slower side (inner) (Dietz et al., 1994).

1.2. Functionality of interlimb reflexes

Interlimb reflexes are known to be relevant for postural stability (Dietz, 2002). The displacement of one leg during quite stance elicits rapid bilateral activation and co-contraction of the non-displaced leg, probably in order to increase stability and compensate for the perturbation (Dietz et al., 1989). During walking, bilateral leg muscle EMG responses are elicited by unilateral rotational hip or knee joint displacements, and these responses are much stronger when the displacement is directed against the physiological movement trajectory; it was therefore suggested that these response patterns are meant to restore the physiological movement trajectory (Dietz et al., 2004). Moreover, different unilateral perturbations elicited bilateral EMG responses specific for the type of perturbation and dependent on the phase of the gait cycle in which the perturbation occurred (Berger et al., 1984). For instance, treadmill deceleration evoked a bilateral tibialis anterior activation while acceleration evoked an ipsilateral gastrocnemius and contralateral tibialis anterior activation. Electrical stimulation of the tibial nerve during the swing phase was instead followed by an ipsilateral tibialis anterior and contralateral gastrocnemius activation. Even if different responses were observed, the same functional mechanism was proposed to govern these responses; it was suggested that early ipsilateral responses repositioned the displaced leg, while the early contralateral and late ipsilateral responses compensated for body displacement (Berger et al., 1984).

During phase transitions from stance to swing or vice versa, the role of interlimb reflexes is even more evident; bilateral responses are more prominent when a perturbation is delivered during phase transitions than when it occurs at other times (Berger et al., 1984; Dietz et al., 2004). This seems to indicate that at these times there is an increased need for interlimb coordination to

compensate for external perturbations. For instance, an ipsilateral knee extension joint rotation during the late stance phase evokes a facilitation in the contralateral biceps femoris (Stevenson et al., 2013). Since at this time, the contralateral leg is preparing to sustain the full body weight, the response in the contralateral biceps femoris may indicate a preparation of the contralateral leg for early load bearing and slowing the forward progression of the body, in order to maintain postural stability during the phase transition (Stevenson et al., 2013).

Interlimb reflexes have been widely described using EMG recordings, from which their functionality is often inferred. However, none of these studies investigated the effects of interlimb reflexes on the Center of Pressure (CoP), which is commonly used to evaluate balance and postural control; such measurement would provide direct evidence for the functional significance of these responses.

1.3. Spinal structures underlining interlimb communication

1.3.1. Commissural interneurons

In the cat, a group of interneurons crossing the spinal cord and projecting directly or via interneurons to contralateral motor neurons has been described (see Jankowska, 2008 for review). These interneurons are referred to as commissural interneurons (comINs) and have been suggested to contribute to the coordination between the two sides (Jankowska, 2008). ComINs have been identified within the spinal cord of the anesthetized cat in the dorsal horn of lamina IV, V (Matsushita, 1970), and VIII (Matsushita, 1970; Jankowska and Noga, 1990; Edgley et al., 2003).

Effects on contralateral motorneurons have been observed after stimulation of several ipsilateral afferents. Although comINs were not always directly identified as a mediator of the response, the short latency of the responses and spinal transection indicates that the responses in the contralateral motorneurons are mediated by comINs. Inhibitory or excitatory short-latency

connections mediated by di, tri or poly synaptic pathways have been observed from group Ia and group Ib afferents (Jankowska, 2008). Following stimulation of group II afferents, inhibitory postsynaptic potentials (IPSPs) or no responses were recorded in the majority of contralateral motorneurons in cats with intact spinal cord (Arya et al., 1991). A group of comINs has been shown to be facilitated by contralateral group I and II afferent stimulation, indicating that these comINs project to contralateral group Ia/Ib and II interneurons (Jankowska et al., 2005b, 2009; Cabaj et al., 2006; Jankowska, 2008).

The actions of comINs on contralateral motorneurons are strongly modulated by inputs from supraspinal areas. This aspect can be evaluated in animal studies where the spinal cord is completely transected. In cats with a transected spinal cord, a reversal of responses from IPSPs to excitatory postsynaptic potentials (EPSPs) in extensor contralateral motorneurons compared to the spinally intact cat was observed from any ipsilateral group II afferent while the effect on flexor contralateral motorneurons remained IPSPs (Arya et al., 1991). However, after administration of serotonin (5-hydroxytryptamine; 5-HT) to the spinal transected cat, an IPSPs was again observed in extensor contralateral motorneurons and following administration of a 5-HT antagonist the IPSPs was again substituted by an EPSPs. These results indicate that a tonic descending drive involving 5-HT neurotransmitters may be necessary to generate IPSPs in contralateral motorneurons through comINs with group II afferent input (Aggelopoulos et al., 1996).

The supraspinal areas providing input to comINs have been investigated in the cat; input to comINs has been observed from ipsilateral and contralateral reticulospinal tracts, vestibulospinal tract (Jankowska et al., 2005a) and rubrospinal tract (Stecina et al., 2008). In particular, when the mesencephalic locomotor region is stimulated, IPSPs and EPSPs in the ipsilateral and contralateral flexors and extensors of the hindlimb have been observed (Noga et al., 2003). Moreover, after transection superior to the mesencephalon, stimulation of the mesencephalic locomotor region

results in walking, running and trotting (Shik and Orlovsky, 1976). These studies indicate that the reticular formation and mesencephalic locomotor region may have an important role in the control of comINs and interlimb coordination.

1.3.2. Crossed responses and their reversal in animals

Spinal connections such as comINs have been proven to be active during walking (Frigon and Rossignol, 2008). For instance, an inhibition followed by facilitation was observed in the contralateral triceps surae following ipsilateral inferior tibial nerve stimulation in a walking cat (Frigon and Rossignol, 2008). In specific situations, and only in animal studies, it is possible to observe opposite responses in the contralateral limb in the intact animal (Magnus, 1909, 1910; Grillner and Rossignol, 1978; Duysens et al., 1980; Rossignol and Gauthier, 1980). For example, a reversal from extensor to flexor responses has been observed in intact cats when stimuli were delivered at the end of the contralateral stance phase (Duysens *et al.*, 1980).

The position of the contralateral limb has been suggested to be the main factor determining the direction of the crossed response (Magnus, 1909, 1910; Grillner and Rossignol, 1978; Rossignol and Gauthier, 1980), so that the responses are directed toward the muscle that is more stretched (Uexküll, 1904; Magnus, 1909, 1910). Specifically, contralateral muscle receptors have been suggested to play a predominant role in the control of the crossed reflex reversal (Rossignol, 1977). The contralateral response to a knee tap in chronic spinal dogs is an extension or a flexion depending on whether the contralateral limb was initially flexed or extended (Magnus, 1909, 1910). Similarly, when the position of the contralateral hind limb was altered (Grillner and Rossignol, 1978; Rossignol and Gauthier, 1980), a reversal from crossed flexion to crossed extension was observed in spinal cats treated with clonidine, a noradrenergic receptor stimulant. The responses were still present after tenotomy of both antagonist muscles suggesting that other intact pairs of muscles acting on the same joint also contribute (Grillner and Rossignol, 1978; Rossignol and

Gauthier, 1980). In addition, the most commonly evoked response seems to be a crossed extension, unless the flexor muscles are stretched in which case a crossed flexion is evoked (Rossignol & Gauthier, 1980). This idea is supported by the observation that after complete deafferentation, crossed flexor responses disappear while crossed extensor responses persist (Rossignol & Gauthier, 1980).

From the functional perspective, it is possible that the most frequently observed crossed response in animals following ipsilateral stimulation is an extension that has the purpose of sustaining the additional weight when the center of gravity shifts to the contralateral leg (Rossignol & Gauthier, 1980). Such response would however be inappropriate when the contralateral leg is not in a position to sustain weight, e.g. at the end of the ipsilateral swing phase in the walking cat when the contralateral leg is fully extended (Duysens *et al.*, 1980). This condition would be signaled by the stretch of the flexor muscles (Rossignol & Gauthier, 1980). Therefore, the appropriate response seems to be at least partially selected on the basis of the peripheral input. However, the observation that an ipsilateral reflex reversal can be evoked during fictive locomotion in paralyzed cats (Andersson et al., 1978) suggests a possible central control of reflex reversal during locomotion. Thus, an integration of peripheral and central control seems to occur to appropriately adapt reflex responses during locomotion (Rossignol & Gauthier, 1980).

1.3.3. Short latency crossed responses in humans

In humans, the evidence for comINs is more indirect due to the inability to perform similar invasive studies as in animals. However, the existence of spinally mediated interlimb reflex pathways from muscle afferents has been proposed based on responses observed following perturbations such as loading and unloading (Bachmann et al., 2008), whole body perturbations (Dietz et al., 1989), hold and release perturbations (Dietz et al., 1986) and treadmill acceleration/deceleration (Berger et al., 1984); though, it is also possible that in the case of

mechanical perturbations, the observed responses may originate from the contralateral leg if the mechanical stimuli altered the position of both legs. Following stimulation of cutaneous nerves at the ankle, responses in the contralateral muscles have also been observed (Delwaide et al., 1981; Burke et al., 1991; Duysens et al., 1991; Haridas and Zehr, 2003); this methodology reduces the possibility of contralateral responses arising from the contralateral leg.

The latencies of the reported crossed responses ranged from 60 to 120 ms; it is thus possible that some of the observed responses are mediated by cortical or sub-cortical areas. However, recent studies demonstrated that short-latency inhibitory responses can be elicited in the contralateral SOL (cSOL) with a latency of 40 ms following ipsilateral tibial nerve (iTN) stimulation (Stubbs and Mrachacz-Kersting, 2009; Stubbs et al., 2011a, 2011b); this latency is too short for the response to be mediated by a transcortical pathway. The minimum latency for a transcortical pathway's contribution to ipsilateral responses in the tibialis anterior muscle, which is located at a similar distance from the cortex as the triceps surae, was indeed reported to be 79 ms (Petersen et al., 1998a). Therefore, the results reported by Stubbs and colleagues (Stubbs and Mrachacz-Kersting, 2009; Stubbs et al., 2011a, 2011b) support the theory that comINs are present in humans.

It was suggested that these comINs receive input from ipsilateral muscle or tendon afferents from the homologous muscle as no similar crossed responses are elicited by stimulation of the ipsilateral sural nerve (iSuN) and medial plantar nerve (iMpN) (Stubbs and Mrachacz-Kersting, 2009; Stubbs et al., 2011b) and since ischemic block significantly delayed the time of the minimum of the inhibition (Stubbs and Mrachacz-Kersting, 2009). The application of ischemia would block large diameter group Ia and Ib afferents; however, there is some overlap of the diameter of group I and II afferents, thus it is possible that some group II afferents are blocked, too. Consequently, as ischemia only partially altered the inhibition, it was suggested that group I and/or group II afferents may be a source of the response (Stubbs and Mrachacz-Kersting, 2009).

This short-latency crossed spinal inhibitory response seems to be modulated by supraspinal areas. The contribution of this input was evident as the response was impaired in patient populations and significantly more variable in subacute than in chronic stroke patients, at least during sitting, probably due to the reorganisation of cortical networks that become more stable from three to six months following the occurrence of injury (Stubbs et al., 2012).

The short-latency crossed spinal inhibitory response seems to have a functional purpose. The iTN stimulation provokes a sudden plantar flexion of the ipsilateral swinging foot that might indicate a threat to the stability of the body. The threat to balance is greater when it occurs at the gait transition phase from swing to stance while the foot is supposed to be dorsi-flexing. This was also the time when the largest responses were reported. Therefore, the inhibition in the cSOL EMG activity could have the purpose of stopping the forward progression of the contralateral leg toward the source of the disturbance until supraspinal pathways have time to voluntary choose the appropriate reaction (Frigon and Rossignol, 2008; Stubbs et al., 2011b). Thus, the impaired crossed responses observed in patients could indicate an inability to appropriately coordinate the legs in the case of a mechanical disturbance to the ipsilateral limb; this inability might contribute to the increased incidence of falls in patient populations (Stubbs et al., 2012).

1.4. Aims

The aim of the current thesis was to further investigate and elucidate neural pathways at the base of interlimb coordination, focusing on the possible interlimb connections between gastrocnemii muscles. Previous studies reported crossed responses in the contralateral gastrocnemius during walking with latencies ranging from 65 to 112 ms after ipsilateral nerve stimulation or mechanical perturbation (Berger *et al.*, 1984; Dietz *et al.*, 1986, 1989; Duysens *et al.*, 1991). It is predictable that if the short-latency inhibitory response in cSOL has the functional purpose of preventing the push off of the contralateral foot and the progress of the perturbed step, this would be only one of the

responses activated by ipsilateral muscle afferents through iTN stimulation in order to maintain stability. Other responses mediated by pathways underling interlimb coordination would thus be expected.

The goals of the current thesis can be thus summarized by the following questions (Figure 1):

1. Are short-latency crossed responses (SLCR) in the gastrocnemius muscle functional?
2. Which afferent fibers from the ipsilateral leg mediate the response?
3. Does contralateral afferent feedback have a role in the generation of SLCR in the gastrocnemius?
4. Do supraspinal structures have a role in the modulation of SLCR in the gastrocnemius?

These questions have been addressed in 4 studies:

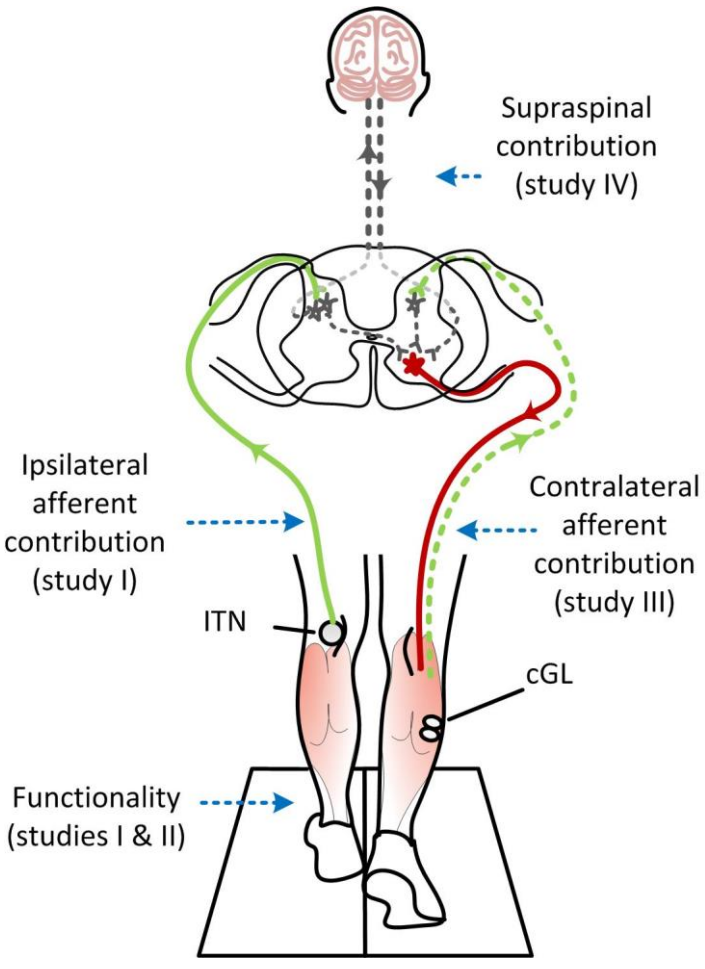

Figure 1: ***Goals of the current thesis divided in four studies.*** Study I and II aimed at assess the functionality of the responses. In addition study I attempted to clarify whether ipsilateral afferents mediating the response are of muscular or cutaneous origin. Study III investigated whether contralateral afferents contribute to the modulation of the response. Study IV aimed at the supraspinal contribution to the SLCR. The red and green traces represent respectively efferent and afferent pathways. Dashed lines represent possible but unknown contributions to the pathway mediating the SLCR.

The first study aims at assessing the functionality of interlimb connections between gastrocnemii muscles by investigating the possibility of eliciting a crossed reflex reversal. A reversal in the response is expected when an opposite reaction would be required, but a crossed reflex reversal has never been observed in humans before. Therefore, the response observed in the contralateral gastrocnemius lateralis (GL) after iTN stimulation was quantified and its behavior was investigated in two walking tasks in which an opposite functional response was expected. A condition requiring an opposite reaction compared to normal walking was created with the use of a hybrid walking task, in which the subject walked on a split belt treadmill with the belts running at the same speed but in opposite directions, thus disturbing the natural coupling of the legs during normal locomotion. During normal walking, a facilitation in the gastrocnemius muscle after the inhibition observed in the cSOL at the end of the ipsilateral swing phase, could be beneficial in order to reinforce the stability of the ankle and knee joints (Duysens et al., 1991). During hybrid walking, at 40 or 50% of the ipsilateral gait cycle, the ipsilateral foot is pushing off and the contralateral foot is not yet completely on the ground; and thus an extension of the contralateral knee forcing the heel to the ground could represent the more appropriate response. Therefore, a crossed inhibition in the gastrocnemii could support such a reaction preventing the knee from flexing. Moreover, in order to investigate the contribution of cutaneous afferents to the SLCR, the responses elicited by iSuN and iMpN were compared to those evoked by iTN. SuN and MpN were stimulated since these two nerves are primarily cutaneous nerves that adjoin to the TN.

However, without any kinetic measurement, the functionality of the SLCR in the cGL can only be speculated. The second study further investigates the functionality of these pathways by observing the effects of crossed responses on CoP and pressure distribution under the contralateral foot. The CoP is a measure commonly used in clinical research to evaluate balance and postural control. It was hypothesized that the facilitation observed in the cGL following a perturbation of the

ipsilateral leg could be a method to stop the progression of the contralateral leg and keep the body weight on it by inducing a flexion of the knee. Accordingly, it was expected that the SLCR would induce a shift of the contralateral foot's CoP backwards in comparison to the unperturbed trajectory.

The last two studies aim at elucidating some of the neural mechanisms contributing to these interspinal pathways. The third study quantifies the SLCR in the contralateral gastrocnemius medialis (cGM) and investigates whether contralateral afferent activity contributes to the modulation of the SLCRs observed in the gastrocnemius muscle during human walking. Indeed, comINs can be facilitated by contralateral group I and II afferent stimulation (Jankowska et al., 2005b, 2009; Cabaj et al., 2006; Jankowska, 2008) and contralateral muscle receptors have been suggested to play a predominant role in the control of the crossed reflex reversal (Rossignol, 1977). Thus, an inverse dynamics model and mathematical models previously used to predict spindles firing of in mammalian triceps surae were integrated to estimate spindles primary ending ("group Ia afferent"), spindles secondary ending ("group II afferent"), and Golgi tendon organ (GTO) ("group Ib afferent") afferent activity that was then compared to the modulation of the SLCR.

The fourth and last study investigated the cortical contribution to the SLCR in the contralateral gastrocnemius muscle. Supraspinal structures are relevant in interlimb coordination and have been shown to modulate the action of comINs. However, to our knowledge, there is no evidence sustaining or rejecting a supraspinal contribution to the investigated interspinal pathways. Moreover, the minimum latency for a transcortical pathway's contribution to ipsilateral responses was reported to be 79 ms for tibialis anterior muscle, which is located at a similar distance from the cortex as the triceps surae (Petersen et al., 1998a). This latency is longer than the latency of the investigated responses (see Results). Transcranial Magnetic Stimulation (TMS) is one of the adopted methods to investigate the role of supraspinal centers in modulating reflexes in lower

limb muscles (Christensen et al., 2001; Mrachacz-Kersting et al., 2006; Stevenson et al., 2013). Therefore, the modulation of contralateral motor evoked potentials elicited by TMS with and without conditioning electrical stimuli applied to the iTN was examined while subjects walked on a treadmill. The observation of a response that when both electrical stimulation and TMS are performed is more prominent than the sum of the responses obtained when a single stimulation is performed would prove the convergence between stimulation of ipsilateral afferent via iTN stimulation and activation of the corticospinal cells by TMS.

CHAPTER 2

2. Methods

This chapter will discuss the methods applied in this PhD thesis. After a description of the general methodology common to all the studies, a discussion of the techniques applied in the specific studies will follow.

Healthy subjects 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 Nord-Jutland (approval number: N-20090037; N-20110040).

2.1. General experimental protocol

The subjects were asked to walk on a split belt treadmill (Woodway GmbH, Weil am Rhein, Germany) that allowed independent control of the two belts. The experimental sessions started with 2-3 minutes of familiarization with the treadmill, during which the subjects were asked to select their natural walking speed. A self-selected speed was adopted since stability and adaptability of the gait cycle have been proved to be enhanced at preferred speeds (Jordan et al., 2007). The same selected speed was then maintained for the duration of the experiment. In study I, III and IV, a pressure-sensitive trigger was placed under the left heel of the subject's shoe and used to trigger the sampling to the computer.

At the beginning of the experimental session, data for 20-30 walking cycles were collected and the average stride time extracted. Stride time was defined as the time between two consecutive touchdowns of the leg ipsilateral to the stimulation and the gait cycle percentage was defined so that 0% corresponded to the ipsilateral touch down and 100% corresponded to the next ipsilateral

touch down. To reduce differences between subjects, all participants wore the same model of shoes (Biltema, Super Star), unless stated otherwise. To limit the inter-step variability, the subjects were asked to maintain the same cadence throughout the testing period and were provided with verbal feedback whenever their stride times deviated. Stride times were then analyzed off line and strides differing from the mean gait cycle duration by more than its 10% were excluded from the analysis.

In order to investigate the functionality of short-latency interlimb reflex pathways connecting gastrocnemius muscles, responses elicited during normal forward walking at 80 - 90 % of the ipsilateral gait cycle were compared with responses elicited during hybrid walking at 40 - 50 % of the ipsilateral gait (study I). During hybrid walking, the two belts of the treadmill ran at the same speed but in opposite directions, causing the subject to walk forward with the ipsilateral leg and backward with the contralateral leg. The same speed selected for normal walking was maintained for the hybrid walking task (studies I and III). Subjects wore a harness for safety purposes; this did not provide weight support to the subject. In order to reduce stride time variability due to the adaptation process, subjects were exposed to familiarization to the hybrid walking task before the data collection started. The familiarization lasted 15 minutes, as 10 minutes have been observed to be sufficient to adapt to a split belt walking task (Choi and Bastian, 2007). Subjects started randomly with normal or hybrid walking and a resting period of approximately five minutes was given to the subject between the two tasks. The average stride time was computed also for the hybrid walking task using 30 walking cycles. The subjects were allowed to take a break or stop at any time.

2.1.1. Electrical stimulation

Electrical stimulation of the iTN was used to elicit the short–latency crossed response in the contralateral gastrocnemius muscle. An isolated stimulator (Noxitest IES 230, Aalborg, Denmark) was used for iTN stimulation with single monopolar stimuli of 1 ms duration. The stimulating

electrodes were placed while standing; the cathode (PALs Platinum round electrode, Model No. 879100, 3.2 cm diam, Axelgaard Man, Lystrup, Denmark) was placed over the popliteal fossa (Figure 2A) and the anode (PALs Platinum rectangular electrode, Model No. 895240, 5cm x 9cm Axelgaard Man, Lystrup, Denmark) at the anterior aspect of the knee joint just above the patella. In order to find the optimal location for the electrode placement, stimuli were delivered every 4 to 7 s and the cathode's position was adjusted until a direct motor response (M-wave) (Pierrot-Deseilligny and Burke, 2005) was observed.

To identify the optimal stimulation intensity the iSOL M-wave peak-to-peak amplitude was observed, starting at the stimulation intensity of 0 mA and increasing with increments of 5 mA every 3 stimuli. When no more increases in the M-wave amplitude were observed, the corresponding M-wave peak-to-peak amplitude was labeled as the M-max. The stimulation intensity that elicited 85% of M-max was then identified using increments of 1 mA. This stimulation intensity was chosen as it evoked prominent short-latency response in the cSOL (Stubbs et al., 2011b). Using this intensity, a total of 30-40 gait-cycles for normal walking (study I, II, III and IV) and 40-60 gait-cycles for hybrid walking condition (larger N due to a visually observable larger step variability) were recorded (study I and III). A trial was collected every 2 to 4 steps, randomly alternating gait cycles with stimulation with the same number of "no stimulation" control gait cycles.

2.1.2. Surface electromyography

Surface EMG (sEMG) and electrical stimulation were used for quantifying the crossed response in cGM and cGL and in study III for quantifying the relative activation of the cSOL, cGL and cGM muscles (see 2.4.2). sEMG was recorded with a single differential configuration following appropriate skin preparation and using single use surface electrodes (Neuroline 720 silver/silver-chloride, AMBU A/S, Denmark). Electrodes were placed on the SOL muscle in accordance with

the recommendations of Cram et al., (1998) and on the contralateral gastronemius lateralis (cGL) and cGM in accordance with the SENIAM recommendations (Hermens et al., 1999) (Figure 2A). A reference electrode was placed over the tibial bone. Data were acquired using the Labview based acquisition tool Mr. Kick II 2.3 and III (Knud Larsen, Center for Sensory-Motor Interaction, Aalborg University, Denmark). sEMGs were pre-amplified and sampled at 2 kHz.

All data were analyzed off-line using the computing environment MATLAB R2010b (MathWorks, USA). Signals were band pass filtered between 25 and 400 Hz, rectified, low pass filtered with a cuff-off frequency of 40Hz, if not stated otherwise, and averaged to obtain an average gait cycle for each subject.

2.1.3. Quantification of the short-latency crossed response (SLCR)

To evaluate the magnitude of the response, the root mean square value (RMS) in a defined time window was computed from the stimulated gait cycle and expressed as a percentage of the RMS in the same window of the control gait cycle. In study I, a facilitation of cGL was observed by visual inspection during the normal walking task. The occurrence of its peak was computed in a time window of 68 to 88 ms after the stimulation. Consequently, a time window of 20 ms centered on this peak was adopted to evaluate the magnitude of this response. In the following studies, since the cGL crossed responses showed a latency of 69.6 ± 9.3 ms and a peak at 81.1 ± 6.6 ms after the stimulation (Gervasio et al., 2013), a time window from 60 to 90 ms after the stimulation was selected for the quantification of the response in cGL and cGM (study II and III). In all studies, the onset of the response was evaluated as the time in which the averaged stimulated gait cycle exceeded the value of the averaged control gait cycle for an amount of two times the standard deviation (SD) of the averaged control gait cycle computed in a time window of 25 to 120 ms after the stimulation. The duration of the response was evaluated as the time at which the averaged stimulated gait cycle remained above this threshold. The response was discarded if its duration

was shorter than 10 ms. Onset and duration of possible crossed responses were quantified at 80% of the gait cycle, when iTN stimulation was observed to elicit the most prominent facilitation in both heads of the gastrocnemius (study I and III). Only in study I, was iTN stimulation delivered at 80% and 90% in of the ipsilateral gait cycle.

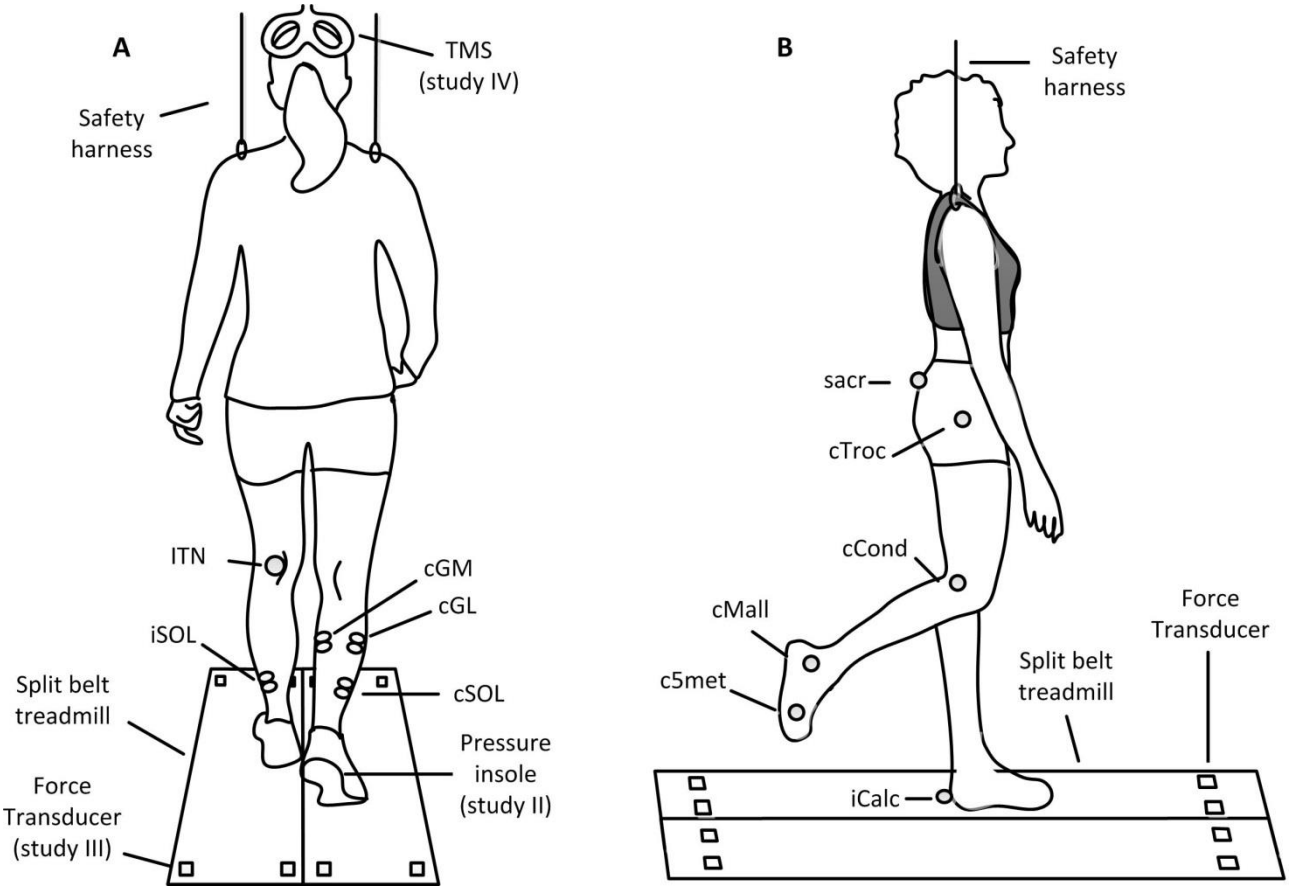

Figure 2: ***Experimental set up.*** **A:** The experimental set up adopted is displayed showing the subject from the back. The subjects walked on an instrumented split belt treadmill where the direction of the two belts could be controlled independently. Subjects wore a safety harness that did not alter their' body weight support. In all studies crossed responses in the contralateral gastrocnemius (cGL: study I, II, IV; cGM: study III) were elicited by iTN stimulation (**A**). iSOL sEMG signal was also collected in order to evaluate the correct stimulation point and intensity. cSOL sEMG signal was collected in study III to evaluate the relative activation of triceps surae muscles. In study I, the direction of the belt under the leg contralateral to the stimulation was inverted in order to compare the SLCR evoked during normal and during hybrid walking. In study II, the change in pressure provoked by the SLCR under the contralateral foot was recorded using pressure sensitive insoles (**A**). In study III, kinematics and kinetics data were used to estimate afferent feedback. The vertical component of the ground reaction force (GRF) was recorded through 8 built in force transducers placed at the corners under each belt. Retroreflective ball-shaped markers (**B**) were placed on the skin of the subject's over the ipsilateral calcaneus (iCalc) and on contralateral leg over the fifth metatarsal joint (c5met), on the tip of the

lateral malleolus (cMall), on the lateral epicondyle of the femur (cCond), on the great trochanter (cTroc) and on the sacral bone (sacr). The position of the markers is illustrated in **B**. In study VI, TMS (**A**) was applied to investigate the cortical contribution to the SLCR.

2.2. Study 1: Crossed reflex reversal during locomotion

Fourteen subjects (11 males and 3 women, age 31 ± 5 years) took part in this study in which short-latency responses elicited in by iTN stimulation cGL were compared between normal walking (at 80 or 90% of the ipsilateral gait cycle) and hybrid walking (at 40 or 50% of the ipsilateral gait cycle) (see 2.1). Please, refer to 2.1.1 for stimulation's procedure. SLCRs in cGL were recorded and quantified from sEMG signals as described in 2.1.2 and 2.1.3.

In five subjects (2 males and 3 women, mean age 29 ± 3 (SD)) iTN, iSuN and iMpN stimulation were performed during normal and hybrid walking. The procedure followed for eliciting and quantifying the SLCR elicited by iTN stimulation is described in 2.1. Surface stimulating electrodes (PALs Platinum round electrodes, Model No. 879100, 3.2 cm diam., Axelgaard Man) were placed, posterior and inferior to the medial malleolus for MpN stimulation, and between the lateral malleolus and calcaneal tendon, posterior and inferior to the lateral malleolus for SuN stimulation. The optimal location of the electrodes was determined delivering a train of 3 pulses, of 1ms duration each and with an inter-pulse interval of 3 ms, at an intensity above the subject's perception threshold (PT) (between 2 and 5 mA). The location of the electrodes was moved until the subject described a spreading on the plantar side of the foot to the first and second metatarsal for MpN stimulation, and a spreading on the lateral side of the foot toward the fifth metatarsal the desired sensation for the SuN stimulation.

The PT was found increasing the stimulation intensity from 0 with 0.5 mA increments until the subjects reported feeling the stimulation while the stimulation. Increments and decrements of 0.1 mA were then used to find the exact PT, which was identified if the subject reported the same

threshold three times. The subject was then asked to walk on the treadmill (either normal or hybrid walking). Three consecutive stimuli, 1 ms duration, at an interval of 3 ms (Nielsen et al., 1997a), were applied at the time at which a SLCR was elicited by iTN stimulation (either 80 or 90 % of the ipsilateral gait cycle) every 3 to 5 steps at an intensity of 1x, 2x, and 3x PT, randomly selected. A control condition of "no stimulation" was also acquired.

2.3. Study 2: Effect of SLCR on CoP

Eight subjects (5 males and 3 females, age 20-22 years) took part to this study. SLCRs were elicited in the contralateral triceps surae muscles (Gervasio et al., 2013) through iTN stimulation (see 2.1.1 for stimulation's procedure). In order to investigate the functionality of these responses, the Center of Pressure (CoP) location and the pressure distribution on the sole of the contralateral foot was recorded continuously using instrumented insoles Pedar-x (99 capacitive sensors per insole, Novel Gmbh).

The insoles were calibrated according to the manufacturer's instructions. Pressure sensitive insoles of the proper size were inserted between the subjects' socks and soles of the shoes. The Pedar-x system indicated the time of ipsilateral foot contact which was used to synchronize the data acquisition for the pressure data and the sEMG (see 2.1.2 for sEMG recording procedure). The trigger level to obtain a valid ipsilateral foot contact's signal was set according to the subject's body weight. Pressure data were sampled at 100 Hz and stored for off line analysis.

A total of 30 electrical stimuli were delivered at 80 % of the gait cycle during normal walking, when iTN stimulation was observed to elicit a prominent facilitation in both heads of the gastrocnemius (study I and III).. A control condition of "no stimulation" was interspersed between the stimulation trials separated from a stimulated gait cycle by 3-4 steps. Using the Pedar-x software, the pressure values recorded by each of the 99 sensors, the instantaneous location of the

in-shoe CoP throughout the stance phase of the gait cycle and the stance and swing duration for every gait cycle of the left and the right foot were obtained. Pressure values and CoP location were segmented for each gait cycle, time normalized as a percentage of the gait cycle and averaged for each condition (stimulation or control). The CoP location was normalized to the length of the foot for each subject while the pressure values were normalized to the maximum pressure during the gait cycle for each subject. Even though CoP's trajectories were collected continuously during a gait cycle, anterior-posterior and medial-lateral location of the CoP of the perturbed and unperturbed gait cycle were compared at selected time points before and after the stimulation, which occurred at 80% of the ipsilateral gait cycle. The selected time points were 2 %, 6 %, and from 72 to 100 % of the ipsilateral gait cycle with increments of 2% of the gait cycle duration; at these times the contralateral foot was on the ground. The pressure recorded by each sensor in the two conditions was compared at the time when the maximal displacement in CoP location was observed.

2.4. Study 3: Contralateral afferent contribution to the SLCR

Twenty-six subjects participated in this study in which the modulation of the amplitude of cGM crossed responses was quantified at 10 different times (0, 10, 20, 30, 40, 50, 60, 70, 80, 90%) during the ipsilateral gait cycle and compared with the estimated contralateral afferent activity of cGL and cGM. The modulation of cGM SLCR was quantified in twelve subjects (6 females, age 24 ± 3 years) using the procedures described in sections 2.1.1-2.1.3. Contralateral afferent output was estimated (see 2.4.2) in eight age and size matched females: age 24 ± 1 years, height 1.68 ± 0.13 m, body weight 62.4 ± 9.2 kg, shank length 0.41 ± 0.02 m (mean ± SD).

2.4.1. Kinematics and kinetics data: acquisition and processing

Data was acquired during one minute recording. Retroreflective ball-shaped markers were placed on the skin of the subject, over the ipsilateral calcaneus and over the following contralateral anatomical landmarks: fifth metatarsal joint, the tip of the lateral malleolus, the lateral epicondyle of the femur, the great trochanter and the sacral bone (Figure 2B) (Voigt et al., 1998). A motion analysis system with eight infrared digital video cameras (ProReflex MCU 240, Qualisys, Gothenburgh, Sweden) and QTM Qualisys Track Manager version 2.5 software was used for acquiring the markers' trajectory. A sampling frequency of 240 Hz was used. To allow a better tracking, all participants walked barefoot. Kinetic data were acquired at 1200 Hz together with the kinematic data. The vertical component of the ground reaction force (GRF) was measured by eight built in force transducers (quartz force-sensing elements, type 9321B, Kistler Instrument Corp., USA) placed at the corners under each belt (Figure 2B).

Data were low-pass filtered with a cut-off frequency off 6 Hz, segmented for each stride, interpolated at 50 samples, and averaged in order to obtain an average gait cycle time for each subject. Heel strike was identified as the time in which the vertical position of the heel marker reached its lowest spot for each gait cycle. Inverse dynamic analysis was applied on a four link segment model of the leg (Winter, 1990; Voigt et al., 1998). The horizontal component of the GRF was estimated from the subject's body weight and the acceleration of the center of body mass; the latter was obtained from the kinematic data of the sacral bone marker (Gard et al., 2004) as during human walking the center of gravity is located in the region just anterior to the top of the second sacral segment (Schafer, 1983). Center of Pressure (CoP) coordinates were derived from the force measures.

2.4.2. Estimation of muscle spindle and GTO activity

To estimate the muscle spindles afferent output, the assumption that the muscle spindles are located in parallel with the muscle fibers was made. Muscle fascicle length changes were calculated as the difference between the origin to insertion length change and the tendon length change (Voigt *et al.*, 1998). The length obtained considering the fascicles parallel to the tendon was divided with the cosine of the pennation angle for the GM, extracted from the data of Lichtwark *et al.* (2007). This was done to take into account the pennation of the muscle fibers. The pennation angle for GL was derived from the pennation of GM taking into account the mean value for GM/GL pennation ratio, that is 1.97 (derived from data of Kawakami *et al.*, 2013), as no data about the pennation angle for GL during walking could be obtained from the literature.

Origin to insertion length changes were obtained through a transfer function from the joint angle (Grieve et al., 1978). The tendon length changes were attained from a non-linear tendon model (Voigt *et al.*, 1998) based on the calculation of the tendon force. The model parameters were adjusted to fit the subject population of the current study; for this purpose values of 1.7 GPA (Arya and Kulig, 2010) and 46 mm^2 (Magnusson *et al.*, 2003) were used respectively for the Young's modulus for the tendinous tissue and for the cross sectional area of the tendon. The Achilles tendon force was obtained by dividing the net ankle moment by the moment arm, which was calculated differentiating the origin to insertion length changes of SOL with respect to the ankle joint angle. The force produced by the individual muscles depends on their relative physiological cross-sectional areas (PCSAs) and activation levels; however, no significant differences ($F_{(2,51)}$ = 1.40, P = 0.26) were found in the activation level of the triceps surae (SOL, GM and GL) during the stance phase in 6 subjects (4 females, age 29 ± 3 years). Thus, Achilles tendon force was distributed to the muscles in relation to the PCSA of the muscles, namely 26% and 12% of the total cross-sectional area of triceps surae for GM and GL respectively (Albracht et al., 2008).

To estimate the ensemble activity for muscle spindles primary and secondary afferent fibers, mathematical models previously used to predict spindles firing of in mammalian triceps surae providing the best fit to experimental data were adopted (Prochazka and Gorassini, 1998). For the primary afferents activity (Ia), the following model was used:

$$Ia = 4.3 \cdot v^{0.6} + 2 \cdot l + b + f(EMG) \quad (1)$$

$$f = EMG \cdot 120 \cdot (s + 1)/(s + 20) \quad (2)$$

where l is the muscle fascicles length changes, v is the velocity of length changes, b is an offset value and f(EMG) is a signal representing the EMG-linked fusimotor action. EMG is the rectified, averaged and normalized sEMG signal for the investigated muscle and s is the Laplace operator.

Spindles secondary afferents activity (II) was estimate using the following model:

$$II = 13.5 \cdot l + b + 20 \cdot EMG \quad (3)$$

The last components is a signal representing the EMG-linked fusimotor action, obtained by scaling up the rectified, averaged and normalized sEMG of the investigated muscle by a factor of 20. To take into account that spindles afferents are never completely silent during a gait cycle (Prochazka and Gorassini, 1998), the offset parameters b were assigned the value of 285 for muscle spindles primary afferents and 190 for muscle spindles secondary afferents. Different values for b would not have affected the statistical results of the current paper. To estimate the ensemble afferent activity from single fibers firing, the number of muscle spindles in human gastrocnemius muscle, that is 156 muscle spindles (Voss, 1971), was taken into account.

The ensemble GTO activity (Ib) was derived from the muscle force (F), using the following relationship obtained for mammalian gastrocnemius (Mileusnic & Loeb, 2009):

$$Ib = 10^{0.4939 \cdot log_{10}(F) + 3.2154}. \quad (4)$$

The average afferent activity across all subjects was computed and expressed in pulses per second (pps).

2.5. Study 4: Cortical contribution to the short latency crossed reflex

Nine subjects (6 male, 3 female; aged 23-25 years) took part in this study where the modulation of contralateral motor evoked potentials (MEP) elicited by TMS with and without conditioning electrical stimuli applied to the iTN was examined while subjects walked on a treadmill (see 2.1 for the procedure used for eliciting and quantifying the crossed response).

TMS was applied using a Magstim 200 (Magstim Company, Dyfed, UK) with a focal figure of eight double cone coil (110 mm diameter). MEPs were elicited in the cGL muscle using single pulses with a posterior to anterior directed current. The subject's vertex was marked for the placement of the TMS coil while the subject was seated. The initial TMS intensity to find the optimal site for evoking a MEP in the cGL was set at approximately 50% of stimulator output. The best spot for stimulation, the hot-spot, was chosen as the point at which the most prominent MEP (measured as the peak to peak amplitude) was elicited in the cGL for a given stimulus intensity. This site was approximately one to two centimeters anterior to the vertex for all subjects. The coil was then fixed on the selected hot spot by a custom made brace (Balgrist Tec, Zurich, Switzerland; (Schubert et al., 1997; Petersen et al., 1998b)).

Subjects were then asked to walk and a SLCR was elicited through iTN and quantified. The intensity of the TMS was set to induce a MEP with amplitude similar to that of the crossed response in the cGL for each subject.

Subsequently, iTN and TMS were combined with a total of 10 different interstimulus intervals (ISIs) so that MEPs occurred before (−30 and −15 ms), at (0 ms), or after (+5, +10, +15, +20, +30, +45 and +200 ms) the onset of the SLCR. ISIs were varied randomly. In addition, a control condition in

which no stimuli occurred as well as one in which only iTN stimuli were applied and one in which only TMS was applied, were interspersed with the combined stimuli. The time between consecutive trials was set to 5-7 seconds.

Since two peaks were observed in the crossed response for most of the subjects, sEMG signals were only high pass filtered at 20 Hz, in order to better distinguish the possible two components of the response. The response in the averaged and rectified cGL was quantified as the RMS in a window from MEP onset to MEP offset for the TMS only and for the combined TMS and iTN conditions. For the iTN only condition the onset of the window was altered to reflect the window in which the MEP would arrive. To obtain the expected level of convergence from combining iTN and TMS, the algebraic sum of the iTN only and TMS only condition was calculated from the cGL data for each subject after subtracting to each condition the background level. The algebraic sum was then compared to the response elicited by the combination of iTN and TMS at the different ISI, to which the background level was also subtracted.

2.6. Statistical analysis

To establish the significance of the SLCR, a two tailed paired sample *t*-test was used to compare the RMS of the averaged stimulation gait cycle in the defined time window and RMS of the averaged control gait cycle in the same time window.

In study I, to ensure that the stimulation was the same during the two walking tasks, the M-wave peak-to-peak amplitude was computed from the non-rectified averaged iSOL sEMG signal in a time window from 5 to 25 ms after the stimulation, normalized to the M-max of the respective walking task and compared between normal and hybrid walking conditions. Moreover, to compare the muscle activation level between normal and hybrid walking, a paired sample t-test was performed between RMS values of the control cGL signal during the two walking tasks. The

RMS was computed in a time window of 20 ms centered on the stimulation onset. To evaluate whether a relation exists between the size of the responses and the muscle activation level, computed as the RMS of the control signal in the same time window in which the responses occurred, Spearman correlation coefficients were computed.

Responses elicited by TN and each singular intensity of SuN and MpN stimulations (1×, 2×, and 3× PT) were compared-way using a 1-way ANOVA. A post hoc Fishers LSD multiple comparisons test was performed to establish the nature of the difference in case a significant difference was identified.

In study II, significant displacement in CoP location between stimulation and control condition were established through a two-way repeated measure ANOVA, using as factors the condition and the percentage of the gait cycle. A post-hoc pairwise comparisons with Bonferroni correction was used to establish the location of the difference in case a significant interaction was observed between the two factors (gait cycle percentage × condition). Difference in the pressure values at the time at which the most prominent CoP displacement was observed were assessed through a two tailed paired sample *t*-test. Stance phase duration of a stimulated step was compared with the stance phase duration of the previous and following step using a one-way repeated measure ANOVA. The same procedure was adopted to compare the swing phase duration and repeated for both legs.

In study III, the relation between amplitude modulation of the cGM responses and the afferent output of cGM and cGL was assessed using Spearman correlation and Student's t distribution for a transformation of the correlation was used to obtain *P*-values.

In study IV, to evaluate whether the combined iTN and TMS condition evoked a response larger than the algebraic sum of the responses evoked by iTN and TMS separately (Petersen et al. 1998a),

two-tailed paired sample Student's t tests was used for the time course data. Besides, a repeated measure (condition; control, perturbation only, TMS only, perturbation and TMS) ANOVA was performed on the EMG data to compare the amplitude of the response between conditions (control, iTN only, TMS only, iTN and TMS with onset of crossed response and of MEP occurring at the same time and the algebraic sum of the responses elicited separately to which the background activity was subtracted). Since a second peak was observed in most of the subjects, the same procedure was adopted to for the second component of the response.

The results of this thesis were considered significant when the obtained P-value was less than 0.05. All results are expressed as mean ± SD.

CHAPTER 3

3. Results

This current thesis investigates the SLCR elicited in the gastrocnemius muscle by iTN stimulation during walking. In all studies a significant facilitation was observed compared to the control signal (studies I and II, P = 0.01; study III, P=0.012; study IV, P = 0.001). Figure 3B shows the cGL sEMG for a representative subject in which a facilitation of magnitude of 165.0 % of the control signal was elicited. Onset, duration and magnitude of the responses measured in the individual studies are reported in Table 1.

	Study I	Study II	Study III	Study IV
Muscle	GL	GL	GM	GL
N Subjects	12/14	7/8	12/12	7/9
Subjects' age (years)	31 ± 5	21 ± 1	24 ± 3	24 ± 1
Onset (ms)	69.6 ± 9.3	57.6 ± 9.5	58.3 ± 15.9	62.6 ± 3. 3
Duration (ms)	32.4 ± 10.2	30.4 ± 6.6	36.6 ± 20.3	47.1 ± 7.1
Magnitude (% control)	138.1 ± 24.5	146.8 ± 29.3	183.63 ± 51.61	199.9 ± 79.6

Table 1: ***Characteristics of the SLCR.*** Onset, duration and magnitude of the response in the gastrocnemii quantified in the individual studies are here reported. The table indicates also the specific muscle in which the responses were quantified, the number of the subjects showing a response out of all the subjects that participated in the studies and the average age of the subjects.

3.1. Difference in SLCR between normal and hybrid walking

In study I, the responses elicited during normal and hybrid walking were compared as in these two conditions an opposite response was expected. As reported in previous studies in which hybrid walking was performed (Choi and Bastian, 2007), the subjects were easily able to walk with the two legs moving in opposite directions. The activation levels of gastrocnemius between the

two tasks at the chosen timings were comparable and such that either a facilitation or an inhibition in the sEMG signals could be detected.

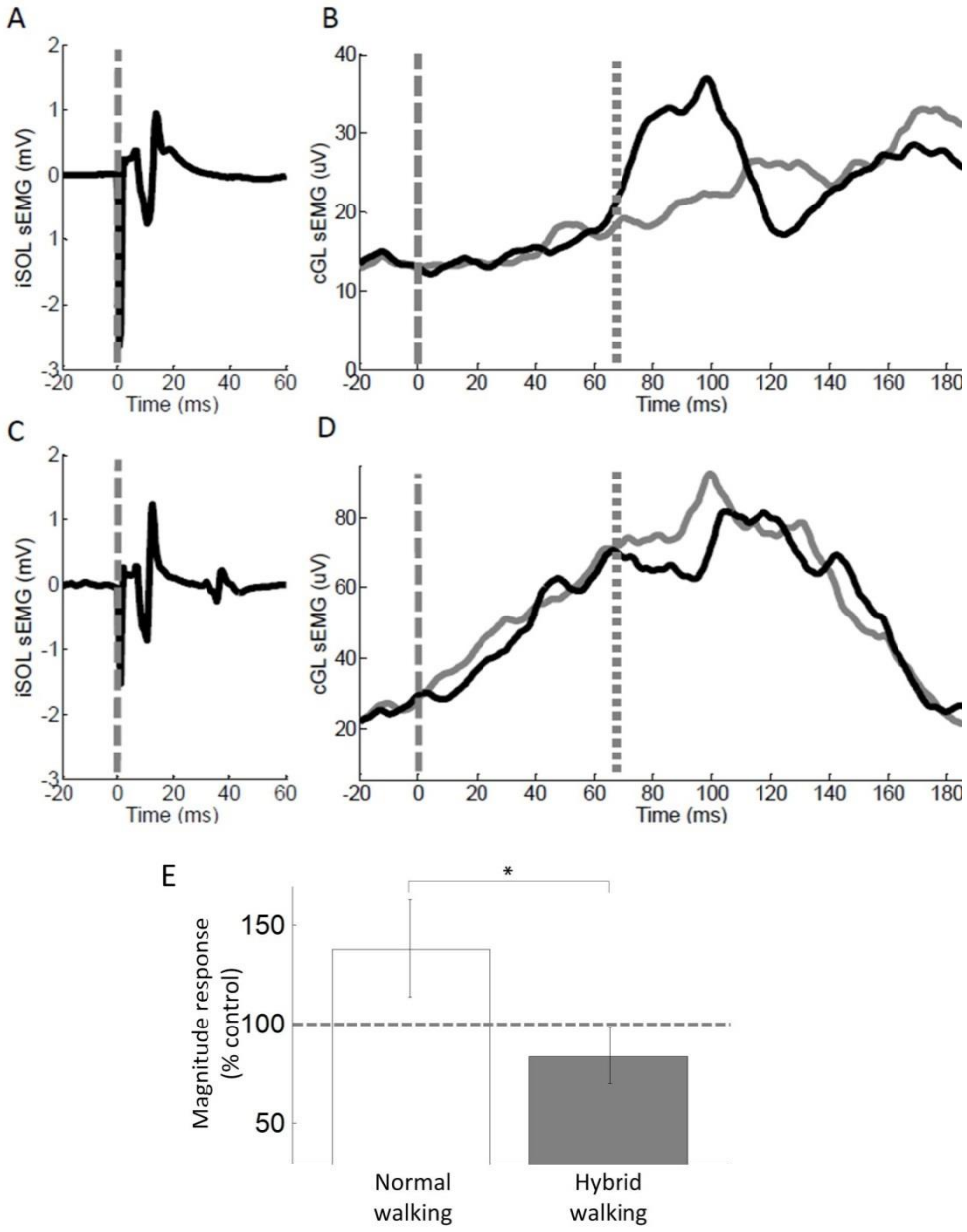

Figure 3: ***Short-latency responses in the cGL after iTN stimulation and their magnitude during normal and hybrid walking.*** **A** and **C** show respectively the iSOL sEMG after the iTN stimulation for normal and hybrid walking. A comparable M-wave can be seen in the two signals. **B** and **D** show the rectified sEMG for the cGL during normal (**B**) and hybrid walking (**D**). The onset of the stimulation corresponds to 0 ms and is further indicated by the dashed vertical lines (**A**, **B**, **C**, **D**). In **B** and **C**, the black and gray traces represent the averaged test signal and the average of the control, respectively. A facilitation occurs in the cGL when iTN is delivered during normal walking (**B**). The onset of the facilitation is at 65 ms after the stimulation and is signaled by the vertical dotted gray line. During hybrid walking (**D**), iTN elicits an inhibition in the same time window. **E**: The magnitude of the responses (mean ± SD, 11 subjects) is

expressed as a percentage of the control signal during normal (left) and hybrid walking (right). The dotted horizontal line signals 100% of the control. cGL responses elicited after iTN changes significantly from a facilitation during normal walking to an inhibition during hybrid walking.

11 out of 12 subjects that showed a facilitation during normal walking, revealed a significant reduction ($P = 0.009$) in the cGL sEMG when performing hybrid walking. The magnitudes of the responses were significantly different between normal and hybrid walking ($P < 0.001$) with a mean amplitude of 138.1 ± 24.5 % of control during normal walking and of 84.0 ± 13.9 % of control during hybrid (Figure 3E). The inhibition elicited in the cGL for a representative subject during hybrid walking is shown in Figure 3D. For this subject, the inhibition was 83.1 % of the control signal.

A similar normalized M-wave peak to peak amplitude was observed between the normal and hybrid walking task ($P = 0.127$) (Figure 3A,C). Similarly, the background activation of cGL at the time when the stimulation was delivered did not significantly differ between normal and hybrid walking ($P = 0.378$). No correlation was observed between the magnitude of the response and the background activity. The Spearman correlation coefficients during normal walking and hybrid walking were $r = 0.195$ and $r = 0.831$ respectively.

The iMpN and iSuN stimulation were performed in five subjects and the effects on cGL where compared with the SLCR elicited by iTN in cGL. However, one subject did not show a facilitation in cGL, thus this subject was excluded from further analysis. 1-way-ANOVA test revealed a difference in the responses elicited by the stimulation of the different nerves ($P < 0.01$, $F_{(2,33)} = 16.163$). Significant differences were found between iTN and iSuN at all the stimulation intensities ($P < 0.01$) and with iMpN stimulation at 1 × PT and 2 × PT ($P < 0.01$) but not with iMpN at 3 × PT ($P = 0.346$). During hybrid walking, no significant difference in cGL responses were found between TN, SuN or MpN stimuli ($P = 0.867$, $F_{(2)} = 0.143$).

3.2. Changes in CoP location, pressure distribution and step parameters

In study II, the effect of the SLCR on CoP location and pressure distribution under the contralateral foot were investigated. Out of eight subjects that participated to the study, one did not show a SLCR in the cGL; thus this subject was excluded from further analysis. The average recorded gait cycle duration was 1.25 ± 0.07 s and iTN stimulation was delivered on average at 80.7 ± 2.7% of the ipsilateral gait cycle.

CoP trajectories for the stimulated and control gait cycle in the medial-lateral direction for a representative subject are show in Figure 4A. For this subject, the maximum displacement of CoP's location toward the medial direction was of 1.5 mm and occurred at 96% of the ipsilateral gait. When analyzing the changes in medial-lateral CoP trajectory for all subjects, a significant interaction ($F_{(16)} = 7.22$, $P < 0.01$) was found between the factors condition (stimulation or control) and the percentage of the gait cycle (the time points at which the two conditions were compared). The post hoc pairwise comparison revealed a significant displacement of the CoP location toward the medial direction from 92% to 100% of the ipsilateral gait cycle ($P < 0.03$ for all time points between 92% and 100%). The maximum medial-lateral deviation from the control condition was of 1.0 ± 0.7 % of the foot length and occurred at 98% of the ipsilateral gait cycle. Figure 4B shows the mean difference in contralateral foot CoP trajectory between stimulated and control gait cycle in the medial-lateral direction for 7 subjects that showed a significant SLCR in cGL.

The stimulated and control CoP trajectories in the anterior-posterior direction for a representative subject are show in Figure 5A. As for the medial-lateral direction, the maximum displacement of CoP's location in the anterior-posterior location for this subject occurred at 96% of the ipsilateral gait cycle. This maximal deviation from the unperturbed trajectory was of 3.1 mm toward the anterior direction. When comparing the contralateral foot CoP location between the stimulation

and control condition for all subjects, a significant interaction ($F_{(16)}$ = 2.12, P = 0.01) between the factors condition (stimulation or control) and the percentage of the gait cycle (the time points at which the two conditions were compared) was observed. A significant displacement of the CoP location toward the anterior direction from 90% to 98% of the ipsilateral gait cycle (P < 0.048) was revealed by post hoc pairwise comparison. The maximum deviation from the control condition in this direction was 3.7 ± 3.0 mm and occurred at 92% of the ipsilateral gait cycle. Figure 5B shows the mean differences in the contralateral foot CoP trajectory between the perturbed and the unperturbed gait cycle in the anterior-posterior direction for 7 subjects showing a significant SLCR in cGL.

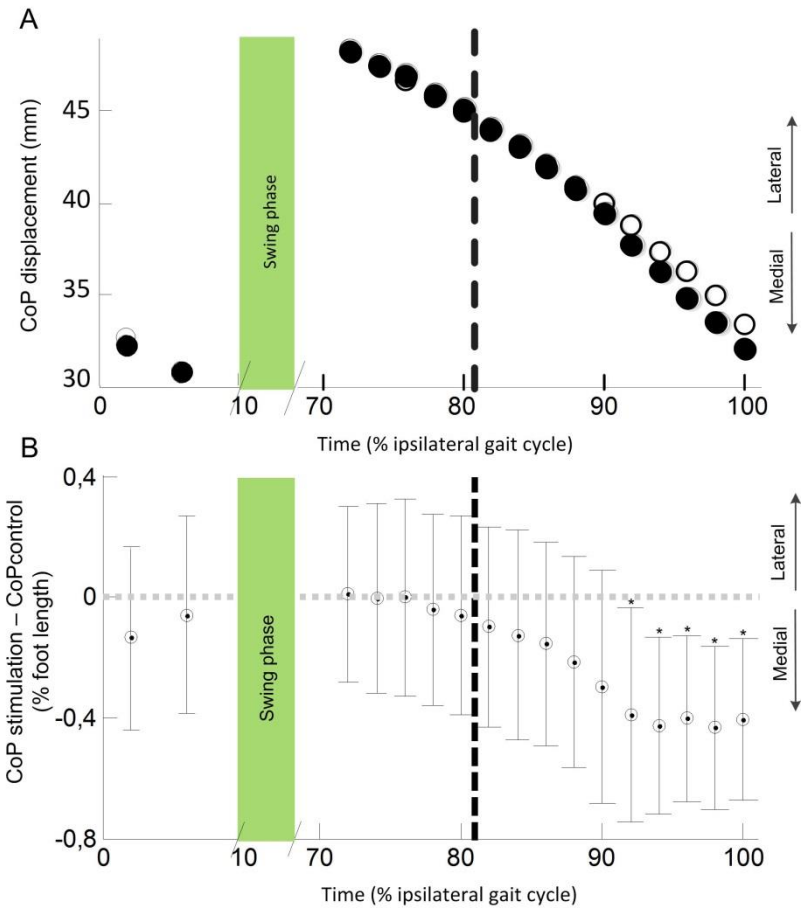

Figure 4: ***CoP displacement in the medial-lateral direction.*** CoP for different timings during the ipsilateral gait cycle. Data are from the contralateral foot where the SLCR was observed. The vertical dashed black line indicates the average time when the stimulation was delivered. **A**: The location of CoP is shown for a representative subject. Measurements are the average of 30 gait cycles for each condition (stimulation or control). Empty and full dots represent the CoP location during the unperturbed and stimulated gait cycle, respectively. **B:** Mean values and SD of the difference between the perturbed and unperturbed CoP's trajectory (expressed as percentage of the subject's foot length) is shown for a pool of 7 subjects that showed a significant short-latency facilitation in the cGL. The horizontal dotted gray line represents "no deviation" from the unperturbed trajectory.

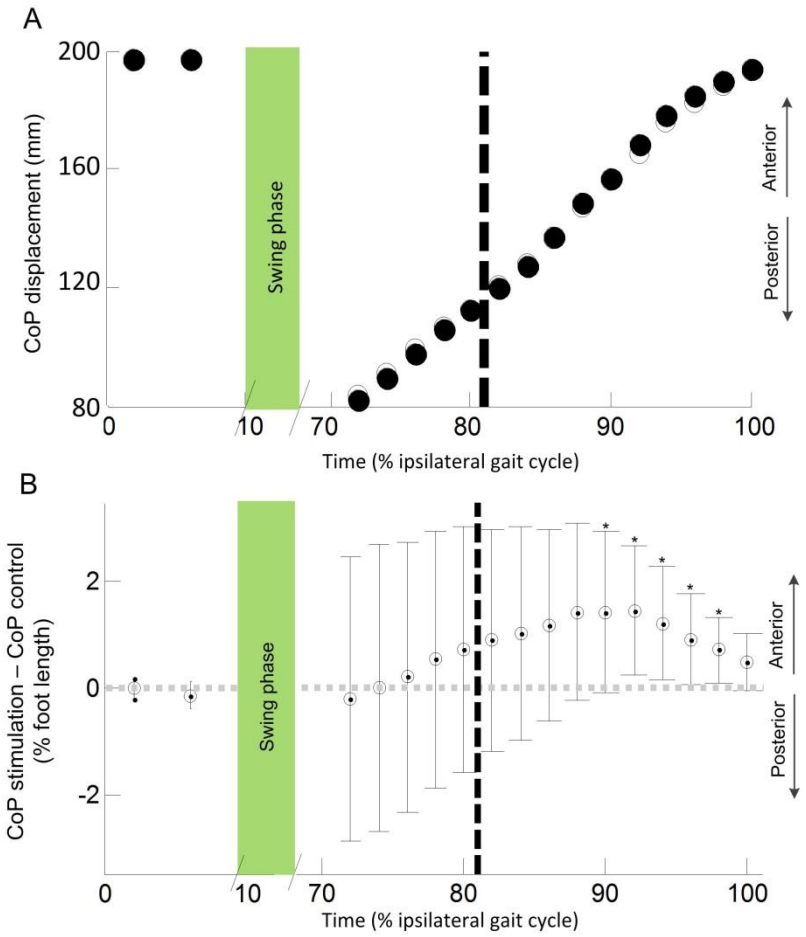

Figure 5: ***CoP displacement in the anterior-posterior direction.*** CoP for different timings during the ipsilateral gait cycle. Data are from the contralateral foot, where the SLCR was observed. The vertical dashed black line indicates the average time when the stimulation was delivered. **A**: The displacement of CoP is shown for a representative subject. Measurements are the average of 30 gait cycles for each condition (stimulation or control). Empty and full dots represent the CoP location during the unperturbed and stimulated gait cycle, respectively. **B**: Mean value and SD of the difference between perturbed and unperturbed CoP's trajectory (expressed as percentage of the subject's foot length) is shown for a pool of 7 subjects that showed a significant short-latency facilitation in the cGL. The horizontal dotted gray line represents "no deviation" from the unperturbed trajectory.

Since the average maximal displacement of the CoP trajectory occurred at 98 % and at 92 % of the ipsilateral gait cycle in the medial-lateral and in the anterior-posterior direction respectively, pressure measured at 95 % of the gait cycle by each of the 99 sensors was compared between the two conditions, stimulation and control. A significant difference between the pressure recorded during the stimulation and control condition was revealed at 6 sensor locations (all $P < 0.05$). Figure 6A indicates these locations. The colors white, black and gray indicate that the sensor in those locations recorded respectively a significant increase ($P < 0.042$), a significant decrease ($P < 0.05$) or no significant differences in pressure between the two conditions. Figure 6B displays the absolute difference in pressure values between stimulated and control condition for one representative subject.

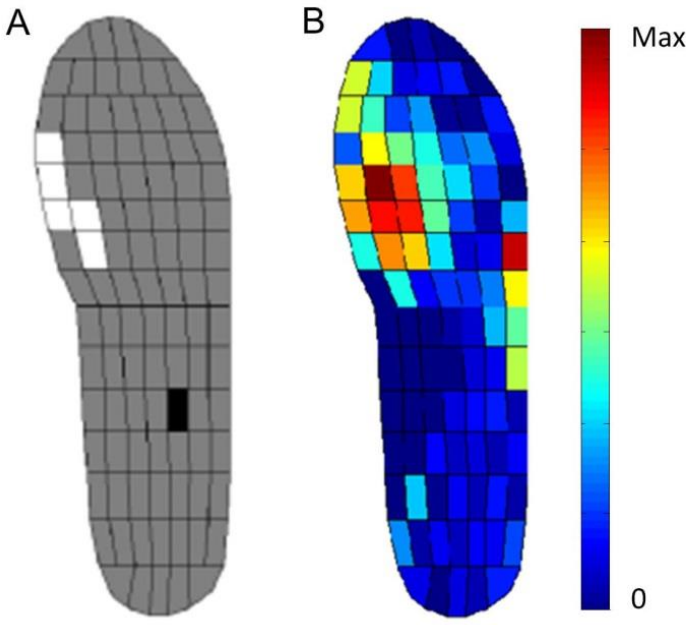

Figure 6: ***Changes in pressure distribution.*** **A:** The significant different values for the pressurepads between stimulation and control condition at 95 % of the ipsilateral gait cycle, when the average maximum displacement of CoP location was recorded in both directions is here displayed. The white, black and gray color indicates respectively significant increase ($P < 0.042$), significant decrease ($P = 0.049$) and no significant differences in pressure between stimulation and control condition ($P > 0.05$). Data were recorded from a pool of 7 subjects that showed a significant short-latency facilitation in the cGL following iTN stimulation. **B**: Absolute difference in pressure values between stimulated and control condition for one representative subject at 95 % of the ipsilateral gait cycle.

Stance and swing duration of the stimulated step were compared with the ones of the previous and following step for both legs (Figure 7). No significant differences were found in stance or in swing duration between the step before, during and after stimulation ($P > 0.2$) for the contralateral leg.

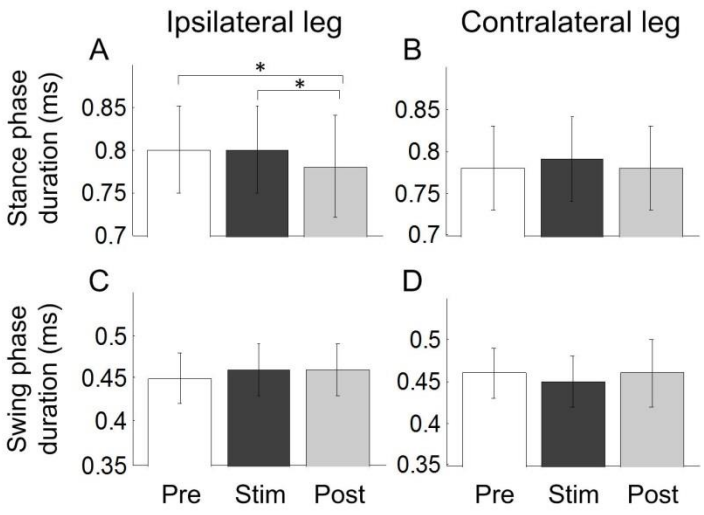

Figure 7: ***Alteration in gait parameters.*** Mean values (and SD) of stance and swing duration for the step before (Pre), during (Stim) and after (Post) stimulation are here displayed. Data are from 7 subjects that showed a significant short-latency facilitation in the cGL following iTN stimulation. Gait parameters for the leg ipsilateral to the stimulation are reported on the left (**A**, **C**), while gait parameters for the contralateral leg (where the SLCR was observed) are reported on the right (**B**, **D**).

For the ipsilateral leg, no significant differences ($F_{(1.61)} = 0.66$, $P = 0.51$) were revealed between swing phase duration of the steps before, during and after stimulation. However, stance phases for this leg had significant different durations ($F_{(1.39)} = 12.56$, $P = 0.005$). The post hoc test revealed a

significantly shorter stance phase for the step successive to the stimulation compared to the stance phase of the stimulated step and of the previous step (P = 0.01) while no difference (P = 0.58) was observed between stance duration of the stimulated step and of the previous one (Figure 7).

3.3. Relation between contralateral afferent contribution and SLCR

The relation between crossed responses and contralateral muscle afferent activity during normal walking was investigated in study III. The modulation of the magnitude of short-latency responses in cGM was quantified and as for the cGL (Gervasio et al., 2013), the strongest facilitation occurred at 80% of the gait cycle (Figure 8E,L).

The magnitude of the cGM responses was significantly correlated with the estimated group Ia afferent activity of cGM and cGL (in both cases P = 0.02, r = 0.71). Similarly, the magnitude of the cGM responses showed a significant correlation with the estimated group II ensemble afferent activity of the same muscle (P = 0.04, r = 0.64) but not with the group II ensemble afferent activity of cGL (P = 0.07, r = 0.59). No significant correlation was found between the magnitude of the cGM responses and the estimated group Ib ensemble afferent activity for cGM and cGL (P = 0.24, r = 0.41).

Group Ia (Figure 8B,G), group Ib (Figure 8C,H) and group II (Figure8D,I) estimated afferent outputs for the cGM (Figure 8B,C,D) and for cGL (Figure 8G,H,I) are shown in Figure 8 to allow comparison with the modulation of the response in cGM (Figure 8E,L).

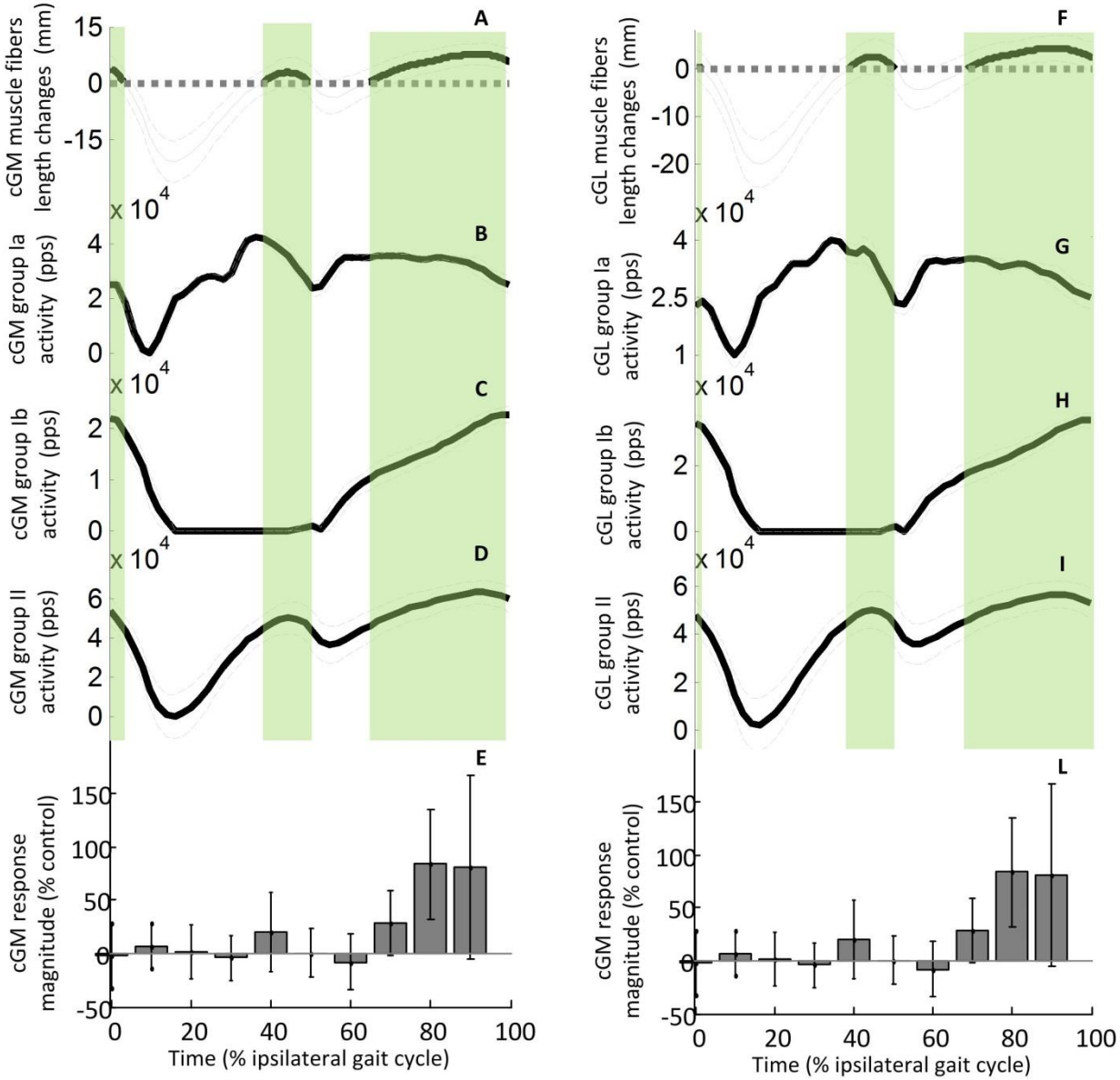

Figure 8: ***Modulation of gastrocnemius afferent feedback excitability and SLCR in cGM during walking***. Muscle fascicle length changes (**A**,**F**) and ensemble group Ia (**B**,**G**), Ib (**C**,**H**) and II (**D**,**I**) afferent activity estimated for cGM (left) and cGL (right) are shown to allow a comparison with the modulation of SLCR in cGM (**E**,**L**) during a normal ipsilateral walking gait cycle. Muscle fascicle length changes (**A**,**F**) are expressed in relation to the reference position, with both ankle and knee joints at an angle of 90° (Grieve et al., 1978). The full and dashed lines represent respectively the average and the SD of the estimated values across all subjects. Since animal studies suggest that after a perturbation, a flexion in the contralateral leg is evoked only when the flexor muscles are stretched (Rossignol & Gauthier, 1980), only afferent activity associated with a state of stretch of the gastrocnemius muscle, which is a flexor of the knee, compared to the reference position was compared to the magnitude of the response elicited in cGM; the state of stretch of the muscle is indicated by the green zones. In **E** and **L**, the magnitude of the response elicited at different timings during the walking cycle is expressed (mean and SD) as a percentage of the control (no stimulation). The RMS of the control signal has been subtracted from the amplitude of the response so that the 0 indicates no difference between the stimulation and the control trial. Positive values indicate a facilitation and negative values indicate an inhibition of the muscle activation.

3.4. Conditioning MEP evoked by TMS with iTN stimulation

Since in several subjects it was possible to distinguish two components of the SLCR (see Figure 3B and Figure 9B), study IV attempted to investigate whether spinal or supraspinal pathways contributed to the gastrocnemius SLCR, or to a part of it. The latency of the SLCR in cGL is reported in Table 2. The latency of the second component in relation to the onset of the first component was inspected in the individual studies and is reported in Table 2.

	Study I	Study II	Study IV	**Total**
N Subjects	4/12	5/7	7/7	**16/26**
Onset (ms)	17.5 ± 3.3	15.3 ± 0.5	13.0 ± 4. 3	**15.1 ± 3.7**

Table 2: ***Onset of the second component of the SLCR.*** Onset of the second component of the SLCR elicited by iTN in the cGL has been investigated in the individual studies and the latency in relation to the onset of the first component are here reported. The number of subjects in which was possible to distinguish the second component from the first, out of the number of subjects that showed a SLCR, is also reported.

TMS was applied so that MEPs occurred before (−30 and −15 ms), at (0 ms), or after (+5, +10, +15, +20, +30, +45 and +200 ms) the onset of the SLCR, elicited by iTN stimulation at 80% of the ipsilateral gait cycle, and the changes in MEP size in cGL were investigated. The mean stimulation intensity used was 50 ± 5 % of maximum stimulator output, and MEPs were elicited with mean onset latency of 28.6 ± 1.8 ms. Figure 9 shows the cGL sEMG (average of 15 trials) from one representative subject for the control condition (no iTN nor TMS, Figure 9A), iTN stimulation alone (Figure 9B), TMS alone (Figure 9C), a combination of iTN stimulation and TMS applied so that the onset of MEPs in the cGL occurred at the same time as the onset of the SLCR (Figure 9D), and a combination of iTN stimulation and TMS applied so that the onset of MEPs occurred 15 ms after the onset of the SLCR (Figure 9E). The last condition has the purpose of evidencing a possible cortical contribution to second component of the SLCR starting in average 15.1 ± 3.7 ms after the onset of the first component.

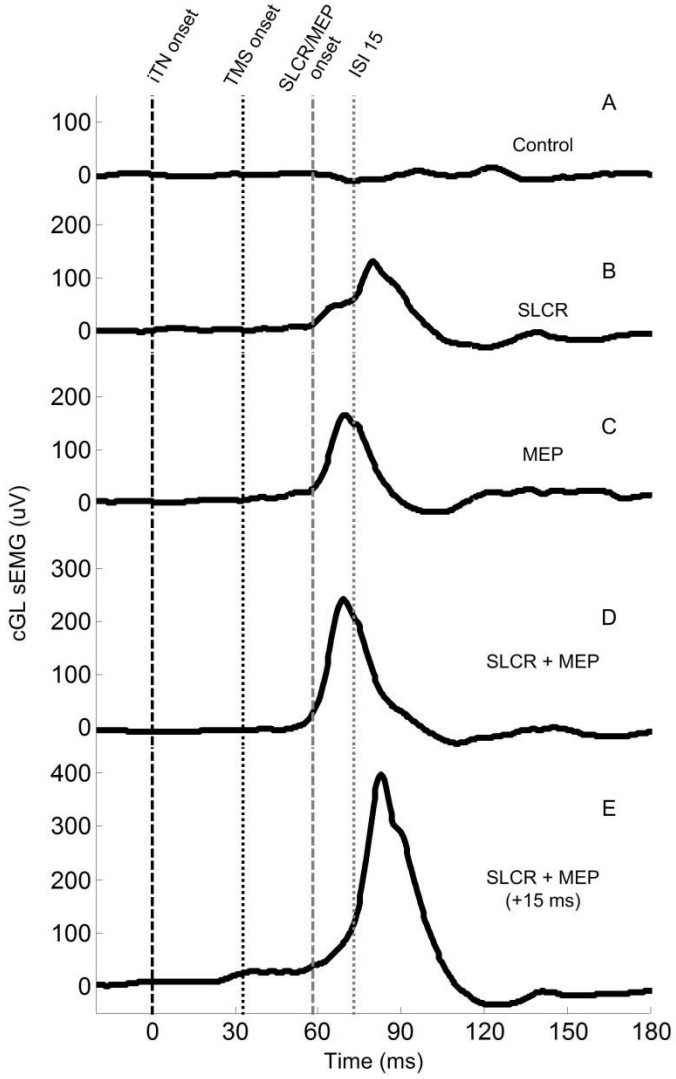

Figure 9: ***Combination of SLCR and MEP for a single subject.*** Mean rectified cGL sEMG for one subject for a control steps (no stimulations, **A**), following iTN stimulation (**B**), following TMS only (**C**), following a combination of iTN and TMS timed so that the MEP onset occurred at the same time of the SLCR's onset (**D**) and following a combination of iTN and TMS timed so that the MEP onset occurred 15ms after the SLCR's onset (**E**). Vertical lines represent iTN stimulation onset, TMS onset, SLCR onset, and 15 ms after the SLCR onset, respectively.

When TMS was timed so that the onset of the MEP and of the SLCR coincided, repeated measure ANOVA revealed a significant difference between the control, the SLCR alone, the MEPs alone, the algebraic sum of these two and the combination of the SLCR and the TMS ($F_{(4)} = 9.10$, $P < 0.001$) for all subject. The post hoc test confirmed a similar amplitude of the SLCR and the MEP ($P = 0.12$) and that both were different from the control ($P < 0.03$). However the combination of the two stimuli did not produce a response different from the MEP alone ($P = 0.09$) nor from the algebraic sum of the SLCR and MEP elicited separately ($P = 0.35$).

When the TMS was timed so that the onset of the MEP occurred 15 ms after the onset of the SLCR, which is when in average the onset of the second component seems to occur, a significant difference is again revealed by repeated measure ANOVA between the MEPs alone, the control, the SLCR alone, the algebraic sum of these two and the combination of the SLCR and the MEP ($F_{(4)}$ = 9.96, P < 0.001). In this case, however, the combined response was larger than the control and the single SLCR and MEP elicited separately (P < 0.02) as well as larger that the algebraic sum of the SLCR and the MEP elicited separately (P = 0.02) (Figures 9 and 10).

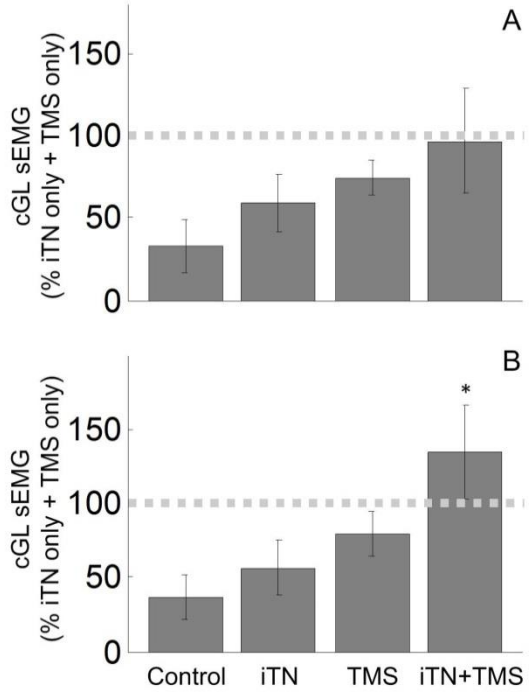

Figure 10: ***Combination of SLCR and MEP.*** Group mean (and SD) cGL sEMG data computed on the window where the MEP occurs in the four conditions (control, iTN only, TMS only and combination of iTN and TMS) for seven subjects. In **A** the MEP is timed to arrive at the same time of the SLCR while in **B** it arrives 15ms after the onset of the SLCR. Data were normalized to the algebraic sum of SLCR and MEP elicited separately (horizontal dashed lines). When the MEP occurred 15 ms after the onset of the SLCR, the MEP elicited by the combined iTN and TMS was significantly larger than the algebraic sum of SLCR and MEP elicited separately.

When looking at the MEP size following the combined iTN stimulation and TMS at different ISI, the MEP elicited by the combination of TMS and iTN was significantly less than the algebraic sum of the SLCR and the control MEP when the MEP arrived 30 ms before the onset of the SLCR (−30 ms, $t_{(6)}$ = 2.75, P = 0.03), and when the MEP arrived 30 ms (+30 ms, $t_{(6)}$ = 2.94, P = 0.03), 45ms (+45 ms, $t_{(6)}$ = 2.69, P = 0.04) and 200 ms after (+250 ms, $t_{(6)}$ = 2.57, P = 0.04) the SLCR onset. The MEP size following the combined iTN stimulation and TMS was not different from the algebraic sum of control MEP and SLCR when the MEP occurred 15 ms before (-15 ms), at (0 ms), 5 ms (+5 ms) and

20 ms (+20 ms) after the onset of the SLCR (P > 0.08). However, when MEPs were evoked either 10 ms (+10 ms, $t_{(6)}$ = -3.10, P = 0.02) or 15 ms (+15 ms, $t_{(6)}$ =-3.38, P =0.01) following the SLCR onset, the conditioned MEPs were significantly greater than the algebraic sum of the SLCR and the control MEPs (Figure 11).

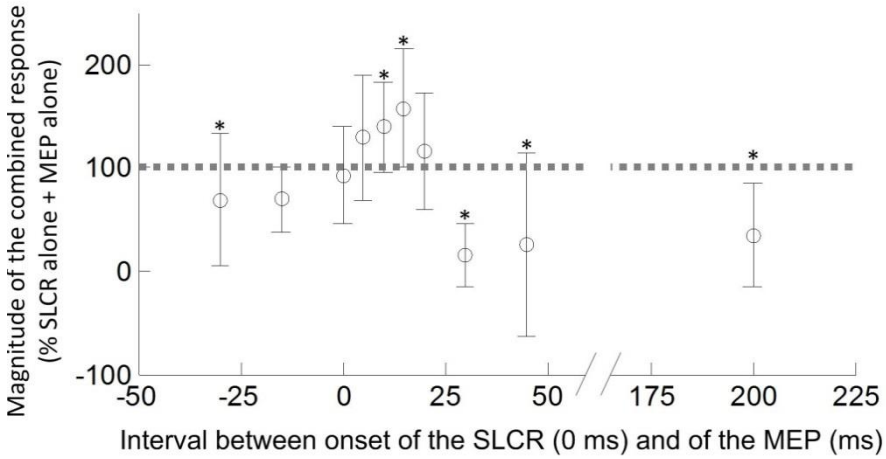

Figure 11: ***Time course of the MEPs in the cGL induced by TMS following iTN stimulation.*** MEPs were evoked by TMS at different timings (−30, −15, 0, +5, +10, +15, +30 +45 and +200 ms) relative to the SLCR onset in cGL (occurring at time 0). Mean values (and SD) of the magnitude of conditioned MEPs (iTN and TMS condition) are expressed as a percentage of the algebraic sum (indicated by the horizontal dashed line) of the SLCR and MEP elicited separately for each time interval. The asterisks indicate that the combined iTN and TMS condition resulted in MEPs significantly different (P < 0.05) than the sum of SLCR and the control MEP elicited separately. There was a significant increase in the MEPs only when these were timed to arrive 10 and 15 ms after the onset SLCR.

CHAPTER 4

4. Discussion

The current thesis investigated interlimb neural pathways connecting gastrocnemii muscles during human locomotion. Facilitations were evoked in the sEMG of cGL and of cGM by iTN stimulation during walking. The latencies of the SLCR (see Table 1) are in accord with the results of previous studies in which crossed responses have been elicited in the contralateral gastrocnemius from 65 to 112 ms after ipsilateral nerve stimulation or mechanical perturbation during walking (Berger *et al.*, 1984; Dietz *et al.*, 1986, 1989; Duysens *et al.*, 1991). The latencies of the responses and the mean age of the subjects for the individual studies (Table 1) suggest that crossed-responses might have a longer latency in older subjects, as also observed for the SLCR in cSOL (Stubbs et al., 2012).

The facilitation in the gastrocnemius occurred around 30 ms after the short-latency crossed inhibition elicited in the cSOL using the same stimulation. Different behavior of SOL and gastrocnemius muscles has previously been observed both in intact walking cats (Duysens and Loeb, 1980) and in walking humans (Duysens *et al.* 1991) and a separate control of SOL and GM was proposed (Duysens *et al.* (1991). The triceps surae muscles differ in terms of anatomy, action and motor units' property, although they all act on the ankle joint (Nardone *et al.*, 1990). For instance, the SOL is a monoarticular and purely plantarflexor muscle, while the gastrocnemius muscles are biarticular and contribute to knee flexion (Gravel *et al.*, 1987).

Not all the participants displayed a SLCR in the gastrocnemius (5 out of 43) and in those that showed a response, the latency with which it occurred varied with the subject population participating in the different studies (see Table 1). Similarly, the SLCR in the cSOL was not present in every subject (5-8 out of 21, depending on the time during the gait cycle) and the percentage of the gait cycle in which the response appeared varied between subjects (Stubbs et al. 2011). Stubbs

et al. (2011) suggested that a diverse expression of the cSOL response could be caused by the difference in feedback produced by different walking strategies adopted by the subjects. Chronic recordings in cats have shown that during walking reflexes and muscle activation are very consistent in the same animal but vary from one animal to another (Loeb, 1993). Even if genetics is a strong component in the generation of the locomotor program (Yang et al., 2004), inter-individual differences could arise during development, due to different activity dependent mechanisms and interactions with the environment (Frigon, 2011). Confirming this hypothesis is the observation that conditioning the soleus H-reflex can induce persistent changes in the spinal circuitry of adult rats (reviewed in Wolpaw and Tennissen, 2001; Wolpaw, 2007). Therefore, inter-individual variability influencing the SLCR in gastrocnemius could be reason of the response not being present in all subjects and the variability in latencies.

4.1. Possible pathways and neural mechanisms

Muscle afferents likely mediate the SLCR in gastrocnemius muscle during normal walking. Indeed, as for the SLCR in cSOL (Stubbs and Mrachacz-Kersting, 2009), stimulation of cutaneous afferents (through iMpN and iSuN stimulations) did not produce similar responses (Gervasio et al., 2013). An exception is when iMpN stimulation is performed at an intensity of 3 x PT; at this intensity iMpN elicits responses similar to those elicited by iTN stimulation. Since MpN is not a pure cutaneous nerve, it is possible that, at this intensity, also muscle fibers were activated (Gracies et al., 1994; Nielsen et al., 1997a). Therefore, muscle afferents remain the likeliest source for SLCR in cGL. However, during hybrid walking, when the response was reverted from facilitation to inhibition, the responses evoked by iTN, iSuN and iMpN stimulation did not differ. Although cutaneous inputs are not essential in locomotion (Sherrington, 1910; Grillner and Zangger, 1979), experiments on cats whose cutaneous nerve were transected demonstrated that information provided by these afferents might be crucial in tasks more demanding than normal walking, as

walking on a ladder or inclines (Bouyer and Rossignol, 2003). It is thus possible that cutaneous afferents play a more relevant role during human hybrid walking, due to the uncertainty of the task.

Characterizing the neural mechanism behind the crossed reflex reversal would help to further our understanding of the networks controlling lower interlimb coordination during locomotion. Unfortunately, this is a difficult task in the intact human. It is unlikely that the reverse in the response was caused by a different stimulation, as comparable iSOL M-wave peak-to-peak amplitude indicated that the same efferent fiber population was stimulated, although this does not confirm that the afferent volley was constant during the two tasks. Moreover, the reversal in the response is not influenced by the muscle's background activation since the level of muscle activity was similar in the two conditions and cGL.

The reversal mechanism is likely controlled at a premotoneuronal level since there was no correlation between the magnitude of the response and the background activity level. By performing motor unit recordings to investigate the reflex reversal in the ipsilateral tibialis anterior during locomotion, De Serres et al. (1995) suggested that parallel excitatory and inhibitory pathways from cutaneous afferents to single motoneurons are involved in the reversal and that a shift between the two pathways occurs during the gait cycle. It is thus possible that parallel excitatory and inhibitory pathways from different afferent populations in the ipsilateral gastrocnemius and to single motorneurons in the contralateral gastrocnemius are activated in the two tasks. However, the excitability provoked by the state of stretch of muscles acting on the same joint in the contralateral leg seems also to contribute to the modulation of the response and might contribute to its reversal. This aspect will be discussed in the next paragraph.

4.1.1. Contralateral afferent contribution to crossed responses

A mathematical model based on inverse dynamics was adopted to estimate muscle spindle and GTO ensemble afferent activity for the human gastrocnemius muscles and used to estimate the contribution of contralateral muscle afferents to the SLCR. The amplitude of the SLCR in cGM was correlated with the ensemble group II afferent activity of cGM and with group Ia afferent activity of both heads of the contralateral gastrocnemius, but not with cGL group II activity. Thus, contralateral afferent feedback is likely involved in the neural mechanism generating the SLCR.

Animal research showed that the main factor determining whether the crossed response produced by an ipsilateral non-specific perturbation results in extension or flexion is the position of the contralateral limb (Magnus, 1909, 1910; Grillner and Rossignol, 1978; Rossignol and Gauthier, 1980), and that the response is directed toward the muscle that is more stretched (Uexküll, 1904; Magnus, 1909, 1910). The response pattern seems to be due to an ensemble afferent activity from the receptors in the muscles of the leg (Grillner and Rossignol, 1978; Rossignol and Gauthier, 1980). The results of the current thesis suggest that a similar mechanism might exist in humans as ipsilateral stimuli produce a crossed facilitation in the cGL when the contralateral knee was extended and muscle fascicles were stretched. Moreover, the responses in cGM were correlated not only with spindle activity of the same muscle but also with the spindle primary afferents of cGL (although a contribution of cGL spindle secondary afferents to the regulation of the response in cGM cannot be excluded due to the not significant but low P value of this correlation). The SLCR might therefore depend on autogenic feedback from the same muscle and heterogenic feedback from other muscles of the same limb.

Two classes of heterogenic short-latency feedback mechanisms have been distinguished in the cat according to architectural features of the musculoskeletal system (Eccles et al., 1957; Nichols, 1999; Wilmink and Nichols, 2003). Length related feedback, likely mediated by group Ia afferents,

although a role for group II afferents is not excluded (Nichols, 1999), leads to excitation of synergistic muscles crossing the same joint (Wilmink and Nichols, 2003) and to inhibition of antagonistic muscles (Nichols and Koffler-Smulevitz, 1991; Nichols, 1999). Force related feedback is mediated by group Ib fibers and has inhibitory effects. However, in the cat, this feedback was found to extend from gastrocnemius muscles to SOL but not between GL and GM (Bonasera and Nichols, 1994; Nichols, 1999). Moreover, no correlation was observed between the SLCR and group Ib activity; therefore it is unlikely that force related feedback contributes to the SLCR.

The close relation between the SLCR and spindle afferents indicates a main contribution of length related feedback. This feedback seems to regulates joint stiffness, enhance force output during stance (Stein *et al.*, 2000; Mazzaro *et al.*, 2005, 2006), strengthen the coupling between the ankle and knee joints (Wilmink and Nichols, 2003), and contribute to the coordination of muscles during responses to postural disturbances (Bonasera and Nichols, 1994; Nichols, 1999). The biomechanical information provided by muscle spindles and the high conduction velocity of these afferents make them suitable to provide fast, appropriate information that is needed for corrective responses during postural disturbance (Honeycutt *et al.*, 2012). The observation that group I afferents seem to be suppressed (Dietz *et al.*, 1984) during gait, probably through presynaptic inhibition (Eccles, 1964; Schmidt, 1971), would suggests a main contribution of group II afferents to the SLCR in the gastrocnemius. Nevertheless, group Ia afferents contribute to reflex responses when an unexpected perturbation occurs (Nielsen and Sinkjær, 2002), and the activity of these afferents was correlated to the amplitude of the cGM SLCR. Thus, group Ia afferent might also be involved in the generation of SLCR.

The contribution of contralateral cutaneous feedback, which regulates the ongoing locomotor output during walking (Zehr and Stein, 1999; Duysens et al., 2000) to the SLCR was not investigated and cannot be excluded.

4.1.2. Cortical contribution to crossed responses

The short-latency of the crossed responses observed in the current thesis compared to the reported minimum latency of 79 ms for a transcortical pathway's contribution to ipsilateral reflexes in the tibialis anterior muscle (Petersen et al., 1998a) would suggest that these SLCR might be spinally mediated. Indeed the tibialis anterior is located at a similar distance from the cortex as the gastrocnemius. This thesis investigated whether SLCR in the gastrocnemius muscles were mediated by purely spinal pathways or whether there was a transcortical contribution to the response or to part of it. Indeed, in several subjects a second component of the response starting 15.1 ± 3.7ms after the onset of the first component was observed and the latency of this second component is consistent with a transcortical pathway.

The minimum latency for a transcortical pathway includes afferent conduction time, efferent conduction time and intracortical relay time. The mean afferent conduction time to the cortex after tibial nerve stimulations at the ankle level has been reported to be 33.9 ms (Jones and Small, 1978). To this value the conduction time from ankle to knee must be subtracted as in our case the tibial nerve was stimulated at the popliteal fossa. MEPs elicited by magnetic stimulation of the motor cortex occurred in the gastrocnemius muscle with a mean onset latency of 29 ms. Adding to this value the estimated time for central processing (3–10 ms, Nielsen et al., 1997b; Kurusu and Kitamura, 1999) and considering a somatosensory evoked potential (SEP) latency of 39 ms (Van de Wassenberg et al., 2008) as the afferent conduction time, a minimum latency of 71 ms can be obtained for a transcortical pathway's contribution to gastrocnemius muscles after tibial nerve stimulation at the popliteal fossa. The response in gastrocnemius, at least the first component, showed latency often too short for a transcortical pathway (see Table 1).

When MEPs elicited in cGL by magnetic stimulation of the primary motor cortex were timed to coincide with the onset of the SLCR evoked by iTN stimulation at 80% of the ipsilateral gait cycle,

they were not different from the algebraic sum of the responses elicited separately (Figure 9D, Figure 10A). However, when the MEPs were timed to coincide with the onset of the second component of the SLCR in cGL (around 15 ms after the onset of the first component, Table 2) they were significantly larger than the algebraic sum of the separately elicited responses (Figure 9E, Figure 10B). The results could be interpreted as an increase in cortical excitability following the iTN, supporting the hypothesis for a pathway that is at least in part mediated transcortically, as for the long latency stretch reflex response in the tibialis anterior muscle (Petersen et al., 1998a).

Moreover, when MEPs in the cGL were timed to arrive from 30 ms before, to 5 ms after the onset of the SLCR in cGL, the MEPs were not different from the algebraic sum of responses elicited separately (Figure 11). Previously we considered a mean latency of SEP following iTN as 39 ms. Adding the central processing time (3–10 ms, Nielsen et al., 1997b; Kurusu and Kitamura, 1999), we obtain that the realistic time for sensory information to influence the excitability of the cortical cells controlling the cGL after iTN stimulation is 42 ms. Considering, the mean latency of the SLCR in study VI (62.6 ms) and mean MEP latency (28.6ms), TMS was delivered on average 34 ms following iTN stimulation. Convergence at the cortical level between the afferent input from the iTN stimulation and TMS was indeed observed in the present study when TMS was delivered on average 44 and 49 (+10 and +15 ms) following iTN stimulation onset, but not when TMS was delivered on average from 4 to 39 ms before (−30, −15, 0, +5 ms) and from 54 to 234 ms after (+20, +30, +45, +250 ms) iTN stimulation (Figure 11). This evidence further supports the hypothesis of a transcortical pathway contributing to the second component of the SLCR.

4.2. Functionality of the crossed responses

Interlimb reflexes have a significant role in maintaining postural stability (Dietz, 2002). This is more critical during human walking and during phase transitions (from swing to stance or from stance to swing) and indeed when a perturbation is delivered at these times of the gait cycle it elicits more prominent ipsilateral and contralateral responses (Berger et al., 1984; Dietz et al., 2004). In this thesis, when iTN was delivered at 80% of the normal ipsilateral gait cycle, a contralateral facilitation was observed in both heads of the gastrocnemius and for the GM this facilitation was shown to be more prominent than when iTN was delivered at other timings during the gait cycle. At 80% of the gait cycle, the ipsilateral foot is in swing phase and approaching heel strike while the contralateral leg is in terminal stance. It was initially hypothesized that the short-latency inhibition observed in cSOL (Stubbs et al., 2011b) coupled with the facilitation in the knee flexor gastrocnemius might have had the purpose of preventing the push off of the contralateral foot, maintaining the body weight on the contralateral leg (Gervasio et al., 2013). However, pressure measurements under the contralateral foot showed a displacement of CoP location toward the medial and anterior direction, with a substantial increase in the pressure distribution under the first metatarsal head at 95% of the ipsilateral gait cycle. Such displacement of the contralateral foot's CoP could be interpreted as a method to accelerate the propulsion phase. The large synchronous afferent volley to the spinal cord elicited by the stimulation could indeed indicate a mechanical disturbance to the ipsilateral leg. With the latter approaching heel strike when the stimulation occurs, the functional result of the response could be aimed at preparing the contralateral leg to a faster step, in case the ability of the ipsilateral leg to sustain the body weight was compromised. This hypothesis is sustained by the observation that the stance phase of the ipsilateral leg has a significantly shorter duration in the step immediately following the iTN stimulation step.

Considering that the iTN stimulation was delivered on average at 80.7 % of the gait cycle and that the first significant displacement in CoP location can be observed at 90% of the gait cycle, it takes around 9.3% of the gait cycle to observe any chances in pressure. The average recorded gait cycle duration was approximately 1.25 s, thus the displacement in CoP was observed on average 116 ms after stimulation; this latency is too long for the displacement to be caused by the iTN itself. The main latency of the SLCR for these subjects was 58 ms (Table 1, study II); to obtain the time for the SLCR to produce changes in pressure, this time must be added to the time for electromechanical delay, associated with EMG onset and transmission of muscle force. Considering an electromechanical delay of approximately 50 ms (Zatsiorsky, 2000), which falls within the range reported in the literature (10–100ms, Corcos et al., 1992; Guimaraes et al., 1995) and has been previously used for electromechanical delay in human gastrocnemius medialis, we obtain that the time for the SLCR to produce changes in pressure would be 108 ms. This time shows a good correspondence with the CoP displacement observed around 116 ms following the stimulation which therefore seems to be due to the SLCR itself.

When an opposite reaction is required, a reversal of the SLCR is observed in the cGL. In the current thesis cGL SLCR during normal walking at 80-90% of the gait cycle was compared to the responses elicited during hybrid walking at 40-50% of the gait cycle. The latter timings during hybrid walking were chosen since they correspond again to a transition phase, where appropriate interlimb coordination is required to react rapidly to a disturbance of balance; at these timings, indeed, the ipsilateral leg is pushing off while the contralateral leg is touching down. The contralateral leg is not yet completely on the ground and might not be prepared to support the possible body weight shift provoked by the iTN stimulation. An inhibition in cGL may contribute to prepare the contralateral leg to support the body weight, forcing the heel to the ground by accelerating the knee extension. The observed reversal of the cGL SLCR from facilitation during

normal walking to inhibition during hybrid walking provides the first evidence for reflex reversal in the contralateral limb in humans and confirms the task dependence of the SLCR.

Sensory information that signals whether the contralateral leg is in a condition to sustain the body weight is crucial to generate an appropriate crossed response. This theory is sustained by the observation that SLCR are modulated during locomotion (study III, Stubbs et al., 2011) and that a reflex reversal occurs when an opposite reaction is required (Gervasio et al., 2013). When the iTN stimulation is delivered at 80% during hybrid walking, the stretch of the gastrocnemii signals the state of the contralateral knee and contributes to generate a facilitation in this muscle group. Moreover, the second component of the SLCR appears to be mediated by a transcortical pathway, as the interlimb reflexes produced in the contralateral biceps femoris by knee joint rotations during walking (Stevenson et al., 2013). These reflexes could be integrated with concurrent sensory information and may therefore potentially be better adjusted to the specific situations than purely spinal reflexes (Christensen et al., 2001; Zuur et al., 2009).

4.3. Methodological consideration

The responses investigated in the current thesis are evoked by iTN electrical stimulation. This stimulation elicits an artificial synchronous afferent volley that differs from the asynchronous volleys that would be evoked by a physiological stretch of the plantarflexors. However, the use of electrical stimulation allowed a specific stimulation of the tibial nerve afferents and a better identification of the afferent mediating the response. Furthermore, although ipsilateral spinal reflexes elicited by electrical stimulation are processed differently than a tendon tap (Morita et al., 1998), ipsilateral plantarflexion induced by mechanical rotation of the ankle joint produced similar responses to the one elicited by electrical stimulation of the tibial nerve (Mrachacz-Kersting, unpublished data).

A direct measure of the afferent activity from muscles is not currently available in the intact human. The accuracy of the fascicle length changes estimation, used to investigate muscle spindles afferent activity, depends on the accuracy of the parameters selected for the model; these were selected with respect to age and gender of the investigated population. The use of a mathematical model has been preferred over the use of ultrasound scanning; the latter technique raises indeed some significant issues as non-applicability of 3D measurement to dynamic situations and burden introduced by the use of cast and strapping to attach the probe to the subject's skin.

The result of the current thesis suggests the involvement of a transcortical pathway to the second component of the gastrocnemius SLCR. However, TMS activate fibers of the whole corticospinal tract thus changes in TMS MEP size could indicate changes at cortical and/or at subcortical level (Petersen et al., 2001). An experimental protocol that includes both TMS and transcranial electrical stimulation (TES) should be considered in order to confirm the involvement of cortical cells. Indeed, while MEPs elicited by TMS are produced by the excitability of cortical cells, MEPs elicited by TES are not or not as much. An alternative method would be using weak TMS stimuli which have been shown to activate only inhibitory cortical interneurons that inhibit the activity of the corticomotoneuronal cells. Such weak stimuli have been shown to "knock out" the corticospinal contribution to the EMG activity during human walking (Petersen et al., 2001) demonstrating that the activity of the motor cortex is involved in the control of the muscles during human walking

4.4. Future perspectives

The results of the current thesis provide insight into the neural pathways mediating the SLCR in the gastrocnemius muscle, suggesting for instance that at least part of the response might be transcortically mediated. However, as previously suggested, the use of both TMS and TES could allow conclusive proof for the involvement of cortical cells. Moreover, the contribution of other

supraspinal areas such as the brain stem and cerebellar structures cannot be excluded and therefore should be investigated. To estimate the degree of involvement of supraspinal areas it might be of interest to assess the status of the SLCR in patients with lesions in those areas. Moreover, to further elucidate on the spinal pathways involved in the SLCR the use of medication aimed at reducing specific afferent activity and elucidating the intake of certain neurotransmitter agonists/antagonists (such as 5-HT, tizanidine, benzodiazapines, L-DOPA) could be considered.

Finally, as the results of the current thesis sustain the functionality of the gastrocnemius SLCR as a method to maintain dynamic stability following a perturbation, future perspectives should include investigating the possibility of adopting reflex conditioning protocols to increase functional stability. It has been shown that, in people with impaired locomotion caused by incomplete spinal cord injury, down-conditioning the soleus H-reflex in one leg (the most affected one) increased the modulation of EMG activity during the cycle in both legs and this effect was accompanied with a faster and more symmetrical locomotion (Thompson et al., 2013). The SLCR in the cSOL is impaired in stroke patients. This impairment might indicate an inability to properly coordinate the legs following a disturbance to the ipsilateral leg and thus contribute to the increased incidence of falls in patient populations (Stubbs et al., 2012). Investigating whether the interlimb pathway mediating the gastrocnemius SLCR is impaired in patients with reduced supraspinal input, such as stroke or spinal cord injury patients, would represent the first step to evaluate the possibility of using SLCR conditioning protocols as innovative rehabilitation approach.

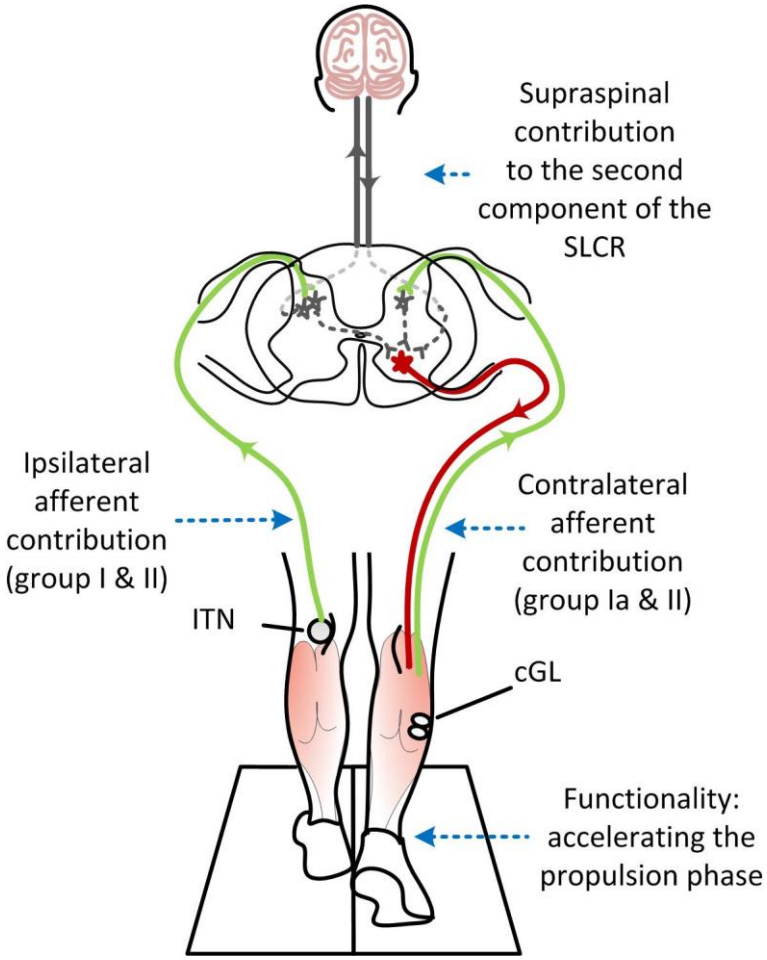

Figure 12*: Main outcomes of the current thesis.* The SLCR in gastrocnemius seems to be a method to accelerate the propulsion phase of the contralateral leg and therefore preparing for a faster stride in case the ipsilateral leg in not able to sustain the body weight after the stimulation. Ipsilateral muscle afferents seems to mediate the response. Contralateral afferents activity contributes to the modulation of the SLCR during the gait cycle. A supraspinal contribution to the second component of the response is likely. The red and green traces represent respectively efferent and afferent pathways. Dashed lines represent unknown neural pathway mediating the SLCR.

CHAPTER 5

5. Conclusions

This PhD thesis explored interlimb neural interlimb pathways connecting gastrocnemii muscles during human locomotion by investigating SLCRs elicited in the contralateral gastrocnemius by iTN stimulation. The main outcomes of the current thesis are summarized in Figure 12. Study I demonstrate that a reversal of SLCR mediated by these pathways is reverted when an opposite response is expected. This reversal is thus functionally relevant during human walking. Study II further confirms the functionality of the SLCR showing that the response produces a displacement in CoP location aimed at preparing the contralateral leg to a faster step, in case the perturbed ipsilateral leg was not able to sustain the body weight. Study III demonstrated that sensory feedback from the contralateral limb, and not only from the one that has been perturbed, is essential to generate a proper reaction. Study IV investigated the cortical contribution to the SLCR in gastrocnemius, suggesting that at least part of the response might be mediated by a transcortical pathway. These results provide new insight into the neural mechanism behind interlimb coordination during human locomotion. The possibility of adopting training protocols for the reflex conditioning of the gastrocnemius SLCR should be investigated in order to increases the functional stability. This perspective would be beneficial both for the rehabilitation of patients with locomotor impairment and for the sport field.

About the author

Sabata Gervasio was born in Rome, Italy, August 22th 1984. During her master education at the Roma TRE University, Rome, Italy, she was granted a scholarship for Thesis Research Abroad. The research was performed at the Center of Sensory Motor Interaction, Aalborg University. She graduated, summa cum laude, as an electronic biomedical engineer (M.Sc. E.E.) in May 2010 at the Roma TRE University. From October 2010 to January 2014 she was enrolled as a PhD student at the Center for Sensory-Motor Interaction at Aalborg University, Denmark. Her research interests include neurophysiology of movement and motor control, biomechanics and movement analysis, neuromuscular modeling, electrophysiology and electrical stimulation, gait rehabilitation. In August 2012 she was appointed with the MPP (Most Promising Proposal) Award at the Expert Scientific Meeting (ESM) 2012.

References

Aggelopoulos NC, Burton MJ, Clarke RW, Edgley S a. Characterization of a descending system that enables crossed group II inhibitory reflex pathways in the cat spinal cord. *The Journal of Neuroscience* 16: 723–9, 1996.

Albracht K, Arampatzis A, Baltzopoulos V. Assessment of muscle volume and physiological cross-sectional area of the human triceps surae muscle in vivo. *Journal of Biomechanics* 41: 2211–8, 2008.

Andersson O, Forssberg H, Lindqvist M. Phase dependent modulation of the transmission in reflex pathways during "fictive locomotion". *Acta Physiologica Scandinavica* 102: 8A, 1978.

Arshavsky YI, Gelfand IM, Orlovsky GN, Pavlova GA, Popova LB. Origin of Signals Conveyed by the Ventral Spino-Cerebellar Tract and Spino-Reticulo-Cerebellar Pathway. *Experimental Brain Research* 54: 426–431, 1984.

Arya S, Kulig K. Tendinopathy alters mechanical and material properties of the Achilles tendon. *Journal of Applied Physiology (Bethesda, Md. : 1985)* 108: 670–5, 2010.

Arya T, Bajwa S, Edgley SA. Crossed reflex actions from group II muscle afferents in the lumbar spinal cord of the anaesthetized cat. *Journal of Physiology* 444: 117–131, 1991.

Bachmann V, Müller R, Van Hedel HJ a, Dietz V. Vertical perturbations of human gait: organisation and adaptation of leg muscle responses. *Experimental Brain Research* 186: 123–30, 2008.

Berger BYW, Dietz V, Quintern J. Corrective reaction to stumbling in man: neuronal co-ordination of bilateral leg muscle activity during gait. *Journal of Physiology* 357: 109–125, 1984.

Bonasera SJ, Nichols TR. Mechanical actions of heterogenic reflexes linking long toe flexors with ankle and knee extensors of the cat hindlimb. *Journal of Neurophysiology* 71: 1096–1110, 1994.

Bouyer LJG, Rossignol S. Contribution of cutaneous inputs from the hindpaw to the control of locomotion. I. Intact cats. *Journal of Neurophysiology* 90: 3625–39, 2003.

Burke BYD, Dickson HG, Skuse NF. Task-dependent changes in the responses to low – threshold cutaneous volleys in the human lower limb. *The Journal of Physiology* 432: 445–458, 1991.

Bussel B, Roby-Brami A, Néris OR, Yakovleff A. Evidence for a spinal stepping generator in man. Electrophysiological study. *Acta Neurobiologiae Experimentalis* 56: 465–8, 1996.

Cabaj A, Stecina K, Jankowska E. Same Spinal Interneurons Mediate Reflex Actions of Group Ib and Group II Afferents and Crossed Reticulospinal Actions. *Journal of Neurophysiology* 95: 3911–3922, 2006.

Calancie B, Needham-Shropshire B, Jacobs P, Willer K, Zych G, Green B. Involuntary stepping after chronic spinal cord injury: Evidence for a central rhythm generator for locomotion in man. *Brain* 117: 1143–1159, 1994.

Choi JT, Bastian AJ. Adaptation reveals independent control networks for human walking. *Nature Neuroscience* 10: 1055–62, 2007.

Christensen LO, Andersen JB, Sinkjaer T, Nielsen J. Transcranial magnetic stimulation and stretch reflexes in the tibialis anterior muscle during human walking. *The Journal of Physiology* 531: 545–57, 2001.

Corcos DM, Gottlieb GL, Latash ML, Almeida GL, Agarwal GC. Electromechanical delay: An experimental artifact. *Journal of Electromyography and Kinesiology* 2: 59–68, 1992.

Cram J, Kasman G, Holtz J. *Introduction to Surface Electromyography*. Gaithersburg, MD: Aspen: 1998.

Van de Crommert HW, Faist M, Berger W, Duysens J. Biceps femoris tendon jerk reflexes are enhanced at the end of the swing phase in humans. *Brain Research* 734: 341–4, 1996.

Delwaide PJ, Crenna P, Fleron MH. Cutaneous nerve stimulation and motoneuronal excitability: I, soleus and tibialis anterior excitability after ipsilateral and contralateral sural nerve stimulation. *Journal of Neurology, Neurosurgery, and Psychiatry* 44: 699–707, 1981.

Dietz V, Colombo G, Müller R. Single joint perturbation during gait: neuronal control of movement trajectory. *Experimental Brain Research* 158: 308–16, 2004.

Dietz V, Horstmann G a, Berger W. Interlimb coordination of leg-muscle activation during perturbation of stance in humans. *Journal of Neurophysiology* 62: 680–93, 1989.

Dietz V, Quintern J, Berger W. Cerebral evoked potentials associated with the compensatory reactions following stance and gait perturbation. *Neuroscience Letters* 50: 181–186, 1984.

Dietz V, Quintern J, Boos G, Berger W. Obstruction of the swing phase during gait: phase-dependent bilateral leg muscle coordination. *Brain Research* 384: 166–9, 1986.

Dietz V, Zijlstra W, Duysens J. Human neuronal interlimb coordination during split-belt locomotion. *Experimental Brain Research* 101: 513–20, 1994.

Dietz V. Do human bipeds use quadrupedal coordination? *Trends in Neurosciences* 25: 462–7, 2002.

Dimitrijevic M, Gerasimenko Y, Pinter M. Evidence for a spinal central pattern generator in humans. *Ann N Y Acad Sci* 860: 360–76, 1998.

Dobkin B, Harkema S, Requejo P, Edgerton V. Modulation of locomotor-like EMG activity in subjects with complete and incomplete spinal cord injury. *Journal of Neurologic Rehabilitation* 9: 183, 1995.

Dominici N, Ivanenko YP, Cappellini G, D'Avella A, Mondì V, Cicchese M, Fabiano A, Silei T, Di Paolo A, Giannini C, Poppele RE, Lacquaniti F. Locomotor primitives in newborn babies and their development. *Science* 334: 997–9, 2011.

Duysens J, Clarac F, Cruse H. Load-regulating mechanisms in gait and posture: comparative aspects. *Physiological Reviews* 80: 83–133, 2000.

Duysens J, Van de Crommert HWAA. Neural control of locomotion; Part 1: The central pattern generator from cats to humans. *Gait & Posture* 7: 131–141, 1998.

Duysens J, Loeb GE, Weston BJ. Crossed flexor reflex responses and their reversal in freely walking cats. *Brain Research* 197: 538–42, 1980.

Duysens J, Loeb GE. Modulation of ipsi- and contralateral reflex responses in unrestrained walking cats. *Journal of Neurophysiology* 44: 1024–37, 1980.

Duysens J, Tax AAM, Van der Doelen B, Trippel M, Dietz V. Selective activation of human soleus or gastrocnemius in reflex responses during walking and running. *Experimental Brain Research* 87: 193–204, 1991.

Eccles BJC, Eccles RM, Lundberg A. The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. *Journal of Physiology* 137: 22–50, 1957.

Eccles J. Presynaptic inhibition in the spinal cord. In: *Progress in Brain Research*, edited by Eccles J, Schade J. Amsterdam: Elsevier, 1964, p. 65–91.

Edgley SA, Jankowska E, Krutki P, Hammar I. Both dorsal horn and lamina VIII interneurones contribute to crossed reflexes from feline group II muscle afferents. *Journal of Physiology* 552: 961–74, 2003.

Faist M, Blahak C, Duysens J, Berger W. Modulation of the biceps femoris tendon jerk reflex during human locomotion. *Experimental Brain Research* 125: 265–70, 1999.

Forssberg H, Grillner S, Halbertsma J. The locomotion of the low spinal cat I. Coordination within a hindlimb. *Acta Physiologica Scand.* 108: 269–281, 1980.

Frigon A, Hurteau M-F, Thibaudier Y, Leblond H, Telonio A, D'Angelo G. Split-belt walking alters the relationship between locomotor phases and cycle duration across speeds in intact and chronic spinalized adult cats. *The Journal of Neuroscience* 33: 8559–66, 2013.

Frigon A, Rossignol S. Short-latency crossed inhibitory responses in extensor muscles during locomotion in the cat. *Journal of Neurophysiology* 99: 989–98, 2008.

Frigon A. Chapter 7-Interindividual variability and its implications for locomotor adaptation following peripheral nerve and/or spinal cord injury. In: *Progress in Brain Research*. Elsevier BV., 2011, p. 101–18.

Fukunaga T, Kubo K, Kawakami Y, Fukashiro S, Kanehisa H, Maganaris CN. In vivo behaviour of human muscle tendon during walking. *Proceedings. Biological sciences / The Royal Society* 268: 229–33, 2001.

Gard S, Miff S, Kuo A. Comparison of kinematic and kinetic methods for computing the vertical motion of the body center of mass during walking. *Human Movement Science* 22: 597–610, 2004.

Gervasio S, Farina D, Sinkjaer T, Mrachacz-Kersting N. Crossed reflex reversal during human locomotion. *Journal of Neurophysiology* 109: 2335–2344, 2013.

Gracies JM, Pierrot-Deseilligny E, Robain G. Evidence for further recruitment of group I fibers with high stimulus intensities when using surface electrodes in man. *Electroencephalography and Clinical Neurophysiology* 93: 353–357, 1994.

Gravel D, Arsenault A, Lambert J. Soleus-gastrocnemius synergies in controlled contractions produced around the ankle and knee joints: an EMG study. *Electromyography and Clinical Neurophysiology* 27: 405–413, 1987.

Grieve D, Pheasant S, Cavanagh PR. Prediction of gastrocnemius length from knee and ankle joint posture. In: *Biomechanics VIa, Interntional Society of Biomechanics*. 1978, p. 205–412.

Grillner S, Rossignol S. Contralateral reflex reversal controlled by limb position in the acute spinal cat injected with clonidine i.v. *Brain Research* 144: 411–4, 1978.

Grillner S, Zangger P. On the central generation of locomotion in the low spinal cat. *Experimental Brain Research* 34: 241–261, 1979.

Grillner S. Neurobiological bases of rhythmic motor acts in vertebrates. *Science* 228: 143–9, 1985.

Guimaraes AC, Herzog W, Allinger TL, Zhang YT. The EMG-force relationship of the cat soleus muscle and its association with contractile conditions during locomotion. *The Journal of Experimental Biology* 198: 975–87, 1995.

Haridas C, Zehr EP. Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. *Journal of Neurophysiology* 90: 2850–61, 2003.

Harkema SJ, Hurley SL, Patel UK, Requejo PS, Dobkin BH, Edgerton VR. Human lumbosacral spinal cord interprets loading during stepping. *Journal of Neurophysiology* 77: 797–811, 1997.

Hermens H, Freriks B, Merletti R, Stegeman D, Blok J, Rau G, Disselhorst-, Klug C, Hägg G. *European Recommendations for Surface Electromyography: Results of the Seniam Project (SENIAM).* Enschede, The Netherlands: Roessingh Research and Development: 1999.

Honeycutt CF, Nardelli P, Cope TC, Nichols TR. Muscle spindle responses to horizontal support surface perturbation in the anesthetized cat: insights into the role of autogenic feedback in whole body postural control. *Journal of Neurophysiology* 108: 1253–61, 2012.

Jankowska E, Bannatyne B a, Stecina K, Hammar I, Cabaj A, Maxwell DJ. Commissural interneurons with input from group I and II muscle afferents in feline lumbar segments: neurotransmitters, projections and target cells. *The Journal of Physiology* 587: 401–18, 2009.

Jankowska E, Edgley SA, Krutki P, Hammar I. Functional differentiation and organization of feline midlumbar commissural interneurones. *The Journal of Physiology* 565: 645–58, 2005a.

Jankowska E, Krutki P, Matsuyama K. Relative contribution of Ia inhibitory interneurones to inhibition of feline contralateral motoneurones evoked via commissural interneurones. *The Journal of Physiology* 568: 617–28, 2005b.

Jankowska E, Noga BR. Contralaterally projecting lamina VIII interneurones in middle lumbar segments in the cat. *Brain Research* 535: 327–30, 1990.

Jankowska E. Spinal interneuronal networks in the cat; elementary components. *Brain Research Reviews* 57: 46–55, 2008.

Jones SJ, Small DG. Spinal and sub-cortical evoked potentials following stimulation of the posterior tibial nerve in man. *Electroencephalography and Clinical Neurophysiology* 44: 299–306, 1978.

Jordan K, Challis JH, Newell KM. Walking speed influences on gait cycle variability. *Gait & Posture* 26: 128–34, 2007.

Kato M. Walking of cats on a grid: performance of locomotor task in spinal intact and hemisected cats. *Neuroscience Letters* 145: 129–132, 1992.

Kawakami Y, Ichinose Y, Fukunaga T. Architectural and functional features of human triceps surae muscles during contraction Architectural and. *Journal of Applied Physiology* 85: 398–404, 1998.

Kurusu K, Kitamura J. Long-latency reflexes in contracted hand and foot muscles and their relations to somatosensory evoked potentials and transcranial magnetic stimulation of the motor cortex. *Clinical Neurophysiology* 110: 2014–9, 1999.

Lichtwark GA, Bougoulias K, Wilson AM. Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. *Journal of Biomechanics* 40: 157–64, 2007.

Loeb G. The distal hindlimb musculature of the cat: interanimal variability of locomotor activity and cutaneous reflexes. *Experimental Brain Research* 96: 125–140, 1993.

Magnus R. Zur Regelung der Bewegungen durch das Zentralncrvensystem. Mitteilung I. *Pfliigers Arch. ges. Physiol.* 130: 219–252, 1909.

Magnus R. Zur Regelung der Bcwegungen durch das Zentralnervensystem. Mitteilung III. *Pfliigers Arch. ges. Physiol.* 134: 545–583, 1910.

Magnusson SP, Beyer N, Abrahamsen H, Aagaard P, Neergaard K, Kjaer M. Increased Cross-sectional Area and Reduced Tensile Stress of the Achilles Tendon in Elderly Compared With Young Women. *Journal of Gerontology: Biological Sciences* 58: 123–127, 2003.

Matsushita M. The axonal pathways of spinal neurons in the cat. *Journal of Comparative Neurology* 138: 391–417, 1970.

Matsuyama K, Drew T. Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. II. Walking on an inclined plane. *Journal of Neurophysiology* 84: 2257–76, 2000.

Mazzaro N, Grey MJ, Do Nascimento OF, Sinkjaer T. Afferent-mediated modulation of the soleus muscle activity during the stance phase of human walking. *Experimental Brain Research* 173: 713–23, 2006.

Mazzaro N, Grey MJ, Sinkjaer T. Contribution of afferent feedback to the soleus muscle activity during human locomotion. *Journal of Neurophysiology* 93: 167–77, 2005.

Mileusnic MP, Loeb GE. Force estimation from ensembles of Golgi tendon organs. *Journal of Neural Engineering* 6: 036001, 2009.

Miller S, Van der Burg J, Van der Meché F. Coordination of movement of the hindlimbs and forelimbs in different forms of locomotion in normal and decerebrate cats. *Brain Research* 91: 217–237, 1975.

Morita H, Petersen N, Christensen LO, Sinkjaer T, Nielsen J. Sensitivity of H-reflexes and stretch reflexes to presynaptic inhibition in humans. *Journal of Neurophysiology* 80: 610–20, 1998.

Mrachacz-Kersting N, Grey MJ, Sinkjaer T. Evidence for a supraspinal contribution to the human quadriceps long-latency stretch reflex. *Experimental Brain Research* 168: 529–40, 2006.

Nardone A, Corrà T, Schieppati M. Different activations of the soleus and gastrocnemii muscles in response to various types of stance perturbation in man. *Experimental Brain Research* 80: 323–32, 1990.

Nichols TR, Koffler-Smulevitz D. Mechanical analysis of heterogenic inhibition between soleus muscle and the pretibial flexors in the cat. *Journal of Neuro* 66: 1139–1155, 1991.

Nichols TR. Receptor Mechanisms Underlying Heterogenic Reflexes Among the Triceps Surae Muscles of the Cat. *Journal of Neurophysiology* 81: 467–478, 1999.

Nielsen J, Petersen N, Fedirchuk B. Evidence suggesting a transcortical pathway from cutaneous foot afferents to tibialis anterior motoneurones in man. *The Journal of Physiology* 501 (Pt 2): 473–484, 1997a.

Nielsen J, Petersen N, Fedirchuk B. Evidence suggesting a transcortical pathway from cutaneous foot afferents to tibialis anterior motoneurones in man. *The Journal of Physiology* 501 (Pt 2: 473–84, 1997b.

Nielsen JB, Sinkjær T. Reflex excitation of muscles during human walking. *Advances in Experimental Medicine and Biolody* 508: 369–375, 2002.

Nielsen JB. How We Walk: Central Control of Muscle Activity during Human Walking. *The Neuroscientist* 9: 195–204, 2003.

Noga BR, Kriellaars DJ, Brownstone RM, Jordan LM. Mechanism for activation of locomotor centers in the spinal cord by stimulation of the mesencephalic locomotor region. *Journal of Neurophysiology* 90: 1464–78, 2003.

Pang MYC, Yang JF. The initiation of the swing phase in human infant stepping: importance of hip position and leg loading. *The Journal of Physiology* 528 Pt 2: 389–404, 2000.

Pang MYC, Yang JF. Sensory gating for the initiation of the swing phase in different directions of human infant stepping. *The Journal of Neuroscience* 22: 5734–40, 2002.

Petersen N, Christensen LO, Morita H, Sinkjaer T, Nielsen J. Evidence that a transcortical pathway contributes to stretch reflexes in the tibialis anterior muscle in man. *The Journal of Physiology* 512 (Pt 1): 267–76, 1998a.

Petersen N, Christensen LO, Nielsen J. The effect of transcranial magnetic stimulation on the soleus H reflex during human walking. *The Journal of Physiology* 513 (Pt 2): 599–610, 1998b.

Petersen NT, Butler JE, Marchand-Pauvert V, Fisher R, Ledebt A, Pyndt HS, Hansen NL, Nielsen JB. Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. *The Journal of Physiology* 537: 651–6, 2001.

Pierrot-Deseilligny E, Burke D. *The Circuitry of the Human Spinal Cord*. Cambridge University Press, 2005.

Prochazka a, Gorassini M. Ensemble firing of muscle afferents recorded during normal locomotion in cats. *The Journal of Physiology* 507 (Pt 1): 293–304, 1998.

Rossignol S, Gauthier L. An analysis of mechanisms controlling the reversal of crossed spinal reflexes. *Brain Research* 182: 31–45, 1980.

Rossignol S, Saltiel P, Perreault M-C, Drew T, Pearson K, Bélanger M. Intralimb and interlimb coordination in the cat during real and fictive rhythmic motor programs. *Seminars in Neuroscience* 5: 67–75, 1993.

Rossignol S. The control of crossed extensor and crossed flexor responses. In: *Soc. Neurosci. Abstr.* 1977.

Schafer RC. Body alignment, posture, and gait. In: *Clinical Biomechanics: Musculoskeletal Actions and Reactions*. Lippincott Williams & Wilkins, 1983.

Schmidt R. Presynaptic inhibition in the vertebrate central nervous systems. *Erg Physiol Biol Exp Pharmacol* 63: 20–101, 1971.

Schubert M, Curt A, Jensen L, Dietz V. Corticospinal input in human gait: modulation of magnetically evoked motor responses. *Experimental Brain Researchrain research* 115: 234–46, 1997.

De Serres SJ, Yang JF, Patrick SK. Mechanism for reflex reversal during walking inhuman tibialis anterior muscle revealed by single motor unit recording. *Journal of Physiology* 488.1: 249–258, 1995.

Sherrington C. Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *Journal of Physiology* 40: 28–121, 1910.

Shik ML, Orlovsky GN. Neurophysiology of locomotor automatism. *Physiological Reviews* 56: 465–501, 1976.

Stecina K, Slawinska U, Jankowska E. Ipsilateral actions from the feline red nucleus on hindlimb motoneurones. *The Journal of Physiology* 586: 5865–84, 2008.

Stein RB, Misiaszek JE, Pearson KG. Functional role of muscle reflexes for force generation in the decerebrate walking cat. *The Journal of Physiology* 525 Pt 3: 781–91, 2000.

Stephens MJ, Yang JF. Loading during the stance phase of walking in humans increases the extensor EMG amplitude but does not change the duration of the step cycle. *Experimental Brain Research* 124: 363–70, 1999.

Stevenson AJT, Geertsen SS, Andersen JB, Sinkjær T, Nielsen JB, Mrachacz-Kersting N. Interlimb communication to the knee flexors during walking in humans. *The Journal of Physiology* 591: 4921–35, 2013.

Stubbs PW, Mrachacz-Kersting N. Short-latency crossed inhibitory responses in the human soleus muscle. *Journal of Neurophysiology* 102: 3596–605, 2009.

Stubbs PW, Nielsen JF, Sinkjaer T, Mrachacz-Kersting N. Crossed spinal soleus muscle communication demonstrated by H-reflex conditioning. *Muscle & Nerve* 43: 845–50, 2011a.

Stubbs PW, Nielsen JF, Sinkjær T, Mrachacz-Kersting N. Phase modulation of the short-latency crossed spinal response in the human soleus muscle. *Journal of Neurophysiology* 105: 503–11, 2011b.

Stubbs PW, Nielsen JF, Sinkjær T, Mrachacz-Kersting N. Short-latency crossed spinal responses are impaired differently in sub-acute and chronic stroke patients. *Clinical Neurophysiology* 123: 541–9, 2012.

Swinnen SP, Duysens J, editors. Part I Interlimb Coordination: Neural Networks for Locomotion and Processes of Functional Recovery. In: *Neuro-Behavioral Determinants of Interlimb Coordination: A multidisciplinary approach*. Springer Science+Business Media, LLC, 2004.

Thompson AK, Pomerantz FR, Wolpaw JR. Operant conditioning of a spinal reflex can improve locomotion after spinal cord injury in humans. *The Journal of Neuroscience* 33: 2365–75, 2013.

Ting LH, Kautz SA, Brown DA, Zajac FE, Hyngstrom A, Onushko T, Chua M, Schmit BD, Reisman DS, Bastian AJ, Morton SM. Contralateral Movement and Extensor Force Generation Alter Flexion Phase Muscle Coordination in Pedaling. *Journal of Neurophysiology* 83: 3351–3365, 2000.

Ting LH, Raasch CC, Brown D a, Kautz S a, Zajac FE. Sensorimotor state of the contralateral leg affects ipsilateral muscle coordination of pedaling. *Journal of Neurophysiology* 80: 1341–51, 1998.

Uexküll J von. Die ersten Ursachen des Rhythmus in der Tierreihe. *Ergebn Physiol* 3: 1–11, 1904.

Vallis LA, Patla AE, Adkin AL. Control of steering in the presence of unexpected head yaw movements. *Experimental Brain Research* 138: 128–134, 2001.

Voigt M, Dyhre-Poulsen P, Simonsen EB. Modulation of short latency stretch reflexes during human hopping. *Acta physiologica Scandinavica* 163: 181–94, 1998.

Voss VH. Tabelle der absoluten und relativen Muskelspindelzahlen der menschilichen Skelettmuskulatur. *Anat Anz* 129: 562–572, 1971.

Van de Wassenberg WJG, Kruizinga WJ, Van der Hoeven JH, Leenders KL, Maurits NM. Multichannel recording of tibial-nerve somatosensory evoked potentials. *Neurophysiologie clinique* 38: 277–88, 2008.

Wilmink RJH, Nichols TR. Distribution of heterogenic reflexes among the quadriceps and triceps surae muscles of the cat hind limb. *Journal of Neurophysiology* 90: 2310–24, 2003.

Winter DA. *Biomechanics and Motor Control of Human Movement*. 2nd edn. New York: Wiley, 1990.

Wolpaw JR, Tennissen AM. Activity-dependent spinal cord plasticity in health and disease. *Annual Review of Neuroscience* 24: 807–843, 2001.

Wolpaw JR. Spinal cord plasticity in acquisition and maintenance of motor skills. *Acta Physiologica* 189: 155–69, 2007.

Yanagihara D, Udo M, Kondo I, Yoshida T. Rapid communication during perturbed locomotion in decerebrate cats. *Neuroscience Research* 18: 241–244, 1993.

Yang J, Lam T, Pang M, Lamont E, Musselman K, Seinen E. Infant stepping: a window to the behaviour of the human pattern generator for walking. *Canadian Journal of Physiology and Pharmacology* 82: 662–674, 2004.

Yang JF, Stephens MJ, Vishram R. Infant stepping: a method to study the sensory control of human walking. *The Journal of Physiology* 507 (Pt 3: 927–37, 1998.

Zatsiorsky V, editor. *The Encyclopaedia of Sports Medicine: An IOC Medical Commission Publication, Biomechanics in Sport: Performance Enhancement and Injury Prevention*. Wiley-Blackwell, 2000.

Zehr EP, Stein RB. What functions do reflexes serve during human locomotion? *Progress in Neurobiology* 58: 185–205, 1999.

Zuur AT, Christensen MS, Sinkjaer T, Grey MJ, Nielsen JB. Tibialis anterior stretch reflex in early stance is suppressed by repetitive transcranial magnetic stimulation. *The Journal of Physiology* 587: 1669–76, 2009.