EFFECTS OF TRANS-SPINAL DIRECT CURRENT STIMULATION ON SPINAL AND CORTICOSPINAL EXCITABILITY DURING GAIT AND QUIET STANDING

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ABSTRACT

Jarske, H. 2020. Effects of trans-spinal direct current stimulation on spinal and corticospinal excitability during gait and quiet standing. University of Jyväskylä, masters' thesis in Biomechanics, 80 s.

Trans-spinal direct current stimulation (Ts-DCS) is a novel method aimed at modulating neural activity in the ascending and descending pathways of the spinal cord. Therefore, it may serve as a functional modulator for different pathological conditions. However, the results have not always been consistent across studies. Sources for these discrepancies can range from factors that are not only dependent on stimulation polarity but also of neural orientation, cell size, electrode location and genetic predisposition. Furthermore, activity of the neurons under stimulation may contribute. As previous studies in humans have administered and measured the effects of Ts-DCS during rest, they cannot be taken to reveal modulation effects in active conditions with different underlying neural activity.

This masters' thesis was conducted to reveal the effects of anodal Ts-DCS administered during gait with protocols that included measurement with matching neural activity. A total of eight (8) subjects participated in three measurement session. Anodal or sham Ts-DCS was administered during treadmill gait with preferred speed in three protocols with measurements pre and post stimulation. During gait with matched neural activity, spinal excitability changes were measured with soleus Hoffmann reflex (H-reflex) during three phases of stance. During quiet standing, recruitment curves with H-reflex and transcranial magnetic stimulation (TMS) were constructed to reveal Hmax/Mmax and corticospinal excitability modulation across intensities. Anodal and sham stimulation paradigms with all measurements are referred as AG and SG, respectively. Protocol R with anodal Ts-DCS excluded additional gait during spinal excitability measures as gait itself is a possible modulator.

Results indicate that anodal Ts-DCS could not induce significant changes in a systematic manner. No changes were revealed for H/M relationship during gait or standing. Although a reduction of Mmax amplitudes were seen in standing conditions, the change was accompanied with concurrent H-reflex depression. No systematic Mmax reduction was found at early, mid or late stance. In protocol R during standing measurement, there was an increase in MEPs at 90 % of active motor threshold (aMT) and a reversal of modulation direction at 120 % aMT between SG and R protocols with no other significant results although a similar trend was visible. Despite individual significant results, it is concluded that the effects of Ts-DCS seem not to affect spinal and corticospinal excitability in a systematic manner that prevails over normal neural activity. However, further research is warranted.

Key words: Spinal excitability, corticospinal excitability, trans-spinal direct current stimulation

TIIVISTELMÄ

Jarske, H. 2020. Trans-spinaalisen tasavirtastimulaation vaikutukset spinaaliseen ja kortikospinaaliseen herkkyyteen kävelyn ja seisomisen aikana. Liikuntatieteellinen tiedekunta, Jyväskylän yliopisto, biomekaniikan pro gradu -tutkielma, 80 s.

Trans-spinaalinen tasavirtastimulaatio (Ts-DCS) on uusi menetelmä, minkä tarkoituksena on muokata selkäytimen nousevien ja laskevien hermoratojen aktiivisuutta. Hermoratojen muokkaaminen voi olla hyödyllistä erilaisissa patologisissa sairauksissa. Stimulaation tulokset eivät kuitenkaan ole olleet systemaattisia eri tutkimuksissa. Erot voivat johtua stimulaation polariteetista, mutta myös stimuloitavan hermon asennosta ja etäisyydestä sähkökenttään nähden, sen koosta, elektrodien asettelusta sekä geneettisestä altistuksesta. Näiden tekijöiden lisäksi hermojen aktiivisuudella stimulaation aikana voi olla merkitystä tuotettuun muutokseen. Aikaisemmat tutkimukset ovat antaneet stimulaatiota levon aikana. Johtopäätökset levon aikana annetusta stimulaatiosta eivät kuitenkaan kerro, miten stimulaatio vaikuttaa aktiiviseen hermostoon.

Tämän tutkielman tarkoitus oli selvittää kävelyn aikana annetun anodaalisen Ts-DCS;n vaikutuksia spinaaliseen ja kortikospinaaliseen herkkyyteen aktiivisissa tilanteissa. Koehenkilöitä rekrytoitiin tutkimukseen yhteensä kahdeksan (8). Anodaalista tai lumestimulaatiota annettiin juoksumatolla kävelyn aikana kaikissa kolmessa protokollassa. Mittaukset suoritettiin ennen ja jälkeen stimulaation. Kävelyn aikana spinaalisen herkkyyden muutoksia tarkasteltiin Hoffmannin refleksin (H-refleksi) avulla, mikä yhdistää Ts-DCS:n antohetken ja mittauksen aikana olleen hermoaktiivisuuden. Muutoksia tarkasteltiin tukivaiheen ajalta kolmesta eri vaiheesta. Seisomisen aikana rekrytointikäyrät rakennettiin niin spinaalisen ja kortikospinaalisen radan osalta ja mitattiin H-refleksin ja transkraniaalisen mageettistimulaation (TMS) avulla. Anodaaliseen ja lumestimulaatioon viitataan protokollissa, jotka sisältävät kaikki mittaukset, lyhenteillä AG (anodaalinen) ja SG (lume). Anodaalinen protokolla R ei sisältänyt kävelyn aikana mitattua spinaalista herkkyyttä, koska kävely ennen stimulaatiota voi itsessään vaikuttaa saatuihin tuloksiin.

Tutkimuksen tulokset viittaavat, että Ts-DCS ei pystynyt muokkaamaan spinaalista tai kortikospinaalista herkkyyttä systemaattisella tavalla. Muutoksia H/M suhteen ei havaittu kävelyn eikä seisomisen aikana. Seisten mitatut Mmax arvot laskivat merkittävästi, mutta muutos oli samankaltainen H-refleksin kanssa. Mmax ei laskenut systemaattisesti kävelyn alku-, keski-, tai lopputukivaiheen aikana. Protokollassa R seisomisen aikana nähtiin suuremmat MEP-arvot intensiteetillä, joka oli 90 % aktiivisesti motorisesta kynnysarvosta (aMT). Intensiteetillä 120 % aMT muutoksen suunta näyttäytyi vastakkaisena SG ja R protokollan välillä ilman muita merkittäviä tuloksia, vaikka samanlaisesta trendi näyttäytyi. Yksittäisistä tuloksista huolimatta, tulokset viittaavat siihen, että muutokset eivät esiinny systemaattisina normaalin aktiivisuuden aikana. Lisätutkimuksia kuitenkin tarvitaan.

Avainsanat: Spinaalinen herkkyys, kortikospinaalinen herkkyys, trans-spinaalinen tasavirtastimulaatio

CONTENTS

ABBREVIATIONS

aMT	Active motor threshold
CNS	Central nervous system
COP	Centre of pressure
CST	Corticospinal tract
DC	Direct current
EMG	Electromyography
GM	Gastrocnemius medialis
GTO	Golgi tendon organs
Hmax	Maximal H-reflex
H-reflex	Hoffmann reflex
IO	Input-output
MEP	Motor evoked potential
Mmax	Maximal M-wave
MVC	Maximal voluntary contraction
RMS	Root mean square
rMT	Resting motor threshold
SCI	Spinal cord injury
SE	Standard error
SD	Standard deviation
SICI	Short interval intracortical inhibition
SOL	Soleus
TA	Tibialis anterior
TMS	Transcranial magnetic stimulation
Ts-DCS	Trans-spinal direct current stimulation

1 INTRODUCTION

Walking is a highly automated movement, which requires a merging of sensory information from multiple sources (Dietz 2010). Although descending commands from the cortical levels control our goal directed movements (Kandel 2013, 739-740) afferent information from muscles, skin, joints, and tendons help in reacting to unexpected events and to initiate appropriate muscle activity. The spinal cord level is of special interest for it serves as an integration point of afferent and descending information, which are converged on common spinal interneurons. (Dietz 2010.) Modulation or disruption of these circuits can have a dramatic effect on walking abilities. For example, humans with spinal cord injuries have demonstrated downregulated reflexes during walking (Knikou et al 2015) and patients who demonstrate spasticity are reported to exhibit weaker presynaptic inhibition (Morita et al 2001) and post-activation depression (Grey et al 2008).

Trans-spinal direct current stimulation (Ts-DCS) is a relatively new method aimed at modulating the neuronal pathways of the spinal cord. Evidence has suggested that lower thoracic stimulation can modulate ascending sensory pathways (Cogiamanian et al 2008), presynaptic inhibition (Kaczmarek et al 2017) post-activation depression (Winkler et al 2010) and the excitability level of the monosynaptic pathway (Kuck et al 2018; Murray et al 2018). In addition to spinal levels, DCS may affect descending information processing as modulation of the corticospinal pathway has also been reported. (Bocci et al 2015a; Murray & Knikou 2019). Thus, Ts-DCS may be a powerful tool to induce modulation of neural circuits and thus, improve motor performance in pathological conditions as well as in healthy humans. However, the exact mechanisms are yet known to full extent.

Research of the effects of Ts-DCS have focused on both the spinal and cortical modulations. However, testing of the effects and the administration of Ts-DCS has usually been conducted during resting conditions (Winkler et al 2010; Bocci et al 2015ab; Kuck et al 2018), which neglects the contributions of active neural circuits. Indeed, Capaday (1997) argued that the best way to investigate motor control is during natural movement or functions that the human motor system is designed for. Stein and Thompson (2006) affirmed the notion in their review by asserting that test results obtained in resting condition may not represent control in functional movements. During gait, reflex excitability is known to be modulated in a phasic manner

(Knikou et al 2015) and the amplitude differs between that of quiet standing, lying prone and sitting (Simonsen & Dyhre-Poulsen 1999). Furthermore, DCS is suggested to modulate active neurons more easily than those at rest (Bikson & Rahman 2013) indicating possible activity dependent modulation. Thus, the effects of Ts-DCS may vary with differences in neural activity and body position indicating a rationale for matching intervention and testing protocols.

Studies using Ts-DCS have reported a variety of results that in some cases are found contradictory. Some of these differences can be attributed to differences in methods such as electrode locations (Kuck et al 2018; Priori et al 2014). Interindividual differences have also raised questions beyond methodology with possible differential modulation that can stem from genetic predisposition (Lamy & Boakye 2013). Additionally, animal studies have provided important information that might further elucidate differential responses. Indeed, it has been revealed that the electrical field associated with DC-stimulation can modulate neurons differently for example depending on cell size, its orientation and distance (Ahmed 2014).

The aim of the present thesis was to study the effects of anodal Ts-DCS on both monosynaptic spinal reflexes and corticospinal excitability of the soleus muscle (SOL). The administration of Ts-DCS was done during treadmill gait with testing protocols during standing and gait, which was done to match neural activity during administration and testing. Activity dependence of the stimulation protocol would provide important information for the application of Ts-DCS in a clinical environment and could bring about important information of the mechanisms of the stimulation. To the knowledge of the author, studies have yet to investigate the effects of Ts-DCS during gait in humans.

2 FUNCTIONAL ANATOMY OF THE MOTOR SYSTEMS

It is evident that the motor system is a complex and integrative system where each part can influence our movements. In natural voluntary movements, muscles receive a number of excitatory impulses from motoneurons that are translated into muscle contractions. (Enoka 2015, 220.) The origin of those impulses arises from cortical areas of the brain. Impulses descend from the cortical level to the spinal cord, exit through the ventral column and polarize skeletal muscles appropriate for the wanted movements. Furthermore, afferent information that arises from peripheral receptors enter the spinal cord through the dorsal horn and synapse with a multitude of ascending and descending neurons. (Latash 1998, 145-151.) Thus, afferent information can influence our motor behaviour in the spinal cord for example by affecting the excitability of motor axons (Dietz 2010).

In this section the anatomy of cortical and spinal systems and the most essential parts of the descending and ascending pathways are reviewed. In addition to neural pathways in the central nervous system, essential receptors are introduced to the reader. These receptors reside in the peripheral system and provide the body with sensory feedback of the environment and the body itself. It is to be noted that in addition to the pathways and receptor presented here there are many others that provide important contributions to normal human movement. The ones presented are those that have been recognized to be of essential importance but do not represent the movement processing system as a whole, which would require a far more comprehensive and detailed description.

2.1 Motor cortex and the corticospinal tract

The cortex is organized in a six-layer laminar structure. In this structure, there are many neurons with a predominance of two types that are most common. These are the stellate cells, which are in essence interneurons in the cortex, and the pyramidal cells. Pyramidal cells unlike the stellate cells have dendrites that leave the cortex and provide connections to many structures in the central nervous system. The most known pyramidal tract is the corticospinal tract (CST) (see figure 1). It arises primarily from the frontal motor areas but has origins from the parietal somatosensory brain areas (see figure 2). The mostly myelinated neurons in this tract travel down to the spinal cord and thus pose control over skeletal muscles in voluntary movements.

The second pyramidal tract is known as the corticobulbar tract. This tract innervates cranial nerves and thus, controls face and neck muscles. (Latash 2012, 192-193.)

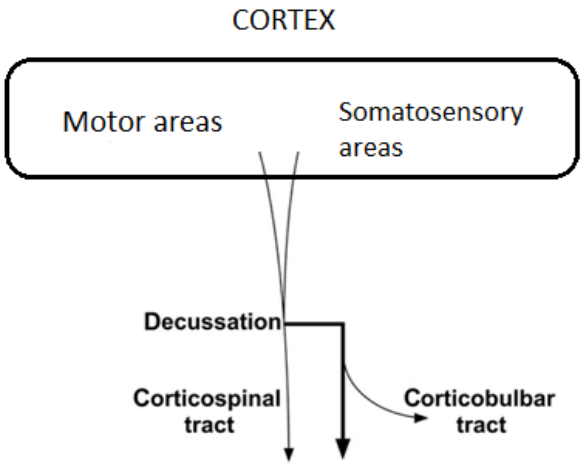

FIGURE 1. An illustration of the pyramidal tract. Pyramidal cells from the frontal motor areas and primary somatosensory areas form the corticospinal tract and the corticobulbar tract. Some of the neurons decussate at the junction of brainstem and spinal cord. (Modified from Latash 2012, 192)

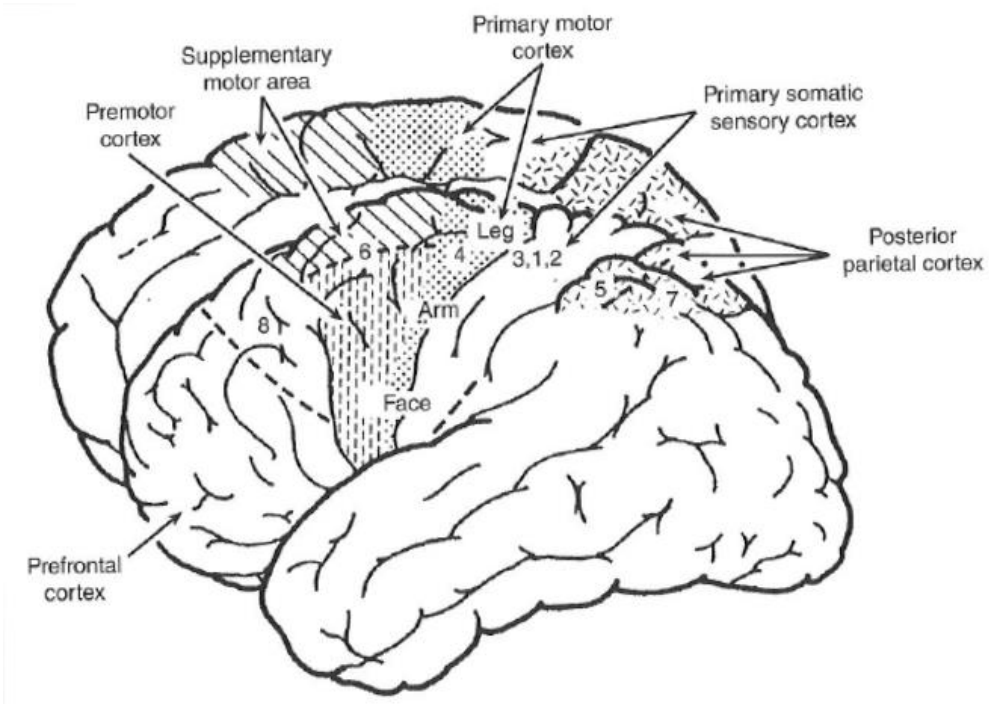

FIGURE 2. Cerebral cortex. Numbers refer to different Brodmann areas. (From Enoka, 2015, 305)

In humans, most axons of the CST cross the body's midline, decussate, at the junction between the brainstem and spinal cord, which serves an essential function in our movement. For example, bilateral connections of all the CST axons would result in the inability to produce asymmetric movements (Welniarz, Dusart & Roze 2016), which are common in humans and most other mammals. Some axons however do not cross the midline and thus project in an ipsilateral manner to the same side of the body. These axons innervate mainly proximal muscles such as those that control trunk muscles. These axons are known as the ventral corticospinal tract while the tract that decussates is known as the lateral CST. (Latash 1998 148.)

The motor cortex is often presented to have a somatotopic organization, which means that each body part is represented in the cortex by a specific area. This revelation was first observed by Wilder Graves Penfield (1891-1976) in the 50's. The representation of these body parts in the motor areas are visualized by a distorted human figure, the homunculus man. However, the notion that the motor cortex is divided into specific parts has been counterproven and the representation areas of the cortex are much more intermixed, mosaic in nature, than thought before. Specifically, studies have revealed that single neurons can pose control over different body parts and to a single muscle, there might be cortical neurons that are located in different brain areas. (Latash 2012, 193.)

In cat experiments, it has been well demonstrated that the corticospinal tract is primarily non-monosynaptic and thus controls movement via interneurons. Because these interneurons receive input from afferent neurons that convey sensory signals, cortical commands are modulated continuously by the environment and intrinsic movement. (Gracies et al 1994.) In humans, connection of the CST neurons onto spinal alpha motoneurons have been found to be monosynaptic in nature (De Noordhout et al 1999) and the most prominent monosynaptic connections are onto the muscles that control finger movements (Latash 1998, 125). However, non-monosynaptic modulation is also evident in human movement (Gracies et al 1994). The corticospinal tract is known to have pathways to the dorsal horn (Lemon 2008), from where it can control sensory information via spinal interneurons (De Noordhout et al 1999).

2.2 Spinal cord and afferent pathways

The spinal cord is an important machinery for movement that resides between the peripheral system and the higher brain areas. As such, it transmits descending information to motor

neurons and conversely receives signals from peripheral receptors that can be transmitted further upwards to higher brain areas. However, the functions of spinal cord are not just mediation of signals but also houses reflex machineries and further can influence our movements in complex manners. (Latash 2012, 172.)

The spinal structure can be divided into segments that withhold 8 cervical, 12 thoracic, 5 lumbar and 5 sacral bony vertebrae. The nerve fibres of spinal cord start from the medulla and travel down these segments as an elongated structure ending at the lumbar vertebrae. The inner parts of the spinal cord are consisted of the so-called grey matter that is organized in a butterfly-like structure. A depiction of these structures is visualised in figure 3 below. Grey matter consists of neuron bodies and some shorter neuron fibres. Grey matter is surrounded by white matter that consists of long neural fibres that transmit signals towards the higher brain areas as well as to lower segments of the spinal cords. (Latash 2012, 172-173.) White matter gets its light appearance from the lipid myelin sheath that it is enveloped in (Purves et al 2012, 14).

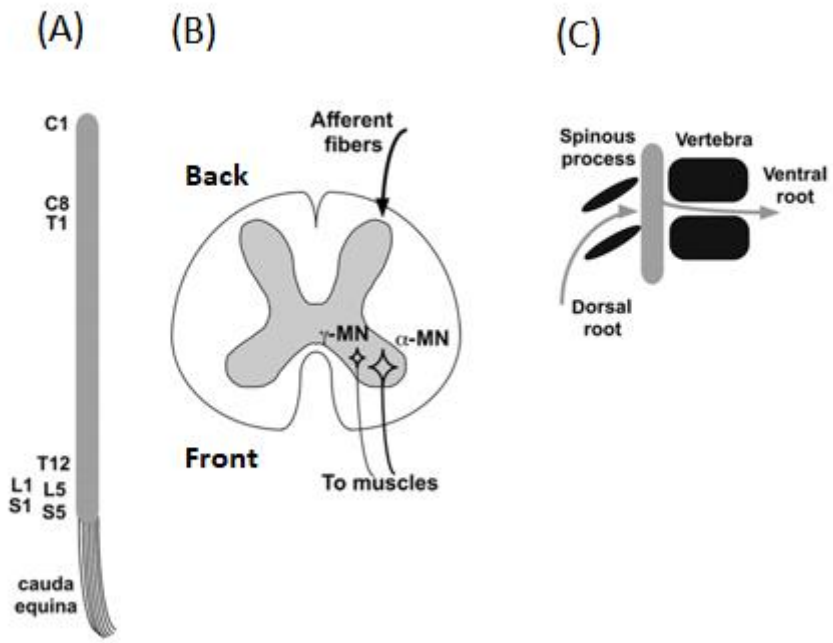

FIGURE 3. Illustration of the anatomical structure of the spinal cord. (A) Picture shows the elongated form of the spinal cord with the spinal segments. (B) The cross-sectional image of the spinal cord with the butterfly-like organization of the spinal grey matter. (C) As illustrated in (B), afferent fibres enter the cord through the dorsal horn in-between spinous processes and efferent fibres exit the ventral horn between vertebrae. (Modified from Latash 2012, 173-174)

As axons of the motor neurons, also known as efferent neurons, exit the ventral parts they travel further down to skeletal muscles and thus can control its force production. Sensory fibres, or afferent neurons, of the same area enter the spinal cord through the dorsal roots of the, more or less, same vertebral level. The bodies of the afferent neurons, unlike the efferent neuron bodies, reside in the spinal ganglia outside the spinal cord. Neuron that reside within the spinal cord are called interneurons (see figure 4). Their functions are of great importance for the reflex system as they transmit signals between neurons in the cord. However, only a small part of interneurons is described to this day in detail, which is why this part of the spinal functions is sometimes describes as a "black-box". (Latash 2012, 172-173.)

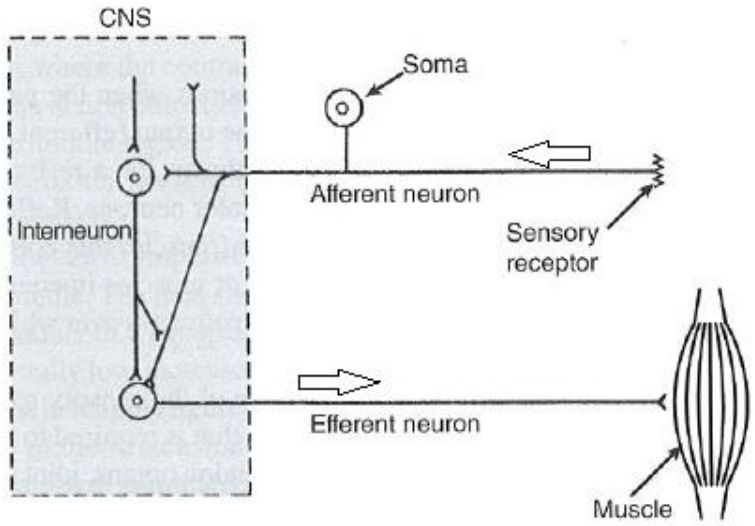

FIGURE 4. Interneurons reside in the spinal cord. Descending commands synapse with interneurons, which then modulated efferent motoneurons to skeletal muscles. Afferent information from the periphery travel towards the spinal cord and make connections with interneuron that can influence both ascending and descending signals. (Modified from Enoka 2015, 255.)

The neurons in the spinal cord are organized in a somatotopic manner. That is, neurons that innervate axial muscles such as those that control for our posture are located in the most medial parts. Further away from the midline lie the neurons of progressively more distal muscles in a way that the most lateral parts of the spinal cord hold the neurons of finger muscles and toes. This organization reflects the framework of how the movements of the body and different body parts are controlled. The more axial muscles receive commands from long projection systems of the medial and ventral white matter. Muscles that provide for more skilled behavious, such

as finger muscles, receive their commands from lateral aspect of white matter. Local spinal circuits are organized to control for these different neural tracts differently. (Purves et al 2012, 355-356.)

There are multiple different pathways that carry different sensory information to a variety of brain areas such as the spinocervical pathway, spinothalamic pathway, and the spinocerebellar tracts (Latash 1998, 147-149). These pathways have some common features. In particular, the relay nuclei, such as the thalamus, are structures that combine a variety of information from both the ascending and the descending pathways. (Latash 2012, 190.) That is, these ascending pathways enter the spinal cord through the dorsal roots of vertebrae and ascend usually to the thalamus or cerebellum. Sensory information is then processed and further distributed to other brain areas where a decision for appropriate action is ultimately formed. (Latash 1998, 147-149.)

The anterolateral system is a collective term used to describe multiple ascending pain pathways. The most important one is considered to be the spinothalamic tract. (Diaz & Morales 2016.) The spinothalamic tract projects information about pain but also of touch, pressure and temperature from the periphery to the cortex. The pathway travels from dorsal and intermediate grey matter of the dorsal horn up towards the thalamus on the contralateral side of the spinal cord. (Latash 1998, 147.) More specifically, at the same level of entry or a few segments above the pathway crosses over via the anterior white commissure and travels to the ventroposterior-lateral thalamic nuclei. Thus, patients with lesions or abnormalities of this tract exhibit pain and temperature deficits of the contralateral side. (Diaz & Morales 2016.)

The spinocerebellar tracts carry information of mainly unconscious proprioception (Diaz & Moralez 2016; Latash 1998, 147). That is, information deriving from muscle receptors such as muscle spindles and Golgi tendon organs ascend through these pathways. Importantly, this tract conveys sensory information mainly from the hindlimbs (Bosco & Poppele 2001; Latash 1998, 148) while information from the forelimbs ascend through the cuneocerebellar tract (Latash 1998,148). The spinocerebellar tracts are commonly divided into the ventral and dorsal tracts (Bosco & Poppele 2001). While the dorsal tract consists of mainly fast-conducting myelinated axons and receive information from muscle and joint receptors, such as muscle spindles and Golgi tendon organs, the ventral consists of smaller axons and receives input from mainly flexion reflex afferents (Latash 1998, 147-148).

2.3 Sensory receptors

Information from the environment and the sensations arising from the body itself are sensed with an array of different stimuli. This information is of essential importance as it is used to plan appropriate movement and control for unexpected events. Sensory receptors are cells or subcellular structures that can differentiate between these different modalities of stimuli and thus contribute to neural processes essential for normal movement. (Latash 2012, 35.) Sensory receptors also form an important piece of the spinal reflex system. That is, spinal reflexes are automatic responses that can respond to environmental signals fast bypassing the cortical voluntary circuits (Enoka 2015, 255).

The information deriving from sensory receptors if often unconscious as it is not actively perceived. This kind of information can derive from internal organs (interoceptive information). Information from other body parts such as the human eye can deliver information about the environment (exteroception) and those that give us a sense of our own position and the position of a body parts, with regards to others, are derived from yet another types of receptors that are called proprioceptors. (Latash 2012, 35-36.) As there are many different types of sensory stimuli and sensory receptors in our bodies, it poses a complex system.

In this section we only focus on two sensory receptors: the muscle spindle and the Golgi tendon organ. These receptors are well described and known in literature and their functions are of great importance in out movement. The reflex systems that are associated with these receptors are presented here. However, a more detailed description of their effects in human movement is referred in the section 5 named as “Natural modulation of spinal reflexes”. Thus, the reader is suggested to refer between these sections if needed.

2.3.1 Muscle spindle

Muscle spindles are receptors that reside within the muscles and provide information about their changes in length and velocity (Latash 1998, 36-38). They are an essential part of sensory reflexes such as the muscle stretch reflex and reciprocal inhibition. A familiar representative of the simple stretch reflex is the tendon tab, where for example the patellar tendon is tapped with a hammer. This tap stretched the quadriceps muscle and activates muscle spindles, which in

turn cause the muscle to contract thus producing a kick-like movement. (Purves et al 2012, 364.)

Muscle spindles consist of specialized muscle fibres, which are called intrafusal fibres Intrafusal fibres contain two types of cells, the nuclear bag fibres that are few in numbers and the more numerous nuclear chain fibres (figure 5). They differ with regards to their arrangement of nuclei, architecture, and their sensitivity to stretch. (Purves et al 2012, 362.) As the muscle spindle resides in skeletal muscles, fibres of that muscle are known as extrafusal fibres. Intrafusal fibres are organized in a parallel manner with the extrafusal fibres, which serves an important purpose. That is, intrafusal fibres in parallel can move similarly to extrafusal fibres and thus can monitor the different states of the muscle. (Latash 1998, 36-38.)

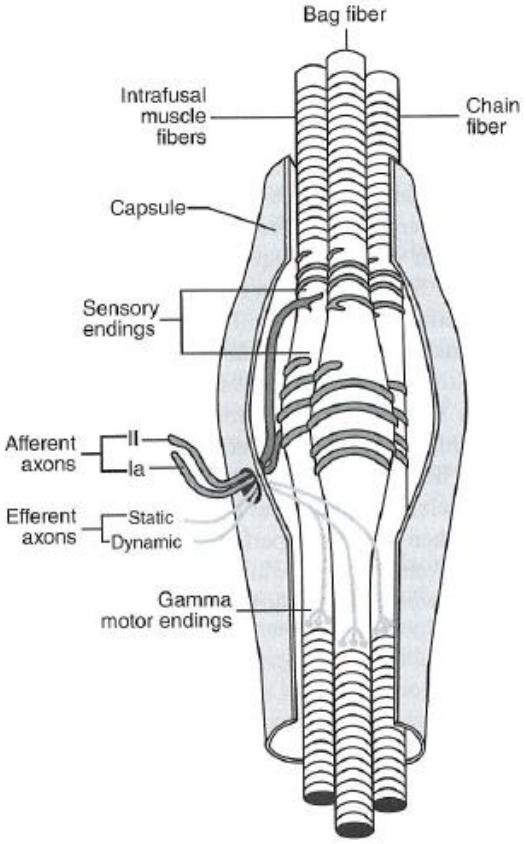

FIGURE 5. Muscle spindle. Intrafusal fibres are innervated by afferent and efferent nerve fibres.

Muscle spindles contain two different types of afferent neurons which are the group Ia known as the primary spindle endings and the group II called secondary endings. Primary spindle endings are fast conducting cells that are sensitive to both velocity and length while secondary

endings are slower and only sensitive to length. (Latash 1998, 36-38.) Axons of the Ia afferents innervate the intrafusal fibres at the central parts and are coiled around them. Group II afferents, however, innervate the fibres just outside this centre. (Purves et al 2012, 362.) Some spindles do not contain group II afferents at all, but all do include Ia fibres. However, both groups can transmit information through mono- or polysynaptic pathways to the skeletal muscles and contribute to the ascending white matter tracts. (Enoka 2015, 257-261.)

The Ia afferents impose their effects on movement through excitatory connections to other neurons. The Ia make monosynaptic connections onto motoneurons that derive from the same muscle. That is, firing of the Ia afferents can increase the force production of the muscle by excitatory monosynaptic connections as seen in figure 6. Additionally, they make monosynaptic connections to other muscles, which are known as heteronymous connections. The connections to the same muscle of origin are known as a homonymous connection. The Ia afferents also exhibit excitatory connections with interneurons. These interneurons play an important part in the mechanisms of spinal circuits as for example they provide an inhibitory effect on antagonist muscles in disynaptic manner. This inhibitory system is known as reciprocal inhibition (see figure 7 for the reciprocal pathway). (Enoka 2015, 257-261.)

In contrast with Ia, pathways of group II afferents are more diverse and mainly contain polysynaptic connections with interneurons. (Enoka 2015, 260-261). They have been known to control for muscle tone, which is the resting level of tension seen in muscles (Purves et al 2012, 364). Muscle tone is an important characteristic as it helps maintaining posture during activities such as walking. Additionally, muscle tone helps to store mechanical energy (Purves et al 2012, 397), absorb power, which is a common feature for the triceps surae muscle group during walking (Whittle 2007, 69-70).

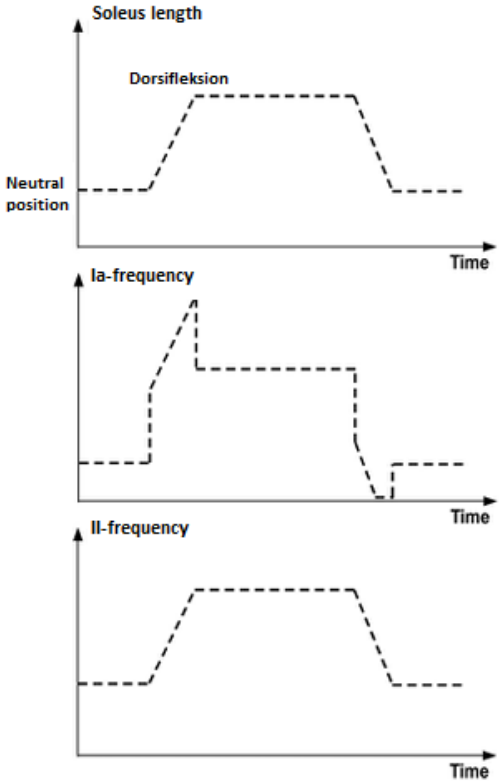

FIGURE 6. Spindle afferent discharge responses due to muscle length changes of the soleus muscle. As the ankle moves to dorsiflexion, soleus muscle length increases. Middle panel shows the activation frequency of Ia muscle spindle fibres to the length change and the lower one for the type II fibres. (Modified from Latash 2012, 38.)

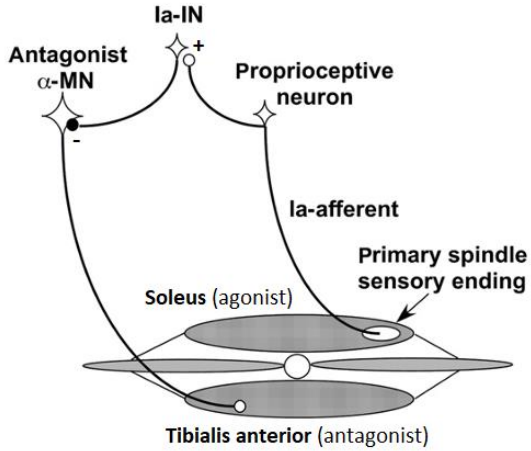

FIGURE 7. Reciprocal inhibition between antagonistic muscles soleus and tibialis anterior. Ia afferents from an agonist muscle make excitatory synaptic connections (excitatory synapse indicated by symbol +) with spinal interneurons (Ia-IN) that inhibits (-) the motor nerves innervating antagonist muscles. (Modified from Latash 2012, 44.)

An important functional aspect regarding the muscle spindles is the gamma system (see figure 8). Gamma neurons belong to the class of motoneurons but in terms of size and conduction speed they are smaller and slower. They innervate intrafusal fibres of the spindles and as such impose effects on their sensitivity. That is, their main effect is to increase spindle firing speed. (Latash 1998, 38-39.) Gamma neurons innervate intrafusal fibres at the spindle ends, which is where the contractile proteins are located. Thus, when gamma neurons activate, they act to contract the spindle ends, which in turn stretches the middle parts. This effect increases the firing speed of Ia afferents compared to the state of firing without its contribution to length changes or velocity. (Enoka 2015, 257.)

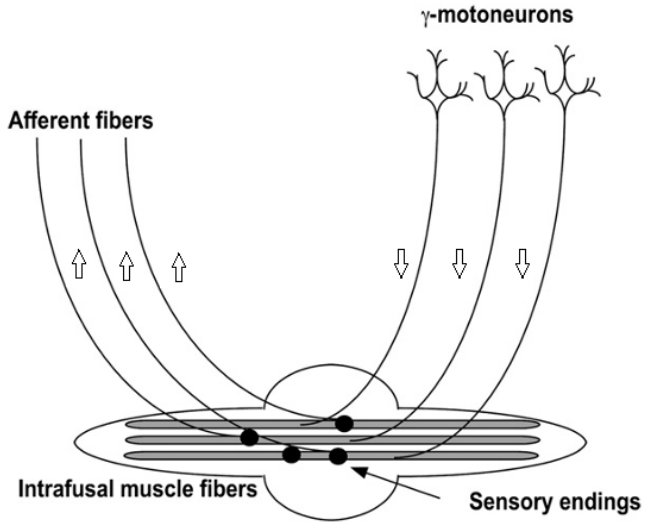

FIGURE 8 Muscle spindles are innervated by efferent gamma-motoneurons. Arrows up indicate the signal direction to be from the spindle towards spinal cord and arrows down from the spinal cord to muscle spindle. (Modified from Latash 2012 39.)

It has been noted that that activity of the gamma-motoneurons coincide with the activity of the alpha motoneurons depicting the term alpha-gamma coactivation. By this coactivation the spindle level of activity depends on the length and velocity of the muscle but also depends on the muscle activity in question. (Latash 1998, 195.) That is, the while the alpha motoneurons produce to forces needed for an action, the gamma system sets the desired feedback level. (Enoka 2015, 258) Indeed, it has been noted that the gain in gamma motoneuron activity is increased in certain types of movements. Movements that are difficult or demands high execution speed and precision increase gamma motoneuron activity and thus increase muscle spindle responsiveness. Same effect is also evident in unpredictable situations. Thus, the activity of the gamma neurons can be adjusted by descending control and by local spinal circuitry. (Purves et al 2012, 364-365.)

2.3.2 Golgi tendon organ

Golgi tendon organs (GTO) are located in human tendons and muscle-aponeurosis junctions (see figure 9). Unlike the activation of the muscle spindle, GTOs are activated by muscle deformation such as muscle contraction. (Pierrot-Deseilligny & Burke 2012, 215-217). They provide information about forces and is activated differently when the force experienced by the organ is active or passive. That is, GTOs react to active forces much more sensitively. (Enoka 2015, 261.) When a passive muscle is stretched the deformation happens mainly in the muscle tissue. However, when the muscle is actively contracted forces are also transmitted to the tendon and thus, induce a compression of the intertwined receptors engaging them. (Purves et al 2012, 365.)

The afferents that arise from GTOs are called Ib afferents (Pierrot-Deseilligny & Burke 2012, 215-217). Ib afferents pose inhibition on the homonymous and synergist muscles, contrary to the muscle spindles, and are effective through di- and trisynaptic pathways (see figure 10). They pose an inhibitory effect on the agonist muscle through one inhibitory interneuron and an excitatory effect on the antagonist muscle through two interneurons. (Enoka 2015, 262.) In this manner, the GTOs can protect the muscle against exceptionally large forces by inhibiting its activation. However, they are also effective with lower force levels as this system helps in maintaining steady muscle force levels. (Purves et al 2012, 365-366.)

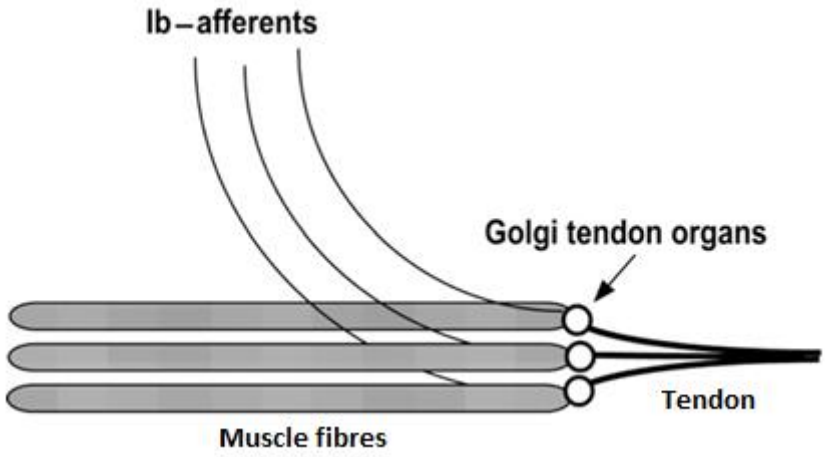

FIGURE 9. Golgi tendon organs lie at the junction between tendon and muscle. (Modified from Latash 2012, 39.)

Besides muscle force levels, Ib afferents receive monosynaptic excitation also from corticospinal and rubrospinal tracts and can be activated by connections from other afferents (Pierrot-Deseilligny & Burke 2012, 215-217). These afferents include those of cutaneous receptors, muscle spindles and join receptors. Join receptors for example produce signals about the state of hyperextension or -flexion of the joint and with its connection to GTOs, can inhibit muscle force production in these situations to protect against potentially harmful effects (Purves et al 2012, 366.) Therefore, Ib afferents are influenced by several sensory inputs but are also under descending control.

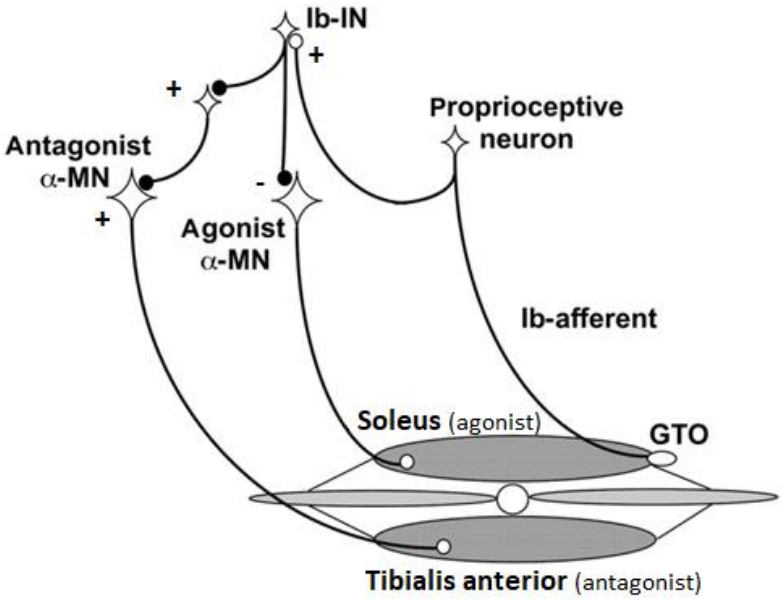

FIGURE 10. Ib inhibitory and excitatory pathway. Ib afferent arising from soleus GTOs form disynaptic inhibitory connections with agonist muscles and trisynaptic facilitatory connections to the antagonist. (From Latash 2012, 45.)

3 DESCRIPTION OF HUMAN LOCOMOTION

Human locomotion is composed of repeated cyclic body movements that move our bodies centre of mass in the direction of progression. Although it seems to be an easy task of everyday life, it requires the coupling of multiple individual neuromuscular systems to function smoothly. Through the combination of these systems, it is no surprise that humans have adopted multiple different ways of walking, all differing in some aspect. However, these idiosyncratic ways of walking have gross similarities that are easily identified and described as human. (Rose & Gamble 2006.)

Human locomotion can be viewed from multiple perspectives such as kinematics, kinetics and the neural systems that drive the movement. Although all three are important aspects to understand, when a full description of human locomotion is wanted, not all are reviewed here. This section presents a basic description of human gait focusing on the movements of the shin and foot with a description of the tibialis anterior and triceps surae muscle activity and the movement of the ground reaction force vector. Later, differences related to treadmill gait are introduced, due to its frequent application in scientific protocols, and compared to over ground walking.

3.1 The gait cycle

Movement of the body and joints happen in all planes of motion. However, mainly the sagittal plane is usually described as it is the plane where most of the movements occur. For example, the hip and knee joints produce flexion and extension movements during gait cycles and as such most of the movement occurs in the plane of progression, the sagittal plane. The ground reaction forces (GRF) and the force vector relative to the joint position produce moments about joints. During gait, GRF are produced in three dimensions: vertical, lateral and anteroposterior. The force vector combines two of these, the vertical and anteroposterior GRF. This vector is thus a projection of these two force components and as such provides a good description of moments about a joint as the lateral forces are usually very small (see figure 11). However, they are to be interpreted as approximations as they neglect segment masses and acceleration. (Whittle 2007, 58-81.)

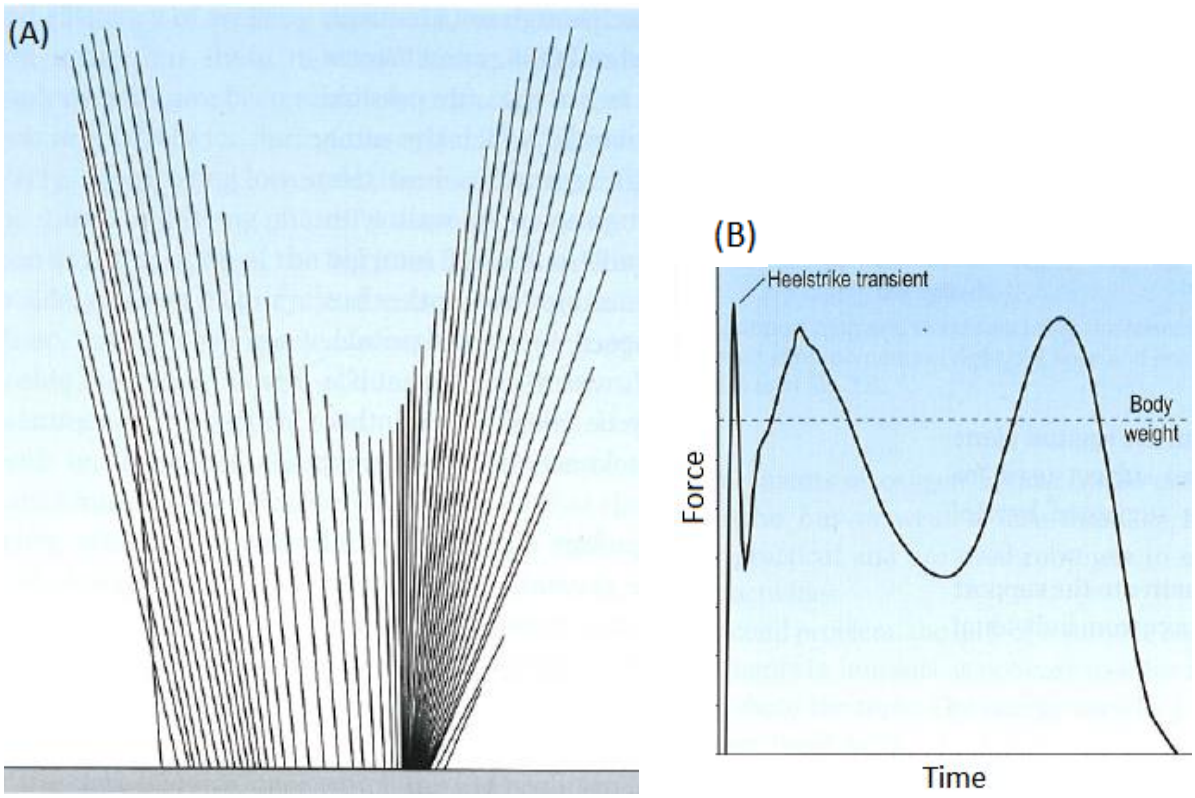

FIGURE 11. (A) Force vector during gait. (B) Vertical ground reaction forces. (Modified from Whittle 2007, 61, 83)

The basic gait cycle can be described to include two phases, the stance phase when the leg is restricted by the ground and the swing phase when the leg is moved forward. Furthermore, the cycle can be separated in four distinctive events that describe the transitioning phases: 1) heel strike 2) contralateral leg toe-off 3) contralateral heel strike 4) toe off. In terms of numeral description, it is more common to transform the cycle into percentage values of the full cycle rather than depicting its temporal values. Thus, the heel strike can be labelled as 0 % and the last toe off as 100 %. (Rose & Gamble, 2005.)

Heel contact marks the beginning of loading response, which is the early part of stance phase. Timing of this phase is usually at around 0-12 % of the cycle. At heel contact, tibia is in a backwards tilted position with the ankle usually at its neutral position between dorsi- and plantarflexion. Thus, the toes point slightly upwards due to the position of the tibia, like the foot, which is also usually slightly supinated so that the lateral aspects of the heel are first on the ground. The forces exerted on the ground during contact produces the first vertical peak of GRF (see figure 11B “heelstrike transient”) and a braking force in the posterior direction. The vector resides posterior to the ankle joint, which is met by an activation of ankle dorsiflexors.

That is, to hold the forefoot from dropping to the ground uncontrolled, tibialis anterior is activated and holds activity during early stance. (Whittle 2007, 64-81.)

After heel contact, the initial dorsiflexion of the foot is decreased and changed to a net plantarflexion movement, which remains under the control of TA muscles eccentric contraction. Additionally, the initial supination is decreased with an internal rotation of the tibia. Thereafter, the foot progresses towards a foot flat position while the contralateral foot is lifted from the ground. Foot flat position can occur at about 8 % of the entire gait cycle while the contralateral toe off happens just before at 7 %. The vertical GRF is peaked just after contralateral toe off from where it starts to decline as the acceleration of the body changes. Furthermore, the braking component starts to decline. (Whittle 2007, 66-81.)

Mid stance is described as the phase between contralateral toe off to ipsilateral heel rise. During this phase, the tibia is rotated forward about the stationary ankle joint as the ankle angle changes from a position of plantarflexion to dorsiflexion. Until now the ground reaction force vector has remained in a slightly backwards tilted position with the largest forces under the heel. The vector position in the mid stance moves progressively forward along the foot. This movement changes the initial TA activation to an activity of the triceps surea as it starts to contract eccentrically at first and absorbs power. Furthermore, the tibia externally rotates with a concurrent supination of the foot. (Whittle 2007, 69-70.)

Heel rise marks the end of mid-stance. The time of heel rise can vary between subjects markedly and its timing is further modulated by walking speed. The peak ankle joint dorsiflexion angle is reached usually just after heel rise. When the ipsilateral knee starts to flex, the ankle joint angle remains for a time and then starts to plantarflex at late terminal stance. The tibia further rotates externally, and the foot supinates. The force vector moves in front of the tibia with a trend of tilting further forward now starting to produce a propulsion force in the line of progression. The muscle activity at the triceps surae is initially eccentric with power absorption. Further towards the contralateral heel contact, the ankle plantarflexion moment increases with a change to a concentric muscle activation. The vertical GRF starts to rise after heel rise and produces the second peak in the vertical component. (Whittle 2007, 71-81.)

Toe off happens usually at or around 60 % of gait cycle. The ankle joint reaches its peak plantarflexion angle with a concurrent decrease in muscle force production. This is

accompanied by an increase in TA activation, which brings the ankle joint to a neutral position during the swing phase. As the foot is lifted from the ground, reaction forces decline, and fall to zero with a continuation, of course, of the contralateral foot. (Whittle 2007, 75-76.) An example of the changes in knee and ankle joint movements is presented in figure 12, with concurrent EMG activity patters.

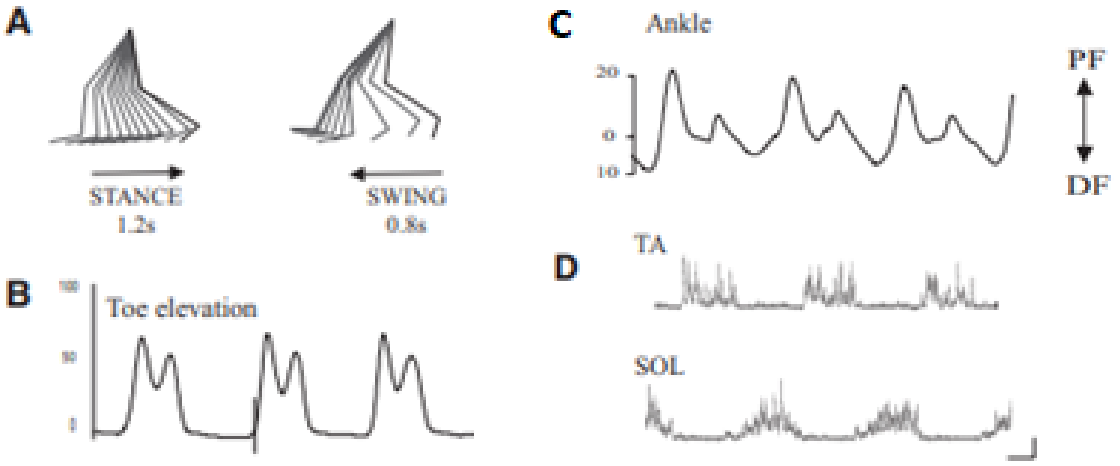

FIGURE 12. Picture A shows the pattern of movement in the lower legs at different time points at stance and swing phase while B depicts the movement of the toes from the ground and C the angular changes of the ankle joint (PF = plantarflexion, DF = dorsiflexion) in the anterior-posterior plane. D shows the relative muscle activity patterns associated with the stance and swing phase. (Modified from Barthélemy et al 2010.)

In addition to leg motions that are the basis of forward progression, specific arm, head and torso movements are distinctive to human locomotion. The question of why arm swing is so tightly incorporated to normal gait has developed many different hypotheses. These include such as stability optimization, lowering energy consumption and remnants of evolution from quadrupedal locomotion (Meyns et al 2013.) Whatever the cause, it is known that the arm movements are not merely a passive product of movement from the torso, pelvis and lower limbs but needs also active control. Indeed, activity in the arm muscles has been reported to control for the direction of arm swing and to control its amplitude. (Goudriaan et al 2014.)

3.2 Treadmill walking

Treadmills are frequently used in research and testing due to its advantages in controlling the speed and place of the subject. However, there seems to be subtle differences in the way humans walk on treadmills. Differences are unlikely to stem from different air flow compared to treadmill gait but instead are contributed to the subtle belt speed changes and perhaps the subject's awareness of the reduced dimensions of the treadmill. (Whittle 2007, 133.) Despite many studies that have analysed these differences, there still remains contradictory results and disagreements in the field (Lee & Hidler 2008).

Lee and Hidler (2008) showed that even with a similar temporal pattern of walking, there are differences in how the pattern is produced. More specifically, they found no differences in vertical and lateral reaction forces but a smaller braking reaction force at heel contact during treadmill walking. A similar trend of the vertical forces was seen in Riley et al (2007). This effect was most likely due to a slight slowing down of the treadmill belt at initial contact (Lee & Hidler 2008; Riley et al 2007). Furthermore, the pathway of the centre of pressure (COP) movement immediately after heel contact was also seen to be different in the recent study by Lu et al (2017) and was suggested to be the result of the moving belt.

The slight slowing down of the treadmill belt can also cause a reduction of the ankle dorsiflexor moment, which is accompanied by a reduced EMG activity of the tibialis anterior muscle compared to over ground walking. Muscle activity was also noted to be lower in the gastrocnemius during stance phase but larger in terminal swing. Furthermore, the activity in hamstrings, vastus medialis and adductor longus was noted to be lower during the swing phase on the treadmill but increased during terminal swing. (Lee & Hidler, 2008.)

Lu and colleagues (2017) reported no changes in the parameters of cadence, step length and width when the speed was matched for over ground and treadmill tests. These results however are partly contrary to Alton et al (1998) who reported a decrease in cadence while the stride length was constant. Furthermore, they found an increase in stance time during treadmill walking accompanied with larger hip range of motion. That is, subjects used a larger hip flexion angle with less extension. The differences in results might stem from methodological differences.

4 CORTICOSPINAL EXCITABILITY - ASPECTS OF MEASUREMENT

Electrical stimulation of brain structures can be a useful tool to discover their contributions to movement. However, this has not gained particular success as the electrical stimulations produce rather inconvenient side effects, such as pain (Latash 2012, 309), which may eliminate many possible subjects. Thereafter, a method of transcranial magnetic stimulation (TMS) has been introduces with the advantage of allowing the stimulation to reach brain tissue with less pain and discomfort (Latash 2012, 309). Electrical stimulation however is still used in the examination of spinal excitability. The first technique to investigate the spinal pathways and their contributions to human movement was in the context of the simple monosynaptic reflex. However, this reflex was later shown to have many confounding factors and thus its description and attached methodology seems not that simple after all. (Pierrot-Deseilligny & Burke 2012, 1.)

This section introduces the methods of transcranial magnetic stimulation and the Hoffman reflex with discussion of methodological considerations. These methods are well reviewed and used in the literature for the assessment of corticospinal and spinal excitability. Although the basic principles of the methods do seem rather simple, the physiology of what tissues and how they stimulate is complex. Thus, this section provides information about these concepts in order to understand results gained in literature.

4.1 Transcranial magnetic stimulation

Transcranial magnetic stimulation can be used in multiple different ways in the field of research and that of clinical settings. Those include testing parameters that aim to reveal changes in corticospinal excitability and those that aim to modulate excitability in itself. The differences in these cases can be represented by the amount of stimulation, their respective temporal parameters and intensity. In most cases, however, the basic procedure is the same. That is, the method aims at producing electromagnetic pulses to brain areas which depolarize neurons when a sufficient intensity is used. (Rossi et al 2009.) If the simulation is administered to the motor cortex, a muscle contraction can be seen in the EMG response of the affected muscle, which is known as a motor evoked potential (MEP). Eliciting MEPs at a threshold of a resting muscle thus reflects excitability of the corticospinal pathway. (Hallett 2000.)

The electromagnetic pulse is delivered by a coil that is held over the appropriate brain area. A simple coil consists of copper wire in loops that is connected to a large electrical capacitance. The capacitance has the ability to deliver large currents in less than 1 ms that is translated as an electromagnetic pulse at the coil. This current is further delivered to brain tissue with little attenuation by the skull and other overlying tissues (see figure 13). The induced current activates preferably neuron axons but is also dependent of its orientation and membrane properties. Axons that are most likely stimulated lie parallel to the current direction. (Rossini et al 2015.) Neurons are also inclined to activate when its axon bends out of the current circle, which produces a large change in electric field potentials (Rothwell 1997).

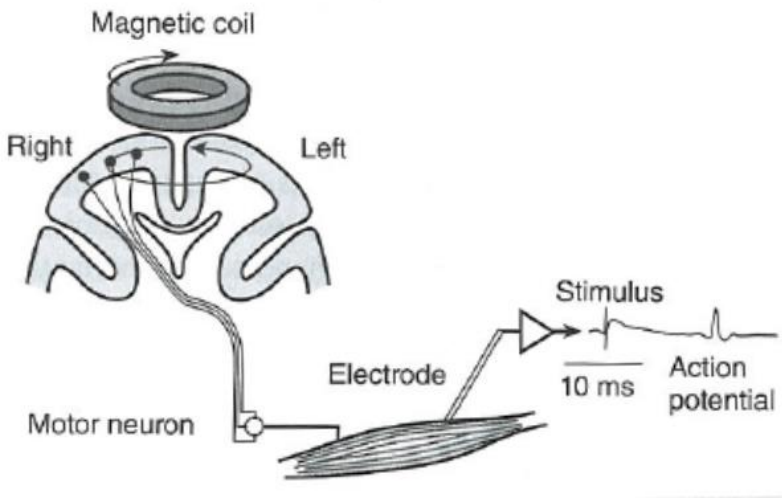

FIGURE 13. Transcranial magnetic pulses activate cortical pyramidal cells. The response of the stimulation in a muscle, a motor evoked potentials (MEP), is seen as a visible muscle contraction or an EMG response. (From Enoka 2015, 309.)

There are two main coil types that have been used: a single round coil and a double coil shaped as a figure-eight. The most significant difference in these two types is the focality and depth of stimulation. The round coil produces a current flow underneath in a diameter of about 8-12 cm. (Rothwell 1997.) However, the current intensity drops fast with distance and thus a circular coil does not stimulate deeper dwelling neurons (Rossini et al 2015). The figure-eight coil produces a more focal stimulation under the coil junction centre that is also twice in strength, which effectively increases the depth of stimulation (Rothwell 1997).

The motor threshold (MT) is the intensity that produces reliable EMG response in the target muscle, i.e. a motor evoked potential, at the lowest intensity (Rossini 2015). Thus, it represents

the stimulus intensity that is needed to activate the neurons that are most excitable (Devanne et al 1997). Furthermore, resting motor threshold (rMT) corresponds to a threshold value when the muscle is at rest and active motor threshold (aMT) when active. In active conditions, the stimulation usually is administered when the target muscle is about 20 % of maximal muscle strength (Rossini 2015) but has been also used during free standing and treadmill walking (Capaday et al 1999). As the background EMG levels contribute to corticospinal excitability it is important to keep the EMG levels at constant. Furthermore, care must be taken of other technical and environmental aspects such as the constancy of coil positioning, environmental noise, and the arousal level of the participant. (Rossini 2015.)

The relationship of the input-output (IO) properties, the recruitment curve, of TMS responses is a relevant parameter for the assessment of corticospinal excitability. It is formed from the MEP responses when a brain area is stimulated with increasing intensity until saturation of the responses. The shape of the curve is sigmoidal. It is known to not depend on the properties of single motoneurons but are the result of summation of multiple motor unit action potentials with contributions of other components of the corticospinal volley. That is, increasing the strength of stimulation recruits new neurons with increasing motor unit potentials. Furthermore, influencing factors also arise from other properties of the neural circuitry, such as motor unit synchronization, in combination determine the shape and steepness of the curve. Importantly, the maximal values gained from the curve, the plateau values, do not necessarily represent the maximal response of the corticospinal volley but instead represent the balance of inhibitory and excitatory components. (Devanne et al 1997.)

Stimulation of the same brain area with matched coil position results in the activation of the same motoneurons that lie beneath the coil with the principle of orderly recruitment. However, the responses may differ when the circuitries are modulated. That is, the curve might show a steeper rise, which contributes to an increase in gain. This effect is evident when the responsive muscle is activated and thus the neurons are more easily activated by the same TMS pulse. However, it can also represent a more synchronous motor unit discharge. (Devanne et al 1997.)

The magnetic pulse, however, can spill onto other muscles besides that, which was targeted due to the lack of specific focality (Kesar et al 2018b) and due to the mosaic nature of the motor cortex in humans (Latash 2012, 193). Stimulating the representation area of tibialis anterior for example, likely activates SOL, and due to spatial proximity, the rectus femoris and biceps

femoris as well. This issue is further raised when it comes to stimulation during active conditions. That is, the effects of activation of the antagonist muscles, synergist muscles or even the activity of the contralateral limb muscles might contribute to MEP amplitudes in other muscles. The consequences of these factors are not known to full extent and thus, need further investigating. (Kesar et al 2018b.)

4.2 The Hoffman reflex

Monosynaptic reflexes were the first to be studied in the attempt to investigate spinal pathways and thereafter have been under extensive research (Dietz 2010). In short, Hoffmann reflexes (H-reflex) asses the efficacy of synaptic transmission from Ia afferent fibres to motoneurons by percutaneous electric stimulation (Capaday 1997) and can be elicited both during dynamic movement and resting conditions. Therefore, it is a prominent method to investigate spinal pathways and motor control in natural movements without invasive methods as are used in reduced animal studies. (Pierrot-Deseilligny & Mazevet 2000.)

Ia fibres are the only ones to synapse directly to the motoneurons of the same muscle with a short latency of about 40 ms. (Capaday & Stein 1986). Being bigger in diameter, they have a lower threshold for electrical stimulation and therefore can be depolarized with a lower intensity than alpha motoneurons (Simonsen & Dyhre-Poulsen 1999) particularly for stimuli of relatively long duration (Pierrot-Deseilligny & Burke 2012, 3). At rest, H-reflexes can be elicited in the soleus muscle, quadriceps, and flexor carpi radialis muscles. However, during activation of the test muscle, reflexes can be recorded in virtually all muscles of the limbs with a peripheral nerve accessible to percutaneous stimulation due to the potentiation of the reflex. (Pierrot-Deseilligny & Mazevet 2000.)

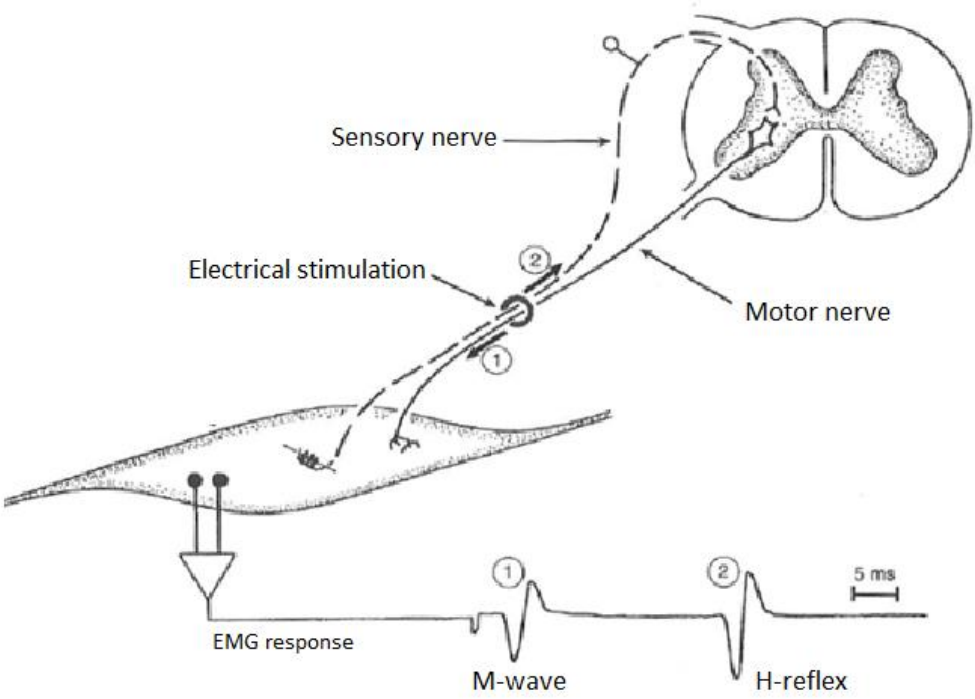

FIGURE 14. Sketch of the H-reflex stimulation pathway. Ia afferents from muscle spindle primary endings (dashed line) have monosynaptic projections to alpha motoneurons innervating the muscle of origin. The H-reflex is produced by electrical stimulation of Ia afferents and as such bypasses muscle spindles. (Modified from Enoka 2015, 265.)

The method of progressively increasing the stimulus intensity produces the H-reflex/M-wave recruitment curve. When stimulation intensity (mA) is low, only the H-reflex can be visualised in the EMG signal. Increasing stimulus intensity will increase the H-reflex amplitude by progressively recruiting new motor units by the afferent impulse. It has been noted that the order of motoneuron activation by the Ia input is from the smallest to the largest as according to the size principle. Eventually the stimulation intensity will also reach the threshold of motoneurons directly under the simulation site, which forms a separate event, an M-wave, as seen in figure 14. In the EMG signal, M-wave responses are produced before the H-reflex because of its shorter latency. Further increase in the intensity will start to suppress the H-wave (and increase the M-waves) due to the antidromic volley of action potentials from the motoneuron towards the spinal cord, which eliminates some of the potentials rising from Ia afferents. When stimulus intensity is high enough, the M-waves reach their maximum responses and the curve plateaus while the H-reflex is suppressed completely. (Pierrot-Deseilligny & Burke 2012, 4-7.)

The H-reflex and M-wave recruitment curves, IO-curves, thus represent the recruitment of additional motor units by the increasing peripheral stimulus (Pierrot-Deseilligny & Burke 2012, 4-7). The curves are often presented as a function of stimulus intensity and is used mainly if a current monitor is available. However, if a current monitor is not available, the H-reflex amplitude can be plotted relative to the concurrent size of the M-wave (see figure 15). There are many determinants that can be investigated from the ascending limb of the H-reflex. These include factors such as the maximal H-reflex (Hmax), H-reflex threshold and the slope of the ascending curve. Modulation can happen differentially in all three aspects. (Klimstra & Zehr 2008).

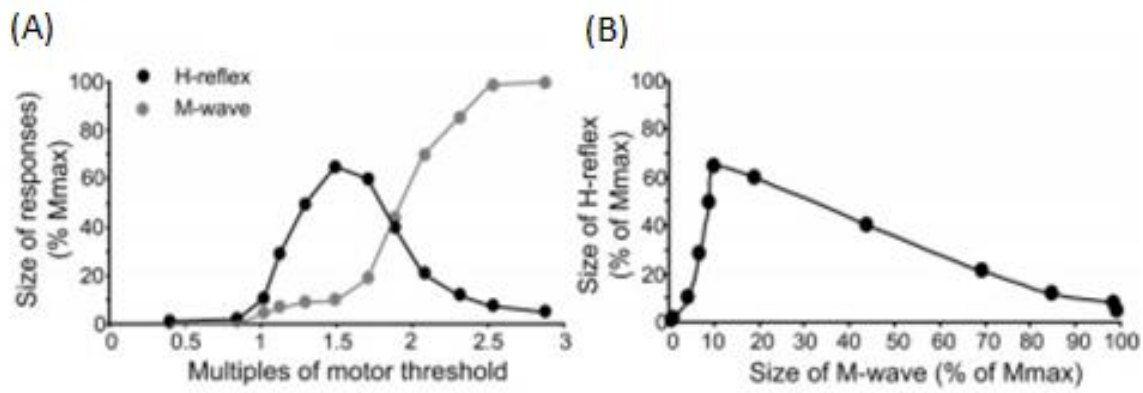

FIGURE 15. Recruitment curve of the H-reflex and M-wave plotted as a function of the current intensity (A) and as a function of the concurrent M-wave (B) (Modified from Knikou 2008).

Maximal M-wave (Mmax) is an estimate response of the proportion of motor neuron pool tested by this method. However, as muscles have synergistic muscles that can contribute to the force productions of others and are also stimulated by the supramaximal stimulation, Mmax value can be considered as an overestimate. Nonetheless, reflex responses should always be normalized to the Mmax value to overcome the differences in muscle architecture and the responsiveness of the motor neuron pool for example due to muscle contraction. (Klimstra & Zehr 2008; Pierrot-Deseilligny & Mazevet 2000).

When studying the modulation of the H-reflex due to an intervention there are important methodological aspects to understand. First, it is to be noted that the largest motor neurons reach their threshold first due to peripheral stimulation but at the same time are the ones last stimulated by the Ia afferents (Knikou 2008; Pierrot-Deseilligny & Mazevet 2000). Therefore,

if stimulating the nerve with a larger intensity corresponding to the descending curve of the H-reflex the response in the EMG is produced mainly by small diameter motoneurons due to the antidromic volley that cancels out most others (Pierrot-Deseilligny & Mazevet 2000).

Secondly, electrical stimulation does not only stimulate the Ia afferents and motoneurons. For example, Ib afferents rising from Golgi tendon organs have the same approximate size and are stimulated equally efficiently. Consequently, afferents with disynaptic or oligosynaptic connections to the motoneuron pool, and fast conduction velocity, may affect the reflex amplitude. Again, this effect can be seen mainly in the descending part of the curve. (Burke et al 1983.) Thus, the stimulation of the H-reflex, when regarding studies investigating reflex modulation, should take place on the ascending portion due to its sensitivity to changes in inhibition and facilitation (Knikou 2008; Pierrot-Deseilligny & Mazevet 2000).

Furthermore, there are other methodological aspects that should always be regarded and controlled in H-reflex studies. These include the stimulus duration and shape, electrode locations and stimulus frequency. Duration of the stimulus can affect the relative recruitment of sensory and motor fibres and therefore affect the results. In subjects without neurological problems, the usual duration has been a 1 ms square pulse elicited with a constant current stimulator (Simonsen & Dyhre-Poulsen 1999; Simonsen & Dyhre-Poulsen 2011). However, there are a few studies that have used a different duration. Lavoie et al (1997) used a 0,5 ms stimulus for stimulating the posterior tibial nerve. This duration may have been used for its less unpleasant sensation but still producing an adequate separation between afferent fibre and motoneuron recruitment (Capaday 1997). Despite some differences, the most generally recommended duration for healthy subjects seems to be 1 ms according to Pierrot-Deseilligny and Burke (2012). With this duration, an increased range of controlled measurements can be made (Capaday 1997).

Stimulation frequency can be considered a compromise between the time constraints of the data collection sessions due to a depression of the subsequent reflex amplitude with short interstimulus rates. This phenomenon is known as post-activation depression. (Knikou 2008.) Post-activation of the H-reflex is increased when the rate is above 0.1 Hz and it takes 10 s for complete attenuation of the previous stimulus. (Pierrot-Deseilligny & Burke 2012, 6-7.) However, the attenuation of the reflex responses can occur even without its effects. That is, during long measurements, amplitudes of maximal M-waves are reported to decrease with a

similar decrease in H-reflex amplitude (Crone et al 1999.) indicating a need to control for the Mmax amplitude during long measurement (Pierrot-Deseilligny & Burke 2012, 8).

Placement of the recording electrodes should always be kept constant to counteract issues of differential spatial distribution of responses. However, even if the electrode placement over the skin is kept constant, the muscle architecture and position can change underneath the skin. This is evident especially during dynamic movements such as gait. (Pierrot-Deseilligny & Burke 2012.) Indeed, knee flexion moves the electrode away from the nerve and in extension the electrode moves back towards it. Therefore, the intensity of the stimulus must be adjusted in each phase of gait. That is, the stimulus intensity is fine-tuned to produce a constant M-wave response, which is calculated as a percentage of Mmax. (Simonsen & Dyhre-Poulsen 2011.) Thus, only those H-reflexes that coincide with the M-wave of appropriate size are accepted to ensure test reproducibility. (Pierrot-Deseilligny & Burke 2012, 8).

5 NATURAL MODULATION OF SPINAL EXCITABILITY

The discussion so far has been focusing on the description of neural pathways, human gait and methods to study corticospinal and spinal mechanisms. We move then further to a more integrative motor system where even more inhibitory and excitatory forces area at work. In our everyday life we move our bodies by means of different descending outputs and by effect of afferent input from our bodies and environments. All this activity produces changes in the excitability levels of our spinal motoneurons, which can be describes as the final common path. Thus, excitability of this path is the final determinant that modulates to what extent descending information is transmitted as movement. (Pierrot-Deseilligny & Burke 2012, 1.)

During normal movements, the degree of inhibition is modulated in a behaviourally dependent manner (Seki et al 2003). The knowledge of what modulates the excitability of motoneurons is of importance in understanding the mechanisms of normal movement and therefore also the mechanisms of pathological conditions. It is known that neurological disorders such as a spinal cord injury can interfere with the normal processing of spinal reflexes. For example, patients with spinal cord injuries (SCI) have upregulated responses compared to healthy counterparts in different body positions ranging from semi reclined to standing and walking. (Phadke et al 2010.)

5.1 Inhibitory modulators

The role of excitatory and inhibitory feedback during movement are highly complementary. Excitatory forces assist in force production in propulsion and posture. On the other hand, inhibitory forces promote joint stability especially during movements with high velocities and accelerations. (Nichols 2018.) As the body receives a multitude of afferent inputs from different organs, information processing of these inputs could be overwhelming. Thus, the amount of input must be reduced, or some of it totally neglected in the central nervous system for us to be able to concentrate on the most relevant ones. (Rudomin & Schmidt 1999.) For example, by means of sensory inhibition, humans can adjust the excitability of afferent inputs to aid in movement generation and reduce unwanted interferences from the periphery. (Seki et al 2003).

From a cellular point of view, a neuron can receive potentials from multiple sources that modulate the postsynaptic cell excitability. That is, presynaptic cells aim to depolarize or hyperpolarize the postsynaptic cell and so induce either an excitatory or inhibitory postsynaptic potential. A presynaptic cell can connect to a postsynaptic cell anywhere along its length for example the dendrites, cell body or axon. However, the most potential modulation usually connects near the axon hillock, as the amplitude of the action potential will decay over distance. (Enoka 2015, 189.) Mediators of these inhibitory potentials include presynaptic inhibition and neural circuits known as reciprocal and recurrent inhibition.

5.1.1 Presynaptic inhibition

Presynaptic inhibition depicts an event that produces an inhibitory postsynaptic potential on the presynaptic cell. Figure 16 provides a more visual description of this phenomenon. The figure displays a synapse between a sensory neuron (presynaptic cell) and a motoneuron (postsynaptic cell) with an interneuron that synapses near the axon hillock of the presynaptic cell. This interneuron is an inhibitory interneuron. Action potential from the interneuron, therefore, can induce an inhibitory postsynaptic potential on the presynaptic cell, the sensory neuron, hyperpolarizing it and decreasing its excitability and probability to transmit an action potential on the motoneuron. Conversely, presynaptic facilitation depicts an event when an action potential from the interneuron causes an increase in neurotransmitter release and prolong the postsynaptic potential. (Enoka 2015, 191-192.) Presynaptic inhibition takes place early in the sensory pathways for reasons yet unknown (Kandel et al 2013).

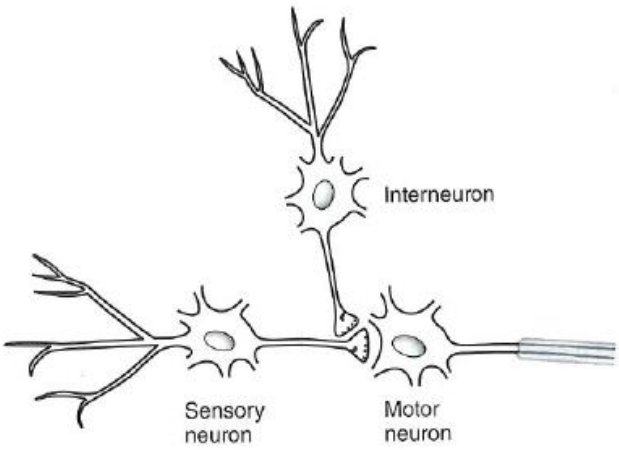

FIGURE 16. Presynaptic inhibition. (From Enoka 2015, 192)

There are two main sources of presynaptic inhibition in humans. That of cortical origin and that derived from peripheral afferent fibres (Seki et al 2003). In the case of cortical input, studies have revealed a selective decrease of presynaptic inhibition on Ia afferents involved in muscle contraction (Meunier 1999; Rudomin & Schmidt 1999) and an increase in inhibition on muscles not involved (Meunier 1999). This pathway involves, amongst others, the corticospinal pathway (Rudomin & Schmidt 1999). As Ia afferents arise from muscle spindles, a decrease of presynaptic inhibition in this context would produce an increase in the responsiveness of the muscle to changes in velocity and stretch (Latash 1998, 36-38). However, it has been speculated, that although effects of the stretch reflex are vital for the lower limb muscles, and thus a decrease of presynaptic inhibition would be of functional benefit, it is not the case regarding wrist movements (Meunier & Pierrot-Deseilligny 1998). Indeed, during voluntary wrist movements cutaneous afferents are affected by an increase in presynaptic inhibition that precedes muscle activity (Seki et al 2003).

Presynaptic inhibition arising from peripheral afferents can adjust motoneuron excitability. For example, mechanical stimulation by an activation of peripheral cutaneous afferents reduces presynaptic inhibition allowing for more afferents to take effects. For example, when countering an unexpected obstacle with your foot sole, the reduction of presynaptic inhibition would allow your muscles to be more responsive. (Rudomin & Schmidt 1999.) Peripheral afferents belonging to groups Ia and Ib from flexor muscles can produce Ia presynaptic inhibition onto practically all ipsilateral muscles. However, those originating from extensors affect mainly other extensors. (Enoka 2015, 273.) Furthermore, afferents arising from skin, skeletal muscle and joints also have the ability to modulate excitability of other afferents. This modulation seems to be strong especially by large diameter primary afferents such as group Ib arising from Golgi tendon organs and those of group II. (Rudomin & Schmidt 1999.) Their effects, however, are restricted mainly to the same segment of origin and thus only affect in a highly localized manner (Riddell et al 1995).

In humans, the evidence of presynaptic inhibition must always be assessed with care as direct evidence for cannot be attained (Meunier & Pierrot-Desilligny 1998) due to the need of reduced preparations. Thus, more direct evidence from the functional benefits of presynaptic inhibition comes from animal studies that have utilized lesions or other invasive methods to interfere of remove presynaptic inhibition. For example, in a study conducted in mice, Fink et al (2014) used genetic ablation of interneurons that elicit the characteristics of presynaptic inhibition at

sensory synapses. Removing and interfering with these interneurons increased the gain of proprioceptive feedback. Their findings provide evidence of the importance of presynaptic inhibition in ensuring smooth and controlled movements as the mice showed forelimb oscillations that were evident only during movement and present even in the presence of intact postsynaptic circuits. Evidence in humans, despite being mainly derivative, indicate similar effects. For example, the functionality of a decrease in Ia presynaptic inhibition, whether cortical of peripheral origins, is to increase the excitability of the monosynaptic pathway making it more responsive at the onset (Meunier 1999) and during movement (Enoka 2015, 273) while increasing motor selectivity (Meunier 1999).

5.1.2 Reciprocal inhibition

During voluntary movements, modulation of the reciprocal Ia inhibition has been reported to ensure appropriate activation of antagonistic muscles (Pierrot-Deseilligny & Burke 2012, 528). That is, excitation of the Ia inhibitory interneuron from for example the soleus muscle spindle inhibits the activation of tibialis anterior (Enoka 2015, 257-261). However, in addition to this peripherally induced modulation, cortical input can also modulate Ia excitability, which can exhibit a similar function of reciprocal inhibition. Therefore, the term of reciprocal inhibition in itself does not distinguish the mechanism behind it but is more a representative of the functional aspects of the term. (Lavoie et al 1997).

The effects of modulation of reciprocal inhibition deriving from different origins can be demonstrated with a look at the inhibition profile of H-reflex. Descending presynaptic inhibition on the Ia inhibitory interneuron is suggested to play an important part before the onset of EMG activity of the antagonist muscle. Indeed, the H-reflex has been noted to be depresses 50 ms before EMG activity. However, during ongoing activation, peripheral input maintains the activity of the interneuron and therefore the depression the H-reflex is further continued. (Enoka 2015, 268-270).

Reciprocal inhibition is suggested to be more prominent during dynamic movement than during tonic voluntary activity such as maintaining posture. However, it seems that this inhibition is also modulated in a task dependent manner, which is likely cortical of origin, i.e. the modulation is presented in anticipation of movement. (Lavoie et al 1997). However, inhibition between antagonistic muscles may not be symmetrical. The amount of inhibition from SOL and GM to

TA muscle has been reported to be four-fold in magnitude compared to the inhibition from TA to triceps surae muscles. Although the exact reasons for these differences are not known, it may be dependent on the input from muscle spindles but can be also influenced by Renshaw cell activation. (Yavuz et al 2018.) Indeed, Renshaw cells have been suggested to influence inhibition strength (Lavoie et al 1997). An illustration of the pathways of control that have been proposed to account for reciprocal inhibition on soleus alpha motoneurons is presented in figure 17 below.

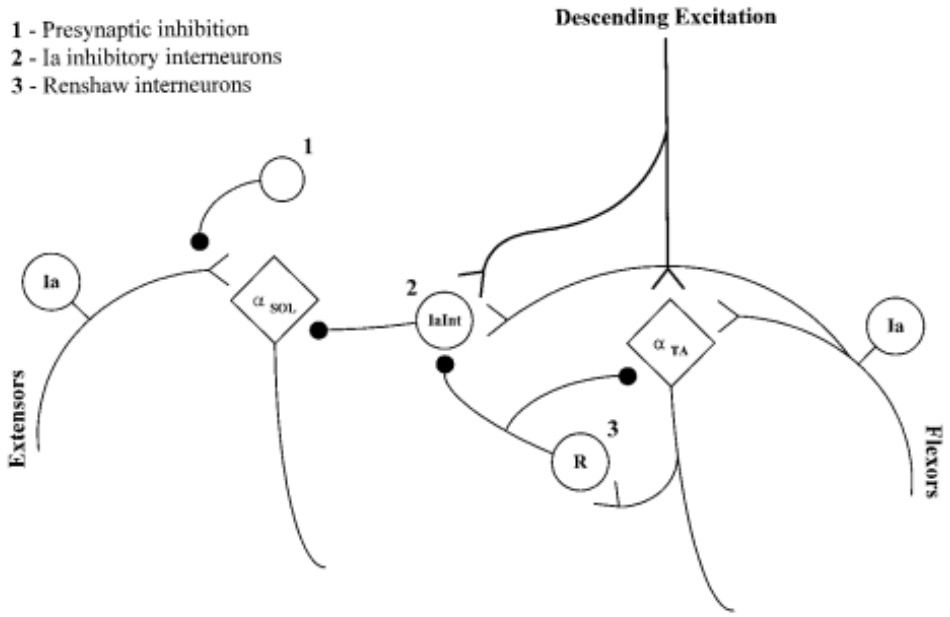

FIGURE 17. The suggested pathways behind the modulation of reciprocal inhibition. (From Lavoie et al 1997)

5.1.3 Recurrent inhibition

Renshaw cells are inhibitory interneurons that contribute to the amount of afferent feedback onto the spinal cord. Renshaw cells are activated by three main sources that include homonymous alpha motoneurons, afferent fibres belonging to group II-IV, and descending commands from supraspinal regions. These inputs can modulate the cell at numerous sites along its path. Because the motor axons that activate Renshaw cells are called recurrent collaterals, this inhibitory pathway has been given the term recurrent inhibition. (Enoka 2015, 270-271.) Recurrent inhibition has been observed in all tested proximal muscles (Pierrot-Deseilligy & Burke 2012, 153) but not in the muscles of the foot and hand. (Enoka 2015, 270-271).

Renshaw cells induce inhibition onto both alpha and gamma motoneurons as well as Ia inhibitory interneurons (Enoka 2015, 270-271). They are also reported to make inhibitory connections onto the same motor nerve that excites them. (Kandel 2013, 797.) However, the relative amount of inhibition to these three vary as the inhibition to gamma motoneurons is far weaker than that of alpha motoneurons. Furthermore, as mentioned in the previous section, due to the connections of Ia inhibitory interneurons, recurrent pathway can also contribute to reciprocal inhibition between antagonists (Lavoie et al 1997). Indeed, although motoneurons from the antagonist do not contribute to the activation of Renshaw cells, they do have connections to the antagonist muscle. By this connection they can induce recurrent facilitation. This facilitation induces a reduction of reciprocal inhibition. (Enoka 2015, 270-271.)

The functionality of recurrent pathways is evident in low contractions and coactivation of antagonist muscles. During stronger contractions descending inputs inhibit the activation of Renshaw cells and thus ensured the force production of the muscles. In situations when coactivation is needed, such as free standing, facilitation of the recurrent pathway inhibits reciprocal inhibition and reduces the effects of Ia afferents onto motor neurons. In this manner, recurrent inhibition can ensure appropriate muscle activation. In contrast, Renshaw cells are more inhibited during dynamic contractions. (Enoka 2015, 271.)

5.2 Modulation during natural movements

It has been demonstrated that spinal reflexes are modulated depending on the position of the body (Cecen et al 2018; Cattagni et al 2014) and the functional task at hand (Lavoie et al 1997). This of course is no great surprise as muscle activity is linked to corticospinal and spinal excitability (Nielsen 2002). However, even with similar muscle activity, differences have been reported to exist indicating to neural modulation of these circuits beyond peripheral muscle activity (Koceja et al 1995; Cattagni et al 2014). This subsection reviews studies that have reported different ways and possible mechanisms behind neural excitability during different body position and movement. The inhibitory modulators such as presynaptic inhibition, reciprocal inhibition and recurrent inhibition are neural pathways that have been recognised to be of importance in this matter.

It is to be noted that for the most parts, forthcoming sections consider changes of excitability between body position and changes from one activity to another with little regards to the muscle

action type and contraction strength behind the activity. For example, it has been reported that different muscle action types are controlled differently by the spinal and cortical levels (Valadão et al 2018) and increasing contraction effort during isometric contractions increases the H-reflex amplitude but not during concentric actions (Oya & Cresswell 2008). That being said, the excitability changes between muscle action types and a more detailed review of the modulatory effects of increasing contractions are beyond the scope of this thesis.

5.2.1 Position dependent modulation

Different body positions have been reported to regulate soleus H-reflexes in healthy individuals (Phadke et al 2010). That is, reflex excitability has been found to be downregulated during standing compared to sitting or lying down (Tokuno et al 2007; Cecen et al 2018; Cattagni et al 2014; Koceja et al 1995). Some studies, however, have reported conflicting results. That is, soleus reflex excitability in standing conditions has been reported to be upregulated, downregulated and having no effects compared to other positions. The reason for these differences has been suggested to stem from small differences in methodologies. These include means of controlling the level of EMG activation levels and the stimulus intensity as well as controlling for the effects of other receptors. (Cecen et al 2018.)

However, recent studies that have controlled for said factors may demonstrate results in favour of downregulation of H-reflexes during standing (Cecen et al 2018; Cattagni et al 2014.) Cattagni and colleagues (2014) for example compared Hmax/Mmax relations in two conditions of sitting (active and passive) and standing. During sitting, activation of the soleus muscle was found to reduce Hmax/Mmax while it was further reduced in standing with comparable EMG activity levels. Their results are in agreement with an earlier study by Koceja, Marcus and Trimble (1995) as they reported decreased H-reflexes in standing compared to prone. Although they did not match the level of EMG activity, the results showed a decrease despite increased EMG activity during standing. (Koceja et al 1995.) Study conducted by Cecen and others (2018) also reported reduced reflex excitability during standing. However, they concluded that Hmax was reduced during standing compared to prone position, but no differences were seen comparing standing to sitting conditions.

Methodological differences, however, are likely to explain these differences. Cecen and other (2018) reduced the level of background EMG to a minimum during their experiments as the

tested leg was suspended from the ground during standing and sitting. However, as Cattagni and others (2014) found a depression of reflex excitability during weak contractions compared to resting situation, the reflex control in these situations are likely different. Differences can stem from presynaptic inhibition. (Cattagni et al 2014.) However, as the effects of recurrent inhibition are comparatively strong during low contractions such as are produces during standing (Enoka 2015, 271) it has been suggested to play an important part in the reduction of reflex excitability at this position (Cattagni et al 2014). Furthermore, suspending the leg from the ground may differentially activates the cutaneous afferents with the addition of increasing weight bearing on the contralateral leg (Cecen et al 2018). Indeed, it has been reported that differential activation of contralateral cutaneous afferents can induce changes in the tested ipsilateral H-reflex (Suzuki et al 2016).

Reduced excitability of the SOL muscle has been noted as well in the context of corticospinal excitability. Capaday and others (1999) compared SOL MEPs between sitting and stance phase of gait and revealed that during stance SOL MEPs are reduced by 26 % of that of voluntary contraction during sitting with matched background EMG levels. Interestingly, TA amplitudes showed the opposite modulation. Thus, they hypothesized that soleus activity could be more under the control of spinal circuits during gait while the TA is more under cortical influence. (Capaday et al 1999.) Further evidence comes from a study by Barthélemy and colleagues (2010) who showed that TA activation in SCI patients is correlated to the transmission of signals from supraspinal levels through the corticospinal tract and thus, other pathways are suggested to contribute to a lesser extent.

5.2.2 Posture control

There are two main components of posture: balance and orientation. To maintain one's balance, we must counteract external forces that are posed on our bodies. Gravity being the most substantial one. By postural orientation, however, body segments are aligned according to the forces. Body orientation can depend on the gravitational vertical or other determinants such as support surface. However, the body is still mechanically unstable, and humans exhibit a small sway during quiet standing. Thus, to not fall we must control the motion of our centre of mass located in our abdomen at about L2-vertebral level by direction specific muscle activation. (Kandel et al 2013, 936.) This muscle activation is not only tonic in nature (Vieira et al 2012). Rather, the control of posture requires several sensory systems that must be continuously

adjusted as tonic activation of antigravity muscles would not be sufficient to maintain balance (Kandel et al 2013, 936).

Not surprisingly, the amplitude of H-reflex is affected by postural sway in freely standing humans. Specifically, the soleus H-reflex is greater with more anterior displacement and reduced when posterior with more TA activation. (Johannsson et al 2015; Tokuno et al 2007.) This modulation was in the range of 12 % in different positions of sway for soleus and 23 % for the gastrocnemius medialis (Tokuno et al 2007). The reason for postural sway modulation has been previously suggested to be due to changes in Ia presynaptic inhibition but was not directly addressed until 2015 when Johannsson and colleagues conducted a study using conditioning methods designed for this purpose.

Contrary to previous evidence, Johannsson and colleagues (2015) concluded that the modulation of H-reflexes during postural sway was more likely originating from the postsynaptic level and not from Ia presynaptic inhibition. They proposed that as background EMG activation level increases it would bring the membrane potential closer to excitation and thus, a larger H-reflex gain is present. Secondly, reciprocal inhibition originating from the tibialis anterior might influence the reflex as a larger activation in the TA in the backward position would reduce soleus H-reflex. (Johannsson et al 2015.)

The anteroposterior sway seems to induce a similar neural strategy for both soleus and the gastrocnemius medialis. This similar control strategy may reflect minimal mechanical requirements needed for this task (Tokuno et al (2007) as different strategies for neural modulation responses are suggested to be involved in tasks with more complex requirements (Cattagni et al 2014). Although these muscles might respond similarly to the direction of sway based on H-reflex modulation (Tokuno et al 2007) soleus and gastrocnemius medialis do have differences in motor unit activity. That is, soleus activity is largely tonic while the activity of GM is more phasic in both standing and voluntary contractions. (Héroux et al 2014.) Indeed, the activity of GM is mainly present during forward sways (Vieira et al 2012). The reason behind these differences are suggested not to derive from differences in descending drive but rather to functional roles of the muscles based on their muscle unit properties. Furthermore, the activity of gastrocnemius lateralis is relatively absent during standing and shows a significantly larger recruitment threshold. These might indicate a differential architecture and/or mechanical advantage of the muscles during this task. (Héroux et al 2014.)

Interestingly, a co-contraction of the antagonist muscles during standing has been reported to affect H-reflex and corticospinal excitability differently. Kesar and others (2018a) showed that when transitioning from free standing with only the SOL activated to a co-contraction of the TA muscle SOL, MEPs are increased. As the facilitated TA MEPs are likely due to the activation of the muscle, SOL MEPs increased despite changes in the background activity level. Similar results have been obtained elsewhere. Indeed, Geertsen, Zuur and Nielsen (2010) reported that SOL MEPs at 1.2 MT were enhanced already 50 ms prior to dorsiflexion while the soleus H-reflex showed the opposite modulatory pattern. Similar trend, however in a slightly smaller way, was reported prior to dorsiflexion. Furthermore, as cervicomedullary stimulation showed no differences with TMS, the origin of this modulation is suggested to stem from subcortical regions. The authors speculated that this motor programme of increasing agonist excitability concurrently with antagonist prior to activation could function to ensure that movements direction could be changed in a more efficient and swift manner. (Geertsen et al 2010.)

H-reflex excitability is also differently modulated by muscle action type in passive (Pinniger et al 2001) and active conditions (Valadão et al 2018). That is, eccentric actions have shown to have a reduced reflex excitability than that of isometric condition (Valadão et al 2018) and increased in shortening contractions (Pinniger et al 2001). The results are in accordance with the effects seen in standing. During forward sway, SOL and GM exhibit a shortening contraction (and increased H-reflexes) and lengthening contraction during swaying backwards (with decreased H-reflexes). However, based on the observation that muscle length is changed independently of centre of mass during standing it is suggested that balance could be based on anticipatory displacements rather than mere reflexes. (Loram et al 2005).

Indeed, it has been noted from the evidence of animal studies that balance and posture are two different mechanisms that have different neural circuits. Cats with complete spinal transection can support the weight of their hindlimb but cannot maintain balance. Especially head movements cause the cats to lose their balance although they are able to activate their muscles against gravity. Humans with SCI have also demonstrated similar effects as some can produce antigravity support but not balance support. Therefore, it is postulated that antigravity support can be of spinal contributions, but balance requires a more cortical contribution. (Kandel 2012, 951-953.) Posture control is therefore a combination of both. However, cortical strategy for balance does not exclude the role of reflexive actions, which are considered as an effective part

of balance as they might respond to disturbances that exceed the ability of the anticipatory strategy (Enoka 2015, 281).

5.2.3 Phase dependent modulation

Phase dependent modulation of monosynaptic reflexes during gait has been studied in healthy individuals (Simonsen & Dyhre-Poulsen 1999; Nair et al 2014) and in patients with SCI (Knikou et al 2015). The pattern of H-reflex during a gait cycle is seen in figure 18, where the soleus H-reflex is relatively silent during the swing phase and progressively increasing during the first part of stance and then decreases towards toe off. (Simonsen & Dyhre-Poulsen 2011.) As can be seen from the concurrent EMG activity of soleus and tibialis anterior, the excitability of H-reflex follows a classic pattern of inhibition from agonist to antagonist i.e. the reciprocal inhibition. (Schneider et al 2000.) Indeed, it has been demonstrated that reciprocal Ia inhibition is modulated during different phases of gait. Reciprocal inhibition is inhibited in the stance phase and facilitated during the swing phase. In this way reciprocal inhibition helps to inactivate the antagonist in gait and help ensure unhindered activation of appropriate muscles in each phase (Petersen et al 1999).

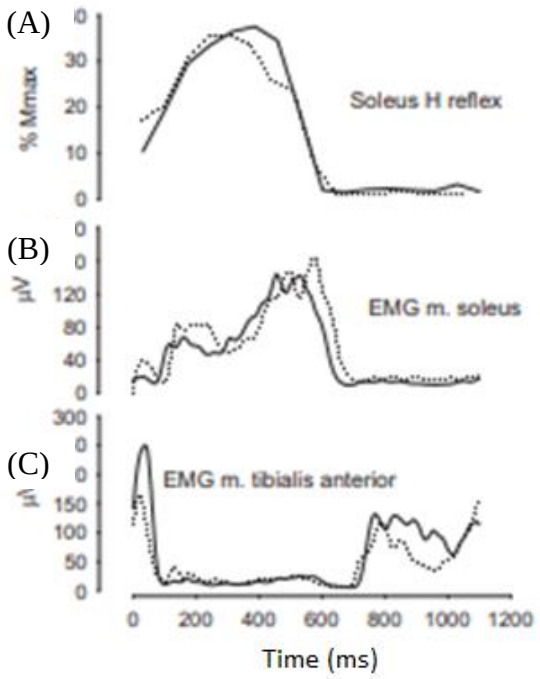

FIGURE 18. Modulation of the H-reflex during gait cycle. Timepoint 0 refers to heel contact. (A) H-reflex modulation in different gait phases. (B) Soleus EMG activity. (C) Tibialis anterior EMG activity. (Modified from Simonsen & Dyhre-Poulsen 2011.)

Although peripherally controlled disynaptic reciprocal inhibition has been a noteworthy theory behind reflex modulation, regarding the concurrent EMG activity pattern, it seems to be operation to a lesser extent. Lavoie and others showed in 1997 that during gait, modulation of H-reflex is attained in most cases before the onset of EMG activity rather than after and that the strength of reciprocal inhibition is independent of the antagonist level of activity. Therefore, it seems that the phase dependency in the case of gait does stem for the most part from cortical centres. However, cortical contributions do not exclude the possibility of peripheral inhibition, which can ensure reciprocal inhibition to endure. (Lavoie et al 1997.)

Furthermore, TMS measures of corticospinal excitability during gait has also been demonstrated to follow the same pattern. That is, SOL and gastrocnemius medialis (GM) MEPs are progressively increased during the stance phase of walking and reaches its peak when at late stance. Similarly, TA showed the highest amplitudes at heel contact with concurrent TA-activation, progressively decreased at stance and was again facilitated at swing phase. (Pulverenti et al 2019.) The modulation of MEPs with EMG levels, however, is not surprising as MEP amplitudes depend on the excitability of spinal motoneurons that are under various inhibitory and excitatory interneurons. Therefore, evidence of corticospinal MEP modulation cannot be taken to argue for the dominant role of this drive to motoneurons as the origins of gait modulation. (Nielsen 2002.)

When studying spinal contributions to gait modulation, the effects of afferent feedback from cutaneous receptors is also to be noted. Suzuki and others (2016) revealed that contralateral stimulation of cutaneous afferents modulated the soleus H-reflex in a phasic manner. When transitioning from swing to stance it had an inhibitory effect and from stance to swing it facilitated the H-reflex. No significant modulations were seen on other phases of gait. Based on their results, Suzuki and colleagues concluded that this modulation between limbs may contribute to the sophistication and sensitivity of locomotor systems in humans in the transition phases. However, a significant drawback of the methodology is that the specific pathways responsible for this modulation cannot be directly identified. This is due to the relatively long interstimulus interval of 100 ms between contralateral stimulation, and thus multiple central pathways may be involved. (Suzuki et al 2016) Indeed, the role of cutaneous afferents in motor activity may be expressed through shared pathways for they converge on interneurons of other muscle afferents and descending tracts. (Pierrot-Desilligny & Burke 2012, 334.)

Taken together, the origin of phase dependent H-reflex modulation is considered to derive from cortical and peripheral origins, however, relative contributions are not yet clear. Cortical centres contribute to the modulation of presynaptic inhibition of soleus Ia afferents with contributions from reciprocal pathways of peripheral (Mummidisetty et al 2013) and cortical origins (Lavoie et al 1997). Indeed, despite the inability to exactly point out the origin of the modulation in each phase, the pattern does serve functional benefits. That is, reciprocal inhibition helps to inactivate the antagonist in gait and therefore help to ensure unhindered activation of appropriate muscles in each phase. (Petersen et al 1999.) Furthermore, cutaneous afferents may contribute to the sophistication of gait but as these afferents are known to converge onto many others, their specific pathways are not yet known. (Suzuki et al 2016).

6 TRANS-SPINAL DIRECT CURRENT STIMULATION (Ts-DCS)

Trans-spinal direct current stimulation is a potential tool for neuromodulation, which has gained attention in the research community in the last few decades. If effective, this stimulation could possibly facilitate spinal plasticity that is of interest in many types of injuries to the spinal cord. (Kuck et al 2018.) However, to this date researchers have reported a variety of results ranging from no effects to moderate and significant results depending on the target of observation. Some of the discrepancies most likely result from differences in methodologies such as the differences in current stimulation protocols. In any case, the specific effects of stimulation are not yet fully clear and thus, new studies are warranted.

In this section, the results of relevant published research papers are reviewed. First, in order to give a more comprehensive understanding of direct current stimulation, electrophysiological aspects are introduced with a closer look at neural behaviours in these electric fields. Studies in this section include mainly those that have used modelling methods and reduced animal studies for obvious reasons. Moving further, effects on spinal and cortical processes are reviewed respectively in human and animal studies.

6.1 Neurophysiology of direct current stimulation

Ions move from one place to another based on potential differences. This phenomenon allows the ions in our bodies to move into and out from cells and thus is the basis of all neural activity. Resting membrane potential refers to the potential difference across a cell membrane when the nerve cell is in rest and no stimuli has activated ion-gated membrane channels. In the case of a nerve cell, resting membrane potential is typically around -65 mV. (Enoka 2015, 166-173.)

The movement of ions, which indicates the rate at which ions move from more positive to more negative areas, or vice versa, can be expressed as a unit of measurement called amperes (A). In other words, current (I) is a product of charge (Q) divided by time (t) (see equation 1). The currents that normally take place in our cells are small and as such are expressed usually as mA, nA and pA. (Enoka 2015, 166-167.) In the case of DCS, a unit of current density is usually reported as the size of the stimulating electrode affects the average current produced underneath and expressed as A/m2. It has been concluded from animal studies that injury can occur at

current densities of 6.3-13 A/m2, which is significantly higher than those used in conventional DCS studies (Bikson et al 2016).

$$l = \frac{Q}{t}$$

EQUATION 1. Current (l) equals charge (Q) divided by time(t).

In Ts-DCS, the polarity of the stimulation is determined by the spinal electrode (Winkler et al 2010). Thus, the basic assumption that the stimulation modality, anodal or cathodal alone, induces the inhibitory or excitatory modulation in nerves is an appealing statement. However, this might be an over-simplification (Bikson & Rahman 2013). Indeed, although many studies have found mainly differential results for cathodal and anodal stimulation paradigms (Cogiamanian et al 2008; Winkler et al 2010) others have found similar results for both (Kaczmarek et al 2017).

We already know that axons that are stimulated lie mainly parallel to the current direction. That is, neurons are activated by an extrinsic current preferably when the anode and the cathode lie parallel to the nerve orientation. (Rossini 2015.) However, there may be other determinants that affect what structures are stimulated and how. The resulting effects of direct current stimulation have been tied to the polarity of the stimulation, duration of the stimulation, temporal domain, orientation of the neuron with regards to the current direction, their respective distance (Ahmed 2014), neuron cell size (Ahmed 2016) and activity (Bikson & Rahman 2013).

In rat experiments, Ahmed (2016) showed that during anodal stimulation most examined gamma motoneurons exhibited a reduced firing rate making the muscle spindles less sensitive to muscle stretches. With cathodal paradigm most showed an increase in firing rate. However, both modalities exhibited one neuron that showed the opposite effect. In the case of alpha motoneurons, anodal paradigm showed a main excitatory effect on firing rate while the opposite was revealed with cathodal. The opposite effects of the same stimulation to gamma and alpha motoneurons were found to be due to differences in cell size. That is, those with a larges cell body showed an increased effect with anodal stimulation and vice versa. (Ahmed 2016.) Furthermore, findings by Kabakov et al (2012) suggest that axonal orientation beneath the current also has effects on whether the nerve shows inhibitory or excitatory effects.

It has been suggested that transcranial Direct Current Stimulation (tDCS), a cortical equivalent of the Ts-DCS, preferably modulated neurons that are active at the time of stimulation while the resting neural networks would stay outside its effects. The reason for this is suggested to stem from the threshold of neural activation as the resting membrane potential in quiet neurons is higher and the stimulation induced would not be strong enough. Furthermore, the amount and result of stimulation may vary from subject to subject as there might be anatomical variations that are not accounted for in the stimulating protocol (Bikson & Rahman 2013.) This is of importance also in the case of spinal stimulation as the amount tissue in-between stimulating electrode and neuron bundles may vary.

When it comes to spinal stimulation, a modelling study by Parazzini and colleagues (2014) demonstrated that the field amplitude in the area of stimulation is distributed in the spinal ventral and dorsal neural tracts with a low level of variation. Thus, both structures experience the same intensity of stimulation. However, according to Ahmed (2011) the nerves in close proximity to the stimulating electrode in the dorsal aspects of the spinal cord could be modulated differently to those that are in the ventral aspects (see figure 19). That is, the electric field near the stimulating electrode for example an anodal electrode could produce an effect of hyper-polarization while in further parts the stimulus would produce a net effect of depolarization. Stimulation effects can also be biphasic as the effects during stimulation might differ from those after the stimulation. (Ahmed 2011.)

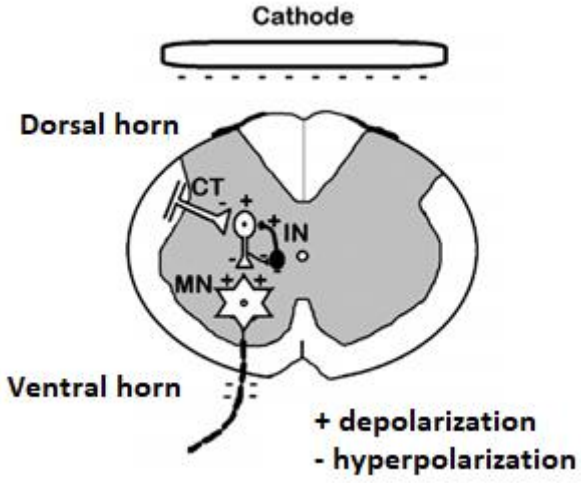

FIGURE 19. The electric current from a cathode at the dorsal side of the spinal cord induces a depolarization of nearby neurons and conversely, a hyperpolarization to further lying neurons at the ventral side. (Modified from Ahmed 2011).

Furthermore, differences can be also due to electrode placement. According to Priori et al 2014, influences of different placements should be further investigated as the specific mechanisms are not yet fully understood. Stimulation intensity and duration on the other hand, has been quite uniform across studies ranging from 2-2,5 mA and 15 to 20 minutes (Priori et al 2014) indicating that the diverse effects of stimulation would not likely contribute to stimulation intensity in humans studies at least. However, evidence still indicated to a multitude of factors that influence the effects of neural modulation under electric fields. Indeed, as is the case of H-reflex, the mechanisms under DC-stimulation are far from simple.

6.2 Spinal modulation

Presynaptic inhibition and post-activation depression are important mechanisms that affect spinal inputs to motoneurons and the lack or disruption of their normal processes can lead to various motor disorders (Kaczmarek et al 2017). More specifically, post-activation depression has been found to be impaired in spastic patients and correlated with its severity (Grey et al 2008). Thus, modulation of these pathways might be of great advantage. A study conducted in anesthetized rats aimed at revealing these possibilities and succeeded in demonstrating that trans spinal stimulation facilitated presynaptic inhibition and post-activation depression with similar results with both anodal and cathodal protocols (Kaczmarek et al 2017).

Similar results were obtained in another study in mice with spasticity. That is, post-activation depression was facilitated with repeated anodal stimulation with concurrent improvements in locomotion indicating a restoration of spinal inhibition. (Mekhael et al 2019.) In humans however, anodal Ts-DCS was found to decrease post-activation depression while cathodal stimulation increased it. (Winkler et al 2010). Differences can be related to many factors ranging from stimulation intensity to neural orientation and distances, which were introduced in more detail in the previous section.

Hmax/Mmax ratio has been reported to remain unchanged after anodal and cathodal stimulation. Furthermore, as Winkler, Hering and Straube found concurrent changes in the post-activation depression in their study, their results suggest that stimulation changes the efficiency of Ia-motoneuron synapse but does not influence the excitability of the motoneuron in itself. (Winkler et al 2010.) Similar results were also reported by Bocci and colleagues (2015a) as they found no changed in H-reflex threshold of Hmax. However, Kuck and others (2018)

investigated the effects with three different electrode configurations and found that in one, in which the anode is placed over Th11 and the cathode under Th11 with similar distance, Hmax and Hmax50% was reduced. Thus, electrode placement can be a noteworthy cause in why H-reflexes were not modulated in other studies. Murray and colleagues (2018) also found decreased H-reflex excitability with cathodal stimulation with yet another configuration and a larger 4.0 mA stimulus intensity.

Additionally, there are other aspects of the recruitment curve that have been reported to be modulated after Ts-DCS. Lamy and Boakye (2013) showed that anodal Ts-DCS produced a leftward shift in the stimulus-response curve of H-reflex. This modulation was further replicated in a later study but was found to be only evident when grouping subjects to those with BDNF (brain-derived neurotropic factor) Val homozygote genotype and without. Thus, they suggested that effects of stimulation may also vary with genetic dependencies. These results would seem rather logical as BDNF is one of the genes that is thought to influence synaptic plasticity amongst other factors. (Lamy & Boakye 2013.) However, the results could not be repeated by Kuck and colleagues (2018). Indeed, the different genotype groups showed no differences and no leftward shift was observed in either groups. Differing results were suggested to derive from experimental and subject specific factors. (Kuck et al 2018.)

Transcutaneous spinal direct current stimulation was also used by Hubli and colleagues (2013) to uncover spinal reflex excitability changes produced by either Ts-DCS or assisted locomotion in healthy subjects and those with spinal cord injuries. Spinal reflex excitability was investigated by non-noxious cutaneous stimulation, which can be used as a marker for the function and modulation of locomotor circuitries. With a stimulation duration of 20 min and an intensity of 2.5 mA (total charge of 0,1008 C/cm2) they found that in SCI patients the stimulation induces increased reflex amplitudes (84 %) with anodal condition, which was not observed in cathodal, sham or locomotion training conditions. In addition, reflex threshold was found to be lower in both anodal stimulation protocol and locomotor training groups. (Hubli et al 2013.)

However, the results were different for heathy individuals. That is, sham protocol induced a reduction in the cutaneous reflex in itself and was hypothesized to stem from immobility during the stimulating protocol. Similar reduction was seen with the cathodal condition. Comparatively, anodal stimulation and locomotion did not show a reduction of reflex

excitability compared to baseline. Thus, it seems that anodal stimulation and locomotion could in fact counteract the observed reduction in cutaneous evoked reflexes. The differences in the results comparing SCI subjects and healthy humans might be dependent on the general lower spinal excitability levels presented by SCI patients and thus modulatory effects might have more effects. (Hubli et al 2013.)

Ts-DCS has also been found to influence the ascending sensory pathways. Anodal stimulation at the thoracic level reduced somatosensory potentials for at least 20 min after stimulation end, which indicated changes in the ascending sensory pathways in the human spinal cord. Conversely, cathodal could not influence the potential significantly despite a tendency to increase. (Cogiamanian et al 2008.) Furthermore, anodal stimulation increased pain tolerance measured by a foot cold pressor test suggesting its effectiveness in impairing conductance in the ascending pain pathways. These results are thought to take place in the spinal cord. (Truini et al 2011.)

The modulation effects do diminish over time. However, not many studies have addressed the temporal patterns of stimulation. Indeed, Corgiamanian and colleagues (2008) found their results to persist at least for 20 min after stimulation end but did not extent their study to reveal later responses. Albuquerque and others (2018) found Hmax/Mmax to persist at 30 min testing point. However, as their protocol included a 20 min walking bout after stimulation making the actual timepoint of measurement to be at 50 min after stimulation end. Unfortunately, they did not control for the effects without walking making the interpretation of its effects on the temporal pattern remain speculative. (Albuquerque et al 2018.) However, Kaczmarek et al (2017) did show similar results as they found the effects of DC to prevail for at least 45-60 min. These indicating a longer modulatory effect.

6.3 Cortical modulation

Since the spinal cord hosts a multitude of different descending and ascending neural pathways, it is no far-fetched notion that direct current stimulation at the spinal level could also modulate cortical processes. (Ahmed 2011.) Indeed, rMT has been found to be increased with anodal stimulation while left unchanged with cathodal. Cathodal stimulation showed a mean excitatory effect on MEP areas at 120 % of rMT. (Bocci et al 2015a.) Similar results were seen in Murray et al (2018) as cathodal protocol increased MEPs in the IO recruitment curve. Furthermore,

they found this result to be position dependent as effects were seen only when testing is done in a supine position compared to sitting. The increase in cortically induces muscle contractions (MEPs) were later repeated by the same team (Murray & Knikou 2019).

As with spinal measurement, cortical responses to stimulation might differ in subjects with neurological disorders. Ardolino et al (2018) used a similar testing protocol as Bocci et al (2015a) with the distinction that the subjects had hereditary spastic paraplegia. They found reductions in the Ashworth scale of spasticity in the anodal protocol but could not contribute the effects to changes to modulation of H-reflex or MEPs at 120 % of rMT.

MEP amplitudes with a paired pulse technique at different interstimulus intervals have also been reported to be modulated. (Bocci et al 2015b; Murray & Kinkou 2019.) This technique has the ability to reveal cortical circuitry modulation by local pathways or afferent circuits from other brain areas. (Hanajima & Ugawa, 2008). Cathodal stimulation has been reported to increase MEP amplitudes with 3 ms interstimulus intervals (short intracortical inhibition; SICI) while a decrease was seen with anodal. No effects were reported for MEPs at a 10 ms interval thus, depicting no changes in intracortical facilitation. Results thus indicated a specific site of modulation in the M1 that can be functional for a number of disorders. (Bocci et al 2015b.) Indeed, abnormal measures of SICI have been reported in various pathological conditions (Hanajima & Ugawa, 2008) indicating possible functional outcomes of stimulation. Murray and Knikou (2019) later reported similar results as they reported a decreased intracortical inhibition at stimulus intervals of 2 ms and further an increased intracortical facilitation at 25 and 30 ms with cathodal Ts-DCS.

In light of these studies presented here, Ts-DCS has the ability to influence activity in the ascending sensory pathways, segmental motor systems (Cogiamanian et al 2012) with effects that influence also corticospinal excitability (Bocci et al 2015a) and intracortical inhibition (Bocci et al 2015b). However, studies have shown differences in testing protocols that might have contributions to at least parts of the discrepancies seen in outcomes. Thus, there seems to be a need for research with more uniform protocols and thus, methodological aspects need to be further studied.

7 PURPOSE AND DESIGN OF THE STUDY

Trans-spinal direct current stimulation has the potential to induce neural modulation on spinal and corticospinal pathways. However, the majority of studies conducted so far represent conditions of rest in humans and/or animal studies. The function of human neural pathways however is known to be complex and to be modulated by a multitude of factors including posture and different functional tasks. It has also been suggested that direct current stimulation may modulate active neurons more than those at rest. Therefore, modulation may be evident in an activity dependent manner. Lacking the information about how Ts-DCS affects humans in active conditions narrows the possible advantages of the method.

The purpose of the study was to examine whether anodal Ts-DCS induces spinal and/or corticospinal modulations on the soleus muscle during treadmill walking and quiet standing. Anodal or sham Ts-DCS was administered during gait with preferred speed. Spinal excitability changes were assessed with the soleus H-reflex during the stance phase of gait. Three different phases were selected that represented early, mid and late stance. During standing, the H-reflex and TMS recruitment curves were constructed to reveal Hmax/Mmax modulation and the modulation of corticospinal excitability. Furthermore, measurement protocols were constructed to further give information about the possible modulatory effects of an additional 30 min of walking before Ts-DCS on the modulation of Hoffmann reflexes.

Although previous studies have failed to show consistent effects of Ts-DCS on H-reflex during resting conditions, it is unclear whether and how activity influences the results especially when it is administered and tested in the same functional task. On corticospinal excitability, as previous studies have measured modulation in only one intensity due to time constraints, it is of interest to see possible protocol dependent changes in the entire recruitment curve. Some studies have used voluntary contraction to reveal cortical modulation, no studies have yet used standing protocols. Furthermore, as different phases of the gait cycle are studied, differences in their modulation, if seen, can demonstrate the effects on different mechanisms.

8 METHODS

8.1 Subjects

8 healthy subjects participated in the study with a mean age of 26-years with a standard deviation (SD) of ±4. Six subjects were female and two males. All subjects were informed about the procedures and signed consent forms before participating in any measurement. Risk factor questionnaire for TMS was filled out and no subjects reported any contradictions for measurements. For Ts-DCS the skin under the electrode locations were checked and stimulation was only administered if skin was intact and showed no signs of other irritation. Subjects did not have any musculoskeletal pain or diseases during measurements and at least 6 months prior. Participants were instructed to restrain from consuming caffeine before measurement and not participate in any exhaustive or unaccustomed exercise bouts for the previous 24 h. All procedures were approved by the ethics committee of University of Jyväskylä and conducted according to the Declaration of Helsinki.

8.2 Experimental protocols

All subjects completed three measurement sessions that lasted for 2-3 h depending on the protocol (see figure 20). Two sessions included all measurement protocols pre and post DC-stimulation applied during treadmill walking. Ts-DCS was administered with either sham or anodal protocols, further referred as sham-gait (SG) and anodal-gait (AG), respectively. In protocol (R), gait stimulation was not done as it served as a control for the effects of additional 30 min of walking prior to anodal DC-stimulation. This protocol consists of 20 min of walking with the DC-stimulation while others had an additional 30 min of walking in pre and post measurements with H-reflex stimulation. Sessions were separated with at least 7 days in order to count out possible carry-over effects. Sessions were carried with a similar starting time to counteract possible effects of modulation depending on the time of day. As effects have been demonstrated to prevail for 45-60 min after stimulation end (Kaczmarek et al 2017) all post measurement were finished within an hour of stimulation end.

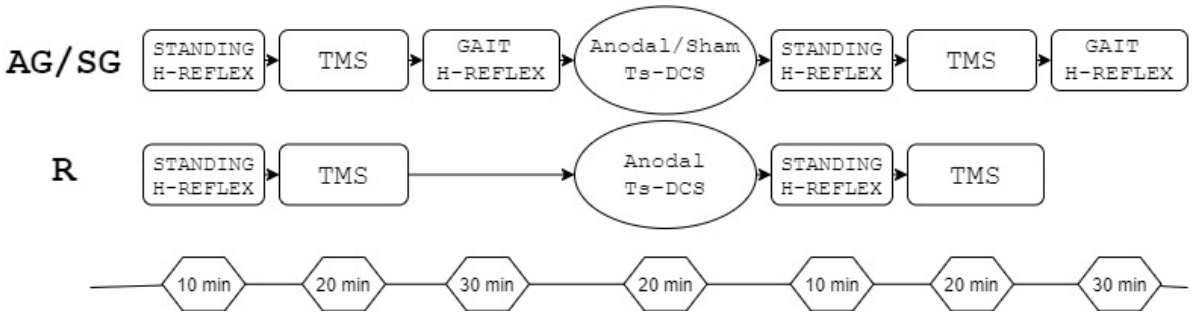

FIGURE 20. Measurement protocol. SG refers to sham stimulation protocol with gait testing and AG similarly to anodal. Anodal stimulation is administered in protocol R but includes no gait stimulation.

8.3 EMG recordings

Electromyographic recordings were measured and collected from the right soleus and tibialis anterior muscles by a portable EMG recording system (Noraxon TeleMyo 2400R G2 Receiver, TeleMyo 2400T G2 Transmitter, USA). EMG was pre-amplified, low pass filtered at 500Hz and sampled at 1500Hz. Delay was set to 200ms to ensure no data loss during measurement. All data was recorded, stored, and later analysed with Spike2 software (version 6.17, CED Ltd, Cambridge, UK).

Prior to attaching Ag-AgCl electrodes (Ambu BlueSensor N-00-S/25, diameter 6 mm) with a bipolar setting the skin area was shaved, abraded, and cleaned with alcohol to improve conductance. The soleus electrodes to measure H-reflex amplitude were positioned centrally and distally near the Achilles tendon (Botter & Vieira 2017). The electrodes in tibialis anterior were positioned according to SENIAM guidelines (see http://www.seniam.org for references) and the ground electrode was placed over the tibial bone. Electrodes were positioned according to the underlining muscle fibre orientation. Interelectrode resistance was checked before measurements and accepted when under 2kΩ. Self-adherent bandage were used over all electrodes and the attached wires in order to secure and reduce movement artifacts (see figure 21). Care was taken not to restrict blood flow or movement. Comfort of movement with this setup was monitored from subjects during measurement.

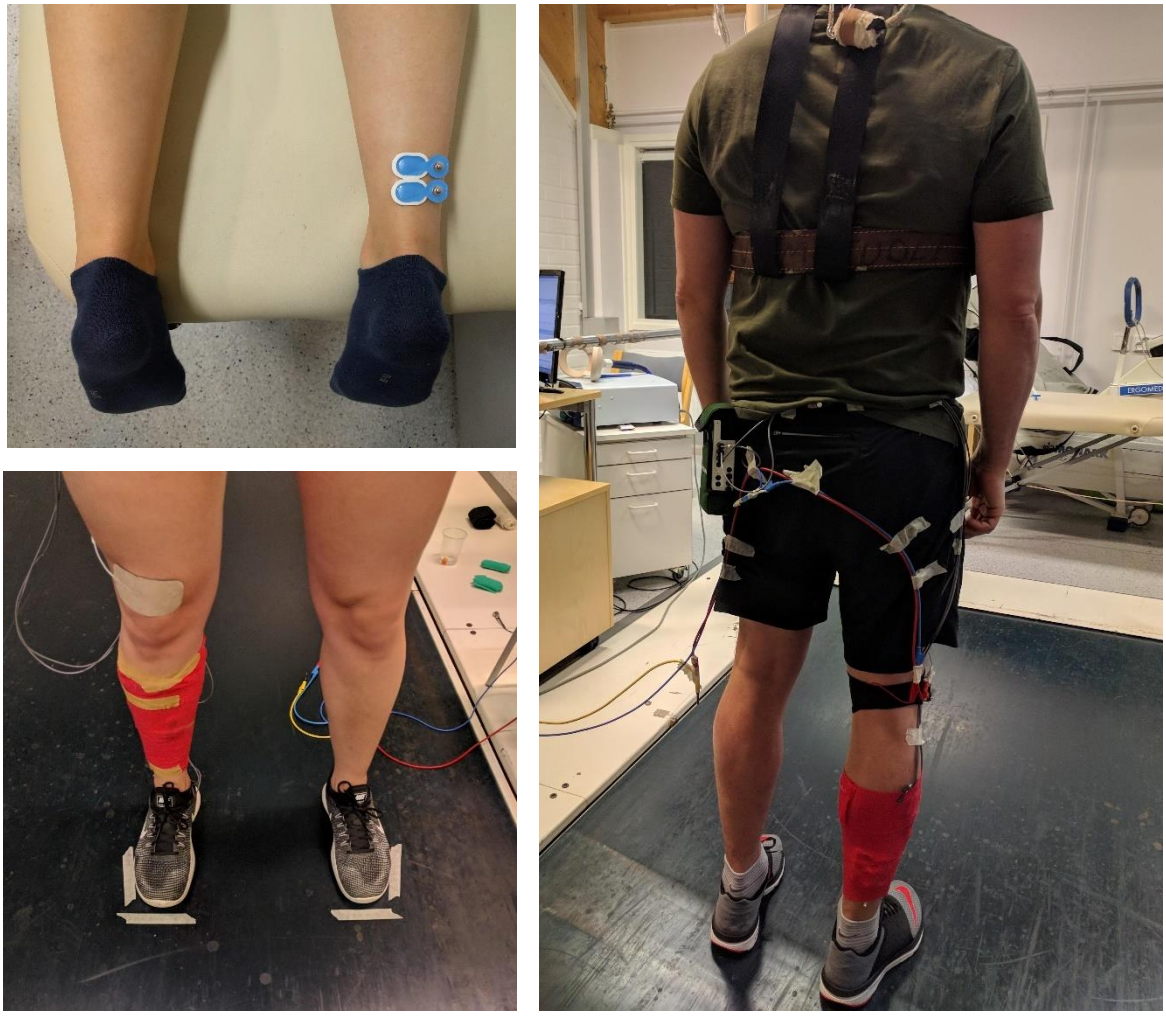

FIGURE 21. Measurement setup. Soleus EMG electrodes are placed distally near the Achilles tendon. Electrodes and wiring are fixed with elastic wrap and tape.

8.4 Evoking soleus H-reflex

H-reflex recruitment curve. Soleus H-reflex recruitment curve was measured during unsupported standing. The posterior tibial nerve stimulation site was identified from the popliteal fossa by a hand-held probe during standing. The optimal position was identified when the M- and the H-waves had a similar shape and the H-reflex could be elicited without a visible M-wave with lower intensities. (Simonsen & Dyhre-Poulsen 2011.) After optimal location was found a permanent (2 cm) stimulation electrode was attached and an anode of 5x7 cm in diameter was attached above the patella (Nair et al 2014). With this set up, the current passes transversely through the posterior tibial nerve (Pierrot- Deseilligny & Mezeves 2000). In order

to apply constant pressure on the cathode an elastic wrap was used. Standing width and foot positions were marked and kept constant throughout measurements.

Throughout the standing measurements, background EMG activity of the soleus and TA was monitored, and directions were given to the subject if levels were visibly changed. The subject was informed to stand relaxed and as steady as possible. Stimulations were given with 10-15 s in between to remove the effects of post-activation depression. Current intensity was increased with 1 mA steps to the point where the M- and H-waves crossed and from that point a 2 mA step increments were used due to time constraints. Stimulation was continued until the M-wave plateaued. 4 % and 8 % of the Mmax value were then calculated and manually checked that the resulting H-wave was on the ascending limb. In some subjects, this value was already at around Hmax values. If so, the lowest possible intensity was used in gait stimulations that still resulted in a visible M-wave.

H-reflex gait stimulation. Preferred walking speed was timed for each subject before treadmill measurements with photocell sensors over ground. Natural preferred walking speed was chosen to reduce possible effects of variation in the temporal domain of gait phases. Subject were then familiarised to the walking speed on the treadmill and small changes were made if needed. After familiarization, treadmill speed was kept constant in all successive measurement. Before H-reflex measurement, each subject walked on the treadmill until average swing and stance phases were stable for at least two consecutive minutes. Stance and swing phase times were monitored by footswitches attached to the heel and forefoot (Noraxon, USA) and average times were calculated automatically from 1 min time windows with a script specially written for Spike2 software (see data example in figure 22).

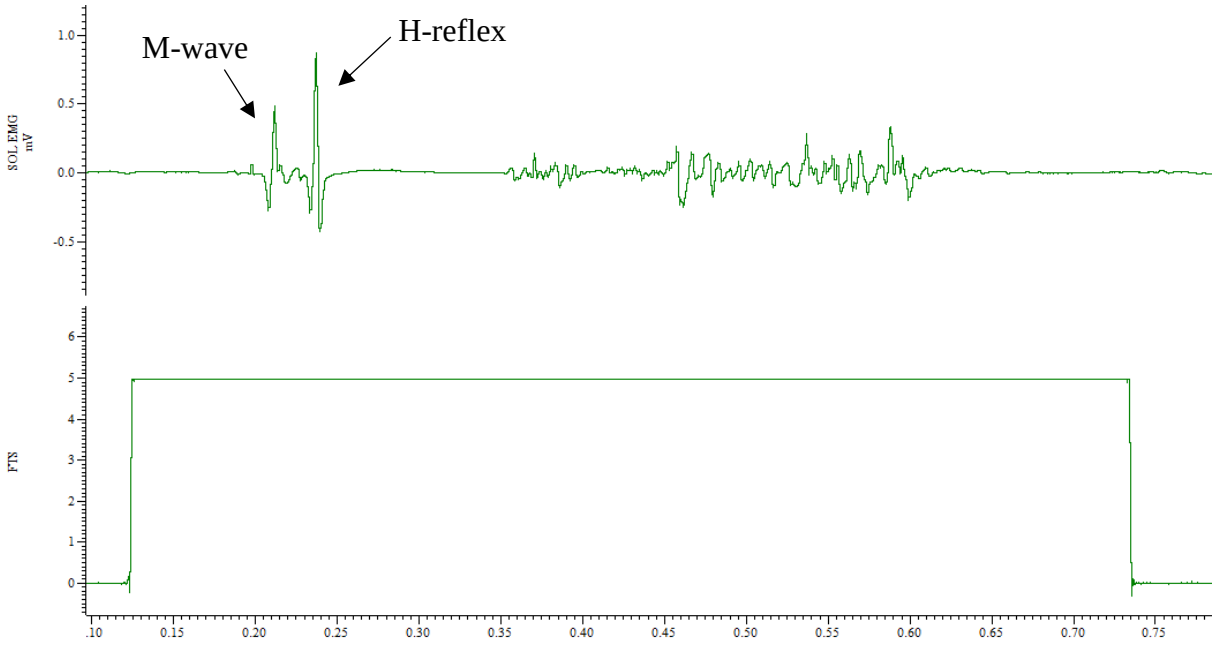

FIGURE 22. Data example from early stance stimulation. Upper channel shows soleus EMG data with visible M-waves and H-reflexes. Lower channel shows foot switch data (FTS) that has a value of 0 at swing phase and 5 during stance.

Stimulation was given in three different phases: the early stance phase corresponding to an average of 10 % of the stance phase, mid-stance at 50 % and late stance at 80 %. These phases were selected to present different H-reflex excitability levels and different levels of EMG background activity. Stimulation order was randomised but executed in one phase at a time. Mmax peak-to-peak amplitudes were first determined with an intensity that corresponded to at least 2-times of what was needed for standing Mmax. At least three maximal stimulations were given first to obtain phase dependent Mmax-values. H-reflexes were stimulated with an intensity that produced an M-wave usually corresponding to a range of 4-8 % Mmax amplitudes. If M-waves were visible against the background EMG levels, this range was used for all measurement. The accepted range was increased, but always kept within 4 %, if M-waves were not visible. Same ranges were always used for later measurements. Stimulation trains were given with a separation of 9-14 s. At least 30 stimulations were given in each phase to get at least ten stimulations at the appropriate range. Picture example of the gait trials can be seen below in figure 23.

FIGURE 23. Treadmill walking with concurrent H-reflex stimulation.

8.5 Transcranial magnetic stimulation

Transcranial magnetic stimulation testing was done with the subject standing without support and delivered using a single pulse, monophasic Magstim 200 stimulator and Bistim unit equipped with a figure-8 coil (Magstim, Dyfed, UK) (see figure 24). Current direction was oriented in a posterior–anterior direction and optimized for soleus MEPs. The Cz-area of the skull was measured and marked while the subject was sitting. When the largest soleus MEPs with the least tibialis anterior activity was identified, the area was marked clearly on the scalp. The area was then checked with the subject standing and adjustments were made accordingly. Standing width and foot positions were marked on the floor and thus controlled throughout the measurement.

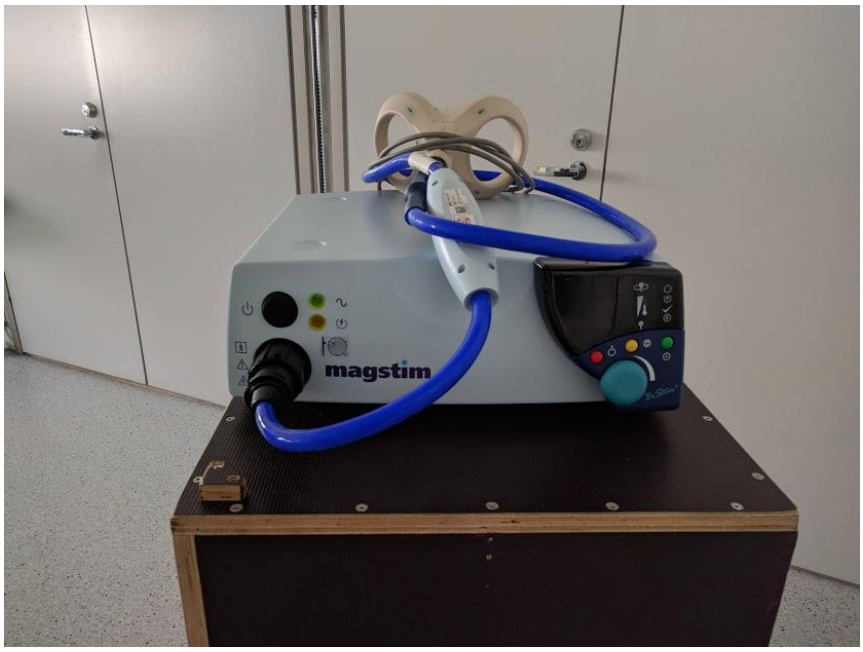

FIGURE 24. Magstim TMS stimulator (Dyfed, UK) with a figure-eight coil.

Active motor threshold (aMT) was defined as the lowest intensity that gave visible MEPs over the background EMG-activity in at least 3 out of 5 times. Thereafter, 10 stimulation were given with the aMT intensity in order to confirm its accuracy. This was done to avoid time loss due to erroneous intensity values, which can be significant with regards to the temporal pattern of modulation caused by Ts-DCS. When the aMT value was determined accurate, 10 stimulations were given at intensities of 90 %, 110 %, 120 %, 130 % and 140 % of aMT respectively with a randomized order. Subjects rested in between stimulation trains and moving was encouraged in order to reduce fatigue and dizziness. However, two subjects complained about dizziness during the TMS protocol or during standing H-reflex testing, which was likely due to standing still for long periods of time. The same background EMG levels, as in H-reflex testing, were targeted and visually confirmed by both the researcher and the subject. That is, throughout the stimulation subject were asked to self-monitor soleus EMG levels on a computer screen placed in front of them. The level was visualised as an RMS signal with a reference line to help visualise target levels.

8.6 Trans-spinal direct current stimulation

Anodal or sham stimulation was administered with HDCstim® direct current stimulator (Newronica, Cologno Monzese, Italy). Subjects and researcher were naive to the stimulation protocol with the exception of R-protocol, in which the stimulation was always anodal. Th11

spinosus process was palpated and marked on the skin as the centre place for the stimulation electrode (see figure 25). Stimulation electrodes were elastic rubber electrodes with sponge pockets. The sponges were soaked in physiological solution (0,9 %) and attached to the lower back and the right shoulder. Care was taken to make sure sponges were moist throughout stimulation. Electrodes were attached by tape and elastic wraps to keep the contact to the skin as constant as possible.

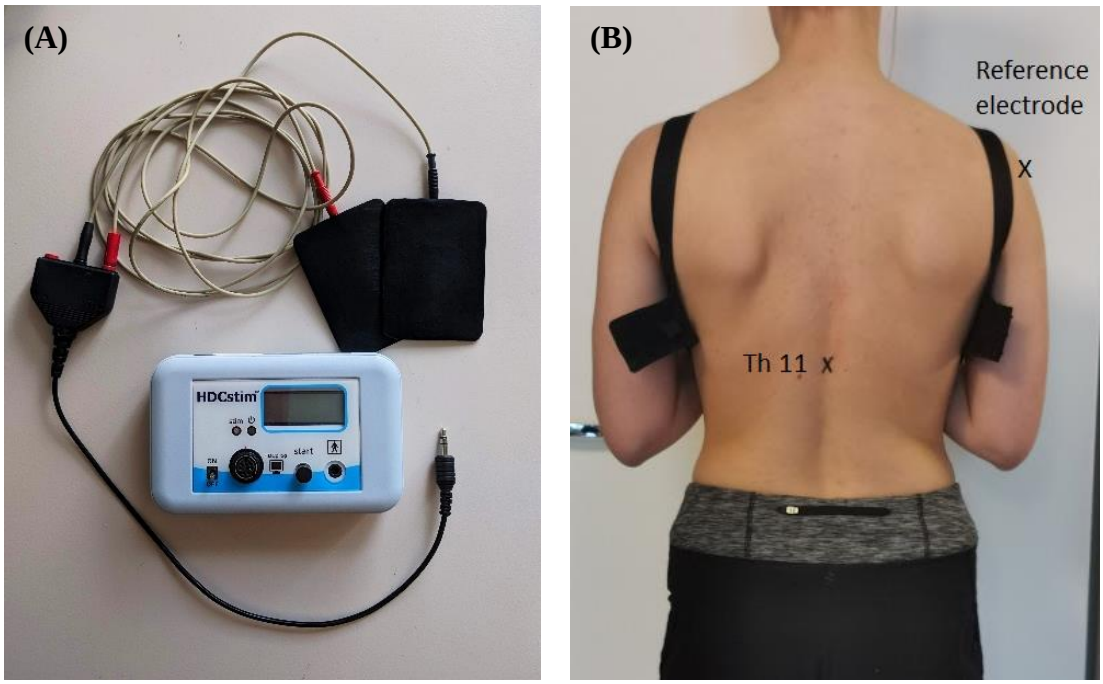

FIGURE 25. (A) HDCstim® direct current stimulator. (B) Anodal electrode was placed over Th 11 and the reference electrode on the right shoulder.

Stimulation intensity was set to 2 mA with a ramp up of 30 s and duration to 20 min. In the sham protocol stimulation was given for 1 min and then turned off. Subject walked with the stimulation for 20 min on the treadmill with the predetermined speed. As stimulation parameters were set to 2 mA for 20 min, and the stimulating electrodes were the size of 7 x 5 cm (35 cm2) the resulting current density was 0,057 mA/cm2 with a total charge of 68,4 mC/cm2. Current density that was used is significantly lower than values that have been seen to cause tissue damage (Bikson et al 2016). Subject were not able to confidently differentiate between anodal and sham conditions. Some subjects reported tingling sensations at the beginning of stimulation and exhibited slight redness under the electrode, which subsided quickly after stimulation.

8.7 Data analysis

All data was processed and exported for further analysis with Spike2 software (CED Ltd, Cambridge, UK). The EMG signal was band pass filtered with 20-500Hz (FIR-filter). Fast Fourier transformations were used in order to check prior noise levels and confirm appropriate filtering results. In standing measurement for TMS and standing H-reflex measurement average background EMG levels (RMS) were analysed from a time window of 100 ms before the stimulus.

Mmax and Hmax values were identified and analysed as Hmax/Mmax and separately for Mmax for both standing and gait results. MEPs were calculated as the mean area under 10 stimuli and normalized to Mmax values and compared within and between protocol. A post hoc analysis was made to determine whether the sum of the TMS MEP areas across intensities would reveal significant differences.

In gait measurements, the relative values from each stimulus were accepted for further analysis if two conditions were met. First, an acceptance range of ±5 % was used to acquire H-reflexes and M-waves in the same stance phase similarly as reported by Simonsen et al (2002). This time frame corresponded to a total time range of 60 ms in a stance phase of 600 ms. Secondly, M-waves were targeted to be within a distribution of 4 % of the predetermined M-wave amplitude. In one case, M-waves during gait were only visible with exceptionally large intensities. Due to concurrent low or absent H-reflex amplitudes we could not confirm that the stimulation was done on the ascending part of the curve and thus was excluded from further analysis. Furthermore, one dataset was excluded from AG pre 80 % and one from SG pre 10 % due to violations of aforementioned criteria.

The soleus H/EMG-gain analysis was conducted to reveal H-reflex amplitude modulation relative to the background RMS analysed from a 60 ms time frame before stimulus artifacts. Furthermore, another analysis was made from the same data after normalizing both the EMG and H-reflex with Mmax. This was done to correct for changes that stem from movement of the muscle fibres relative to the skin electrodes (Ferris et al 2001).

8.8 Statistical analysis

Statistical data analysis was done with IBM SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). Due to the low sample size and non-normal distribution of some of the data revealed with the Shapiro-Wilk test, non-parametric statistical methods were used. The Wilcoxon signed-rank test was used for dependent samples pair analysis and Friedman's test was used when appropriate. All data is presented as means and visualised in line or bar charts with standard deviation in bar charts and standard error in line charts (SD; SE). Significance level was set as <0.05 in all comparisons.

9 RESULTS

H-reflex recruitment curve. The mean RMS values of the rectified and filtered EMG signal 100 ms before the stimulus artifact were compared between the protocol pre and post values with Wilcoxon signed ranks test. The pre and post values were found to be similar in both cases and no statistical differences were found (AG p = 0,484, SG p = 0,779, R p = 0,484). Table 1 shows the mean RMS values with SD and SE for each protocol.

TABLE 1. Mean RMS values mV for each protocol pre and post values with standard deviation.

	Mean	Std. Deviation	Minimum	Maximum
AG_PRE	0,0098425	0,0050044	0,00581	0,02022
AG_POST	0,0094665	0,00563723	0,00356	0,02177
SG_PRE	0,0097459	0,00527985	0,00243	0,0202
SG_POST	0,0102087	0,00676175	0,00191	0,02377
R_PRE	0,0102307	0,00754862	0,00368	0,02586
R_POST	0,0107811	0,0079803	0,00115	0,02728

Mmax values were found to be significantly different in the post measurement in all condition protocols (AG p = 0,050; SG p = 0,050; R p = 0,012) analysed with the Wilcoxon signed ranks test. Post values were revealed to be lower in amplitude and to the extent of 14 % for R protocol and 13 % for AG and SG. Furthermore, the Hmax mean values were revealed to be lower in the post condition with statistical significance (AG p = 0,025; SG p = 0,012; R p = 0,050). However, the Hmax/Mmax relation did not differ between protocols or pre and post values indicating the Mmax values and Hmax amplitudes were indeed decreased in post measurements but changed in a similar manner and did not differ between protocols.

TMS recruitment curve. No statistical differences were found between the mean RMS values (100 ms before stimulus) between the pre and post measurements analysed with the Wilcoxon signed ranks test (Table 2). The aMT threshold did not change between pre and post values (AG p = 0,084; SG p = 0,527; R p = 0,257) and no changes were found between the protocols. TMS areas normalized to Mmax values are found to be significantly different between the pre and post R condition in 90% aMT with the post values being larger in 6 out of 8 instances (see figure 26). No statistical differences were found for RMT values for that protocol and intensity although a slightly larger RMS values were indeed discovered for the post values (pre mean =

,0097 mV; post mean = ,0103 mV). No significance was found in other intensities. Furthermore, the sum of MEP areas across intensities did not reveal statistically significant results.

TABLE 2. Mean RMS mV values for each protocol pre and post values with standard deviation

	Mean	Std. Deviation	Minimum	Maximum
AG PRE	0,0115969	0,00663284	0,00226	0,02354
AG POST	0,0106452	0,00603413	0,00208	0,02226
SG PRE	0,0121021	0,00748098	0,00155	0,02758
SG POST	0,0104723	0,00695062	0,00149	0,0248
R PRE	0,0106793	0,00762173	0,00171	0,02488
R POST	0,0113003	0,00927607	0,00165	0,02911

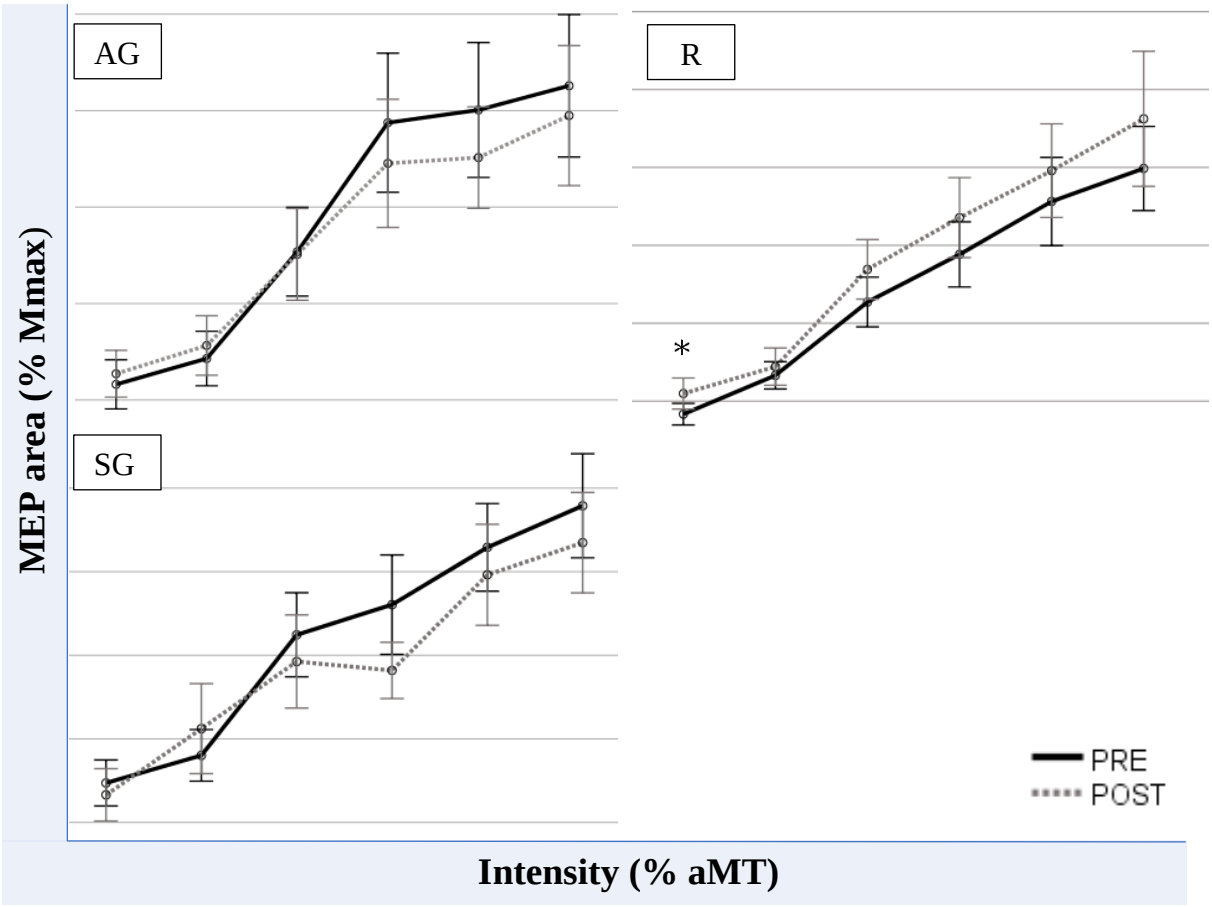

FIGURE 26. TMS recruitment curves. Asterix indicates significance between pre and post values.

Differences between protocols were compared by comparing the percentual changes between protocols. Statistical difference was found between SG and R at 120 % aMT stimulation intensity (p= 0,036). Thus, anodal stimulation at 120 % without the additional walking (protocol R) showed decreased MEP areas in the post condition while the converse was true in SG. This suggests a differential effect of the stimulation direction with anodal stimulation and sham stimulation but only when no additional walking is done before anodal stimulation. No other stimulation intensity showed significant results although a similar trend between R and AG/SG is seen at 110%, 130% and 140%.

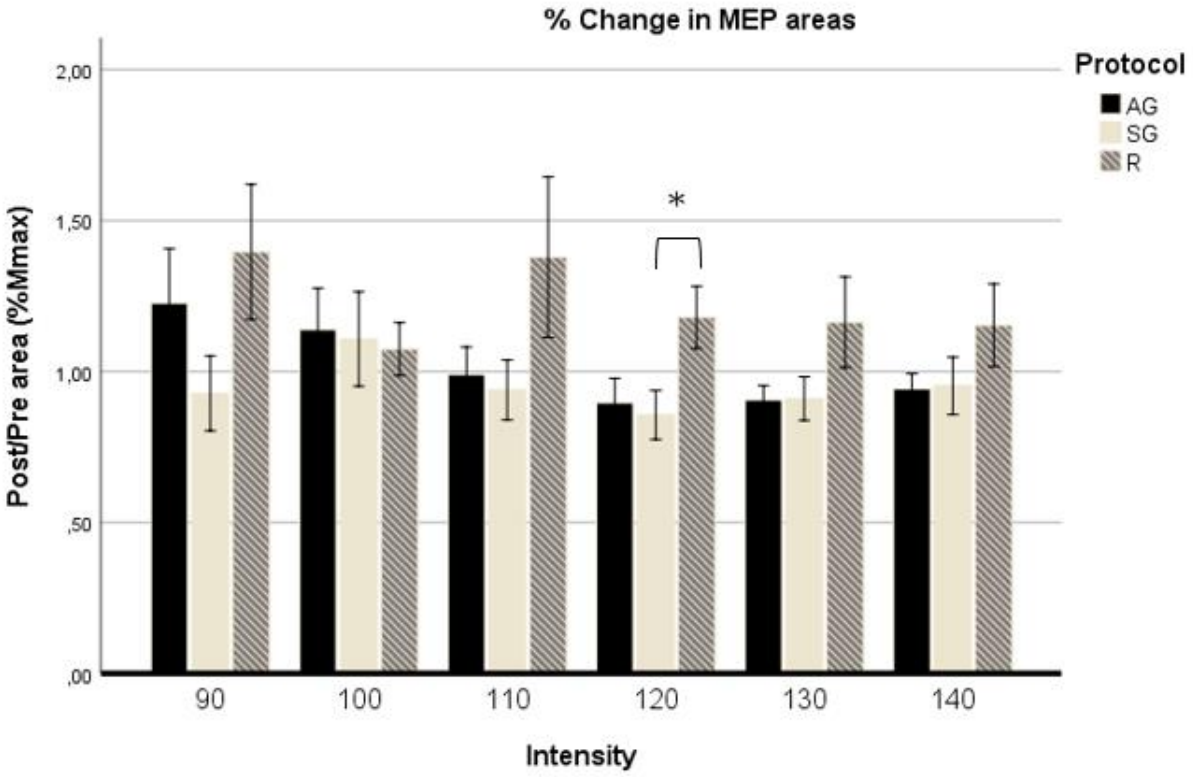

FIGURE 27. Percentual changes in post/pre values between protocols. Values over 1,00 indicate higher post values and under 1,00 indicated lower values after DC-stimulation.

H-reflex gait measurements. In gait protocols (AG and SG), Mmax values were not statistically different between pre and post values in any of the three gait phases. In most subjects, post values showed an effect of depression (table 3.) Interestingly, one subject showed an effect of excitation in both conditions and in all gait phases. Furthermore, the relative change in H/Mmax relation did not reach significance in any phase (see figure 28). Similar results were obtained when comparing the H/Mmax percentual changes as no relative changes were found between the two protocols.

TABLE 3. Subject specific results of the direction of change in Mmax values. Arrow up depicts an increase in Mmax value and an arrow down a decrease.

AG

	Early	Mid	Late
A	↘	↘	↘
B	↘·	↘	↘
C	↘	↘	↘
D	↘	↘	↗
E	↗	↗	↗
F	↗	↘	↗
G	↘	↘	↘

SG

Early	Mid	Late
↘	↘	↘
↘	↘	↗
↘	↘	↗
↘	↘	↘
↗	↗	↗
↘	↘	↘
↗	↘	↘

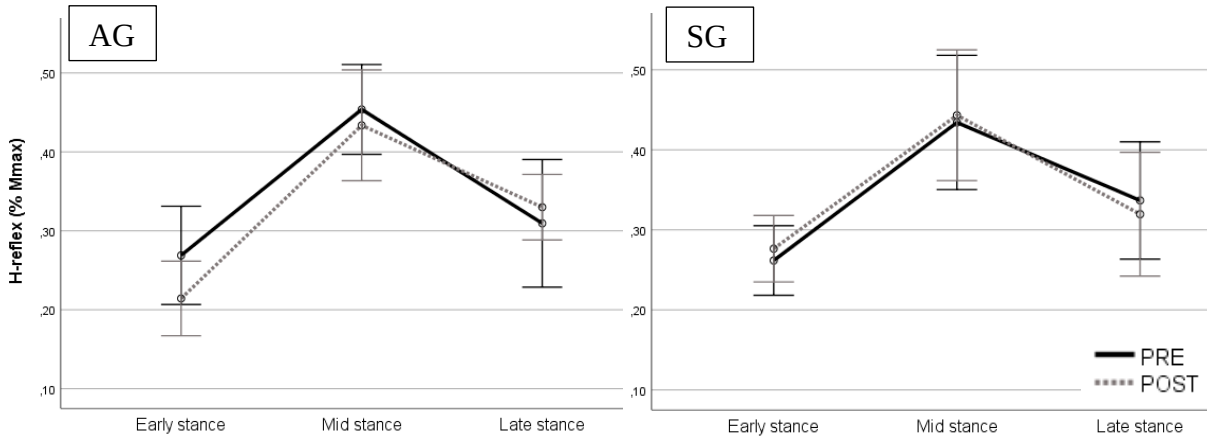

FIGURE 28. Phase dependent H/Mmax values were not statistically changed in AG and SG protocols. The overall shape indicated largest values at mid stance and lowest at early stance.

The H/EMG gain analysis revealed no statistical differences in pre and post values (AG: 10 % p= 0,310; 50 % p= 0,612; 80 % = 0,753) (SG: 10 % p=0,917; 50 % p= 0,866; 80 % = 0,866) or between post/pre relations between protocols. Similar results were attained with normalisation of the H-reflex and RMS to phase dependent Mmax value indicating no changes in H-reflex gain during gait.

10 DISCUSSION

To the knowledge of the author, this study was the first to investigate modulatory changes of low thoracic Ts-DCS in humans during gait. The main results indicate that Ts-DCS could not induce changes in H-reflex during stance phase of walking. Thus, activity during the administration of Ts-DCS could not affect the results over the normal modulatory pattern derived from gait itself. Furthermore, standing H-reflex and corticospinal excitability did not show modulatory effects in a systematic manner despite some results reaching significance. Indeed, a reversal of modulatory direction was seen at 120 % aMT between R and SG conditions and a statistically larger MEPs were at 90 % aMT in R condition. However, this study was unable to reveal effects in any other intensity downplaying the overall implications of these results.

Spinal excitability. The Mmax values during standing conditions were reduced in all conditions with no differences between protocols. As similar changes were found in Hmax values, no changes were seen in the relative Hmax/Mmax indicating that the excitability of the monosynaptic pathway remained unchanged. Result are in accordance with a study conducted by Winkler, Hering & Straube (2010) and Bocci and colleagues (2015). Furthermore, results are in accordance to Kuck and others (2018) as they found Hmax/Mmax to decrease only with one electrode configuration but not with the one closest to the configuration of this study. Thus, the electric field produced by this electrode configuration may not be optimal for inducing regulation of the H-reflex even with active neural circuits that could have potentiated the modulatory effects. However, modulation still might be present in other determinants of H-reflex excitability such as the threshold intensity that were not included in the analysis of this thesis.

Mmax values measured during the three gait phases (early, mid and late stance) did not show significant differences between pre and post values. Although in most cases the trend was a reduction in peripheral excitability, some subjects showed increased Mmax values. Furthermore, the H-reflex excitability (either as a percentage of Mmax or H/EMG gain) was not affected by stimulation in any protocols or between protocols indicating no effect of stimulation on motoneuron pool excitability. The inability of the stimulation to induce changes can stem from many different reasons. Stimulation has been suggested to influence the

threshold levels of neurons differently depending on their orientation, cell size, distance (Ahmed 2014; 2016) and possibly genetic predisposition (Lamy & Boakye 2013).

Interestingly, Mmax values were reduced in standing measurement but not in gait measurements. The reduction of Mmax in itself has been demonstrated during the course of long measurements (Crone 1999), which is why Mmax was controlled before every measurement pre and post stimulation. Crone and colleagues found that in some subjects, the reduction of Mmax values might be caused by the stimulation itself. This notion might support our observations. That is, if reductions would be caused early by the pre standing measurements no changes would be revealed between pre and post gait measurement if no further reduction or modulation is present. Furthermore, similar to our results, Crone and others (1999) noted differences between subjects as some did not show depression at all and some at other instances. The amount and timing of the change in Mmax amplitudes thus likely contributed to the reported interindividual responses of Mmax depression. Possible reasons for the Mmax depression may include changes in the underlying muscle architecture due to walking as tendon tissue compliance has been reported to increase during walking (Cronin et al 2009).

Although the continuous walking bouts were for 20-30 min at a time with rest in between, it is possible that fatigue could have impacted measurement as it was not directly controlled for. In submaximal conditions, fatigue induces an increase in the EMG-force coefficient (Avela & Komi 1998). EMG levels in this study were seen to remain constant between pre and post conditions at least in the pre stimulus timeframe. However, forces were not investigated. Thus, reductions in ground reaction forces during gait, if present, could still suggest induced fatigue even with similar EMG levels. Furthermore, Avela and Komi (1998) suggested that fatigue after a marathon run is likely influenced by a reduction of the sensitivity of the stretch reflex. Also, repeated stretch shortening cycles during walking are reported to induce an increase in the compliance of tendinous tissue and thus cause concurrent changes in sensory feedback. That is, as extrafusal muscle fibres would experience less stretch with more compliant tendon, muscle spindle responsiveness would decrease (Cronin et al 2009).

Thus, continued walking would be hypothesized to induce a reduction of the H/M relationship (Hoque et al 2018) with fatigue (Avela & Komi 1998) and without (Cronin et al 2009). Our protocol, however, did not show such effects, which can be caused by the protocol not being optimized for the purpose of revealing specifically gait related depression (as opposed to DC

dependent effects). H-reflex stimulation was done in one phase at a time with randomized order. For example, the stimulation for late stance phase in premeasurements might be stimulated starting from 20 min after first stimulations and as such H/Mmax could already be depressed showing no large differences between post measurements. As mentioned, results indicated to large interindividual differences which might therefore be somewhat related to this issue. However, depression has been reported to be further reduced at 40 min compared to 20 min (Hoque et al 2018), which might have shown in results. However, our protocol did consist of standing measurement in between gait interrupting constant walking bouts, which also might have affected inability to see gait dependent H/Mmax depression.

Corticospinal excitability. It can be suggested based on these results, that anodal stimulation may affect the direction of excitability differently when compared to gait related modulation seen in sham condition. Indeed, anodal stimulation increased post MEPs and sham stimulation decreased them. However, only one stimulation intensity managed to reach significance (120 % aMT). Nonetheless, there seems to be a similar trend towards increased MEPs at post condition at intensities ranging from 110-140 during anodal stimulation with paradigm R. One might thus hypothesize that additional walking could overrun the modulation as no differences were seen in AG compared to SG and they did not exhibit a similar trend. Furthermore, as no changes were seen in protocol SG, gait itself did not affect corticospinal excitability. However, as large interindividual differences were present and no other significant results were obtained, the overall modulatory effects should be further investigated with larger sample sizes and/or increased homogeneity of the test population.

Previous studies have reported an increase in MEPs after cathodal stimulation at 120 % rMT (Bocci 2015; Murray et al 2018) and an increase in rMT values with anodal stimulation while leaving MEP areas unchanged. (Bocci et al 2015). Our result did not show changes in aMT as seen in the study by Bocci and colleagues (2015) but similar results were obtained for MEP areas, thus, indicating results to be partly in accordance to earlier studies. At subthreshold intensities, however, anodal R stimulation was found to increase EMG area. Subthreshold TMS has been reported to induce a suppression of EMG in active muscles due to activation of inhibitory neurons. (Davey et al 1994). Thus, a reduction of these inhibitory neurons may increase the EMG area as reported here and thus could indicate cortical modulation. However, in our experiments, the timeframe for analysis may have included a few small MEPs with subthreshold aMT value and thus may not directly compare to Davey and others (1994).

Therefore, it would be interesting to see whether results would remain with more subthreshold intensities even in the absence of MEPs.

It has been suggested that active neurons could be more predisposed to modulation (Bikson & Rahman 2013). However, a study by Murray and colleagues (2018) revealed position dependent changes in modulatory results with Ts-DCS as they were only seen during lying down and not during sitting. Furthermore, as H-reflexes have been demonstrated to be downregulated in standing compared to sitting or lying down with similar background EMG-levels (Cecen et al 2018; Cattagni et al2014.), it could be that modulatory effects are not strong enough to induce changes in active circuitries with increased inhibition. Based on our results, however, no direct conclusions can be made on the effectivity of standing vs lying down and activity vs resting conditions. Specific studies to reveal these influences should be conducted in the future.

Limitations. The effects of anodal trans-spinal direct current stimulation were studied for the first time in active conditions. This setup poses some limitation and possible larger error margins compared to studies that use testing during rest. Slight changes in background EMG levels might contribute to seen effects even though the mean values of EMG levels were comparable between conditions. This could be a potential contributor essentially when modulatory responses are small. Thus, errors associated with measurement during activity might be a potential reason behind why only some of the measures showed statistical significance while most other did not. Further studies with more strict methods should be conducted to reveal these contributions. Furthermore, the step cycle can change during treadmill walking with concurrent changes in muscle activity, which could influence the effects seen here. However, normalisation of the H-reflexes to EMG levels did not influence the results and as such it is unlikely that changes in background EMG levels contributed to the results.

The start of the descending part of the H-reflex recruitment curve can sometimes precede the rise of the M-wave (Pierrot-Deseilligny & Mazevet 2000), which was also seen in some subjects in this study. Indeed, as has been demonstrated that H-reflex not only depends on the excitability of monosynaptic pathways, polysynaptic pathways have able time to contribute (Burke et al 1983) especially for higher threshold motoneurons. Marchand-Pauvert and others (2002) provided evidence that disynaptic inhibition originating from the test volley can limit the size of the H-reflex. In relation to this effect, in some cases, the stimulation of H-reflexes could not be confirmed to be on the ascending curve during gait but rather near or at Hmax. As

Hmax has been criticized not to be sensitive to inhibition and facilitation (Knikou 2008; Pierrot-Deseilleigny & Mazevet 2000) further studies are needed to shed more light on the matter. The results thus pose limitation of the effect of H-reflex monosynaptic modulation.

During free standing, the H-reflex has been reported to be inhibited or facilitated depending on the direction of postural sway (Johannsson, Duchateau & Baudry 2015; Tokuno et al 2007). The measurement protocol here, however, did not account for changes in centre of pressure as would have been possible with force plates. While Johannsson, Duchateau & Baudry (2015) reported only a 2 % change in H/Mmax values due to changes in displacement, Tokuno and colleagues (2007) reported a more significant 12 % change in SOL Hmax/Mmax. Thus, although real time EMG signals were observed at all stimulation instances counteracting for large changes background EMG, changes associated by the effects of sway cannot be totally neglected.

Temporal pattern, more specifically, the diminution of modulation responses by the function of time is also a possible reason for the lack of effects during walking. Albuquerque et al (2018) showed modulatory responses to persist for at least 50 min after stimulation end when a 20 min walking bout was done after stimulation. However, they did not control for the effects without walking so no conclusion about the effects of walking can be directly made. Nonetheless, stimulation effects in other studies have reported to persist for 20 min (Cogiamanian 2008) 30 min (Lamy & Boakye 2013) and up to 45-60 min after stimulation end according to Kaczmarek and others (2017). However, activity in between stimulation times can be a possible modulator for the temporal pattern, thus, the direct comparison of previous studies may not be favourable.

11 CONCLUSIONS

Trans-spinal direct current stimulation is a relatively new method that has been reported to influence ascending (Cogiamanian 2008; 2011) and descending neural pathways (Bocci et al 2015a; Murray et al 2018). Results, however, have highlighted its modulatory effects only when administered during rest and tested in resting muscles. Thus, there is no direct indication in humans how the active nervous system would affect results during administration of Ts-DCS. This study was conducted to reveal if modulation would persist during matched neural activity. Results show that Ts-DCS could not influence the H/M relationship of the H-reflex gain during gait or standing conditions indicating no changes in the monosynaptic pathway. However, results mainly highlight that no modulation was observed of Hmax/Mmax, thus, modulation on other variables of the H-reflex might still be present, which warrants further research.

TMS measurements during standing revealed an increase in EMG area after anodal Ts-DCS at 90 % aMT only without gait measurements suggesting that additional walking before stimulation might override modulatory effects or otherwise affect them. No changes were seen in other intensities. The effects of Ts-DCS on subthreshold transcranial stimulation on EMG depression should be further investigated with methods more equipped for that purpose. Although a trend towards increased MEPs in post measurement were seen in R conditions and decreased MEPs for SG and AG, differences reached statistical power only at 120 % between R and SG. Despite these results, it is concluded that Ts-DCS did not modulate corticospinal or spinal excitability in a largely systematic manner.

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