AALBORG UNIVERSITY
DENMARK

The effect of walking supported by elicitation of the nociceptive withdrawal reflex on the corticospinal pathways

4th semester master, in Biomedical Engineering and Informatics, Master Thesis

Group 17gr10404
Marjolein E.G. Thijssen and Petr Šipka

Aalborg University
Department of Health Science and Technology
Spring 2017

In the present study the following tools and software were used to produce results and figures: MATLAB®, IBM SPSS©, Mr. Kick III and Brainsight®.

Department of Health
Science and Technology
Aalborg University, Denmark
http://www.smh.aau.dk

AALBORG UNIVERSITY
STUDENT REPORT

Title:
The effect of walking supported by elicitation of the nociceptive withdrawal reflex on the corticospinal pathways

Theme:
Master's thesis

Project Period:
Spring Semester 2017

Project Group:
17gr10404

Participants:
Marjolein E.G. Thijssen
Petr Šipka

Supervisors:
Erika Spaich
Natalie Mrachacz-Kersting

Page Numbers: 84

Date of Completion:
7th of June 2017

Abstract

Introduction: Despite the high incidence of stroke, the mortality following stroke is decreasing [Schmidt et al., 2014]. The increasing number of stroke survivors is causing an increased demand for new rehabilitation techniques improving their quality of life [Bushnell et al., 2014]. One of the most limiting impairments of stroke survivors are gait impairments. Regaining the ability to walk is therefore one of the main goals of the rehabilitation [Olney and Richards, 1996]. New techniques are being proposed and one of them is electrical stimulation on the sole of the foot, which elicits the withdrawal reflex, to initiate and facilitate the swing phase [Spaich et al., 2014]. This technique was found to be effective in rehabilitating gait in stroke patients [Spaich et al., 2014]. However, the effect of the activation of the withdrawal reflex on the cortical and subcortical pathways is unknown. Better understanding of the underlying mechanism could lead to improvements of the treatment. In the present study, the effect of walking with electrical stimulation to activate the withdrawal reflex on the corticospinal and spinal pathways in healthy subjects was investigated.

Methods: A total of 17 healthy subjects participated in the experiment. 9 subjects were placed in the intervention group and walked 30 minutes on a treadmill with electrical stimulation on the sole of the foot at heel-off which activated the withdrawal reflex. 8 subjects were placed in the control group and walked 30 minutes on a treadmill without stimulation. Measurements of corticospinal and spinal excitability were carried out before, immediately after and 30 minutes after treadmill walking. All measurements were recorded from the tibialis anterior (TA). Single pulse TMS (transcranial magnetic stimulation) was used to asses conrticospinal pathways which led to I/O (input/output) curves. The different parameters of the I/O curves were analyzed. Stretch reflexes were mechanically induced to asses spinal excitability. The amplitude and latency of the first and the second component of the stretch reflex were analyzed. Two-way repeated measures ANOVA was used to analyze the effect of time and group on the parameters of the I/O curves and on the amplitude and latency of the first two components of the stretch reflex.

Results: The rMT (resting motor threshold) showed a significant decrease between measurements immediately post and 30 minutes post intervention (Mean difference (MD): 3.7 $\pm$ 4.4 %). The MEP_{max}-value of the I/O curve showed a significant decrease immediately post (MD: 127,2 $\pm$ 206.1 μV) and 30 minutes post (MD: 110.6 $\pm$ 120.9 μV) intervention compared to the baseline measurements. Other parameters (Slope K, S_{50}) of the I/O curves did not show any significant differences over time. No significant effect of group on any of the outcome measures was found. Analysis of the first component of the stretch reflex showed a significant decrease of the peak amplitude immediately (MD: 85,2 $\pm$ 93.3 μV) and 30 minutes post (MD: 74 $\pm$ 70.4 μV) intervention compared to baseline. Analysis of the second component of the stretch reflex showed a significant decrease in peak amplitude between pre and 30 minutes post intervention (MD: 108.4 $\pm$ 185.3 μV). The latencies of both analyzed stretch reflex components did not show any significant differences over time and no significant difference was found between the groups for any of the outcome measures of the stretch reflex.

Conclusion: The study did not found any difference between the two groups for any of the measured outcomes, but did found changes over time for the MEP_{max}-value of the Boltzmann fit and the first and second component of the stretch reflex. This suggests that treadmill walking itself can modify (decrease) the excitability of corticospinal/spinal pathways. However, further research to investigate the effects of longer training period is needed to see if changes occur as a consequence of the activation of the withdrawal reflex.

Contents

List of Figures

Page

List of Tables

Page

Preface

This project was conducted during the last semester of the master in Biomedical Engineering and Informatics at Aalborg University. The project was carried out from February 2017 until June 2017.

The purpose of the project was to investigate the effect of walking with the activation of the withdrawal reflex of the lower limb on the corticospinal pathways.

The Harvard method was used for references within the present report. Reference can be used as an active part of the sentence, e.g. *"The figure was derived from Petersen et al. [1998]."*. The reference can also be found before the full stop if it is referring to the sentence or after the full stop if it is referring to the section. The following examples demonstrate the applied rules.

When citing a sentence, the reference will be found before the full stop. Example:

> *One of the consequences of hemiparesis is a disturbance and impairment of the normal human gait and reduced ability to walk for long distances [Dickstein et al., 2004].*

When all the content of a paragraph belongs to one source, the reference is found after the paragraph. Example:

> *The withdrawal reflex can be activated by applying an electrical, mechanical or radiant heat stimuli. Categorization of the stimulus being painful or tactile is often used to determine which fibers are activated. However, when either an electrical or mechanical stimulus is applied, which causes a painful sensation, both the nociceptive (aδ) afferents as well as the mechanoreceptive afferents are elicited. [Pierrot-Deseilligny and Burke, 2012]*

Figures without any references in the caption are self-made.

Acknowledgment

We would like to express our gratitude for both of our supervisors, Erika G. Spaich and Natalie Mrachacz-Kersting, for their positive feedback and help during the set-up of the experiments. Moreover, we would like to thank all the subjects who participated in this study. Without them this project would not have been possible.

List of Abbreviations

- ALS - amyotrophic lateral sclerosis
- ANOVA - analysis of variance
- AP - action potential
- APA - American Psychological Association
- BCI - brain-computer interface
- BGA - background activity
- CPG - central pattern generators
- CS - conditioning stimulus
- EEG - electroencephalogram
- EMG - electromyography
- FES - functional electrical stimulation
- H-reflex - Hoffman reflex
- I/O curve - input/output curve
- ICF - intracortical facilitation
- ISI - interstimulus interval
- K - slope of the Boltzmann fit
- LICI - long-interval intracortical inhibition
- M - mean
- MD - mean difference
- MEP_{max} - maximum MEP of I/O curve
- MEP - motor evoked potential
- MT - motor threshold
- MVC - maximum voluntary contraction
- NMDA - N-methyl-D-aspartate
- PCF - proprioceptive neuromuscular facilitation
- PET - positron emission tomography
- RMS - root mean square
- RMSE - root-mean-square error
- S_{50} - intensity needed for eliciting 50% of MEP_{max}
- SCI - spinal cord injury
- SD - standard deviation
- SICF - short-interval intracortical facilitation
- SICI - short-interval intracortical inhibition
- SP - silent period
- SSE - sum of squared errors
- TA - Tibialis Anterior
- TASS - transcranial magnetic stimulation adult safety screening
- TMS - transcranial magnetic stimulation
- TS - test stimulus
- aMT - active motor threshold
- cSP - contralateral silent period
- fMRI - functional magnetic resonance
- fNIRS - near-infrared spectroscopy
- iSP - ipsilateral silent period
- ppTMS - paired pulse transcranial magnetic stimulation
- rMT - resting motor threshold
- rTMS - repetitive transcranial magnetic stimulation

Chapter 1
Introduction

Stroke ranks in the top five among all causes of death in the USA [Koton et al., 2014; Kleindorfer et al., 2010]. According to Go et al. [2014] stroke was the cause of death in 1 out of 19 deaths in the USA in 2010. Even though stroke incidence has decreased in the past two decades [Koton et al., 2014], the numbers are still high. In the USA, approximately 800.000 people experience a stroke every year of which 75 % are new events and 25 % are recurrent [Go et al., 2014; Koton et al., 2014]. In Europe, the incidence rate of stroke in the 21st century is 95-290/100.000 inhabitants per year [Béjot et al., 2016]. Despite the high incidence rate, the mortality following stroke is rapidly decreasing. In Denmark, the 30-day post stroke mortality rate dropped by approximately 45 % for ischemic stroke and by 35 % for hemorrhage between the years 1994-1998 to 2009-2011 [Schmidt et al., 2014].

Due to the decreasing mortality rate following stroke, increasing attention is being given to improve the quality of life for stroke survivors [Bushnell et al., 2014]. Patients after stroke suffer from impairments in both psychological and physical quality of life. Stroke survivors often feel as a burden for their family, are discouraged about their future, have troubles remembering things and some report changes in personality. They also experience difficulties with performing daily work around the house, taking a bath or buttoning buttons. One of the main impairments affecting their independence is a gait impairment. [Reeves et al., 2015]

Regaining the ability of normal walking is therefore one of the main goals for post-stroke rehabilitation [Olney and Richards, 1996]. Although there are many conventional physiotherapeutic approaches which are currently being used, they only provide limited recovery [McCrimmon et al., 2015]. Researchers are therefore looking for new methods and approaches in rehabilitation which would be more effective and speed up the recovery after stroke [Belda-Lois et al., 2011]. Most of the newly investigated approaches, such as electromechanical assisted training [Mehrholz et al., 2013] (e.g. robotic gait training [Pennycott et al., 2012]), virtual reality therapy [Lohse et al., 2014] or therapeutic electrical stimulation [Bosch et al., 2014], are used in addition to physiotherapy.

In the area of electrical stimulation, it is mainly functional electrical stimulation (FES) which is being used and investigated. FES in gait rehabilitation after stroke is for example used for stimulation of motorneurons of the muscles which are responsible for dorsiflexion [Kottink et al., 2004]. The dorsiflexion muscles are often impaired after stroke and many rehabilitation techniques are therefore targeting them [Kottink et al., 2004]. Another effective way of achieving dorsiflexion with electrical stimulation is eliciting the withdrawal reflex on the sole of the foot [Andersen et al., 1999; Spaich et al., 2004b,a]. The combination of physiotherapy and electrical stimulation eliciting the withdrawal reflex was found to improve the general walking ability of sub-acute hemiparetic patients with severe gait impairments [Spaich et al., 2011, 2014].

Even though the effect of rehabilitation using electrical stimulation is unknown, various forms of training with electrical stimulation were found to drive cortical changes [Kimberley et al., 2004; Michou et al., 2014; Rushton, 2003]. However, the effect of training with electrical activation of withdrawal reflex on corticospinal pathways has not been investigated and thus the mechanism underlying the effectiveness of this treatment is unknown. It is unknown if similar cortical changes happen after electrical stimulation of the withdrawal reflex as was found in studies of Kimberley et al. [2004]; Michou et al. [2014]; Rushton [2003] which used various forms of electrical stimulation as well (e.g. FES [Rushton, 2003], paired associative stimulation [Michou et al., 2014] or neuromuscular electrical stimulation [Kimberley et al., 2004]). Further research could give a better understanding of the underlying mechanism which could lead to improvements of the treatment.

This introduction initiates the following questions which will be answered in the problem analysis of this report:

- *How does stroke affect the normal gait?*
- *What rehabilitation techniques are being used?*
- *How does the activation of withdrawal reflex work?*
- *What techniques are used to asses (sub)cortical changes?*

Part I

Problem Analysis

Chapter 2
Locomotion

2.1 Normal Human Gait

Locomotion in general is the process of moving from one position to another (walking, running, crawling, hopping, etc.). It is composed of various transient activities which all together create a basic pattern. In walking this pattern is a periodical motion of the lower limbs ensuring a constant movement of the whole body. Unlike most animals, who are quadrupedal and can therefore use the stability of a tripod during walking, human gait is bipedal. Upright gait requires higher neural control as well as instinct and prolonged intensive learning in comparison to the quadrupedal gait. [Rose and Gamble, 2006]

There are differences in gait among individuals as we can recognize a person by his/her manner of walking. The manner of walking of tall people differs from short people and it can be also altered e.g. by wearing shoes with heels. Despite the fact that gait is highly individual, there are characteristic patterns which are common to all people and those will be described in following section. [Troje et al., 2005]

2.1.1 Kinematics of Normal Human Walking

Human locomotion can be divided into three stages. The first is a *development stage* which is describing the transition from rest to velocity. The second is a *rhythmic stage*, describing the movement with a constant mean velocity and the third is a *decay stage* describing the transition back into rest. The most studied stage is the rhythmic stage of people walking with their preferred speed. It was found to be very consistent over time with individual optimal efficiency. In addition, different periodically repeating events were identified and those together create the gait cycle. [Rose and Gamble, 2006]

The gait cycle is defined as the period during which a set of events is completed. Any event could be chosen as a start of the cycle, however, initial contact of the foot with the ground is generally considered as the starting point. Usually it is the contact of the heel. However, in pathological gait it can be another area of the foot such as the toe which is the initial contact. [E.Ayyappa, 1997]

The whole gait cycle consists of two phases; stance phase and swing phase. The gait cycle is often described in percentage, rather than in time intervals. The events occurring during the cycle can be seen in figure 2.1. [Gage et al., 1995]

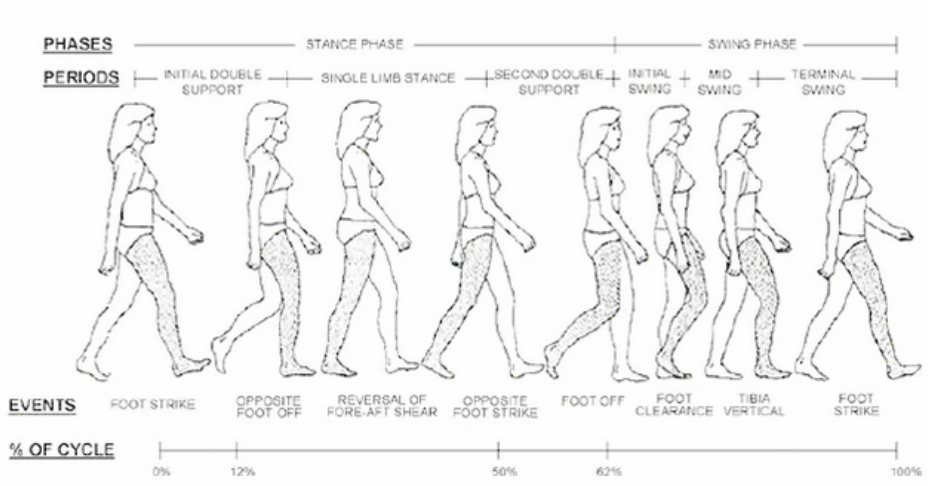


Figure 2.1: Illustration of the events occurring during the normal gait cycle. Figure was adopted from Rose and Gamble [2006].

The stance phase is the time when the foot is in contact with the ground and it lasts for approximately 62 % of the whole gait cycle. The stance phase is composed of three intervals. The first is the *initial double support* when the front limb (i.e. the one which just landed on the floor) is rapidly loaded with weight after the initial contact. The foot usually gets into a flat position and the knee absorbs the energy. Simultaneously, the opposite foot is preparing for swing and the load on the other limb is further increased. The moment when the opposite foot leaves the ground the second interval, *single limb stance*, begins. As the opposite foot is in its swing phase, the load on the weight-bearing foot is increased to maximum. Single limb stance takes up to 30 % of the whole cycle and once the opposite foot terminates the swing phase and makes contact with the ground, the third interval of stance, *second double support* begins. Weight is being transferred on the opposite limb and the first limb is preparing for swing. [Hughes and Jacobs, 1979; Gage et al., 1995]

The end of the stance phase, when the leg is preparing for the swing, is known as push-off. Push-off consists of heel-off and toe-off [Hughes and Jacobs, 1979]. At this stage, the ankle is actively plantar flexed which leads to the heel-off. The more the opposite limb is being loaded, the weaker is the plantarflexion [Winter et al., 1990]. Due to the plantarflexion of the toes, the knee is passively flexed. In the end of the push-off, the knee is rapidly flexing to which further results in toe-off [E.Ayyappa, 1997]. At the same time, the hip flexes into neutral position and the leg is ready for the initial swing.

The swing phase is the time when the foot is in the air and it lasts for the remaining time of the whole cycle (38 %). Swing can as well be subdivided into three intervals. During the first, *initial swing*, the foot is leaving the ground due to the action of pretibial muscles, long toe extensor and additional knee flexion. During the second, *mid swing*, the limb is moved forward in front of body and in the third, *terminal swing*, the limb slows down and prepares for a new weight load. [Hughes and Jacobs, 1979]

2.1.2 Motor Control

Walking requires a synchronous activation and deactivation of many muscles. This is allowed through a complex neural network which is innervating the muscles. All voluntary movements result from the neural activity of the central nervous system. At first, there is the imagination of the movement at the cerebral cortex which communicates with the motor cortex. The motor cortex is located in the precentral gyrus and it is the place where the muscle activating action potentials (AP) are generated. The organization of the different body parts in the sensory-motor cortex is known and it is graphically represented by sensory-motor homunculus as displayed in figure 2.2. [Kandel, 2013]

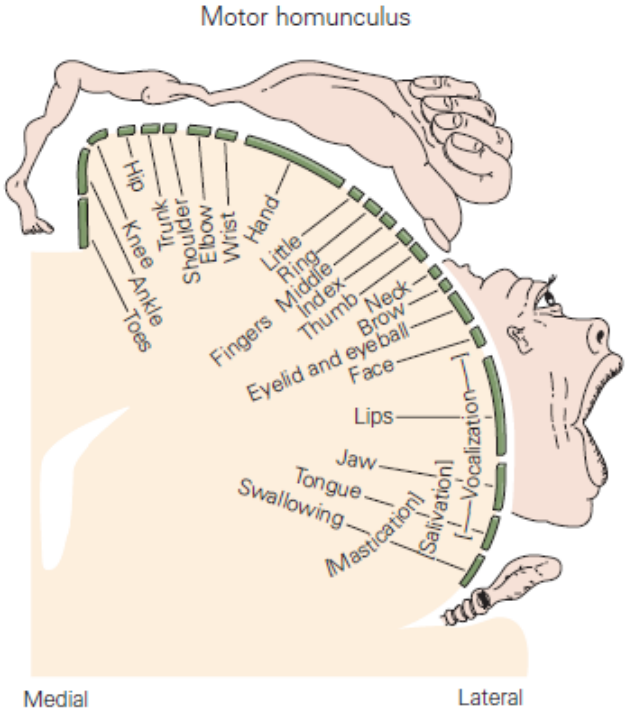


Figure 2.2: Illustration of the homunculs in motor cortex with all body parts encoded there. Figure was adopted and adjusted from Kandel [2013].

Once the AP is generated in the motor cortex, the AP travels in the motor neuron through the brainstem and spinal cord where it is connected with a second motor neuron in the spinal cord. From this place, the signal travels further into the α-motor neurons which are connected with the muscle or muscle group. The signal can be modified at each level of the process. [Rose and Gamble, 2006]

The development and decay stages of walking are coordinated from supraspinal regions. However, for rhythmic movements such as the rhythmic stage of walking, where certain patterns are repeated, the supraspinal or sensory inputs are not necessary. It was indeed proven in several studies that decerebrated cats or cats with spinal cord injuries were able to walk once the walking was externally initiated (e.g. lateral hypothalamic stimulation [Grillner and Rossignol, 1978]) [Rossignol, 2000; Jordan, 1998; Grillner and Rossignol, 1978; Shik and Orlovsky, 1976]. However, in humans and primates the evidence of only spinal control is not so clear and it is therefore suggested that there is also supraspinal contribution [Nielsen, 2003]. These rhythmic patterns can be produced by the neuronal circuits in the spinal cord and can still be modified by supraspinal brain signals. Furthermore, the neuronal circuits in the spinal cord do not require sensory inputs for generation of rhythmic patterns. These networks are called 'central pattern generators' (CPG). For safe walking, it is important to receive sensory feedback. Based on the sensory input, the walking pattern can be prolonged and modified e.g. prolonging steps to avoid obstacles. It can also contribute to the corrective reflexes, which are following sudden deviations. [Nielsen, 2003; Kandel, 2013]

2.2 Gait Impairments Following Stroke

Stroke is a consequence of a hemorrhage or thrombosis affecting the blood supplies, usually of one side of the brain [Olney and Richards, 1996]. The lack of blood supplies results in damage of brain cells and pathways of the central nervous system [Olney and Richards, 1996]. About 88 % of stroke survivors suffer from hemiparesis, which is a weakness on one side of the body due to damage of the motor cells [Bonita and Beaglehole, 1988]. One of the consequences of hemiparesis is a disturbance and impairment of the normal human gait and reduced ability to walk for long distances [Dickstein et al., 2004]. In the first week after stroke, about 37 % of stroke survivors can walk independently and

50 % is unable to walk, while after 11 weeks of rehabilitation 50 % can walk independently and 18 % is still unable to walk [Balaban and Tok, 2014].

A common post-stroke impairment is a decreased strength in the muscles. The affected side shows lower amplitudes than the unaffected [Olney et al., 1991]. Some even lack the ability to produce voluntary muscle contractions [Olney et al., 1991]. This can be due to decreased capacity to activate the motor units [McComas et al., 1973] or reduced firing rate of motor units [Tang and Rymer, 1981]. Especially the weakness in the TA is a major problem causing 'drop foot', which is the inability to dorsiflex the foot during the swing phase leading to additional compensatory movements, restricted mobility and increased risk of falls [Kluding et al., 2013].

In addition Den Otter et al. [2007] found a prolonged muscle activation of biceps femoris and tibialis anterior (TA) in hemiparetic subjects when compared to healthy controls. The increased activations were found on both, paretic and nonparetic leg. The duration of muscle activity during the different phases of the gait cycle is prolonged for the muscles of the paretic leg [Den Otter et al., 2007].

Gait impairments are manifested in several parameters. The walking speed of post-stroke patients is radically reduced to 0.18 - 1.03 m/s, [Wade et al., 1987; Knutsson and Richards, 1979; Olney et al., 1994] while comfortable walking speed of healthy adults was found to range between 1.2 m/s and 1.5 m/s [Bohannon, 1997]. Furthermore, the step length of both limbs is approximately the same in healthy adults, whereas in post-stroke patients, the step length of the two limbs is asymmetric [Balasubramanian et al., 2007]. There has not been observed any pattern in the asymmetry, some patients have longer steps lengths on the paretic leg while others on the non-paretic leg and it is assumed that it is due to different compensatory strategies [Kim and Eng, 2003]. The temporal characteristic is also changed. The stance phase is prolonged on both the affected and unaffected sides and it also occupies a large proportion of the whole gait cycle in comparison to healthy adults [Olney et al., 1991]. The difference in duration of stance phase was also found between the affected and unaffected side, where the stance phase of unaffected side is longer and occupies large proportion of the cycle in comparison to the affected side [Olney et al., 1991]. In addition, in healthy subject the time of single support during the gait cycle is also the same for both limbs, whereas in patients after moderate stroke the time of the single support of the unaffected limb is prolonged in comparison to the affected limb [Wall and Turnbull, 1986].

As a consequence of the impaired mobility, stroke survivors also report decreased quality of life. According to Hackett et al. [2005] 33 % of all stroke survivors suffer from depression due to lower functional independence. Regaining the ability of independent walking and improving the impaired walking parameters is the objective of gait rehabilitation.

Chapter 3
Gait Rehabilitation in Stroke Patients

The main goal of the rehabilitation programs of post-stroke patients is the gait recovery so the patient is able to walk independently and manage to perform daily activities such as doing daily work around the house or taking a bath [Reeves et al., 2015].

The classical gait rehabilitation techniques, such as different physical therapies, are based on bottom-up approaches, meaning that they work on the physical level, trying to influence the neural system. All these techniques require individually designed exercises assisted by a physical therapist or under the supervision of a physical therapist. An alternative to the bottom-up approaches are top-down approaches, when the rehabilitation therapy is defined based on the state of the brain after the stroke. [Belda-Lois et al., 2011]

Since conventional gait training based on physiotherapy does not restore normal gait in many post stroke patients, new ways of rehabilitation were developed [Dohring and Daly, 2008]. The following chapter analyzes the most used methods of gait rehabilitation beside the conventional physical therapy.

3.1 Neurophysiological Techniques

In neurophysiological techniques, the patient is a passive recipient, while the physiotherapist is the one who supports the correct movement patterns and acts as the decision maker and problem solver. The most popular treatment approach, but not superior to others, is the Bobath method. The Bobath method tries to inhibit muscle spasticity, which is seen in 30 % of stroke patients [Thibaut et al., 2013], by activation of tactile and proprioceptive stimuli. [Kollen et al., 2009]

3.2 Motor Learning Techniques

Motor learning techniques are based on active practicing of context-specific motor tasks and related feedbacks which induce motor learning and support the recovery. The training is targeting only specific tasks relevant to the goal of the rehabilitation. One of these methods is the Perfetti method. [Langhorne et al., 2011]

A Cochrane review by Pollock et al. [2007] identified many neurophysiological and motor learning rehabilitations techniques which are being used. Some examples of the neurophysiological techniques are e.g. Bobath method, Brunnström method, Rood method and Proprioceptive Neuromuscular Facilitation (PNF) approach [Pollock et al., 2007]. There are also various forms of motor learning techniques as used by e.g. Carr and Shepherd [1989], Salbach et al. [2004], McClellan and Ada [2004], Dean and Shepherd [1997]. The review by Pollock et al. [2007] found that these techniques and their mix are more effective than no treatment. However, it could not be concluded that any of the approaches is more effective than others. Furthermore, there is an evidence that intensive high repetitive task-specific and task-oriented training rapidly enhance the trained function or activity [Veerbeek et al., 2014; Salbach et al., 2004]. In addition, it is broadly accepted that the rehabilitation should start as soon as possible after stroke [Bernhardt et al., 2009].

3.3 Electromechanical Devices

Another approach using high repetitive task-specific and task-oriented training in gait rehabilitation is the use of electromechanical devices which is mainly used in the rehabilitation of post-stroke pa-

tients with mild to severe motor impairments. Electromechanical devices allow safe task-oriented training with the reduction of the need for physical assistance of a therapist. Electromechanical devices also provide a precise control of the executed movement, objective quantification of the patients performance or increased motivation due to the use of interactive biofeedback. [Belda-Lois et al., 2011]

The general goal for electromechanical devices is to assist or correct the movements of the subject. The devices for gait rehabilitation often focus on task-specific repetitive movements which increase muscular strength, coordination of the movement and retrain locomotion [Fasoli et al., 2004]. The devices can be constructed as simple walking aids; treadmill with body weight support [Sousa et al., 2011], Gait Trainer [Hesse et al., 2000], KineAssist [Peshkin et al., 2005], Robot-Aided Treadmill training [Riener et al., 2005]. But it can also be constructed as complex electromechanical exoskeleton; e.g. leg exoskeleton ALEX [Banala et al., 2009], exoskeleton Lokomat [Mayr et al., 2007].

Electromechanical and robotic devices showed successful improvements in the walking speed, endurance, balance, motor recovery and improvements in the gait properties such as stride length or double stance time. [Morone et al., 2017]

3.4 Electrical Stimulation

Another approach used in gait rehabilitation, beside or in combination with conventional training, is electrical stimulation. Broadly used is functional electrical stimulation (FES). In addition, new ways of using the electrical stimulation are being investigated. [Belda-Lois et al., 2011]

3.4.1 Functional Electrical Stimulation

FES is based on delivering an electric current through electrodes into the targeted muscle, where it can activate motorneurons and therefore elicit a contraction of the muscle [Robbins et al., 2006]. Selective stimulation of the muscle helps the patient to contract this muscle and thus aids with walking [Daly et al., 2011]. Especially multichannel FES, where several muscles are stimulated throughout the gait cycle was found to be effective in improving the gait performance in post-stroke patients. FES among others is stimulating the TA, peroneus longus, gastrocnemius lateral head, biceps femoris short head, semimembranosis, semitendenosis, vastus lateralis and/or gluteus medius [Daly et al., 2011]. Furthermore, the combination of FES and other rehabilitation techniques proved to be faster in reducing the gait impairments than if used independently [Bogataj et al., 1995, 1997].

FES can also be coupled with electromyography (EMG) [Chae, 2003], electroencephalogram (EEG) functional magnetic resonance imaging (fMRI), positron emission tomography (PET) or functional near-infrared spectroscopy (fNIRS) [Daly and Wolpaw, 2008]. In case of EMG the stimulus is delivered once the signal is detected in the muscle. This helps in situations when the patients are not able to elicit big enough response in the muscle. Combination with the other techniques (EEG, fMRI, PET, fNIRS), for stroke patients with more severe impairments, can be used in BCI (Brain-Computer Interface) systems, where only the intention of making the movement can trigger the stimulation. [Daly and Wolpaw, 2008]

3.4.2 Activation of the Withdrawal Reflex

Another use of electrical stimulation in gait rehabilitation of stroke patients is the activation of the withdrawal reflex which is elicited on the sole of the foot [Spaich et al., 2006]. Stimulation at heel-off activates the reflex which helps to dorsiflex the foot. This method will be described in detail in chapter 4.

Chapter 4
The Withdrawal Reflex

The nociceptive withdrawal reflex of the lower limb has been widely investigated and proven useful in the rehabilitation of gait impairments following disorders like stroke [Spaich et al., 2014] and spinal cord injuries [Granat et al., 1991]. This chapter will explain the mechanism of the withdrawal reflex and the use of this reflex in gait rehabilitation.

4.1 The Function of the Withdrawal Reflex

The withdrawal reflex is activated by an external stimulus and results in the withdrawal of the specific body part [Marieb and Hoehn, 2010]. The organization of the withdrawal reflex is task dependent, meaning that different situations will elicit different muscle activation patterns [Pierrot-Deseilligny and Burke, 2012]. Withdrawal action is performed to keep the body safe from a possible threat. An often heard example of the withdrawal reflex is the withdrawal of a hand when touching something hot or the withdrawal of the foot when stepping on a piece of glass.

The withdrawal reflex is a polysynaptic reflex. Polysynaptic means that a sensory stimulus does need at least one interneuron (in the spinal cord) to transfer the electrical signal, generated by a stimulus, from a sensory to a motor neuron [Martin and Hine, 2008]. The withdrawal reflex has the ability to overrule the spinal pathways and other reflexes because it is a protective reflex, however it can be overridden by the descending pathways coming from the brain. [Marieb and Hoehn, 2010]

During the withdrawal reflex it is not only the muscles of the leg which needs to be withdrawn from the external stimuli, but also the muscles from the opposite leg. This cooperated response of the two limbs consists of the ipsilateral leg which shows a withdrawal reflex and the contralateral leg which shows an extensor reflex. The pathway of the combination of both limbs can be seen in figure 7.2.2. In the figure, it can be seen that the sensory input coming from the foot also elicits motor neurons in the other leg. The function of this is mainly to maintain balance. [Emborg, 2010]

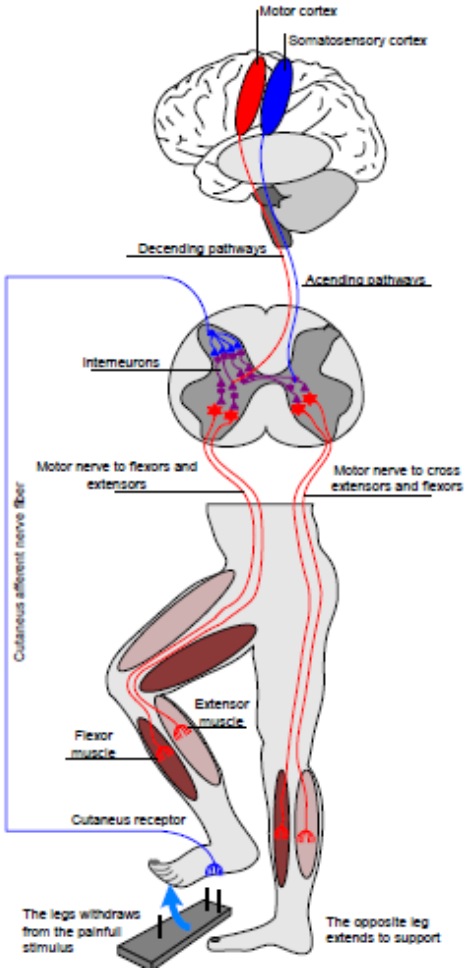


Figure 4.1: Here it can be seen that a noxious stimulus does not only elicit a reflex in the ipsilateral leg, but also elicits a reflex in the contralateral leg. Derived from [Emborg, 2010]

The withdrawal reflex can be grouped into two different groups:

- early reflexes which have a latency of <100 ms [Pierrot-Deseilligny and Burke, 2012]. These reflexes probably have a spinal pathway. Several arguments support this theory, such as: the latency decreases from 65 ms to 35 ms when the stimulation site is moved from the sole of the foot to the upper parts of the limb [Pierrot-Deseilligny and Burke, 2012]; in people with a complete spinal cord injury similar muscles responses can be measured when compared to the responses of people without a spinal cord injury [Shahani and Young, 1971].
- long-latency response reflexes which have a latency of >120 ms [Pierrot-Deseilligny and Burke, 2012]. This long-latency response often follows the early reflex when the intensity of the stimulus is high, indicating that the long-latency response has a higher threshold than the early reflex. Due to the long latency, it is assumed that there is a supraspinal involvement in the long-latency response. Pierrot-Deseilligny and Burke [2012]

4.2 Activation of the Withdrawal Reflex for Gait Rehabilitation Purposes

The withdrawal reflex can be activated by applying an electrical, mechanical or radiant heat stimuli. Categorization of the stimulus being painful or tactile is often used to determine which fibers are activated. However, when either an electrical or mechanical stimulus is applied, which causes a painful sensation, both the nociceptive (aδ) afferents as well as the mechanoreceptive afferents are elicited. [Pierrot-Deseilligny and Burke, 2012]

The stimulation of the withdrawal reflex at the sole of the foot has a big clinical relevance in rehabilitation of gait in several disorders. The effect of eliciting the withdrawal reflex is very dependable on the site of stimulation. Stimulating on different sites can activate different muscles as can be seen in figure 4.2 [Pierrot-Deseilligny and Burke, 2012]. If a stimulation is applied on the ball of the foot, all lower limb muscles will contract: figure 4.2 A, D. When stimulation to the hollow of the foot is applied, dorsiflexion is the outcome, figure 4.2 B. This is dorsiflexion of the toes and flexion in both the hip, knee and ankle. When stimulation is applied on the heel of the foot, there is plantar flexion of the toes, extension of the ankle and flexion in both the knee and hip, figure 4.2 C. More specifically, stimulation on the hollow of the foot causes excitation of the TA and stimulation on the heel causes inhibition of this muscle and excitation of the gastrocnemius medialis. Stimulating the forefoot causes inhibition of the soleus. The biceps femoris is excited with stimulation on the entire sole of the foot [Andersen et al., 1999; Pierrot-Deseilligny and Burke, 2012].

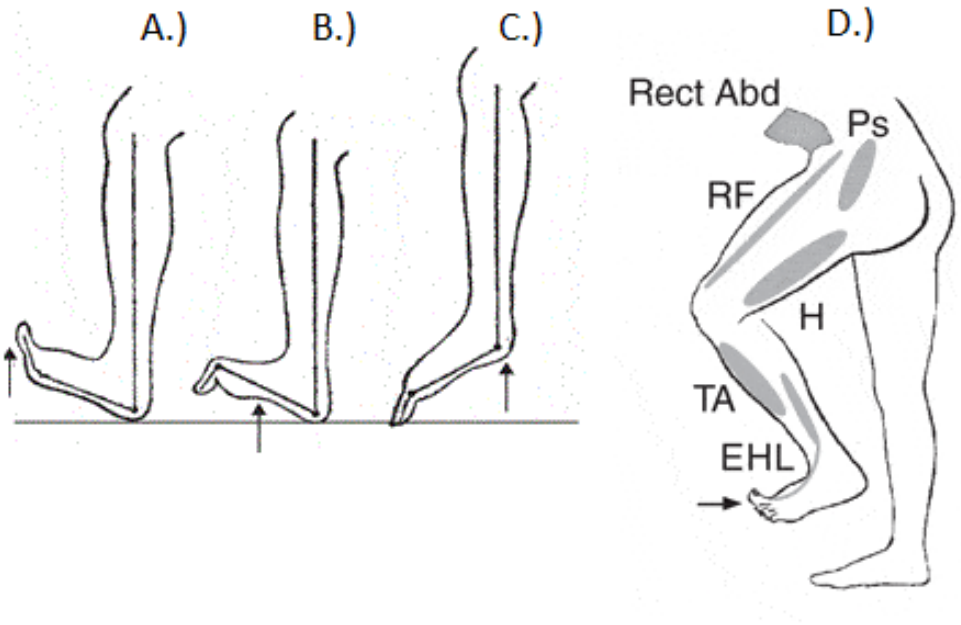


Figure 4.2: The response of a stimulus applied at different places on the sole of the foot. A.) Ball of the toes, B.) Hollow of the foot, C.) Heel of the food, D.) represent which muscles are active during the withdrawal reflex. Figure modified from [Pierrot-Deseilligny and Burke, 2012]

When looked at different time points in the different phases of the gait cycle when the stimulus is applied, it was found that closer to the toe-off the main response of the withdrawal reflex is dorsiflexion of the ankle, while when stimulation closer to the heel-off the main response is knee and hip flexion [Emborg et al., 2009]. It makes stimulation of the withdrawal reflex site and phase modulated [Spaich et al., 2004a, 2006; Richard et al., 2015] which is seen in healthy subjects. In stroke patients, it is only phase modulated and is independent of site [Spaich et al., 2006]. This information can be used while addressing different problems which certain patient groups experience while walking. What is often seen in different studies about gait rehabilitation is that eliciting the withdrawal reflex is used as an initiator and facilitator of the swing phase [Emborg et al., 2009; Granat et al., 1988; Spaich et al., 2014]. It is mainly used during the initiation of the swing phase because toe clearance is often reduced in stroke patients [Matsuda et al., 2017] which causes a higher incidence of falling [Barrett et al., 2010]. Important advantage of using the withdrawal reflex in rehabilitation after stroke is that it can reduce foot-drop which is of big functional importance for patients with gait impairments after stroke [Spaich et al., 2006].

Next to site and phase modulation, is the withdrawal reflex also dependent on the amount of bursts in a train [Arendt-Nielsen et al., 1994]. It was found that after 4-6 bursts the reflex response diminished even though the bursts were still perceived as painful. These different bursts were delivered at 3 Hz.

A study of Spaich et al. [2009] also investigated the effect of frequency on the kinematic response of the withdrawal reflex in healthy subjects. This study found that a stimulation frequency of 15 Hz was more effective in eliciting the kinematic response than at 30 Hz. However, as discussed in this article, this can be caused by the longer stimulation duration which is accompanied with a lower frequency.

A study of Spaich et al. [2014] investigated the effect of gait training with electrically eliciting the nociceptive withdrawal reflex in the lower limb at heel-off for subacute stroke patients. In total 30 patients received a total of 20 sessions of gait training (one half of the patients with and the other half without activation of the withdrawal reflex) for 5 days a week, 40 minutes per day. They found that after these 20 training sessions the subjects with activation of the withdrawal reflex had a better self-chosen walking speed and a better maximum walking speed when compared to the group without activation of the withdrawal reflex. They showed a bigger improvement in symmetry-index when compared to the control group, indicating that they better lengthened their stance phase. The intervention group also showed a shorter duration of the total gait cycle after the 20 training sessions.

However, the mechanism of these improvements remains unknown. Even though research investigating the mechanism of FES in rehabilitation found that the FES drives cortical changes [Kimberley et al., 2004; Michou et al., 2014; Rushton, 2003], to our best knowledge there was not conducted research investigating the effects of stimulation of the withdrawal reflex. It is therefore not known whether the stimulation affects cortical or spinal levels of the neural system nor if there are any changes happening in these pathways as a consequence of training with electrical elicitation of the withdrawal reflex while walking.

Chapter 5

Investigating Corticospinal Excitability

As described in section 4.2, the mechanism behind gait rehabilitation with elicitation of the withdrawal reflex has not been studied before. The first step in investigating the mechanism is to find which parts of the nervous system are modulated by the intervention. Therefore, at first it needs to be differentiated between the cortical and subcortical levels. One approach which has been intensively used for identifying the effects of an intervention is to measure the excitability of related pathways. In order to evaluate the corticocortical and corticospinal pathways, TMS has been extensively used [Nitsche and Paulus, 2000; Ziemann, 2004; Badawy et al., 2012; Bunse et al., 2014]. To evaluate the spinal pathways, e.g. H-reflex [Weaver et al., 2012] or stretch reflex [Ritzmann et al., 2013; Mrachacz-Kersting and Stevenson, 2017] are used. The first part of the following chapter therefore introduces the concept of TMS as the tool for evaluation of the corticocortical and corticospinal excitability. The second part is introducing the possible ways of measuring spinal excitability.

5.1 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is based on Faraday's principle of electromagnetic induction. A pulse of electric current flowing through a wire coil generates a rapidly changing magnetic field. The change in the magnetic field produces a secondary electric current in a conductor located nearby. In case of TMS, a stimulating coil is placed on the scalp, where it generates a powerful and rapidly changing current when triggered. The changing current induces a variable magnetic field perpendicular to the plane of the coil and penetrates the cranium which further generates a secondary current in the membranes of neurons flowing in a plane parallel to that of the coil, but in the opposite direction in comparison to the original current. In the neurons, it elicits action potentials. The power of the induced magnetic field decreases exponentially with the distance from the coil and therefor the superficial brain areas are predominantly exposed to TMS. TMS is restricted to the depth of 1.5-2 cm from the scalp. [Rotenberg et al., 2014; Terao and Ugawa, 2002]

The simplified circuit of a single-pulse TMS device can be seen in the figure 5.1. The TMS device consists of the voltage source V which is charging the capacitor C with high voltage. The capacitor allows a quick discharge controlled by the thyristor switch T. [Rotenberg et al., 2014]

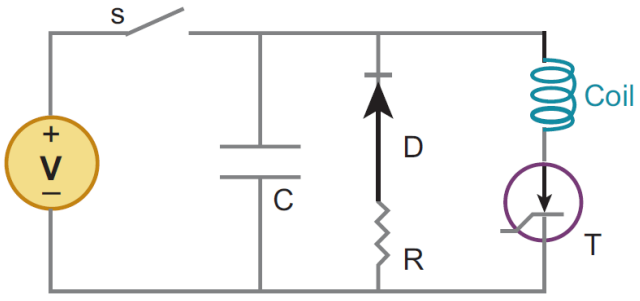


Figure 5.1: Simplified circuit of a single-pulse TMS device, where V stands for voltage source, s for switch, C for capacitor, D for diode, R for resistor and T for thyristor. The figure was taken from Wagner et al. [2007].

Part of the circuit is also the stimulating coil, which shape and size modifies the shape, strength and focality of the resulting magnetic field and the secondary electric field. The oldest design is the circular coil which generates a spherical magnetic field perpendicular to the coil. However, a circular coil is not very focal. The most used coil type is the figure-of-eight coil. It is made of two circular coils merged together. The focus point is the contact point between the two circular coils and the resulting

combined magnetic field is strongest there. It is assumed that the figure-of-eight coil can achieve a spatial resolution of 5 mm^3. To increase the focality, different types of the figure-of-eight coils can be used. the smaller is the angle between the two circular coil the more focal the magnetic field is. [Rotenberg et al., 2014]

The device output is usually expressed as the percentage of the maximal stimulator output, because the values of absolute current are based on the coil parameters such as number of wire loops, coil geometry or distance of the coil from cortex. [Thielscher and Kammer, 2004]

5.1.1 TMS of the Motor Cortex - D, I Waves

Stimulation of the motor cortex evokes two types of waves. The first are D waves which result from the direct stimulation of the pyramidal tract axons. When the intensity of stimulation is increased, three types of I waves follow the D wave in intervals of about 1.5 ms. I waves are assumed to result from trans-synaptic (indirect) activation of pyramidal tract neurons and are termed I1, I2 and I3 based on their latencies [Di Lazzaro et al., 2012]. Furthermore, the I1 waves are supposed to result from activation of monosynaptic corticocortical connections projecting to the corticospinal neurons and the I2 and I3 waves are assumed to result from indirect activation of the output cells through more than one synapse. [Lazzaro et al., 2008]

Transcranial magnetic stimulation evokes predominantly I waves. Only with high intensities above the motor threshold the D waves are also evoked [Lazzaro et al., 2008]. As the corticospinal fibers run horizontally in the motor cortex, the coil orientation influences the type of elicited waves. A lateromedial current induces predominantly D waves, while posterioranterior current induces predominantly the I waves [Werhahn et al., 1994]. It was also found that not only the current orientation but also its direction is important; TMS with medially and anteriorly directed current in the brain evokes I1 waves, whereas laterally and posteriorly directed current preferentially evokes I3 waves [Sakai et al., 1997].

In addition, the TMS of the leg areas tend to produce D waves more readily than I waves [Terao et al., 2000], while in the hand areas the I waves dominate [Sakai et al., 1997]. Lastly, cortical TMS can elicit both, excitatory and inhibitory effects [Terao and Ugawa, 2002].

5.1.2 Safety in TMS Usage

When using TMS for research and/or clinical purposes, there are a couple of issues which need to be considered to maintain a safe environment for subjects/patients. The more important or often discussed issues will be described shortly in this section. Only the risks single-pulse TMS will be discussed.

Heating of the Tissue

The heating of tissue around the site of stimulation is not found to be a considerable safety risk as it only heats less than 0.1 °C after a single-pulse TMS. However, the localized electric current induced after magnetic stimulation can heat up implants and surface electrodes and thus causes skin burns [Rotenberg et al., 2007]. The amount of increased temperature is dependent on several factors e.g. shape, size and conductivity of the implant or electrodes itself, but it can also be dependent on the TMS coil, position of the coil and stimulation parameters. [Rossi et al., 2012]

Attractive and Repulsive Forces

The magnetic field induced by the TMS coil induces attractive forces on ferromagnetic objects and repulsive forces on non-ferromagnetic objects. This effect of the magnetic field could be dangerous for subjects/patients wearing implants, because they could potentially be moved as a consequence of

the stimulation (dependent on the material the implants are made of [Rotenberg et al., 2007]). [Rossi et al., 2012]

Voltage

Extra safety consideration needs to be taken when working with people with active implants in the brain, because the magnetic field induced by the TMS coil can induce voltage in electronic devices and wires nearby. This cannot only cause a malfunction, but also can cause undesired stimulation in e.g. deep brain stimulators. [Rossi et al., 2012]

Side Effects

Hearing loss is a rare side effect which is experienced by some users as a consequence of TMS. Some adults reported an increase in their auditory threshold [Loo et al., 2001]. This is caused by the acoustic artifact induced when stimulated. This acoustic artifact can exceed the safety level of 140 dB [Counter and Borg, 1992]. [Rossi et al., 2012]

Another rare, but more serious side effect of TMS is the induction of seizures. Seizures happen when there is an imbalance in inhibitory and excitatory synaptic activity in the gray matter which causes a hypersynchronized discharge of neuron groups. A factor which have been found to induce the possibility of getting a seizure is the usage of pro-epileptogenic medication. There are also other factors which could increase the risk of seizures e.g. a history of seizures, medication decreasing seizure threshold and diseases which possibly affect cortical excitability. [Rossi et al., 2012]

More often appearing side effect of TMS is syncope, which in general is a common reaction to anxiety and psychophysical discomfort. However, the symptoms can be quite similar to the symptoms which arise when having a seizure. The biggest difference between the two is that syncope only last for seconds whereas a seizure last longer than that. [Rossi et al., 2012]

Discomfort or even pain is the most often reported side effect of TMS. However, most people can tolerate TMS. The amount of discomfort/pain experienced during TMS is different from person to person and can also vary based on location, coil design, intensity and frequency of the stimulation. Headache is also often reported during and after a session of TMS as well as neck pain which could possibly be explained by the forced posture and head immobilization during TMS. [Rossi et al., 2012]

Medication usage should also be considered before starting a TMS session, due to their potentially negative effects when combined with TMS. A list of possible medications, which have an effect, can be found in appendix A. Most of the medications on the list are known to be seizure threshold reducing and this is the reason for being on the list. It should be considered that the list is not complete and thus there could be other medication having a negative effect when used in combination with TMS. [Rossi et al., 2012]

A questionnaire (appendix B) is available to evaluate the possible risk of the people undergoing TMS. When someone answers positively on any of the questions in the questionnaire the subject should only be used when the benefits of this specific person exceed the risks. The questionnaire is only an indication and cannot completely rule out any risks when all questions are answered negatively. [Rossi et al., 2012]

5.2 TMS Stimulation Methods

The following section summarizes the most frequently used TMS techniques and stimulation protocols in current research and describes the different ways of evaluating the outcomes.

5.2.1 Single Pulse TMS

TMS pulses applied to the primary motor cortex evoke motor evoked potentials (MEP) in the target muscle [Farzan, 2014]. MEPs are typically measured by EMG and are the main outcome which is being evaluated. MEPs induced by single pulse TMS provide information about overall integrity of corticospinal pathways and do not differentiate between the spinal and cortical mechanisms [Farzan, 2014]. Therefore, single pulse TMS is often conjoined with other techniques which focus specifically on spinal pathways such as TES [Valle et al., 2007] or induction of spinal reflexes e.g. H-reflex [Hosoido et al., 2009] or stretch reflex [Ogawa et al., 2012].

To evaluate the MEP, different features can be used. One feature is the latency of onset. It is defined as the time between the delivery of the single TMS impulse on the scalp and the appearance of MEP at the periphery. Latency reflects the number of synapses between the stimulation site and the target muscle. In addition, it also reflects information about the integrity of white matter fibers such as diameter and thickness of myelin sheaths. Latency is measured in milliseconds and the results can be used to approximate the speed of the combined central and spinal conduction. [Farzan, 2014]

Another feature of MEPs is the magnitude, which is often measured as the peak-to-peak amplitude expressed in μV or mV. Sometimes the magnitude can be measured from the baseline to the maximum positive or negative value. Magnitude can also be measured as the area under the MEP curve, which has an advantage that it is able to distinguish two MEPs with the same peak-to-peak amplitude but different durations. [Farzan, 2014]

The sections below describe the most used stimulation protocols used with single pulse TMS.

Motor Threshold (MT)

Motor threshold (MT) measures are often used for evaluation of the corticospinal excitability as a baseline measure which guides the intensity for other TMS protocols. There are two ways of measuring MT. First is a resting MT (rMT) which is identified for the target muscle at rest. rMT is often defined as the minimum intensity needed for producing MEPs with peak-to-peak amplitude higher than 50 μV in 50 % of the trials when 10 consecutive single pulses are applied by the TMS coil on the hot spot of target muscle (relative frequency method). It has a high degree of inter-subject variability. [Rossini et al., 2015]

The second is identified during voluntary contraction of the target muscle and is therefore called an active MT (aMT). It is obtained the same way as the rMT, except with low voluntary contraction when stimulated. The aMT is usually lower than the rMT. [Groppa et al., 2012]

Silent Period

The inhibitory effect of TMS pulse can be investigated when TMS is applied during voluntary contraction of the target muscle and the inhibitory effect is seen as a suppression of the background EMG activity, which is normally following the MEP. Periods of suppression are called the silent periods (SP). Depending whether it is induced in contralateral or ipsilateral muscles we distinguish ipsilateral SP and contralateral SP. [Farzan, 2014]

Contralateral Silent Period (cSP) is the suppression of the EMG activity following the TMS pulse applied to the motor cortex during voluntary contraction of the contralateral target muscle [Fuhr et al., 1991]. In healthy subject the intensity needed for inducing the silent period is the same needed for induction of MEPs. However, in pathological conditions the silent periods can be seen even though MEPs are not observed and in some cases the silent periods can be shortened. Results are usually reported as the peak-to-peak amplitude of MEP and the duration of the silent period. It is assumed that the first 50 ms of the SP is due to spinal mechanism and the remaining time due to cortical inhibition. [Rossini et al., 2015]

Ipsilateral Silent Period (iSP) is the suppression of EMG activity following the TMS pulse applied to the motor cortex during voluntary contraction of the ipsilateral target muscle [Wassermann et al., 1991]. It is suggested that iSP results from the transcallosal pathways between the target and contralateral hemispheres. It is further assumed that iSP is reflecting the integrity of the corticospinal tract from the non-stimulated cortex to the muscle ipsilateral to the stimulated motor cortex. [Farzan, 2014]

Input/Output (I/O) Curves

When using TMS, the higher is the stimulation intensity the stronger is the descending excitatory drive, which is resulting in gradually increasing MEP amplitude. The relationship between the amplitude of MEPs and the stimulation intensity is sigmoidal; for low intensities below the MT the amplitude is below 50 μV, then it increases with approximately linear tendency until the plateau is reached and the MEPs do not further increase even though the stimulation intensity is being increased. The sigmoidal function can be modeled using a Boltzmann equation. [Devanne et al., 1997]

The outcome measures of the I/O curves are the maximum slope of the curve (K), the maximum MEP amplitude or the MEP when plateau phase begins (MEP_{max}), and the stimulus intensity needed to obtain an MEP with amplitude 50 % of the MEP_{max} (S_{50}) [Devanne et al., 1997]. Changes in these parameters indicate changes in excitability of the descending paths of the corticospinal tract. The shape of the I/O curves is different for various muscles presumably due to differences in strength of corticospinal projections [Chen et al., 1998].

In practice, the I/O curves are obtained by measuring amplitudes of MEPs for different TMS pulse intensities. The procedure starts with sub-threshold intensities and continues until the 100 % output of the TMS machine is reached or the plateau phase is reached and the amplitude is not increasing anymore. [Farzan, 2014]

5.2.2 Paired Pulse (pp) TMS

While single pulse TMS is used to investigate the excitability of corticospinal pathways, ppTMS is used to investigate the excitability of corticocortical pathways. During ppTMS two stimuli are applied to the same location with variable inter stimulus intervals (ISI). The first stimuli is called a conditioning stimulus (CS) and the second one is called a test stimulus (TS). [Vahabzadeh-Hagh, 2014]

ppTMS can induce both inhibitory and excitatory responses depending on the intensities of CS and TS and on the length of ISI. There are four circuits that can be activated; two inhibitory and two facilitatory circuits. Inhibitory circuits are short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI). The facilitatory circuits are intracortical facilitation (ICF) and short-interval intracortical facilitation (SICF). [Vahabzadeh-Hagh, 2014]

In practice, at first the MT is obtained. Based on the motor threshold, CS and TS can be derived depending on what circuit is being activated. To activate different circuits, also different ISIs are used. The different intracortical inhibition and facilitation protocols reflect the excitability of separate populations of interneurons. [Vahabzadeh-Hagh, 2014]

SICI is widely used to investigate the inhibitory circuits. It is assumed that SICI reflects the inhibition mediated through GABA receptors. To induce SICI, subthreshold CS, supratreshold TS and ISI of 1-6 ms are used. However, the highest inhibition was seen when using ISIs of 1-2.5 ms. The ISIs of 3-5 ms tend to inhibit only I3 waves, but not I1 waves. In addition, reduced SICI was found to indicate the deficient motor cortical inhibition. [Epstein et al., 2012]

For ICF are used the same CS and TS as for SICI but the ISI is longer, 8-30 ms. In comparison

to SICI, ICF has not been extensively studied. However, it was found that ICF consists of both, weak inhibition and predominant facilitation, which is thought to be mediated by NMDA (N-methyl-D-aspartate) receptors and ICF thus affects mainly NMDA receptor-dependent interneural circuits. [Epstein et al., 2012]

SICF uses a suprathreshold CS followed by a subthreshold TS with ISIs of 1-5 ms. Furthermore, SICF is assumed to originate from the activation of the neural pathways involved in I-wave generation. The facilitation was found in I2- and I3- waves, but never in I1 waves. Lastly, for LICI are used a suprathreshold CS and TS with ISIs of 50-200 ms. The increase in LICI seems to correlate with bradykinesia in patients. [Epstein et al., 2012]

5.3 Spinal Cord Excitability

The MEP generated by TMS shows a combination of both cortical and sub-cortical influences [Pirio Richardson et al., 2009; Quartarone et al., 2005]. If more information is needed about the effect of sub-cortical influences, a different method needs to be used to get a more specific image of changes happening at the spinal level. This section will address some of the methods which are used to measure the spinal cord excitability.

5.3.1 F-waves

A F-wave is a motor response, represented by the α-motorneurons, to stimulation of the nerve which causes an antidromic (impulse) activation of spinal motor neurons [Mercuri et al., 1996; Mesrati and Vecchierini, 2004]. A F-wave only occurs when the stimulation activates a motor axon directly and the reponse is likely to only be caused by the activation of large motorneurons [McNeil et al., 2013]. The reason for this is the antidromic nature of the response which causes a collision with the smaller motorneurons and the antidromic volley before the cell body (some).

F-waves can be elicited in many different muscles and are elicited by electrical stimulation of the peripheral motor nerve. Often surface electrodes are used and intensities are delivered around 125 % of the threshold for eliciting a direct motor response. The muscle can be activated along the whole pathway of the nerve, but the most distal part is often chosen. [McNeil et al., 2013]

Excitability of the spinal cord and the motorneuron pool has been measured previously with the F-wave by several different articles (e.g. [Mercuri et al., 1996; Takemi et al., 2015; Pirio Richardson et al., 2009]. The following parameters are used; the area under the curve of the F-wave [Pirio Richardson et al., 2009], the amplitude of the F-wave [Mercuri et al., 1996; Takemi et al., 2015] and the frequency of occurrence of the F-wave [Takemi et al., 2015].

F-waves however come with some limitation and things which needs to be taken into consideration when measuring the F-wave. One of these limitations is that the latency of the F-wave is associated with the length of the limb [Lachman et al., 1980; Panayiotopoulos and Chroni, 1996; Nobrega et al., 2004] which can cause big inaccuracies when measuring latencies. Another article found that the F-wave increases when movement of the limb is imagined [Hara et al., 2010] which could indicate that attention to the certain limb can also influence the measured F-wave. There is also a big within-subject variety in shape and size of the F-wave [Lin and Floeter, 2004], therefor large number of measurements need to be carried out. Moreover, there are also several articles [Kudina and Andreeva, 2017; Balbi et al., 2014; Espiritu et al., 2003] which have indicated that the measuring the F-wave as an indication of spinal excitability is incorrect/flawed. It is stated that the increase in F-wave amplitude can be caused by neural excitability as well as by inhibition [Balbi et al., 2014]. One of the other articles found that the motorneurons were not able to recurrently fire during the most excitable part of a specific target interval [Kudina and Andreeva, 2017] which is another indication that F-waves are not the best measurement for spinal excitability.

5.3.2 H-reflex

Another technique for investigating the excitability of motor neurons is the Hoffman reflex (H-reflex), which can be evoked by electrical stimulation of peripheral nerve [McNeil et al., 2013]. More specifically, the H-reflex originate from stimulation of the Ia afferent axon [Rizzo et al., 2014; Pierrot-Deseilligny and Burke, 2012]. The AP elicited in the Ia afferent axon travels into the spinal cord and can result in muscle activation [Aagaard et al., 2002]. When eliciting the H-reflex, it is accompanied by the M-wave which occurs first and represents the muscle compound action potential [Hosoido et al., 2009]. The recruitment of the motorneurons following the stimulation is according to Henneman size principle, smallest to largest motorneurons [McNeil et al., 2013]. The amplitude of H-wave increases with stimulation intensity until it reaches its maximum, which happens either because the increasing intensity is not increasing the excitatory input into motor neurons or because further increase of intensity reduces the H-reflex and increases the M-wave [McNeil et al., 2013]. During relaxation, the H-reflex can be evoked only in several muscles (e.g. soleus and quadriceps). Therefore, during the experiment, the target muscle is precontracted at a certain level of maximum voluntary contraction (MVC) [McNeil et al., 2013]. In general, the H-reflex showed to be constant over time [Zhang et al., 2015].

However, the H-reflex is confounded by oligosynaptic contributions from the disynaptic Ib afferents inhibiting the H-wave which can be seen from the long rising time of the reflex. The pathway of the H-reflex does not take into account the sensory endings of muscle spindles (because of the direct stimulation of the muscle afferents) and thus the gamma-motor neurons. [Misiaszek, 2003]

An increase in amplitude of the H-reflex is often used to describe spinal excitability, however this can be easily misinterpreted, because the increase in amplitude can have 3 different reasons [Misiaszek, 2003]:

- An increase in excitability of the motor neurons
- More neurotransmitters released by the afferents terminals which can be caused by presynaptic inhibition and post-activation depression
- A difference in intrinsic properties of the motorneurons

Beside the possible misinterpretations, there are also other disadvantages of measuring the H-reflex. One of the disadvantages of the H-reflex is that it can be influenced by the subject itself. If they show a high level of attention the H-reflex can be increased [Pierrot-Deseilligny and Burke, 2012]. A study from Weaver et al. [2012] found that the H-reflex decreases when the attention is directed away from the posture. Also, the posture itself can affect the amplitude of the H-reflex [Misiaszek, 2003]. Thus, in general, the H-reflex is highly affected by both the physical and mental state of the subject [Misiaszek, 2003].

5.3.3 Transcranial Electrical Stimulation

Transcranial electrical stimulation is a non-invasive method where electrical current is sent through the brain by electrodes. This is different from TMS for which a coil is used as can be seen in figure 5.2 [Lozano and Hallett, 2013]. Direct stimulation of the scalp (TES) can give more information about the excitability of the subcortical pathways [Valle et al., 2007]. TES induces a direct activation of the corticospinal neurons and thus an activation of the D-wave and not of the indirect I-waves [Quartarone et al., 2005]. This makes the resulting muscle activation independent of the motor cortex [Quartarone et al., 2005], because the D-wave only has information about spinal excitability and is not affected by the excitability of the cortex. This method however produces discomfort on the scalp and contractions of the scalp muscles [Deletis and Shils, 2002; Rothwell, 1997].

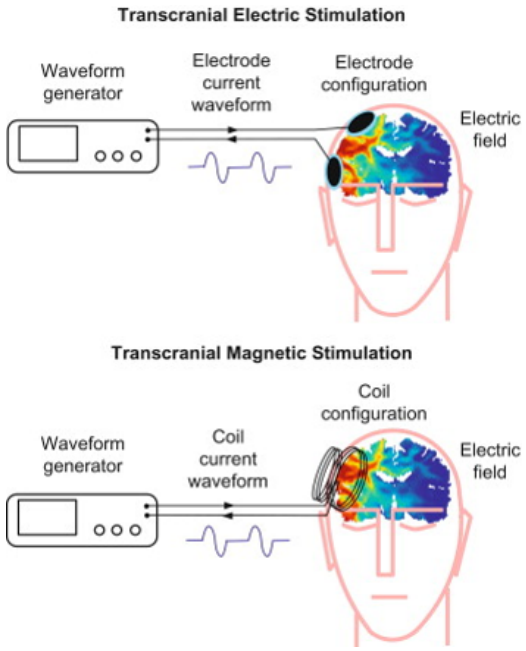


Figure 5.2: Diagram of transcranial electrical stimulation (top) and transcranial magnetic stimulation (button). TES induces an electrical current with electrodes placed on the scalp and TMS induces magnetically, with a coil, an electrical current in the brain. Derived from Lozano and Hallett [2013]

The biggest difference between TES and TMS is that with TES the action potentials are elicited in deeper layers of the cortex, whereas TMS only elicits the cells in a closer distance to the cell soma [Perez et al., 2004]. Currents induced by TES also travel in all directions, both tangentially and radially. Whereas TMS-induced currents are highly directional [Di Lazzaro and Rothwell, 2014].

5.3.4 Stretch Reflex

A spinal stretch reflex is a consequence of a quick stretch of a muscle which is mechanically elicited [Ritzmann et al., 2013] and sensed by the velocity-sensitive muscle spindles [Stein and Oğuztöreli, 1976]. Its pathway consists of the receptors (muscle spindles), Ia-afferent fiber going from the muscle to the spinal cord and the synapses in the spinal cord to the alpha-motorneurons to the muscles which quickly stretches. The stretch reflex can give information about the excitability of the spinal motorneuron pool [Ogawa et al., 2012] and the sensitivity of the muscle spindles and alpha gamma linkage [Ritzmann et al., 2013]. [Ogawa et al., 2012]

The stretch reflex consists of 3 different bursts. The different bursts are a representation of the different mechanisms which are named M1, M2 and M3 and are specified by their onset latency [Petersen et al., 1998]. These bursts start at a latency of respectively 44, 69 and 95 ms for the TA. M1 represent the mono-synaptic Ia pathway to the spinal motorneurons. The M2 represent the group II afferents with a spinal origin. The M3 burst involves a pathway going to cortical levels. An example of the EMG response following ankle joint rotation is shown in the figure 5.3. [Petersen et al., 1998; Christensen et al., 2001]

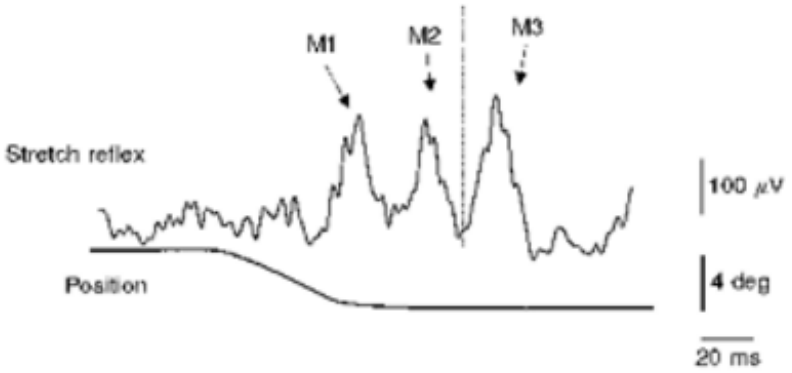


Figure 5.3: The EMG response of the stretch reflex measured in the TA. The different bursts (M1, M2 and M3) are clearly distinguishable. Derived from Petersen et al. [1998].

Inducing a stretch reflex in a subject is done by giving the targeted joint a perturbation. This perturbation needs to be fast enough to elicit a stretch reflex [Stevenson et al., 2015] For healthy subjects the velocities lies approximately between 200-360 degrees/s (e.g. in studies of Nakazawa et al. [2003]; Mrachacz-Kersting and Stevenson [2017]). A study of Berardelli et al. [1982] found that the amplitude of the measured response in the muscle increases when the velocity is increased. The amplitude of the measured response in the muscle does also increase when the amplitude of the mechanical elicited stimulus is increased [Evans et al., 1983]. The study of Evans et al. [1983] used a sinusiodal stretch and found increased peak-to-peak amplitudes with increased amplitudes of the stimulus. The increase of peak-to-peak amplitude however was not linear but reached a plateau after a certain point, i.e. the response saturated.

Chapter 6
Problem Statement

In the problem analyses it can be found that a stroke can cause both physical and psychological impairments. Gait impairment is one of the main issues limiting the independence of stroke patients. There are several different therapies addressing gait recovery in stroke patients and one of them is a conventional physiotherapy combined with electrical stimulation on the sole of the foot which elicits the nociceptive withdrawal reflex.

Gait of stroke patients is different from gait of healthy people in both spatio-temporal and kinematic parameters. Some examples of gait impairments after stroke are reduced walking speed, asymmetry in their walking and a reduced hip, knee and ankle flexion. Electrical stimulation on the sole of the foot elicits the withdrawal reflex which causes ankle dorsiflexion, knee flexion and hip flexion and thus gives a more functional gait pattern. It has been found that this intervention improves several gait parameters like walking speed, symmetry ratio and duration of the stance phase of the affected limb in stroke patients.

However, there has not been carried out any research addressing the effect of this intervention on the corticospinal pathways. It is unclear whether possible changes are happening on a cortical level or on a spinal level. The first step in the investigation of the effect is to identify where the possible changes occur in healthy adults. Measurements which have been intensively used to investigate the cortical and sub cortical pathways are TMS and the stretch reflex which are both used to evaluate the excitability of related pathways. TMS gives information about the corticospinal and the cortico-cortical pathways and the first two components of the stretch reflex give information about the spinal pathways alone. Therefore, the following research question was formulated:

Is there any change in the excitability of the corticospinal pathways following walking with the activation of the withdrawal reflex of the lower limb?

Part II

Solution

Chapter 7
Methods

To address the question about the effect of walking with activation of the withdrawal reflex on the corticospinal excitability, two groups of healthy subjects were recruited. One group walked with electrical stimulation on the sole of the foot, which elicited the withdrawal reflex during every heel-off and the other group walked without stimulation. Two groups enable to distinguish between the effect of walking itself and the intervention. To asses the changes, the excitability of the corticospinal pathways was measured prior to, immediately after and 30 minutes after walking using TMS. In addition, to distinguish between the corticospinal and spinal changes, the excitability of spinal pathways was measured at the same time points using the stretch reflex. Comparison of the data obtained prior to walking with the data obtained immediately after allows to see the immediate effect of the intervention on the excitability of corticospinal and spinal pathways while the comparison with the data obtained 30 minutes post intervention allows to see if the intervention induced any persistent plastic changes in the corticospinal or spinal pathways.

7.1 Subjects

In total 26 subjects were recruited for the experiment. Of these 26 subjects a total of 9 (35 %) subjects were excluded because they did not meet the inclusion and exclusion criteria which are listed below, some of these exclusion criteria where tested with a TASS (transcranial magnetic stimulation adult safety screening) questionnaire which subjects filled in before coming (appendix B). Out of the 9 subjects, 2 (22 %) were not eligible for TMS, 2 (22 %) dropped out of the experiment due to adverse effects of TMS and 5 subjects (56%) had a threshold which was above 80% of the maximum stimulator output. A flow chart of the subject recruitment and group outcome can be found in figure 7.1.

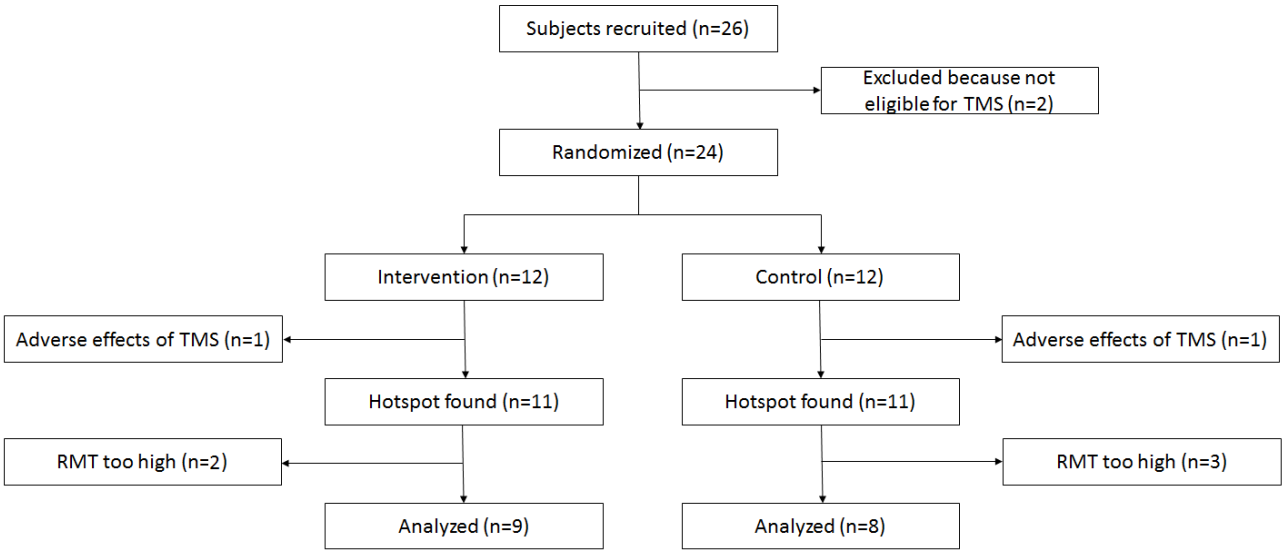


Figure 7.1: Flow chart of subject recruitment and retention.

Inclusion criteria:

- age >18 years old
- Able to walk for 30 consecutive minutes
- Being able to give informed consent

Exclusion criteria:

- Pregnancy
- Epilepsy
- Metals in head
- History of seizures
- 1st degree family with epilepsy
- Medical, neurological or psychiatric illness
- Using pain medication
- Stimulator in the body
- Skin conditions at the site of the electrodes
- Not being able to tolerate the stimulus intensity needed to elicit the withdrawal reflex
- Adverse effects as a consequence of TMS
- Having a motor threshold $>$ 80 % of the maximum stimulator output with TMS (Otherwise limited amount of data would be available for the I/O curves.)

Subjects were asked to eat before the session and to get enough sleep the night before. Sleep deprivation can influence the inhibition-facilitation balance in the primary motor cortex which could affect the results of TMS stimulation [Cantello et al., 2000].

A complete overview of subject information can be found in table 7.1.

Table 7.1: Subject information

Subject #	Gender	Age	Dominant leg	Intervention/ Control group	Stimulation intensity (mA)
1	Male	24	Right	Control	
2	Female	24	Right	Control	
3	Male	24	Right	Intervention	9
4	Female	24	Right	Control	
5	Female	24	Right	intervention	8
6	Male	23	Right	Control	
7	Male	26	Right	Intervention	10
8	Male	24	Right	Intervention	10
9	Male	25	Right	Intervention	12
10	Female	24	Right	Control	
11	Female	29	Right	Intervention	5
12	Male	25	Right	Control	
13	Male	27	Right	Intervention	11
14	Male	24	Right	Control	
15	Male	25	Left	Intervention	9
16	Male	20	Right	Control	
17	Female	24	Right	Intervention	8
Total	**11M/6F**	**24.5 ± 1.8**	**16R/1L**	**9I/8C**	

7.2 Training Protocol

7.2.1 Treadmill Walking

The experiment consisted of a single training session which lasted for 30 minutes. Subjects had to walk on a treadmill (TECHNOGYM, Italy) with a speed of 2.5 km/h. Subjects were strapped in a harness

for safety reasons, however, this did not support any of the body weight. For the first minute of the training, subjects were allowed to hold on to the rail in front of them/at the side for security. After 1 minute subjects were asked to release the rail, because this could affect the walking as stated by Chen et al. [2005]. Both subject groups had the same training protocol on the treadmill. However, the subjects of the intervention group walked while having their withdrawal reflex elicited with electrical stimulation as described in section 7.2.2.

7.2.2 Eliciting the Withdrawal Reflex

For the intervention group the stimulation electrode (Ambu® Neuroline 700, 20 mm x 15 mm) was placed at the highest point of the arch of the dominant foot. The anode (Axelgaard manufacturing co., LTD. PALS® neurostimulation electrodes, 7.5 cm X 10 cm) was placed on the dorsum of the foot. This electrode placement will elicit dorsiflexion and flexion in both the hip, knee and ankle [Andersen et al., 1999; Pierrot-Deseilligny and Burke, 2012]. This electrode placement was also used by [Spaich et al., 2014]. The stimulation was given during every gait cycle at heel-off to facilitate the initiation and execution of the swing phase [Spaich et al., 2014]. The heel-off was detected with a force sensor. The stimulation train used to elicit the withdrawal reflex had a frequency of 15 Hz and consisted of 4 stimuli. Each stimulus in the train consisted of five 1 ms wide pulses delivered with a frequency of 200 Hz. These settings are chosen based on Spaich et al. [2009] which were found to be optimal for eliciting the largest dorsiflexion. A graphical overview of the delivered stimuli can be seen in figure 7.2. The stimulation was delivered with a current controlled stimulator (Isolated Stimulator, NoxiTest IES 230, "NoxiStim") which was controlled by the software program Mr. Kick III (Knud Larsen, SMI, Aalborg University).

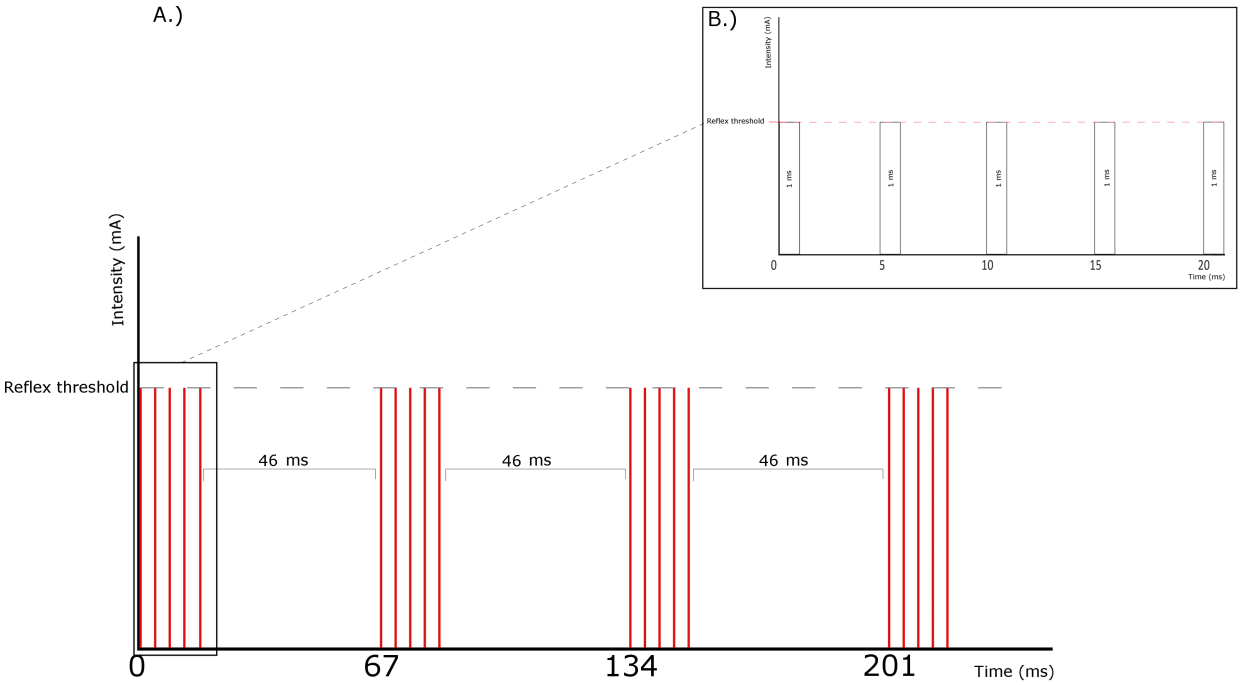


Figure 7.2: A.) The pulse train used to elicit the withdrawal reflex. B.) the stimulation bursts.

To familiarize the subjects with the stimulation, the subjects were first allowed to trigger the stimulus themselves while sitting down. Once the subjects felt comfortable with the stimulation, they were asked to stand up and the intensity needed to elicit the withdrawal reflex was determined. During the procedure of finding the optimal withdrawal reflex response the subjects were still allowed to trigger the stimulation themselves to reduce the possibility of eliciting a startle reflex instead of the withdrawal reflex which is often seen in combination with the withdrawal reflex [Álvarez-Blanco et al., 2009; Dowman, 1992]. The initial amplitude of the stimulation was 1 mA. The amplitude was increased with random steps of 1-3 mA until a sufficient kinematic response was reached. A sufficient kinematic response should have included flexion of the hip and knee and dorsiflexion of the foot.

When habituation occurred during treadmill walking the initial stimulation intensity for eliciting the withdrawal reflex was increased with steps of 1 mA.

Stimulation of the feet was applied to the dominant leg of the subject. Dominance was determined with the revised Waterloo Footedness Questionnaire by Elias et al. [1998]. The results were calculated with equation 7.1 which is based upon the principle of The Edinburgh Handedness Inventory, described by Oldfield [1971].

$$footedness = (R - L)/(R + L) \tag{7.1}$$

Where:
R = Amount of points given to the right leg based on the Waterloo Footedness Questionnaire
L = Amount of points given to the left leg based on the Waterloo Footedness Questionnaire

7.3 Evaluation of Excitability

7.3.1 TMS

To asses changes in corticospinal excitability before, immediately after and 30 minutes after the intervention, MEPs were elicited using TMS. For the TMS measurements subjects were seated in a chair in a comfortable position with their knee in a 90 degree angle. They were asked to relax their dominant leg, try to avoid movements and keep their eyes open during stimulation. Two surface electrodes (Ambu® Neuroline 720) were placed on the TA and the third, reference electrode, was placed on the tibia. The electrode placement was based upon the SENIAM placement recommendations [Stegeman and Hermens, 1998]. Surface EMG was recorded from 100 ms before stimulation (to detect undesirable pre-activation) to 300 ms after the stimulation. The raw EMG signal was recorded with a sampling frequency of 3 kHz. The raw EMG signal was first pre-amplified (SENS-003-001, Rogue Research Inc.) and differentially amplified (Brainsight® 2 EMG Isolation Unit, Rogue Research Inc.). Then, the EMG signal was band-pass filtered at 16 Hz-550 Hz. Brainsight® (Rogue Research, 2012), was used to record and track the stimulations.

To ensure preciseness of the location of stimulation, a tracking system (Brainsight TMS Navigation from Rogue Resolutions, Cardiff, UK) was used. Prior to the TMS experiment, a tracker was placed on the subject's forehead and the tracking system was calibrated to the head. In addition, a coil tracker was placed on the handle of the coil to track the coil movements. This setup allowed for a more precise search for the hotspot.

To elicit MEPs in the TA, a monophasic transcranial stimulator (Magstim 200^2, Magstim, Inc., Morrisville, US) with a focal figure-of-eight stimulating coil (custom-made Magstim Alpha Coil Flat Range (Coated), 1.5 T, diameter of each loop: 70mm) was used. To find the hotspot for targeting the TA, the subject was asked to point to the top of his/her head. The center of the coil was positioned on this spot in posterior-anterior direction with the handle facing laterally as seen in figure 7.3. This placement elicited secondary electric current in the primary motor cortex in medial-lateral direction. Subsequently, the coil was moved around and in each location three TMS pulses with 65% of the stimulator output were applied. The location where stimulation elicited the largest MEPs, which were reasonably consistent, was defined as the hotspot [Sowman et al., 2014]. If no clear hotspot was found, the intensity of the stimulator output was increased by 5 % until the hotspot was found. When no muscle responses were found with an intensity of 80 %, the experiment was stopped and the subject was excluded. After finding the hotspot, the edges of the coil were marked with a permanent marker on the subject's head to assure correct placement of the coil when removing the subject tracker after finding the hotspot.

Figure 7.3: Figure-of-eight coil positioned in posterior-anterior direction. Figure taken and modified from Smith et al. [2017].

The resting motor threshold was determined as the lowest stimulation intensity needed to evoke a MEP with a peak-to-peak amplitude of at least 50 μV in 5 out of 10 consecutive stimulations as done by [Taylor and Murphy, 2008]. The basic principle of searching for the rMT was as followed; at first the intensity used to define the hotspot was used (e.g. 65 %). If it was a subthreshold intensity (less than 5 out of the 10 stimuli evoked a MEP with peak-to-peak amplitude of at least 50 μV), the intensity of the following stimuli was increased by 5 % (to 70 %). In case the MEPs were suprathreshold the intensity was decreased by 4% (to 66%) and if the MEPs were subthreshold, the intensity was increased again by 3 % (to 69 %). This alternation method was used until the threshold was found. In case that one intensity (e.g. 67 %) was subthreshold and another which was 1 % higher (68 %) was suprathreshold, the higher value was used as the rMT.

The next step was obtaining the I/O curve to examine the corticospinal excitability. The stimulations were given at intensities of 90 % - 140 % of the rMT with steps of 10 %. The order of the stimulation was randomized and 8 stimuli were delivered for each intensity with an interval between two consecutive stimuli of at least 5 s. In case the threshold increased during the process, additional stimulation was given at intensity of 150 % of the rMT.

7.3.2 Stretch reflex

To asses changes in spinal excitability as a consequence of the intervention, prior to, immediately after and 30 minutes after the training, stretch reflexes were elicited. For elicitation of the stretch reflex the subject was asked to stand in an upright position on two pedals on a servo-controlled hydraulic actuator (MTS Systems Corporation, Minnesota, USA). The upright position was chosen because there is already some level of pre-contraction and the subject does not need to contract the muscle with a certain percentage of the maximum voluntary contraction. Both feet were strapped on the pedals and the TA of the dominant leg was stretched mechanically while surface EMG was measured from the TA with the same electrode configuration as during TMS (described in section 7.3.1). The ankle of the dominant leg was aligned with the axis of rotation of the actuator.

Thirty stretches were performed with random intervals of 5-7 s between the single stretches, so the subject could not predict when the stretch was applied. The amplitude of the stretch was 6 degrees and the rise/fall time was 24 ms. The hold time in the stretched position was 460 ms. The waveform with which the stretch reflex was performed was sine-hold-sine. The parameters used to elicit the stretch reflex are similar to the ones used in the study of Mrachacz-Kersting and Stevenson [2017].

Surface EMG was recorded from 100 ms before the perturbation until 1 s after the perturbation. The raw EMG was recorded with a low pass filter of 1 kHz and a high pass filter of 5 Hz and was amplified with a gain of 10000. The computer program, Mr. Kick III (Knud Larsen, SMI, Aalborg University) was used to initiate the perturbations and record the muscle response.

7.4 Data Analysis

The data collected during the experiments consisted of two parts, the stretch reflex and TMS data. The following sections describe how the data was treated and is divided into two subsections.

7.4.1 TMS

The data collected during single pulse TMS consisted of the information about the rMT and of the MEPs recorded at different intensities from the TA. The rMT and MEPs were recorded prior to, immediately after and 30 minutes after the cessation of the intervention.

The peak-to-peak amplitude of each individual MEP was quantified and the mean was calculated for every intensity measured for the three different time points. Since the resting motor threshold was different between pre, post and 30 min post intervention, the intensities for the I/O curve were adjusted to the new respective rMT. Thus, the stimulation intensities differed between the pre, immediately post and 30 minutes post intervention. Therefore, a comparison of the same intensities across the sessions for individual intensities was not possible. Instead, the data was fitted to a Boltzmann equation using the Trust-Region algorithm in the Curve Fitting Toolbox of MATLAB. The Boltzmann equation (equation 7.2) was derived from Kouchtir-Devanne et al. [2012] and is structured as follows:

$$MEP(S) = y_0 + \frac{MEP_{max}}{1 + e^{(S_{50} - S)/k}} \tag{7.2}$$

The equation consists of four parameters; MEP_{max} is the maximum value of the curve or the plateau of the I/O curve, S_{50} is the stimulus intensity needed to obtain a MEP with an amplitude of 50 % of the MEP_{max} and it is also the place with the maximum slope, K is the slope parameter, y_0 is the floor of the curve which is the minimum.

In the present study, y_0 was individually set to the minimum of all the mean intensities for the specific subject. Because there were only six measured intensities in the I/O curve, in some cases the plateau phase of the curve was not reached. Therefore, in order to obtain a better fit to the Boltzmann equation (7.2), an assumption was made that the plateau of the Boltzmann fit could not exceed the highest average amplitude from the MEPs measured during any of the six intensities. This was implemented in the curve fitting process by putting a lower and upper bound to the MEP_{max} coefficient. The lower bound was the lowest measured average MEP amplitude for the six different intensities and the upper bound was the highest measured average MEP amplitude for the six different intensities.

The outputs of this process were three I/O curves for each subject, one for prior to the intervention, one immediately after and one 30 minutes after the intervention. For the purposes of the statistical analysis K-, S_{50}- and MEP_{max}-values were derived from each I/O curve.

The goodness of the fit was measured by calculating the sum of squared errors (SSE), R-square and the root-mean-square error (RMSE) for every Boltzmann fit. The average of these values was calculated and reported.

An additional fit was done. In the Curve Fitting Toolbox of MATLAB a least square fit of a straight line 7.3 (polynomial with 1 degree) to the data was performed:

$$y = mx + b \tag{7.3}$$

The equation has two parameters, m the slope of the line and b the intercept of the line with the y-axis. The outputs were three lines for each subject, one for prior to the intervention, one for immediately after and one for 30 minutes after the intervention. Out of these lines the values of the slope were extracted for the statistical analysis.

7.4.2 Stretch Reflex

For every subject, three sets of data were obtained prior, immediately post and 30 minutes post intervention. Each of these data sets consisted of 30 EMG signals obtained during 30 sweeps.

Prior to the analysis, the raw EMG signals were rectified and filtered with a Butterworth low pass filter with a cut-off frequency of 40 Hz. After the rectification and filtering of the raw EMG signals, the individual sweeps were visually analyzed to ensure that the signals did not include artifacts or noise and those which did were excluded. The raw and preprocessed signals can both be seen in figure 7.4.

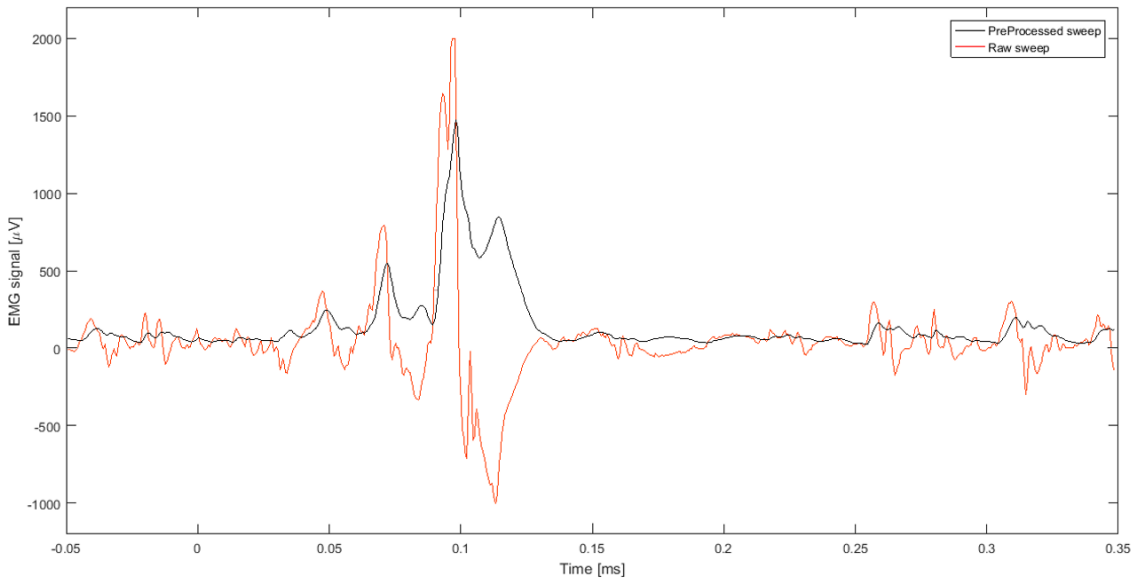


Figure 7.4: Example of the raw sweep and its preprocessed version of subject 11 from the intervention group. The black line represents the preprocessed sweep and the red line represents the raw sweep.

A mean of the remaining EMG signals was obtained for each data set resulting in three averaged signals; S1 (prior), S2 (immediately post) and S3 (post 30 minutes). To perform a comparison of the three EMG data sets, the root mean square (RMS) of the baseline (recorded 100 ms prior to onset of the perturbation) of the S2 and S3 had to be in a range of ±3 times the standard deviation (SD) of the baseline of S1. In case the RMS of S2 and S3 were not in the range, the individual sweeps with abnormal (much higher or much lower than baseline) RMS were not included in the calculation of the mean of the data sets. If the RMS values of the baseline of S2 and S3 were still not in the range of ±3 times the SD of the baseline of S1, abnormal (the ones furthest away from the mean) individual sweeps were also excluded from S1. This was done to ensure that the baseline of the 3 different data sets did not vary too much from each other and did not influence the results. Higher pre-contraction could cause higher reflex amplitudes due to the automatic gain theory while lower baseline EMG values will cause a decrease in reflex size [Ogawa et al., 2012].

The preprocessed averaged signals (S1, S2 and S3) were visually analyzed and the first two peaks (M1 and M2) of the stretch reflex response were identified. For the identification, it was assumed that the onset of the first peak should not occur before 40 ms after the perturbation and the onset of the second peak could not occur after 90 ms after onset of the pedal perturbation. This was based on the literature suggesting that the first peak should occur around 44 ms [Petersen et al., 1998] after the perturbation and the second peak around 69 ms [Petersen et al., 1998] after the perturbation. For proper identification, individual EMG traces were also inspected visually for the two peaks (M1 and M2) before looking at the average signal to ensure that the assumed peaks occur throughout all the samples. Once the M1 and M2 were found, the peak amplitude and the peak latency were

extracted. Signal preprocessing and data extraction was done in the software program Mr. Kick III (Kund Larsen, SMI, Aalborg University).

7.5 Statistical Analysis

A two-way repeated measures ANOVA was carried out to evaluate the effect of time and group on the outcome measures (rMT and, K-value, MEPmax-value and X50-value of the Boltzmann fit, the slope of the line fit and, peak latency and peak amplitude of the first two components of the stretch reflex). Time (pre, post and post 30) was used as an independent within-subject variable and group (intervention and control) was used as a between-subject variable. All the outcome measures were tested separately

First the different outcome measures were tested on normality and sphericity to see if the assumption for performing an analysis of variance (ANOVA) were not violated. The Shapiro-Wilk test was used to test if the data of every within-subject variable had a normal distribution. The Shapiro-Wilk test was used instead of the Kolmogorov-Smirnov test because of the relatively small sample size. Sphericity of the data was tested with Mauchly's test of sphericity. When sphericity could not be assumed ($P<0.05$), then the data was adjusted by using the Huynh-Feldt estimate.

If a significant effect ($P<0.05$) of the interaction between time and group (time*group) was found a paired-samples t-test was performed. If only a significant effect of time was found, a paired-samples t-test was performed without taking into account the between-subject variable (group). If only a significant effect of group was found, an independent t-test was performed. An independent t-test was also performed to check for difference between the two groups (intervention and control) at baseline (pre-measurements). The t-tests were corrected for the multiple tests of significance. These corrections where carried out based on an article of Rom [1990].

All statistical tests were carried out using IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corporation.

Chapter 8
Results

In this chapter, the results obtained during the experiments will be presented. The chapter will be divided into three different sections. The first section (section 8.1) will present the results of the rMT for the three different time points. The second section (section 8.2) will present the results of the I/O curves obtained by fitting two different equations (Boltzmann equation and the equation for a straight line) to the TMS data. The third section (section 8.3) will present the results of the stretch reflex measurements. All data was tested for a significant difference between the two groups (intervention and control) at baseline. It showed that there was no significant difference between groups at baseline.

8.1 Resting Motor Threshold

Most of the data for the rMT was found to have a normal distribution. Only the data for the intervention group obtained 30 minutes after the intervention was not found to be normally distributed ($p<0.05$, Shapiro-Wilk test). The results of the normality tests can be found in appendix C. The mean rMT was calculated for the three time points for both groups separately. The resulting means with their standard deviations can be seen in table 8.1.

Table 8.1: Mean values and standard deviation for the rMT for the three time points for both groups (control and intervention) separately. SD represents standard deviation and N represents number of subjects

Group		Mean [%]	SD	N
Threshold pre	**Control**	60.88	7.28	8
	Intervention	56.44	8.13	9
Threshold post	**Control**	63.63	4.81	8
	Intervention	57.33	6.86	9
Threshold post 30	**Control**	57.63	6.63	8
	Intervention	55.67	6.60	9

The two-way repeated measures ANOVA for the rMT showed significance for the main effect of time on the rMT; $F(2,30)=4.810$, $p=0.015$. The main effect of group yielded an F-ratio of $F(1,15)=1.998$, $p=0.178$ indicating that the effect of group was not significant. The interaction between time and group was not found to have a significant effect on the rMT; $F(2,30)=1.546$, $p=0.230$.

Paired-samples t-test performed to analyze the significant effect of time on the rMT found a significant difference between the mean rMT immediately post intervention (M=60.3 %, SD=6.6) and the mean rMT 30 minutes post intervention (M=56.6 %, SD=6.5); $t(16)=3.452$, $p=0.003$. The mean difference was 3.7 % (SD=4.4). This significant difference is marked in figure 8.1 which shows the summary of the results for the rMT, where the mean values and its standard deviations are visualized. The significant difference is shown with the asterisk symbol (*).

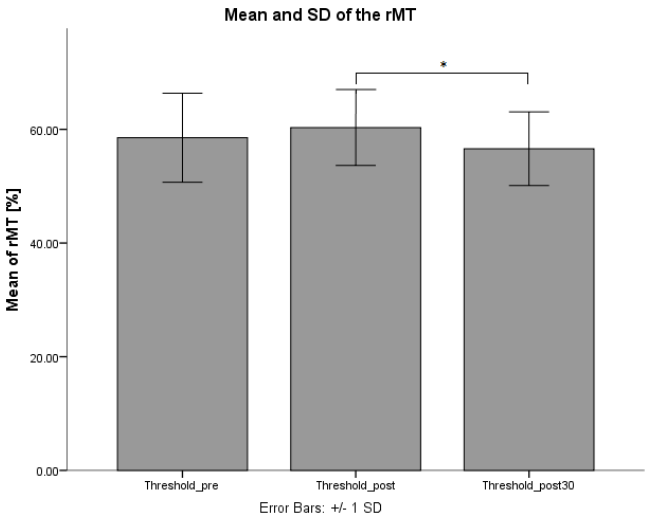


Figure 8.1: The mean values and standard deviations of the rMT for both groups over the three measured time points. Significant interaction is marked with the asterisk symbol (*).

8.2 Input/Output Curves

The following section presents the results of fitting the data to the Boltzmann equation and to a straight line. An example of the single MEPs obtained with intensity of 120 % of the rMT for the three time points can be seen in figure 8.2. The MEPs of two subjects are displayed, one from the control group and one from the intervention group. The MEPs which were closely reflecting the mean peak-to-peak amplitude (μV) of the specific intensity (120 % of rMT) were chosen to be displayed.

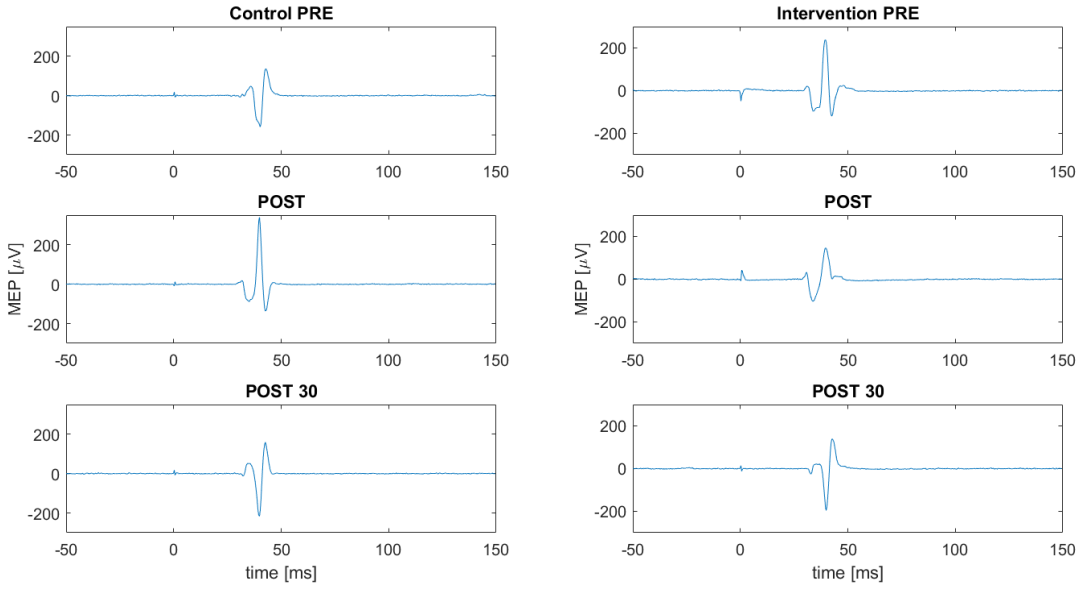


Figure 8.2: MEPs of subject 1 from the control group on the left and MEPs of subject 9 from the intervention group on the right for the three different time points. MEPs were obtained with intensity of 120 % of rMT.

The results of both fits are presented in separate subsections below.

8.2.1 Boltzmann Fit

The following subsection presents the results of the analysis of the I/O curve which was derived by fitting the data to a Boltzmann equation. An example of the Boltzmann fit can be seen in figure 8.3. Figure 8.3 shows the measured data points and the corresponding Boltzmann fit from a subject from

the control group (subject 12) and from a subject from the intervention group (subject 15) for the three different time points.

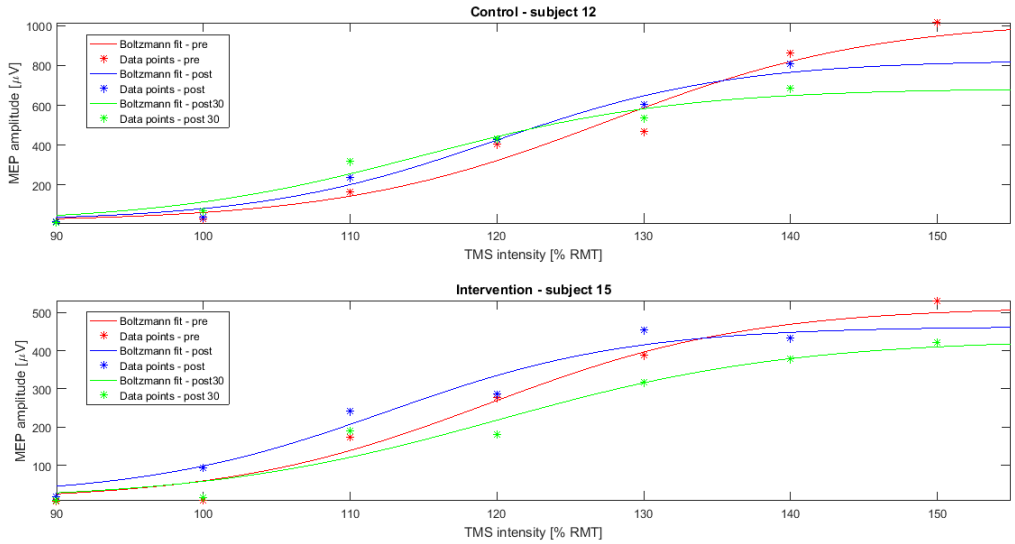


Figure 8.3: I/O curves from a subject from the control group and a subject from the intervention group for the 3 different time points. The stars represent the measured data points and the lines represent the Boltzmann fit. Red represents the pre-measurements, blue represents the post measurements and green represents the post 30 measurements.

The overall goodness of the Boltzmann fit can be seen in table 8.2. Here the root-mean-square error, the sum of squared errors and the R-squared are given for the Boltzmann fits for the different groups and time points.

Table 8.2: Goodness of the fit for the Boltzmann equation fitted to the data point obtained during TMS stimulation. R^2 indicates r-squared, RMSE indicates root-mean-square error, SSE indicates the sum of squared errors and SD indicates standard deviation.

Group	Time	$R^2 \pm$ SD	RMSE $\pm$ SD	SSE $\pm$ SD
Control	**Pre**	0.93 $\pm$ 0.06	67.80 $\pm$ 43.59	21881.75 $\pm$ 24150.44
	Post	0.97 $\pm$ 0.03	35.98 $\pm$ 22.32	5191.80 $\pm$ 4681.48
	Post 30	0.911 $\pm$ 0.09	43.52 $\pm$ 23.12	7085.2 $\pm$ 6319.13
Intervention	**Pre**	0.94 $\pm$ 0.08	46.40 $\pm$ 35.20	7741.42 $\pm$ 8655.39
	Post	0.90 $\pm$ 0.13	43.90 $\pm$ 43.60	10854.3 $\pm$ 19230.19
	Post 30	0.86 $\pm$ 0.144	38.28 $\pm$ 18.79	5568.56 $\pm$ 5747.99

The Slope K of the Boltzmann Fit

The data for the slope K of the Boltzmann curve was found to be normally distributed. The results of the normality tests for the slope K can be found in appendix C. The mean values of slope K were calculated for both groups and the three different time points. The resulting means with their standard deviations can be seen in table 8.3.

Table 8.3: Mean values and standard deviation for the K-value of the Boltzmann fit for the three different time points and for both groups (control and intervention) separately. SD represents standard deviation and N represents number of subjects.

		Mean	SD	N
K_pre	**Control**	6.56	3.39	8
	Intervention	5.78	2.98	9
K_post	**Control**	6.70	2.22	8
	Intervention	6.04	3.81	9
K_post30	**Control**	7.43	3.82	8
	Intervention	5.09	4.40	9

The main effect of time on the slope K yielded an F-ratio of F(2,30)=0.016, p=0.984, indicating that the effect of time was not significant. The main effect of group yielded an F-ratio of F(1,15)=1.311, p=0.270 indicating that the effect of group was also not significant. The interaction between time and group was not found to have a significant effect on the slope of the Boltzmann fit; F(2,30)=0.337, p=0.717.

A summary of the results for the slope K can be seen in the figure 8.4. Here the mean values and its standard deviations are visualized. No significant effects were found.

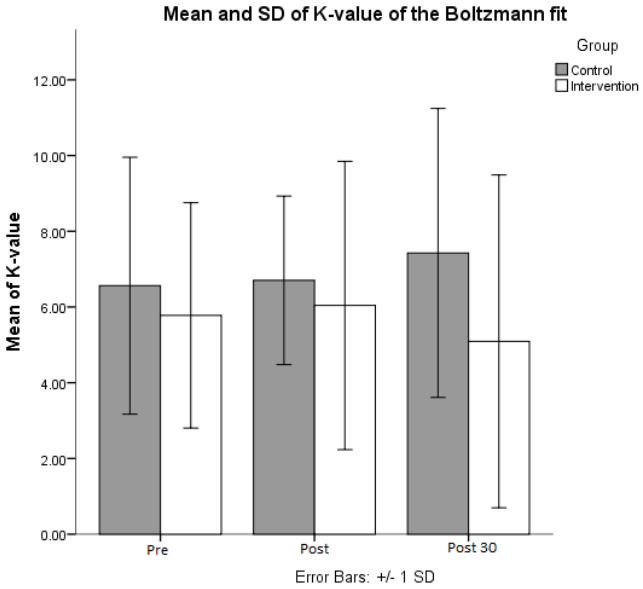


Figure 8.4: The mean values and standard deviations of the slope K of the Boltzmann fit for both groups for the three different time points.

MEP_{max}-value of the Boltzmann Fit

Most of the data for the MEP_{max} value of the Boltzmann fit was found to have a normal distribution. Only the data obtained 30 minutes after the intervention of the intervention group was not found to be normally distributed (p<0.05, Shapiro-Wilk test). The results of the normality tests for the MEP_{max} can be found in the appendix C. The mean values of the MEP_{max} were calculated for both groups and the three different time points. The resulting means with their standard deviations can be seen in table 8.4. The normalized mean values for MEP_{max} were also calculated. These values represent the difference compared to the baseline (MEP_{max}-pre) in percentage. A negative number indicates a decline compared to baseline and a positive number indicates an increase compared to baseline.

Table 8.4: Mean values and standard deviation for the MEP_{max} value of the Boltzmann fit for the three different time points and both groups (control and intervention) separately. SD represents standard deviation, N represents number of subjects and NM represents the normalized mean.

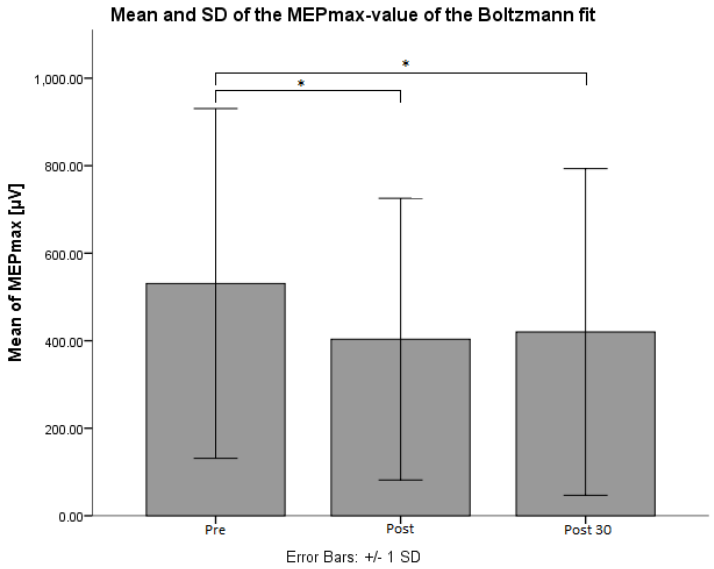
		Mean [μV]	SD	NM [%]	SD	N
MEPmax pre	**Contr.**	639.15	478.97			8
	Interv.	434.43	310.90			9
MEPmax post	**Contr.**	539.18	422.51	-13.25	21.07	8
	Interv.	282.94	127.75	-23.08	22.83	9
MEPmax post30	**Contr.**	526.17	446.69	-22.66	20.66	8
	Interv.	325.89	287.86	-25.14	36.15	9

The two-way repeated measures ANOVA conducted for the MEP_{max} value of the Boltzmann fit showed a main effect of time, which yielded an F-ratio of F(2,30)=0.4.637, p=0.018, indicating that the effect of time was significant. The main effect of group yielded an F-ratio of F(1,15)=1.746,

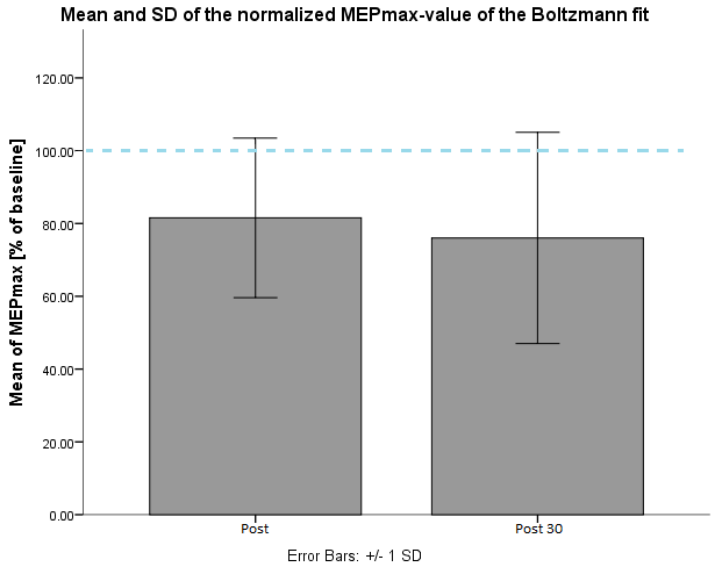
p=0.206 indicating that the effect of group was not significant. The interaction between time and group was not found to have a significant effect on the MEP_{max}; F(2,30)=0.238, p=0.790.

Paired-samples t-test performed to analyze the significant effect of time on the MEP_{max} found a significant difference between the mean MEP_{max} prior to the intervention (M=530.8 μV, SD=399.7) and the mean MEP_{max} immediately post intervention (M=403.5 μV, SD=322); t(16)=2.546, p=0.022. The mean difference was 127.2 μV (SD=206.1). A significant difference was also found between the mean MEP_{max} prior to the intervention (M=530.8 μV, SD=399.7) and the mean MEP_{max} 30 minutes post intervention (M=420.1 μV, SD=373.3); t(16)=3.774, p=0.002. The mean difference was 110.6 μV (SD=120.9).

The significant differences are marked in figure 8.5 which shows the summary of the results for the MEP_{max}. Here the total mean values and its standard deviations are visualized. Also the normalized data is shown here, which represents the change compared to the baseline (MEP_{max}-pre) measurements. 100 % indicates that no change has occurred, >100 % indicates an increase in MEP_{max} and <100 % indicates a decline in MEP_{max}. The significant interactions are shown with the asterisk symbol (*).



(a) raw data



(b) Normalized data

Figure 8.5: The mean values and standard deviations of the MEP_{max} of the Boltzmann fit for both groups for the three measured time points. (a) is a representation of the raw data. (b) is a representation of the normalized data. The normalized data is calculated as a percentage change with the baseline (represented by the dashed blue line). 100 % means that no change happened.

S_{50}-value of the Boltzmann Fit

The data for the S_{50}-value of the Boltzmann curve was found to be normally distributed. The results of the normality tests for the S_{50}-value can be found in appendix C. The mean values of the S_{50} were calculated for both groups and the three different time points. The resulting means with their standard deviations can be seen in table 8.5.

Table 8.5: Mean values and standard deviation for the S_{50}-value of the Boltzmann fit for the three different time points for both groups (control and intervention) separately. SD represents standard deviation and N represents number of subjects.

		Mean [% rMT]	SD	N
S50_pre	**Control**	116.74	9.48	8
	Intervention	118.56	9.32	9
S50_post	**Control**	115.50	8.61	8
	Intervention	113.21	8.18	9
S50_post30	**Control**	112.40	7.13	8
	Intervention	109.99	13.70	9

The two-way repeated measures ANOVA conducted for the S_{50}-value for the Boltzmann fit showed a main effect of time, which yielded an F-ratio of F(1.519,22.780)=3.345, p=0.065, indicating that the effect of time was not significant even though a tendency was present. The main effect of group yielded an F-ratio of F(1,15)=0.066, p=0.801 indicating that the effect of group was not significant even though a tendency was present. The interaction between time and group was not found to have a significant effect on the S_{50}; F(1.519,22.780)=0.465, p=0.581.

A summary of the results for the S_{50}-value of the Boltzmann fit can be seen in figure 8.6. Here the mean values and its standard deviations are visualized. No significant effects were found.

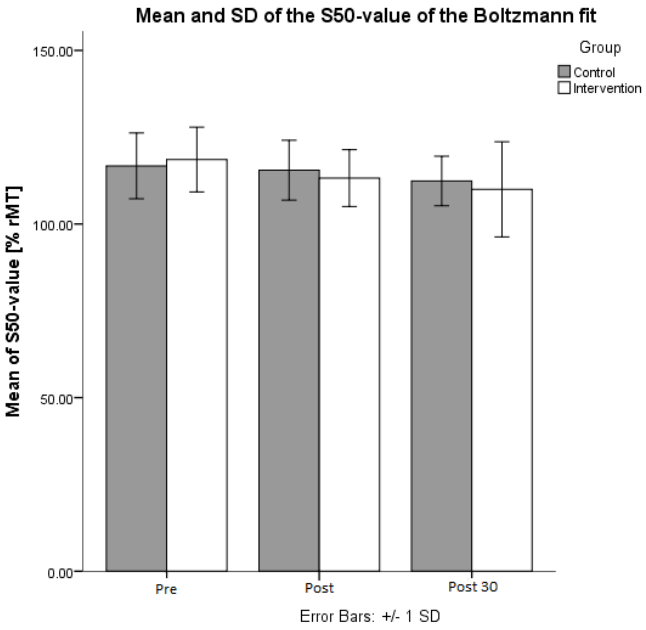


Figure 8.6: The mean values and standard deviations of the S_{50} of the Boltzmann function for both groups for the three measured time points.

8.2.2 Line Fit

The following subsection presents the results of the data fitted to a straight line. An example of the line fit can be seen in figure 8.7. Figure 8.7 shows the measured data points and the corresponding line fit of a subject from the control group (subject 12) and data of a subject from the intervention group (subject 15) for the 3 different time points.

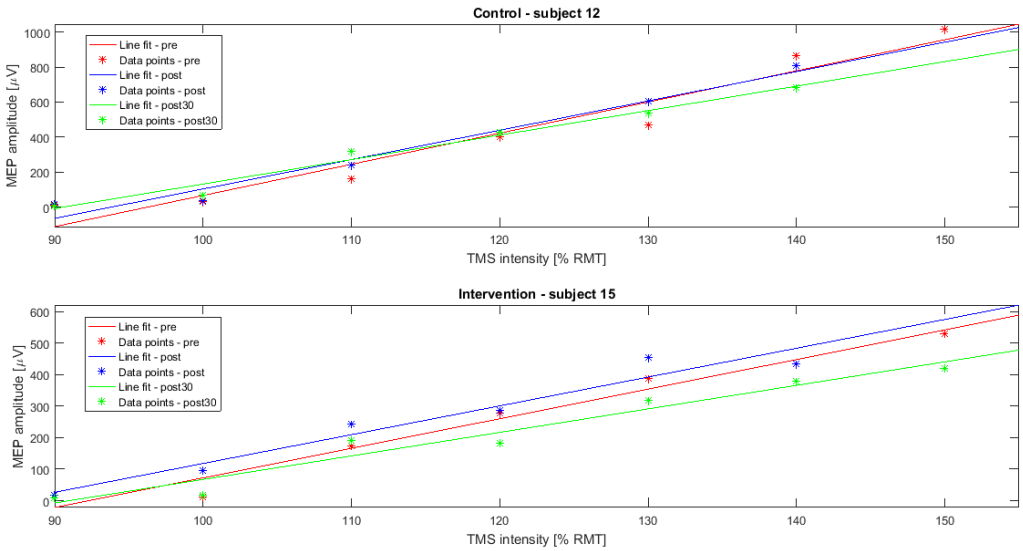


Figure 8.7: Line fits from a subject from the control group and a subject from the intervention group for the 3 different time points. The stars represent the measured data points and the lines represent the line fit. Red represents the pre-measurements, blue represents the post measurements and green represents the post 30 measurements.

The overall goodness of the line fit can be seen in table 8.6. Here the root-mean-square error, the sum of squared errors and the R-squared are given for the line fits for the different groups and time points.

Table 8.6: Goodness of the fit of the line fitted to the data point obtained during TMS stimulation. R^2 indicates r-squared, RMSE indicates root-mean-square error, SSE indicates the sum of squared errors and SD indicates the standard deviation.

Group	Time	$R^2 \pm$ SD	RMSE $\pm$ SD	SSE $\pm$ SD
Control	**Pre**	0.86 $\pm$ 0.14	92.27 $\pm$ 86.45	67489 $\pm$ 101017.8
	Post	0.89 $\pm$ 0.09	63.89 $\pm$ 48.43	20510.19 $\pm$ 36318.79
	Post 30	0.88 $\pm$ 0.08	84.43 $\pm$ 105.48	67465.79 $\pm$ 153915.8
Intervention	**Pre**	0.72 $\pm$ 0.14	80.07 $\pm$ 95.68	47911.7 $\pm$ 104902.4
	Post	0.70 $\pm$ 0.23	50.79 $\pm$ 46.28	18323.3 $\pm$ 37910.19
	Post 30	0.29 $\pm$ 0.29	48.13 $\pm$ 29.61	13252.6 $\pm$ 13701.42

Slope of Line Fit

Most of the data for the slope of the line fit was found to have a normal distribution, besides the data immediately post intervention for the control group (p<0.05, Shapiro-Wilk test). The results of the normality tests for the slope of the straight line can be found in appendix C. The mean values of the slope of the line were calculated for both groups and the three time points. The resulting means with their standard deviations can be seen in the table 8.7.

Table 8.7: Mean values and standard deviation for the slope of the line for the three different time points for both groups (control and intervention) separately. SD represents standard deviation and N represents the number of subjects.

		Mean	SD	N
Slope_pre	**Control**	12.15	10.52	8
	Intervention	8.38	5.32	9
Slope_post	**Control**	8.42	9.08	8
	Intervention	7.73	4.55	9
Slope_post30	**Control**	10.64	10.86	8
	Intervention	6.90	5.41	9

The two-way repeated measures ANOVA conducted for the slope of the line fit showed a main effect of time, which yielded an F-ratio of F(2,30)=3.192, p=0.055, indicating that the effect of time was

not significant even though a tendency was present. The main effect of group yielded an F-ratio of $F(1,15)=0.548$, $p=0.471$ indicating that the effect of group was not significant. The interaction between time and group was not found to have a significant effect on the slope of the line; $F(2,30)=1.984$, $p=0.155$.

The summary of the results for the slope of the line can be seen in the figure 8.8. Here the mean values and its standard deviations are visualized. No significant effects were found.

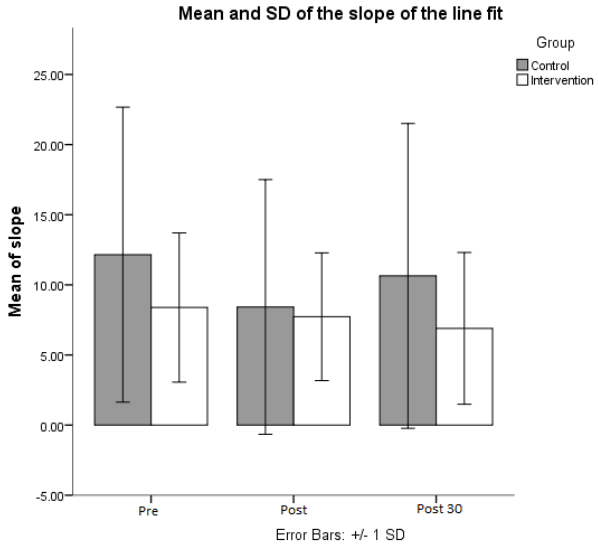
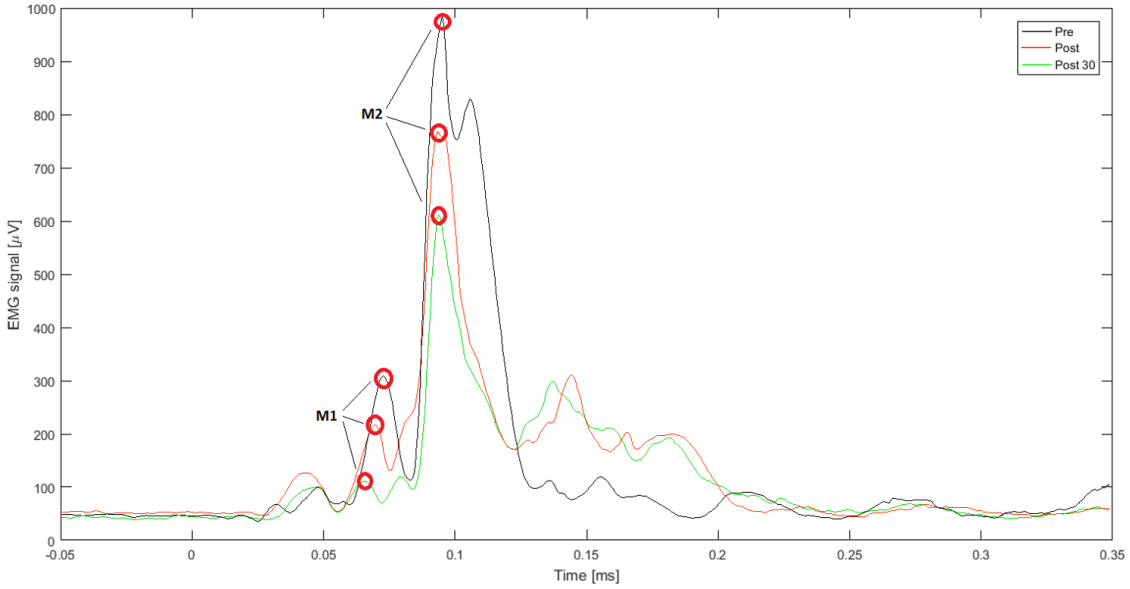


Figure 8.8: The mean values and standard deviations of the slope of the line for both groups for the three measured time points.

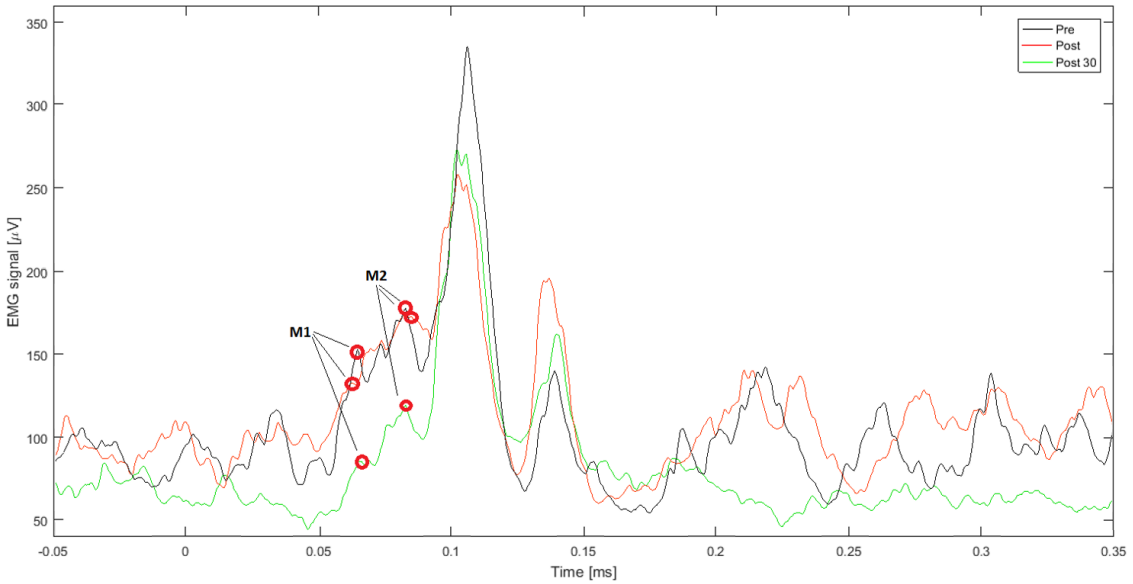
8.3 Stretch Reflex

The following section presents the results of the stretch reflex. The data of subject 10 of the control group was excluded from the data set because of saturation of the signal. Also subject 8 was not included, because the M1 and M2 peak were not successfully identified. The first peak (M1) was not found in one subject from the control group. The second peak (M2) was not identified for one subject from the intervention group. Thus, the final data for the analysis of the M1 peak consisted of 14 subjects (6 control, 8 intervention) and the data for the analysis of the M2 peak also consisted of 14 subjects (7 control, 7 intervention).

An example of the mean signals from two subjects (one from the control group and one from the intervention group) is shown in figure 8.9, where the identified peaks are marked with red circles.



(a) Subject 11 - intervention group



(b) Subject 12 - control group

Figure 8.9: Superimposed view of the mean signal for the three time point for two test subjects. The identified peaks are marked with red circles. Black represents the pre-measurements, red represents the post measurements and green represents the post 30 measurements.

The following parts of the section present the results of analysis of the identified peaks M1 and M2. Peak amplitude and peak latency were analyzed for both peaks.

8.3.1 First Component of the Stretch Reflex

Amplitude of the M1 Peak

Most of the data for the amplitude of the M1 peak was found to have a normal distribution, besides the data 30 minutes post intervention for the intervention group (p<0.05, Shapiro-Wilk test). The results of the normality tests for the amplitude of the M1 peak can be found in appendix C. The mean values of the amplitude of the M1 peak were calculated for both groups and the three different time points. The resulting means with their standard deviations can be seen in table 8.8. The normalized mean values for the amplitude of the M1 peak were also calculated. These values represent the difference compared to the baseline (amplitude of M1 peak-pre) in percentage. A negative number indicates a

decline compared to baseline and a positive number indicated an increase compared to baseline.

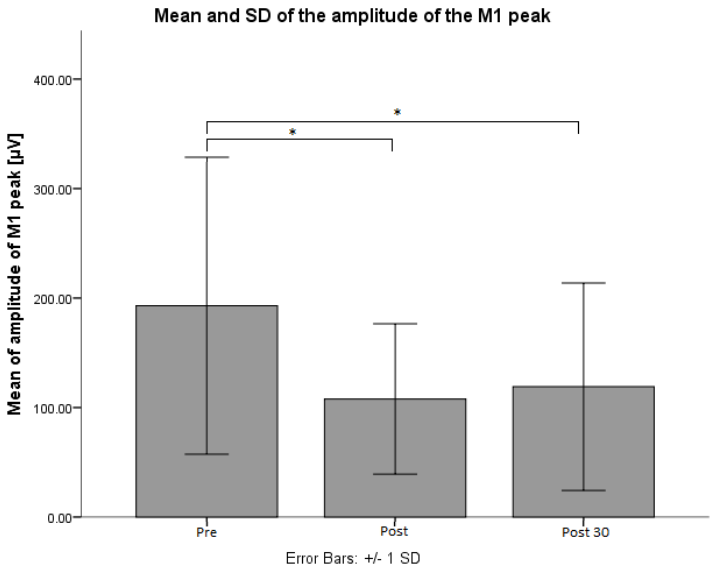
Table 8.8: Mean values and standard deviation for the amplitude of the M1 peak for the three different time points for both groups (control and intervention) separately. SD represents standard deviation, N represents the number of subjects and NM represents the normalized mean.

		Mean [μV]	**SD**	**NM [%]**	**SD**	**N**
Amplitude M1 pre	**control**	199.87	146.48			6
	intervention	187.91	137.04			8
Amplitude M1 post	**control**	88.84	52.57	-45.41	30.06	6
	intervention	122.10	79.09	-32.88	14.44	8
Amplitude M1 post30	**control**	120.19	87.09	-36.82	26.70	6
	intervention	118.15	105.98	-35.16	24.03	8

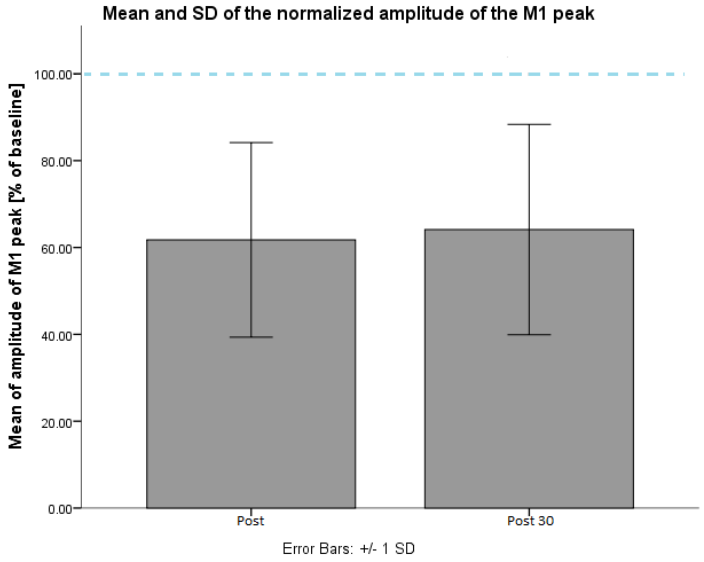
The two-way repeated measures ANOVA conducted for the amplitude of the M1 peak showed a significance for the main effect of time, which yielded an F-ratio of $F(2,24)=9.712$, $p=0.001$. The main effect of group yielded an F-ratio of $F(1,12)=0.015$, $p=0.904$ indicating that the effect of group was not significant. Neither the interaction between time and group was found to have a significant effect on the amplitude of M1 peak; $F(2,24)=0.606$, $p=0.554$.

Paired-samples t-test performed to analyze the significant effect of time on the amplitude of M1 found a significant difference between the mean M1 amplitude prior to the intervention (M=193 μV, SD=135.7) and the mean M1 amplitude immediately post intervention (M=107.8 μV, SD=68.7); $t(16)=3.417$, $p=0.005$. The mean difference was 85.2 μV (SD=93.3). Significant difference was also found between the mean M1 amplitude prior to the intervention (M=193 μV, SD=135.7) and the mean M1 amplitude 30 minutes post intervention (M=119 μV, SD=94.7); $t(16)=3.939$, $p=0.002$. The mean difference was 74 μV (SD=70.4).

The significant differences are marked in figure 8.10 which shows the summary of the results for the amplitude of M1 peak. Here the mean values and its standard deviations are visualized. Also, the normalized data is shown which represents the change compared to the baseline (amplitude of M1 peak-pre) measurements. 100 % indicates that no change has occurred, >100 % indicates an increase in amplitude of the M1 peak and <100 % indicates a decline in amplitude of the M1 peak. The significant interactions are shown with the asterisk symbol (*).



(a) raw data



(b) Normalized data

Figure 8.10: The mean values and standard deviations of the amplitude of the M1 peak for both groups for the three measured time points. Significant interactions are marked with asterisk symbol (*). (a) is a representation of the raw data. (b) is a representation of the normalized data. The normalized data is calculated as a percentage change with the baseline (represented by the dashed blue line). 100 % means that no change happened.

Latency of the M1 Peak

The data for the latency of the M1 peak was found to be normally distributed. The results of the normality tests for the latency of the M1 peak can be found in appendix C. The mean values of the latency of the M1 peak were calculated for both groups and the three different time points. The resulting means with their standard deviations can be seen in table 8.9.

Table 8.9: Mean values and standard deviation for the latency of the M1 peak for the three different time points for both groups (control and intervention) separately. SD represents standard deviation and N represents the number of subjects.

		Mean [ms]	SD	N
Latency M1 pre	**control**	52.67	7.86	6
	intervention	59.19	3.94	8
Latency M1 post	**control**	51.83	8.59	6
	intervention	57.19	3.69	8
Latency M1 post30	**control**	52.67	7.65	6
	intervention	56.75	5.89	8

The main effect of time yielded an F-ratio of F(2,24)=0.893, p=0.422, indicating that the effect of time was not significant. The main effect of group yielded an F-ratio of F(1,12)=2.910, p=0.114 indicating that the effect of group was not significant. The interaction between time and group was not found to have a significant effect on the latency of M1; F(2,24)=0.564, p=0.576.

A summary of the results for the latency of the M1 peak can be seen in the figure 8.11. Here the mean values and its standard deviations are visualized. No significant effects were found.

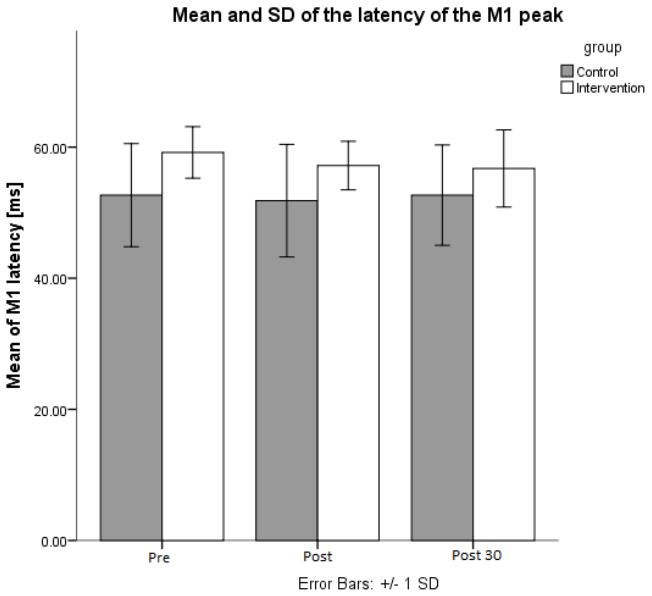


Figure 8.11: The mean values and standard deviations of the latency of the M1 peaks for both groups over three measured time points.

8.3.2 Second Component of the Stretch Reflex

Amplitude of the M2 Peak

Most of the data for the amplitude of the M2 peak was found to have a normal distribution, besides the data prior to the intervention for the intervention group (p<0.05, Shapiro-Wilk test). The results of the normality tests for the amplitude of M2 peak can be found in appendix C. The mean values of the amplitude of the M2 peak were calculated for both groups for the three different time points. The resulting means with their standard deviations can be seen in table 8.10. The normalized mean values for amplitude of the M1 peak were also calculated. These values represent the difference compared to the baseline (amplitude of M2 peak-pre) in percentage. A negative number indicates a decline compared to baseline and a positive number indicated an increase compared to baseline.

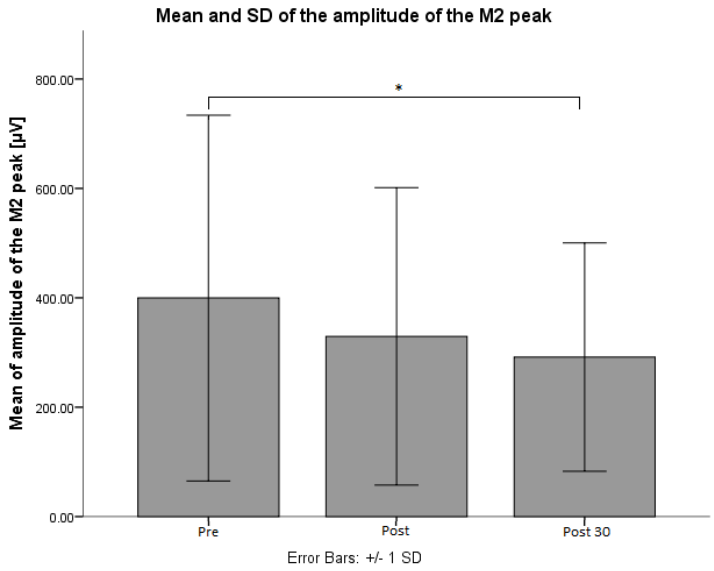
Table 8.10: Mean values and standard deviation for the amplitude of the M2 peak for the three different time points for both groups (control and intervention) separately. SD represents standard deviation, N represents the number of subjects and NM represents the normalized mean.

		Mean [μV]	**SD**	**NM [%]**	**SD**	**N**
Amplitude M2 pre	**control**	521.76	317.86			7
	intervention	278.09	327.41			7
Amplitude M2 post	**control**	370.21	310.25	-30.78	30.17	7
	intervention	288.74	245.25	18.00	61.48	7
Amplitude M2 post30	**control**	339.34	230.25	-34.17	30.00	7
	intervention	243.76	189.91	5.19	49.52	7

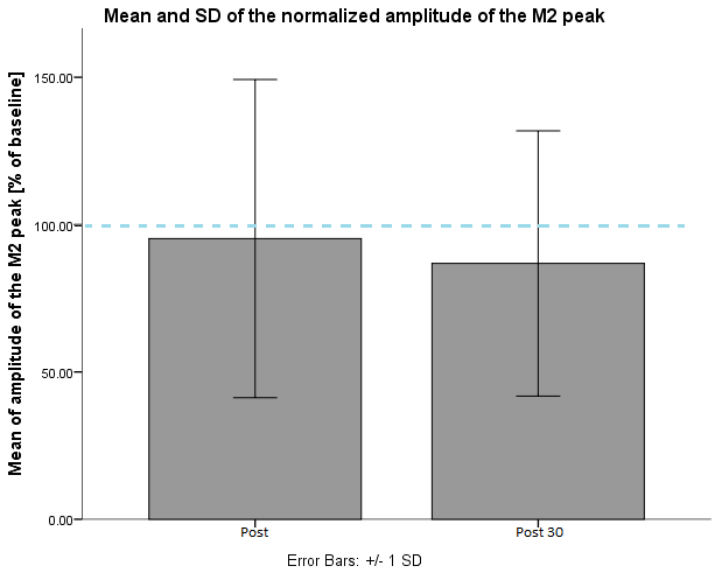
The two-way repeated measures ANOVA conducted for the amplitude of the M2 peak showed a significance for the main effect of time, which yielded an F-ratio of $F(2,24)=3.608$, $p=0.043$. The main effect of group yielded an F-ratio of $F(1,12)=1.015$, $p=0.334$ indicating that the effect of group was not significant. The interaction between time and group was not found to have a significant effect on the amplitude of the M2 peak; $F(2,24)=2.408$, $p=0.111$.

Paired-samples t-test performed to analyze the significant effect of time on the amplitude of M1 found a significant difference between the mean M1 amplitude prior to the intervention (M=399.9 μV, SD=334.8) and the mean M1 amplitude 30 minutes post intervention (M=291.6 μV, SD=208.7); $t(16)=3.939$, $p=0.048$. The mean difference was 108.4 μV (SD=185.3).

The significant difference is marked in figure 8.12 which shows the summary of the results for the amplitude of the M2 peak. Here the mean values and its standard deviations are visualized. Also, the normalized data is shown which represents the change compared to the baseline (amplitude of M2 peak-pre) measurements. 100 % indicates that no change has occurred, >100 % indicates an increase in amplitude of the M2 peak and <100 % indicates a decline in amplitude of the M2 peak. The significant interaction is shown with the asterisk symbol (*).



(a) raw data



(b) Normalized data

Figure 8.12: The mean values and standard deviations of the amplitude of the M2 peaks for both groups for three measured time points. Significant interactions are marked with asterisk symbol (*). (a) is a representation of the raw data. (b) is a representation of the normalized data. The normalized data is calculated as a percentage change with the baseline (represented by the dashed blue line). 100 % means that no change happened.

Latency of the M2 Peak

The data for the latency of the M2 peak was found to be normally distributed. The results of the normality tests for the latency of the M2 peak can be found in appendix C. The mean values of the latency of the M2 peak were calculated for both groups for the three different time points. The resulting means with their standard deviations can be seen in table 8.11.

Table 8.11: Mean values and standard deviation for the latency of the M2 peak for the three different time points for both groups (control and intervention) separately. SD represents standard deviation and N represents the number of subjects.

		Mean [ms]	SD	N
Latency M2 pre	**control**	78.36	15.86	7
	interven	79.21	9.62	7
Latency M2 post	**control**	78.71	16.27	7
	interven	80.29	6.42	7
Latency M2 post30	**control**	78.79	15.21	7
	interven	80.36	7.31	7

The main effect of time yielded an F-ratio of F(2,24)=0.565, p=0.576, indicating that the effect of time was not significant. The main effect of group yielded an F-ratio of F(1,12)=0.041, p=0.843 indicating that the effect of group was not significant. The interaction between time and group was not found to have a significant effect on the latency of the M2 peak; F(2,24)=0.127, p=0.881.

A summary of the results for the latency of the M2 peak can be seen in the figure 8.13. Here the mean values and its standard deviations are visualized. No significant effects were found.

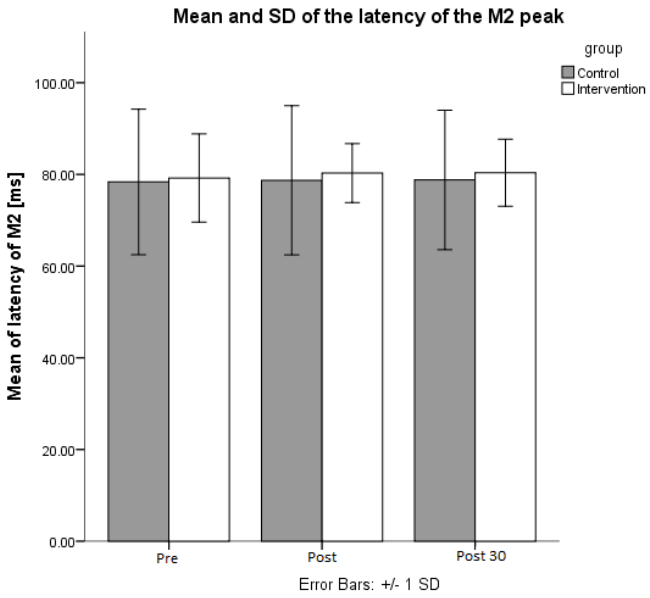


Figure 8.13: The mean values and standard deviations of the latency of the M2 peaks for both groups for three measured time points.

Part III

Synthesis

Chapter 9
Discussion

In this chapter, the results presented in the previous chapter (chapter 8) will be discussed. In the first section (section 9.1), the results will be put into context and will be compared with finding from other studies. The second section (section 9.2) will focus on the methodological aspect of this study and will compare the methodologies used in the current study with methodologies used in other studies. The last section (section 9.2.4) will shortly give some recommendations for future studies.

Although some of the data did not have a normal distribution, a two-way repeated measures ANOVA was used to analyze the data. However, the non-normality was usually found only for one data set of the specific outcome measure. It is however not expected that this is influencing the data because Ito [1980] states that the ANOVA is robust enough for data which does not have a normal distribution when the data of both groups is of similar size.

9.1 Results

9.1.1 Changes in rMT

In the present study, a significant decrease in rMT was found between the measurements immediately post and 30 minutes post intervention. No difference was found between the groups. A decrease in threshold can indicate that the neurons in the nearby location of the coil (i.e. the neurons with a low threshold for TMS) increased in excitability [Perez et al., 2004] which possibly indicates a higher cortical excitability as stated by Hsu et al. [2015]. However, it could also be another part of the corticospinal pathway which is responsible for a change in rMT. In patients (e.g. stroke, SCI, ALS), for which the location of the change in the pathway is known, the change in the rMT was found to be related to either spinal excitability or cortical excitability [Groppa et al., 2012]. This indicates that the change could be caused by an increase in excitability at a cortical level but also by an increase in excitability at a spinal level.

When looking at the changes in the rMT on an individual basis, big changes in rMT over time can be seen. However, the direction of change differed from subject to subject. This is in line with earlier research from Wassermann [2002] and Tranulis et al. [2006]. They both found a big inter- and intra- individual variability in rMT. Wassermann [2002] found an inter-session variability of 12 $\pm$ 7 % for the rMT, however the sessions were spread out over a month. The study of Tranulis et al. [2006] found a similar variability (9.0 $\pm$ 5.6 %) and their rMT measurements were spread out over 90 minutes, which is comparable to the present study. It is thus possible that not updating the stimulation intensities, needed for the I/O curve, to the rMT could affect the data.

9.1.2 Corticospinal Excitability

Three parameters of the I/O curves were analyzed, S_{50}, slope K and the MEP_{max}. In addition, the slope of the straight line fitted to the data was analyzed as an alternative to the slope K of the Boltzmann fit.

A significant decrease in the MEP_{max}-value over time was found, while no significant differences between groups were found. This observation of MEP_{max} reduction is in line with previous studies investigating arm muscles [Samii et al., 1996] as well as leg muscles [Temesi et al., 2014; Brasil-Neto et al., 1993; Schubert et al., 1998; Brasil-Neto et al., 1994]. In these studies the decrease in MEP_{max} was linked to physical fatigue induced by walking. In the study by Temesi et al. [2014] the decrease

was found after ultra-trail running of 110 km, while in the study of Schubert et al. [1998] the decrease in MEP_{max} was observed after 15 minutes walking with comfortable pace for both, test and control groups. Furthermore, the study by Brasil-Neto et al. [1993] beside using TMS, also used TES which did not show any post-exercise decrease in the MEPs. The neuronal system behind the MEP_{max} decrease is therefore assumed to be related to the fibers presynaptic to the corticospinal neurons [Schubert et al., 1998], which are excited by the TMS, since the TES is exciting the corticospinal neurons directly [Day et al., 1987]. In contrary with these results is the study by Kido Thompson and Stein [2004] which did not found any changes in MEP_{max} as well as in S_{50} following walking with a speed of 4.5 km/h. However, in the study of Kido Thompson and Stein [2004] the time of walking was only 10 minutes and this might be the reason why no decrease was observed, since it might not have been long enough to induce some level of fatigue. Results of the present study, which are in line with the literature, seem to indicate that it is indeed a certain level of fatigue caused by the treadmill walking which is reducing the excitability of the fibers presynaptic to the corticospinal neuron. However, fatigue after walking was not evaluated and it can be questioned whether it is possible to induce fatigue when walking 30 minutes with a speed of 2.5 km/h.

The effects of time and group were not found to significantly affect the two other parameters of the I/O curves, the slope K and S_{50}-value. A tendency was however seen in S_{50} for both the effect of time (p=0.065) and group (p=0.066). This could be explained by high inter-subject variability and therefore high standard deviations which affect the statistical test. Higher number of subjects could help to reduce the SD and show different statistical result. All the parameters of the I/O curves are not dependent on the behavior of a single motorneuron but are rather reflecting multiple descending volleys of the corticospinal tract [Devanne et al., 1997]. Changes in these parameters reflect changes in the excitability of the related pathways if one of these parameters is changed [Devanne et al., 1997]. Based on the fact that the K-value, S_{50} and the slope of the line were not significantly different throughout the time or between groups and since the MEP_{max} decreased over time, it can be assumed that the excitability of the corticospinal pathways decreased. Since no effect of group on any of the measured parameters was seen, it can be assumed that the electrical stimulation eliciting the withdrawal reflex was not the cause of the observed change.

Moreover, the analysis of the slope of the straight line did not show any significant differences which is in line with the observations of the slope of the Boltzmann fit. A tendency was seen for the main effect of time (p=0.055). Interpretation of line fit results and this tendency needs to be taken with caution, because the goodness of the fit was low, especially for the intervention group.

The study by Spaich et al. [2014] found that the nociceptive withdrawal reflex based therapy for rehabilitation of hemiparetic patients was effective in improving gait impairments. Nevertheless, the study by Perez et al. [2004] found that only subjects following motor skill training showed increased corticospinal excitability, whereas passive and non-skilled training did not cause any changes in the excitability. Similarly, the study by Jayaram et al. [2011] suggested that the changes in the excitability of the motor cortex are more likely to be a result of a performance of a complex locomotor task rather than motor adaptation process. It could be assumed that walking with activation of the nociceptive withdrawal reflex will increase the corticospinal excitability, however for healthy subjects this might not have been a motor learning task or complex locomotor task and therefore no increase in the excitability was observed in the current study. For stroke patients undergoing the treatment as described by Spaich et al. [2014] it is actually an intensive motor learning (relearning) task which in their case could be enhancing the corticospinal excitability.

9.1.3 Spinal Excitability

First Component of the Stretch Reflex

The data for the amplitude of the M1 peak showed a significant reduction between the measurements prior to the intervention and the measurements post intervention (both immediately after and 30

minutes after). No difference between the groups was found. No changes in the peak latency were seen for the M1 peak for both groups.

The significant reduction of the amplitude over time indicates a change (reduction) in the excitability of the pathway of the M1 wave which includes the muscle spindles, Ia afferent fibers going to the spinal cord, its synapse to the alpha-motorneurons and the alpha-motorneuron projections to the muscle [Ogawa et al., 2012]. A change in this pathway can indicate several things, but it does not include a change on a cortical level which is not included in the M1 pathway [Mrachacz-Kersting and Stevenson, 2017]. One of the possible sub-cortical mechanisms which can explain a reduction in amplitude of the M1 peak is a change in the monosynaptic excitation of the motorneurons in the spinal cord [Matthews, 1986] which would suggest that the treadmill walking used in this study can induce spinal plasticity.

Other explanations for a change in the amplitude of the M1 peak need to be considered as well. One of them is that a post synaptic change in the properties of membrane or dendrites could alter the responsiveness of the motorneuron to the input derived from the Ia-afferents [Wolpaw et al., 1983]. Also a change in the recruitment order of the population of motorneurons could change the amplitude of the M1 peak because of the difference in sensitivity for Ia-afferent input [Wolpaw et al., 1983]. This, however, seems to be unlikely because in general the recruitment order stays similar [Henneman et al., 2011], although significant changes have been reported before [Burke et al., 2011]. Another explanation could be that the recruitment order stayed the same, but that the timing of the different motor-units changed [Wolpaw et al., 1983]. A reduction in sensitivity of the muscle spindles affected by the gamma-motorneurons could be another explanation for the decreased amplitude of the M1 peak [Wolpaw et al., 1983].

Another fact which could explain the reduction in peak amplitude of the M1 wave is a reduction in background EMG which is called automatic gain compensation [Ogawa et al., 2012]. However, during the data analysis there was corrected for fluctuations in mean background EMG. Moreover, a reduction in amplitude of the M1 peak was also seen in subjects with a slightly increased background EMG, so it seems unlikely that this explains the reduction found in amplitude of the M1 peak.

9.1.4 Second Component of the Stretch Reflex

The amplitude of the second peak (M2) of the stretch reflex was significantly decreased 30 minutes after the intervention in comparison to the measurements prior to the intervention while no effect of group was found. No changes in latency were found. The second peak is assumed to be mediated by the group II muscle afferents [Grey et al., 2001]. The result indicates a reduction in excitability of these group II muscle afferents. To our best knowledge the effect of walking on the M2 peak of the stretch reflex was not studied, there are however human and primate studies that confirmed that different types of training were able to reduce as well as increase the amplitude of both spinal components of the stretch reflex depending on the concept of training [Khan et al., 2016; Wolpaw et al., 1983]. In addition, a study by Dietz et al. [1994] found that the M2 component of the stretch reflex in arm flexors and extensors can be modulated by various tasks. A possible explanation could be that walking itself is a type of training decreasing the excitability of the group II afferents and other spinal pathways, as the decrease was also observed for the amplitude of the M1 peak and inhibitory effects were also seen on the MEP_{max}-value.

High inter-subject variability (both in raw and normalized data) in the amplitudes of M2 peaks, which was reflected in high standard deviations, arises a question whether the same effect would be seen also in a bigger study population.

9.2 Study Design

9.2.1 Experimental Setup

The subjects were asked to walk with or without elicitation of the withdrawal reflex for 30 minutes and the experiment consisted of only one session. However, no significant effect of the intervention was found when assessing the excitability of the corticospinal tract since the effect of group was never significant. It might not necessary mean that the intervention itself has no effect on the corticospinal pathway. Only one session of 30 minutes walking was investigated, prolonged intervention or prolonged training composed of several sessions might be more likely to show any differences, since Spaich et al. [2014] found that the gait of hemiparetic patients significantly improved after 20 daily sessions with maximum of two consecutive days without training. The reason can be that the stimulation on the sole of the foot is not able to modulate the corticospinal pathways during such a short time. Another reason, also related to the intervention duration is the limited dose of stimuli. A supporting fact of this statement is that repetitive TMS (rTMS) delivering 1800 stimuli with frequency of 5Hz was able to induce changes in the excitability of the corticospinal pathways for at least 30 minutes, while 150 stimuli caused no effect and 900 stimuli induced only short term (5-10 minutes) MEP facilitation [Peinemann et al., 2004]. In the present study, the subjects received approximately 900 stimuli, but those were targeted into the peripheral nervous system and not directly into the central nervous system as with rTMS. Moreover, the frequency of the stimulation could play a role. rTMS was delivered with frequency of 5 Hz, while in the current study it was approximately 0.5 Hz. It is therefore plausible that this was not enough to induce any changes in the corticospinal pathway, however, some changes were found on a spinal level.

Another important factor of the present study was the recruitment of healthy subjects. As already pointed out in subsection 9.1.2 of the discussion, walking with continuous elicitation of the withdrawal reflex might not be as complex motor learning task as for stroke patients who undergo gait 'relearning'. In addition, the corticospinal pathways in healthy subjects are not affected by stroke while stroke survivors were found to have a decreased capacity to activate motor units [McComas et al., 1973] and reduced firing rates of motor units [Tang and Rymer, 1981] as a consequence of stroke. Therefore, elicitation of the withdrawal reflex causing a more appropriate kinematic response might help to strengthen the pathway of stroke patients, whereas the pathway of healthy subject does not need strengthening since the appropriate response is already present. In stroke patients, this aided walking also requires high level of concentration and effort to execute the task which may be controlled by supraspinal regions, while for healthy subjects this might be a highly automated task which does not require much special effort and might therefore be controlled primarily from subcortical structures. Indeed, as observed during the experiment an increased concentration of healthy subjects was required only for the first couple of steps when the electrical stimulation on the sole of the foot was a new input for the body. With time the subjects were quickly getting used to the stimulation and sometimes the intensity had to be increased several times throughout the experiment to maintain an appropriate kinematic response approximately the same during the whole walking period.

Finally, it is also possible that the lack of significant differences in the I/O curves was due to the small sample size. The high inter-subject variability resulted in high SDs. This is supported by the fact that some tendencies were seen for some of the parameters such as the S_{50}-value from the Boltzmann fit.

9.2.2 Withdrawal Reflex - Methodology

During the intervention (i.e. treadmill walking) no EMG signals were recorded from the intervention and control group. EMG could have been recorded to measure if possible fatigue occurred in the muscle [Graham et al., 2015] which is discussed in section 9.1.2 as a possible reason for finding a reduction in the MEP_{max}-value of the Boltzmann fit. Moreover, EMG measurements could have been taken from the TA to see if any changes in the muscle activity occurred during treadmill walking in both

groups. In the current study, subjects were walking with a relatively slow speed (2.5 km/h, whereas normal walking speed in adults is around 5 km/h [Nymark et al., 2005]). Slow walking is known to alter the preferred walking cadence, which is associated with optimal oxygen consumption and thus with optimal efficiency [Holt et al., 1991, 1995]. Slow walking is also known to induced more conscious effort to remain balanced [Nymark et al., 2005]. Furthermore, it is also known that a slow walking speed reduces muscle activity [Nymark et al., 2005; Yang and Winter, 1985; Russell and Apatoczky, 2016]. It seems likely that this would be the case in this study as well, because the same walking speed was used. This all could indicate that the changes found by corticospinal and spinal measurements could have been an effect of slow walking instead of the intervention which is also supported by the fact that no difference was found between the control and intervention group in the current study. It is however uncertain if changes in muscle activity can induce changes in either the corticospinal and/or spinal measurements.

The withdrawal reflex elicited in the intervention group was quantified visually which is a subjective way of quantification of the elicited kinematic response of the withdrawal reflex. A more objective measure would make the elicited kinematic response more uniform throughout the whole study population. A more objective quantification of the withdrawal reflex could have been implemented with the use of goniometers which has been used in several different studies to quantify the kinematic response more objectively [Emborg et al., 2009; Andersen et al., 1999; Spaich et al., 2004b; Serrao et al., 2012]. In these studies, the goniometers were mounted to the ankle, knee and hip joints to monitor the kinematic response elicited by the electrical stimulation on the sole of the foot [Emborg et al., 2009; Spaich et al., 2004b]. Andersen et al. [1999] only placed the goniometer on the ankle joint and Serrao et al. [2012] placed the goniometers on the knee and ankle joints. The study of Spaich et al. [2006] used another method to quantify the withdrawal reflex. They were also targeting the TA in their study and used EMG for quantification of the minimum reflex response. In this study, the lowest intensity needed to elicit an appropriate withdrawal reflex was quantified as an increment of at least 100 % of EMG activity in the interval of 60-300 ms after stimulation.

Both methods for quantification of the withdrawal response could have made the kinematic response of the elicited withdrawal reflex more uniform throughout the whole intervention group. Another advantage of using these methods would be that the point of habituation to the stimuli, while walking on the treadmill, could have been objectively quantified and increments in the intensity while walking could have been done more systematically.

9.2.3 TMS - Methodology

Another point of the discussion is the method used for collecting the TMS data. When collecting the data, the rMT was measured at the beginning of every TMS session (prior, immediately post and 30 minutes post intervention). Every time the stimulation intensities needed for the I/O curve were recalculated based on the current rMT, while other studies use the same stimulation intensities throughout the whole experiment [Mrachacz-Kersting and Stevenson, 2017; Carroll et al., 2001; Peinemann et al., 2004]. The approach used in the current study does not allow direct comparison of the MEP amplitudes for different intensities as done by Peinemann et al. [2004], because the intensities changed throughout the time as the rMT was changing for the single subjects. On the other hand, this direct comparison does not take into account the variability of the rMT which was found to vary throughout days [Wassermann, 2002] as well as within 90 minutes [Tranulis et al., 2006]. Therefore, the data was instead fitted into the Boltzmann equation [Kouchtir-Devanne et al., 2012]. However, only six different intensities were measured in the current study, which might not be enough to obtain an optimal fit. In many cases the upper plateau is not found due to the limited number of data points. Measuring the MEPs for more stimulation intensities could therefore result in a better fit of the Boltzmann equation and thus reflect the behavior of the corticospinal tract in a more precise manner. In fact, it is not uncommon to use intensities starting from 10-20 % below rMT increasing until the plateau phase is reached or up to 100 % of the stimulator output, which gives more data points and a better fit [Houdayer et al., 2008; Suzuki et al., 2012; Talelli et al., 2008].

Additional information could be possibly obtained if also the silent period (SP) of the MEPs was evaluated. The study of Kido Thompson and Stein [2004] found that the silent periods were slightly prolonged 20 minutes after walking which could indicate that walking could reflect some cortical inhibition. Since the SPs might originate from different cortical neurons than the MEP amplitude, as suggested by Ikeda et al. [2000]; Trompetto et al. [2001]; Ziemann et al. [1996], more information about the effects of the electrical stimulation eliciting the withdrawal reflex could be obtained.

9.2.4 Stretch Reflex - Methodology

In the current study, stretch reflex measurements from the TA were taken while subjects were quietly standing. Some other studies have been using a sitting posture for measuring the response of the TA to the muscle stretch [Mrachacz-Kersting and Stevenson, 2017; Obata et al., 2010]. The sitting posture gives the possibility to better control the pre-contraction levels because subjects can be asked to contract the muscle with a certain percentage of the MVC as is done in the study of Mrachacz-Kersting and Stevenson [2017]. These pre-contraction levels however do have an influence on the measured amplitudes of the different components of the stretch reflex [Ogawa et al., 2012]. The measured amplitudes can change if background activity is induced [Ogawa et al., 2012] as it is in the sitting procedure.

Not only the background noise is something which needs to be considered when measuring the stretch reflex. If the stretch reflex is measured in a standing position, as is done in the present study, ankle joint stabilization is something which is influencing the reflex response as well [Nakazawa et al., 2003; Obata et al., 2012]. Ankle joint stiffness is influenced in the standing position because it is modulated to maintain balance [Bock et al., 2004]. In general, the whole posture is something which needs to be taken into account when measuring stretch reflexes, because a study of Nakazawa et al. [2003] found that a significantly greater stretch reflex can be elicited in an upright standing posture when compared with a supine posture. Moreover, as discussed by Bock et al. [2004] a standing posture is a more natural posture and thus gives a more meaningful result compared to other postures used to measure stretch reflexes.

Another factor which needs to be taken into account while doing measurements in the standing position is the standing posture of the subject itself. Only leaning slightly forward or backward can induce a change in the ankle angle and induce significant changes in the measured stretch reflex [Yamamoto et al., 2003]. The TA is less sensitive for joint angle changes compared with the ankle extensor muscles (e.g. soleus) [Yamamoto et al., 2003]. This is because the soleus is known to show inhibition of the Ia-motorneurons as a consequence of position, whereas the TA is not showing similar inhibitions [Katz et al., 1988]. However, it is something which needs to be considered when measuring stretch reflexes, because it can still be a factor changing the excitability found over the different time points. Goniometers could have been used for controlling the posture of the body.

The peak identification was done by visually identifying the peaks, other studies have used a more objective way for the identification process. A study of Obata et al. [2010] defined the onset of the short latency peak (M1) as the first time the background activity (BGA) exceeded mean BGA + 3SD. The onset of the M2 component was defined as 20 ms after the onset of the M1 component [Obata et al., 2010, 2012]. A study of Nakazawa et al. [2003] used the same method for identifying the onset of the M1 component and found that their onset corresponded with the onsets found in earlier literature. These methods could have reduced the possibility of identifying the M1 and M2 peaks incorrectly by the visual method as was used in the present study.

Future Study

This is the first study investigating the effects of walking with electrically eliciting the withdrawal reflex on corticospinal pathways. Future study should run the experiments either with longer walking time or with several sessions per week and try to investigate how much time is needed to induce

possible plastic changes in the corticospinal pathways. Future study should also consider using bigger sample size than the present study to see if the same effects occur again or diminish with an increased sample size. Close attention should be given to the parameters which showed tendencies in a certain direction. A power analysis could be performed to calculate the statistical power of results observed in the presents study and based on it determine the appropriate number of subjects needed in both groups. If the study is also extended with more sessions, the expected drop-out rate should be taken into account as well.

In case of prolonged training, adding measurements of silent periods, SICI and SICF could bring more information about different pathways. It would also be interesting to run the experiment with hemiparetic patients undergoing rehabilitation, because there any possible changes could be more obvious in comparison to healthy subjects.

Chapter 10
Conclusion

The electrical stimulation eliciting the nociceptive withdrawal reflex was found to help hemiparetic patients with gait rehabilitation. It is unknown how the stimulation is affecting the patients on a spinal and/or cortical level. The current study was therefore investigating the effect of electrical stimulation eliciting the withdrawal reflex on the excitability of corticospinal and spinal pathways.

A significant decrease was found for the MEP_{max}-value of the Boltzmann fit. This decrease however, seems to originate from walking induced fatigue and not from the walking with electrical stimulation because no difference between the groups was found. No other significant changes following the intervention were found for the other investigated parameters (slope K, S_{50}, slope of straight line) of the I/O curve. A significant decrease over time was found for the rMT.

The investigation of the spinal pathways by the stretch reflex showed a significant decrease in the amplitude of the first peak (M1). A significant decrease was also found in the amplitude of the second peak (M2). No changes were found between the groups nor the time points of the peaks latencies.

The results suggest that 30 minutes of walking with electrical stimulation on the sole of the foot was not enough to induce changes which could be detected by the TMS procedure since no differences were found between the two groups. Results however indicate that walking itself was able to decrease the excitability of the spinal pathways since a decrease was observed in amplitudes of the first two components of the stretch reflex. Further research is needed to see the effect of walking with activation of the withdrawal reflex on the corticospinal pathways when the training program is extended.

Bibliography

Aagaard, P., Simonsen, E. B., Andersen, J. L., Magnusson, P., and Dyhre-Poulsen, P. (2002). Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. *Journal of Applied Physiology*, 92(6):2309–2318.

Álvarez-Blanco, S., Leon, L., and Valls-Solé, J. (2009). The startle reaction to somatosensory inputs: different response pattern to stimuli of upper and lower limbs. *Experimental Brain Research*, 195(2):285–292.

Andersen, O. K., Sonnenborg, F. A., and Arendt-Nielsen, L. (1999). Modular organization of human leg withdrawal reflexes elicited by electrical stimulation of the foot sole. *Muscle and Nerve*, 22(11):1520–1530.

Arendt-Nielsen, L., Brennum, J., Sindrup, S., and Bak, P. (1994). Electrophysiological and psychophysical quantification of temporal summation in the human nociceptive system. *European Journal of Applied Physiology and Occupational Physiology*, 68(3):266–273.

Badawy, R. A. B., Loetscher, T., Macdonell, R. A. L., and Brodtmann, A. (2012). Cortical excitability and neurology: insights into the pathophysiology. *Functional neurology*, 27(3):131–45.

Balaban, B. and Tok, F. (2014). Gait disturbances in patients with stroke. *PM & R: the journal of injury, function, and rehabilitation*, 6(7):635–42.

Balasubramanian, C. K., Bowden, M. G., Neptune, R. R., and Kautz, S. A. (2007). Relationship Between Step Length Asymmetry and Walking Performance in Subjects With Chronic Hemiparesis. *Archives of Physical Medicine and Rehabilitation*, 88(1):43–49.

Balbi, P., Martinoia, S., Colombo, R., and Massobrio, P. (2014). Modelling recurrent discharge in the spinal α-motoneuron: Reappraisal of the F wave.

Banala, S., Seok Hun Kim, Agrawal, S., and Scholz, J. (2009). Robot Assisted Gait Training With Active Leg Exoskeleton (ALEX). *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, 17(1):2–8.

Barrett, R., Mills, P., and Begg, R. (2010). A systematic review of the effect of ageing and falls history on minimum foot clearance characteristics during level walking. *Gait & Posture*, 32(4):429–435.

Béjot, Y., Bailly, H., Durier, J., and Giroud, M. (2016). Epidemiology of stroke in Europe and trends for the 21st century. *La Presse Médicale*, 45(12):e391–e398.

Belda-Lois, J.-M., Mena-del Horno, S., Bermejo-Bosch, I., Moreno, J. C., Pons, J. L., Farina, D., Iosa, M., Molinari, M., Tamburella, F., Ramos, A., Caria, A., Solis-Escalante, T., Brunner, C., and Rea, M. (2011). Rehabilitation of gait after stroke: a review towards a top-down approach. *Journal of neuroengineering and rehabilitation*, 8(1):66.

Berardelli, A., Hallett, M., Kaufman, C., Fine, E., Berenberg, W., and Simon, S. R. (1982). Stretch reflexes of triceps surae in normal man. *Journal of neurology, neurosurgery, and psychiatry*, 45(6):513–25.

Bernhardt, J., Thuy, M. N., Collier, J. M., and Legg, L. A. (2009). Very Early Versus Delayed Mobilization After Stroke. *Stroke*, 40(7):e489–e490.

Bock, P. J., Kearney, R. E., Forster, S. M., and Wagner, R. (2004). Modulation of stretch reflex excitability during quiet human standing. *Conference proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference*, 7:4684–7.

Bogataj, U., Gros, N., Kljajić, M., Aćimović, R., and Maležič, M. (1995). The Rehabilitation of Gait in Patients With Hemiplegia: A Comparison Between Conventional Therapy and Multichannel Functional Electrical Stimulation Therapy. *Physical Therapy*, 75(6):490–502.

Bogataj, U., Gros, N., Kljajic, M., and Acimovic-Janezic, R. (1997). Enhanced rehabilitation of gait after stroke: a case report of a therapeutic approach using multichannel functional electrical stimulation. *IEEE Transactions on Rehabilitation Engineering*, 5(2):221–232.

Bohannon, R. W. (1997). Comfortable and maximum walking speed of adults aged 20—79 years: reference values and determinants. *Age and Ageing*, 26(1):15–19.

Bonita, R. and Beaglehole, R. (1988). Recovery of motor function after stroke. *Stroke*, 19(12):1497–1500.

Bosch, P. R., Harris, J. E., and Wing, K. (2014). Review of Therapeutic Electrical Stimulation for Dorsiflexion Assist and Orthotic Substitution From the American Congress of Rehabilitation Medicine Stroke Movement Interventions Subcommittee. *Archives of Physical Medicine and Rehabilitation*, 95(2):390–396.

Brasil-Neto, J., Pascual-Leone, A., Valls-Sol, J., Cammarota, A., Cohen, L., and Hallett, M. (1993). Postexercise depression of motor evoked potentials: a measure of central nervous system fatigue. *Experimental Brain Research*, 93(1):181–184.

Brasil-Neto, J. P., Cohen, L. G., and Hallett, M. (1994). Central fatigue as revealed by postexercise decrement of motor evoked potentials. *Muscle & Nerve*, 17(7):713–719.

Bunse, T., Wobrock, T., Strube, W., Padberg, F., Palm, U., Falkai, P., and Hasan, A. (2014). Motor Cortical Excitability Assessed by Transcranial Magnetic Stimulation in Psychiatric Disorders: A Systematic Review. *Brain Stimulation*, 7(2):158–169.

Burke, R. E., Burke, and E., R. (2011). Motor Units: Anatomy, Physiology, and Functional Organization. In *Comprehensive Physiology*. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Bushnell, C., Reeves, M., Zhao, X., Pan, W., Prvu-Bettger, J., Zimmer, L., Olson, D., and Peterson, E. (2014). Sex differences in quality of life after ischemic stroke. *Neurology*, 82(11):922–93.

Cantello, R., Civardi, C., Cavalli, A., Varrasi, C., Tarletti, R., Monaco, F., and Migliaretti, G. (2000). Cortical Excitability in Cryptogenic Localization-Related Epilepsy: Interictal Transcranial Magnetic Stimulation Studies. *Epilepsia*, 41(6):694–704.

Carr, J. H. and Shepherd, R. B. (1989). A Motor Learning Model for Stroke Rehabilitation. *Physiotherapy*, 75(7):372–380.

Carroll, T. J., Riek, S., and Carson, R. G. (2001). Reliability of the input–output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. *Journal of Neuroscience Methods*, 112(2):193–202.

Chae, J. (2003). Neuromuscular electrical stimulation for motor relearning in hemiparesis. *Physical medicine and rehabilitation clinics of North America*, 14(1 Suppl):S93–109.

Chen, G., Patten, C., Kothari, D. H., and Zajac, F. E. (2005). Gait deviations associated with post-stroke hemiparesis: Improvement during treadmill walking using weight support, speed, support stiffness, and handrail hold. *Gait and Posture*, 22(1):57–62.

Chen, R., Tam, A., Bütefisch, C., Corwell, B., Ziemann, U., Rothwell, J. C., and Cohen, L. G. (1998). Intracortical Inhibition and Facilitation in Different Representations of the Human Motor Cortex. *Journal of Neurophysiology*, 80(6):2870–2881.

Christensen, L. O. D., Andersen, J. B., Sinkjaer, T., and Nielsen, J. (2001). Transcranial magnetic stimulation and stretch reflexes in the tibialis anterior muscle during human walking. *The Journal of Physiology*, 531(2):545–557.

Counter, S. A. and Borg, E. (1992). Analysis of the coil generated impulse noise in extracranial magnetic stimulation. *Electroencephalography and clinical neurophysiology*, 85(4):280–8.

Daly, J. J. and Wolpaw, J. R. (2008). Brain–computer interfaces in neurological rehabilitation. *The Lancet Neurology*, 7(11):1032–1043.

Daly, J. J., Zimbelman, J., Roenigk, K. L., McCabe, J. P., Rogers, J. M., Butler, K., Burdsall, R., Holcomb, J. P., Marsolais, E. B., and Ruff, R. L. (2011). Recovery of Coordinated Gait. *Neurorehabilitation and Neural Repair*, 25(7):588–596.

Day, B. L., Thompson, P. D., Dick, J. P., Nakashima, K., and Marsden, C. (1987). Different sites of action of electrical and magnetic stimulation of the human brain. *Neuroscience Letters*, 75(1):101–106.

Dean, C. M. and Shepherd, R. B. (1997). Task-Related Training Improves Performance of Seated Reaching Tasks After Stroke. *Stroke*, 28(4):722–728.

Deletis, V. and Shils, J. L. (2002). *Neurophysiology in neurosurgery: a modern intraoperative approach*. Academic Press, 1 edition.

Den Otter, A., Geurts, A., Mulder, T., and Duysens, J. (2007). Abnormalities in the temporal patterning of lower extremity muscle activity in hemiparetic gait. *Gait & Posture*, 25(3):342–352.

Devanne, H., Lavoie, B. A., and Capaday, C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Experimental Brain Research*, 114(2):329–338.

Di Lazzaro, V., Profice, P., Ranieri, F., Capone, F., Dileone, M., Oliviero, A., and Pilato, F. (2012). I-wave origin and modulation. *Brain Stimulation*, 5(4):512–525.

Di Lazzaro, V. and Rothwell, J. C. (2014). Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex. *The Journal of Physiology*, 592(19):4115–4128.

Dickstein, R., Dunsky, A., and Marcovitz, E. (2004). Motor imagery for gait rehabilitation in post-stroke hemiparesis. *Physical therapy*, 84(12):1167–77.

Dietz, V., Discher, M., and Trippel, M. (1994). Task-dependent modulation of short- and long-latency electromyographic responses in upper limb muscles. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 93(1):49–56.

Dohring, M. E. and Daly, J. J. (2008). Automatic synchronization of functional electrical stimulation and robotic assisted treadmill training. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, 16(3):310–313.

Dowman, R. (1992). Possible startle response contamination of the spinal nociceptive withdrawal reflex. *Pain*, 49(2):187–197.

E.Ayyappa (1997). Normal human locomotion, part 1: basic concepts and terminology. *Journal of Prosthetics and Orthotics*, 9(1):1–10.

Elias, L. J., Bryden, M., and Bulman-Fleming, M. (1998). Footedness is a better predictor than is handedness of emotional lateralization. *Neuropsychologia*, 36(1):37–43.

Emborg, J. (2010). *Modulation of the nociceptive withdrawal reflex and its use in rehabilitation of gait of stroke patients*. Phd thesis, Aalborg university.

Emborg, J., Spaich, E. G., and Andersen, O. K. (2009). Withdrawal reflexes examined during human gait by ground reaction forces: Site and gait phase dependency. *Medical and Biological Engineering and Computing*, 47(1):29–39.

Epstein, C. M., Wassermann, E. M., and Ziemann, U. (2012). *The Oxford handbook of transcranial stimulation*. Oxford University Press.

Espiritu, M. G., Lin, C. S.-Y., and Burke, D. (2003). Motoneuron excitability and the F wave. *Muscle & Nerve*, 27(6):720–727.

Evans, C. M., Fellows, S. J., Rack, P. M., Ross, H. F., and Walters, D. K. (1983). Response of the normal human ankle joint to imposed sinusoidal movements. *The Journal of Physiology*, 344(1):483–502.

Farzan, F. (2014). Single-Pulse Transcranial Magnetic Stimulation (TMS) Protocols and Outcome Measures. In Rotenberg, A., editor, *Transcranial Magnetic Stimulation*, pages 69–115. Springer Science + Business Media, New York, NY.

Fasoli, S. E., Krebs, H. I., Stein, J., Frontera, W. R., Hughes, R., and Hogan, N. (2004). Robotic therapy for chronic motor impairments after stroke: Follow-up results. *Archives of physical medicine and rehabilitation*, 85(7):1106–11.

Fuhr, P., Agostino, R., and Hallett, M. (1991). Spinal motor neuron excitability during the silent period after cortical stimulation. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 81(4):257–262.

Gage, J. R., Deluca, P. R., and Renshaw, T. S. (1995). Gait Analysis: Principles and Applications. *The Journal of Bone & Joint Surgery*, 77(10):1607 –1623.

Go, A. S., Mozaffarian, D., Roger, V. L., and Benjamin, E. J. (2014). Executive summary: Heart Disease and Stroke Statistics - 2014 Update: A report from the American Heart Association. *Circulation*, 129(3):399–410.

Graham, R. B., Wachowiak, M. P., and Gurd, B. J. (2015). The assessment of muscular effort, fatigue, and physiological adaptation using EMG and wavelet analysis. *PLoS ONE*, 10(8):1–13.

Granat, M., Smith, A., Phillips, G., Kirkwood, C., Barnett, R., and Andrews, B. (1988). Characterization of the electrically excited flexion withdrawal response used in restoration of locomotion in spinal cord injured paraplegics. *IEEE Engineering in Medicine & Biology Society 10th annual international conference*, pages 1975–6.

Granat, M. H., Nicol, D. J., Baxendale, R. H., and Andrews, B. J. (1991). Dishabituation of the flexion reflex in spinal cord-injured man and its application in the restoration of gait. *Brain Research*, 559(2):344–346.

Grey, M. J., Ladouceur, M., Andersen, J. B., Nielsen, J. B., and Sinkjaer, T. (2001). Group II muscle afferents probably contribute to the medium latency soleus stretch reflex during walking in humans. *The Journal of Physiology*, 534(3):925–933.

Grillner, S. and Rossignol, S. (1978). On the initiation of the swing phase of locomotion in chronic spinal cats. *Brain Research*, 146(2):269–277.

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L. G., Mall, V., Kaelin-Lang, A., Mima, T., Rossi, S., Thickbroom, G. W., Rossini, P. M., Ziemann, U., Valls-Solé, J., and Siebner, H. R. (2012). A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clinical Neurophysiology*, 123(5):858–882.

Hackett, M. L., Yapa, C., Parag, V., and Anderson, C. S. (2005). Frequency of Depression After Stroke: A Systematic Review of Observational Studies. *Stroke*, 36(6):1330–1340.

Hara, M., Kimura, J., Walker, D. D., Taniguchi, S., Ichikawa, H., Fujisawa, R., Shimizu, H., Abe, T., Yamada, T., Kayamori, R., and Mizutani, T. (2010). Effect of motor imagery and voluntary muscle contraction on the F wave. *Muscle & Nerve*, 42(2):208–212.

Henneman, E., Mendell, L. M., Henneman, E., and Mendell, L. M. (2011). Functional Organization of Motoneuron Pool and its Inputs. In *Comprehensive Physiology*. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Hesse, S., Uhlenbrock, D., Werner, C., and Bardeleben, A. (2000). A mechanized gait trainer for restoring gait in nonambulatory subjects. *Archives of Physical Medicine and Rehabilitation*, 81(9):1158–1161.

Holt, K. G., Hamill, J., and Andres, R. O. (1991). Predicting the minimal energy costs of human walking. *Medicine and science in sports and exercise*, 23(4):491–8.

Holt, K. G., Jeng, S. F., Ratcliffe, R., and Hamill, J. (1995). Energetic Cost and Stability during Human Walking at the Preferred Stride Frequency. *Journal of Motor Behavior*, 27(2):164–178.

Hosoido, T., Motoyama, S., Goto, M., Mori, F., Tajima, T., Hirata, H., and Wada, N. (2009). Characteristics of H- and M-waves recorded from rat forelimbs. *Neuroscience letters*, 450(3):239–241.

Houdayer, E., Degardin, A., Cassim, F., Bocquillon, P., Derambure, P., and Devanne, H. (2008). The effects of low- and high-frequency repetitive TMS on the input/output properties of the human corticospinal pathway. *Experimental Brain Research*, 187(2):207–217.

Hsu, W.-Y., Kuo, Y.-F., Liao, K.-K., Yu, H.-Y., and Lin, Y.-Y. (2015). Widespread inter-ictal excitability changes in patients with temporal lobe epilepsy: A TMS/MEG study. *Epilepsy Research*, 111(201):61–71.

Hughes, J. and Jacobs, N. (1979). Normal human locomotion. *Prosthetics and orthotics international*, 3(1):4–12.

Ikeda, A., Ohara, S., Matsumoto, R., Kunieda, T., Nagamine, T., Miyamoto, S., Kohara, N., Taki, W., Hashimoto, N., and Shibasaki, H. (2000). Role of primary sensorimotor cortices in generating inhibitory motor response in humans. *Brain : a journal of neurology*, 123(8):1710–21.

Ito, P. (1980). 7 Robustness of ANOVA and MANOVA test procedures. In *Handbook of Statistics*, pages 199–236. 1 edition.

Jayaram, G., Galea, J. M., Bastian, A. J., and Celnik, P. (2011). Human locomotor adaptive learning is proportional to depression of cerebellar excitability. *Cerebral Cortex*, 21(8):1901–1909.

Jordan, L. M. (1998). Initiation of Locomotion in Mammals. *Annals of the New York Academy of Sciences*, 860(1 NEURONAL MECH):83–93.

Kandel, E. R. (2013). *Principals of Neural Science*. McGraw-Hill Professional, New York, fifth edit edition.

Katz, R., Meunier, S., and Pierrot-Deseilligny, E. (1988). Changes in presynaptic inhibition of Ia fibres in man while standing. *Brain : a journal of neurology*, pages 417–37.

Khan, A. S., Patrick, S. K., Roy, F. D., Gorassini, M. A., and Yang, J. F. (2016). Training-Specific Neural Plasticity in Spinal Reflexes after Incomplete Spinal Cord Injury. *Neural Plasticity*, 2016.

Kido Thompson, A. and Stein, R. B. (2004). Short-term effects of functional electrical stimulation on motor-evoked potentials in ankle flexor and extensor muscles. *Experimental Brain Research*, 159(4):491–500.

Kim, C. and Eng, J. J. (2003). Symmetry in vertical ground reaction force is accompanied by symmetry in temporal but not distance variables of gait in persons with stroke. *Gait & Posture*, 18(1):23–28.

Kimberley, T. J., Lewis, S. M., Auerbach, E. J., Dorsey, L. L., Lojovich, J. M., and Carey, J. R. (2004). Electrical stimulation driving functional improvements and cortical changes in subjects with stroke. *Experimental Brain Research*, 154(4):450–460.

Kleindorfer, D. O., Khoury, J., Moomaw, C. J., Alwell, K., Woo, D., Flaherty, M. L., Khatri, P., Adeoye, O., Ferioli, S., Broderick, J. P., and Kissela, B. M. (2010). Stroke Incidence Is Decreasing in Whites But Not in Blacks. *Stroke*, 41(7).

Kluding, P. M., Dunning, K., O'Dell, M. W., Wu, S. S., Ginosian, J., Feld, J., and McBride, K. (2013). Foot Drop Stimulation Versus Ankle Foot Orthosis After Stroke: 30-Week Outcomes. *Stroke*, 44(6):1660–1669.

Knutsson, E. and Richards, C. (1979). Different types of disturbed motor control in gait of hemiparetic patients. *Brain : a journal of neurology*, 102(2):405–30.

Kollen, B. J., Lennon, S., Lyons, B., Wheatley-Smith, L., Scheper, M., Buurke, J. H., Halfens, J., Geurts, A. C., and Kwakkel, G. (2009). The Effectiveness of the Bobath Concept in Stroke Rehabilitation. *Stroke*, 40(4):e89–e97.

Koton, S., Schneider, A. L. C., Rosamond, W. D., Shahar, E., Sang, Y., Gottesman, R. F., and Coresh, J. (2014). Stroke Incidence and Mortality Trends in US Communities, 1987 to 2011. *JAMA*, 312(3):259.

Kottink, A. I., Oostendorp, L. J., Buurke, J. H., Nene, A. V., Hermens, H. J., and IJzerman, M. J. (2004). The Orthotic Effect of Functional Electrical Stimulation on the Improvement of Walking in Stroke Patients with a Dropped Foot: A Systematic Review. *Artificial Organs*, 28(6):577–586.

Kouchtir-Devanne, N., Capaday, C., Cassim, F., Derambure, P., and Devanne, H. (2012). Task-dependent changes of motor cortical network excitability during precision grip compared to isolated finger contraction. *Journal of Neurophysiology*, 107(5):1522–1529.

Kudina, L. P. and Andreeva, R. E. (2017). F-wave of single firing motor units: correct or misleading criterion of motoneuron excitability in humans? *Neurological Sciences*, 38(3):465–472.

Lachman, T., Shahani, B. T., and Young, R. R. (1980). Late responses as aids to diagnosis in peripheral neuropathy. *Journal of neurology, neurosurgery, and psychiatry*, 43(2):156–62.

Langhorne, P., Bernhardt, J., and Kwakkel, G. (2011). Stroke rehabilitation. *The Lancet*, 377(9778):1693–1702.

Lazzaro, V. D., Ziemann, U., and Lemon, R. N. (2008). State of the art: Physiology of transcranial motor cortex stimulation. *Brain Stimulation*, 1(4):345–362.

Lin, J. Z. and Floeter, M. K. (2004). Do F-wave measurements detect changes in motor neuron excitability? *Muscle & Nerve*, 30(3):289–294.

Lohse, K. R., Hilderman, C. G. E., Cheung, K. L., Tatla, S., and Van der Loos, H. F. M. (2014). Virtual Reality Therapy for Adults Post-Stroke: A Systematic Review and Meta-Analysis Exploring Virtual Environments and Commercial Games in Therapy. *PLoS ONE*, 9(3):e93318.

Loo, C., Sachdev, P., Elsayed, H., McDarmont, B., Mitchell, P., Wilkinson, M., Parker, G., and Gandevia, S. (2001). Effects of a 2- to 4-week course of repetitive transcranial magnetic stimulation (rTMS) on neuropsychologic functioning, electroencephalogram, and auditory threshold in depressed patients. *Biological Psychiatry*, 49(7):615–623.

Lozano, A. M. and Hallett, M., editors (2013). *Handbook of Clinical Neurology*. Elsevier B.V., 116 edition.

Marieb, E. N. and Hoehn, K. (2010). *Human Anatomy & Physiology*. Benjamin Cummings, 8 edition.

Martin, E. and Hine, R. (2008). *A Dictionary of Biology*. Oxford University Press, 6 edition.

Matsuda, F., Mukaino, M., Ohtsuka, K., Tanikawa, H., Tsuchiyama, K., Teranishi, T., Kanada, Y., Kagaya, H., and Saitoh, E. (2017). Biomechanical factors behind toe clearance during the swing phase in hemiparetic patients. *Topics in Stroke Rehabilitation*, 24(3):177–182.

Matthews, B. Y. P. B. C. (1986). Observations on automatic compensagtion of reflex gain on varying the pre-excisting level of motor discharge in man. *Journal of Physiology*, 374(1):73–90.

Mayr, A., Kofler, M., Quirbach, E., Matzak, H., Frohlich, K., and Saltuari, L. (2007). Prospective, Blinded, Randomized Crossover Study of Gait Rehabilitation in Stroke Patients Using the Lokomat Gait Orthosis. *Neurorehabilitation and Neural Repair*, 21(4):307–314.

McClellan, R. and Ada, L. (2004). A six-week, resource-efficient mobility program after discharge from rehabilitation improves standing in people affected by stroke: Placebo-controlled, randomised trial. *Australian Journal of Physiotherapy*, 50(3):163–167.

McComas, A. J., Sica, R. E., Upton, A. R., and Aguilera, N. (1973). Functional changes in motoneurones of hemiparetic patients. *Journal of neurology, neurosurgery, and psychiatry*, 36(2):183–93.

McCrimmon, C. M., King, C. E., Wang, P. T., Cramer, S. C., Nenadic, Z., and Do, A. H. (2015). Brain-controlled functional electrical stimulation therapy for gait rehabilitation after stroke: a safety study. *Journal of NeuroEngineering and Rehabilitation*, 12(1):57.

McNeil, C. J., Butler, J. E., Taylor, J. L., and Gandevia, S. C. (2013). Testing the excitability of human motoneurons. *Frontiers in Human Neuroscience*, 7(152).

Mehrholz, J., Elsner, B., Werner, C., Kugler, J., and Pohl, M. (2013). Electromechanical-Assisted Training for Walking After Stroke: Updated Evidence. *Stroke*, 44(10):e127–e128.

Mercuri, B., Wassermann, E. M., Manganotti, P., Ikoma, K., Samii, A., and Hallett, M. (1996). Cortical modulation of spinal excitability: an F-wave study. *Electroencephalography and clinical neurophysiology*, 101(1):16–24.

Mesrati, F. and Vecchierini, M. (2004). F-waves: neurophysiology and clinical value. *Neurophysiologie Clinique/Clinical Neurophysiology*, 34(5):217–243.

Michou, E., Mistry, S., Jefferson, S., Tyrrell, P., and Hamdy, S. (2014). Characterizing the Mechanisms of Central and Peripheral Forms of Neurostimulation in Chronic Dysphagic Stroke Patients. *Brain Stimulation*, 7(1):66–73.

Misiaszek, J. E. (2003). The H-reflex as a tool in neurophysiology: Its limitations and uses in understanding nervous system function. *Muscle and Nerve*, 28(2):144–160.

Morone, G., Paolucci, S., Cherubini, A., De Angelis, D., Venturiero, V., Coiro, P., and Iosa, M. (2017). Robot-assisted gait training for stroke patients: current state of the art and perspectives of robotics. *Neuropsychiatric Disease and Treatment*, 13:1303–1311.

Mrachacz-Kersting, N. and Stevenson, A. J. T. (2017). Paired Associative Stimulation Targeting the Tibialis Anterior Muscle using either Mono or Biphasic Transcranial Magnetic Stimulation. *Frontiers in Human Neuroscience*, 11.

Nakazawa, K., Kawashima, N., Obata, H., Yamanaka, K., Nozaki, D., and Akai, M. (2003). Facilitation of both stretch reflex and corticospinal pathways of the tibialis anterior muscle during standing in humans. *Neuroscience Letters*, 338(1):53–56.

Nielsen, J. B. (2003). How we walk: central control of muscle activity during human walking. *The Neuroscientist: a review journal bringing neurobiology, neurology and psychiatry*, 9(3):195–204.

Nitsche, M. A. and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of physiology*, 527(3):633–639.

Nobrega, J. A. M., Pinheiro, D. S., Manzano, G. M., and Kimura, J. (2004). Various aspects of F-wave values in a healthy population. *Clinical Neurophysiology*, 115(10):2336–2342.

Nymark, J. R., Balmer, S. J., Melis, E. H., Lemaire, E. D., and Millar, S. (2005). Electromyographic and kinematic nondisabled gait differences at extremely slow overground and treadmill walking speeds. *The Journal of Rehabilitation Research and Development*, 42(4):523.

Obata, H., Kawashima, N., Akai, M., Nakazawa, K., and Ohtsuki, T. (2010). Age-related changes of the stretch reflex excitability in human ankle muscles. *Journal of Electromyography and Kinesiology*, 20(1):55–60.

Obata, H., Kawashima, N., Ohtsuki, T., and Nakazawa, K. (2012). Aging effects on posture-related modulation of stretch reflex excitability in the ankle muscles in humans. *Journal of Electromyography and Kinesiology*, 22(1):31–36.

Ogawa, T., Kawashima, N., Suzuki, S., and Nakazawa, K. (2012). Different modulation pattern of spinal stretch reflex excitability in highly trained endurance runners. *European Journal of Applied Physiology*, 112(10):3641–3648.

Oldfield, R. C. (1971). The Assessment And Analysis of Handedness: The Edinburgh Inventory. *Neuropsychologia*, 9(1):97–113.

Olney, S. J., Griffin, M. P., and McBride, I. D. (1994). Temporal, Kinematic, and Kinetic Variables Related to Gait Speed in Subjects With Hemiplegia: A Regression Approach. *Physical Therapy*, 74(9):872–885.

Olney, S. J., Griffin, M. P., Monga, T. N., and McBride, I. D. (1991). Work and power in gait of stroke patients. *Archives of physical medicine and rehabilitation*, 72(5):309–14.

Olney, S. J. and Richards, C. (1996). Hemiparetic gait following stroke. Part 1: Characteristics. *Gait & Posture*, 4(2):136–148.

Panayiotopoulos, C. and Chroni, E. (1996). F-waves in clinical neurophysiology: a review, methodological issues and overall value in peripheral neuropathies. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control*, 101(5):365–374.

Peinemann, A., Reimer, B., Löer, C., Quartarone, A., Münchau, A., Conrad, B., and Roman Siebner, H. (2004). Long-lasting increase in corticospinal excitability after 1800 pulses of subthreshold 5 Hz repetitive TMS to the primary motor cortex. *Clinical Neurophysiology*, 115(7):1519–1526.

Pennycott, A., Wyss, D., Vallery, H., Klamroth-Marganska, V., and Riener, R. (2012). Towards more effective robotic gait training for stroke rehabilitation: a review. *Journal of NeuroEngineering and Rehabilitation*, 9(1):1–13.

Perez, M. A., Lungholt, B. K. S., Nyborg, K., and Nielsen, J. B. (2004). Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Experimental Brain Research*, 159(2):197–205.

Peshkin, M., Brown, D., Santos-Munne, J., Makhlin, A., Lewis, E., Colgate, J., Patton, J., and Schwandt, D. (2005). KineAssist: A Robotic Overground Gait and Balance Training Device. In *9th International Conference on Rehabilitation Robotics, 2005. ICORR 2005.*, pages 241–246. IEEE.

Petersen, N., Christensen, L. O. D., Morita, H., Sinkjær, T., and Nielsen, J. (1998). Evidence that a transcortical reflex pathway contributes to stretch reflexes in the tibialis anterior muscle in man. *Journal of Physiology*, 512(1):267–276.

Pierrot-Deseilligny, E. and Burke, D. (2012). *The Circuitry of the Human Spinal Cord*. Cambridge University Press, Cambridge.

Pirio Richardson, S., Bliem, B., Voller, B., Dang, N., and Hallett, M. (2009). Long-latency afferent inhibition during phasic finger movement in focal hand dystonia. *Experimental Brain Research*, 193(2):173–179.

Pollock, A., Baer, G., Langhorne, P., and Pomeroy, V. (2007). Physiotherapy treatment approaches for the recovery of postural control and lower limb function following stroke: a systematic review. *Clinical Rehabilitation*, 21(5):395–410.

Quartarone, A., Bagnato, S., Rizzo, V., Morgante, F., Sant'Angelo, A., Battaglia, F., Messina, C., Siebner, H. R., and Girlanda, P. (2005). Distinct changes in cortical and spinal excitability following high-frequency repetitive TMS to the human motor cortex. *Experimental Brain Research*, 161(1):114–124.

Reeves, S. L., Brown, D. L., Baek, J., Wing, J. J., Morgenstern, L. B., and Lisabeth, L. D. (2015). Ethnic differences in poststroke quality of life in the brain attack surveillance in corpus christi (BASIC) project. *Stroke*, 46(10):2896–2901.

Richard, M. A., Spaich, E. G., Serrao, M., and Andersen, O. K. (2015). Stimulation site and phase modulation of the withdrawal reflex during gait initiation. *Clinical Neurophysiology*, 126(12):2282–2289.

Riener, R., Lunenburger, L., Jezernik, S., Anderschitz, M., Colombo, G., and Dietz, V. (2005). Patient-Cooperative Strategies for Robot-Aided Treadmill Training: First Experimental Results. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, 13(3):380–394.

Ritzmann, R., Kramer, A., Gollhofer, A., and Taube, W. (2013). The effect of whole body vibration on the H-reflex, the stretch reflex, and the short-latency response during hopping. *Scandinavian Journal of Medicine and Science in Sports*, 23(3):331–339.

Rizzo, V., Terranova, C., Crupi, D., Sant'Angelo, A., Girlanda, P., and Quartarone, A. (2014). Increased transcranial direct current stimulation after effects during concurrent peripheral electrical nerve stimulation. *Brain Stimulation*, 7(1):113–121.

Robbins, S. M., Houghton, P. E., Woodbury, M. G., and Brown, J. L. (2006). The Therapeutic Effect of Functional and Transcutaneous Electric Stimulation on Improving Gait Speed in Stroke Patients: A Meta-Analysis. *Archives of Physical Medicine and Rehabilitation*, 87(6):853–859.

Rom, D. M. (1990). A Sequentially Rejective Test Procedure Based on a Modified Bonferroni Inequality. *Biometrika*, 77(3):663–665.

Rose, J. and Gamble, J. G. (2006). *Human Walking*. Lippincott Williams & Wilkins, Philadelphia, 3rd edition.

Rossi, S., Hallett, M., Rossini, P. M., and Pascual-Leone, A. (2012). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology*, 120(12):323–330.

Rossignol, S. (2000). Locomotion and its recovery after spinal injury. *Current Opinion in Neurobiology*, 10(6):708–716.

Rossini, P., Burke, D., Chen, R., Cohen, L., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P., George, M., Hallett, M., Lefaucheur, J., Langguth, B., Matsumoto, H., Miniussi, C., Nitsche, M., Pascual-Leone, A., Paulus, W., Rossi, S., Rothwell, J., Siebner, H., Ugawa, Y., Walsh, V., and Ziemann, U. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology*, 126(6):1071–1107.

Rotenberg, A., Harrington, M. G., Birnbaum, D. S., Madsen, J. R., Glass, I. E. S., Jensen, F. E., and Pascual-Leone, A. (2007). Minimal heating of titanium skull plates during 1Hz repetitive transcranial magnetic stimulation. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, 118(11):2536–8.

Rotenberg, A., Horvath, J. C., and Pascual-Leone, A. (2014). The Transcranial Magnetic Stimulation (TMS) Device and Foundational Techniques. In Rotenberg, A., editor, *Transcranial Magnetic Stimulation*, pages 3–13. Springer Science + Business Media, New York.

Rothwell, J. C. (1997). Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. *Journal of Neuroscience Methods*, 74(2):113–122.

Rushton, D. (2003). Functional Electrical Stimulation and rehabilitation—an hypothesis. *Medical Engineering & Physics*, 25(1):75–78.

Russell, D. M. and Apatoczky, D. T. (2016). Walking at the preferred stride frequency minimizes muscle activity. *Gait & Posture*, 45:181–186.

Sakai, K., Ugawa, Y., Terao, Y., Hanajima, R., Furubayashi, T., and Kanazawa, I. (1997). Preferential activation of different I waves by transcranial magnetic stimulation with a figure-of-eight-shaped coil. *Experimental Brain Research*, 113(1):24–32.

Salbach, N., Mayo, N., Wood-Dauphinee, S., Hanley, J., Richards, C., and Côté, R. (2004). A task-orientated intervention enhances walking distance and speed in the first year post stroke: a randomized controlled trial. *Clinical Rehabilitation*, 18(5):509–519.

Samii, A., Wassermann, E. M., Ikoma, K., Mercuri, B., and Hallett, M. (1996). Characterization of postexercise facilitation and depression of motor evoked potentials to transcranial magnetic stimulation. *Neurology*, 46(5):1376–1376.

Schmidt, M., Jacobsen, J. B., Johnsen, S. P., Botker, H. E., and Sorensen, H. T. (2014). Eighteen-year trends in stroke mortality and the prognostic influence of comorbidity. *Neurology*, 82(4):340–350.

Schubert, M., Wohlfarth, K., Rollnik, J. D., and Dengler, R. (1998). Walking and fatigue in sclerosis: The role corticospinal system. *Muscle and Nerve*, 21(8):1068–1070.

Serrao, M., Spaich, E. G., and Andersen, O. K. (2012). Modulating effects of bodyweight unloading on the lower limb nociceptive withdrawal reflex during symmetrical stance. *Clinical Neurophysiology*, 123(5):1035–1043.

Shahani, B. T. and Young, R. R. (1971). Human flexor reflexes. *Journal of Neurology, Neurosurgery and Psychiatry*, 34(5):616–627.

Shik, M. L. and Orlovsky, G. N. (1976). Neurophysiology of locomotor automatism. *Physiological Reviews*, 56(3):465–501.

Smith, M. C., Stinear, J. W., Alan Barber, P., and Stinear, C. M. (2017). Effects of non-target leg activation, TMS coil orientation, and limb dominance on lower limb motor cortex excitability. *Brain Research*, 1655:10–16.

Sousa, C. O., Barela, J. A., Prado-Medeiros, C. L., Salvini, T. F., and Barela, A. M. (2011). Gait training with partial body weight support during overground walking for individuals with chronic stroke: a pilot study. *Journal of NeuroEngineering and Rehabilitation*, 8(1):48.

Sowman, P. F., Dueholm, S. S., Rasmussen, J. H., and Mrachacz-Kersting, N. (2014). Induction of plasticity in the human motor cortex by pairing an auditory stimulus with TMS. *Frontiers in Human Neuroscience*, 8(June):398.

Spaich, E. G., Andersen, O. K., and Arendt-Nielsen, L. (2004a). Tibialis Anterior and Soleus Withdrawal Reflexes Elicited by Electrical Stimulation of the Sole of the Foot during Gait. *Neuromodulation: Technology at the Neural Interface*, 7(2):126–132.

Spaich, E. G., Arendt-Nielsen, L., and Andersen, O. K. (2004b). Modulation of lower limb withdrawal reflexes during gait: a topographical study. *Journal of neurophysiology*, 91(1):258–66.

Spaich, E. G., Emborg, J., Collet, T., Arendt-Nielsen, L., and Andersen, O. K. (2009). Withdrawal reflex responses evoked by repetitive painful stimulation delivered on the sole of the foot during late stance: Site, phase, and frequency modulation. *Experimental Brain Research*, 194(3):359–368.

Spaich, E. G., Hinge, H. H., Arendt-Nielsen, L., and Andersen, O. K. (2006). Modulation of the withdrawal reflex during hemiplegic gait: Effect of stimulation site and gait phase. *Clinical Neurophysiology*, 117(11):2482–2495.

Spaich, E. G., Svaneborg, N., and Andersen, O. K. (2011). Withdrawal reflex-based gait training in the subacute post-stroke phase: Preliminary results. *Biomedical Engineering & Medical Physics, IFMBE Proceedings 34,*, 34 IFMBE:230–232.

Spaich, E. G., Svaneborg, N., Jørgensen, H. R., and Andersen, O. K. (2014). Rehabilitation of the hemiparetic gait by nociceptive withdrawal reflex-based functional electrical therapy: a randomized, single-blinded study. *Journal of Neuroengineering and Rehabilitation*, 11(1):81.

Stegeman, D. and Hermens, H. (1998). Standards for surface electromyography: The European project Surface EMG for non-invasive assessment of muscles (SENIAM). In Hermens, H., Rau, G., Disselhorst-Klug, C., and Freriks, B., editors, *Surface Electromyography Application Areas and Parameters. Proceedings of the Third General SENIAM Workshop on Surface Electromyography*, pages 108–112. Aachen.

Stein, R. B. and Oğuztöreli, M. N. (1976). Does the velocity sensitivity of muscle spindles stabilize the stretch reflex? *Biological Cybernetics*, 23(4):219–228.

Stevenson, A. J., Mrachacz-Kersting, N., van Asseldonk, E., Turner, D. L., and Spaich, E. G. (2015). Spinal plasticity in robot-mediated therapy for the lower limbs. *Journal of NeuroEngineering and Rehabilitation*, 12(1):81.

Suzuki, M., Kirimoto, H., Onishi, H., Yamada, S., Tamaki, H., Maruyama, A., and Yamamoto, J.-i. (2012). Reciprocal changes in input–output curves of motor evoked potentials while learning motor skills. *Brain Research*, 1473:114–123.

Takemi, M., Masakado, Y., Liu, M., and Ushiba, J. (2015). Sensorimotor event-related desynchronization represents the excitability of human spinal motoneurons. *Neuroscience*, 297:58–67.

Talelli, P., Waddingham, W., Ewas, A., Rothwell, J. C., and Ward, N. S. (2008). The effect of age on task-related modulation of interhemispheric balance. *Experimental Brain Research*, 186(1):59–66.

Tang, A. and Rymer, W. Z. (1981). Abnormal force–EMG relations in paretic limbs of hemiparetic human subjects. *Journal of neurology, neurosurgery, and psychiatry*, 44(8):690–8.

Taylor, H. H. and Murphy, B. (2008). Altered Sensorimotor Integration With Cervical Spine Manipulation. *Journal of Manipulative and Physiological Therapeutics*, 31(2):115–126.

Temesi, J., Rupp, T., Martin, V., Arnal, P. J., Féasson, L., Verges, S., Millet, G., and Y. (2014). Central Fatigue Assessed by Transcranial Magnetic Stimulation in Ultratrail Running. *Medicine & Science in Sports & Exercise*, 46(6):1166–1175.

Terao, Y. and Ugawa, Y. (2002). Basic mechanisms of TMS. *Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society*, 19(4):322–43.

Terao, Y., Ugawa, Y., Hanajima, R., Machii, K., Furubayashi, T., Mochizuki, H., Enomoto, H., Shiio, Y., Uesugi, H., Iwata, N. K., and Kanazawa, I. (2000). Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. *Brain Research*, 859(1):137–146.

Thibaut, A., Chatelle, C., Ziegler, E., Bruno, M.-A., Laureys, S., and Gosseries, O. (2013). Spasticity after stroke: Physiology, assessment and treatment. *Brain Injury*, 27(10):1093–1105.

Thielscher, A. and Kammer, T. (2004). Electric field properties of two commercial figure-8 coils in TMS: calculation of focality and efficiency. *Clinical Neurophysiology*, 115(7):1697–1708.

Tranulis, C., Guéguen, B., Pham-Scottez, A., Vacheron, M. N., Cabelguen, G., Costantini, A., Valero, G., and Galinovski, A. (2006). Motor threshold in transcranial magnetic stimulation: Comparison of three estimation methods. *Neurophysiologie Clinique*, 36(1):1–7.

Troje, N. F., Westhoff, C., and Lavrov, M. (2005). Person identification from biological motion: Effects of structural and kinematic cues. *Perception & Psychophysics*, 67(4):667–675.

Trompetto, C., Buccolieri, A., Marinelli, L., and Abbruzzese, G. (2001). Differential modulation of motor evoked potential and silent period by activation of intracortical inhibitory circuits. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, 112(10):1822–7.

Vahabzadeh-Hagh, A. (2014). Paired-Pulse Transcranial Magnetic Stimulation (TMS) Protocols. In Rotenberg, A., editor, *Transcranial Magnetic Stimulation*, pages 117–127. Springer Science + Business Media, New York, NY.

Valle, A. C., Dionisio, K., Pitskel, N. B., Pascual-Leone, A., Orsati, F., Ferreira, M. J. L., Boggio, P. S., Lima, M. C., Rigonatti, S. P., and Fregni, F. (2007). Low and high frequency repetitive transcranial magnetic stimulation for the treatment of spasticity. *Developmental Medicine & Child Neurology*, 49(7):534–538.

Veerbeek, J. M., van Wegen, E., van Peppen, R., van der Wees, P. J., Hendriks, E., Rietberg, M., and Kwakkel, G. (2014). What Is the Evidence for Physical Therapy Poststroke? A Systematic Review and Meta-Analysis. *PLoS ONE*, 9(2):e87987.

Wade, D. T., Wood, V. A., Heller, A., Maggs, J., and Langton Hewer, R. (1987). Walking after stroke. Measurement and recovery over the first 3 months. *Scandinavian journal of rehabilitation medicine*, 19(1):25–30.

Wagner, T., Valero-Cabre, A., and Pascual-Leone, A. (2007). Noninvasive Human Brain Stimulation. *Annual Review of Biomedical Engineering*, 9(1):527–565.

Wall, J. C. and Turnbull, G. I. (1986). Gait asymmetries in residual hemiplegia. *Archives of physical medicine and rehabilitation*, 67(8):550–3.

Wassermann, E. M. (2002). Variation in the response to transcranial magnetic brain stimulation in the general population. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, 113(7):1165–1171.

Wassermann, E. M., Fuhr, P., Cohen, L. G., and Hallett, M. (1991). Effects of transcranial magnetic stimulation on ipsilateral muscles. *Neurology*, 41(11):1795–9.

Weaver, T. B., Janzen, M. R., Adkin, A. L., and Tokuno, C. D. (2012). Changes in Spinal Excitability During Dual Task Performance. *Journal of Motor Behavior*, 44(4):289–294.

Werhahn, K., Fong, J., Meyer, B.-U., Priori, A., Rothwell, J., Day, B., and Thompson, P. (1994). The effect of magnetic coil orientation on the latency of surface EMG and single motor unit responses in the first dorsal interosseous muscle. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 93(2):138–146.

Winter, D. A., Patla, A. E., Frank, J. S., and Walt, S. E. (1990). Biomechanical Walking Pattern Changes in the Fit and Healthy Elderly. *Physical Therapy*, 70(6):340–347.

Wolpaw, J. R., Braitman, D. J., and Seegal, R. F. (1983). Adaptive plasticity in primate spinal stretch reflex: initial development. *Journal of Neurophysiology*, 50(6):1296–1311.

Yamamoto, S., Kumagai, T., Obata, H., Koyama, H., Komeda, T., and Nakazawa, K. (2003). Effect of different joint angle on stretch reflex sensitivity in lower leg muscles. *Proceedings of the 25th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (IEEE Cat. No.03CH37439)*, 2:1483–1486.

Yang, J. F. and Winter, D. A. (1985). Surface EMG profiles during different walking cadences in humans. *Electroencephalography and Clinical Neurophysiology*, 60(6):485–491.

Zhang, S., Holmes, M., and Li, L. (2015). Reliability of nerve function assessments for people with peripheral neuropathy. *International Journal of Neuroscience*, 125(3):201–207.

Ziemann, U. (2004). Cortical threshold and excitability measurements. In *Handbook of Clinical Neurophysiology*, volume 4, pages 317–335.

Ziemann, U., Rothwell, J. C., and Ridding, M. C. (1996). Interaction between intracortical inhibition and facilitation in human motor cortex. *The Journal of physiology*, 496(3):873–81.

Part IV

Appendix

Appendix A

List of Medication which Should not be Used in Combination with TMS

The following list consist of medication/drugs which should not be used in combination with TMS, mostly because they are known to have a seizure threshold lowering effect [Rossi et al., 2012]:

- BCNU
- MDMA, ecstasy
- alcohol
- amitriptyline
- amphetamines
- ampicillin
- anticholinergics
- antihistamines
- aripiprazole
- barbiturates
- benzodiazepines
- bupropion
- cephalosporins
- chloral hydrate
- chlorambucil
- chloroquine
- chlorpromazine
- clozapine
- cocaine
- cyclosporin
- cytosine arabinoside
- doxepine
- duloxetine
- fluoxetine
- fluphenazine
- fluvoxamine
- foscarnet
- gammahydroxybutyrate (GHB)
- ganciclovir
- haloperidol
- imipenem
- imipra-mine
- isoniazid
- ketamine
- levofloxacin
- lithium
- maprotiline
- mefloquine
- meprobamate
- methotrexate
- metronidazole
- mianserin
- mirtazapine
- nortriptyline
- olanzapine
- paroxetine
- penicillin
- phencyclidine (PCP, angel's dust)
- pimozide
- quetiapine
- reboxetine
- risperidone
- ritonavir
- sertraline
- sympathomimetics
- theophylline
- venlafaxine
- vincristine
- ziprasidone

Appendix B
Questionnaires filled in by subjects

TASS Questionnaire (English Version)

ID Number:
Date:
Age:

Please answer the following. Have you ever:

Had an adverse reaction to TMS?	Yes	No
Had a seizure?	Yes	No
Had an electroencephalogram (EEG) for medical purposes?	Yes	No
Had a stroke?	Yes	No
Had a serious head injury (include neurosurgery)?	Yes	No
Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips, or fragments from welding or metalwork?	Yes	No
Do you have any implanted devices such as cardiac pacemakers or medical pumps?	Yes	No
Do you suffer from frequent or severe headaches?	Yes	No
Have you ever had any other brain-related condition?	Yes	No
Have you ever had any illness that caused brain injury?	Yes	No
Are you taking any medications?	Yes	No
If you are a woman of childbearing age, is there a chance that you could be pregnant?	Yes	No
Does anyone in your family have epilepsy?	Yes	No
Do you need further explanation of TMS and its associated risks?	Yes	No

If you answered yes to any of the above, please provide details (use reverse if necessary):

Waterloo Footedness Questionnaire---Revised

Instructions: Answer each of the following questions as best you can. If you *always* use one foot to perform the described activity, circle Ra or La (for **right always or left** always). If you **usually** use one foot circle Ru or Lu, as appropriate. If you use **both** feet **equally often, circle Eq.**

Please do not simply circle one answer for all questions, but imagine yourself performing each activity in turn, and then mark the appropriate answer. If necessary, stop and pantomime the activity.

1. Which foot would you use to kick a stationary ball at a target straight in front of you?	La	Lu	Eq	Ru	Ra
2. If you had to stand on one foot, which foot would it be?	La	Lu	Eq	Ru	Ra
3. Which foot would you use to smooth sand at the beach?	La	Lu	Eq	Ru	Ra
4. If you had to step up onto a chair, which foot would you place on the chair first?	La	Lu	Eq	Ru	Ra
5. Which foot would you use to stomp on a fast-moving bug?	La	Lu	Eq	Ru	Ra
6. If you were to balance on one foot on a railway track, which foot would you use?	La	Lu	Eq	Ru	Ra
7. If you wanted to pick up a marble with your toes, which foot would you use?	La	Lu	Eq	Ru	Ra
8. If you had to hop on one foot, which foot would you use?	La	Lu	Eq	Ru	Ra
9. Which foot would you use to help push a shovel into the ground?	La	Lu	Eq	Ru	Ra
10. During relaxed standing, people initially put most of their weight on one foot, leaving the other leg slightly bent. Which foot do you put most of your weight on first?	La	Lu	Eq	Ru	Ra
11. Is there any reason (i.e. injury) why you have changed your foot preference for any of the above activities?		**YES**		**NO**	
12. Have you ever been given special training or encouragement to use a particular foot for certain activities?		**YES**		**NO**	

13. If you have answered **YES** for either question 11 or 12, please explain:12, please explain:

NAME _____

SIGNATURE _____

DATE _____

$$\text{Score} = \frac{\text{Right} - \text{Left}}{\text{Right} + \text{Left}} =$$

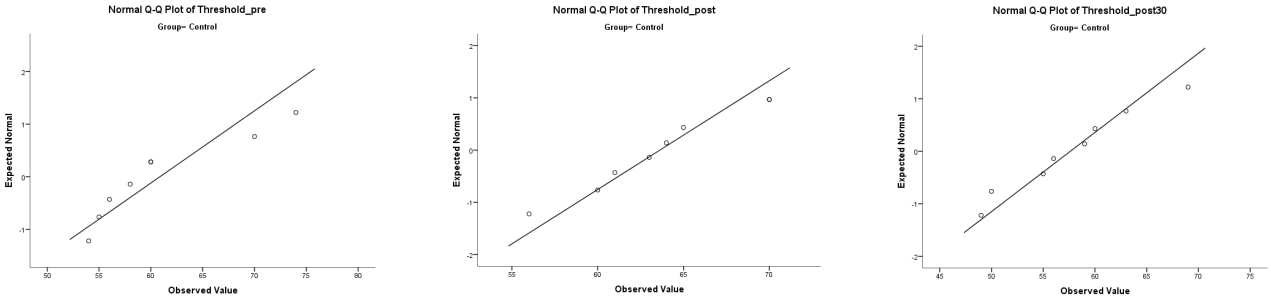
Appendix C
Results of Normality Tests

The following section shows the results of Shapiro-Wilk's test of normality for all the tested data. In the tables df represents the degrees of freedom, Sig, represents the p-value.

Resting Motor Threshold

Table C.1: Shapiro-Wilk test of normality for the resting motor threshold for the control group.

Session	Statistic	df	Sig.
Threshold pre	0.841	8	0.077
Threshold post	0.948	8	0.696
Threshold post 30	0.967	8	0.877

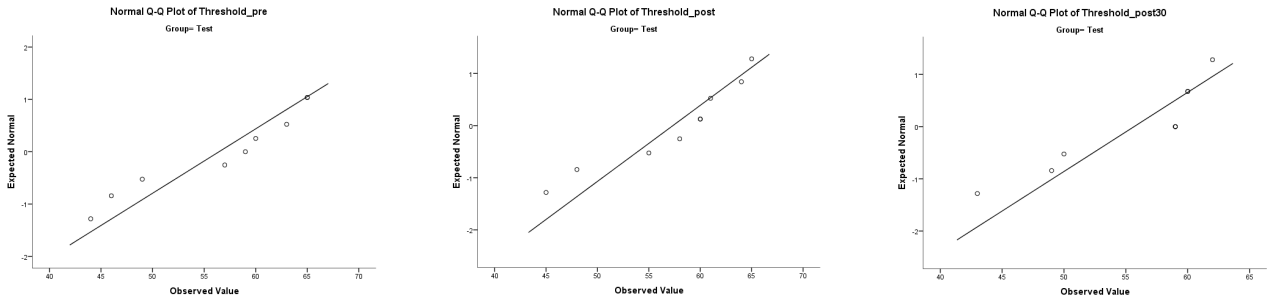


(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.1: Normality plots of the resting motor threshold for the three sessions of the control group.

Table C.2: Shapiro-Wilk test of normality for the resting motor threshold for the intervention group

Session	Statistic	df	Sig.
Threshold pre	0.882	9	0.164
Threshold post	0.895	9	0.223
Threshold post 30	0.805	9	0.023



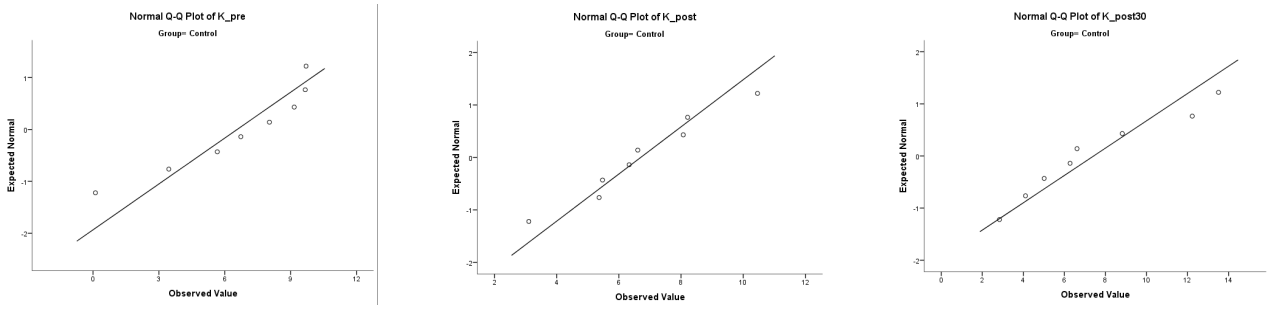
(a) Intervention-Pre **(b)** Intervention-Post **(c)** Intervention-Post30

Figure C.2: Normality plots of the resting motor threshold for the three sessions of the intervention group.

Slope K

Table C.3: Shapiro-Wilk test of normality for the slope K for the control group.

Session	Statistic	df	Sig.
Slope K pre	0.888	8	0.222
Slope K post	0.978	8	0.951
Slope K post 30	0.926	8	0.479

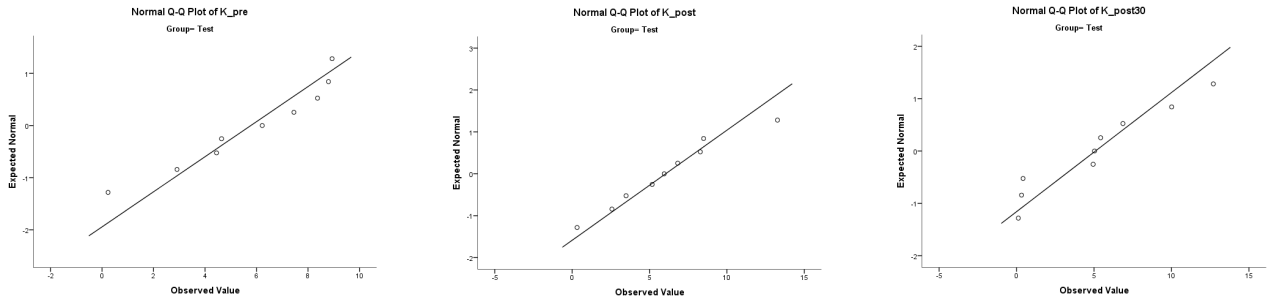


(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.3: Normality plots of the slope K for the three sessions for the control group.

Table C.4: Shapiro-Wilk test of normality for the slope K for the intervention group.

Session	Statistic	df	Sig.
Slope K pre	0.921	9	0.400
Slope K post	0.978	9	0.955
Slope K post 30	0.911	9	0.325



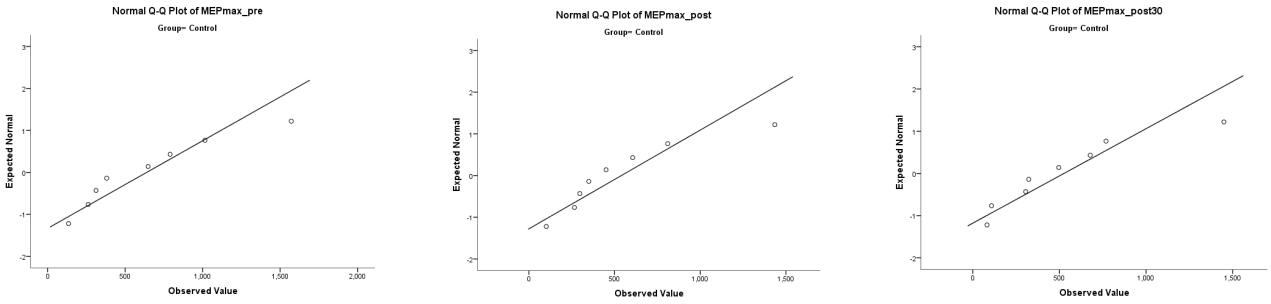
(a) Intervention-Pre **(b)** Intervention-Post **(c)** Intervention-Post30

Figure C.4: Normality plots of the slope K for the three sessions for the intervention group.

MEPmax

Table C.5: Shapiro-Wilk test of normality for the MEP_{max} for the control group.

Session	Statistic	df	Sig.
MEPmax pre	0.907	8	0.336
MEPmax post	0.864	8	0.131
MEPmax 30	0.884	8	0.206

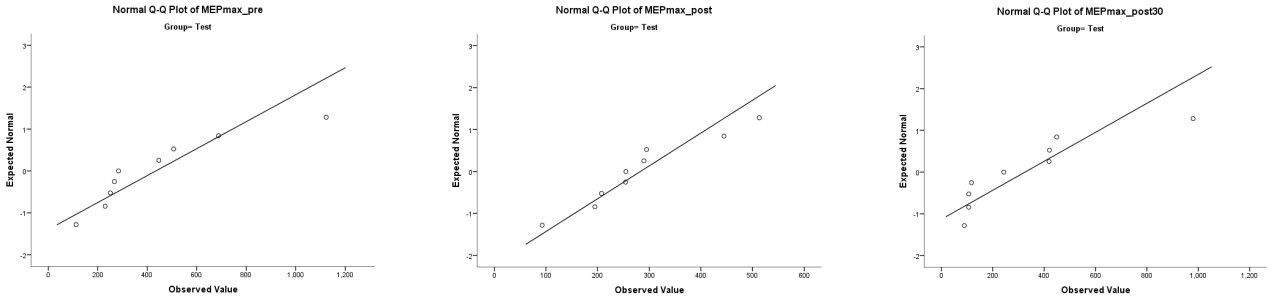


(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.5: Normality plots of the MEP_{max} for the three sessions for the control group.

Table C.6: Shapiro-Wilk test of normality for the MEP_{max} for the intervention group.

Session	Statistic	df	Sig.
MEPmax pre	0.851	9	0.076
MEPmax post	0.934	9	0.516
MEPmax post 30	0.795	9	0.018



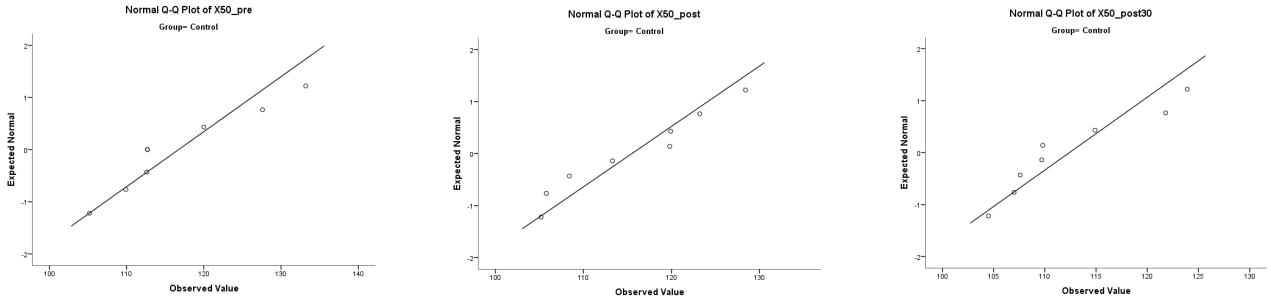
(a) Intervention-Pre **(b)** Intervention-Post **(c)** Intervention-Post30

Figure C.6: Normality plots of the MEP_{max} for the three sessions for the intervention group.

S50

Table C.7: Shapiro-Wilk test of normality for the S_{50} for the control group.

Session	Statistic	df	Sig.
S50 pre	0.903	8	0.307
S50 post	0.926	8	0.480
S50 post 30	0.882	8	0.195

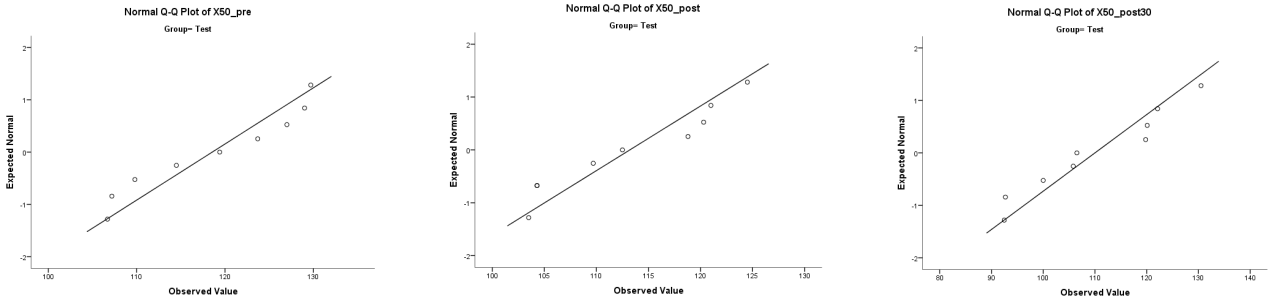


(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.7: Normality plots of the S_{50} for the three sessions for the control group.

Table C.8: Shapiro-Wilk test of normality for the S_{50} for the intervention group.

Session	Statistic	df	Sig.
S50 pre	0.892	9	0.211
S50 post	0.888	9	0.193
S50 post 30	0.924	9	0.423



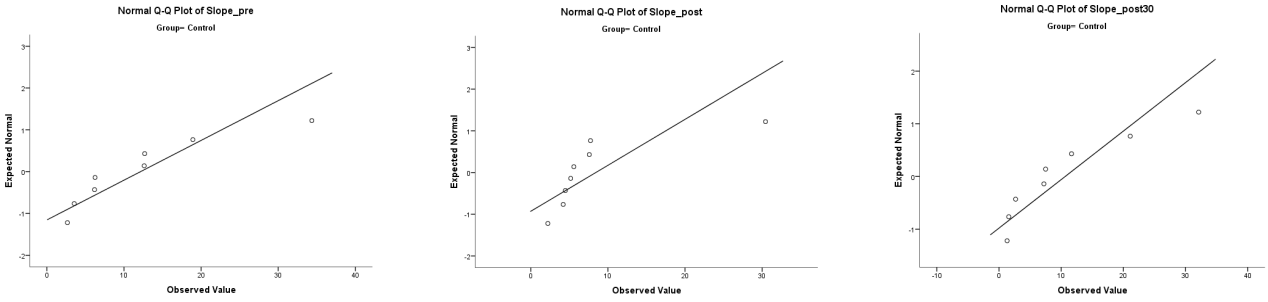
(a) Intervention-Pre **(b)** Intervention-Post **(c)** Intervention-Post30

Figure C.8: Normality plots of the S_{50} for the three sessions for the intervention group.

Line

Table C.9: Shapiro-Wilk test of normality for the slope of the line for the control group

Session	Statistic	df	Sig.
Slope pre	0.843	8	0.080
Slope post	0.611	8	0.000
Slope post 30	0.844	8	0.083

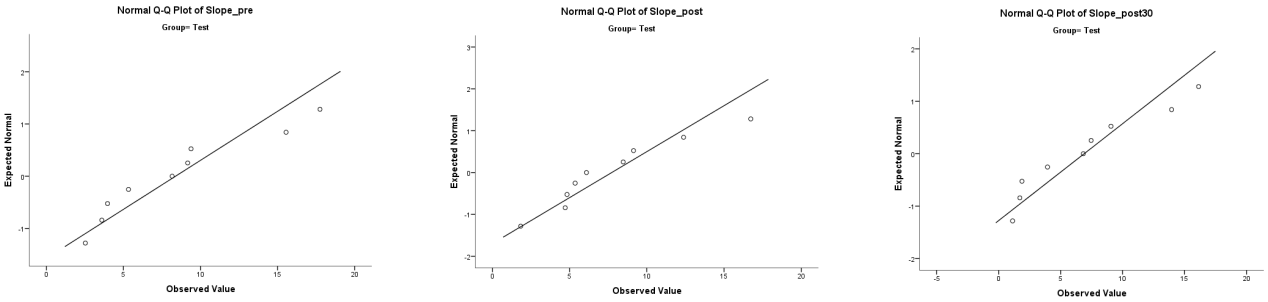


(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.9: Normality plots of the slope of the line for the three sessions for the control group.

Table C.10: Shapiro-Wilk test of normality for the slope of the line for the intervention group

Session	**Statistic**	**df**	**Sig.**
Slope pre	0.899	9	0.244
Slope post	0.928	9	0.467
Slope post 30	0.905	9	0.285



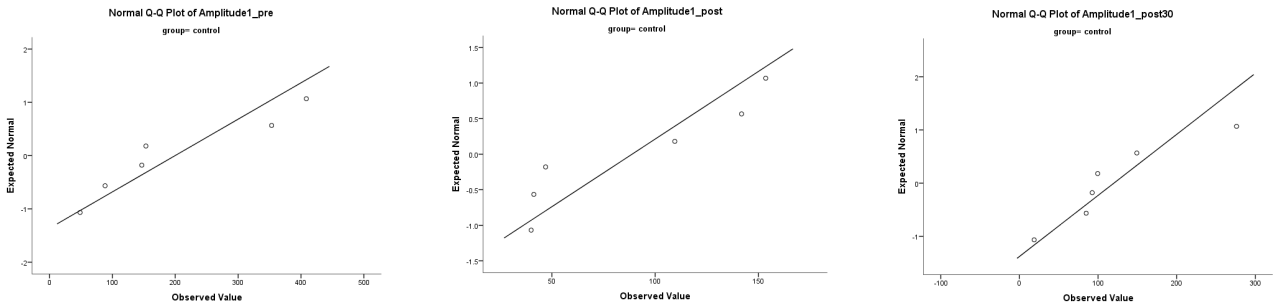
(a) Intervention-Pre **(b)** Intervention-Post **(c)** Intervention-Post30

Figure C.10: Normality plots of the slope of the line for the three sessions for the intervention group.

Amplitude of the M1 peak

Table C.11: Shapiro-Wilk test of normality for the amplitude of te M1 peak of the stretch reflex for the control group.

Session	**Statistic**	**df**	**Sig.**
Amplitude M1 pre	0.874	6	0.243
Amplitude M1 post	0.824	6	0.096
Amplitude M1 post30	0.895	6	0.347

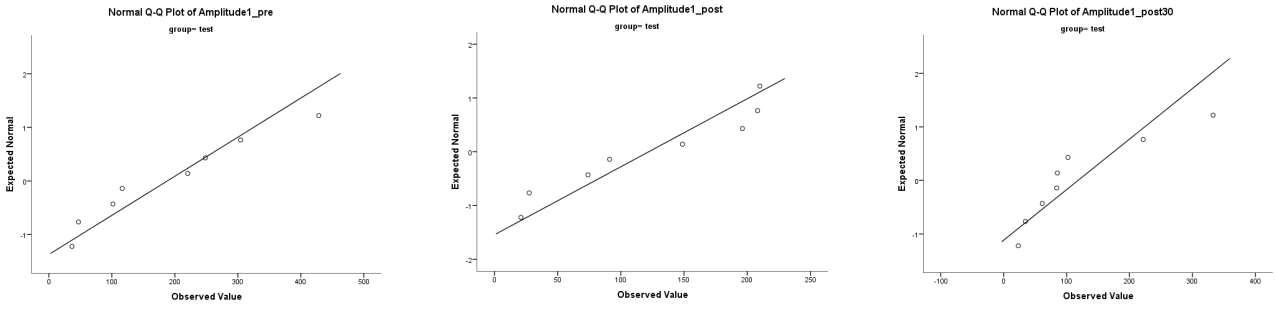


(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.11: Normality plots of the amplitude of the M1 peak of the stretch reflex for the 3 different time points for the control group.

Table C.12: Shapiro-Wilk test of normality for the amplitude of te M1 peak of the stretch reflex for the intervention group.

	Statistic	**df**	**Sig.**
Amplitude M1 pre	0.932	8	0.537
Amplitude M1 post	0.879	8	0.184
Amplitude M1 post30	0.820	8	0.046



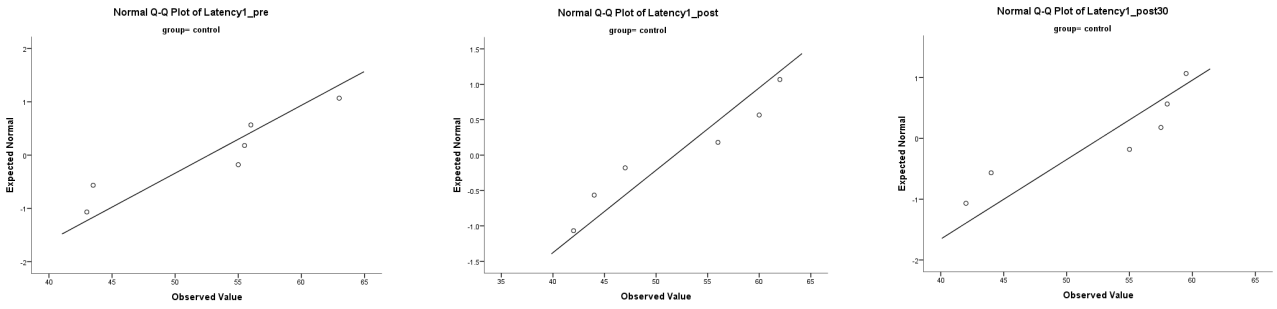
(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.12: Normality plots of the amplitude of the M1 peak of the stretch reflex for the 3 different time points for the intervention group.

Latency of the M1 peak

Table C.13: Shapiro-Wilk test of normality for the latency of te M1 peak of the stretch reflex for the control group.

	Statistic	**df**	**Sig.**
Latency M1 pre	0.872	6	0.235
Latency M1 post	0.893	6	0.332
Latency M1 post30	0.810	6	0.072

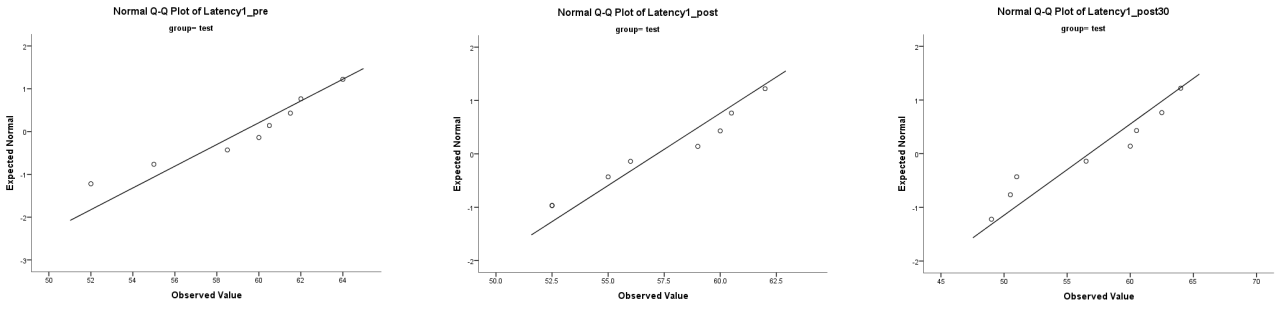


(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.13: Normality plots of the latency of the M1 peak of the stretch reflex for the 3 different time points for the control group.

Table C.14: Shapiro-Wilk test of normality for the latency of te M1 peak of the stretch reflex for the intervention group.

	Statistic	df	Sig.
Latency M1 pre	0.927	8	0.491
Latency M1 post	0.911	8	0.358
Latency M1 post30	0.895	8	0.261



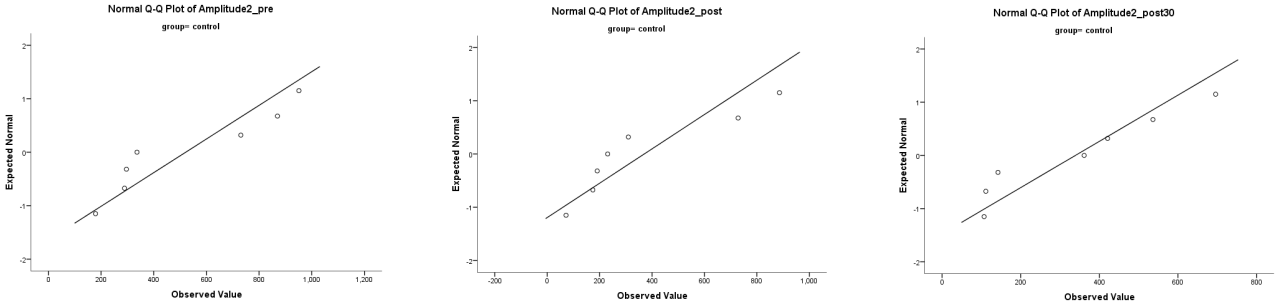
(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.14: Normality plots of the latency of the M1 peak of the stretch reflex for the 3 different time points for the intervention group.

Amplitude of the M2 peak

Table C.15: Shapiro-Wilk test of normality for the amplitude of te M2 peak of the stretch reflex for the control group.

	Statistic	df	Sig.
Amplitude M2 pre	0.852	7	0.127
Amplitude M2 post	0.830	7	0.080
Amplitude M2 post30	0.901	7	0.335

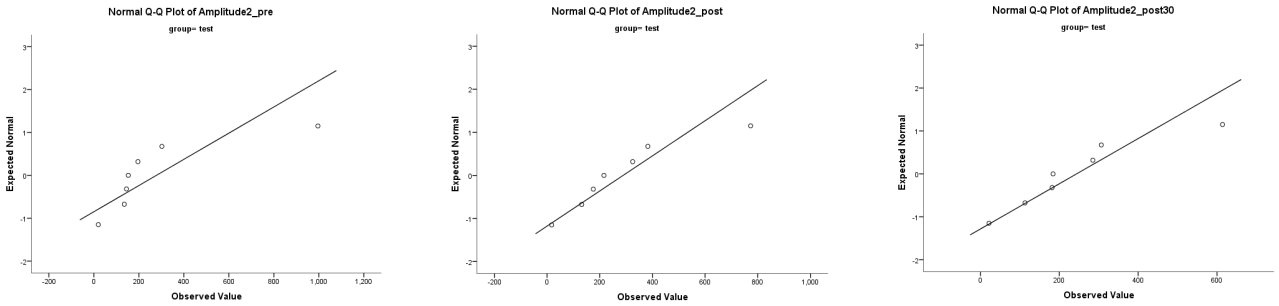


(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.15: Normality plots of the amplitude of the M2 peak of the stretch reflex for the 3 different time points for the control group.

Table C.16: Shapiro-Wilk test of normality for the amplitude of te M2 peak of the stretch reflex for the intervention group.

	Statistic	**df**	**Sig.**
Amplitude M2 pre	0.690	7	0.003
Amplitude M2 post	0.893	7	0.293
Amplitude M2 post30	0.903	7	0.352



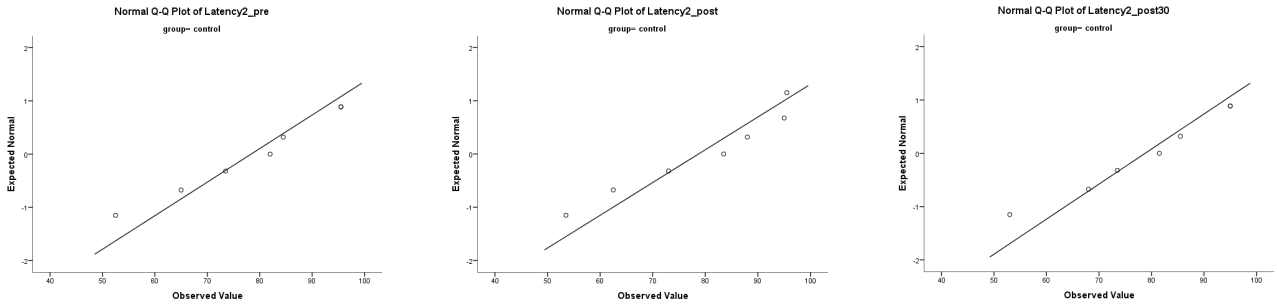
(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.16: Normality plots of the amplitude of the M2 peak of the stretch reflex for the 3 different time points for the intervention group.

Latency of the M2 peak

Table C.17: Shapiro-Wilk test of normality for the latency of te M2 peak of the stretch reflex for the control group.

	Statistic	**df**	**Sig.**
Latency M2 pre	0.936	7	0.604
Latency M2 post	0.916	7	0.440
Latency M2 post30	0.936	7	0.607

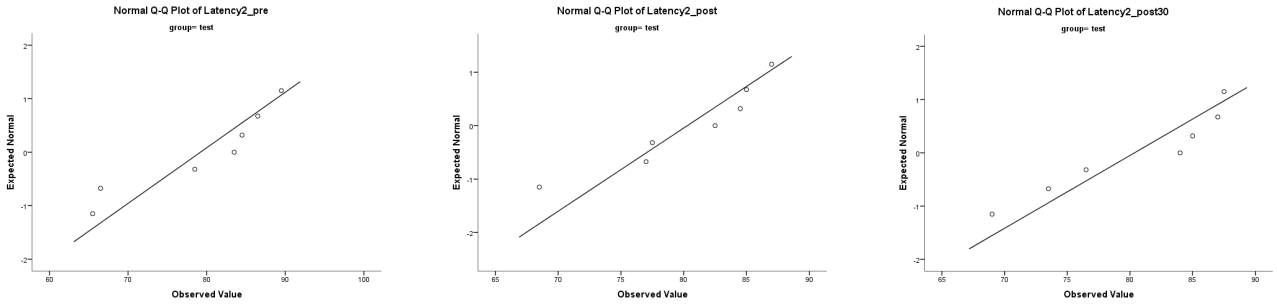


(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.17: Normality plots of the latency of the M2 peak of the stretch reflex for the 3 different time points for the control group.

Table C.18: Shapiro-Wilk test of normality for the latency of te M2 peak of the stretch reflex for the intervention group.

	Statistic	**df**	**Sig.**
Latency M2 pre	0.860	7	0.153
Latency M2 post	0.902	7	0.343
Latency M2 post30	0.882	7	0.233



(a) Control-Pre **(b)** Control-Post **(c)** Control-Post30

Figure C.18: Normality plots of the latency of the M2 peak of the stretch reflex for the 3 different time points for the intervention group.