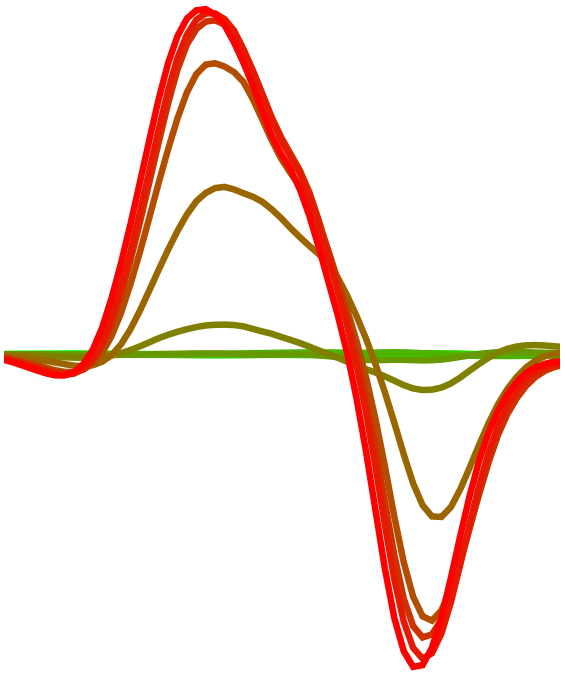
A Novel Sciatic Nerve Stimulation Technique for Assessing Phase Modulation of the H-reflex in the Hamstrings During Human Gait



Master Thesis

Rasmus Elbæk Andersen & Alessandro Ranieri

Aalborg University
Department of Health Science & Technology

This Report was written using LaTeX, figures and simulations were produced in Matlab, Google Docs, and Lucidchart.

AALBORG UNIVERSITY

STUDENT REPORT

Department of Health Science & Technology
Frederik Bajers Vej 7D2
9220 Aalborg East
Telephone (+45) 9940 9940
Fax (+45) 9815 4008
http://www.hst.aau.dk

Title:
A Novel Sciatic Nerve Stimulation Technique for Assessing H-reflex Phase Modulation in the Hamstrings During Human Gait

Theme:
A functional stimulation technique for the sciatic nerve to investigate reflex modulation during gait

Project Period:
Spring Semester 2015

Project Group:
15gr1081

Participants:
Rasmus Elbæk Andersen
Alessandro Ranieri

Supervisors:
Natalie Mrachacz-Kersting
Erika G. Spaich

Page Numbers: 90

Date of Completion:
June 3, 2015

Abstract:

Introduction: Assessing reflex modulation during gait is of interest for research aiming to help people that suffer from gait deficits as a result of injuries to the nervous system. For the hamstring muscles there is a lack of techniques and studies for investigation of the H-reflex. Problem formulation: Can a protocol based on H-reflex techniques targeting the sciatic nerve be used to assess reflex phase modulation in the hamstrings during gait? Problem Solution: Constant current stimulation was applied to a grid of electrodes to find an optimal site based on H and M waves in both biceps femoris and soleus. Stimulation was then administered to ten volunteers, in different parts of the gait cycle. Result: Phase modulation consistent with results from literature were observed. For some subjects cross spinal responses could be elicited. Conclusion: H-reflex techniques can be adapted to the hamstrings in dynamic studies.

Contents

List of Figures

List of Tables

Danish summary - Dansk resumé

Introduktion: Flere typer af nerveskader og sygdomme kan forårsage tab eller nedsættelse af gangfunktion. For personer med gangbesvær forårsaget af nerveskade i forbindelse med bl.a. rygmarvsskader og apopleksi kan rehabilitering forbedre parametre af gang som f.eks. ganguafhængighed. Reflekser er interessante for rehabilitering både i forskning af deres betydning i normal gang og som en klinisk evaluering af patienters nerveskader. En vigtig del af refleksers normale funktion er fasemodulation. Reflekser involveret i gang er ikke altid aktive, men er i visse faser af gang-cyklussen ned reguleret så ingen eller mindre refleksresponse opstår ved sanseinput, der normalvis ville udløse en refleks. For strækreflekser kan modulering testes ved brug af H-refleks teknikker. H-refleksen er en kunstig refleks, der opstår ved elektrisk stimulering af strækreflekskredsløbet langs nerverne der forbinder sanseorganerne i senerne og muskler med rygraden. H-refleks teknikker har været brugt for underbenet, men har stort set ikke været anvendt til test af baglårsmusklernes strækreflekser.

Problemformulering: Kan en protokol baseret på H-refleks teknikker målrettet iskiasnerven bruges til at undersøge fasemodulering af reflekers i baglårsmusklerne under gang.

Problemløsning: Gennem en række pilotforsøg blev en protokol og forsøgsopsætning målrettet H-refleks modulering af iskiasnerven under gang udviklet og en undersøge af tvær-spinal reflekser udarbejdet. 10 forsøgspersoner blev rekrutteret. Protokollen består af 4 trin. 1: Den optimale placering af stimuleringskatoden vælges blandt 9 kandidat katoder i en firkant påbaglåret ud fra H- og M-bølge respons i bicep femoris og soleus musklerne. 2: Input-output kurve for M- og H-bølger optages fra en stilleliggende forsøgsperson ved brug af stimulering mellem 0 og 100 mA monopolære stimulerings impulser med en pulsbredde på 1 ms. 3: Input-output kurver for M- og H-bølger optages for hver 10% af gangcyklussen. 4: Med en konstant 70 eller 80 mA stimuleringsintensitet baseret på soleus H-refleksen, stimuleres iskiasnerven 20 gange for 50% og 90% af gangcyklussen. Herefter optages 20 skridt uden stimulering.

Resultater: Resultaterne fra anden del af protokollen viste både H-reflekser og M-bølger i både biceps femoris og soleus musklerne for størstedelen af de 10

forsøgspersoner. For biceps femoris kunne M-max opnås for 4 ud af 10 forsøgspersoner. For soleus kunne 1 ud af 10 forsøgspersoner stimuleres til M-max. Latenstiden i biceps femoris var 12.24 $\pm$ 1.62ms for M-bølgen og 22.8 $\pm$ 2.19ms for H-refleksen. For soleus var resultaterne henholdsvis 16.66 $\pm$ 1.63ms og 32.3 $\pm$ 2.68ms. Resultaterne fra den tredje del af protokollen viste at ratioen mellem H-refleks og M-bølge var større i soleus end i biceps femoris. Selvom H/M ratioen generelt stiger i takt med baggrunds EMG aktivitetene er dette ikke altid tilfældet. Biceps H/M ratio var størst omkring 90-0% af gangcyklussen mens H/M ratioen for soleus var størst mellem 20-50% af gangcyklussen. Resultaterne for fjerde del af protokollen viste klaret hurtige tvær-spinalereflekser (75 ms) for 1 ud af 7 forsøgspersoner og langsommere respons (100-150 ms) i 2 ud af 7 forsøgspersoner.

Diskussion: Resultaterne for fasemodulering af H-reflekser under gang stemmer overens med resultater opnået ved brug af andre metoder, samt andre stimuleringseleketrodeplaceringer. Dette bekræfter at protokollen er istand til at stimulerer iskiasnerven under gang. Det var dog ikke muligt at opnå M-max for alle forsøgspersoner ved stimulering mellem 0-100 mA. Resultater for undersøgelse tvær-spinalereflekser var kun positive for 1 ud af 7 forsøgspersoner. Da undersøgelse heraf var inkluderet fordi der var en teoretisk mulighed for optagelse af tvær-spinalereflekser eksisterede ved aktivering af disse H-reflekser ses dette som et positivt resultat der åbner muligheden for udvikling af en lignende protokol målrettet netop tvær-spinalereflekser.

Konklusion: En protokol baseret på H-refleks teknikker målrettet iskiasnerven bruges til at undersøge fasemodulering af reflekser i baglårsmusklerne under gang er blevet udviklet og verificeret via fasemodulering og input-output kurver for H-reflekser i både biceps femoris og soleus. Resultaterne viste også at det er plausibelt at videreudvikle teknikken til undersøgelse af hurtige tvær-spinalereflekser men at flere områder af protokollen stadig kan forbedres.

Preface

The model used for learning here at Aalborg University (AAU), The Aalborg Model for Problem Based learning, is based around problem oriented projects. The structure of this report reflects this problem orientation. It has been divided into 5 parts and Appendix.

Part I: Problem Analysis. This part is an analysis of the larger problem area. It is based on a search of relevant literature both by key words and by sources used by other researchers we collaborate with.

Part II: Problem Formulation. This part is a formulation of a more specific problem within the larger area which we would like to find a solution or answer to.

Part III: Problem Solution. This part deals with the methods we used to solve the problem.

Part IV: Solution Evaluation. This part contains the results of the solution we came up with and a discussion of theses and their connection to the larger problem and specific problem and our solution.

Part V: Paper. This is a mock up of a paper with the results of the project which is a more widely used way of communication scientific results.

Citations in the report will be given with numbers in the form [1], and bibliography can be found in the back of the report. The paper in part V has its own bibliography and reference numbering.

Acknowledgements

The authors would like to acknowledge the technical assistance and guidance provided by Andrew J. T. Stevenson, Knud Larsen and Jan Stavnshøj.

Aalborg University, June 3, 2015

Rasmus Elbæk Andersen
<rean08@student.aau.dk>

Alessandro Ranieri
<aranie13@student.aau.dk>

Part I

Problem Analysis

Chapter 1

Introduction

A number of different diseases and injuries which affect the nerve system can cause either impairment or loss of the ability to walk [1], [2]. Impairments to walking and standing can severely hamper independence in a world full of stairs and tall cupboards. This necessitates means to restore gait either through assistive devices or rehabilitation is highly valued by those affected [3, 4].

For some people affected by nerve damage from stroke and spinal cord injuries (SCI), two of the frequent injury types that affect gait, there is potential for improvement in independence of gait through the use of different rehabilitative and assistive strategies [3], [4]. Stroke and SCI affect a large number of people worldwide and without rehabilitation they may become dependent on others for simple day to day tasks or severely hampered when performing those tasks themselves [5], [6].

Currently a number of strategies are being used or developed, aimed at alleviating the severity of gait deficits caused by nerve damage. Assistive devices to compensate for or overcome the impairment such as simple canes, leg braces and even robotic assistive devices such as exoskeletons are all being used, [7], [8]. Different approaches to rehabilitation such as traditional physiotherapy and newer physiotherapy approaches based on neuroplasticity theory and employing new tools such as robotics and electrophysical stimulation are in use or being developed, [4], [7]. The area of restorative medicine is also making attempts at promoting nerve regrowth through pharmacological intervention which may one day help repair nerve damage and restore gait, [9].

With all these old and new possibilities for helping those with gait deficiencies from nerve damage there is a need for tools for assessing the need of individual patients so that the assistive, rehabilitative and restorative strategies used can target the specific patient needs [10]

One such assessment tool could be a test of the circuitry of the stretch reflexes. From experimentation it is known that stretch reflexes play a role in gait of not

just humans but for the different animals used as experimental models during neurological and gait research [11]. The exact role of the stretch reflexes during human gait is still not fully understood although it is of importance to a type of gait training [12] which is of importance to both people with Stroke or SCI [3, 4].

Initially it is therefore interesting to understand what characterises the these two patient groups and which techniques are available to test stretch reflexes.

Chapter 2

Nerve Damage and Gait Deficit

The section below contains commonly accepted anatomy and physiology and as such two sources [13, 14] which are both textbooks on the subject will be cited often.

The nervous system can be divided into two parts. The central and the peripheral nervous system. The central nervous system contains most of the neural circuitry and is responsible for interpreting and responding to inputs from the environment. The peripheral nervous system contains sensory organs and carries signals from these to the central system and from the central system to muscles and other organs which it either entirely or partially regulate [13, 14].

The nervous system is vital for life, through its role in maintaining homeostasis, and virtually all functions of the body. Certain functions can to some extent continue independently for a time and have a degree of autonomy [13, 14].

For animals, such as cats, it has been shown that the lower limbs do have some measure of autonomy and can perform stepping and even some degree of gait in response to external input. Whether this is the case for humans is still a matter of debate and much time and resources have been devoted proving the existence of and describe the a central pattern generator in humans [12, 15, 11].

The brain is situated in the cranium, meninges and suspended in cerebrospinal fluid. The spinal cord is encased in the vertebrae of the spinal column, its own manning and cerebrospinal fluid [13, 14].

The central nervous system can despite its considerable protection be damaged. The protections may not be enough in the case of large external trauma or when blood vessels gets obstructed, rupture or when a tumour form [16].

In these cases, depending on the location and cause, a person can suffer either a stroke [17] or SCI [18]. Strokes can be divided into to two types: ischemic stroke when there is damage due to obstruction of blood vessels, causing a lack of oxygen and hemorrhagic stroke when bleeding occurs due to blood vessel rupture [16]. If the stroke occurs in or affects parts of the brain involved in coordination of gait,

such as the sensorimotor system, gait impairment can occur [1]. Such impairments are often asymmetric, based on the site that the stroke occurred in [19].

The brain is connected to the rest of the body and the peripheral nervous system via twelve cranial nerves and the spinal cord. Apart from muscles and sensory organs in the head, all muscles and sensory organs are connected to the body via the spinal cord [13, 14].

The spinal cord can be damaged due to disease but most often due to trauma beyond the protection of the aforementioned systems. This is termed spinal cord injury [18]. SCI can either temporarily or permanently interrupt the connections of nerves between the brain and parts of the body. This can result in somatosensory loss or alteration as well as paralysis or alterations of motor control. Apart from complete or partial loss, sensation can also become abnormal; pain is especially of great concern [20]. Loss of cerebral control over muscles can result in spasticity when i.e. inhibition of reflexes is absent [21]. Apart from interrupting the connection between the brain and the periphery, damage to the part of the spinal cord where integration occurs can be a problem all in its own [18]. Reflexes needed to maintain posture and respond quickly to external input occur in the gray matter of the spinal cord and damage here can impair these functions [12].

2.0.1 Spinal Cord Organization

The spinal cord is organized into several different regions or columns. Looking at a transversal cut of the spinal cord, a central channel filled cerebrospinal fluid can be seen in the center. Surrounding the central channel is an approximately H-shape region of gray matter. Filling out the regions ventrally, dorsally and on each side of the H are regions of white matter. The white matter is mainly axons sending signals either upwards or downwards [13, 14].

The gray matter regions have cell nuclei and synaptic connections. Here may be more complex function such as reflexes and integration of signals from peripheral sensors and descending from the brain. The gray matter is organized into ventral and dorsal horns separated by intermediate columns. The white matter has a right and left dorsal column separated by a central channel on each side of the H-shaped area, a region of white matter called lateral column. Between and ventrally (towards the stomach) of the ventral horns are two regions called the ventral columns separated by the anterior median fissure [13, 14].

From the body and the peripheral nervous system signals enter the spinal cord via the dorsal root connected to the dorsal horn. From the spinal cord nerves leave from the ventral root to the peripheral nervous system. These nerves innervate skeletal and visceral muscles. Signals between the brain and the periphery of the body travel via different descending and ascending pathways in the spinal column. These are organized with regard to function / which system they belong to and with regard to their destination [13, 14].

2.1 Epidemiology - Incidence

The incidence of both stroke and SCI varies with several factors such as age, gender and geography [22, 23, 17, 24, 25]. Major differences exist between high income countries and middle to low income countries, mostly with regard to outcome and in the reporting. For both stroke and SCI much less is known in terms of number and size of studies for the lower to middle income countries. For stroke, the the number of studies have however been increasing since 2000 [26].

Of the two diseases, stroke is by far the most common. Stroke covers a large number of diseases. In the World Health Organizations (WHO) International Statistical Classification of Diseases and Related Health Problems (ICD10) it covers a large number of diseases in the the Cerebrovascular diseases category; I60-I69 [23]. SCI are classified in a number of different categories such as S14 and S24, which cover injuries to the spinal cord at the neck and thorax from external causes respectively [27]. SCI is special in that it covers all people who have an injury to the spinal cord irrespective of cause, with the special condition that you are only a person with SCI if you live long enough to be classified as such.

For SCI rather than the ICD10 system, The American Spinal Injury Associations (ASIA) International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) is used. This system gives classifications, not based on location, but on the specific neurological impairments. In this system, major divisions are between complete and incomplete as well as between quadriplegia and paraplegia. It also distinguishes between injuries affecting the sensory pathways and the motor pathways [28].

2.1.1 Stroke

WHO has estimated that in 2002 approximately 15.3 million strokes occurred globally, which accounted for ca. 10% of all deaths. [26]. In 2005, this number increased to 16 million people with regard to first-ever stroke. In 2002, 5.5 million strokes resulted in death [26] which, in 2005 had increased to 5.7 million [5]. The rising trend of these two years are expected to continue as prevalence of risk factors such as body weight, age and hypertension continue to rise. If these risk factors are not decreased through clinical or public health interventions, first ever stroke could reach 23 million in 2030 and 7.8 million deaths [17].

There has over the last 40 years been an equalling out of the stroke cases between high and middle to low income countries. High income countries' rates have fallen to 94 stroke cases per 100000 person-years in 2000–2008. This is a fall of 42% over the past 4 decades. Middle to low income countries' rates have increased ca. 100% over the same period to 117 per 100000 person-years [26].

2.1.2 Spinal Cord Injuries

Due to lack of data from many nations or differences in reporting, getting exact and comparable numbers is a difficult task [29]. A paper from 2011 by Cripps et al. found that only data from North America, Western Europe and Australia were sufficient for direct comparison. The incidences of these three regions have been reported with some difference in various studies [24].

In North America, incidence is generally reported as being 40 cases per million. In Western Europe and Australia, the incidence numbers have been reported as being 16 and 15 per million, respectively. The higher incidence rate in North America has been speculated as being due to the higher number of violence related SCI and possibly higher number of traffic related injuries [24].

The incidence rate seems to have increased from the post-World War 2 period but remained stable over the last couple of years with a shift in the age and etiology for some countries. Reports have been made of two spikes in incidence, one among young men in their late teens to early twenties attributed to motor-vehicular accidents and one late in life attributed to fall injuries. There has been a trend observed in some studies of an increasing average age among SCI which might be related to aging population. Changes in incidences may also be attributed to greater survival chance of accidents leading more people to live to be diagnosed [29].

2.2 Epidemiology - Prevalence

For both stroke and SCI, several factors complicate the process of finding the prevalence of these diseases. For both diseases, the variation in incidence and survival rate from country to country and the lack of data from some populations, makes it difficult to get exact numbers. For stroke, the prevalence is tied to the prevalence of other risk factors, such as smoking, hypertension and obesity as well as the age composition of the population, which can be used to estimate numbers [5].

For SCI, getting accurate numbers for prevalence as with incidence is problematic as they are most often based on either surveys of a very rare condition or based on calculations of incidence and life expectancy. The last method is often problematic as neither the incidence nor the life expectancy are well known and so for estimating i.e. old prevalence, new numbers for life expectancy is used with older incidence numbers although the life expectancy has gone up [29].

2.2.1 Stroke

Stroke survivors have globally been estimated to be 62 million in 2005 and been projected to rise to 77 million by 2030 [5]. For stroke survivors, the best measure of its impact is DALYs lost. Stroke globally is associated with 43.7 million lost DALYs, which is the seventh largest at 3.2% of all global lost DALYs. Among some

populations, stroke is a much larger cause of lost DALYs as many of the largest causes of DALYs lost are diseases predominantly affecting children or are highly regional such as HIV/AIDS [26].

2.2.2 Spinal Cord Injuries

The numbers that do exist suggest that 236 persons per million live with SCI in the Kashmir region of India (1986). For Australia, numbers of two studies from 1998 and 1997 suggest 540 and 681 per million, respectively. For Europe, Finland reported 280 per million in 1999, Iceland report 316 per million (1973-1989) and Norway reported 365 per million (2002). In North America, a number of surveys from the US have reported a median value of 853 per million which fit with a 2006 estimate of ca. quarter of a million people living with SCI. In Canada, a 2001–2002 study estimates approximately 1173 per million using numbers of survival rates from the US [24].

2.3 Outcomes after Injury with regard to Gait

For both stroke and SCI, gait is often affected due to the many systems of the brain and spine all needing to interact for gait to function normally [1].

Since the nerves to the legs branch from the lower end of the spine, almost all SCI will affect gait to some degree [24].

2.3.1 Stroke

In the first week after stroke, only 23-37% of people can walk independently. This number rises to somewhere between 50 and 80% of people being able to walk unaided, after 3 weeks or when people are discharged. After rehabilitation 50-60% of patients will still have some degree of motor impairment [19].

By six months, after stroke recovery which is the plateau at which point ca. 85% may be able to walk unaided. Even after the 6 month point those that can walk unaided or independently may still exhibit some deficit that makes them less capable walkers than before stroke, such as hemiparetic gait [19].

2.3.2 Spinal Cord Injuries

For people that have suffered SCI, life will most likely change. A small number can expect to make large recoveries or only suffer small disability. For most etiologies, tetraplegia and paraplegia are very equally likely outcomes. Over all, Tetraplegia makes up 54.1% of cases and Paraplegia 45.2%. On the ASIA scale, 55% of people with SCI score an A, indicating a complete SCI. The ASIA score D accounts for up 24.8% of cases.

In a combination of level of injury and ASIA score into neurological classes, paraplegia incomplete accounts for 19.2%, paraplegia complete 26.1%, tetraplegia incomplete 30.6% and tetraplegia complete 23.4% [24].

For people with SCI, some disabilities are a large focus of rehabilitations. Recovery of gait and other mobility is of large importance for subjects as it is very important in being independent. Control of bowel and bladder functions are also in focus as this is both important to function without the discomfort that inappropriate or untimely discharge can lead to. Lastly, sexual functions both for pleasure and for procreation have been increasingly important and feasible [18]. For non-traumatic injuries, a study of 62 patients found that after initial rehabilitation, 14.5% were capable of Walking unaided, 29.0% of gait using aid to walk > 100 m, 14.5% of gait using aid to walk > 10 m and 41.9 were wheelchair dependent [30].

For people with SCI, mortality is higher than for the general population. Due to the multivariate nature of the people living SCI, getting a clear view of the life expectancy is difficult. Genitourinary disorders, cardiovascular diseases, neoplasms and respiratory system disease make up the largest number, of causes of death for persons with SCI. For all these causes, people with SCI are at greater risk of death in almost all ages and overall the mortality rate is more than doubled for all age groups between 15 and 84 except 65-74 [20].

Chapter 3

Reflex Studies

3.1 Approaches to reflex studies

Two main approaches to induction of reflexes in skeletal muscles are generally employed. The first relies on mechanical stimulation of either a joint, to stretch the muscle, or the tendon directly by percussion, activate the spindles. The second relies on electrical stimulation of the sensory afferents originating from individual muscles or muscle groups. In general, mechanical stimulation is regarded to provide more natural stimulation, although the involvement of the muscle spindles and Golgi organs complicate the comparison of different condition, tasks and subjects as the response depends from joint angle, background activity and other factors. Electrical stimulation, on the other hand, bypasses the proprioceptive organs mentioned above, thus providing more consistent responses. However, stimulation is often applied to mixed nerves, in which different kinds of fibers are bundled together that cannot be selectively activated, except by carefully modulating the stimulation intensity in such a way that only larger fibers (belonging to the Ia group) are activated. [31]

3.2 Previous work

In his Ph.D. thesis, Floy [31] provides a comprehensive overview of reflex studies involving both mechanical and electrical stimulation.

Since then, however, an increasing amount of studies has been published, addressing not only reflexive responses in individual muscles or in agonist-antagonist pairs of certain joints, but also interlimb communication and reflexes. In particular, recent work conducted by the Neural Engineering and Neurophysiology of Movement group showed how mechanical perturbations and electrical stimulation can elicit relatively short latency cross-spinal reflexes [32, 33]

There is a lack of studies dealing with electrical stimulation for the investigation

of reflex modulation in the Hamstrings group, despite all the evidence demonstrating its importance in human gait. Floy proposed a technique for the stimulation of the sacral roots with stimulation intensities as high as two amperes during gait [31]. The next year, a student group developed and performed initial validation of a protocol to investigate the H-reflex in the hamstrings by stimulating the sciatic nerve in the upper thigh between 0 and 100 mA in static conditions (prone position), showing that it was possible to elicit consistent H-reflexes in the hamstrings.

In addition, pilot experiments were conducted with a prototype flexible electrode matrix, produced by Tecnalia (Spain). The matrix electrodes were developed in different form factors and dimensions, and were shown to improve on the results obtained with self-adhesive electrodes [34].

This work is aimed at expanding on the technique to allow its application to dynamic studies, as will be formulated in more detail in the following chapter. The rest of this chapter includes background information relevant to this aim.

3.3 Classification of nerve fibers

The term Nerve fiber refers to the protrusions of neurons that carry signals through the nervous system, and in the context of peripheral nerves, comprises the axon, eventual myelin sheet (and the relative Schwann cells), as well as the endoneurium. Nerve fibers are classified through two nomenclatures: the Erlanger-Gasser classification and the numerical classification, see fig 3.1. Generally, they are ordered by diameter, and consequently, by conduction speed and level of myelination. Motor nerve fibers are named corresponding to the respective motorneurons (or motoneurons), and follow a similar ordering.

E-G	Origin	Numerical
Aα	Motor (somatic), proprioceptive (Golgi, muscle spindles)	Ia/Ib
Aβ	Mechanoceptive	II
Aγ	Motor (spindles)	
Aδ	Nociceptive, thermoceptive	III
B	Visceral sensory, preganglionic fibers	
C	Sympathetic, sensory, slow pain	IV

Table 3.1: Classification of nerve fibers

3.4 The H-reflex

The H-reflex involves Ia afferent fibers and α-motorneurons. When electrical stimulation is applied to a mixed nerve, the larger Ia afferents are activated first; this re-

sults in an afferent volley reaching the spinal cord, where it causes excitatory post-synaptic potentials in the corresponding α-motorneurons' soma. The motorneuron fires in turn, sending a highly synchronous efferent volley to the corresponding muscle, which contracts with intensity proportional to the fraction of motorneurons that were successfully activated by the afferent signal. This contraction can be measured in the EMG signal recorded from the muscle, and is refereed to as the H-wave. As stimulation intensity increases, the slightly smaller axons of the motorneurons are activated at the stimulation site; this results in two action potentials propagating in the prodromic (towards the muscle) and antidromic (towards the spinal cord) directions. The prodromic action potential reaches the muscle in a shorter time than the afferent-mediated H-wave, and can be measured by the same principle. This is referred to as the M-wave. If stimulation intensity is increased further, the antidromic impulse progressively cancels out the afferent-mediated activation, resulting in the progressive reduction of the amplitude of the H-wave and an increase in the amplitude of the M-wave. In some cases, the antidromic impulse can cause the soma of the motorneuron to fire, sending another impulse through the axon and to the muscle: this is referred to as the F-wave, but is not as consistently elicited and is generally of much lower intensity with respect to the M and H waves.

3.5 The Sciatic nerve

The sciatic nerve is the largest and longest nerve in the human body, innervates most of the lower limb and originates from the sacral plexus, a complex of fibers emanating from L4 to S4. The sciatic nerve contains both sensory and motor fibers to and from most of the muscles in the back of the leg and large surfaces of the skin through many smaller branches that arise at various points down the limb. Between the sacral plexus and approximately two thirds of the thigh, the sciatic nerve branches in two larger decurrent nerves, the Tibial nerve and the Common Peroneal nerve. [35]

It is well documented that the branching pattern and the decurrence path of the sciatic nerve itself can vary between individuals, and a few "typical" patterns have been identified. In one study of 420 limbs, the sciatic nerve passed beneath the piriformis in 87.5%, through the piriformis in 12% (peroneal division), and above piriformis in 0.5%.

Although most frequently the sciatic nerve splits into its two main branches about two thirds down the upper leg, it has been observed to branch as high as the sacral plexus itself and as low as below the popliteal fossa; this implicates that depending on the subject, stimulation delivered by positioning the electrodes with the same procedure and landmarks can give rise to radically different results, before even considering other geometrical or anatomical factors [36].

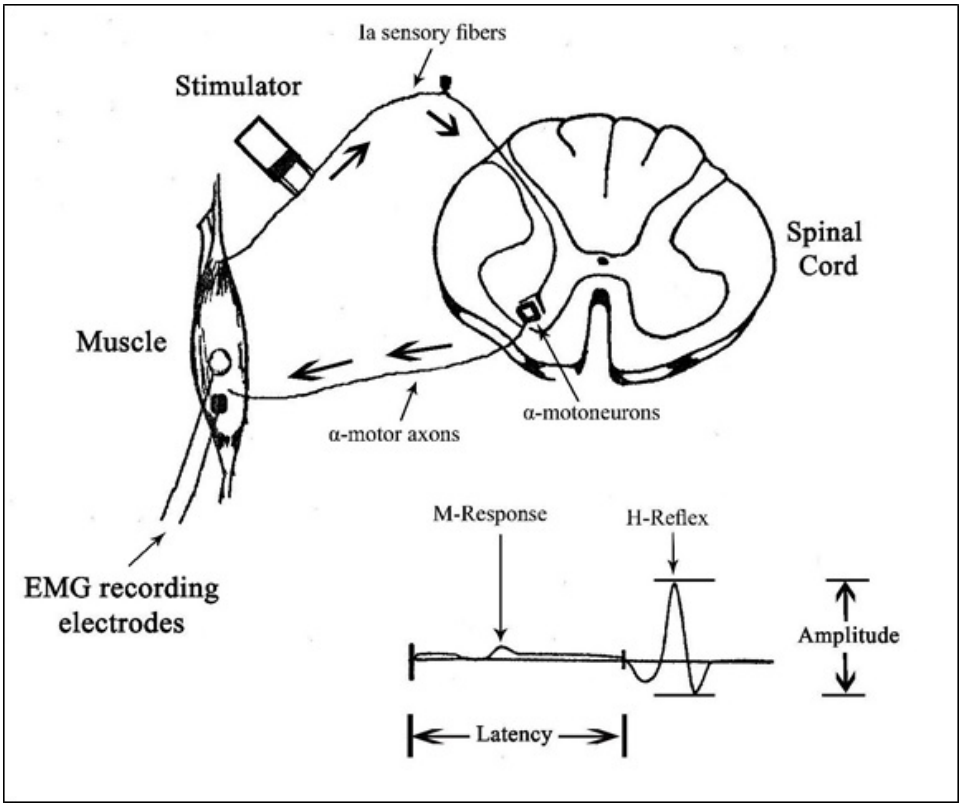


Figure 3.1: Schematic representation of the H-reflex circuit.

The fiber composition of the sciatic nerve is not well documented; in rats the proportion of the various fiber types has been observed to be as follows: 6% myelinated motor axons, 23% myelinated sensory axons, 48% are unmyelinated sensory axons, and 23% unmyelinated sympathetic axons [37].

Despite the relatively high variability in the morphology of the sciatic nerve, a few well established procedures for its location are in place, depending on the particular application based on external landmarks. For instance, the New York school of regional anesthesia provides guidelines for anterior and a posterior approaches for sciatic nerve block, both of which are based on bony landmarks and fixed measurements from them to locate the insertion point for the stimulation/injection probe. The nerve is then located depth-wise by low intensity electrical stimulation, observing twitch patterns in the muscles innervated by the sciatic nerve.

3.5.1 Hamstring muscles

The hamstrings consists of 3 muscles; the long head of biceps femoris, semitendinosus and semimembranosus. These muscles are all connected both below the knee and above the hip. This allows them to both flex the knee and extend the hip.

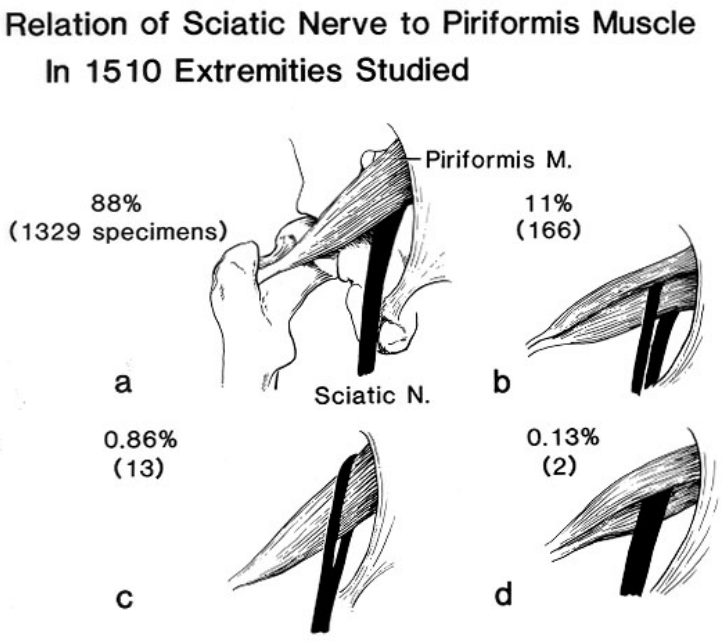
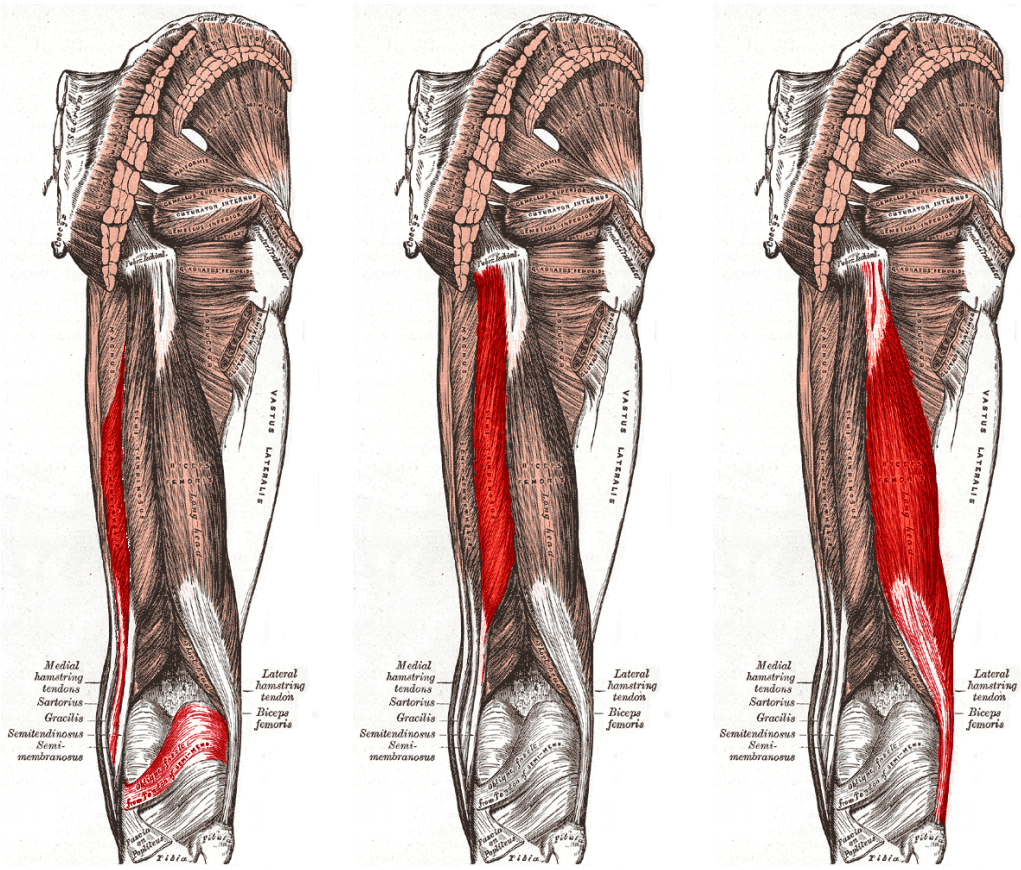


Figure 3.2: Anatomical variations of Sciatic nerve decurrence.

All three hamstring nerves are innervated by the tibial part of the sciatic nerve. When the knee is bend semitendinosus and semimembranosus can rotate the knee medially. Biceps femoris long and short head can together rotate the knee laterally when bend. The hamstrings are antagonists to the quadriceps and together with these play an important role in walking.



(a) Semimembranosus **(b)** Semitendinosus **(c)** Biceps femoris - long head

Figure 3.3: All figures are from public domain version of Gray's anatomy from 1918 uploaded to Wikiversity Journal of Medicine, [38] , by MD Mikael Häggström.

Part II

Problem Formulation

Chapter 4

Problem Formulation

In this chapter, first a summary of the part I will be given and a problem will be formulated and presented along two specific project goals for its solution. Lastly, a series of constraints will be given, that help direct the solution towards a practical application by institutes that could benefit by implementing this technique in their line of research.

4.1 Summary

The assessment the specific nerve damage of individuals suffering from stroke of SCI could be an important part in assessing the specific needs of their rehabilitation. Stretch reflexes and especially their modulation throughout the gait are an important although not yet fully understood part of human gait. Despite this the hamstrings have seen little attention in research of reflex modulation during gait. The ability to assess reflexes in the hamstrings during gait, both to understand their role in human locomotion and to better treat neurological damage affecting these reflexes, is therefore of scientific interest.

Studies have applied mechanical perturbations to the knee to successfully assess reflexive responses in both the ipsilateral and the contralateral leg. These methods provide very specific and natural stimuli to the proprioceptive organs of the hamstrings. The same techniques however present significant technical challenges when transferred to dynamic studies, as the equipment to provide precise and fast dynamics perturbations is often cumbersome and expensive, to the point of altering the features of gait in the subjects, and it is not always consistent between stimulations.

H-reflex based techniques, which are widely used for the lower leg, have been almost entirely neglected in this application. Furthermore, they could be less complicated and costly in their implementation in dynamic studies, especially when considering SCI subjects that often need orthoses or complete robotic devices to

walk in a way that allows for studying reflex modulation.

A technique based on electrical stimulation of the sciatic nerve for the study of H-reflexes in the hamstrings has been introduced in 2013 by a group of Master student at [34]. A flexible electrode array and custom made acquisition hardware and software were developed based on their recommendations. The scope of the project did not include dynamic testing or automation of the optimal site selection procedure.

4.2 Problem formulation:

Can a protocol based on H-reflex techniques targeting the sciatic nerve be used to assess reflex phase modulation in the hamstrings during gait?

4.3 Goals:

1. To reconstruct the matrix electrode based system and test whether the setup and a protocol are able to successfully activate the sciatic nerve. Then begin to modify the protocol and setup to be usable during dynamic movement.

2. To validate the protocol, performing experiments aimed at confirming the purpose of the technique, that is investigating phase modulation of H-reflexes in the Soleus and Biceps Femoris muscles of the ipsilateral leg and to elicit cross-spinal short latency responses in the contralateral leg.

4.4 Constraints:

A technique for studying reflex modulation in the hamstrings during gait is of special interest to the understanding and assessment of people with stroke or spinal cord injury, therefore this project will work towards a solution which can be readily applied to these subjects. The protocol must therefore take into account the potential needs and limitations of this class of subjects, such as fatigue (limiting the number of repetitions or the length of the experiments) and the presence of assistive devices or harnessing (for instance when the subject can only produce natural gait with the aid of a robotic device).

Part III

Problem Solution

Chapter 5

Problem Solution Overview

In order to solve the problem formulated in chapter 4, a number of different tasks have been carried out and documented. The first of these was planning an overall strategy for arriving at a solution or answer within the time frame of the project. This chapter documents this plan as well as the content and structure of the Problem Solution part of the report.

The tasks of the problem solution have been divided into four chapters based on their their purpose and how they relate to other parts. These four chapters are:

- Chapter 5: Problem Solution Overview
- Chapter 6: Pilot Experimentation
- Chapter 7: Experimentation
- Chapter 8: Dataprocessing

Below are descriptions of these chapters and an overall work flow between chapters is shown in figure 5.1. In general, these tasks were conducted in parallel whenever possible. For instance, initial steps in data processing were conducted as soon as any data from a pilot experiment was recorded, so that the gathered information could be used to influence subsequent pilot experiments.

Problem Solution Overview

Purpose of this chapter: Describe the overall plan of the problem solution and how it is structured. This is done to make sure the work the project group carries out is structured, goal oriented, and can be understood by others.

Relationship to the other chapters: Ideally, the work of the other chapters is founded in the overall plan for the Problem Solution and this plan changes as

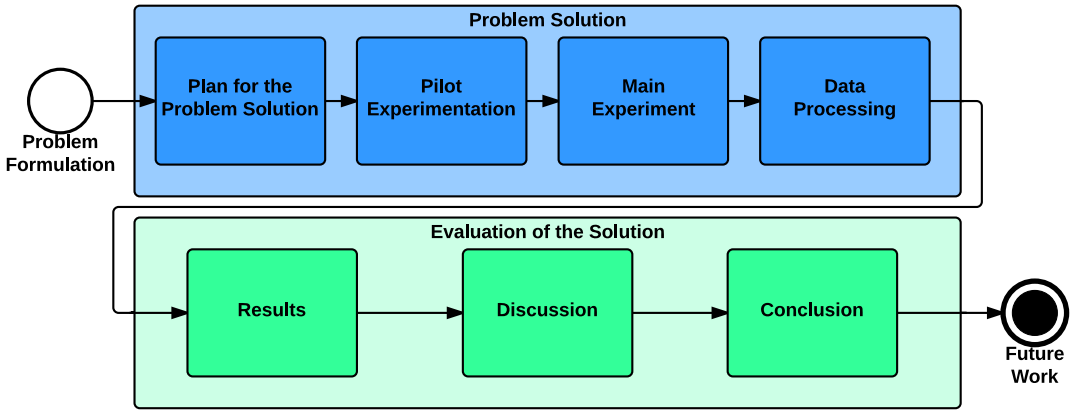


Figure 5.1: General work flow of chapters in the Problem Solution and Solution Evaluation parts. The arrows indicate the overall work flow and how following tasks depend on the information and work in previous tasks.

progress in the other chapters identify the need for unanticipated but necessary work.

Pilot Experimentation

Purpose of this chapter: In the initial phases of the project the objective was to test and understand the protocol carried out by [34], to explore the technical challenges to developing H-reflex techniques of the hamstring during dynamic movement. When challenges were identified, pilot experiments testing possible solutions or improvements were designed and carried out, as an dynamic component of the development process leading to the protocol and equipment for the main experiments. This is a large part of fulfilling the first goal from section 4.3.

Relationship to the other chapters: This section draws on knowledge form the Problem Analysis section. The main experiment is based on the pilot experiments. The pilots also provide important data needed to begin the data processing work and planning.

Experimentation

Purpose of this chapter: Once the setup was finalized in the Pilot Experimentation, an experimental protocol was developed for use with this setup. The experiment was then run on a first group of subjects for assessment of the static and dynamic phase. The protocol was then reviewed and finalized, with the addition of a stimulation session to assess the ability of the technique to elicit cross-spinal responses.

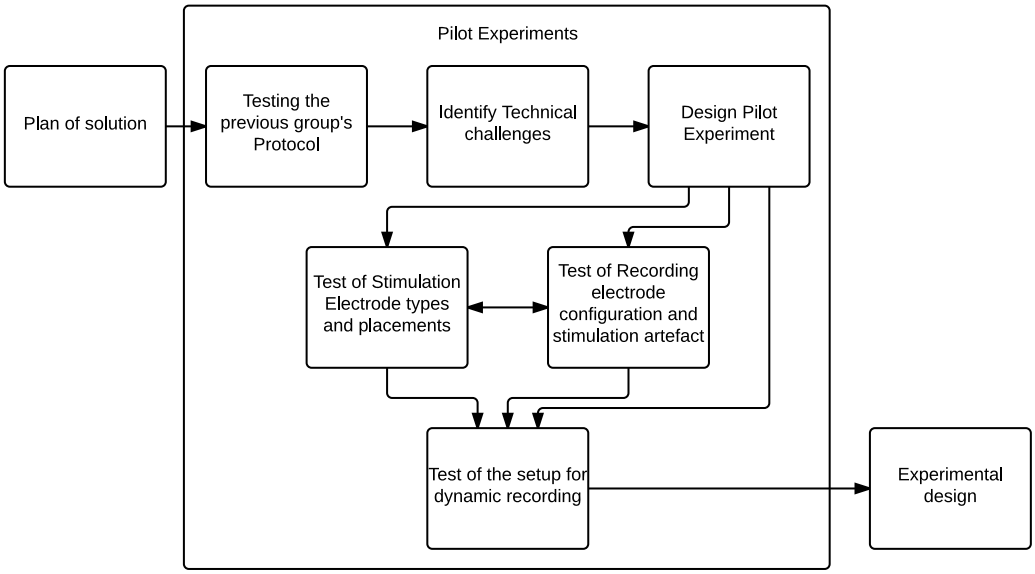


Figure 5.2: Work flow of the Pilot Experimentation chapter. The first step of the pilot experimentation chapter is testing out the protocol of [34] based on which a number of challenges are identified and pilot experiments are designed and carried out to produce a setup capable of hamstring H-reflex recording during dynamic movement.

Relationship to the other chapters: This section is based on the the knowledge from Pilot Experimentation chapter and provides data which is treated in the Work flow of Dataprocessing chapter to arrive at final results whose interpretation is an important part of fulfilling the second goal from section 4.3.

Data Processing

Purpose of this chapter: Describes the processing work carried out to produce results from the data recorded in the experiment. The end products concern the assessment of the successful activation of the sciatic nerve in the right leg, as well as the construction of modulation curves of the H-reflex and the ability of the technique to elicit cross-spinal responses as measured in the controlateral Biceps Femoris.

Relationship to the other chapters: As soon as results from the pilot experiment have been recorded these are used to develop a plan for early prototypes of the data processing work flow. This is also an important part of feedback which allows us to identify technical challenges and design the pilot experiments. Some problems that could be dealt with during data processing is better dealt with by

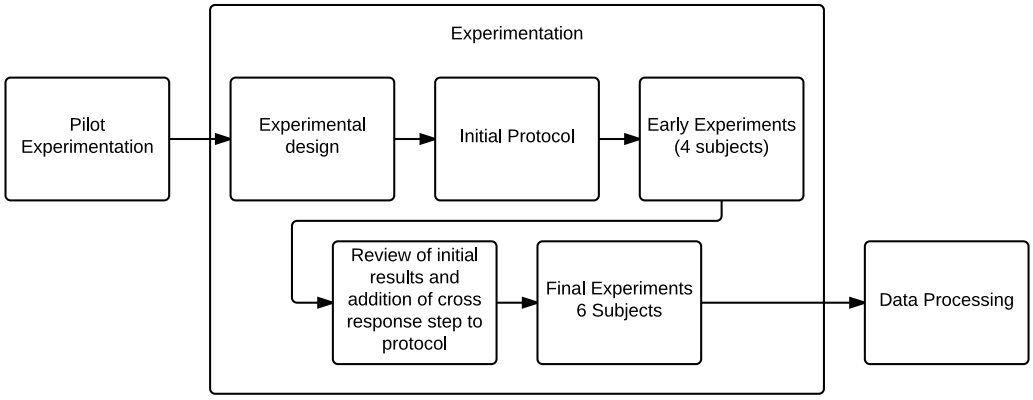


Figure 5.3: Work flow of the Experimentation chapter. The first step is to design an experiment using the setup resulting from the Pilot Experimentation chapter, aimed at investigating H-reflex modulation in different parts of the gait cycle as well as investigating whether cross responses can be elicited. The second step consists of describing the protocol for the designed experiment. Then follows the conduction of the designed experiments. This is divided into two parts by a review of initial results needed to find parameters for the cross response part of the experiment.

changing the setup to arrive at cleaner data before processing. As data is produced in the main experiment it is used to iterate over and refine the data processing work flow 4.3.

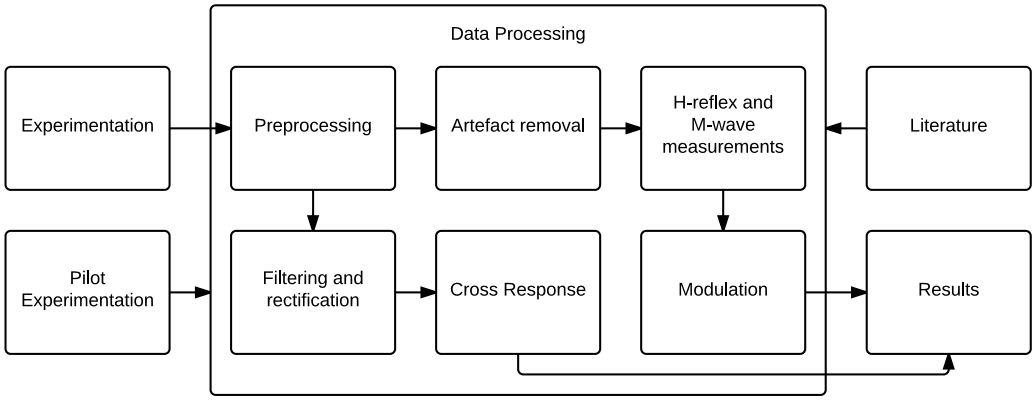


Figure 5.4: Workflow of the Dataprocessing chapter. There are two main paths of processing; the first one begins with artifact removal, followed by extraction of the H- and M-waves from both dynamic and static phases, which are then used to produce modulation results. The second line begins with EMG rectification and is followed up by cross response detection.

Chapter 6

Pilot Experimentation

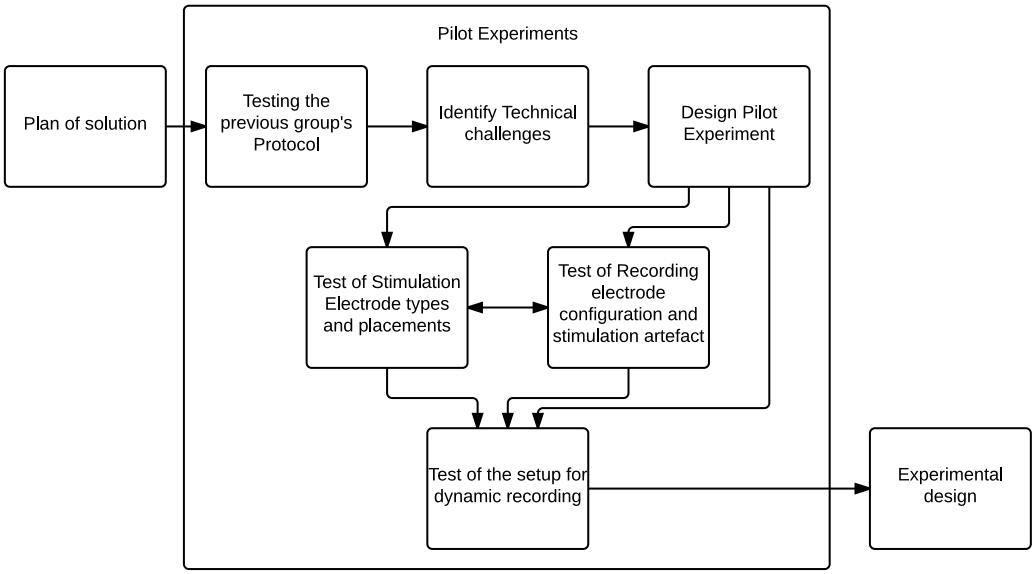


Figure 6.1: Overview of the Pilot experimentation phase

Here at Aalborg University two lines of research for investigating reflexes of the hamstrings are being developed and used. One is a mechanical stretch reflex based protocol using electrical engines and pulley system to mechanically stretch the knee using different setups for static and dynamic studies. The second consists of an H-reflex based approach for which one previous project was carried out in 2013. This work builds on it, and as presented earlier, aims at developing it further to enable its application to dynamic studies in both healthy and SCI subjects. Other H-reflex based investigations have been carried out targeting other more accessible nerves and muscle groups, for instance tibial nerve stimulation in the

popliteal fossa. This chapter reviews how the technique was implemented, tested and refined through pilot experiments conducted in the first half of the semester.

6.1 Testing the static H-reflex protocol

In order to test the protocol of [34] we first put together a setup resembling the one they used. Secondly we used their protocol with the ourselves as subjects. Finally, we evaluated the results and our experience with their protocol.

6.1.1 Setup

The setup we reproduced consisted of two pathways, one carrying stimulation to the hamstring and one recording from a number of muscles. The entire setup was controlled via a computer running a custom made program called Wirex based on Labview, using a DAQ Card (NIDAQ-6024E, National Instruments, USA) both for controlling the stimulation pathway and for sampling the EMG signal.

In the stimulation pathway the NIDAQ was connected to an isolated constant current electrical stimulator (NoxiTest IES 230, Aalborg, Denmark). The stimulator was connected to a custom-built relay capable of selecting between up to 16 output channels pass the stimulate on to. Of the 16 channels 8 were connected to a stimulation electrodes placed on the subject.

In the recording pathway 4 muscles were connected EMG electrodes to using a common reference differential configuration. The EMG electrodes were connected using cables with 1/10 gain custom made preamplifiers to custom-built EMG amplifier. The amplifier were connected to the NIDAQ.

Stimulation electrode placement

Stimulation cathodes described by the previous group is a 4*2 array of 3.2 cm round stimulation electrodes (PALS Platinum, Axelgaard Manufacturing, USA), cut down to square 1.8 by 1.6 cm giving a grid of ca. 8 by 3.5 cm when allowing for gaps in-between electrodes. The anode is a 5x9 cm rectangle platinum weave stimulation electrode (PALS Platinum, Axelgaard Manufacturing, USA) placed on the great trochanter of femur.

The description by the previous group for placing their electrode array is as following:

"The centre of the cathode grid was placed along the line between the ischial tuberosity and the lateral condyle of femur, two centimetres below the buttocks as illustrated in figure 2.5."

The relevant part of "figure 2.5" from the previous group have been reproduced in figure 6.2.

Rather than recreating the exact dimensions of the cut down electrode array we initially chose to use a single round pals electrode which were moved around a grid drawn onto the skin at the location described by the previous group. The grid used was increased to a ca 10x6 cm grid divided into ca 3x3 cm squares.

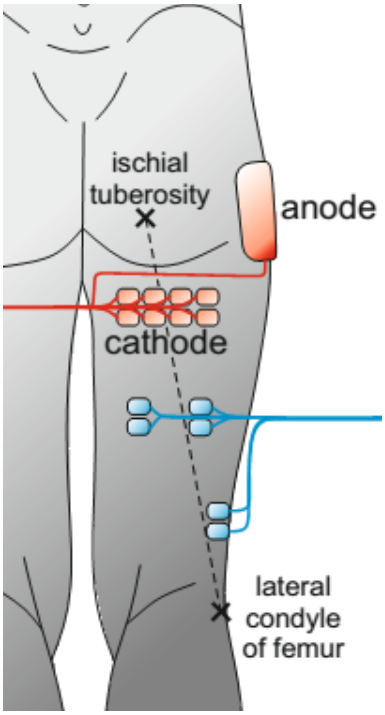


Figure 6.2: Cut down image of electrode placement from previous protocol taken from a larger setup image.

Recording electrodes

Of the five muscles recorded from by the previous group; Long and short head of biceps femoris (LBF and SBF), Semitendinosus (ST), Soleus (SOL) and Tibialis anterior (TA), we chose to record from all except TA. The reference for the a common reference differential configuration was the lateral ankle bone.

6.1.2 Protocol

Preparation

Electrode placement: The skin is wiped clean with an alcohol wipe. EMG electrodes were placed in accordance with the SENIAM project on the muscles listed above. The grid in which the single stimulation cathode were moved around were drawn

as described above. The stimulation anode were placed as described above.

Instrumental setting: The stimulation type used was monopolar square pulses of 1 ms duration. The relay box used to chose between different electrodes was omitted as it does did not serve a purpose when using only one electrode being moved around manually. For the upper leg the EMG amplifier highpass cutoffs were set to DC and lowpass cutoffs to 500 Hz. For the lower leg the the highpass cuttoff were increased to 5 Hz.

Finding optimal site

The subject lies down in a prone position on a medical examination bed. To find the optimal site each of the 6 squares in the drawn grid were stimulated between 0 and 100 mA in increasing steps of 5 mA. When a response were found decreasing steps were also used to find the earliest stimulation intensity at which the response disappeared.

Stimulation of optimal site

For the optimal site 3 recordings of each stimulation intensity were made and saved to be used in data processing development.

6.1.3 Results and experiences

Software: The Wirex custom software that the previous group had used had not been well maintained and suffered from stability issues. It also lacked several features important for dynamic studies. The programmer responsible for making Wirex had since the last project made an Wirex extension for the much more widely used program Mr Kick. We therefore almost immediately shifted to this program. which had all the features essential in Wirex (mainly the ability to control the custom relay box.)

Stimulation location: The description of the optimal placement for the stimulation electrodes were imprecise, prone to arbitrariness from the investigator and variability from inter-subject anatomical variation. The buttocks fold which we have interpreted to be the point that the 2 cm below the buttocks refers to varies from person. The stimulation location also did not yield results which looked like what we, our supervisors and other researchers we consulted expected. It was decided that a more careful investigation of stimulation location should be made covering a larger area.

Noise and Artifacts: Concern arose regarding several noise and artifact components in the recorded signals. The stimulation artifact had a long tailing element in the shape of characteristic of a discharging capacitor. Several normal EMG noise features were also large in the upper leg where the filters highpass cutoff were set to DC. Based on this it was clear that any alterations or additions to limit noise and artifact would be highly desirable especially when movement artifacts from dynamic movements were added to the static ones.

Monitored muscles: It was observed that recordings from Semitendinosus or the short head of Biceps Femoris either did not differ significantly from the recordings in the long head of BF or had a very low signal quality. To simplify the following pilots and to reduce the complexity of the main experiment to the minimum necessary to achieve the goals, it was decided to restrict recordings to the long head of biceps femoris and soleus.

6.2 Technical Challenges

In this section we summarise challenges from the test of the previous protocol and from literature in order to decide on areas we need to investigate improvements for through further pilot experiments. Various challenges are to some degree associated with the anatomy of sciatic nerve and the hamstring. These are general to any protocol wishing to stimulate at the hamstring. The anatomy of hamstring and the sciatic nerve is described in chapter 3. Based on this, and the initial pilot experiment carried out by us a number of challenges have been identified that all relate to the anatomy. The main point of this is that the deep location of the sciatic nerve which create a number of challenges when trying to adapt methods from other h-reflex protocol to this location:

1. Much higher stimulation intensities are required to elicit both h-reflex and M-wave in the hamstrings compared to stimulation at the tibial nerve or other more superficial nerves.

2. Stimulation at the hamstring will also generate M-wave and H-reflexes in the lower leg but not until at least 60 mA and for most not before 70 or 80 mA.

3. The higher stimulation intensity makes painful sensation or discomfort an point of concern. This is to some degree counteracted by the lesser sensitive of the hamstring area compared to the back of the knee.

4. The larger stimulation intensity produces much larger stimulation artefacts compared to other protocols.

5. Even using much higher stimulation intensities, up to 100 mA, some (maybe most) subjects will not produce M-max, this was reported by the previous group and confirmed by us in the pilot.

6. Most techniques for evaluating H-reflexes are based on normalisation based on M-max which necessitates alternative data treatment strategies.

7. The distance from the stimulation site to the spine is shorter than for stimulation at the back of the knee which might result in the m-wave and the h-reflex being poorly separated.

8. The hamstring is relatively large which makes the area where stimulation site could be found larger making the process of finding the optimal site longer.

9. The stimulation site is surrounded by large muscles which makes it more difficult to not stimulate a muscle directly as compared to tibial nerve stimulation the back of the knee.

6.3 Design Of Pilot Experiments

Three main areas of improvement and additions have been identified and will be described in the following sections:

1. Section 6.4: Stimulation Pilot Experiments.

2. Section 6.5: Recording Pilot Experiments.

3. Section 6.6: Dynamic Setup Pilot Experiment.

These will be tested in a number of small pilot experiments. The experiments will be described using the following structure:
Experimental goal: A formulation of the goal of the experiment.
Description: The procedure and setup changes used in the experiment.
Results: The results the experiment.

In the end of the three sections, an combined conclusion will be drawn for those of experiments.

6.4 Stimulation Pilot Experiments

Three points related to stimulation which needs to be tested in pilot experiments have been identified:

1: Improving on the placement of the electrodes. This means the upper hamstring area needs to be re-investigated to check whether a better general location for the optimal site can be found.

2: Deciding on which electrodes to use. The previous group used a set up with small cut-down pals electrodes in an array, based on this design custom made electrode matrices from Technalia were produced. These should all be tested along with the regular pals electrodes.

3: The stimulation site needs a description that is less ambiguous. That means re-evaluating the anatomical landmarks which can be used to describe it and test how these landmarks fit with the subjects through out the experiment.

All experiments use the basic setup described in 6.1 but with the changes mentioned in the end of that section i.e. switching to a new software Mr. Kick and the circumvention of the relay box unless other wise mentioned. From the results of the other series of pilot experiments, the following changes to the recording settings were made: the highpass cut-off had been increased to 0.1 Hz.

6.4.1 Stimulation pilot 1: Investigation of the optimal site location using an electrode probe

Experimental goal:

To investigate the H-reflex response of a larger using a felt tipped stimulation probe to search out two different 10x10cm grids.

Description:

First part: A 10x10 cm grid divided into 1 cm^2 beginning at the fold of the buttocks and centred on the line described in section 6.1.1, was drawn and searched with the felt probe which had been moistened with tap water. This was done for 3 subjects.

Second part: A 10x10 cm grid divided into 1 cm^2 centered on the back of the hamstring region, was drawn and searched with the felt probe which had been wetted. The EMG electrodes were moved down. This was done for 2 subjects.

The felt tipped probe was moved around the the grid and stimulation beginning at 30 mA were followed by increasing stimulation to 100 mA.

Results:

The results were mixed between the subjects in the first part with both the proximal and distal end of the grid giving equal response. In the second part the proximal end of the grid also yielded positive response. Although currents up to 100 mA were used this were only done for some sites to search for responses in soleus which needs higher current that the hamstring. It was also obvious that the probe is a lot more unpleasant to out right painful compared to the pals electrodes used before.

6.4.2 Stimulation pilot 2: Testing the Tecnalia Matrix Electrode

This electrode apart from having the shape and size described by the previous group were also suppose to use a gel interface which produce less painful sensations at higher currents.

Experimental goal:

This pilot were set up to test whether this cable would be good for dynamic movements.

Description:

First part: The custom matrix electrode consisting of 2 rows of 8 stimulation sits ca. 2x2 cm in size made by technalia were placed in accordance with the setup described in section 6.1.1. The cable connected to the matrix electrode fits in the relay box which was therefore reconnected. The 16 stimulation sites were stimulated one after the other between 0 and 100 mA. This was done for 3 subjects lying down in prone position.

Second part: Part one were repeated for 3 subjects walking on a thread mill.

Results:

The results were good for the 3 subjects lying down but the matrix electrode was clearly to wide and apart form the center 6 (2x3) or 8 (2x4) did not yield anything other than painful sensation. For 2 subjects the matrix electrode preformed comparable to the lying position but for one subject no response could be generated despite excellent results in the lying position. The matrix electrodes did have some problems related to movement. The electrodes were very stiff and needed to be taped down a lot.

6.4.3 Stimulation pilot 3: Finding and describing a less ambiguous stimulation site.

Experimental goal:

Choosing a stimulation site and finding a way to describe it and test it.

Description:

First part: Using 2 subjects and 2 different investigates a description of the second grid from Stimulation Pilot 1 were made. Measure the femur bone from the greater trochanter of the femur to the lateral protrusion at the knee and mark out the mid way point. Measure out the length in degrees to the center of the hamstring. The grid were drawn centred on this point (named point (D)).

Second part: 9 pals electrode were placed to cover the grid. Stimulation were applied between 0 and 100 mA to each of the 9 electrodes.

Results:

The method for finding the site worked well. The site were to low as evident by lack of any response M- and H-wave in the biceps but not in the soleus for the center and lower row of electrodes. The procedure were re-tested with the grids lower edge centred on (D). This higher grid performed better, with fewer useless rows. This final grid provides good responses not only for the biceps but also for the soleus.

6.4.4 Stimulation conclusions

A number of conclusions were drawn from our experience with these three pilot experiments:

1. The description of the optimal placement for the stimulation electrodes from the previous group were to imprecise, prone to arbitrariness from the investigator and variability from inter-subject anatomical variation. An investigation of a larger area led us to choose a lower larger grid to search for an optimal site.

2. The probes low surface area and low high resistance makes it incapable of reaching the current levels needed for this stimulation site with our coursing pain and discomfort. A better probe might be used to find the optimal site in the grid but this one is to painful to use.

3. The matrix electrodes although processing many traits which are desirable also had a number of draw backs. Their shape were not optimal for the

possible stimulation locations, too wide, too inflexible for dynamic movement and not sticky enough to stay connected on their own.

4. The new procedure for finding a grid to search worked well after being moved more proximally.

6.5 Recording Pilot Experiments

Two points related to recording which needs to be tested in pilot experiments have been identified:

1: The filter setting needs to be re-evaluated. The DC highpass cut-off used in the 6.1 the filter becomes a problem during dynamic movement. The high cut-off points needs to be tested to evaluate how high it can go before it can no longer be removed in the data processing step.

2: The electrode configuration and placement needs to be tested. Distance influences the size of the stimulation artefact as well as the use of reference electrodes.

3: Lower placement of EMG electrodes along biceps femoris needs to be tested to see how far down the electrodes can be placed.

All experiments use the basic setup described in 6.1 but with the changes mentioned in the end of that section i.e. switching to a new software Mr. Kick and the circumvention of the relay box unless other wise mentioned.

6.5.1 Recording pilot 1: Testing filter settings

Experimental goal:

Testing highpass cutoff between DC and 5 Hz need.

Description:

Using a pals electrode placed during one of the other pilots experiments which produced H- and M-wave were recorded from between 0 and 100 mA using DC, 0.1, 1, 2, and 5 Hz highpass cutoff.

Results:

The DC produced the smallest artefact but not much smaller than 0.1. Beyond 0.1 the long slow part of the stimulation artefact, resembling capacitor discharge, increased a lot. After checking with the performance of the data processing prototypes 0.1 were chose and use for all other experiments. The filters for both the

contralateral biceps and ipsilateral soleus were fare enough away from stimulation to use 5 Hz highpass cutoff.

6.5.2 Recording pilot 2: Testing EMG configurations and lower biceps femoris electrode placement

Purpose:

Test two different common reference configurations along with double differential configuration. Double differential configurations have been used before by researcher at AAU who suggested testing this configuration.

Description:

First part: EMG electrodes are placed as described in section 6.1.1, i.e. common reference with refernce at the lateral bone at the ankle. Stimulation is carried out between 0 and 100 mA. This is repeated with reference at the tibial bone and lateral side of the knee. There after all EMG locations get their own reference by adding a new EMG electrode so 3 electrodes are in a along the muscle fibres with the central electrode as the reference.

Results:

The three common reference configurations all preformed similarly. The double differential configuration preformed somewhat better but also needed less cables which is preferable during dynamic movements.

6.5.3 Recording pilot 3: EMG Biceps Femoris electrode placement

Purpose:

Testing lower placement of EMG electrode on Biceps femoris.

Description:

First part: Double differential EMG electrodes are placed on biceps femoris further down than SENIAM recommendations but along the same line. By feel of touch the lowest place on biceps femoris that still feels like muscles and not tendon are used as placement after which EMG recording of bending of the subjects along with stimulation between 0 and 100 were made.

Results:

The placement gave usable EMG recordings, H- and M-waves. It was how ever difficult to get right and sometimes had to be reapplied for both the first subjects. The stimulation artefact were improved by added distance.

6.5.4 Stimulation conclusions

1. To limit noise and artefacts, especially the stimulation in the hamstring muscles using double differential EMG electrodes instead of common reference mode seemed to give the best results.

2. The biceps femoris EMG electrodes needed to be moved as fare down away from the stimulation site in order to limit the stimulation artefact.

3. The high pass filter in the EMG amplifiers cannot be set higher than 0.1 Hz in terms.

4. Common reference needs wires connecting the different recording sites resulting in more wires that needs to be managed during the dynamic phase.

6.6 Dynamic Setup Pilot Experiment

Based on our pilot experiments with the old protocol and additional pilot experiments we made a number of changes to the setup. Lastly we added hardware needed for recording during gait.

The following is a description of the experiment we intend on using in large points: While the subject lies in a prone position electrodes for electrical stimulation in a grid along with EMG recording electrodes on both biceps femori and ipsilateral soleus are placed. The grid of stimulation electrodes is tested to find the optimal site for stimulation site using 0-100 mA. Then an input-output curve is recorded. After a small break the subject is moved to a treadmill where they are suspended from a safety harness and a foot switch is placed on the ipsilateral foot. Using 4 repetitions each 10% of the gait cycle has an input output curve recorded. Lastly one stimulation intensity is repeated 20 times for each 50 and 90 % of the gait cycle of the ipsilateral leg and 20 recordings without any stimulation is made.

This procedure was tested twice, once for each investigator using the other investigator as a subject. Over all the setup was found to be satisfactory. A number of small alterations were made. Soleus EMG electrodes are placed before lying down. The Inter stimulation interval was increased to avoid miss steps. A strap and ball

contraption capable of applying pressure to the stimulation cathode chosen was added to the experiment.

Chapter 7

Experimentation

The main experiments were conducted in the second half of the semester, after the previously described pilots had been carried out to select equipment and procedures. This chapter details the final design, setup, and procedure that produced the results shown and discussed in later chapters.

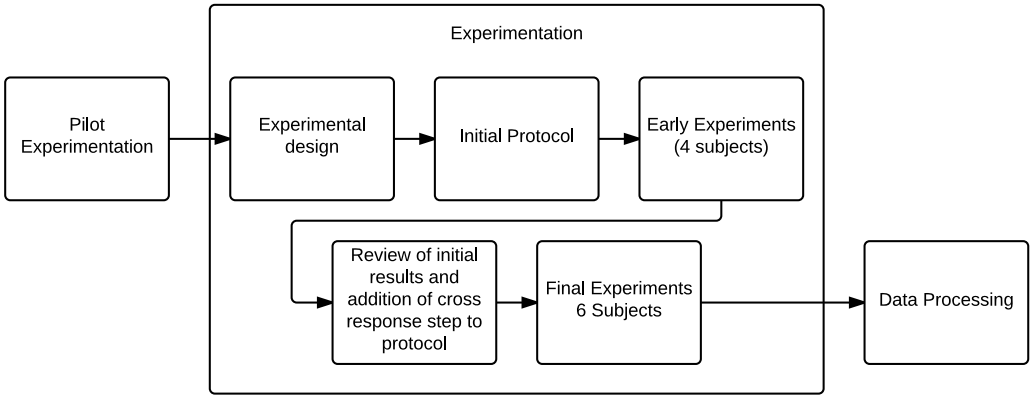


Figure 7.1: Overview of the Experimentation phase

7.1 Experiment Design

The main experiments were carried out at Aalborg univeristy facilities in the spring of 2015. In order to collect enough data to be able to assess the proposed goals, the target length of the experiment session was of approximately two hours.

The experiment is divided into two parts, allowing a break in the middle should the subject require it. The experiment is structured into 4 parts each with their owns goal and structure. The parts depend on each other and their order can not

be changed. Fatigue and other intervening variables can therefore affect all subjects in the same fashion.

Experimental phase	Description
Optimal site selection	Each of the 9 electrodes is in turn selected as a cathode; electrical stimulation is delivered from 0 to 60mA; candidate sites are selected based on features of the response and for each of the candidates stimulation from 0 to 100mA is delivered to confirm the site.
Static	The selected optimal site is used to administer randomized stimulations between 0 and 100mA in steps of 10mA for three repetitions.
Dynamic	Stimulation is delivered at each 10% of the gait cycle sequentially. The same randomized stimulation used in the static phase is administered, with 4 repetitions of each current amplitude.
Dynamic Cross-response	One stimulation intensity is selected based on the previous recordings; 20 stimulations are carried out at 50% and 90% of the gait cycle of the right leg, followed by 20 control steps where no stimulation is conducted.

Table 7.1: Experimental structure

7.1.1 Experimental units / Subjects

Ten healthy volunteers were recruited among students and staff at Aalborg university. Experimentation carried out on fellow students is subject to other rules and not subject to approval from ethical committees.

The protocol and safety measures were approved by supervisors and were in accordance with standard safety requirements for experimentation. All subjects were informed of the goal and procedure of the study. All subjects through the their education were capable of understanding and consenting to the experiment. All subjects were between 20 and 30 years old. The modulation assessment included all 10 subjects (7 male, 3 female). Out of these ten, the cross-response phase of the protocol was applied only to the last seven.

7.1.2 Experimental Variables

In the different parts of the experiment different variables will be used and relevant to interpreting the results. This section gives an overview of some of the variables

mainly Independent Variables and Dependent variables, that is the variables we affect and the variables we measure.

Lastly a number of other kinds of variables that may unintentionally affect the out come are also mentioned such that we may be aware of their possible effect and are better able to reduce their effect. These are Extraneous, Intervening and lurking.

The extraneous variables are the unintended variations between the experimental units which may cause variations i the dependent variables for this experiment the variations in length of the legs may cause variations in timing of the h-reflex.

The Intervening variables are variations caused over the duration of the experiment which may affect the dependent variables. In this experiment tiredness and or discomfort may cause changes over the cause of the experiment.

The lurking variables are unknown or unconsidered variables that may affect the outcome per definition are difficult to deal with and in this experiment we will not do any thing but to state that we and others should be aware that in a relatively new protocol like this and in a field with so much left to explore lurking variables are a real option.

Variable Type	Identified variables
Independent	Stimulation intensity
Dependent	EMG, H-reflex, M-wave

Table 7.2: Experimental variables for the Static phase

Variable Type	Identified variables
Independent	Stimulation intensity, stimulation timing
Dependent	EMG, H-reflex, M-wave

Table 7.3: Experimental variables for the Dynamic phase

Variable Type	Identified variables
Independent	Stimulation vs Non-Stimulation ,Stimulation timing
Dependent	EMG, H-reflex, M-wave

Table 7.4: Experimental variables for the Dynamic Cross-response phase

7.2 Experimental Setup

Stimulation equipment

Variable Type	Identified variables
Extraneous	Hight, leg / step length, gender, age, (Electrode placement)
Intervening	Tiredness, boredom, motivation and, discomfort
Lurking	These are

Table 7.5: General Experimental variables

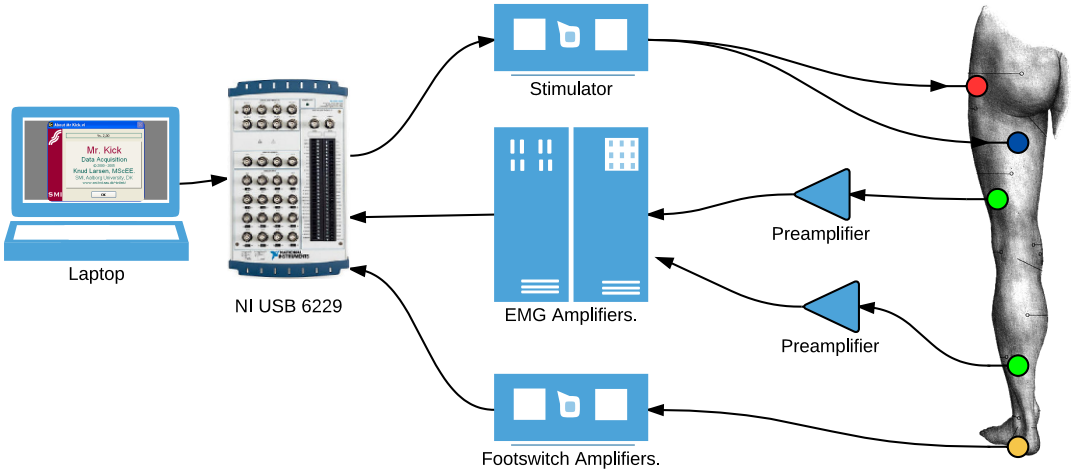


Figure 7.2: The new setup for our dynamic recording experiment.

Electrical stimulation was delivered by an isolated electrical stimulator (Noxi-Test IES 230, Aalborg, Denmark). The stimulator is capable of both constant current and constant voltage delivery, and was used in constant current mode with a maximal intensity of 100mA and a compliance voltage of up to 240V. Stimulation parameters can be set from the up-front controls on the stimulator itself, or by a single control signal to the Analog input port, which controls timing, duration, and amplitude at once.

The control signal was provided by a multifunction data acquisition module (National Instruments, NI USB-6229) through analog output line (BNC cable), and consists of a square pulse of variable amplitude (between 0 and 10V).

All stimulation pulses administered in the experiments were unipolar square pulses of variable amplitude and fixed duration of 1ms. Interstimulus interval in the static phase was set to 4 seconds. In the dynamic phase, the minimum ISI was set to two times and a half the step period.

The cathode matrix consists of 3.2cm diameter flexible cloth electrodes with hydrogel coating (Axelgaard PALS line, Axelgaard, Denmark). The fixed anode was of the same type but dimensioned as a 3.3cm x 5.3cm rectangle.

Recording equipment Disposable, self adhesive wet gel electrodes (Neuroline

720, Ambu, Denmark) were used for all EMG recordings. The electrode cables include a local preamplification stage (approximately 10cm from the contact sites) with a fixed gain of 0.1. The EMG amplifiers were developed at AAU for research purposes, and feature variable gain, bandpass cutoff frequencies, and a triggered blocking feature of adjustable duration.

The amplifiers were set to a 10k gain, for a total gain of 1k. The analog bandpass filter was set to 0.1 to 500hz for Biceps Femoris (left and right) and 5-500hz for Soleus, for noise reduction. The signals were then sampled at 5000hz through the same DAQ module and stored to disk by the control software described below.

For triggering in the dynamic phase flat, round footswitches consisting of a pressure sensor were applied on the subjects' soles, on the posteriolateral edge of the heel. The signal was then routed through a dedicated amplification and thresholding stage, converting the signal to a TTL output routed to the trigger input port of the NI USB-6229 and mirrored to an analog input port for recording and later inspection.

Software and analog IO

The scientific data acquisition software "Mr. Kick", implemented in LabVIEW2011 controlled stimulation and recording. It was developed by Knud Larsen at the Center for Sensory-Motor Interaction (SMI) at Aalborg University. The software allows fine control over all features of stimulation and recording, allowing the user to set class and subclass specific parameters for stimulation, allowing a great degree of flexibility and automation. A plugin was developed to control a rack-mounted demultiplexer for use with electrode arrays that have been tested in the pilot experiments. The software is also capable of performing online data preprocessing and feature extraction (e.g. peak to peak amplitude at predefined intervals for building I-O curves on the fly), allowing the user to better assess the experiment as the sessions are running.

Other equipment In the dynamic phase, the subjects were strapped in a safety harness (Maine Anti-Gravity Systems, US) providing no weight support, to prevent falls. The treadmill used in all pilots and the main experiments was a WoodWay split-belt treadmill (Woodway USA Inc., US).

7.2.1 Aim of the experiment

The experiment consists of four major phases described below:

1. **Optimal site selection** One of the challenges both to us and to others wanting to investigate the h-reflex of the hamstring muscles is the location of an optimal site for stimulating the sciatic nerve. A procedure was developed for consistent placement of the electrode grid, to cover the area where the best site for stimulation in the thigh is expected to be found for most subjects, based on experience from pilot experiments.

2. **Recording input-output curves for H and M-wave in static unloaded conditions** To assess whether the sciatic nerve was successfully stimulated, and investigate its behavior in a situation with as little noise or muscle activity as possible, a static IO curve from 0 to 100mA is constructed. This is also later used to extract a template for stimulation artifact reduction in post-processing.

3. **Dynamic testing for construction of dynamic IO curves and modulation curves for the H-wave** The subject is moved to the treadmill and the dynamic phase of the experiment begins. Stimulation is administered at various time points in the gait cycle to collect the data for the assessment of time-specific H-wave modulation.

4. **Dynamic testing for assessment of cross-spinal responses** From literature and experiments with lower leg H-reflex and mechanically induced hamstring stretch-reflexes, there is evidence of short latency, spinal mediated cross-responses in contralateral muscle groups. This last module is aimed at collecting data from two specific time points in the gait cycle to assess whether the technique can be used to investigate modulation of cross-spinal responses at the same time as modulation in the ipsilateral leg.

7.2.2 Safety

Standard safety guidelines for experiments involving electrical stimulation of human subjects have been followed, including isolation of all equipment through a medical power supply, making full use of software and hardware safety switches on the stimulation pathway, and following a strict protocol with checklists for potentially hazardous phases of the experiment (e.g. moving electrodes or the subject, disconnecting cables during breaks or when moving the subject, etc). The following specific measures have been implemented in the experiments:

- Always make sure the treadmill is powered and the magnetic brakes engaged before subjects are moved to the dynamic phase.

- Always strap in subject in the safety harness when on the treadmill before administering any stimulation.

- Always begin stimulation using low currents to check that those levels are not painful, to avoid causing the subject unnecessary discomfort and allow them to familiarize with the actual sensation of the electrical current.

- Avoid placing stimulation electrodes on wounds, birthmarks or other abnormal skin.

7.3 Experimental protocol

Equipment List:

- Laptop with Mr. Kick software v 3.0 preview build installed.
- DAQ module (NI USB-6229)
- Constant current stimulator (NoxiTest IES 230)
- 9 3.2 cm œcathode electrodes (PALS)
- 1 3.3cm x 5.3cm anode electrode (PALS)
- 9 EMG electrodes (Ambu)
- Footswitch
- 3 EMG amplifiers
- Footswitch amplifier
- Extension/connector box for footswitches and EMG
- Treadmill
- Safety harness
- BNC connection cables and stimulation cable
- Tape
- Safety razors
- Alcohol swabs
- Elastic tube gauze

7.3.1 Placement of stimulation electrodes

To find the best placement the existing protocol was modified to include the placement of an electrode matrix, and standardizing its placement to ensure consistency across subjects and the quick determination of the optimal site for the following phases. The procedure for localizing the electrode matrix is as follows.

The greater trochanter of the femur and the lateral protrusion of the femur at the knee are localized by palpation and marked (7.3, points A and B); the midpoint between the two is measured and marked (point C).

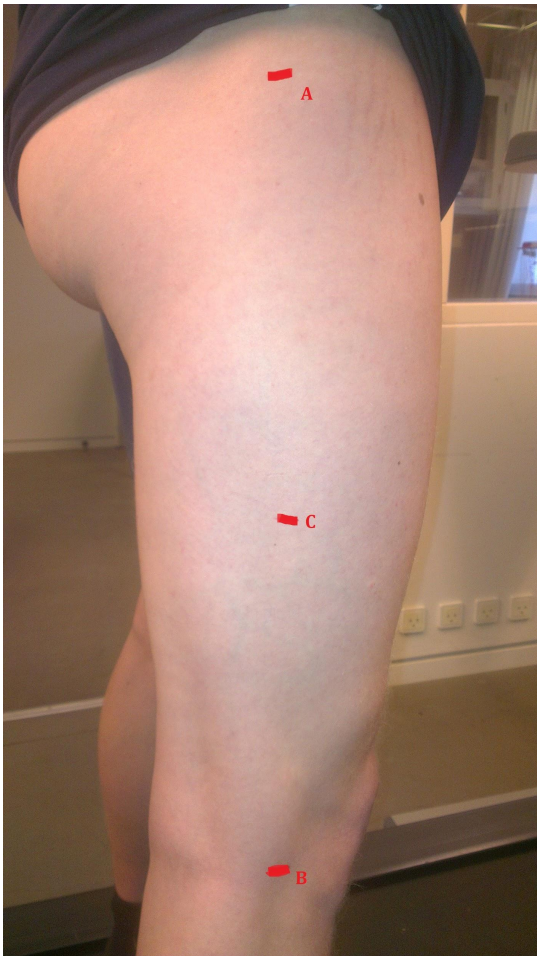


Figure 7.3: An example of the reference points on the upper leg. (A): The greater trochanter of the femur. (B): the lateral protrusion of the femur at the knee. (C): Mid way point between (A) and (B).

The thigh circumference is measured at the level of point C. Between 100 and 110° from the frontal plane (which approximately passes through the line connecting points A and B), moving clockwise towards the rear of the leg, the cleft between the biceps and the semitendinosus muscles is localized by palpation. This marks the center of the lower edge of a 10 by 10cm grid, which is also marked on the skin (see fig. 7.4 and 7.5).

The grid is used as a reference to apply nine round hydrogel-based PALS electrodes (Axelgaard, DK) as the possible cathodes. One electrode is applied on the greater trochanter of the femur (point A) and used as an anode, a 3.3x5.3cm rectangular PALS electrode from the same manufacturer.

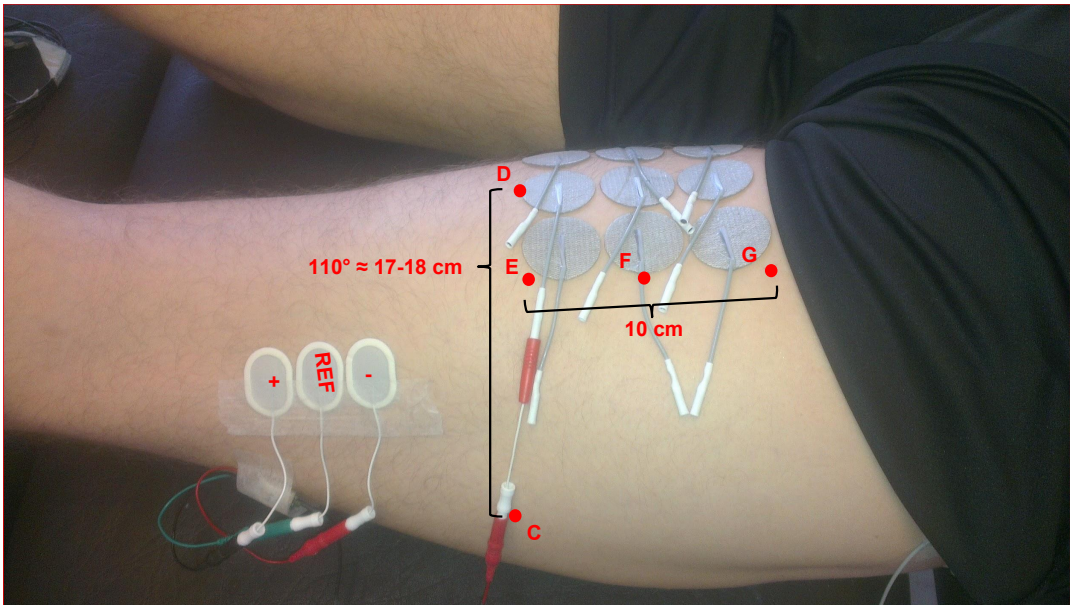


Figure 7.4: The grid of stimulation electrodes along with Biceps femur EMG electrodes seen from lateral direction in relation to the leg. (C): Mid way point between on the femur. (D): The lower center point of the grid found as described in the text ca. 110° in on the back of the leg. The three EMG electrodes in double differential configuration can bee seen with the central reference electrode marked (REF).

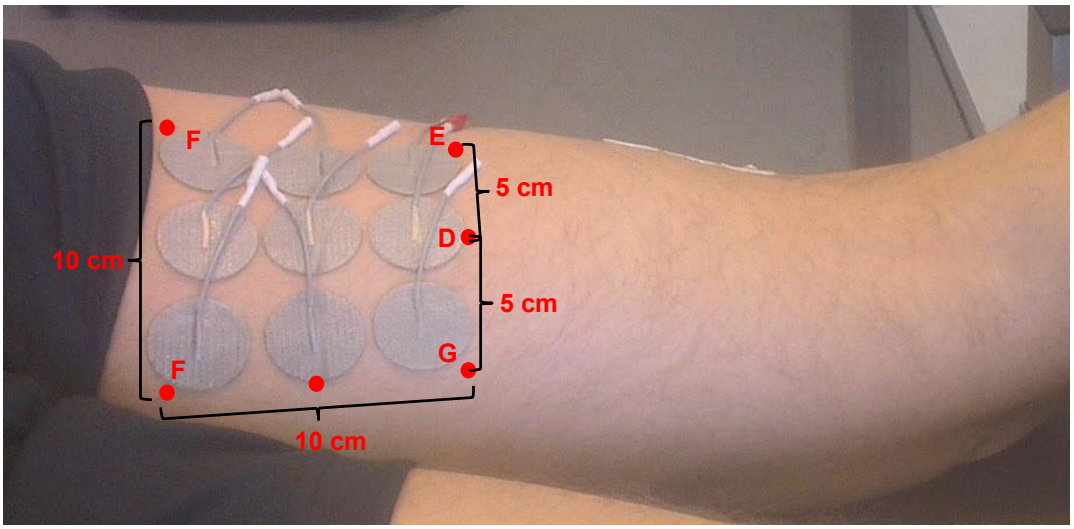


Figure 7.5: The grid of stimulation electrodes along with Biceps femur EMG electrodes seen from medial direction in relation to the leg. (D): The lower center point of the grid found as described in the text ca. 110° degree in on the back of the leg.

7.3.2 Application of recording electrodes

Recording of EMG was carried out at both biceps femoris and at the ipsilateral soleus. Optimal positioning of electrodes in the lower limb is well documented and guidelines such as the SENIAM recommendations should be used whenever possible. However, due to the proximity of the stimulation electrodes to the ipsilateral biceps femoris the electrodes for this muscle were placed further down the thigh, approximately 15cm proximal to the popliteal fossa along the line connecting the distal tendon insertion of Biceps Femoris and the ischial tuberosity of the hip bone. EMG electrodes were applied in a double differential configuration with the three electrodes aligned along the muscle decurrence direction. Electrodes for soleus were applied on the lateral aspect of the lower leg, approximately 20cm from the heel, localizing the muscle by palpation after asking the subject to lift himself on his toes while standing.

7.3.3 Preparation and optimal site selection

Finding a site to stimulate the sciatic nerve without stimulating any muscles or creating a too large artefact is one of the most difficult parts of this experiment and must be done with care. Placing the nine electrodes in an array covering the area where an optimal site is most likely helps but due to individual variation some subjects may not have an optimal or acceptable site in this area. Other subjects are just not suited for this kind of experiment with the current technical limitations. The steps in this part of the experiment are as follows:

Set up the equipment:

- Connect the laptop to the NI USB-6229 and the EMG amplifiers, footswitch amplifiers and constant current stimulator to the NI USB-6229.
- Check the setting on the EMG amplifiers
  - Filter settings: Bandpass - Biceps femoris (left and right): 0.1 - 500 Hz; Soleus: 5 - 500Hz
  - Gain: 10K
- Check the settings on the Constant current stimulator
  - Operation mode: Externally scaled (analog input)
  - Stimulation intensity: knob set to 100 mA (upper limit of stimulation, delivered when the input signal amplitude is 10V)

Electrode placement:

- Wipe the general area where electrode are to be placed with alcohol.

- While the subject is standing, EMG electrodes are placed for the soleus muscle. This placement was chosen due to easier wiring in the dynamic phase.
- The subject is asked to lay face down on an adjustable examining/therapist bed with headrest hole.
- Recording electrodes are placed on left biceps femoris in accordance to SENIAM recommendations (2/3 down the line connecting the ischial tuberosity and tendon insertion in the popliteal fossa).
- Right Femur is measured from the greater trochanter to the lateral protrusion at the knee, the halfway point marked out, circumference at this point measured and the reference grid for the stimulation array is drawn.
- The stimulation electrodes are placed with their wires pointing laterally outwards from the hips. In some short subjects the upper line of electrode might reach the fold of the gluteus maximus. These electrodes do not need to be placed as the site is almost certainly too high and can not be used during dynamic experiment phase as the electrode might detach during stimulation, giving the subject a painful shock as current is pushed through a reduced area.
- The last electrode to be placed is the right biceps femoris electrodes which have to be placed lower than SENIAM recommendations to limit the stimulation artefact. The electrode can't be too low as it might then be placed on the tendon. To find the site it might be easiest to find the tendon of biceps femoris while the subject flexes their knee against against the experimenter's hand. Then by palpation follow it upwards until the muscle begins to develop (softer tissue).
- Check the EMG signal by having the subject bend the knees and ankle and and tapping the electrodes to verify the connections.
- Place a pillow or support under the feet of subject to minimise background activity in soleus due to forced dorsiflection/rotation of the ankle.
- Ask the subject to lay down and to relax to minimize activity in the lower back and in the legs to minimize background noise.

Stimulation procedure

- Begin the experiment with a test run on the first site with amplitude no larger than 30mA to allow the subject to familiarize with the stimulation sensation. If the subject does not report discomfort or pain, proceed with the experiment.

- For each site, stimulate between 0-60 mA with a 1 ms constant current pulse, sequentially for three repetitions.
- After all sites have been stimulated, inspect the data to select 1 to 3 candidates for the optimal site.
- Apply pressure via a soft ball strapped to the leg, to improve contact between the candidate electrodes and the skin.
- Test the candidates as before but in the 0-100 mA range. Be ready to turn of the stimulation if the subject does not wish to continue after experiencing the last high current levels and ask the subjects afterward if the are okay with the highest current levels.
- Choose the optimal site based on the presence of an H-wave in both biceps femoris and soleus, its peak-peak amplitude, noise levels, and correct behaviour of both H and M waves as stimulation intensity increases.

The ideal site will show little noise, well defined waves for the two responses, a fast recovery of the stimulation artifact rebound, and an identifiable M-max in the inspection I-O curve for Biceps Femoris. This was found to be rarely the case however, therefore a tradeoff between noise and desired features is usually to be made.

7.3.4 Recording input-output curves for H and M-wave in static unloaded conditions

- Stimulate the optimal site between 0-100 mA in random order for three repetitions.
- Verify all the expected files have been saved and named properly.

After an optimal site is selected and the static recordings are completed, the subject is given the opportunity to rest and use the restroom.

7.3.5 Dynamic testing for construction of dynamic IO curves and modulation curves for the H-wave

Transfer to treadmill

- Ensure that the treadmill is turned on, the safety switches are armed, and the brakes are engaged.
- Disengage the stimulator and disconnect the stimulation cables. If required, disconnect the EMG leads from the electrodes ensuring the electrodes themselves are not detached from the skin.

- Secure the subject in the safety harness and adjust the height so that the subject is not pulled upwards by the harnessing. The harness should prevent the subject from falling or hitting his head on the rails or other equipment in case of a loss of consciousness or balance.
- Secure the cable extension and connector box to the subject (in a belt bag or directly to the harness).
- Remove the stimulation electrodes not to be used.
- Apply the footswitch on the lateral side of the heel with the lead pointing towards the toes, secure the lead at the arch of the foot and let it run up the side of the shoe and up the leg to the connector box.
- Test the Footswitch and adjust the threshold if needed to ensure proper triggering during gait.
- If the cables to the EMG electrodes were disconnected reconnect them and draw the cables along the legs to the cable connector box. Ensure the preamplifiers are taped to the skin to minimize movement artifacts.
- Apply elastic tube gauze to secure the wires and preamplifiers from moving during the experiment.
- Reconnect the stimulation cables and place the ball and strap onto the stimulation electrode.
- Have the subject swing their legs back and forward to check that the cables are not to short and pulling on the electrodes. Adjust if needed.

Stimulation

- Start the treadmill, gradually increasing speed to 3.4km/h. Arm the stimulator.
- Stimulate at heel strike 4 times between 0-100 mA in steps of 10 mA, in random order.
- Use the previous phase to compute the step period, for subsequent calculation of stimulation delays at other timepoints.
- Repeat the stimulation as before for each timepoint of interest (every 10%) setting the appropriate delay in the software.
- Monitor the recordings and recording equipment, cables and electrodes to ensure movement artifacts are minimized.

7.3.6 Dynamic testing for assessment of cross-spinal responses

- Based on the recordings of the 50% and 90% time points in the previous phase, select a current level for the last part of the experiment. For this experiment, the current level chosen should correspond to the ascending part of the the soleus h-reflex input-output curve (70 or 80 mA).
- Stimulate at 50% of the gait cycle of the ipsilateral leg, for 20 repetitions.
- Stimulate at 90% of the gait cycle of the ipsilateral leg, for 20 repetitions.
- Record 20 steps without stimulation with the same triggering.
- Stop the treadmill, release the subject from the equipment and safety harness, disconnect all cables, finalize and save notes and datafiles.

Chapter 8

Dataprocessing

The data processing consists of a common preprocessing and bookkeeping phase which then branches in two separate lines of elaboration. The first concerns with the process leading to results for the reflex modulation curves, and consists of four steps: Preprocessing, Artefact Removal, H- and M-wave measurement and lastly construction of the individual and aggregate modulation plots.

The second line produces the results for the assessment of cross-spinal responses. As the data structure and the extracted features are different, it consists of three steps: Preprocessing, EMG rectification and filtering, and the output of averaged plots highlighting cross-spinal or other events in the contralateral Biceps Femoris.

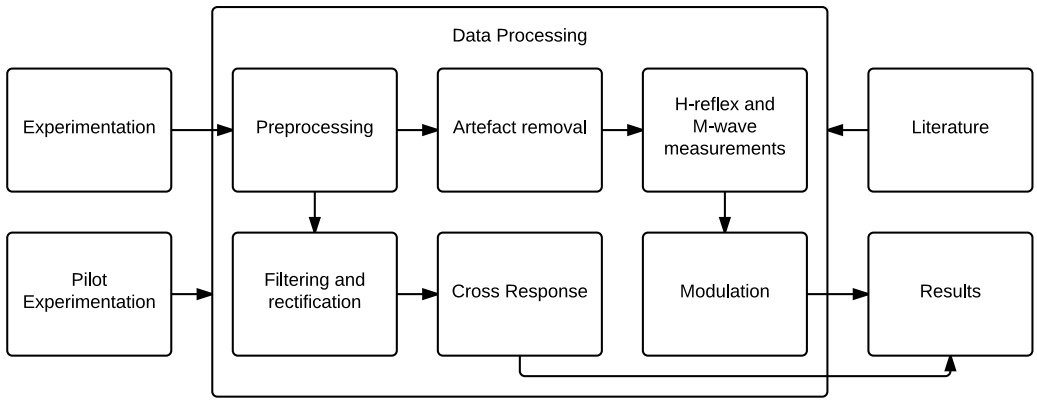


Figure 8.1: Overview of the data processing workflow.

8.1 Preprocessing

This section contains the information pertaining the structure of the output data from Mr. Kick after the experiments, and the elaboration operated on the data in preparation to the dynamic reflex modulation and the cross response studies.

The structure of the data after recording is as follows:

- For each subject a folder in the dataset is created, and contains a Mr. Kick exported .mat file for every session. Each session consists of a variable number of “sweeps”, represented in each file as a separate multidimensional array
- Mr. Kick conveniently saves metadata in appropriate arrays, such as the headers for each stimulation (class, current level or analog output level, timer delay etc)
- In each “sweep” array, the three EMG channels plus the forwarded digital output of the footswitch are stored as column vectors

Therefore, a certain amount of bookkeeping is required to keep track of which sweep belongs to which class, stimulation amplitude, subject, and of all the relevant metadata. This comprises the bulk of the preprocessing phase as it lays out the data for follow up analysis in the two lines of elaboration presented earlier.

The first common step in the preprocessing phase is performed by the helper function createOrderMatrix([n]). It returns a tridimensional vector containing the sweep number of each stimulation class (contact site, when applicable), subclass (stimulation amplitude), and repetition. This structure is consistent across all experiments and allows to quickly sort the randomized recordings and provide an unified method for recalling a particular stimulation from a datafile in the following scripts.

One data file is saved for every stimulation "train" (for example, the ten-sweep sequence in the static phase from 0 to 100mA, or the twenty-sweep sequence for the cross-response phase). During processing in the later steps, it’s necessary to bring up data from different subjects, time points or experimental phases, to extract data or templates from all various steps. A helper function findDataset() can be used to quickly access the data base and return the file handle of a particular recording for immediate use in the rest of the scripts.

8.2 Artefact Removal

The proximity of stimulation and recording electrodes for the right Biceps Femoris muscle introduce a slow recovery rebound just after the stimulation artifact itself, likely caused by the filtering stage in the EMG amplifiers. The amplitude of this

artifact can be large enough to bury the smaller M-waves, causing the measurements to return the amplitude of the underlying slope instead of the peak to peak amplitude of the superimposed M-wave.

To reduce the impact of the stimulation artifact on the measured responses, a correction algorithm is implemented that takes advantage of the apparent linearity of the artifact, subtracting from the recordings a matched and scaled template built individually for each subject. The correction is applied only to the ipsilateral Biceps Femoris recording as this artifact is not evident in other recordings.

For each subject, a template is extracted from the first 60ms after the stimulation artifact peak of a low intensity stimulation from the static phase (10mA), representative of the stimulation artifact in iBF.

For every subsequent recording from the same subject, the template is scaled and translated to match each of the dynamic phase responses, and subtracted from the signal in the same 60ms window.

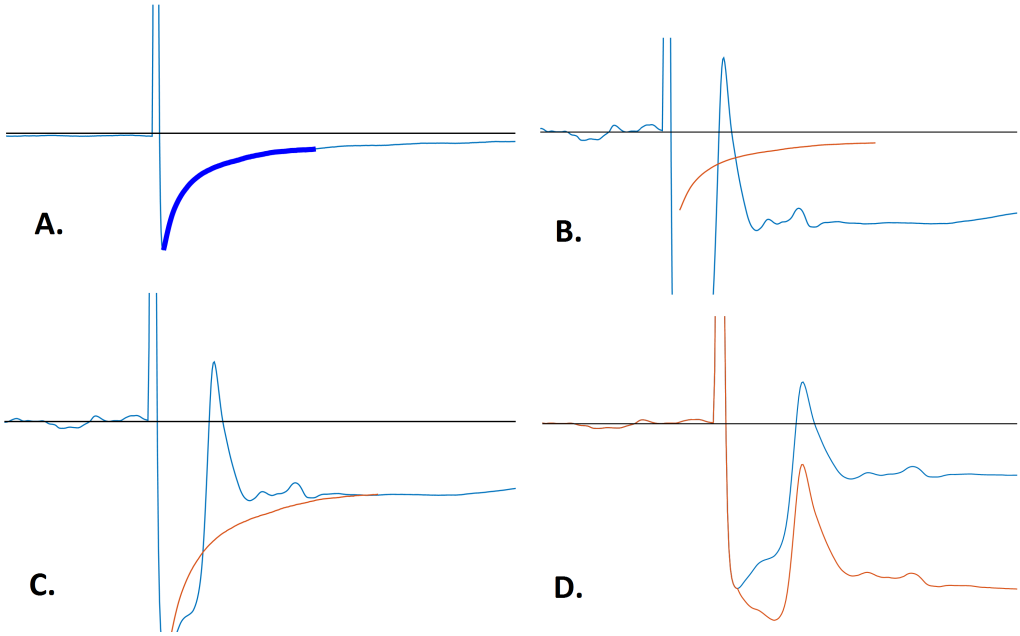


Figure 8.2: The artefact removal procedure. A: a low intensity stimulus in the static phase contains minimal noise, no reflex response, but the typical shape of the stimulation artifact tail. The highlighted segment is extracted and stored as the template. B: The template is aligned with the stimulation in one of the responses from the dynamic or static phases to be corrected. C: the template is scaled to match the signal and connect to the peak just after stimulation and to the signal after 60ms. D: Subtracting the template yields the corrected signal.

8.3 H-reflex And M-wave Measurement

The script responsible for constructing the modulation curves relies on a function that expands on the built in findpeak() functionality offered by Matlab. The same function is used whenever a peak to peak amplitude is to be computed on the signals; the caller is responsible for supplying the correct data interval based on either automatic recognition of features in the recordings or a manually specified time interval.

The function is passed the corrected data from the previous step, and begins by detecting positive and negative peaks. These are then sorted in descending amplitude order; the largest positive and negative peaks are then used for computing the peak to peak amplitude in the interval. This implementation allows to specify additional constraints like prominence or width at half maximum.

The peak to peak amplitude and the latency (local) of the earliest of the two peaks used to calculate it are then returned to the caller.

If a positive and negative peak can not be found the function returns the simple difference between the minimum and maximum value in the given interval, together with a latency of zero so that this case can be detected by the caller.

8.4 Modulation

The overall processing for the reflex modulation part consists of the following steps:

- Latency intervals: intervals for the H-reflex and the M-wave are loaded from a predefined file, specific to each subject.
- Template extraction
- Artifact removal
- Amplitude extraction
- Graphing: input/output curves are constructed from the m and h-wave results for inspection
- Alignment of the I/O curves: to plot the modulation curves for the H-reflex, first the motor threshold for each timepoint that has been stimulated in the dynamic phase is identified. Since methods based on sigmoid fitting or similar to predict the current level corresponding to the motor threshold did not give satisfactory results on a dataset with only ten datapoints, the following method was used:

  - the first four intensities are used to establish a baseline in terms of the mean M-wave in that interval (MeanM) and its standard deviation (StdM)
  - the first current level that gives rise to an M-wave larger than MeanM + 2*StdM is chosen as the reference point for that gait cycle phase
  - the H-wave amplitude corresponding to that current level is then stored in an array This way we obtain a plot representative of the up- and down-regulation of the H-reflex through the gait cycle for the two muscles.

- Modulation curves: finally, an aggregate plot of all subjects' modulation curves is constructed in the form of a boxplot showing per-timepoint median, first and third quartile, and outliers.

8.5 Cross Response

The processing for the cross-response part consists of the following:

- The 20 repetitions from the control stimulations and the stimulations at 50% and 90% of gait cycle are loaded separately and sorted
- The signals are highpass-filtered (15Hz, 4th order butterworth; Fs = 5000Hz) and full-wave rectified
- The recordings are then averaged and the three curves (control, 50% stimulation, 90% stimulation) are displayed.
- For the two intervention curves, the signal is highlighted where it exceeds the control EMG profile by more than two standard deviations (on a point to point basis)

Part IV

Solution Evaluation

Chapter 9

Results

9.1 Static phase - evaluation of the procedure

Evoked responses in the electromyographic recordings presenting all features of both the prodromic M-wave and the afferent mediated second response of the H-reflex were observed in all ten subjects included in the analysis.

Furthermore, the static input-output curves for the two responses in both muscles indicate that it was possible to achieve a maximal M-wave (Mmax), although in 40% of the subjects for Biceps Femoris and in 10% of the subjects for Soleus (fig. 9.1 and 9.2).

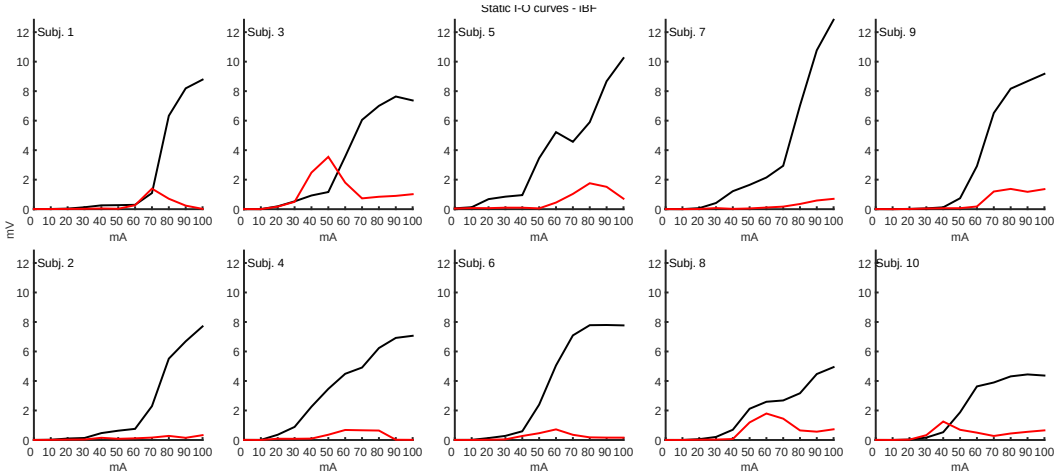


Figure 9.1: Input-output curves from all subjects in the ipsilateral Biceps Femoris. The M-wave is indicated in black, the H-wave in red.

Stimulation-related artifacts have a large impact on the quality of the I-O curves, as indicated in some cases by linear trends superimposed to the expected sigmoid-bell shape of the curves (fig. 9.1, subj. 2, 3). The onset (in terms of stimulation

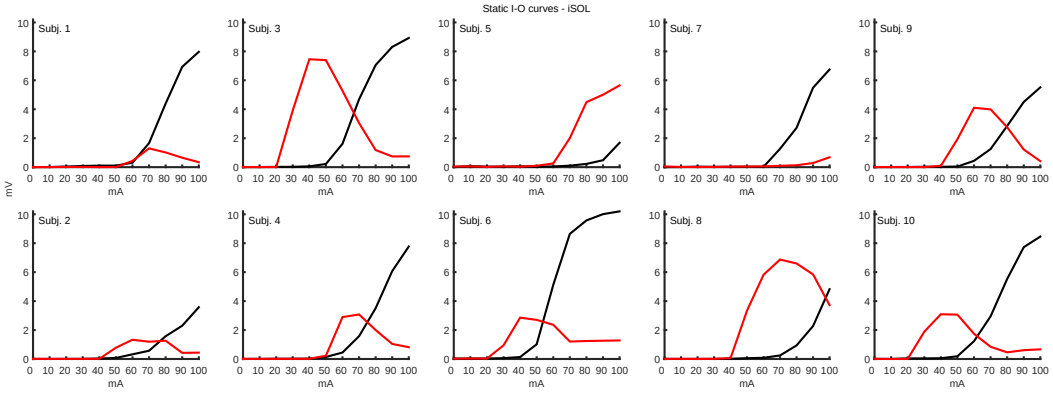


Figure 9.2: Input-output curves from all subjects in the ipsilateral Soleus. The M-wave is indicated in black, the H-wave in red.

amplitude) of the M and H waves vary significantly across different subjects, as do the relative amplitudes of Hmax and Mmax (when applicable).

The I-O curves obtained in the static phase were also used to assess the latencies of H and M waves. Table 9.1 summarizes the mean latencies of both responses in Soleus and Biceps femoris. The time intervals separating the peaks of the first and second responses appear to be different in the two muscles, at approximately 10ms and 16ms for Biceps Femoris and Soleus, respectively. All latencies are referred to the onset of stimulation (accounting for delays built into the software and hardware), that is, the rising front of the TTL command as it is sent by the IO device to the stimulator.

Muscle	M-Wave latency (first peak)	H-Wave latency (first peak)
Biceps Femoris	12.24 ± 1.62ms	22.8 ± 2.19ms
Soleus	16.66 ± 1.63ms	32.3 ± 2.68ms

Table 9.1: First and second response latencies in the static phase.

Figures 9.3 and 9.4 expand on selected subjects, showing the individual responses in the static phase of the experiment along the derived I-O curve. The stimulation artifact is present in a large fraction of the recordings Biceps Femoris, with a noticeable rebound past the zero-line due to the proximity of stimulation that increases linearly with current amplitude. Soleus traces present significantly less noise, although in some cases, the proximity of other muscles such as the two Gastrocnemius heads could have contaminated the recordings. As expected however, as the stimulation intensity increases, the H-wave appears first in the recordings followed by the M-wave at higher intensities.

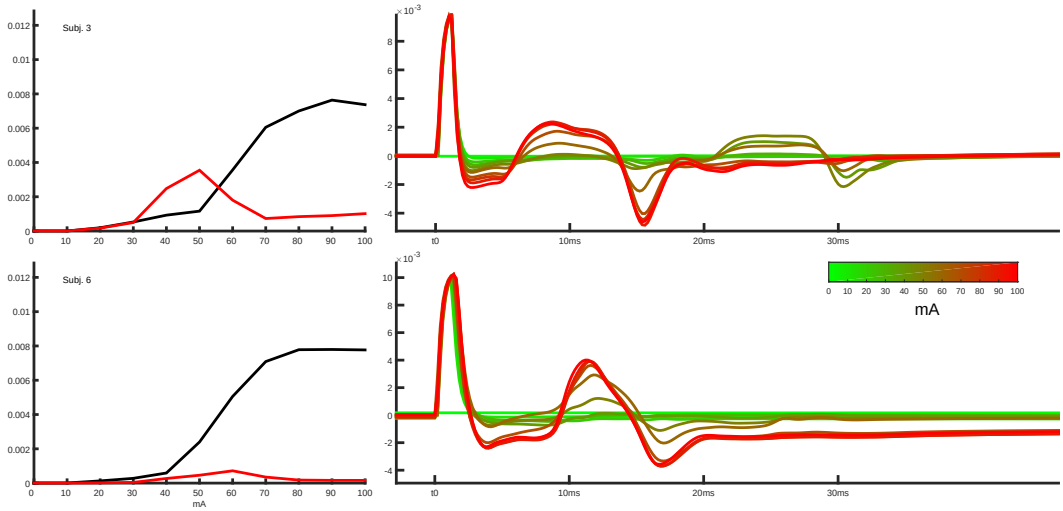


Figure 9.3: Details from subjects 3 and 6 in Biceps Femoris. The averaged responses (by stimulation amplitude) are shown on the right.

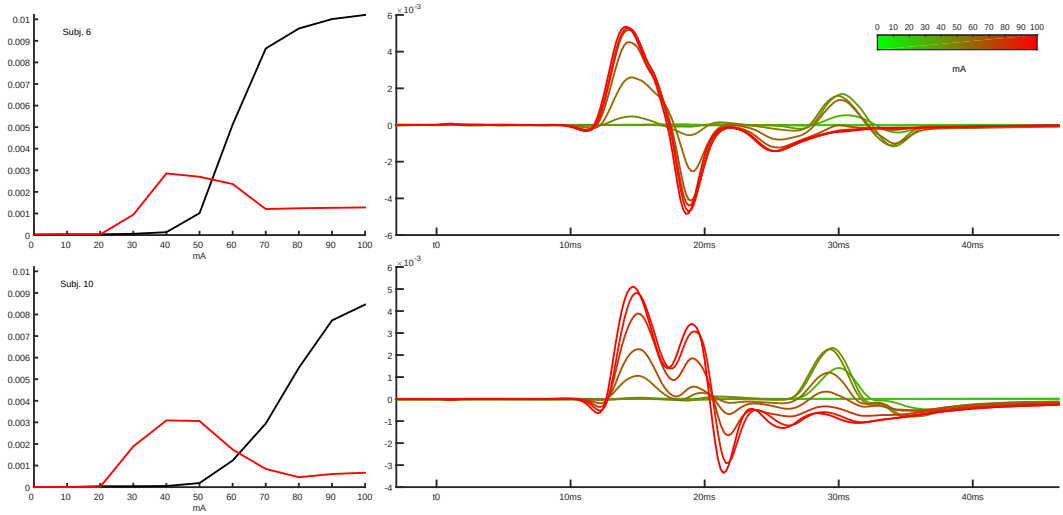


Figure 9.4: Details from subjects 3 and 10 in Soleus.

9.2 Phase modulation

Recordings from the dynamic phase allow evaluating whether phase modulation of the H-reflex in occurred in the monitored muscles. The aggregate results are shown in figure 9.5; the peak to peak amplitudes of the H and M components at the ten time points of the gait cycle are extracted from the processed data, and their ratio is plotted together with the background EMG activity in the corresponding muscle. In general, the H/M ratio is larger in Soleus than in Biceps Femoris, as was

the case in the static recordings. It is also evident that although the modulation follows a similar pattern to the background muscle activity of normal gait, the amplitudes of the reflexes are much larger, and that an increase in the background EMG is not always reflected in an increase of the H/M ratio. This is the case for the early to mid-stance phase in soleus, where upmodulation of the H-reflex does not correlate to increased EMG activity, and in the late stance of Biceps femoris, where minor contractions of the muscle are measured in more than half of the subjects, with no apparent increase in the H/M ratio in the corresponding phase.

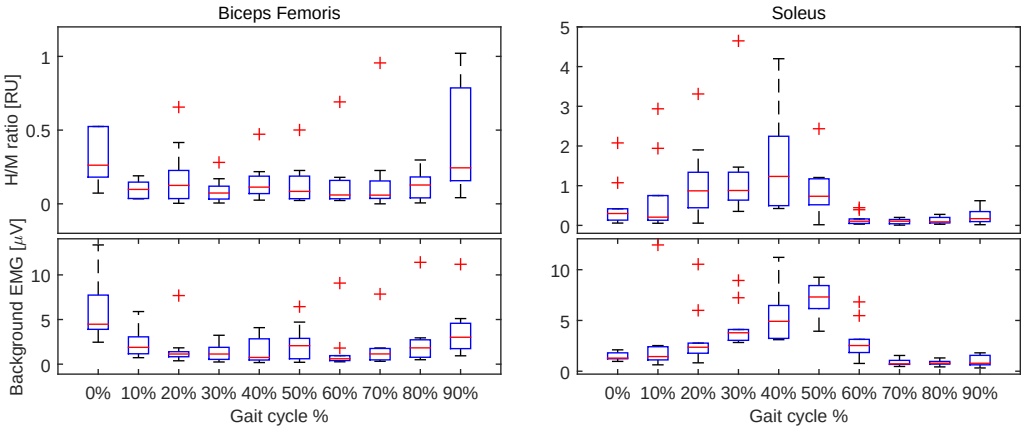


Figure 9.5: Modulation curves. Left: Biceps femoris; right: soleus. On the top half, the ratio of H-wave to M-wave across all subjects to the experiment are shown. The bottom half shows the RMS background activity in the 100ms preceding stimulation for each time point of the gait cycle.

Figure 9.6 shows in more detail the modulation curve from subject 6, along with the ten responses along the gait cycle that have been picked by the algorithm to construct the modulation curve. The modulation is evident, since if there were no time-dependent change in the H-reflex amplitude, should the same M-wave amplitude be recorded at different time points, the same H-wave amplitude should be found.

9.3 Cross spinal responses

We present a subset of the results to illustrate the responses that have been encountered as a proof of concept that the protocol allowed to measure cross-spinal short latency reflexes in the contralateral Biceps Femoris, although in only one subject. The graphs show, on the left and right sides, the responses measured in the left BF to stimulation at 50% and 90% of the right leg respectively, for three different subjects. The black solid line represents the average EMG activity from the control session, and the dotted line indicates the average EMG plus two standard deviations, per-sample. The blue line is the average stimulated response, and has been

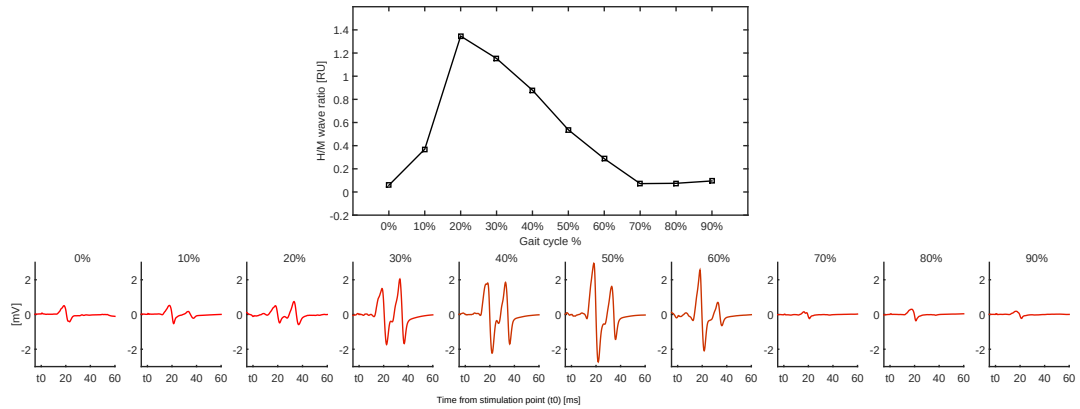
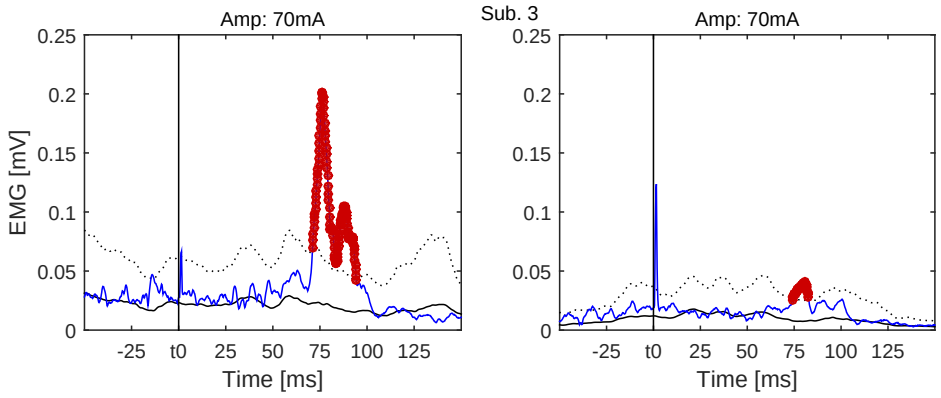


Figure 9.6: The modulation curve in Soleus for subject 5. The individual recordings that have been selected for the construction of the curve are shown in the bottom row, corresponding to each time point in the gait cycle.

highlighted in red when the measured activity increased above the mean plus two standard deviations of the control recordings (normal activity during gait).

In one subject (figure 9.7, top), short-latency facilitation can be observed at 75ms after stimulation, a result consistent with what has been observed with mechanical perturbations of the knee to elicit cross-spinal responses -Stevenson et al-. The response seems to be very prominent at 50% of the right leg's gait cycle and suppressed significantly at 90%. Other subjects (figure 9.7 center and bottom) did not present the same quality of response. Some activity linked to the stimulation can be observed later (around 100-150ms).



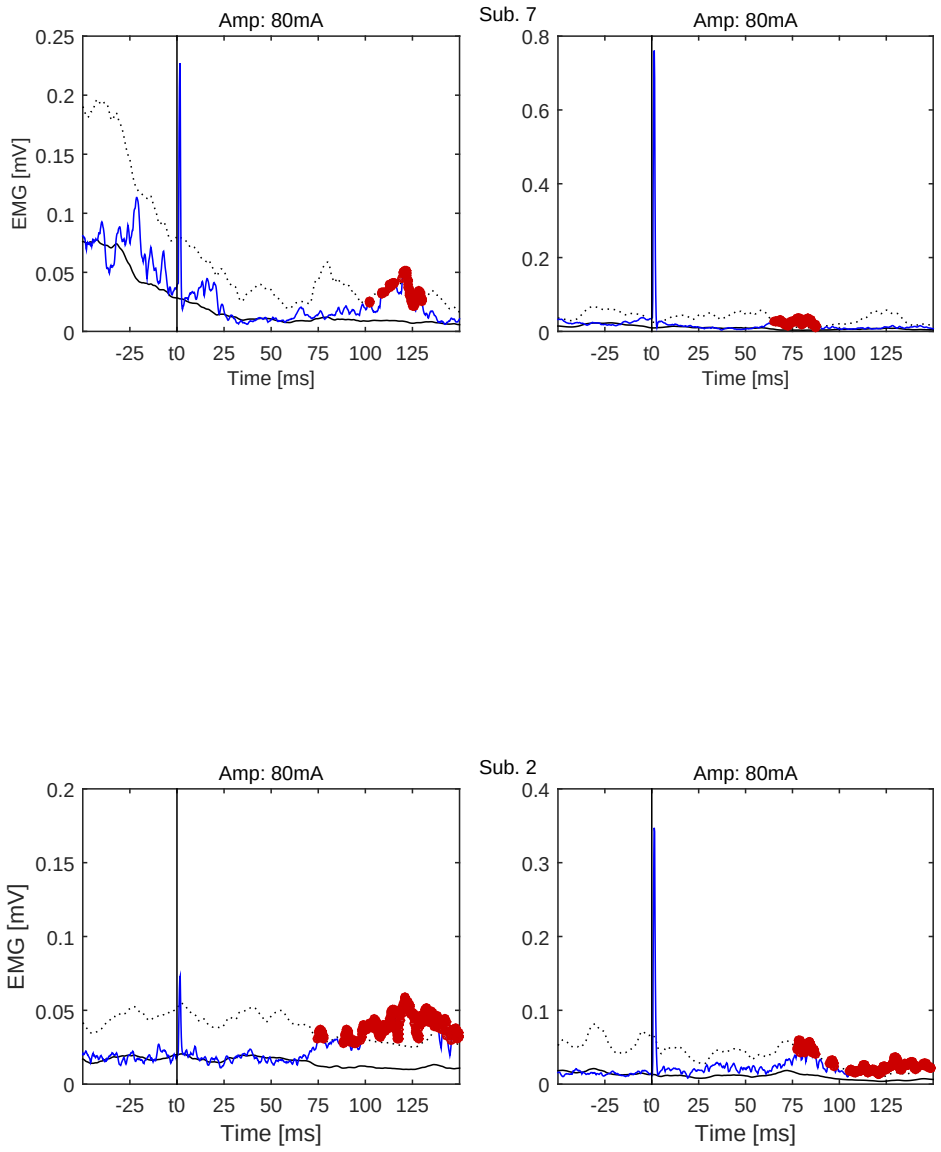


Figure 9.7: Example of responses of the Left Biceps Femoris to stimulation of the right sciatic nerve at 50% (left) and 90% (right) of the gait cycle.

Chapter 10

Discussion

In chapter 4 the problem addressed by this work was formulated as follows:

Can a protocol based on H-reflex techniques targeting the sciatic nerve be used to assess reflex phase modulation in the hamstrings during gait?

Two goals were formulated together with the main problem: **1:** To reconstruct the matrix electrode based system and test whether the setup and a protocol are able to successfully activate the sciatic nerve. Then begin to modify the protocol and setup to be usable during dynamic movement.

2: To validate the protocol, performing experiments aimed at confirming the purpose of the technique, that is investigating phase modulation of H-reflexes in the Soleus and Biceps Femoris muscles of the ipsilateral leg and to elicit cross-spinal short latency responses in the contralateral leg.

10.1 Results

Although very little data exist for phase modulation of reflexes in the human hamstring muscle group, findings from Faist et al. [39] obtained through mechanical stimulation (tendon tap) and Floy [31] through stimulation of the sacral spinal roots seem in agreement with our results in that the M/H ratio or, for mechanical stimulation, the percentage of the maximal reflexive response, are increased around heel strike in Biceps Femoris.

Plenty of evidence is available, on the other hand, for modulation in Soleus [40, 41]. The modulation pattern of the H-reflex in soleus matches closely the results in this work, with a strong correlation between H-reflex amplitude and EMG activity in the muscle as well as suppression of the H-reflex corresponding to activation of the antagonist Tibialis Anterior during swing.

With regards to cross-spinal responses, results showed that only one subject exhibited a clear short latency (around 75ms) response in the contralateral biceps femoris, although recordings from other subjects also show less prominent

responses at the same time or later activation compatible with voluntary contractions in reaction to the perturbation to normal gait caused by the reflex. Although the nature of the stimulus is different, this timing matches closely results obtained by Stevenson et al. through mechanical perturbations of the knee [32]. By further developing the selection criteria for stimulation amplitude in this phase, evoking more consistent short latency trans-spinal responses is likely to be possible.

The timing of the M- and H-wave during the static recordings were 12.24 $\pm$ 1.62ms and 22.8 $\pm$ 2.19ms respectively for biceps femoris and 16.66 $\pm$ 1.63ms and 32.3 $\pm$ 2.68ms for soleus. These latencies fall within previously reported normality ranges for Soleus [42] or are compatible with similar findings for mechanical stimulation of Biceps Femoris [43].

Results for the Input-Output curves for the static recordings showed that in only four out of ten subjects M-max could be reached in biceps femoris with the available stimulation current range; similarly, Mmax was found only in one subject for Soleus. The previous group encountered similar limitations stimulating approximately 10cm higher [34]; sacral root stimulation can require up to two amperes to achieve Mmax [31]. Based on the curves obtained in the static phase, it is likely that by increasing the maximal stimulation current to approximately 140mA it would be possible to consistently reach Mmax.

The results mentioned above demonstrate that H-reflexes can be produced via sciatic nerve stimulation in both biceps femoris and soleus during gait, and that the resulting recordings can successfully be treated to extract information regarding both reflex modulation in the ipsilateral hamstrings and cross-spinal reflexes in the contralateral leg. It is also clear, however, that various technical challenges complicate the successful application of the technique, and that significant experience is required to properly conduct the experiments. Result from such protocol therefore will benefit from accumulated experience of the investigators and the streamlining of clear and repeatable procedures, as well as reliable software for data analysis supporting the operators during the experiments themselves.

10.2 Problem solution

With regard to the protocol and methods in general, a number of improvements can be suggested to enhance the results and to move closer to practical applications.

The most urgent area of improvement is perhaps the quality of recordings in biceps femoris. Several sources of noise and artifacts have been identified and, while mitigation is conducted in the current implementation, artifact reduction consideration must be integral to the development of a revised protocol and software. While the H-reflex is sufficiently distant from the major artifacts, more reliable measures would be possible if Mmax could be consistently achieved in Biceps Femoris, as it would allow cancellation of noise originating from the stimulation ar-

tifact and local contraction by subtracting a scaled supramaximal response instead of a subthreshold template, as was the case in this study. Other techniques such as double stimulation that takes advantage of the refractory period of nerve fibers to only elicit artifacts in the second pulse could also see application, if the parameters of such stimulation could be set so as not to contaminate the reflexive responses [44]. Furthermore, different electrode configurations or materials could lessen the impact of the stimulation artifact on the measurements of the M-wave, which are critical for many steps of the procedure from site selection to the construction of modulation curves. Amplifier stages with built in triggered blocking features could also be implemented to mitigate the stimulation artifact at the added cost of more complex hardware and the associated development and possibly certification of such equipment.

The distribution of stimulation levels used in the experiments can be reviewed in light of these results, and if the software is adapted, could even be individualized based on a short pre-experimentation screening session. Many of the stimulation levels used throughout this work did not give rise to reflexive responses and could be avoided in favor of a higher resolution of the input-output curves where it can be of more use. In a similar fashion, the selection of the points in the gait cycle could be redesigned to gather more information in the phases of gait where modulation is more prominent such as heel strike and the transition between stance and swing.

Selecting the site for stimulation is a critical point in the experiments as it is almost entirely dependent on the experimenter's interpretation of a limited set of data and on his own experience with similar experiments. Depending on the application, different factors can affect the selection as proximity to the recording site for Biceps Femoris and to the muscle belly itself affect the recordings significantly. In general, we have found the tradeoff to be between artifact amplitude and current threshold for eliciting the H and M waves, and while usually more than one site will successfully activate the sciatic nerve (as confirmed by observation of an H-wave in soleus), they seldom offer clean, artifact-free signals in Biceps Femoris. A software module could conceivably be developed to perform artifact correction on the fly based on subthreshold stimulations or just after static recordings using a Mmax template, making the interpretation of the raw results much easier.

As shown previously, the inability to consistently measure Mmax limits the options for artifact removal, but also has an impact on the construction of modulation curves as the selection of H-wave amplitudes to be used is based on shape features of the input-output curve rather than a more reliable calculation of the percentage of Mmax. Ultimately, the limiting factor is the distance between the stimulation electrodes and the target nerve; a simple solution could be that of increasing the stimulation current to increase activation of the sciatic nerve, with associated increases in the discomfort for the subjects and the noise levels. Current steering techniques might help shaping the electrical field in the tissue to direct more cur-

rent to the intended target and reduce dispersion without increasing the current intensity, but its application to a problem of this scale might not be straightforward.

Finally, only one subject appeared to show signs of a trans-spinal short latency response. This might be due to inappropriate selection of the stimulation amplitude for this test; in this project the cross-response part of the experiment was meant as an additional result, and added to an already lengthy protocol testing only one stimulation level. A small experiment to assess a larger range of currents (in relation to the response of the ipsilateral leg) could provide further information, eliciting cross-responses with an increased success rate.

10.3 Perspective

After this experiment a number of questions still remain. These open up possibilities for further studies. Below we will outline the three main future works which this group finds most important.

1. The protocol now that is has been tested in healthy subjects should be used to assess a number of subjects with SCI or stroke. For SCI an investigation of different subjects with spasticity could be used to see if evidence of lack of down modulation can be related to that spasticity. For stroke and investigation of subjects with hemiparetic gait could use to investigate the differences in the affected side and non-affected side.

2. Development of the protocol to more precisely target cross spinal reflexes should be sought. Inter-limb communication is of great interest to research into human gait and a tool to investigate this which can be adapted to existing robotic rehabilitation systems is very relevant to research into inter-limb reflexes in people with Stroke and SCI.

3. Both the biceps femoris and soleus are innervated by the tibial branch of the sciatic nerve. It is therefore unknown whether the common peroneal branch was reach during stimulation. This question along with the possibility to use this protocol to further investigation of the relationship between modulation of reflexes in the upper and lower leg is also an area that should be explored.

Chapter 11

Conclusion

A protocol based on H-reflex techniques targeting the sciatic nerve was developed and used to assess reflex phase modulation in the hamstrings during gait. The results from the assessment showed reflex modulation in both biceps femoris and soleus in agreement with results from other researchers using different techniques. Furthermore it was shown that it is plausible through further development of the protocol to target cross spinal reflexes.

Part V

Paper

MOCK-UP PAPER FOR EXAM PURPOSES - METHODOLOGIES

H-reflex Modulation in the Hamstrings During Gait via Sciatic Nerve Stimulation

Rasmus E. Andersen*†, Alessandro Ranieri †, Erika G. Spaich and Natalie Mrachacz-Kersting

DISCLAMER: This is a mock-up paper for an exam at Aalborg University it is not meant to be published or in any way used as if it was a published and peer-reviewed paper.

Abstract

Background: A number of different types of nerve damage can affect gait. For stroke and spinal cord injuries a number of people will be permanently affected, even with physiotherapy. A better understanding of reflexes role in gait along with better tools for assessing especially whether they are correctly modulated during gait might be very useful. Investigating stretch reflexes during gait can be very difficult. An alternative to assessing stretch reflexes and is use of the Hoffman reflex which uses the same fibers and circuits. This is used for a number of different muscle groups but has been largely ignored for the hamstring muscles. **Aim:** This study aims at investigate H-reflex phase modulation in both the biceps femoris and soleus using stimulation at the hamstrings during gait as a proof of concept this way of stimulating. **Methods:** 10 healthy subjects were recruited. Using the best of 9 possible stimulation electrodes constant currents of 1 ms duration between 0-100 mA were used to produce input-output curves for both static prone position and each 10% of the gait cycle for each subject walking at a speed of 3.5 kmph. **Results:** The H-reflex was down modulated in the period 10-80% of the gait cycle in biceps femoris and in the 60-10% of the gait cycle in soleus. **Conclusion:** Results show that sciatic nerve stimulations can be used to investigate both upper and lower leg reflexes modulation during gait.

Keywords: Hoffman Reflexes; Sciatic Nerve; Human Gait; Reflex Phase Modulation; Electromyography; Functional electrical stimulation; Healthy Subjects; Hamstrings

1 Background

A number of different types of nerve damage may affect gait [1, 2], with stroke and spinal cord injuries (SCI) representing the largest groups that can benefit from rehabilitation [3, 2]. The stroke incidence were 16 million globally causing 5.7 million deaths [4]. For SCI the incidence rates have been reported of 40 per million for the US and 16-15 per million of the EU [5]. For the survivors of both diseases temporary or chronic gait impairments are likely, with only 85% of stroke survivors walking unaided after 6 month and 19.2% of people with SCI suffering incomplete paraplegia [5].

As the understanding of the neural pathways is increasing, individualized rehabilitation protocols are beginning to find their way to the clinic, however gait is a complicated neurological task comprising many different systems and pathways and several parts are not yet fully understood [6]. Afferent feedback from proprioceptory organs sensing muscle stretch or tension in the tendons is known to play a role in gait, both for timing and recovering after perturbation [7, 8]. Activation of stretch reflexes can however be difficult during experimentation or in clinical assessment if the influence of phase modulation is interest. The Hoffman reflex (H-reflex) is a time proven and well understood technique that has been used as an alternative to stretch reflexes since it involves the same Ia afferents and α-motor neurons but is elicited via electrical stimulation along the axon [9], and can find application in the assessment of spinal circuits as well as the descending control during gait. In general the equipment for performing H-reflex studies is cheaper and more readily available than that required for mechanical perturbations of limbs or tendon, and could therefore be integrated with clinical assessment protocols or research when other assistive equipment is necessary for the subjects to perform motor tasks resembling natural gait.

*Correspondence: Rean08@student.aau.dk
Department of Health Science and Technology, Aalborg, Fredrik Bajers Vej 7D2, 9220 Aalborg, Denmark
Full list of author information is available at the end of the article
†Equal contributor

Despite the existence of H-reflex techniques and their established application in investigating various nerves and muscles groups [9, 10], the hamstring muscle group has been largely ignored. One Ph.D. Thesis from Floy [11] proposed a technique for the study of H-reflexes in the hamstrings through stimulation of sacral roots using stimulation intensities between 0 and 2 A during gait. A Master thesis [12] have investigated H-reflexes in the hamstrings via stimulation of the sciatic nerve in the upper thigh between 0 and 100 mA in the prone position.

The limited use and knowledge about hamstring H-reflexes should be improved upon given the large amount of people suffering nerve damage where correct identification of the neurological damage might help developing individualized therapies. H-reflex studies including both the upper and lower leg might also be a useful tool in further understanding the role reflexes and their phase modulation in Human gait.

Aim

This study aims to assess H-reflex phase modulation in both the biceps femoris and soleus muscles through stimulation of the sciatic nerve during gait.

2 Methods

2.1 Design

The experiment consisted of three phases. An initial preparation phase had the subjects lie down to apply an electrode matrix used to identify the optimal stimulation site for activation of the sciatic nerve. Each site was used in turn as a cathode, with a fixed anode over the great trochanter of femur. After locating the optimal site, stimulation was applied in randomized order between 0 and 100 mA in steps of 10 mA, for the construction of a static input-output curve of the H and M waves. After a brief break, the subjects were moved to a treadmill for the dynamic phase. The subjects walked at a speed of 3.4 km/h, and were stimulated at each 10% of the gait cycle over the same current range. Dynamic input-output curves can then be constructed. The outcome of the experiment consisted of modulation curves for two monitored muscles, the ipsilateral Biceps Femoris and Soleus.

2.2 Participants

Seven male and three female healthy volunteers (age mean-std: 25.9±2 years) were recruited among the students and staff of the Health Science and Technology department at Aalborg University. The participants were informed about the the purpose, procedure and risks of the experiment and gave consent for the experimentation. Ethical approval for the study was not sought as this type of student project, under the supervision of researchers and lecturers, is conducted in accordance to standing safety guidelines and does not warrant specific approval.

2.3 Materials and Setup

Stimulation was delivered by a constant current stimulator (NoxiTest IES 230, Aalborg, Denmark), through self-adhesive cloth-hydrogel electrodes (Axelgaard PALS line, Axelgaard, Denmark); the cathodes were round with a diameter of 3.2 cm, while the anode was a rectangular (3.3 x 5.3 cm) electrode of the same line. Stimulation triggering and recording were controlled by a laptop running Mr. Kick, a software developed in-house and running off LabView 2011 (National Instruments Corp, US). The software received triggering pulses from a custom footswitch amplifier (TTL output) and sent an output analog control signal to the stimulator through an USB DAQ module (NI USB-6229, National Instruments Corp). Two rack-mounted custom EMG amplifiers provided with variable gain and analog band-pass filtering stage (lower cutoff range: DC to 50 Hz; upper cutoff range: 500 to 5000 Hz) were connected to a preamplifying stage close to the recording site (gain: 0.1), and in turn to the self-adhesive EMG electrodes (Neuroline 720, Ambu, Denmark). For the dynamic phase, a round (4 cm diameter) pressure transducer was applied under the sole, on the posterior-lateral side of the heel. The subjects walked in a split-belt treadmill (Woodway USA Inc., US), secured to a harness (Maine Anti-Gravity Systems, US) providing no weight support.

2.3.1 Recording Electrode placement

Recording electrodes were placed on right biceps femoris and soleus. All recording electrodes were connected in double differential configuration with short wires connecting the three electrodes to the preamplification stage. The biceps femoris electrodes were placed more distally along the line recommended by the SENIAM project, in order to distance it from the stimulation cathode, see figure 1. The soleus electrodes were placed on the lateral aspect of the muscle, approximately two thirds of the distrance from the knee to the ankle.

2.3.2 Stimulation Electrode placement

Nine round PALS electrodes were placed in a three by three grid included in a square marked on the hamstrings. The location of the square is found by first marking out the halfway point (C) between of the greater trochanter of femur and its lateral protrusion at the knee (see figure 1). The circumference of the thigh at the midpoint was then measured. Approximately 110° towards the back of the knee a point is

marked, representing the center point of the lower edge of the reference square. A 10 by 10 cm is then drawn upwards. The single anode is placed on the greater trochanter of the femur.

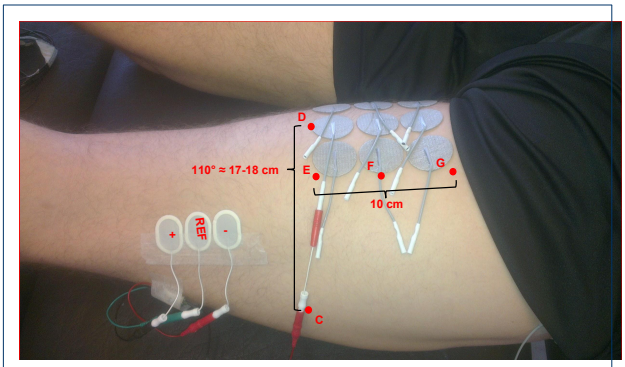


Figure 1 The picture shows the placement of the stimulation cathodes in a square marked above point (D). The EMG recording electrodes for the biceps femoris can be seen marked with (+), (−) and (REF).

2.3.3 Stimulation and recording parameters

All delivered stimulations consisted in unipolar constant current square pulses of 1 ms duration. Amplitude of stimulation varied between 0 and 100 mA. EMG signals recorded from Biceps Femoris were conditioned by analog band-pass filtering between 0.1 and 500 Hz. For Soleus signals the bandwidth was between 5 and 500 Hz. The triggering signal is mirrored to one of the analog input channels and stored along the two EMG tracks. All signals are digitalized at 5000 Hz by the DAQ module.

2.4 Procedure

Electrodes are fitted to the subject, and test stimulations between 0 and 30 mA are administered to verify the setup and allow the subject to familiarize with the sensation. If the subject does not feel pain or discomfort, each of the nine cathodes is used in turn as a cathode, stimulating between 0 and 60 mA in steps of 10 mA, for three repetitions each. After all sites have been stimulated, the recordings are inspected and one to three candidate sites are selected. A pressure strap fitted with a soft ball is applied to the candidate sites in turn, and they are re-tested from 0 to 100 mA in steps of 10 mA. Recordings are assessed once more and a final optimal site is chosen. Ideally, the site should yield well defined waves for the two responses, a fast recovery of the stimulation artifact rebound, and an identifiable M-max in the inspection I-O curve for Biceps Femoris. However, it is more likely that a compromise between noise and quality of the results is to be made. The optimal site is used to stimulate between 0 and 100 mA, in random order for three repetitions, to provide the data for construction of static input-output curves for the two responses. After a short break, the subject is transferred to the treadmill, secured to the harness and tubular elastic gauze is fitted to both legs to secure the electrodes and their leads. The treadmill is started and brought to a speed of 3.4km/h; once the subject has had time to stabilize his or her gait, dynamic tests are initiated. Starting at heel strike, the subject receives four repetitions of the same current amplitudes employed in the static experiment, in random order, for each 10% of the gait cycle.

2.5 Data processing

After reconstructing the randomized data to sort the recordings by subject, stimulation amplitude, and timepoint, artifact removal is performed on all recordings from Biceps Femoris to alleviate the effect of electrical artifacts caused by proximity to the stimulation site, detailed below. Static and dynamic input-output curves are constructed, from which the modulation curve is finally obtained. Background activity in the two muscles is also assessed at each stimulation; the raw signals are digitally filtered separately (High pass, fc = 15 Hz, 4th order butterworth zero-phase filtering) and full-wave rectified. For each stimulation, an interval of 100 ms just before stimulation onset is extracted, and the RMS amplitude of the EMG signal is computed and stored.

2.5.1 Artefact removal

A slow recovery rebound just after the stimulation artifact itself, likely caused by the filtering stage in the EMG amplifiers, can distort measures of the M-wave. To reduce the impact of such artifact, a correction algorithm was implemented, adapted from the one presented in [10] but taking advantage of a subthreshold stimulation instead of one eliciting a maximal M-wave. The method takes advantage of the apparent linearity of the artifact, subtracting from the recordings a matched and scaled template built individually for each subject. The correction is applied only to the ipsilateral Biceps Femoris recording as this artifact is not evident in Soleus. For each subject, a template is extracted from the first 60 ms after the stimulation artifact peak of a low intensity stimulation from the static phase (10 mA), representative of the stimulation artifact in iBF. For every subsequent recording from the same subject, the template is scaled and translated to match each of the dynamic phase responses, and subtracted from the signal in the same 60 ms window.

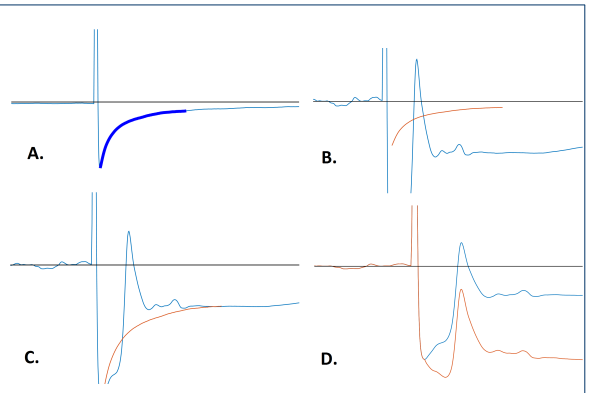


Figure 2 The artefact removal procedure. A: a low intensity stimulus in the static phase contains the typical shape of the stimulation artifact tail. B: The template is aligned with the stimulation. C: the template is scaled to match the signal. D: Subtracting the template yields the corrected signal.

2.5.2 H-reflex And M-wave Measurements

To measure H and M waves, positive and negative peaks are detected in individualized intervals based on preliminary inspection of the data. These are then sorted in descending amplitude order; the largest positive and negative peaks are then used for computing the peak to peak amplitude in the interval. The peak to peak amplitude and the latency of the earliest of the two peaks are stored as the response amplitude and latency.

2.5.3 Modulation

In order to construct the H-reflex modulation, first the motor threshold for each timepoint that has been stimulated in the dynamic phase is identified. Since methods based on sigmoid fitting or similar to predict the current level corresponding to the motor threshold did not give satisfactory results on a dataset with only ten datapoints, the following method was used.

The first four intensities are used to establish a baseline in terms of the mean M-wave in that interval ($MeanM$) and its standard deviation ($StdM$); the first current level that gives rise to an M-wave larger than $MeanM + 2 * StdM$ is chosen as the reference point for that gait cycle phase, and the ratio between the H-wave and M-wave amplitudes corresponding to that current level is then used to construct modulation curves of the H-reflex in the two muscles.

3 Results

All experimental results show that the proposed protocol was able to successfully activate the sciatic nerve, allowing measurement of H and M waves in all subjects and under both static and dynamic conditions.

In order to properly compare different subjects and conditions, however, the maximal efferent-mediated response (MMax) is frequently used to normalize the afferent mediated response. Results indicated that a plateau for the M-wave could be achieved in four out of ten subjects for Biceps Femoris, but only in one out of ten subjects for Soleus, although sigmoid fittings of the I-O curves suggest that in four more subjects MMax could have been reached with a modest increase (20 to 40%) in stimulation amplitude, not allowed by the equipment.

From a subject comfort point of view, the procedure was reported to be painful by only one subject, and only in the relatively brief static phase of the experiments. Cold sweats and uncomfortable sensations were reported by most subjects for the highest stimulation intensities (70mA and upwards), as expected but none decided to interrupt the experiments.

3.1 Static recordings

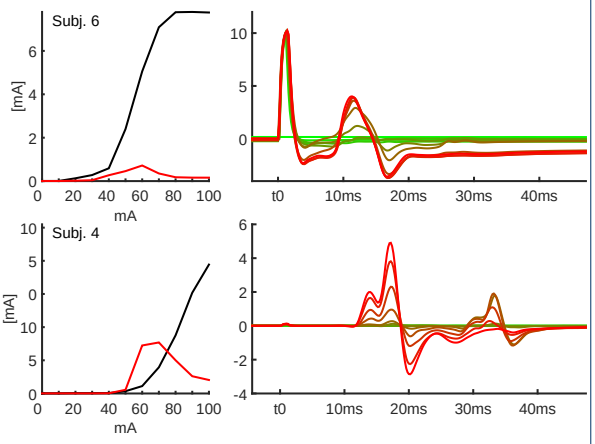


Figure 3 Representative data from Biceps Femoris (upper row) and Soleus (lower row). The input-output curves are shown on the left (red: H-wave; black: M-wave). The averaged responses (by stimulation amplitude, green: 0mA; red: 100mA) are shown on the right.

While the EMG signals from Soleus are remarkably clear and repeatable, with a clear separation of stimulation artifact, M-wave and H-wave, recordings from Biceps Femoris suffer from the proximity to the stimulation site. Two main sources of artifacts were identified. The first is a slow recovery rebound of the stimulation artifact past the zero line, lasting well past the end of the M-wave, which can significantly distort it and compromise the measurements at lower stimulation intensities. The second source of noise appears to be related to direct activation of either the local branch innervating Biceps Femoris or the muscle fibers

Muscle	M-Wave latency	H-Wave latency
Biceps Femoris	12.24 ± 1.62ms	22.8 ± 2.19ms
Soleus	16.66 ± 1.63ms	32.3 ± 2.68ms

Table 1 Latencies in the static phase.

themselves, manifesting itself as a large artifact overlapping the M-wave that can appear for stimulation amplitudes as low as 20mA.

Intervals for the recognition and measurements of the two responses were set individually for each subject, based on inspection of the static phase recordings. The individual intervals were included in the 5-18ms range (M-wave) and 18-45ms range (H-wave) in Biceps Femoris; 9-23ms (M-wave) and 23-45ms (H-wave) for Soleus. Table 1 summarizes the actual latencies of the first peak of each response in the static (prone) sessions.

The previously discussed mitigation technique however alleviated at least the first component, allowing the construction of modulation curves for the H-reflex in both muscles.

3.2 Dynamic Input-Output curves

Input-output curves were constructed for the H and M responses from the dynamic experiments. Representative data is shown in figure 4. The data shows that as the gait cycle develops both M and H curves shift on the stimulation amplitude axis. This effect is canceled by the selection of time-specific H waves for constructing the modulation curves that ensure the degree of neural activation is approximately the same, and that that the only effect left in the modulation curves is that of gait phase.

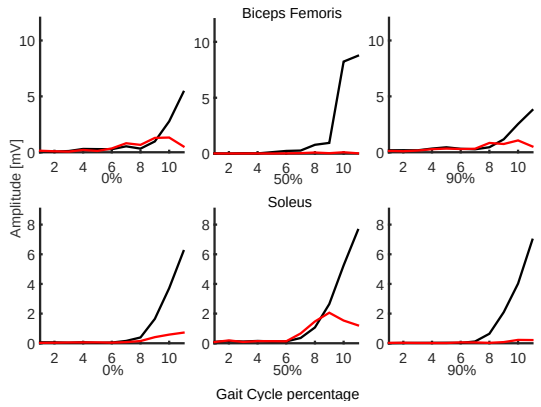


Figure 4 Dynamic input-output curves from one subject in the two muscles. The M-wave is indicated in black, the H-wave in red.

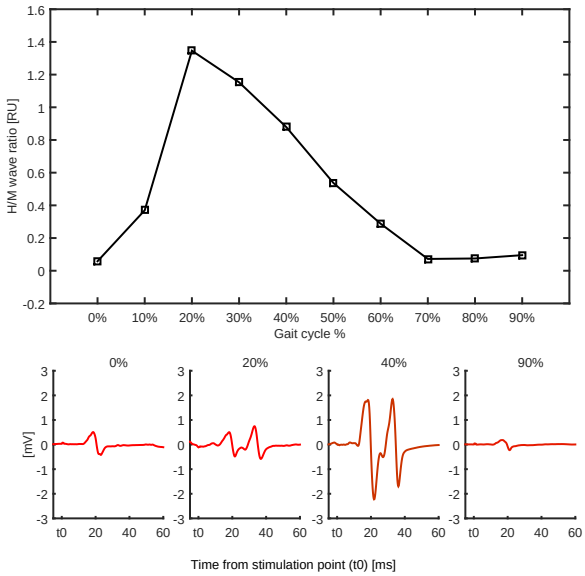


Figure 5 The modulation curve in Soleus for subject 5. A subset of the individual recordings that have been selected for the construction of the curve are shown in the bottom row.

3.3 Phase modulation in Biceps femoris and Soleus

Finally, modulation curves show how the H to M ratio changes over the course of the gait cycle. Figure 5 exemplifies the individual responses picked by the algorithm to construct the modulation curve in Soleus for one participant.

Aggregate modulation plots (figure 6) illustrate how the phase modulation roughly follows the trend of the background EMG activity measured just before stimulation. The modulation patterns roughly match the background EMG pattern; although in Soleus the increase of reflex activity appears to precede the onset of muscular contractions in time.

4 Discussion

The goal of this study was to assess H-reflex phase modulation in both the biceps femoris and soleus muscles through electrical stimulation of the sciatic nerve during gait.

The modulation curves for the H-reflex in both muscles are consistent with previous results from experiments using other stimulation locations [11] and modalities [13, 14].

The timing of the M and H waves during the static recordings were 12.24 - 1.62ms and 22.8 - 2.19ms respectively for biceps femoris and 16.66 - 1.63ms and 32.3 - 2.68ms for soleus. These latencies fall within previously reported normality ranges for Soleus or are

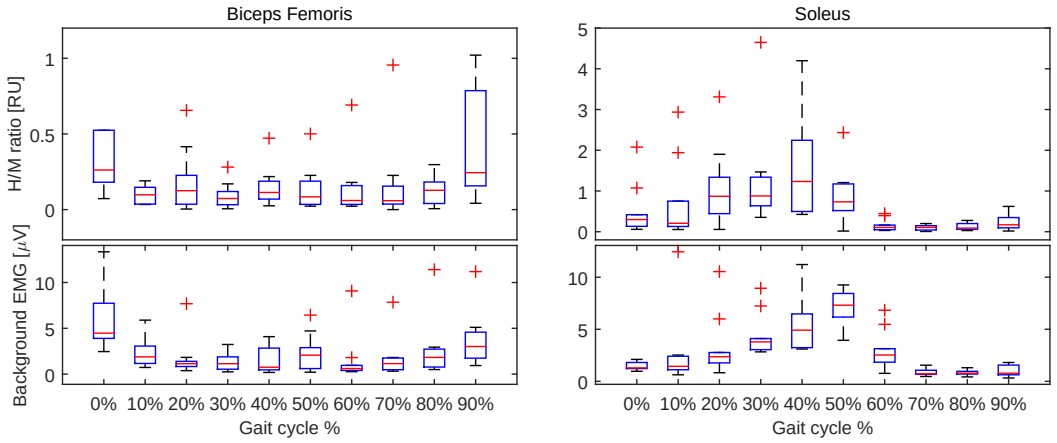


Figure 6 Modulation curves. On the top half, the ratio of H-wave to M-wave across all subjects to the experiment are shown. The bottom half shows the RMS background activity in the 100ms preceding stimulation for each time point of the gait cycle.

compatible with similar findings for mechanical stimulation of Biceps Femoris.

While Mmax could be achieved in a number of subjects, it cannot be consistently used for making comparisons, and as the quality of the modulation curves is directly linked to the selection of H amplitudes based on the level of nerve activation, the current state of the procedure is not the optimal one. An increase of the stimulation amplitude to 140 mA should allow obtaining Mmax in most subjects, at least for Biceps Femoris; the associated discomfort or pain however might limit the practical application of the procedure.

It is also apparent that both the stimulation levels and the timepoints for the analysis could be better distributed, to gather more information in the ranges of interest for the different muscles without lengthening the experiments.

The most urgent area of improvement is the quality of recordings in biceps femoris. Several sources of noise and artifacts have been identified, but depending on the choice of the stimulation site, the mitigation method that was implemented might not be sufficient.

4.1 Conclusion

We believe the results provide enough evidence that stimulation at this location for the study of reflex modulation in the hamstrings is feasible, and that while technical limitations are certainly present, the experience gathered with this work could allow further iterations on the procedure to overcome them and find application as a relatively low cost tool in research and clinical settings.

Competing interests
The authors declare that they have no competing interests.

Author's contributions
REA and AR designed and performed the experiment, analyzed data and wrote the paper; NMK and EGS supervised the project.

Acknowledgements
The authors would like to acknowledge the technical assistance and guidance provided by Andrew J. T. Stevenson, Knud Larsen and Jan Stavnshøj.

References

1. Stolze, H., Klebe, S., Baecker, C., Zechlin, C., Friege, L., Pohle, S., Deuschl, G.: Prevalence of gait disorders in hospitalized neurological patients. Movement disorders : official journal of the Movement Disorder Society **20**(1), 89–94 (2005)
2. Olney, S.J., Richards, C.: Hemiparetic gait following stroke. Part 1: Characteristics. Gait & Posture **4**, 136–148 (1996)
3. Hall, K.M., Cohen, M.E., Wright, J., Call, M., Werner, P., Km, A.H., Me, C., Wright, J., Call, M., Care, S.: Characteristics of the Functional Independence Measure in Traumatic Spinal Cord Injury (1999)
4. Strong, K., Mathers, C., Bonita, R.: Preventing stroke: saving lives around the world. Lancet Neurology **6**(February), 182–187 (2007)
5. Cripps, R.a., Lee, B.B., Wing, P., Weerts, E., Mackay, J., Brown, D.: A global map for traumatic spinal cord injury epidemiology: towards a living data repository for injury prevention. Spinal cord **49**(4), 493–501 (2011)
6. Duysens, J., Van de Crommert HW: Neural control of locomotion; The central pattern generator from cats to humans. Gait & posture **7**, 131–141 (1998)
7. Van de Crommert HW, Mulder, T., Duysens, J.: Neural control of locomotion: sensory control of the central pattern generator and its relation to treadmill training. Gait & posture **7**, 251–263 (1998)
8. Stevenson, A.J.T., Geertsen, S.S., Andersen, J.B., Sinkjæ r, T., Nielsen, J.B., Mrachacz-Kersting, N.: Interlimb communication to the knee flexors during walking in humans. The Journal of physiology **591**, 4921–35 (2013)
9. Capaday, C., Stein, R.B.: Amplitude modulation of the soleus H-reflex in the human during walking and standing. The Journal of neuroscience : the official journal of the Society for Neuroscience **6**(May), 1308–1313 (1986)
10. Larsen, B., Voigt, M.: Quadriceps h-reflex modulation during pedaling. Journal of Neurophysiology **96**(1), 197–208 (2006)
11. Floy, B.W.: "Modulation of hamstrings reflexive responses during human gait." PhD thesis. University of Iowa (2012)

12. Dueholm, S.S., Rasmussen, J.H.: "Intersession reliability study of Hreflexes in the hamstring muscle group elicited by a novel technique to stimulate the sciatic nerve" Master thesis. Aalborg University (2013)
13. Faist, M., Blahak, C., Duysens, J., Berger, W.: Modulation of the biceps femoris tendon jerk reflex during human locomotion. Experimental Brain Research **125**(3), 265–270 (1999). doi:10.1007/s002210050682
14. Faist, M., Dietz, V., Pierrot-Deseilligny, E.: Modulation, probably presynaptic in origin, of monosynaptic ia excitation during human gait. Experimental Brain Research **109**(3), 441–449 (1996). doi:10.1007/BF00229628

Bibliography

[1] Henning Stolze et al. "Prevalence of gait disorders in hospitalized neurological patients." In: *Movement disorders : official journal of the Movement Disorder Society* 20.1 (Jan. 2005), pp. 89–94. ISSN: 0885-3185.

[2] Kathryn L. McCance and Sue E. Huether. *Pathophysiology: The Biologic Basis for Disease in Adults And Children*. 5th ed. Elsevier Mosby, 2006, p. 1780. ISBN: 9780323035071.

[3] Jan Mehrholz et al. "Electromechanical-assisted training for walking after stroke." In: *The Cochrane database of systematic reviews* 7.7 (Jan. 2013), p. CD006185. ISSN: 1469-493X.

[4] Jan Mehrholz, Joachim Kugler, and Marcus Pohl. "Locomotor training for walking after spinal cord injury." In: *The Cochrane database of systematic reviews* 11 (Jan. 2012), p. CD006676. ISSN: 1469-493X.

[5] Kathleen Strong, Colin Mathers, and Ruth Bonita. "Preventing stroke: saving lives around the world". In: *Lancet Neurology* 6.February (2007), pp. 182–187.

[6] Amie B. Jackson et al. "A demographic profile of new traumatic spinal cord injuries: Change and stability over 30 years". In: *Archives of Physical Medicine and Rehabilitation* 85.11 (Nov. 2004), pp. 1740–1748. ISSN: 00039993.

[7] Hugues Barbeau et al. "Walking after spinal cord injury: evaluation, treatment, and functional recovery." In: *Archives of physical medicine and rehabilitation* 80 (1999), pp. 225–235. ISSN: 0003-9993.

[8] I. Benson et al. "Lower-limb exoskeletons for individuals with chronic spinal cord injury: Findings from a feasibility study". In: *Clinical Rehabilitation* (2015). ISSN: 0269-2155.

[9] April Cox, Abhay Varma, and Naren Banik. "Recent advances in the pharmacologic treatment of spinal cord injury". In: *Metabolic Brain Disease* (2014), pp. 473–482. ISSN: 08857490.

[10] Federica Tamburella. ""GAIT RECOVERY IN SPINAL CORD INJURY SUBJECTS: From clinical experience to research developments" PhD thesis". In: *Aalborg University* (2015).

[11] Jacques Duysens and Van de Crommert HW. "Neural control of locomotion; The central pattern generator from cats to humans." In: *Gait & posture* 7 (1998), pp. 131–141. ISSN: 1879-2219.

[12] Van de Crommert HW, Theo Mulder, and Jacques Duysens. "Neural control of locomotion: sensory control of the central pattern generator and its relation to treadmill training." In: *Gait & posture* 7 (1998), pp. 251–263. ISSN: 1879-2219.

[13] Agamemnon Despopoulos and Stefan Silbernagl. *Color atlas of physiology*. 5th ed. Theime, 2003. ISBN: 3135450058.

[14] Frederic H Martini, Judi L Nath, and Edwin F Bartholomew. *Fundamentals of Anatomy and Physiology*. 8th ed. Vol. 7th. Pearson, 2009, p. 1123. ISBN: 0321539109.

[15] P J Whelan. "Control of locomotion in the decerebrate cat." In: *Progress in neurobiology* 49.403 (1996), pp. 481–515. ISSN: 0301-0082.

[16] Kathryn L. McCance and Sue E. Huether. *Pathophysiology: The Biologic Basis for Disease in Adults And Children Fifth Edition*. Mosby, 2005. ISBN: 0323035078.

[17] Debraj Mukherjee and Chirag G. Patil. "Epidemiology and the global burden of stroke". In: *World Neurosurgery* 76 (2011), pp. 85–90. ISSN: 18788750.

[18] John W McDonald and Cristina Sadowsky. "Spinal-cord injury." In: *Lancet* 359.9304 (Feb. 2002), pp. 417–25. ISSN: 0140-6736.

[19] Sandra J Olney and Carol Richards. "Hemiparetic gait following stroke. Part 1: Characteristics". In: *Gait & Posture* 4 (1996), pp. 136–148.

[20] R R Menter et al. "Mortality, morbidity, and psychosocial outcomes of persons spinal cord injured more than 20 years ago". In: 30 (1992), pp. 617–630.

[21] Markus Wirz et al. "Effectiveness of automated locomotor training in patients with chronic incomplete spinal cord injury: a multicenter trial." In: *Archives of physical medicine and rehabilitation* 86.4 (Apr. 2005), pp. 672–80. ISSN: 0003-9993.

[22] S. Claiborne Johnston, Shanthi Mendis, and Colin D. Mathers. "Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modelling". In: *The Lancet Neurology* 8 (2009), pp. 345–354. ISSN: 14744422.

[23] Amanda G. Thrift et al. "Global stroke statistics". In: *International Journal of Stroke* 9.January (2014), pp. 6–18. ISSN: 17474930.

[24] R a Cripps et al. "A global map for traumatic spinal cord injury epidemiology: towards a living data repository for injury prevention." In: *Spinal cord* 49.4 (Apr. 2011), pp. 493–501. ISSN: 1476-5624.

[25] M Wyndaele and J-J Wyndaele. “Incidence, prevalence and epidemiology of spinal cord injury: what learns a worldwide literature survey?” In: *Spinal cord* 44.9 (Sept. 2006), pp. 523–9. ISSN: 1362-4393.

[26] Valery L. Feigin et al. “Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review”. In: *The Lancet Neurology* 8 (2009), pp. 355–369. ISSN: 14744422.

[27] World Health Organization. *ICD-10 Version:2015*. June 2015. URL: http://apps.who.int/classifications/icd10/browse/2015/en.

[28] American Spinal Injury Association. *International Standards for Neurological Classification of SCI (ISNCSCI) Exam*. June 2015. URL: http://www.asia-spinalinjury.org/elearning/ISNCSCI.php.

[29] Amie B. Jackson et al. “A demographic profile of new traumatic spinal cord injuries: Change and stability over 30 years”. In: *Archives of Physical Medicine and Rehabilitation* 85.11 (Nov. 2004), pp. 1740–1748. ISSN: 00039993.

[30] Peter W. New, H.Barry Rawicki, and Michael J. Bailey. “Nontraumatic spinal cord injury: Demographic characteristics and complications”. In: *Archives of Physical Medicine and Rehabilitation* 83.7 (July 2002), pp. 996–1001. ISSN: 00039993.

[31] Brad Wayne Floy. “"Modulation of hamstrings reflexive responses during human gait." PhD thesis”. In: *University of Iowa* (2012). URL: http://ir.uiowa.edu/etd/2871.

[32] Andrew J T Stevenson et al. “Interlimb communication to the knee flexors during walking in humans.” In: *The Journal of physiology* 591 (2013), pp. 4921–35. ISSN: 1469-7793.

[33] Sabata Gervasio et al. “Crossed reflex reversal during human locomotion”. In: *Journal of neurophysiology* 109.9 (2013), pp. 2335–2344.

[34] Søren S. Dueholm and Jesper H. Rasmussen. “"Intersession reliability study of Hreflexes in the hamstring muscle group elicited by a novel technique to stimulate the sciatic nerve" Master thesis”. In: *Aalborg University* (2013).

[35] Henry Gray. *Anatomy of the human body, by Henry Gray. 20th ed.* Lea and Febiger, 1918.

[36] Lindsay E. Beaton and Barry J. Anson. “The relation of the sciatic nerve and of its subdivisions to the piriformis muscle”. In: *The Anatomical Record* 70.1 (1937), pp. 1–5. ISSN: 1097-0185. DOI: 10.1002/ar.1090700102.

[37] H. Schmalbruch. “Fiber composition of the rat sciatic nerve”. In: *The Anatomical Record* 215.1 (1986), pp. 71–81. ISSN: 1097-0185. DOI: 10.1002/ar.1092150111. URL: http://dx.doi.org/10.1002/ar.1092150111.

[38] Wikimedia Foundation. *Wikiversity Journal of Medicine*. June 2015. URL: https://en.wikiversity.org/wiki/Wikiversity_Journal_of_Medicine.

[39] M. Faist et al. "Modulation of the biceps femoris tendon jerk reflex during human locomotion". English. In: *Experimental Brain Research* 125.3 (1999), pp. 265–270. ISSN: 0014-4819. DOI: 10.1007/s002210050682.

[40] C Capaday and R B Stein. "Amplitude modulation of the soleus H-reflex in the human during walking and standing." In: *The Journal of neuroscience : the official journal of the Society for Neuroscience* 6.May (1986), pp. 1308–1313. ISSN: 0270-6474.

[41] M. Faist, V. Dietz, and E. Pierrot-Deseilligny. "Modulation, probably presynaptic in origin, of monosynaptic Ia excitation during human gait". English. In: *Experimental Brain Research* 109.3 (1996), pp. 441–449. ISSN: 0014-4819. DOI: 10.1007/BF00229628. URL: http://dx.doi.org/10.1007/BF00229628.

[42] C.J.M. Frijns et al. "Normal values of patellar and ankle tendon reflex latencies". In: *Clinical Neurology and Neurosurgery* 99.1 (1997), pp. 31 –36. ISSN: 0303-8467. DOI: http://dx.doi.org/10.1016/S0303-8467(96)00593-8.

[43] Sven Bruhn, Christian Leukel, and Albert Gollhofer. "Differential effects of stimulus characteristics during knee joint perturbation on hamstring and quadriceps reflex responses". In: *Human movement science* 30.6 (2011), pp. 1079–1091.

[44] K.C. McGill et al. "On the Nature and Elimination of Stimulus Artifact in Nerve Signals Evoked and Recorded Using Surface Electrodes". In: *Biomedical Engineering, IEEE Transactions on* BME-29.2 (1982), pp. 129–137. ISSN: 0018-9294.