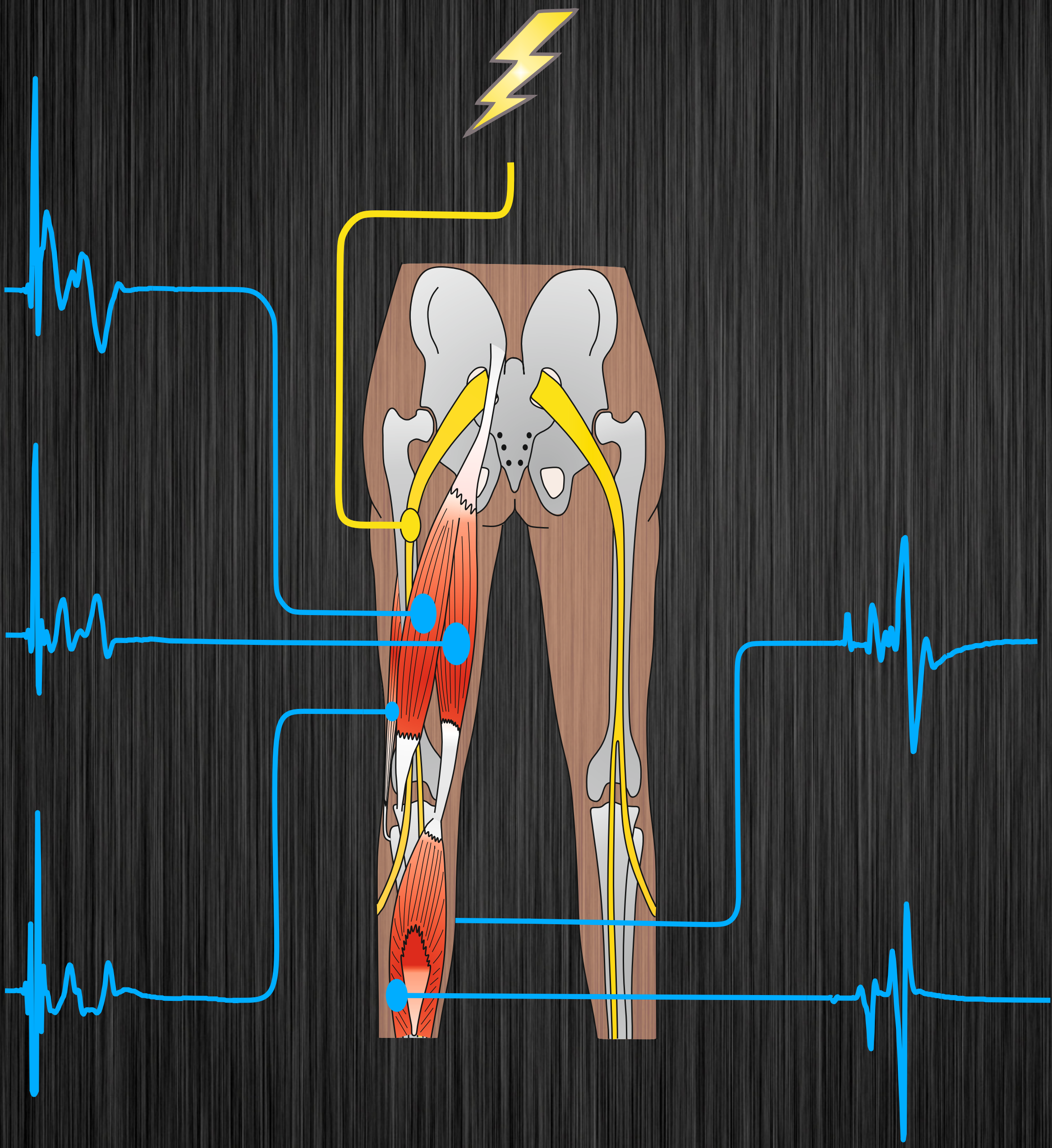
Intersession reliability study of H-reflexes in the hamstring muscle group elicited by a novel technique to stimulate the sciatic nerve



Master thesis • 2013

Biomedical Engineering and Informatics

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Abstract:

H-reflexes are well documented as a tool to estimate the synaptic activity of the motoneuron pool. Only little research has focused on the modulation of H-reflexes in the hamstring muscle group (HMG) despite their importance in human gait. The aim was to design and validate a new technique to stimulate the sciatic nerve and to evaluate the intersession reliability of this techniques' ability to elicit H-reflexes in the HMG. 12 healthy subjects (25.58±0.99 yrs) lying in prone position were applied electrical stimuli through a custom-made 4x2 grid electrode (cathode) placed two cm below the buttocks with anode placed above trochanter major of the dominant leg. Optimal cathode site was found by applying stimuli 25% above the threshold of Ia afferents to each grid site. sEMG was recorded from the long and the short heads of biceps femoris (LH-BF and SH-BF), semitendinosus (SEMI), soleus (SOL) and tibialis anterior (TA). SOL and TA were control muscles. Stimuli were applied at the optimal cathode site (10 to 100 mA) and H_{max}, and the latencies for M-waves and H-reflexes were extracted as parameters. Two identical sessions were conducted one day apart and intraclass correlation coefficients (ICC) were calculated for each parameter within each muscle. Data showed reliable H_{max} ICCs for LH-BF (0.967), SH-BF (0.762) and SEMI (0.736). ICC values for M-wave- and H-reflex latencies for LH-BF (0.931 and 0.993), SH-BF (0.935 and 0.99) and SEMI (0.7 and 0.978) were considered reliable according to the benchmarks of Landis and Koch (1977). H-reflexes were observed in SOL and TA. The novel technique provided reliable H-reflexes in the HMG, and can be used as a tool to investigate synaptic activity in the motoneuron pool. The technique might be able to test the interaction between leg muscles.

Danish summary - Dansk resumé

Det er veldokumenteret at H-reflekser afspejler synaptisk aktivitet af motoriske celler i rygmarven [Misiaszek 2003]. Få studier har fokuseret på modulationen af H-reflekser i hasemusklerne på trods af deres vigtighed under menneskets gangcyklus. Formålet med denne specialeafhandling var at designe og validere en ny teknik til at stimulere iskiasnerven og at evaluere pålideligheden af denne tekniks evne til at udløse H-reflekser i hasemusklerne.

12 mandlige forsøgspersoner (25.58±0.99 år) deltog i et eksperimentelt forsøg. Forsøgspersonerne lå udstrakt på maven mens elektriske stimulationer blev påført igennem et speciallavet 4x2 elektrodenet (brugt som katoder) placeret to cm under balden og en anode placeret på trochanter major på forsøgspersonens dominante ben. Den optimale lokalitet til at stimulere iskiasnerven blev fundet ved at give elektriske stimulanser 25% over aktiveringstærsklen for de Ia afferente fibre, igennem alle katode-anode-konfigurationerne. Elektromyografiske signaler blev målt med overflade-elektroder placeret over det lange- og det korte hoved af m. biceps femoris (forkortet LH-BF og SH-BF), m. semitendinosus (SEMI), m. soleus (SOL) og m. tibialis anterior (TA). De sidste to nævnte muskler blev anvendt som kontrolmuskler. Stimulationer blev påført ved den tidligere fundne optimale katode-lokalitet til at stimulere iskiasnerven med intensiteter varierende fra 10-100 mA. H_{max}, M-wave og H-reflex latencies blev ekstraheret fra data og brugt som fysiologiske parametre. To identiske sessioner blev udført med én dags mellemrum og intraclass correlation coefficients (ICC) blev udregnet indenfor hver fysiologisk parameter for hver muskel.

Data viste pålidelige H_{max} ICC værdier for LH-BF (0.967), SH-BF (0.762) og SEMI (0.736). ICC værdier for M-wave- og H-refleks latencies for LH-BF (0.931 og 0.993), SH-BF (0.935 og 0.99) og SEMI (0.7 og 0.978) ansås for pålidelige ud fra benchmarkene foreslået af Landis og Koch (1977). H-reflekser blev observeret i SOL og TA.

Vores nye teknik til at stimulere iskiasnerven viste pålidelige H-reflekser i hasemusklerne og kan bruges som værktøj til at undersøge den synaptiske aktivitet af de motoriske celler i rygmarven. Denne teknik kan eventuelt bruges i fremtidige studier til at undersøge interaktionen imellem benmuskler, f.eks. under gangcyklys.

Preface

This thesis is made as a completion of the master education in Biomedical Engineering and Informatics. The authors hold a bachelor degree in Biomedical Engineering and Informatics from Aalborg University, Department of Health Science & Technology.

Several persons have contributed academically, practically and with support to this master thesis. We would therefore like to thank our two supervisors Natalie Mrachacz-Kersting and Erika Geraldina Spaich for their time, valuable input and support throughout the entire master period.

Furthermore we would like to thank Knud Larsen for his help in programming and debugging the software used when conducting the experiments.

We would also like to thank Jan Stavnshøj and Søren Wøhlk Nielsen for their big help in constructing and testing electrical hardware.

Finally we would like to thank our family and friends for being helpful and supportive during our time studying Biomedical Engineering and Informatics at Aalborg University.

Aalborg University 2013

Søren Simonsen Dueholm

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Reading instruction

The thesis is divided into four parts and is intended to be read chronologically. The first three parts contain the IMRAD structure (introduction, methodology, results and discussion), while the last part contains appendixes and a bibliography.

The content of the chapters is briefly described in the beginning of each new chapter.

Citations are presented in accordance with the Chicago style and the list of the citations is located at the end of this thesis. The author's surname and publication year are noted in squared brackets, for example, e.g. [Hoffmann 1910]. Publications with two authors are cited as, e.g. [Capaday and Stein 1986]. Publications with more than two authors are cited as, e.g. [Palmieri et al. 2004]. References to tables and figures in the thesis are noted as e.g. "Table 4.1" or "Figure 2.4" where the first number refers to the chapter and the second refers to the sequence in which the figure occurs in the chapter. The first time a frequently used term is used in this thesis, it will be abbreviated and the abbreviation will be used later on.

All figures and tables in this thesis are made by the project group.

Contents

1 Introduction

Neuronal synaptic connections and activity in the spinal cord are difficult to examine, because of their density and complexity. Synaptic activity can be estimated using reflexes. The stretch reflex and the Hoffmann reflex which are monosynaptic reflexes have been used in a vast number of studies to examine the synaptic activity by stimulating afferent fibres and then measure the output from the spinal cord as muscle responses. In this thesis synaptic activity is defined as the excitability of the motoneuron pool in the spinal cord. New research investigating the synaptic activity is providing information about of the human nervous system. Especially the understanding of human reflexes has been expanded due to experimental research and new knowledge within the field of reflexes is continuously introduced as new experimental protocols are developed and tested.

The mentioned reflexes, which are focusing on activation of afferent fibres in order to activate skeletal muscle fibres, are considered fairly simple due to the monosynaptic neural circuitry they use. This is, however, not entirely true as discussed later in this chapter. In the following section the two reflexes will be presented and the similarities and differences of the two will be discussed. First there will be a short description about the historical background of the reflexes where after a section about activation of afferent fibres is presented. Different synaptic connections involved in the reflexes and reflex modulating factors are described. Finally issues when recording the Hoffmann reflexes from the hamstring muscle group, and why this is of interest, will be discussed before the aim of this thesis is formulated.

1.1 Background of the reflexes

The stretch reflex (deep tendon reflex, myotatic reflex, short latency reflex) is an involuntary muscle contraction in response to a muscle stretch. The purpose of the stretch reflex is to automatically regulate the skeletal muscle length and thereby maintain or change body posture and position. [Kandel et al. 2013, chap.35] Liddell and Sherrington (1924) was the first to describe the neural pathway consisting of a single synapse located in the spinal cord transmitting signals from a Ia afferent fibre to an alpha motoneuron (αMN) in the decerebrate cat [Liddell and Sherrington 1924].

The Hoffmann reflex, also called the H-reflex, is often described as an electrically induced analogue to the mechanically induced stretch reflex and is a valuable tool in assessing modulations of synaptic activity in the spinal cord. The H-reflex was first shown by Paul Hoffmann in 1910 and later given his name [Hoffmann 1910, Magladery and McDougal Jr. 1950]. The H-reflex is well studied and described in human and mammalian neurophysiological literature Misiaszek [2003]. H-reflexes can be used as a tool to investigate the synaptic activity and have been used both in research and clinical settings. The fact that H-reflexes are easy to elicit and have been evoked in almost 20 muscles in the human body, mainly muscles of the hand, arm, foot, leg and jaw makes it an attractive clinical tool. [Misiaszek 2003]

1.2 Activation of afferent fibres

One of the main differences between the stretch reflex and the H-reflex is the way Ia afferent fibres are elicited. How the afferent nerves for the two reflexes are activated is presented in the following sections.

The stretch reflex

Tapping on a tendon with a reflex hammer will artificially initiate the stretch reflex in humans due to activation of muscle spindles, which are small encapsulated sensory receptors, located within the muscle (fig. 1.1).

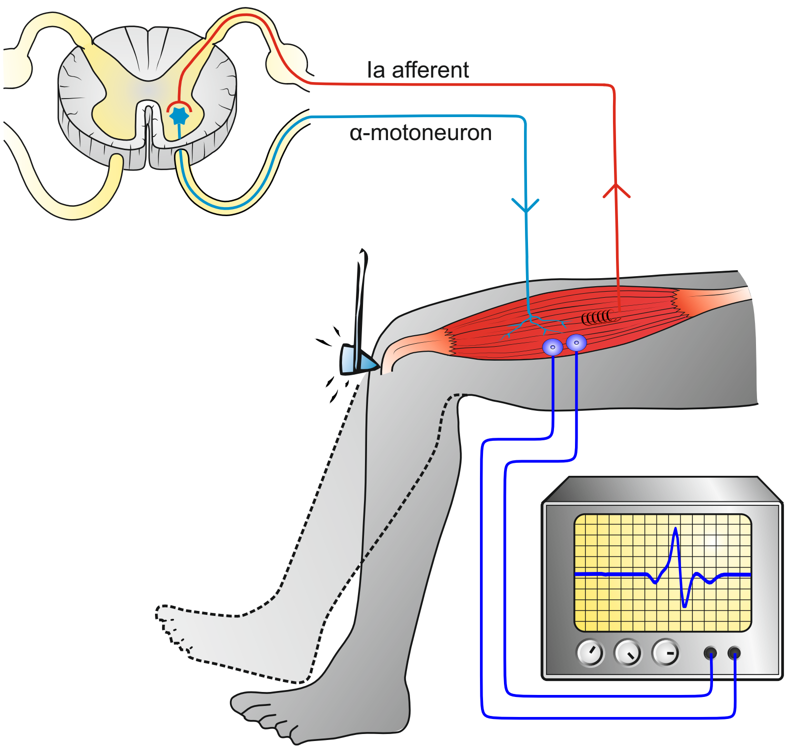

Figure 1.1: Neural pathways of the monosynaptic stretch reflex. Using a reflex hammer to tap above a tendon mechanically stretches the fibres in a muscle. The muscle stretch is registered by muscle spindles (represented as a coil wrapping a muscle fibre). Activation of muscle spindles causes depolarization and firing of action potentials along the Ia afferent fibres, which travels to the spinal cord. At spinal cord level, the action potentials from the Ia afferent fibres activate the αMN through a monosynaptic connection. Action potentials travel to the fibres of the homonymous muscle, causing the muscle to contract. Muscle contraction can be measured and visualised using recording electrodes, hardware and software

When a muscle is stretched the muscle spindles are deformed which lead to a depolarization and firing of action potentials (APs) along the Ia afferent fibres. The Ia afferent fibres synapse to the αMN which projects to the homonyms muscle (primary muscle which muscle spindles initiated the stretch reflex) and synergistic muscles through monosynaptic connections and antagonist muscles are activated through polysynaptic connections. [Kandel et al. 2013, chap.2,35] The more the muscle spindles are deformed (the more the muscle is stretched) the higher the firing rate, and therefore a more powerful muscle contraction is produced in the homonymous muscle.

The H-reflex

The H-reflex is considered the electrical induced analogue to the stretch reflex because both reflexes are using the same neural pathways and one of the main differences between the two reflexes is the way the Ia afferent fibres are activated. The H-reflex is activated by applying percutaneous electrical stimulation to the Ia afferent fibres directly, which bypasses the muscle spindles. A theoretical setup using electrical stimulation to activate a H-reflex is illustrated in figure 1.2.

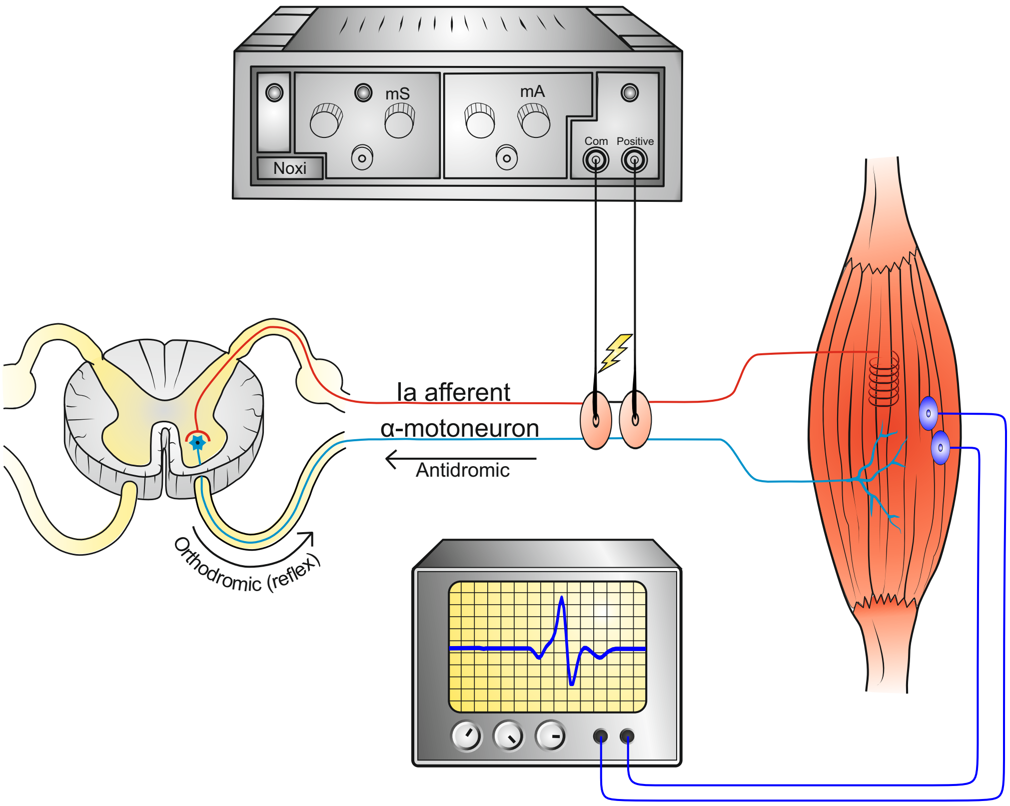

Figure 1.2: An electrical stimulator is used to activate the Ia afferent fibres. At low stimulation intensity only the Ia afferent fibres are activated, resulting in a signal travelling to the MN pool in the spinal cord. When the signal reaches the MN pool, APs are sent along the αMN to the muscle (orthodromic impulse). At high stimulation intensity the αMN is directly activated, resulting in an antidromic impulse moving in the opposite direction of the orthodromic impulse. Orthodromic and antidromic impulses will cancel out each other. Muscle activity are measured and visualised using recording electrodes, hardware and software

The Ia afferent fibres are located in mixed nerves which are spinal nerves containing both afferent (sensory) and efferent (motor) axons. Supra-threshold electrical stimulation will excite the Ia afferent fibres and APs travel to the MN pool within the spinal cord where they are sent through the efferent fibres to the muscle. A muscle response caused by electrical stimulation to the Ia afferent fibres at a certain stimulation intensity, results in a H-reflex (orthodromic signal). At low stimulus intensities only the H-reflex is evoked, since the electrical threshold for activating Ia afferent fibres are lower compared to the threshold of αMNs. If stimulation intensity is increased the H-reflex will increase in amplitude until the stimulation intensity is powerful enough to reach the threshold for αMNs, since stimuli are applied to a mixed nerve. Activation of αMNs generates an early response (denoted M-wave in EMG recordings) and an antidromic impulse, which travels in the opposite direction of the normal orthodromic impulse (fig. 1.2). By progressively increasing the stimulation intensity, the antidromic signal will increase and cancel out the orthodromic signal until the H-reflex is no longer present [Kandel et al. 2013, chap.35][Palmieri et al. 2004]

1.3 Synaptic connections and modulation of afferent inputs

The complexity of reflex arcs are often evaluated by their number of synaptic connections in the spinal cord: the fewer connections the simpler reflex. The stretch reflex and H-reflex consist of a monosynaptic reflex arc to the homonymous and synergist muscles, which is why these reflexes are considered the most simple. Interneurons are neurons distributing information (APs) between afferent and efferent pathways and are located within the brain and spinal cord [Martini and Nath 2009, chap.12]. Interneurons related to the mentioned reflexes are used in polysynaptic connections to modulate the activity of e.g. antagonist muscles. A graphical illustration of a mono- and a polysynaptic connection is seen in figure 1.3.

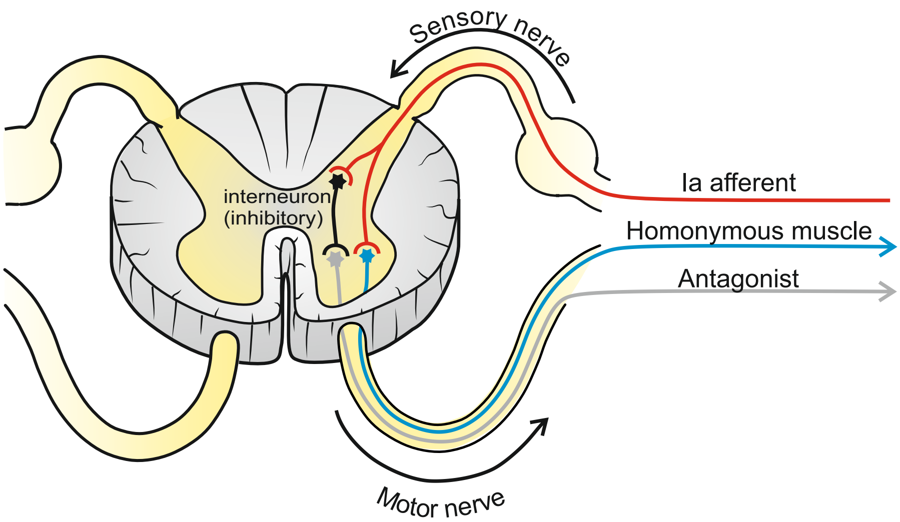

Figure 1.3: Activation of homonymous muscle and inhibition of antagonist muscles after activating the Ia afferent fibres. The αMN to the homonymous muscle is activated by a monosynaptic connection and the αMN to the antagonist is inhibited by an inhibitory interneuron.

Even though the stretch and H-reflexes are considered monosynaptic, several factors can still modulate the reflexes. An important factor in stretch reflex modulation is alterations of the muscle spindles sensitivity, which is controlled by gamma MNs (γMNs). Muscles spindles are not depolarized every time a muscle is stretched, which is an important feature since voluntary contractions of muscles should not result in a sudden muscle contraction (stretch reflex) of the antagonists. The γMNs are activating the intrafusal muscle fibres where the muscle spindles are found. Intrafusal muscle fibres are located deep inside a muscle in parallel with the extrafusal muscle fibres, which are the outermost muscle fibres activated by αMNs (fig. 1.4). [Kandel et al. 2013, chap.35]

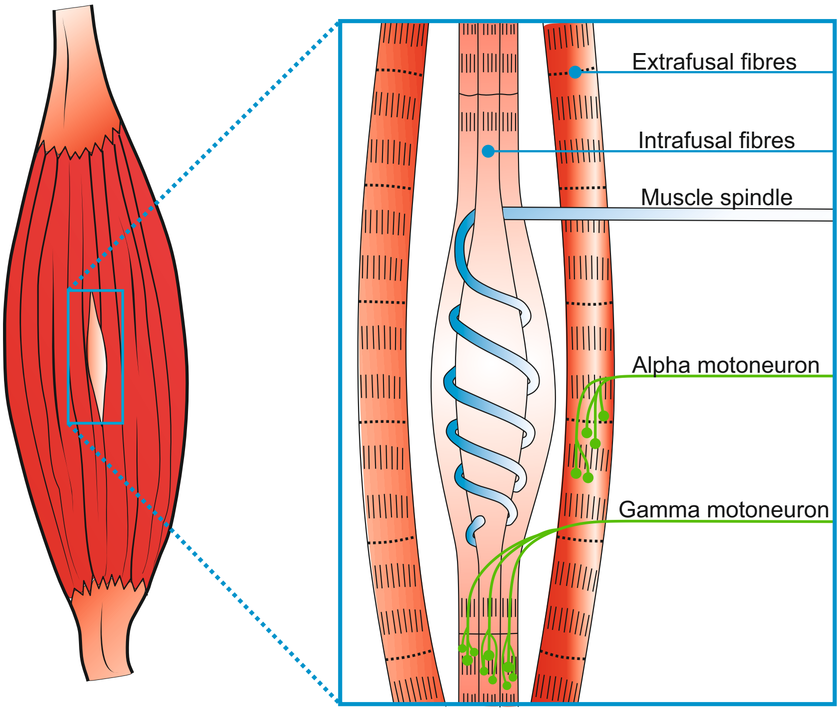

Figure 1.4: The intrafusal- and extrafusal muscle fibres, muscle spindles, αMNs and γMNs. The γMNs are innervating the intrafusal muscle fibres while the αMNs are innervating the extrafusal muscle fibres. The γMNs are controlling the sensitivity of the muscle spindles.

In order to maintain tension of the intrafusial fibres and thereby the sensitivity of the muscle spindles, the γMNs are activated when the αMNs are activating the extrafusal fibres, which is known as alpha-gamma co-activation. The γMNs are contracting the endings of the intrafusal muscle fibres and not the middle part where the muscle spindles are located, when regulating the sensitivity of muscle spindles. γMNs is for this reason a key element in the modulation of the stretch reflex. [Kandel et al. 2013, chap.35] As mentioned earlier the electrical activation of the Ia afferent fibres when evaluating H-reflexes will bypass muscle spindles and muscles spindles are therefore not able to modulate the H-reflex. However, when using the stretch reflex and H-reflex as tools to investigate synaptic activity several afferent feedback factors can affect the synaptic activity, e. g. information from group I, II, III and IV afferent fibres [Kandel et al. 2013, chap.35].

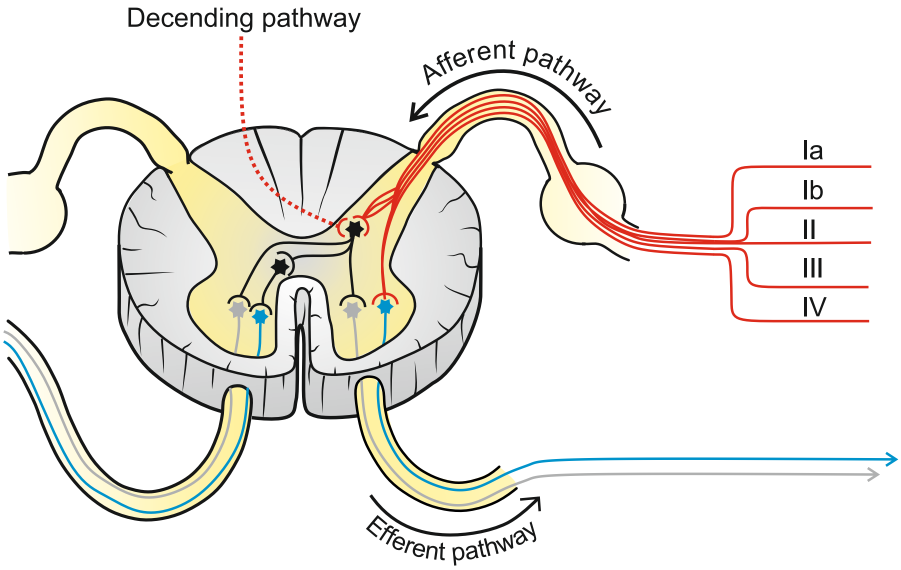

Figure 1.5: The figure shows a series of afferent inputs which can alter the information arriving to the spinal cord. The spinal cord receives also information from descending pathways, which is illustrated with a dotted red line.

The H-reflex has been used in attempts to selectively activate different types of afferent feedback in order to test the contribution of this afferent feedback to the synaptic activity. At low stimulation intensities the Ia afferent fibres are mainly activated due to the large diameter of the axon, but as the stimulation intensity increases the Ib afferent fibres and group II nerves will reach threshold for activation [Misiaszek 2003]. Each type of afferent fibres transmits signals from different sensory receptors. Table 1.1 presents a short overview of the afferent fibres and their type of carried information.

Due to the numerous afferent inputs and information from descending pathways the stretch reflex and H-reflex should not be considered as simple as some presume. When investigating the afferent- and efferent nerves and synaptic activity in research studies, it is important to minimise the number of variables that can produce variation in recorded data [Misiaszek 2003].

Type	Receptor	React on
Ia	Primary ending in muscle spindles	Muscle stretch
Ib	Golgi tendon organ	Muscle tension
II	Secondary ending in muscle spindles	Muscle stretch (non-adapting)
II	Non-spindle nerve endings	Pressure
III	Free nerve endings	Pain, pressure, temperature, chemical stimuli (fast/first pain information)
IV	Free nerve endings	Burning pain, warmth, itch, touch and cramp (secondary pain information)

Table 1.1: The table provides a overview of some of the afferent inputs to the spinal cord. The table is divided into the type of fibre, what type of receptor they are connected to and what kind of stimuli they react on [Kandel et al. 2013].

1.4 Variables in the stretch and H-reflexes

The stretch reflex is an excellent tool to investigate synaptic activity in the MN pool and how afferent feedback can modulate the efferent output [Kandel et al. 2013, chap.35]. However, the interaction of these afferent feedback variables cannot be examined selectively, since it is not possible to completely isolate the contribution from single afferent fibres and investigate the effect [Misiaszek 2003]. The H-reflex is also modulated by these variables; however, the H-reflex is believed to be a more isolated response of synaptic activity since muscle spindles are bypassed. For this reason the H-reflex is an attractive tool for research and clinical neurophysiology [Misiaszek 2003].

An advantage in using H-reflexes compared to the stretch reflex in research and clinical setups is the possibility of controlling the electrical stimulation intensity and stimulation site. When investigating the stretch reflex using e.g. a reflex hammer to activate the patellar reflex (illustrated in fig. 1.1) the point of tapping and force used can be difficult to control. Some studies, however, have designed experimental protocols where tap location and force are controlled [Katz et al. 1977] but these setups can be considered more complex compared to H-reflex setups. In H-reflex studies electrical stimulation of mixed nerves enable researchers and clinicians to activate αMNs directly. This is seen as the early response and by increasing the stimulation intensity it is possible to activate the total number of MNs in the MN pool. This is not possible using the stretch reflex since Ia afferent fibres are not capable of activating the total MN pool (and elicit the M-wave). The H-reflexes have been elicited in many muscles in the human body [Misiaszek 2003]. Some of the vast research and clinical usage of the H-reflex will be described and a gap in the literature concerning the H-reflex in the hamstring muscle group will be described in the subsequent sections.

1.5 H-reflexes in research and clinical setups

The H-reflex can theoretically be elicited in any muscle in which the Ia afferent fibres can be stimulated [Misiaszek 2003]. Table 1.2 and table 1.3 sums up former literature studying the H-reflex in upper and lower musculature, respectively. In research studies, the H-reflex is often presented as the M_{max}/H_{max} ratio which is commonly used to indicate how much of the MN pool is activated when investigating alterations in the H-reflex [Palmieri et al. 2004]. This ratio is commonly used because the synaptic activity is highly variable depending on the subject and state of the nervous system [Palmieri et al. 2004]. The next section will describe the H-reflex and M-wave in more detail.

The H-reflex and the M-wave

The M-wave and H-reflex (fig. 1.6) are two components when studying H-reflexes and are present at different time latencies compared to time of the applied stimulation. The temporal difference between these components is caused by the length of the pathway the signals have to travel. In figure 1.6 the M-wave which is an estimate of the direct activation of the αMN has a latency of approximately 15 ms post stimulation and the H-reflex which have to travel along the Ia afferent fibres to the MN pool and along the αMN before activating the muscle has a latency of approximately 32 ms.

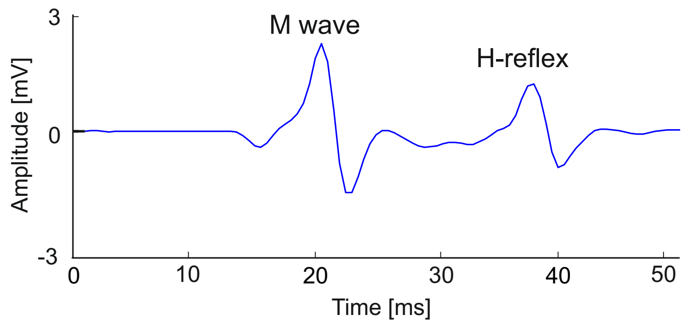

Figure 1.6: The M-wave precedes the H-reflex in the EMG-data due to the differences in distance the signals have to travel. The signal for the M-wave travels directly from the site of stimulation to the muscle fibres, whereas the signal for the H-reflex travels along the Ia afferent fibres to the monosynaptic connection in the spinal cord level and to the muscle extrafusal muscle fibre through the αMNs.

Since the orthodromic (H-reflex) and antidromic signals cancel each other out when progressively increasing the stimulation intensity, it is possible to create an input/output curve (IO curve or recruitment curve) as shown in figure 1.7

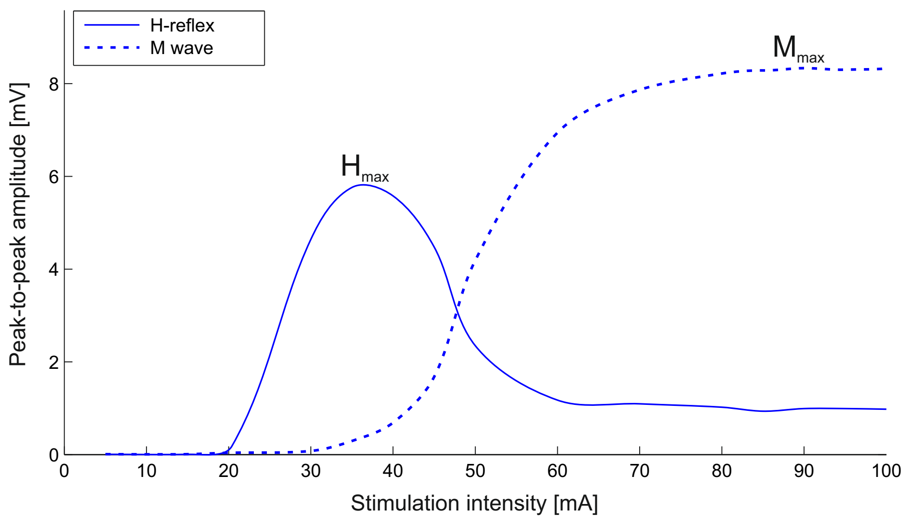

Figure 1.7: The figure shows an IO curve based on stimulus intensity varying from 5 to 100 mA. The H-reflex is present at lower stimulation intensities compared to the M-wave and at a specific stimulus intensity the H-reflex reaches maximal amplitude (H_{max}). When using stimulus intensity above H_{max} the H-reflex decreases and the M-wave continues to increase until the M_{max} is reached.

Due to the lower threshold for activating the Ia afferent fibres compared to the αMNs, the M-wave will appear around the stimulus intensity at which H_{max} is reached. The M_{max} represent activation of the entire MN pool within the spinal cord that is supplying the muscle of interest. For this reason M_{max} is believed to be the highest achievable activating of the muscle of interest [Palmieri et al. 2004, Pierrot-Deseilligny and Mazevet 2000].

Muscle	Stimulated nerve	Reference
UPPER LIMBS:		
Abductor digiti minimi	Ulnar nerve	[Bodofsky 1999]
Abductor pollicis brevis	Median nerve	[Bodofsky 1999]
Biceps brachii	Musculocutaneous nerve	[Bodofsky 1999]
Extensor carpi radialis	Radial nerve	[Miller et al. 1995]
Extensor digit communis	Radial nerve	[Bodofsky 1999]
Flexor carpi radialis	Median nerve	[Bodofsky 1999, Miller et al. 1995]
Flexor carpi ulnaris	Ulnar nerve	[Bodofsky 1999]
Trapezius	Cervical nerves (C3/C4)	[Alexander and Harrison 2002]

Table 1.2: Literature analysing the H-reflexes in different upper musculature.

Muscle	Stimulated nerve	Reference
LOWER LIMBS:		
Abductor hallucis	Tibial nerve	[Versino et al. 2007, Ellrich et al. 1998]
Flexor digitorum longus	Common peroneal nerve	[Hall et al. 1999]
	Tibial nerve	[Hall et al. 1999]
Gastrocnemius	Tibial nerve	[Alrowayeh and Sabbahi 2009, Jusic et al. 1995]
Hamstring muscle group:		
- Biceps femoris long head	Sciatic nerve	[Floy 2012]
- Biceps femoris short head	Sciatic nerve	[Pierrot-Deseilligny et al. 1981]
Peroneal	Sciatic nerve	[Palmieri et al. 2002]
Peroneus longus	Common peroneal nerve	[Nishikawa and Grabiner 1999, Hall et al. 1999]
	Tibial nerve	[Hall et al. 1999]
Soleus	Sciatic nerve	[Palmieri et al. 2002]
	Tibial nerve	[Hopkins et al. 2000, Yang and Whelan 1993, Capaday and Stein 1987] [Pierrot-Deseilligny et al. 1981, Magladery et al. 1951]
Tibialis anterior	Sciatic nerve	[Palmieri et al. 2002]
	Common peroneal nerve	[Brooke et al. 1997, Pierrot-Deseilligny et al. 1981]
Quadriceps muscle group:		
- Rectus femoris:	Femoral nerve	[Garland et al. 1994, Kameyama et al. 1989, Pierrot-Deseilligny et al. 1981]
- Vastus lateralis:	Femoral nerve	[Hopkins and Wagie 2003, Kameyama et al. 1989]
- Vastus medialis:	Femoral nerve	[Kameyama et al. 1989]

Table 1.3: Literature analysing the H-reflexes in different lower musculature.

The synaptic activity can change during different neurological conditions, e.g. radiculopathies (inflamed nerve roots), spinal cord injury and Huntington's disease [Fisher 1992, Braddom and Johnson 1974]. The H-reflex has also been used as a tool to assess responses to different motor tasks, such as alterations in H-reflex peak-to-peak amplitude during running, walking and standing [Capaday and Stein 1987; 1986, Crenna and Frigo 1987, Koceja et al. 1993, Yang and Whelan 1993], in sports injuries like ankle sprain [Hopkins and Palmieri 2004, Hall et al. 1999], ankle and knee joint effusions [Palmieri et al. 2004, Hopkins et al. 2000, Spencer et al. 1984] and pain [Leroux et al. 1995]

H-reflexes have especially been recorded from soleus and tibialis anterior muscles when studying subjects lying, running, walking and standing, since the nerves innervating those muscles (tibial nerve and common peroneal nerve, respectively) are superficial and easy to stimulate in the poptiteal fossa [Palmieri et al. 2004; 2002]. Several studies of the H-reflex in the quadriceps muscle group has also been conducted [Hopkins and Wagie 2003, Garland et al. 1994, Kameyama et al. 1989, Pierrot-Deseilligny et al. 1981]. However, very little research has focused on eliciting H-reflexes in the hamstring muscle group, which is a very important muscle group during human locomotion.

1.6 H-reflexes in the hamstring muscle group

The fact that little research has focused on eliciting H-reflexes in the hamstring muscle group (the long head of biceps femoris, the short head of biceps femoris, semitendinosus and semimembranosus) might be explained by the difficulty in stimulating the sciatic nerve, which innervates this muscle group, because it is located deep to the gluteus maximus and biceps femoris muscle [Chan et al. 2006]. The sciatic nerve is a large nerve in humans originating at the sacral/lumbar plexus and branches into the tibial and the common peroneal nerve [Drake et al. 2008, chap.1]. The sciatic nerve supplies muscles in the upper and lower leg with APs through its branches. However, due to the difficulty of stimulating the sciatic nerve, only little is known about the H-reflexes in the hamstring muscle group, despite the muscle group's major contribution in human locomotion - the hamstring muscle group is especially activated during knee flexion and hip extension and rotation [Martini and Nath 2009, chap.11].

A technique to stimulate the sciatic nerve in order to elicit H-reflexes in the hamstring muscle group has yet to be proposed. If a reliable technique to electrically stimulate the sciatic nerve could be designed it could contribute to more complex studies of the interaction and communication between muscles in the legs during human locomotion. It might, however, be difficult to stimulate the sciatic nerve at the correct spot, since the nerve branches into the tibial nerve and common peroneal nerve in the popliteal fossa [Martini and Nath 2009, chap.13],[Drake et al. 2008, chap.1],[Valerius 2003, chap.4]. Only a few studies have used electrical stimulation of the sciatic nerve to elicit H-reflexes. Those found in the literature are discussed in the next section.

Stimulation of the sciatic nerve

As seen in table 1.3, both Pierrot-Deseilligny et al. (1981), Palmieri et al. (2002) and Floy (2012) have stimulated the sciatic nerve using electrical stimulation. They all use different techniques in order to stimulate the sciatic nerve.

Pierrot-Deseilligny et al. (1981) did not specify the placement of the stimulation electrodes. In order to elicit H-reflexes they used a double stimulation consisting of the first stimulus being subliminal for M-wave activation, and then the second stimulus applied 4 ms after. The consequences of using a double stimulation technique were not discussed in the paper and the reliability of the stimulation technique was not evaluated since no intersession reliability test was conducted.

Palmieri et al. (2002) stimulated the sciatic nerve before it branches into the tibial and common peroneal nerves in the popliteal fossa. H-reflexes were recorded from soleus, peroneal, and tibialis anterior. Palmieri et al. (2002) tested the intersession reliability over two consecutive days, and concluded that the stimulation technique was reliable in order to elicit H-reflexes in soleus, peroneal, and tibialis anterior. By placing the cathode at the superior portion of the popliteal fossa and the anode superior to the patella, Palmieri et al (2002) were capable of evaluating reflexes in the lower leg. However, due to the distal stimulation site compared to the hamstring muscle group this protocol is not suitable to eliciting H-reflexes in this muscle group.

Floy (2012) stimulated the root of the sacral spinal nerves (which was defined as the sciatic nerve). This is only partly true, since the sciatic nerve consist of nerve branches from both sacral and lumbar roots [Drake et al. 2008, chap.1]. H-reflexes were measured from biceps femoris, but intersession reliability of the H-reflexes using this protocol was not tested. H-reflexes were elicited in the hamstrings muscle group, however, due to lack of detail in the methods section and the anatomical site of stimulation, this protocol cannot be described as a reliable technique in our opinion.

The studies of Pierrot-Deseilligny et al. (1981) or Floy (2012) reported no evidence about intersession reliability of their stimulation protocol. Palmieri et al. (2002) applied electrical stimulation close to the point where the sciatic nerve branches in the poptiteal fossa. Some anatomy literature using carcass photos show anatomical variations of the sciatic nerve branching point, where it has been documented to branch around the middle of the thigh and not in the poptiteal fossa [Valerius 2003, chap.4]. The aim of the study of Palmieri et al. (2002) was to elicit H-reflexes in lower leg muscles (soleus, tibialis anterior and peroneal) [Palmieri et al. 2002]. In order to elicit H-reflexes in the hamstring muscle group the sciatic nerve should be stimulated at a more proximal point, because (1) the Ia afferent fibres should be stimulated proximal to the musculature of interest and (2) to minimize the risk of having a point of stimulation after the sciatic nerve has branched into the tibial nerve and the common peroneal nerve.

1.7 Project aim

The use of H-reflexes as a research and clinical tool is well documented and described in section 1.5. However, there is a gap in the literature when it comes to measurement of H-reflexes in the hamstring muscle group, despite this muscles group's importance in locomotion. One issue in eliciting H-reflexes in the hamstring muscle group is the difficulty in stimulating the sciatic nerve, which is explained in the previous section. Therefore,

The aim of the current study was to design and validate a new technique to stimulate the sciatic nerve and to test and evaluate the intersession reliability of this technique's ability to elicit H-reflexes in the hamstring muscle group.

Methods 2

This chapter documents the scientific methods used to design a reliable experimental protocol to fulfil the aim expressed in section 1.7. Two pilot studies were conducted prior to the main experiment in order to test whether it was possible to elicit H-reflexes in the hamstring muscle group and to test the effect of different setup variables. Furthermore, data were collected in order to perform a sample size calculation for the main experiment.

2.1 Pilot studies

The two pilot studies were conducted to provide specific information used to design the main experimental protocol are presented in appendix page 40 and 44. The front page and title of the two conducted pilot studies are seen in figure 2.1.

Figure 2.1: One pilot study is presented as a short article (page 40) while the other pilot study is presented as an abstract (page 44)

The purpose of the first study was to test whether it was possible elicit H-reflexes in the hamstring muscle group after sciatic nerve stimulation, to find the location of stimulation and to test different setup parameters, such as single and double stimulation techniques. The second study was conducted as a pilot study of the designed experimental protocol and to test if elicited H-reflexes in the hamstring muscle group are reliable. Since no data of reliable H-reflexes in the hamstring muscle group were found in the literature, data from the second study were used to calculate the sample size required to obtain reliable measurements in the main experiment.

2.2 Main experiment

In the following sections, the main experiment will be described. The general description will only provide basic information regarding the protocol while the specific experimental design choices are described in depth in the subsequent sections. After the general description, there will be a description of the scientific methods used in the experimental protocol to find the parameters of interest from the measured physiological signals and finally a description of the statistical analysis performed in order to reach the aim of the thesis.

General description of experiment

To evaluate whether the designed stimulation protocol was reliable, and to make sure the sciatic nerve was stimulated, recordings of M-waves and H-reflexes were used. By the use of an electrode grid electric stimulation was applied just below the buttocks. The anode was placed on the greater trochanter of femur. Electromyographic (EMG) signals were recorded from three muscles from the hamstring muscle group.

The electrode grid (cathodes) was used to find the optimal grid site to stimulate the sciatic nerve. Each of the cathodes from the electrode grid was tested. The optimal cathode location was then stimulated with different intensities and M-waves and H-reflexes were acquired to plot an IO curve. Parameters from the IO curve and latencies of the M-wave and H-reflex signal components were then processed and used in further statistical analysis, where the intersession reliability of the stimulation technique was calculated and evaluated.

A detailed experiment protocol will be described in the next section.

Parameters of interest

In order to minimize the risk of misinterpretation, the data should easily be extractable from the raw surface EMG signals. In previous studies the latency [Christie et al. 2005] and the peak-to-peak (pp) amplitude [Palmieri et al. 2002, Christie et al. 2005] of the M-wave and H-reflex has been used to test for H-reflex reliability between sessions. EMG signals were measured from the three hamstring muscle: the long head of biceps femoris (LH-BF), the short head of biceps femoris (SH-BF), the semitendinosus (SEMI). EMG from the soleus (SOL) and the tibialis anterior (TA) were used as control. See figure 2.2. EMG from the semimembranosus, which also is a muscle in the hamstring muscle group, was not collected, due to the deeper laying nature of this muscle [Martini and Nath 2009, chap.11].

The parameters of interest, which were extracted from the EMG, were:

- M_{max}
- H_{max}
- Latency of M-wave
- Latency of H-reflex

The parameters of interest were compared within subjects for two consecutive days in order to evaluate the intersession reliability of these parameters. The statistical analyses performed are described later.

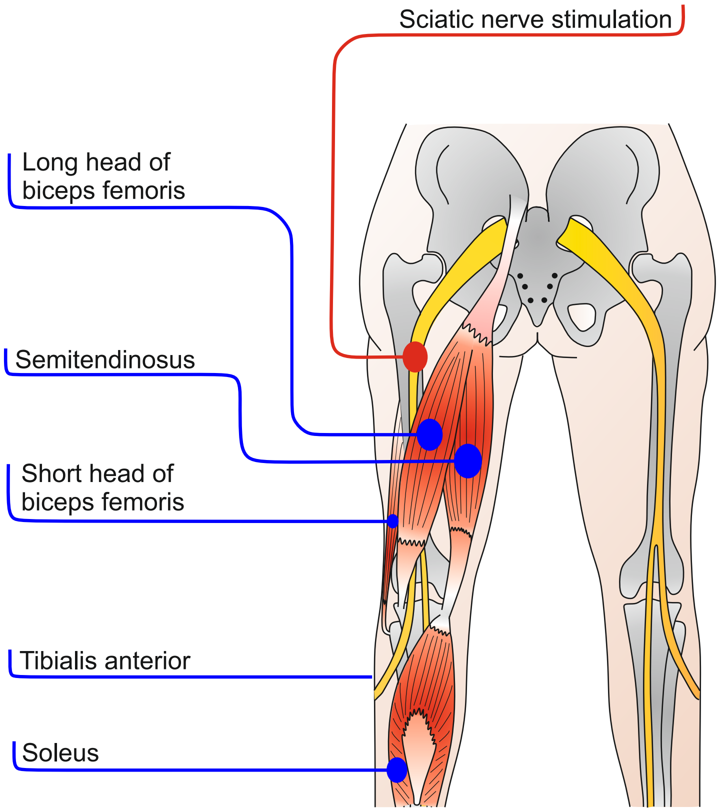

Figure 2.2: The figure shows SH-BF, LH-BF and SEMI along with SOL. TA is placed anterior and is therefore not displayed.

2.2.1 Subjects

The subjects had to satisfy the inclusion criteria in order to participate in the experiment. It is not crucial to choose subjects with similar characteristics, e.g. similar physical activity level. Since, the developed technique was tested within subjects. Findings by Sabbahi and Sedgwick (1982) indicated that signal amplitudes are smaller, and latencies prolonged in elderly (60 to 72 years) compared to younger individuals (19-31 years) [Sabbahi and Sedgwick 1982]. Other findings have shown a dramatic decrease in numbers of motor neurons beyond 60 year of age

[Lexell 1997], so in order to obtain better recordings for the current study, the inclusion criteria was to use subjects between 20-30 years of age. All subjects had to have no prior neurological medical history and not use any medications.

Sample size

Calculation of the minimum sample size (number of subjects) needed is an essential initial part in an intersession reliability study. A study using too many samples to show a specific significant reliability may waste time and economical resources, while too few samples will have little chance in reaching the aim. [Zou 2011] Different sample size calculations are used for specific statistical analysis. In this thesis the statistical analysis, intraclass correlation coefficient (ICC) was used (see section 2.4). Eq. 2.1, designed by Zou (2011) can be used to calculate sample size when performing an ICC analysis.

$$N = 1 + 2\left(z_\alpha + z_\beta\right)^2 \cdot k \left(\ln\left(\frac{(1+(k-1)\cdot\rho)\cdot(1-\rho)^{-1}}{(1+(k-1)\cdot\rho_0)\cdot(1-\rho_0)^{-1}}\right)\right)^{-2} \cdot (k-1)^{-1} \tag{2.1}$$

where,

- N : number of subjects needed
- z_α : z-value of the α value used. The α value is calculated by α =1-confidence interval, where the confidence interval often is set to 95%. The z_α value is then found by table look-up [Zar 2010, p.676]
- z_β : z-value of the β value used. The β value is calculated by β=1-assurance , where the assurance is the probability we wish to ensure that the lower limit of the one-sided confidence interval is no less than ρ_0.
- k : number of sessions
- ρ : expected ICC value
- ρ_0 : lowest acceptable ICC value

Results from earlier studies are often used in order to decide the value of ρ. Since no previous studies have been conducted, trying to find the reliability of H-reflexes in the hamstring muscle group, the second pilot study (page 44) was conducted in order to estimate ρ. This pilot study used the experimental protocol presented in section 2.3.

Six different values for ρ where used in eq. 2.1 based on the results of the pilot study. The values from the pilot study is summarized in table 2.1.

	ICC (used as ρ values)	
	H_{max}	H-reflex latency
LH-BF	0.980	0.996
SH-BF	0.777	0.991
SEMI	0.797	0.939

Table 2.1: ICC findings from the second pilot study (page 44)

In order to calculate the minimum number of subjects needed the following values were used:

z_α : 1.645 (using 95% confidence interval)
z_β : 0.84 (using 80% assurance)
k : 2
ρ : the ICC values from the second pilot study summarized in table 2.1
ρ_0 : 0.71 (midvalue to achieve substantial agreement [Landis and Koch 1977] (table 2.3)

Results from the sample size calculation are summarised in table 2.2.

	Number of subjects needed	
	H_{max}	H-reflex latency
LH-BF	5 (ρ=0.980)	3 (ρ=0.996)
SH-BF	274 (ρ=0.777)	3 (ρ=0.991)
SEMI	151 (ρ=0.797)	10 (ρ=0.939)

Table 2.2: The required number of subjects needed to show reliable ICC values

Large value for ρ results in fewer subjects needed compared to smaller values of ρ. Due to time and financial constraints it was not possible to test neither 274 or 151 subjects, which are indicated if we wanted to evaluate H_{max} from the SH-BF and the SEMI muscle, respectively. In order to evaluate H_{max} from LH-BF and H-reflex latencies for each of the muscles in the hamstring muscle group the sample size had to be at least 10. In the current study, it was chosen to use a few more subjects than the minimum calculated number, therefore the experiment was performed on 12 healthy male volunteers, aged 24-26 years (mean 25.58±0.99 years). Informed consent was obtained from each subject and the study protocol was approved by the Scientific Ethics Committee Northern Jutland, Denmark (reference number: N-20110076).

2.2.2 Stimulation

In the current study eight single stimulation electrodes were used (3.2 cm round PALS Platinum, Axelgaard Manufacturing, USA). The size and shape of the PALS electrodes were customised to a rectangular size of 1.8 cm times 1.6 cm. The eight electrodes were placed in a 4x2 grid configuration (fig. 2.3). Each of the eight cathodes in the single stimulation electrodes were activated in order to find optimal site to stimulate the sciatic nerve. An interstimuli interval of 10 s was used [Misiaszek 2003]. When the optimal stimulation site was found, it was stimulated with 16 different stimulation intensities varying from 10 to 100 mA. Recordings from the different stimulation intensities were used to produce IO curves.

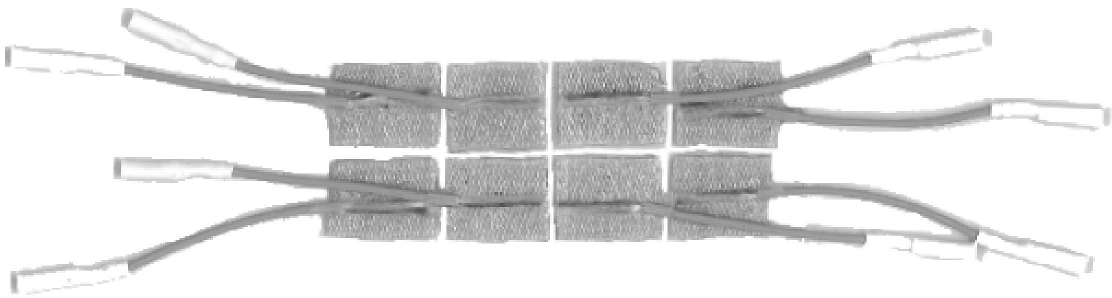

Figure 2.3: The configuration of the 4x2 custom-built stimulation electrode grid used as cathodes

Electrode placement

The cathode grid and the anode was used to stimulate the sciatic nerve in the subject's dominant leg. 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 cathode grid was used to find the optimal stimulation site, due to difficulties in stimulating the sciatic because it lies deep to the gluteus maximus and BF muscles [Chan et al. 2006]. The anode (5x9 cm rectangle PALS Platinum, Axelgaard Manufactoring, USA) was placed on the great trochanter of femur.

Hardware

Electrical stimulation was administered by an isolated constant current electrical stimulator (NoxiTest IES 230, Aalborg University, Denmark). A custom-built relay was used to direct the output from the electrical stimulator to one of the eight sites in the cathode grid. A 1 ms squared stimulation pulse was used based on findings by Panizza et al. (1989) who found the optimal pulse length for eliciting H-reflexes to be 0.5 to 1 ms [Panizza et al. 1989]. A computer controlled the activation of the NoxiTest stimulator and relay through a DAQ card (DAQCard-6024E, National Instruments, USA) mounted in a BNC terminal block (NI BNC-2090, National Instruments, USA). The NoxiTest stimulator, relay, computer and DAQ are seen in figure 2.5.

Software

Custom-built software (Wirex, Aalborg University, Denmark) was used to control the hardware: the relay and the electrical stimulator. The electrical stimulator was triggered by a voltage input sent from the Wirex software, and the output channels in the relay were controlled so the eight sites in the cathode grid were activated alternately in random order.

2.2.3 Recording

The EMG signals were recorded from ipsilateral muscles: LH-BF, SH-BF, SEMI, SOL and TA, using Ag/AgCl surface electrodes (Neurolone 720, Ambu, Denmark). The skin was prepared by scrubbing with disposable alcohol swaps prior electrode placement to lower the skin/electrode impedance [Acierno 1995].

Electrode placement

The three hamstring muscles; LH-BF, SH-BF and SEMI were chosen as the main target muscles. SOL and TA were used as control muscles, because the muscles are innervated by branches of the sciatic nerve (tibial nerve and common peroneal nerve, respectively). EMG electrodes were placed in a bipolar configuration in accordance with the SENIAM project [Hermens et al. 1999] with one common reference placed on the distal end of the medial surface of the tibia bone.

Hardware

EMG signals were amplified and filtered (DC to 500 Hz for LH-BF, SH-BF and SEMI and 5 to 500 Hz for SOL and TA) using custom-built amplifiers and digitized at a sample frequency of 2 kHz using a 12 bit, 16-channel ADC (NI 6024E, National Instruments, USA). The high-pass filter setup was chosen so the capacitors within these filters were bypassed, which removed the smoothing artefact created from the' time constants of these capacitors which affected the distinct separation of stimulus artefact and the M-wave. This capacitor problem was created because the recordings from the hamstring muscle group lies close to the cathode site, which build on experiences from the first pilot study (page 40). Illustrations of the hardware used for recording are seen in figure 2.5.

Software

Wirex was used to acquire, display and save the EMG data from the five muscles. Data were saved as a Matlab-file (`.mat`) and used in later post-hoc offline data processing and statistical analysis.

2.2.4 Extraction of parameters

The first step in the offline data analysis was extraction of parameters of interest. The latencies of M-wave and H-reflex were defined as the onset by which the slope of the EMG signals changed noticeably within a subject-specified time interval (window). Findings in the second pilot study reveal mean H-reflex latencies ranging from 23.6±2.7 (SEMI) to 28±3.3 (SH-BF). The H-reflex time window was defined from 20 to 40 ms, and M-wave window from 6 to 18 ms, which was determined as the end of the stimulus artefact (>5 ms) to the start of the H-reflex window (fig 2.4). However, every data set was manually inspected and the time windows adjusted if needed, due to intersubject variations.

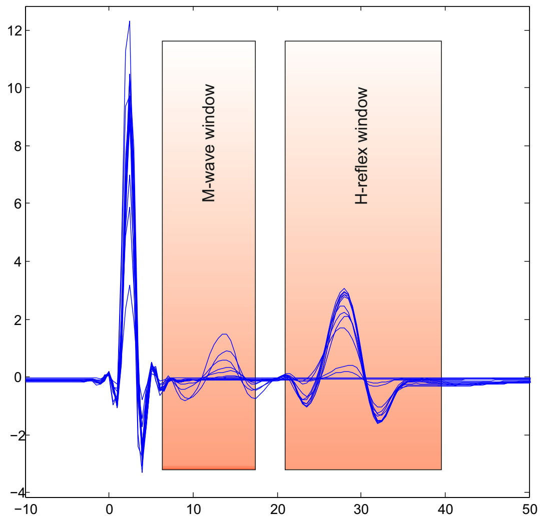

Figure 2.4: The figure illustrates where the windows for M-wave and H-reflexes should be defined. The starts of the windows were defined as the first point in time where the slope of the EMG signals change noticeable. The ends of the windows were defined as the time, the first peak after window start, is back to baseline. The windows were used to define at range from where pp amplitudes were calculated.

After the latencies of M-wave and H-reflex were determined, the pp amplitudes were calculated within the specified time window using a custom-made MATLAB-script. The pp amplitudes from each of 16 stimulus intensities were then used to create an IO curve.

2.3 Experimental protocol

The aim was to design and test a protocol stimulate the sciatic nerve, so the following section described the used protocol and summaries the setup for stimulation and recording and the steps performed to find the optimal cathode site and extract parameters of interest just described in this chapter. The specific steps in the protocol are described in order to make it easier for researchers to able the protocol in future studies.

Preparation

Parameters for stimulation

- Stimulation type: single 1 ms squared pulse
- Starting stimulation intensity: 30 mA
- Interstimuli interval: 10 s
- Electrodes:
  - Anode: PALS Platinum 5x9 cm rectangle (Axelgaard Manufactoring, USA)
  - Cathode: PALS Platinum 3.2 cm round (Axelgaard Manufacturing, USA) customised to rectangular size of 1.8 cm x 1.6 cm

Parameters for recording

- Electrodes: Neuroline 720 (Ambu, Denmark)
- Analogue EMG filter settings:
  - LH-BF, SH-BF and SEMI (highpass: 5 Hz, lowpass: 500 Hz)
  - SOL and TA (highpass: DC, lowpass: 500 Hz)

Electrode placement

- Preparation of subjects skin prior placement of recording electrodes on the dominant leg consiss in removal of hair from the muscle belly of LH-BF, SH-BF, SEMI, TA and SOL and lower the skin/electrode impedance with alcohol
- Placement of EMG recording electrode:
  - A bipolar configuration on muscle belly of LH-BF, SH-BF, SEMI, TA and SOL in accordance with the SENIAM project [Hermens et al. 1999], with an interelectrode distance of 1.5 cm
  - A common reference on the distal end of the medial surface of the tibia bone
- Placement of stimulation electrodes:
  - Anode: the great trochanter of femur
  - Cathode: 4x2 electrode grid with centre along the lead line between the ischial tuberosity and the lateral condyle of femur, two centimetres below the buttock

Finding optimal stimulation site

1. Apply electrical stimulation at each cathode site separately using the pre-set stimulation intensity of 30 mA
2. Record EMG signals and inspect for H-reflexes in the recordings from each muscles

   - If H-reflexes are observed, then the stimulation intensity should be reduced 5 mA
   - If no H-reflexes are observed, then the stimulation intensity shoud be increased 5 mA

3. Apply a new set of electrical stimulations with the new defined stimulation intensity. Continue step 2, until H-reflex threshold is found.
4. Adjust the stimulation intensity to H-reflex threshold + 25%
5. Apply electrical stimulation (three trials) using each cathode sites
6. Average recordings from the three trials for each muscle and each cathode site
7. Measure the pp amplitude of the H-reflexes recorded from each of the eight sites of stimulation
8. The site with the highest pp amplitude is defined as optimal stimulation site

Stimulation of optimal site

1. Apply 16 different stimulation intensities to the optimal stimulation site (10, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 90, 100mA) and record EMG
2. Acquire EMG from all five muscles 200 ms prior to 1 s after stimulation
3. Conduct three trials with an intertrial pause of 1 min

Extraction of parameters

1. Average recordings from the three trials for each muscle and for each cathode site
2. Find latency of M-wave which is defined as the onset of the first distinguished slope in the M-wave window (6-18 ms)
3. Define subject-specific M-wave window if the first distinguished slope is outside the interval of 6-18 ms
4. Find latency for H-reflex which is defined as the onset of the first distinguished slope in the H-reflex window (20-40 ms)
5. Define subject-specific H-reflex window if the first distinguished slope is outside the interval of 20-40 ms
6. Calculate the pp amplitude for M-wave and H-reflex for each stimulation intensity using defined M-wave and H-reflex windows
7. Combine M-wave and H-reflex pp amplitudes to construct an IO curve.
8. Extract H_{max} and M_{max} from the IO curve

After extraction of the parameters of interest the statistical analysis was performed, which is presented in the following section.

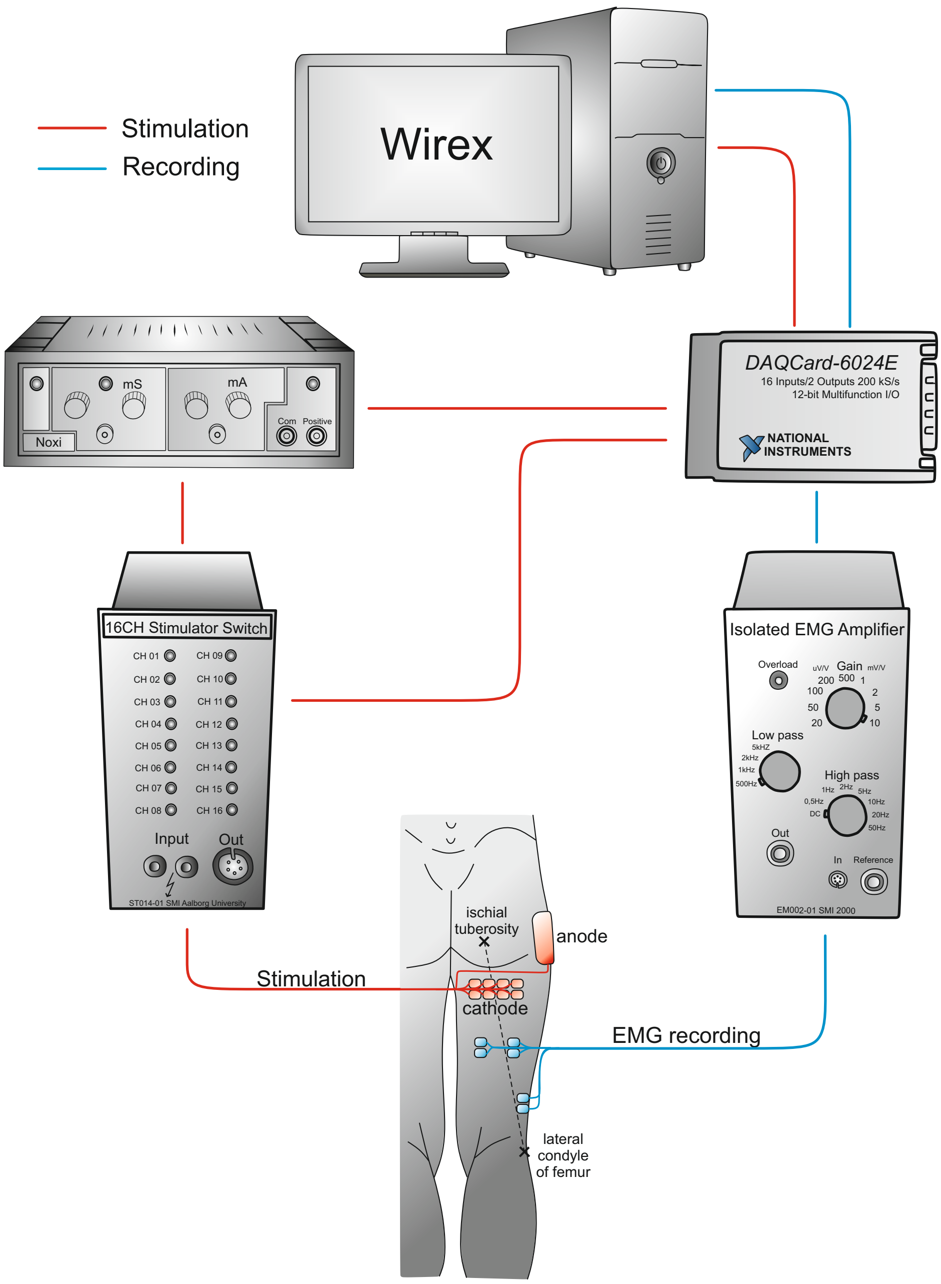

Figure 2.5: The figure illustrates the experimental setup. The red wires are representing the data flow used stimulation and the blue wires represent EMG recording.
Stimulation: Information regarding stimulation intensity, stimulation duration and site of stimulation are send from the computer to the DAQ card where the information regarding stimulation intensity and duration are forwarded to the electrical stimulator and information regarding site of stimulation are forwarded to the relay and then applied to the stimulation site specified by the relay.
Recording: EMG signals were recorded 200 ms prior to 1 s after stimulation. EMG signals were amplified and filtered using a custom-made EMG amplifier, before forwarded to the DAQCard and finally acquired by the computer.

2.4 Statistical Analysis

The statistical analyses used are described in the following section. To ease the description, the flow chart (fig. fig:statisticalAnalysis) illustrates when the different methods were used.

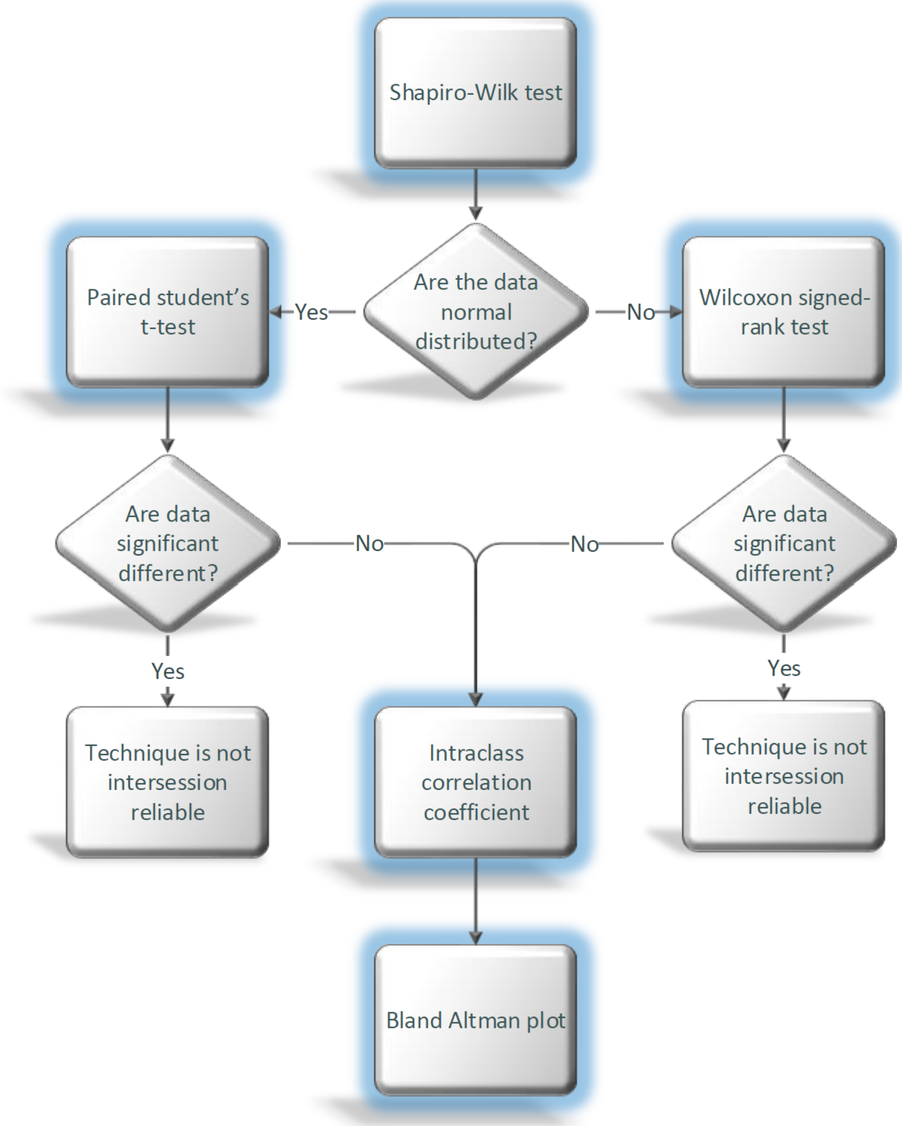

Figure 2.6: The figure illustrate the different statistical analysis used. The specific statistical analyses are highlighted with a blue glow.

Shapiro-Wilk test

The Shapiro-Wilk test was performed to determine whether the data from each parameter of interest were normal distributed. This is important to know when determining the analyses used to test for significant difference between data. The following two test hypotheses were stated using an α value of 0.05. [Shapiro and Wilk 1965]

- H_0 = data are normal distributed → perform a Paired student's t-test
- H_A = data are not normal distributed → perform a Wilcoxon signed rank test

Paired student's t-test

In case the recorded data was normal distributed, the paired student's t-test was used to determine whether a significant difference between data from the two conducted sessions exists. If a statistical significant difference was evident (α = 0.05), the H-reflexes could be considered intersession reliable and no further analysis needed to be done. P-value below α would results in rejecting the null hypothesis in favour of the alternative hypothesis. [Zar 2010]

- H_0 = there is no statistical significant difference
- H_A = there is a statistical significant difference

Wilcoxon signed-rank test

Wilcoxon signed-rank test is an alternative to the paired student's t-test used when data do not follow a normal distribution. The Wilcoxon signed-rank test was used to test for a statistical significant difference between data from the two conducted sessions. If a statistical significant difference was evident (α = 0.05), the H-reflexes could be considered intersession reliable and no further analysis needed to be done. P-value below α would results in rejecting the null hypothesis in favour of the alternative hypothesis. [Zar 2010]

- H_0 = there is no statistical significant difference
- H_A = there is a statistical significant difference

Intraclass correlation coefficient

If the paired student's t-test or Wilcoxon signed-rank test did not present a statistical significant difference between sessions, then an ICC was calculated. ICC can be used as a statistical representation of how well paired data from two groups resemble each other, meaning it can be used to assess reproducibility of quantitative measurements. Unlike traditional correlation analysis, ICC can be set to measure absolute agreement between groups, meaning that data with high correlation can have low ICC values but not vice versa (fig. 2.7). Absolute agreement (table 2.3) is a measure of reliability. [Zou 2011]

Values from ICC are evaluated between 0 and 1 according to Landis and Koch (1977) and will be used to when evaluating the reliability of the data.

ICC values	Strength of Agreement
<0.00	Poor
0.00 - 0.20	Slight
0.21 - 0.40	Fair
0.41 - 0.60	Moderate
0.61 - 0.80	Substantial
0.81 - 1	Almost perfect

Table 2.3: Interpretation of ICC values ranging from 0 to 1 [Landis and Koch 1977].

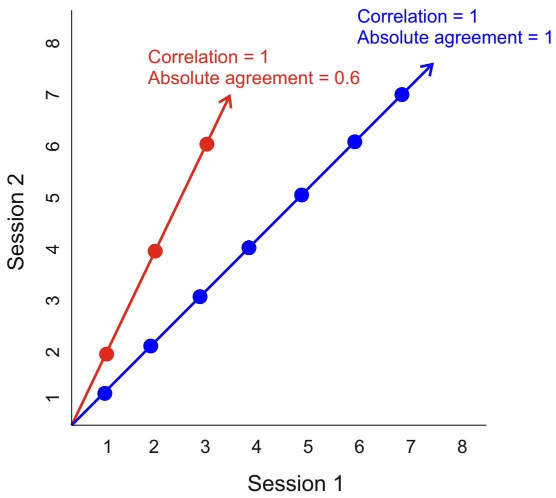

Figure 2.7: Illustration of the difference between correlation and absolute agreement. Correlation evaluate whether there is a relationship between two sessions, which is seen in both the red and blue graph (systematic differences are irrelevant). Absolute agreement evaluates whether the values in each individual pair are the same (systematic differences are relevant).

Bland Altman plot

Bland Altman plots are used as a visual representation of the absolute agreement between data. Figure 2.8 shows an example of a Bland Altman plot. The abscissa is the mean of the data recorded from session 1 and session 2, and the ordinate present the difference between the paired recordings. The mean of the groups differences are shown as a horizontal line and the 95% confidence interval (±1.96 SD) is presented as dashed horizontal lines. [Altman and Bland 1983]

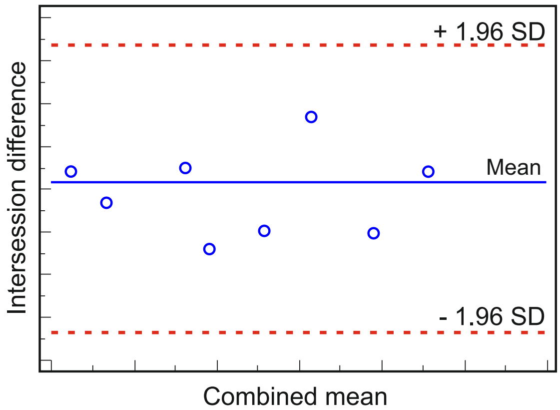

Figure 2.8: Bland Altman plot. Abscissa: the mean of a pair of data. Ordinate: difference between paired data. The blue line presents mean difference among data pairs and the red dotted line present the 95% limits of absolute agreement. Each blue circle is a data pair.

Bland Altman can be used to illustrate if there is a systematic bias or outliers in recorded data by plotting the difference between paired samples from session 1 and 2. If the horizontal line (mean difference) is significantly different from 0, it would indicate bias.

Results 3

This chapter presents the primary and secondary results. For the primary results data from EMG recordings and IO curves from typical subjects will be presented for LH-BF. Data from the two conducted sessions are shown to visualize the absolute agreement between sessions. Mean values and standard deviations (SD) for the extracted parameters of interest are presented before the ICC values are listed and presented in tables. Finally the most interesting Bland Altman plot is shown. The secondary results include representative EMG recordings and IO curves. Table of mean and SD of the extracted data and ICC for SOL and TA is also shown.

3.1 Primary results

Results for parameters of interest

It was possible to extract the following parameters from the data: H_{max}, M-wave latency and H-reflex latency. It was not possible to extract the M_{max}, since the output of the electrical stimulator was insufficient in order to reach a plateau of the M-wave IO function. For this reason it was not possible to calculate the H_{max}/M_{max} ratio. The mean and SD of the extractable parameters are summarized in table 3.1 for LH-BF, SH-BF and SEMI.

	Mean values and SD					
	H_{max} [mV]		M-wave latency [ms]		H-reflex latency [ms]	
	Session 1	Session 2	Session 1	Session 2	Session 1	Session 2
LH-BF	1.44±1.49	1.25±1.42	9.00±2.71	8.86±2.98	25.60±4.35	25.06±4.20
SH-BF	0.60±0.39	0.53±0.27	7.68±1.38	7.82±1.62	26.95±3.25	27.20±3.31
SEMI	2.27±2.23	2.23±1.33	7.33±1.80	7.46±1.18	23.23±2.63	23.55±3.06

Table 3.1: Mean values and SD of H_{max} pp amplitudes and latencies of M-waves and H-reflexes in LH-BF, SH-BF and SEMI

Figure 3.1 shows EMG recordings and IO curves of LH-BF activity from one subject and is vertically divided into sessions and horizontally into EMG recordings (A) and IO curves (B). The time window used to find the pp amplitudes of M-wave and H-reflex are added to the EMG recordings in the two top subfigures. The mean IO curve and SD are shown in the bottom subfigures. Intraclass correlation coefficients (table 3.2) proved to be reliable. According to

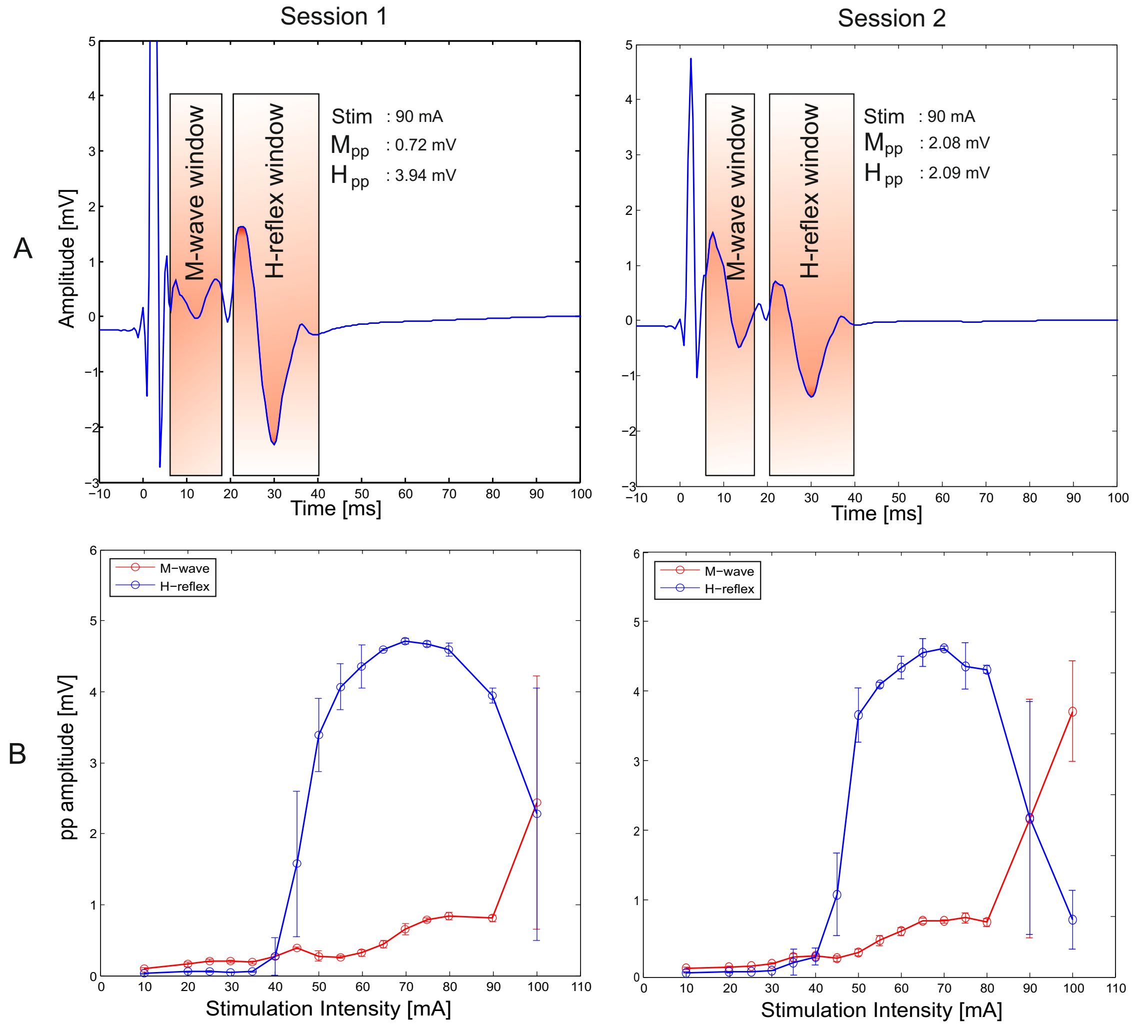

Figure 3.1: Data from LH-BF from one subject, vertically divided into session 1 and session 2. **(A:)** EMG recordings with added M-wave and H-reflex time windows to determine pp amplitudes. **(B:)** mean IO curves and SD

the benchmarks of Landis and Koch (1977) the ICC values for the H_{max} were almost perfect for the LH-BF (0.967) and substantial for the SH-BF (0.762) and SEMI (0.736). ICC values for the M-wave latency were substantial for SEMI (0.700) and almost perfect for LH-BF (0.931) and SH-BF (0.935). The ICC values indicate almost perfect reliability in the H-reflex latencies for the LH-BF (0.993), SH-BF (0.990) and SEMI (0.978).

	ICC values		
	H_{max}	M-wave latency	H-reflex latency
LH-BF	0.967	0.931	0.993
SH-BF	0.762	0.935	0.990
SEMI	0.736	0.700	0.978

Table 3.2: ICC values for H_{max} amplitudes and latencies of M-waves and H-reflexes in LH-BF, SH-BF and SEMI

Bland Altman plots

Bland Altman plots of M-wave latency and H-reflex latency for LH-BF, SH-BF and SEMI, and H_{max} for SH-BF and SEMI had mean value close to zero, indicating no systematic bias. Bland Altman plot for LH-BF H_{max} is presented in figure 3.2 since this plot had mean value different from zero. Even though the mean value for LH-BF H_{max} was different from zero, the data set was not considered biased (see section 4.1 in discussion). Remaining Bland Altman plots calculated on data from LH-BF, SH-BF and SEMI are presented in appendix page 45.

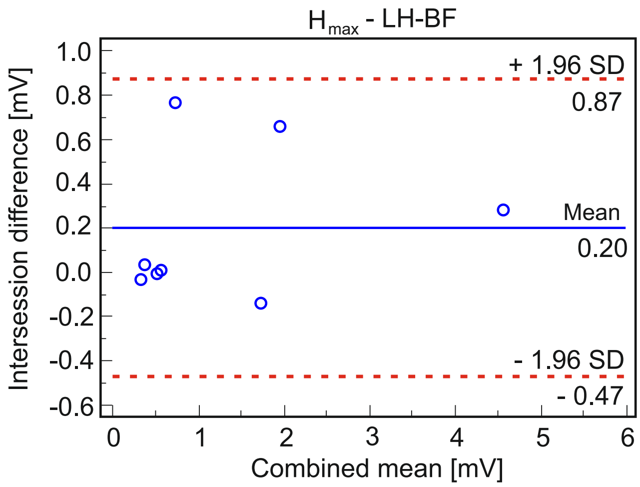

Figure 3.2: Bland Altman plot of absolute agreement between H_{max} recorded from LH-BF

3.2 Secondary results

H-reflex in soleus and tibialis anterior

Recordings from SOL and TA were collected in order to validate that the sciatic nerve was stimulated before branching. The mean H_{max} pp amplitude, M-wave and H-reflex latency and SD for all three parameters are listed in table 3.3

Mean values and SD						
	H_{max} [mV]		M-wave latency [ms]		H-reflex latency [ms]	
	Session 1	Session 2	Session 1	Session 2	Session 1	Session 2
SOL	3.68±3.50	2.27±3.03	13.71±0.78	13.92±0.67	29.17±1.45	29.01±1.58
TA	0.62±0.26	0.60±0.22	12.75±0.97	12.50±0.71	28.13±2.33	28.08±2.29

Table 3.3: Mean values and SD of H_{max} amplitudes and latencies of M-waves and H-reflexes in SOL and TA

SOL and TA EMG recordings from a representative subject are shown in figure 3.3.A and 3.3.B for two sessions and table 3.4 shows the ICC value for SOL and TA.

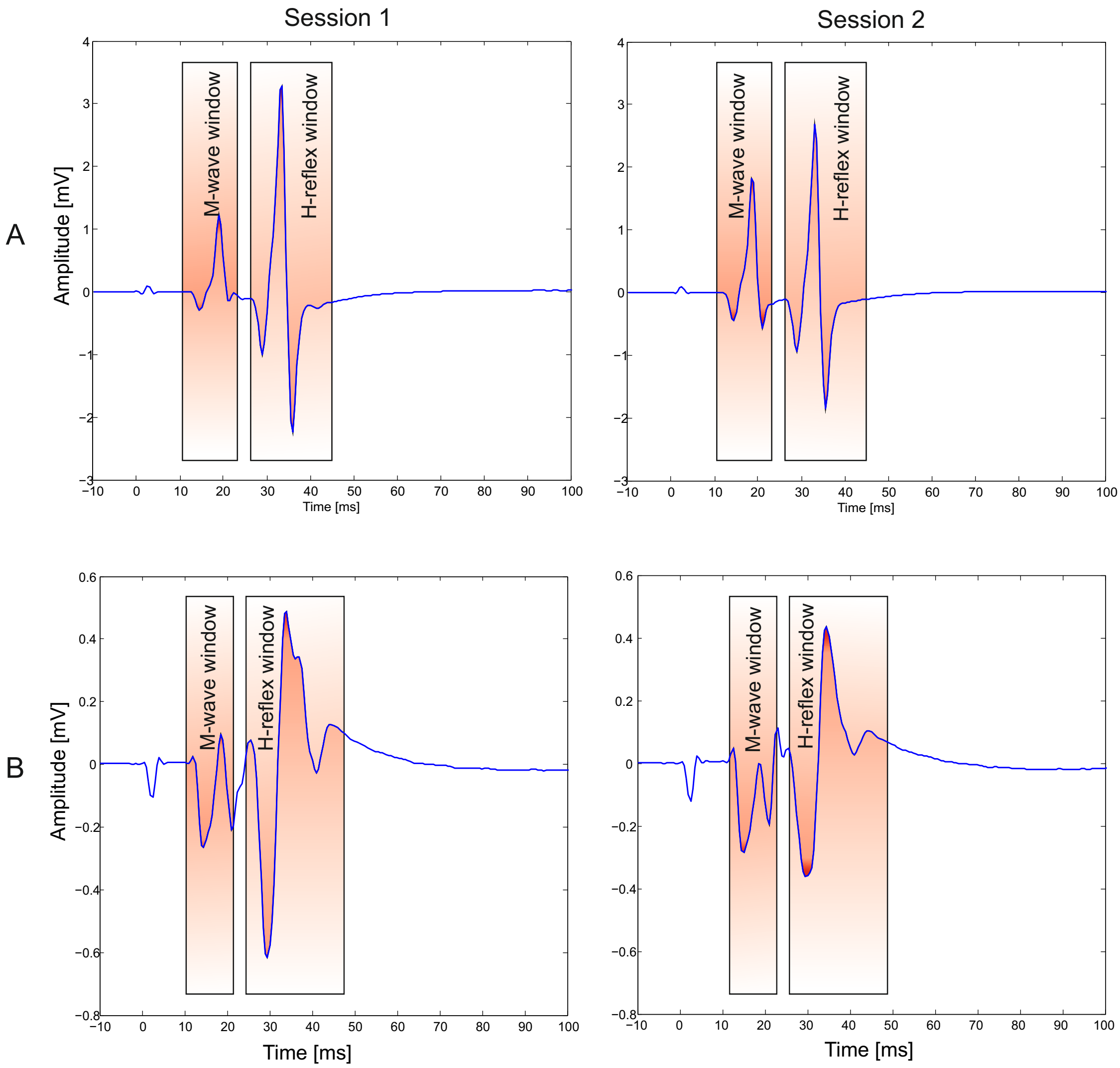

Figure 3.3: Intersession EMG recordings of SOL (A) and TA (B) and M-wave and H-reflex windows used

	ICC values		
	H_{max}	M-wave latency	H-reflex latency
SOL	0.957	0.789	0.769
TA	0.882	0.715	0.951

Table 3.4: ICC values for H_{max} amplitudes and latencies of M-waves and H-reflexes in SOL and TA

Discussion 4

We have designed and validated the proposed technique to stimulate the sciatic nerve and tested the intersession reliability of the technique's ability to elicit H-reflexes in the hamstring muscle group. The primary results in the current study indicate substantial to almost perfect intersession reliability in the hamstring muscle group according to the benchmarks of Landis and Koch (1977), when investigating H_{max} and latencies of the M-waves and H-reflexes.

4.1 EMG recording and input/output curves

The parameters of interest were found by evaluating the EMG pp amplitude within subject-customized time window. Most subjects had easily distinguishable M-waves and H-reflexes, while some had very noisy signals, contaminated by the stimulus artefact and maybe cross-talk activity from adjacent muscles. Especially the M-waves tend to be contaminated by stimulus artefact since these signal components were temporally close to each other in LH-BF and SEMI muscles. The LH-BF and SEMI muscles were the most proximal muscles, meaning that the site of stimulation was located close to the recording sites from these muscles (fig. 2.2). M-wave and H-reflex recordings were easy to distinguish from the stimulus artefact in SH-BF; however it was believed that cross-talk from synergistic muscles, activated by the electric stimulus, were picked up by EMG electrode because of the relative small size of the SH-BF muscle. Cross-talk is further discussed in section 4.3.

Bias and outliners

The Bland Altman plots were used to evaluate whether any systematic bias or outliers were present. A systematic bias would be indicated if the blue horizontal line, representing the mean difference between paired recordings, were significant different from zero. The Bland Altman plots of M-wave latency and H-reflex latency for LH-BF, SH-BF and SEMI, and H_{max} for SH-BF and SEMI indicated no systematic bias. All plots had mean difference close to zero. The Bland Altman plot for H_{max} for LH-BF (fig. 3.2) was inspected in more depth, since the mean difference was different from zero. By inspecting this plot, it was concluded that the mean difference was not caused by systematic bias, but rather caused by two potential outliers. Since the two data sets (potential outliers) only seemed like outliers in one of the nine conducted Bland Altman plots we decided not to exclude the subjects (ICC value without potential outliers for H_{max} for LH-BF would have been 0.997 instead of 0.967). Few other data sets were having values close to the 95% confidence interval limit, but after visual inspection, and comparison with the remaining data sets, they were not considered as clear outliers. No subjects were discarded from the conducted experiment before final data analysis was performed.

4.2 Verification of the protocol

The H-reflexes elicited in the SOL and TA muscle were used to verify the stimulation method since these muscles are innervated by the tibial nerve and the common peroneal nerve branching from the sciatic nerve [Palmieri et al. 2004; 2002]. Recordings showed H-reflexes in SOL and TA, indicating that the sciatic nerve was stimulated before branching into these nerves (fig. 3.3). Palmieri et al. (2002) investigated the reliability of H-reflexes in SOL and TA by applying electrical stimulation over the posterior tibial nerve in the popliteal fossa and over the common peroneal nerve located superior to the popliteal fossa [Palmieri et al. 2002]. Their results showed an intersession reliability of 0.995 (SOL) and 0.859 (TA) when investigating H_{max}. In the current study the ICC for H_{max} was 0.957 (SOL) and 0.882 (TA), which corresponds well with the findings of Palmieri et al. (2002). The comparison with Palmieri et al. (2002) indicates that stimulation of the sciatic nerve can be as reliable as direct stimulation of the tibial nerve and the common peroneal nerve when it comes to activation of H-reflexes in SOL and TA.

Reliability of SOL H-reflex latency

Studies by Ali and Sabbahi (2001) and Hopkins et al. (2000), found intersession reliability of the H_{max} amplitude for the SOL muscle to be 0.970 and 0.932, respectively, after stimulating the tibial nerve. This fits well with our findings (0.957). Although the current study uses a different experimental protocol compared to Ali and Sabbahi (2001) and Hopkins et al. (2000), the results are considered comparable, since all studies attempted to control factors which are known to cause variability in the H-reflex measurements, such as joint angle and position [Hwang 2002, Hwang et al. 2002, Knikou and Rymer 2002, Chapman et al. 1991], muscle length [Hayashi et al. 1992, Garland et al. 1994], muscle contractions [Kameyama et al. 1989]. The studies by Ali and Sabbahi (2001) and Hopkins et al. (2000) suggest that four to five trials are needed to obtain reliable results [Ali and Sabbahi 2001, Hopkins et al. 2000]. The data from the current study along with Palmieri et al. (2002) suggest that reliable measures can be obtained when averaging data from three trials if factors that might cause variability in the H-reflex measurements are controlled [Palmieri et al. 2002].

Latency of H-reflex

The latency of the M-wave and H-reflex is dependent of the nerve conduction velocity, which is a function of the nerve fibre properties, e.g. type of nerve, diameter and length. It is important to know what window to use when examining EMG signals to determine the latency of the M-wave and H-reflex. As described in section 1.2 the H-reflex appears later in time than the M-wave due to the difference in length the two signals have to travel. The closer the muscle or stimulation site is to the spinal cord, the shorter the latency [Palmieri et al. 2004]. Since no prior studies have investigated H-reflexes in the hamstring muscle group after stimulating the sciatic nerve, our results are not directly comparable with other studies.

Studies investigating H-reflex latencies in SOL have reported latencies of 30.8 ± 2.3 ms [Alrowayeh et al. 2011] and 30.7 ± 2.6 ms [Falco et al. 1994] after stimulation in the poptiteal fossa during prone laying. T-tests showed a significant difference in SOL H-reflex latencies when our latencies (session 1: 29.17 ± 1.45 ms, session 2: 29.01±1.58 ms) were compared to results from Alrowayeh et al. (2011) (t-test$_{session1}$, p<0.05, t-test$_{session2}$, p<0.05) and Falco et al. (1994) (t-test$_{session1}$, p<0.01, t-test$_{session2}$, p<0.01). Compared to the two mentioned studies our SOL H-reflex latency differed by approximately 1.7 ms seems reasonable. The difference might be explained by our more proximal site of stimulation.

This latency difference can be estimated by knowing the nerve conduction velocity of the Ia afferent fibres and the distance between the our optimal cathode site and the poptiteal fossa. If we assume a nerve length of 0.2 m between our optimal cathode site and the poptiteal fossa and a nerve conduction velocity (NCV) to be 72-120 m/s [Kandel et al. 2013, chap.22], then the SOL H-reflex latency difference should, theoretically, be 1.7 to 2.8 ms. This theoretical calculation of latency differences corresponds well with the actual latency difference of 1.7 ms.

The results in table 3.1 and 3.3 shows that the H-reflex latencies in SOL and TA are longer compared to the hamstring muscle group, which was expected, considered the longer distance the signals have to travel in order to reach SOL and TA compared to the hamstring muscle group.

Comparison of H-reflex latencies from the hamstring muscle group with results found in literature cannot be done directly, since no earlier studies have investigated the latencies of the hamstring muscle group after sciatic nerve stimulation. However, a study investigating the tendon jerk reflex latency for the LH-BF. Faist et al. (1999) reported a LH-BF stretch reflex latency ranging from 18-24 ms [Faist et al. 1999]. This latency is slightly shorter than our findings of 25 ms (25.06±4.20 to 25.60± ms). Even though the stretch reflex and H-reflex are using some of same neural pathways in order to elicit muscle responses, they are not directly comparable (section 1.4), due to the muscle spindles.

4.3 Methodological considerations and limitations

An intersession reliable experimental protocol to measure H-reflexes in the hamstring muscle group have been designed, however there are some limitations attached to the protocol. The reliability of the protocol have only been evaluated on males. In order to conclude whether the protocol can be used on female gender, ekstra experiments have to be conducted. A consideration, which researchers have to take into account, is the fact that the latencies are defined manually, which is discussed in the following section.

Subjectivity

The manual extraction of the parameters of interest (section 2.2) could add bias to the results. In order to avoid bias caused by the research group the extraction of parameters could have been done by independent evaluators, who did not know about the aim of the current study. By having independent evaluators, the interpretation could be reasonably objective. [Drapeau 2002]

To minimize interpretation bias, we did the data interpretation and extraction within the research group to get independent opinions before agreeing to what parameter values to extract from the data. However, this interpretation and extraction of data might still unconsciously be biased because we knew what the data should be used for and what statistical methods that would be performed on the data. The design of a blinded study where the researchers are interpreting the data in randomized order, meaning that the researchers do not know what subject number or session the current data belongs to would minimize the bias of subjectivity.

Excluded parameters from the signal

Since the M_{max} plateau was not reached due to hardware limitations it was not possible to calculate the H_{max}/M_{max} ratio. In order to reach M_{max} higher stimulation intensity should have been used. In our first conducted pilot study, page 40, we applied two stimulations of up to 100 mA with an interstimuli interval of 4 ms. However using the double stimulation technique, the M_{max} was not reached and the subjects reported discomfort and cold sweats at high stimulus intensities using this stimulation technique. They reported that the double stimulation with intensity higher than 100 mA most likely would be intolerable. The fact that M_{max} was not reached is an important limitation of the experimental setup since we could not estimate how much of the MN pool was activated when measuring H-reflexes. The high stimulation intensity required to reach the M_{max} plateau can be explained by the deep laying nature of the sciatic nerve [Chan et al. 2006].

Cross-talk

Cross-talk from adjacent muscles is a common problem when recording EMG signals. The effect of the cross-talk problem is a combination of muscle size, electrode size and electrode placement. The ICC value imply that we recorded the same signals over two sessions, but it does not provide information whether any cross-talk was present. It is impossible to completely eliminate cross-talk, but it can be reduced [Merlo and Campanini 2010]. In the current study cross-talk was reduced by using the standardized electrode placement recommendations from the SENIAM project [Hermens et al. 1999]. Large electrodes will pick up more signal and thereby increase signal amplitude of the recorded data than smaller electrodes. However, large electrodes will also increase the risk of cross-talk when recording from relatively small muscles [Merlo and Campanini 2010]. In the current study, we chose EMG electrode sizes that fitted to the muscles of interest. Generally, EMG signals are more contaminated with cross-talk when measuring from small muscles adjacent to larger muscles [Merlo and Campanini 2010]. This might explain the difficulty in implicitly define the time latency for H-reflexes in the SH-BF muscle for some subjects. In addition to this, it has been shown that EMG electrodes can move with respect to the skin due to muscle shifts when performing a muscle contraction [Rainoldi et al. 2004]. A muscle shift can alter the recorded signal since the electrode does not constantly measure from the same spot [Rainoldi et al. 2004]. This problem becomes larger when going from low to high stimulus intensities since the muscles contractions becomes progressively stronger, which changes the muscle length causing the muscle to shift. To minimise the risk of electrode movement our subjects were lying prone.

The problem of cross-talk might be reduced in future studies, as a new technique to minimize cross-talk has been developed. Jensen et al. (2013) have developed a technique which uses double differential EMG to detect cross-talk during withdrawal reflex recordings [Jensen et al. 2013]. It would be interesting to use the conduction velocity analysis in future studies in order to improve our designed experimental protocol.

4.4 Perspective

The experimental protocol to stimulate the sciatic nerve proposed in the current study can hopefully be applied to patients suffering from neuromuscular deficits during gait, see e.g. section 1.5 which summarizes some of the applications of the use of H-reflexes in research and clinical settings. The protocol can be used in future studies to obtain quantitative information about the posterior thigh muscle, e.g. applying the stimulation technique in a study of H-reflexes in the hamstring muscle group and their interaction and communication between other muscles in the legs. An interesting next step would be to test the designed protocol during human locomotion.

In addition to future research, it would be of interest to have the 4x2 cathode grid, customised into a fixed matrix electrode. The size of the individual stimulation electrode would then have the same dimensions and inter-electrode distance would be fixed. The matrix electrode should still be placed along the line between the ischial tuberosity and the lateral condyle of femur, two centimetres below the buttock as proposed in the current study.

Another improvement of the designed experimental protocol would be to design a system which automatically could detect the optimal stimulation site after placing the fixed matrix electrode. In order to do that, a software system should be designed to automatically stimulate each of the stimulation sites, store data, calculate H-reflex pp amplitudes for each site, and then presenting the optimal stimulation site. An automatic system to find the optimal stimulation site for the sciatic nerve, can reduce the time and resources needed to conduct scientific experiments when investigating muscles activated by the sciatic nerve e.g. the hamstring muscle group, SOL and TA.

Appendix 5

The appendix contains the following parts:

H-reflexes in the hamstring, soleus and tibialis anterior induced by electrical stimulation to the sciatic nerve

Søren S. Dueholm, Jesper H. Rasmussen, Erika G. Spaich and Natalie Mrachacz-Kersting

Abstract— **H-reflexes are well documented to provide information about the current state of the central nervous system. However, only little research has investigated the modulation of H-reflexes within the hamstring muscle group (hamstring) despite the importance of this muscle in human locomotion. The lack of research might be due to the difficulty of electrically stimulating the sciatic nerve innervating the hamstring. This paper investigates whether H-reflexes can be elicited in the hamstring using three different stimulation techniques; (1) single pulse, (2) double pulse with pre-stimulation sub-threshold to the H-reflex and (3) double pulse of same intensity.**

Two healthy male subjects (26 years) took part in the experiment. Electrical stimulation was applied to the sciatic nerve and sEMG was measured from the short and long head of biceps femoris, semitendinosus, soleus and tibialis anterior muscles. Subjects' sciatic nerve were stimulated using three different techniques: (1) one single stimulation (varied from 10 to 100 mA), (2) double shock technique with the first stimulation fixed to sub-threshold intensity and second stimulation varied from 10 to 100 mA and (3) double shock technique, with both stimulations having the same intensity (10 to 100 mA). Stimulation threshold, latencies and maximum value of the H-reflex and M-wave were extracted as parameters.

During each of the stimulation techniques H-reflexes were recorded in all five muscles. Calculation of the intraclass correlation coefficients between stimulation techniques showed significant reliability for both H-reflex and M-wave threshold (0,975 and 0,960, respectively), H-reflex and M-wave latency (0,956 and 0,959, respectively) and H_{max} (0,985). It was not possible to determine the M_{max} as it did not reach a plateau within the tested stimulation intensities.

Recordings of H-reflexes in the thigh and lower leg muscles reveal that H-reflexes in the hamstring muscles are present after applying electrical stimulating to the sciatic nerve. Furthermore, our data indicates no reason to use double shock technique as a substitute for single stimulation in order to elicit H-reflexes in the hamstring since no significant differences in the evaluated parameters were found and subjects complained about discomfort when using double shock technique. We therefore recommend using one shock stimulation technique, since this reduces discomfort for the subjects.

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I. Introduction

Paul Hoffmann first showed the Hoffmann reflex, also called the H-reflex in 1910 [1]. The H-reflex is often described as an electrical induced analogue to the mechanically induced stretch reflex and has been used as a tool to investigate synaptic activity in the spinal cord [2]. The synaptic activity can changes during different neurological conditions, as spinal cord injury and Huntington's disease [Fisher1992,Braddom1974]. H-reflexes have also been used as a physiological measurement of synaptic activity during running, walking and standing [5-9].

The H-reflex can theoretically be elicited in any muscle in if the peripheral nerve innervationing this muscle can be electrically stimulated. The soleus and tibialis anterior muscles are often used when studying synaptic activity when lying, running, walking and standing, since the peripheral nerves innervating those muscles are superficial and easy to electrically stimulate [2]. However, when using H-reflexes to evaluate muscles role in locomotion, the muscles in the thigh are rarely examined. A muscle group, which plays a crucial role in locomotion, is the hamstring (short and long head of biceps femoris, semitendinosus, semimembranosus), since the hamstring is activated during both knee flexion and hip extension during the gait cycle [10]. One reason for lack in research investigating H-reflex modulations of the hamstring might be that it is difficult to stimulate the deep-lying sciatic nerve, which innervates the hamstring. If recordings of reliable H-reflexes in the hamstring could be obtained, they could be used to learn more about the nervous system, e.g. during gait.

The main aim of our study was to evaluate whether it was possible to elicit H-reflexes in the human hamstring muscles group by applying electrical stimulation above the sciatic nerve.

II. Methods

A. Subject

Two healthy male subjects aged 26 participated in this study. Both subjects did not have any known physical or neurological disorders. Informed consent was obtained from each subject. Informed consent was obtained from each subject and the study protocol was approved by the Scientific Ethics Committee Northern Jutland, Denmark (reference number: N-20110076)

B. Experimental procedure

Subjects were lying in prone position. Electrical stimulation was applied to the sciatic nerve and surface EMG (sEMG) was measured from five leg muscles.

C. Stimulation

Stimulation electrodes were used to stimulate the sciatic nerve in the subjects left leg. The anode (PALS platinum rectangle electrode 5x9 cm, Axelgaard Manufacturing, USA) was placed on the great trochanter of femur and the cathode was a custom-made probe electrode, which was placed along the lead line between the ischial tuberosity and the lateral condyle of femur, two centimeters below the buttock.

Three different stimulation techniques were tested; (1) one pulse stimulation (varied from 10 to 100 mA), (2) double shock technique with the first pulse fixed to sub-threshold intensity and second pulse varied from 10 to 100 mA and (3) double shock technique, with both pulses having the same intensity (10 to 100 mA). Each of the stimulation techniques were applied using 16 different stimulation intensities (varying from 10 mA to 100 mA). Stimulations were applied as rectangular constant square pulses of 1 ms duration. When applying double shock technique the inter-stimuli interval was set to 4 ms. Three repetitions were done within three experimental parts where the subjects were stimulated by one out of three different electrical stimulation techniques. Figure 1 illustrates how electrical stimulation to a peripheral nerve affects both sensory- (Ia afferent) and motor nerves (α-motoneurons).

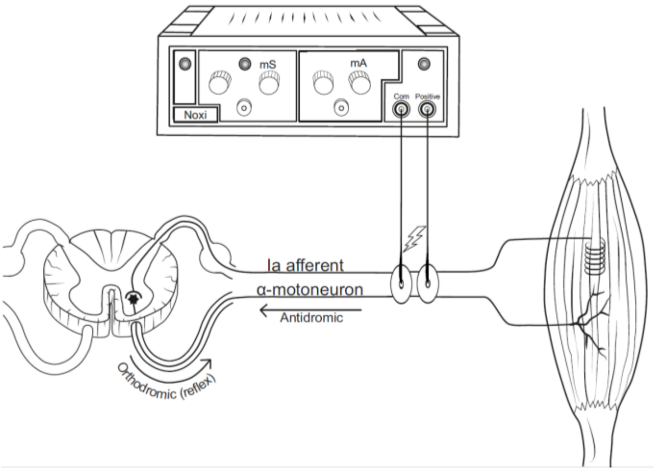

Figure 1 An electrical stimulator was used to activate both the Ia afferent nerve (sensory) and α-motoneuron. At low stimulation intensities, only the Ia afferent nerve is activated, resulting in a signal travelling along the Ia afferent fibres to the MN pool at spinal cord level. When the signal reaches the MN pool, action potentials are elicited and travel along the α-motoneuron to the muscle (orthodromic impulse). At high stimulation intensities, the α-motoneuron starts to be activated directly, resulting in an M-wave and an antidromic impulse. Orthodromic and antidromic impulses will reduce each other's strength when colliding.

D. Recording

Ag/AgCl sEMG electrodes (Neuroline 720, Ambu, Denmark) were placed on the muscle belly of the short head of biceps femoris, long head of biceps femoris, semitendinosus, soleus and tibialis anterior as recommended by SENIAM [11]. A common reference electrode was placed on distal end of the medial surface of the tibia bone. sEMG was recorded from soleus and tibialis anterior in order to verify that the sciatic nerve was stimulated before branching into the tibial nerve and common peroneal nerve.

EMG signals were gained and filtered (DC to 500 Hz for the hamstring and 5 to 500 Hz for soleus and tibialis anterior) using custom-built amplifiers and digitized using a 12 bit, 16-channel ADC (NI 6024E, National Instruments, USA). The bandwidth of the filters was determined so the capacitors in the high-pass filters were bypassed, which removed the smoothing artefact created from these capacitors' time constants affecting the distinct separation of the stimulus artefact and the M-wave. This capacitor problem was created because the recordings from the hamstring muscle group lies close to the stimulation site.

ADC was mounted in a BNC terminal block (NI BNC-2090, National Instruments, USA), forwarding the signals to be acquired, displayed and saved by custum-built software (Wirex, Aalborg University, Denmark) on a computer running Windows 7.

E. Data processing

H-reflex and M-wave peak-to-peak (pp) amplitude values were calculated from each of the five muscles, at each of the 16 stimulation intensities. This was done for each of the three different stimulation techniques. The H-reflex- and M-wave pp values were used to construct an input/output (IO) curve for each muscle for each stimulation technique (15 IO curves for each subject). Parameters of interest from the input/output curves were H-reflex threshold, M-wave threshold, H_{max}, M_{max}, H-reflex latency and M-wave latency.

Due to inter-subject H-reflex and M-wave variability the time windows, used to calculate peak-to-peak values, were manually inspected and adjusted to fit the individual subject.

For the double shock technique using a fixed sub-threshold stimulation, time-zero was defined as the onset of the second stimulus. Time-zero was for the single and the double stimulation techniques (stimuli having same intensity) defined as the onset of the first stimulation. Time-zero was defined in order to evaluate H-reflex- and M-wave latencies.

F. Statistical analysis

Absolute agreement was used to test the reliability between the three stimulation techniques using intraclass correlation coefficients (ICC [2,1]).

III. RESULTS

By the use of the proposed experimental protocol, it was possible to elicit H-reflexes in the hamstring (short head of biceps femoris, long head of biceps femoris and semitendinosus). H-reflexes were also found from soleus and tibialis anterior. H-reflexes were elicited using each of the three different stimulation techniques.

The intraclass correlation coefficients between stimulation techniques indicated almost perfect reliability according to the benchmarks of Landis and Koch (1977) [12]: H-reflex threshold (0.975), M-wave threshold (0.960), H_{max} (0.985), H-reflex latency (0.959) and M-wave latency (0.956). It was not possible to calculate M_{max}, since the subjects did not reach M_{max} plateau.

The subjects were asked to describe the sensation of the three stimulation techniques. It was clear that the double shock technique, having the same stimulation intensities, was the most painful and subjects reported cold sweats.

A sEMG recording, showing stimulation artefact, H-reflex and M-wave can be seen in figure 2 while figure 3 shows an input/output curve, based on peak-to-peak amplitudes.

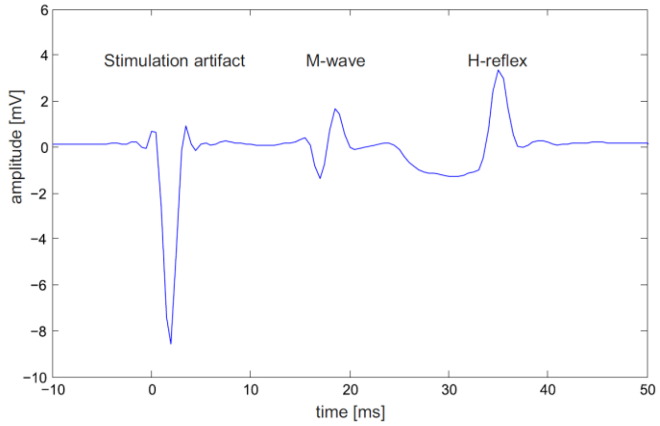

Figure 2 sEMG recording from long head of biceps femoris (hamstring) using one electrical stimulation.

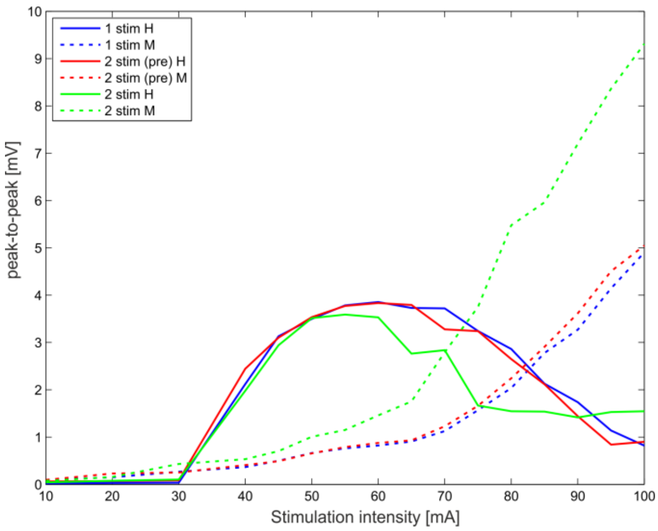

Figure 3 Input/output curve based on H-reflex- and M-wave peak-to-peak values at intensities ranging from 10 – 100 mA, for semitendinosus (hamstring).

IV. Discussion

Stimulation of the sciatic nerve can be difficult; however, three studies claim to have stimulated the sciatic nerve [13-15]. The first study by Pierrot-Deseilligny et al (1981) uses the double stimulation technique in order to elicit H-reflexes, but they do not specify the placement of the stimulation electrodes [13]. The second study [14] stimulates the sciatic nerve just before it branches into the tibial and common peroneal nerves in the popliteal fossa [14]. By placing stimulation electrodes at the superior portion of the popliteal fossa and the other electrode superior to the patella, they were capable of evaluating reflexes in the lower leg (soleus, peroneal, and tibialis anterior). By the use of their protocol, it is not possible to evaluate H-reflexes in the thigh, since these muscles are more proximal to the stimulation site. The third study stimulated the root of the sacral spinal nerves (which in the study was defined as being the sciatic nerve) [15]. The sciatic nerve consists of nerve branches from both sacral and lumbar roots, and therefore this sciatic nerve stimulation site might not stimulate the entire sciatic nerve. H-reflexes were measured from biceps femoris, but it was not specified whether it was from the short head or the long head of biceps femoris. None of the mentioned studies has designed a reliable stimulation technique, which can be used to measure H-reflexes in both thigh and lower leg muscles.

According to the results it is possible to record H-reflexes in both thigh and lower leg muscles by applying electrical stimulating to the sciatic nerve. Furthermore, the data indicates no reason to use double shock technique as a substitute for one pulse stimulation in order to elicit H-reflexes in the hamstring since no significant differences in the evaluated parameters (H-reflex threshold, M-wave threshold, H_{max}, H-reflex latency and M-wave latency) were found. We therefore recommend using one pulse stimulation technique to reduce discomfort for the subjects.

V. Perspective

The results from this study have provides the first indication that H-reflexes are recordable from the hamstring by stimulating the sciatic nerve. In future studies, it would be of interest to evaluate the intersession reliability of this technique.

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A novel technique to elicit H-reflexes in the human hamstring muscle group

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Objective

H-reflexes are well documented to provide information about the current state of the central nervous system (CNS) [1]. However, only little research has been performed on the modulation of H-reflexes in the hamstring muscle group despite their importance in human locomotion [1]. The aim of the current study was to design, test and validate a new technique to elicit the H-reflex in the hamstring muscle group.

Methods

Eight healthy male subjects (25.25±0.71 yrs) participated in a two session experiment. Subjects (lied in prone position) and were applied electrical stimuli through a 4x2 grid electrode (cathode) placed two centimetres below the buttocks. The anode was placed on the greater trochanter of the femur. The best site for cathode placement was found by applying electrical stimuli at each site in the electrode grid with an intensity 25% above the resting motor threshold and measuring peak-to-peak surface EMG amplitudes in the long and short head of biceps femoris (LH-BF and SH-BF), semitendinosus (SEMI), soleus (SOL) and tibialis anterior (TA). SOL and TA were used as control muscles. Electrical stimulation was applied at the best site (intensity: 10 to 100 mA) and input/output responses were plotted. H_{max} and H-reflex latencies were extracted as parameters. Two identical sessions were conducted one day apart and intraclass correlation coefficients (ICC) for each parameter within each muscle were calculated to evaluate intersession reliability.

Results

H_{max} ICC for LH-BF, SH-BF and SEMI were 0.980, 0.777 and 0.797, respectively. H-reflex latencies for LH-BF, SH-BF and SEMI were 26±4.6, 28±3.3 and 23.6±2.7 ms and ICC for H-reflex latencies were 0.996, 0.991 and 0.939, resp. H-reflexes were also observed in SOL and TA.

Conclusions

The ICC values indicated a strong agreement between the parameters measured in both sessions. This indicates that our new technique to elicit the H-reflex in the hamstring muscles group provides reliable results between sessions. The presence of H-reflexes in SOL and TA indicated that the sciatic nerve was stimulated before branching into the tibial nerve and the common peroneal nerve. Reliable H-reflexes in the hamstring muscle group provides a new tool to investigate the CNS, e.g. during gait.

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5.3 Bland Altman plots

Bland Altman plots are presented for H_{max}, M-wave latency and H-reflex latency for long head of biceps femoris, short head of biceps femoris and semitendinosus respectively.

Long Head of Biceps Femoris

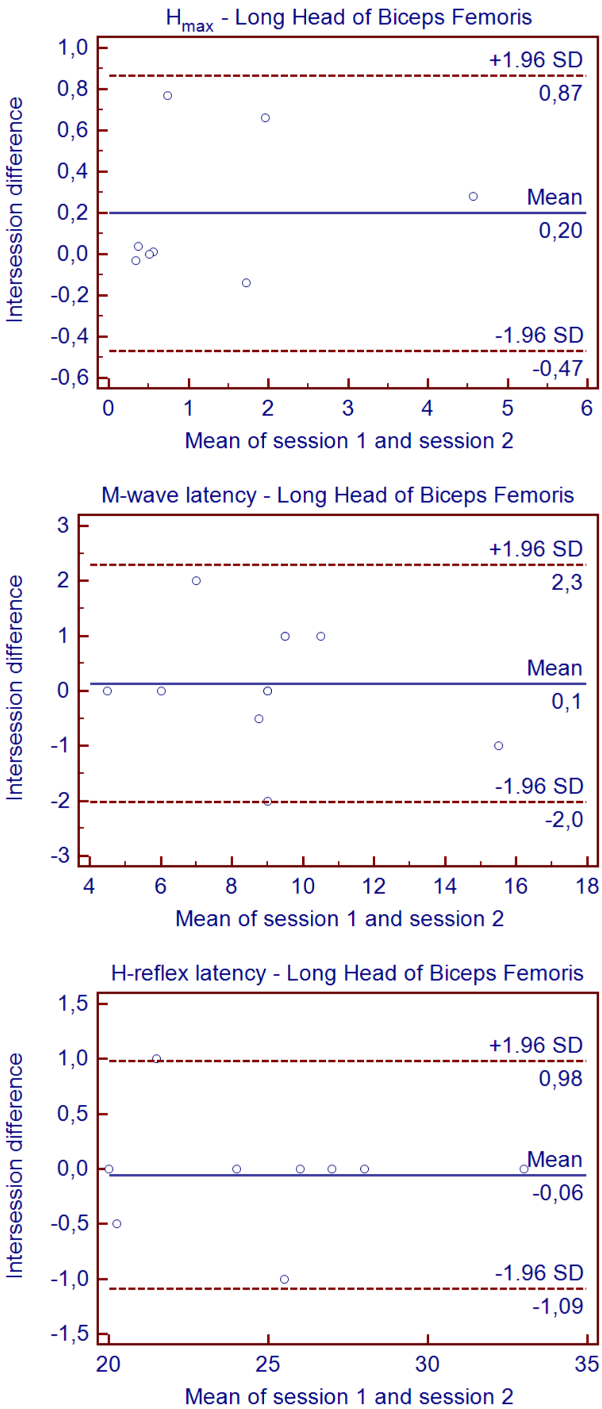

Figure 5.1: Top figure: H_{max} for long head of biceps femoris. Middle figure: M-wave latency for long head of biceps femoris. Bottom figure: H-reflex latency for long head of biceps femoris.

Short Head of Biceps Femoris

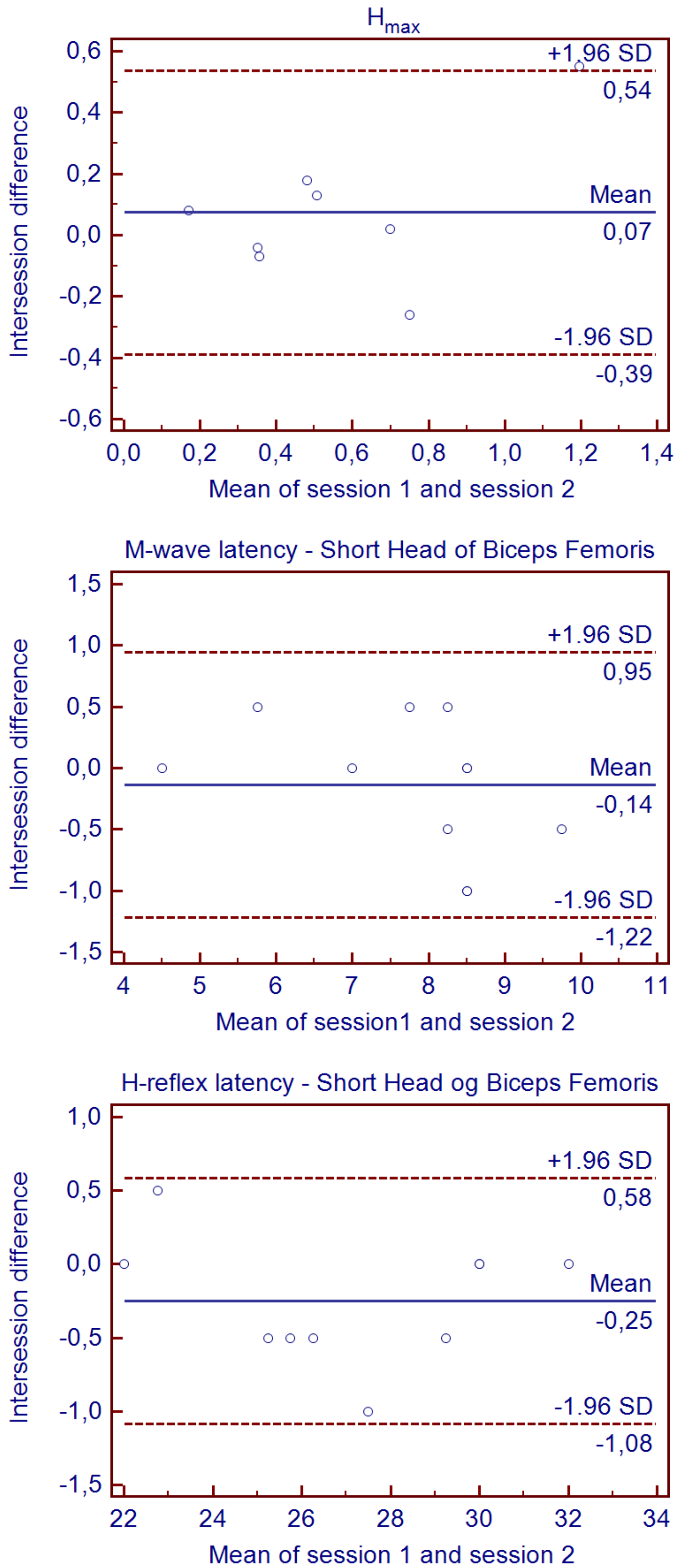

Figure 5.2: Top figure: H$_{max}$ for short head of biceps femoris. Middle figure: M-wave latency for short head of biceps femoris. Bottom figure: H-reflex latency for short head of biceps femoris.

Semitendinosus

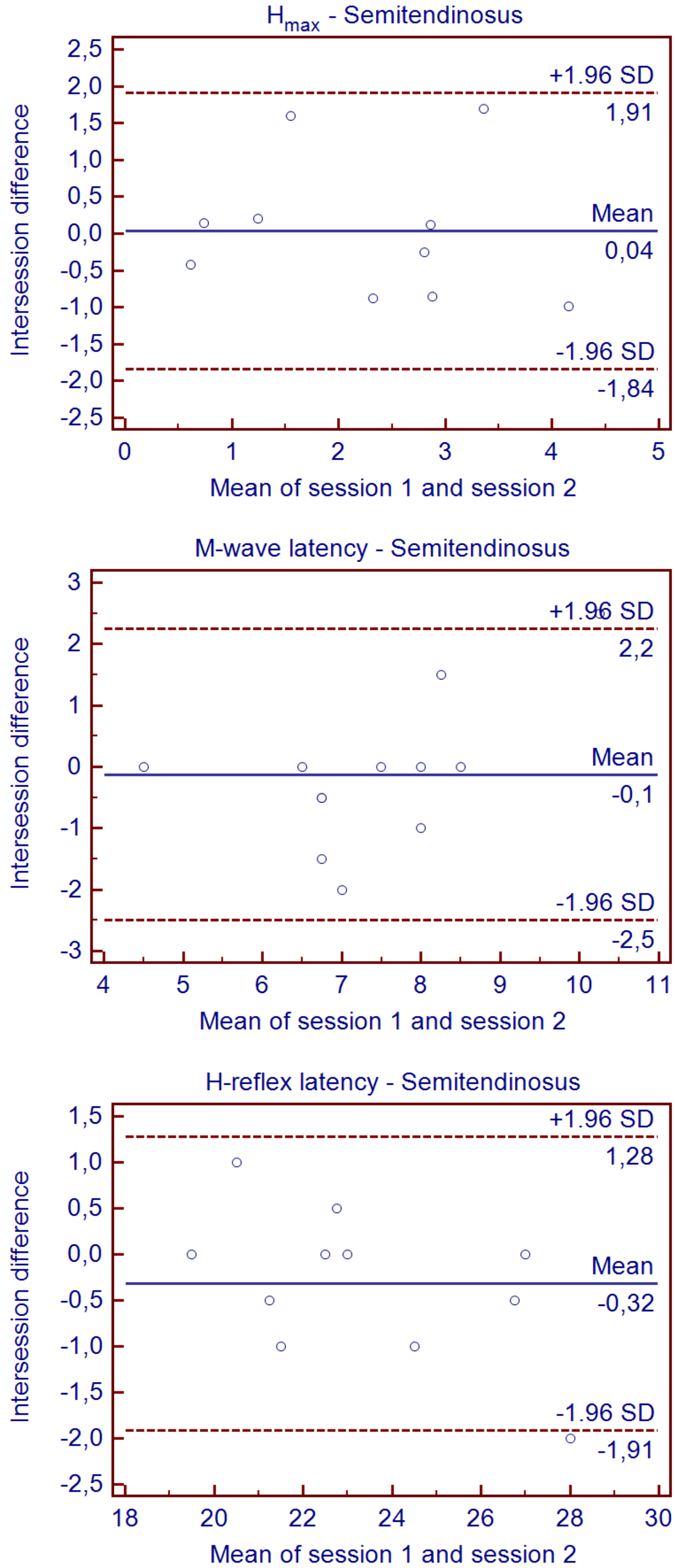

Figure 5.3: Top figure: H_{max} for semitendinosus. Middle figure: M-wave latency for semitendinosus. Bottom figure: H-reflex latency for semitendinosus.

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