Eccentric Exercise Inhibits the H Reflex in the Middle Trapezius Muscle

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AALBORG UNIVERSITET

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Abstract

The aim of this study was 1) to test the reproducibility of the H reflex across consecutive days and 2) to investigate the effect on the H reflex immediately after eccentric exercise and during delayed-onset muscle soreness (DOMS) (24h after eccentric exercise).

H reflexes were recorded from subjects' (n=10) dominant middle trapezius muscle by electrical stimulation of the C3/4 cervical nerve. DOMS was induced by eccentric exercise of the shoulder joint in an experimental setting. H reflexes were obtained in four sessions: "24h before", "Pre", "Post", and "24h after" eccentric exercise. Ratios of maximal H reflex and M wave responses (H_{max}/M_{max}) were compared between sessions. Additionally, ratios of H reflex amplitudes obtained from 75% and 50% of stimulus intensity needed to obtain H_{max} in the session 24h before eccentric exercise (H_{75}/M_{max}, and H_{50}/M_{max}) were also compared between sessions.

Results obtained from sessions before eccentric exercise showed no change in ratios. A decrease in H_{50}/M_{max} ratios was found immediately after eccentric exercise ($P<0.05$). The presence of DOMS 24h after eccentric exercise resulted in a decrease in H_{75}/M_{max} and H_{50}/M_{max} ratios ($P<0.05$).

This study presents evidence that comparison of baseline measures showed acceptable reproducibility of the H reflex with the study's experimental design. Furthermore, stronger stimulus intensity was needed immediately after and 24h after exercise to reach the same magnitude of the H reflex. This modulation of the stimulus-response relationship may be caused by presynaptic inhibition of Ia afferent fibres' input to the motoneuron by group III and IV afferent fibres.

Titel:

Excentriske øvelser inhiberer H refleksen i den midterste trapezius muskel

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Synopsis

Formålet med studiet var 1) at undersøge reproducerbarheden af H refleksen fra dag til dag og 2), at undersøge hvorledes excentriske øvelser påvirker H refleksen både lige efter og 24 timer efter øvelserne, når muskelømhed var indtruffet (eng: delayed-onset muscle soreness (DOMS)).

H reflekser blev udløst ved elektrisk stimulering af den cervikale nerve C3/4 og optaget via EMG målinger fra forsøgspersoners (n=10) dominante midterste trapezius muskel. DOMS blev induceret ved excentriske skulderøvelser i en eksperimentiel opsætning. H reflekser blev optaget i fire sessioner: "24t før", "Præ", "Post", og "24t efter" excentriske øvelser. Ratioer af de maksimale H refleks- og M bølgeresponser (H_{max}/M_{max}) blev sammenlignet mellem sessioner. Derudover blev ratioer fra H refleks-amplituder optaget med 75% og 50% af stimuleringsintenstiteten, som var nødvendig for at opsamle H_{max} i sessionen "24t før" (H_{75}/M_{max} og H_{50}/M_{max}), også sammenlignet mellem sessioner.

Resultater optaget fra sessioner før de excentriske øvelser viste ingen forandring i ratioerne. Et fald i H_{50}/M_{max} ratioer blev fundet lige efter øvelserne (ťťPost", $P<0.05$). Tilstedeværelsen af DOMS "24t efter" excentrisk øvelse resulterede i et fald i H_{75}/M_{max} og H_{50}/M_{max} ratioer ($P<0.05$).

Studiet præsenterer således bevis for, at målinger fra før øvelserne viste en acceptabel reproducerbarhed af H refleksen med studiets eksperimentelle design. Derudover var højere stimuleringsintensitet nødvendig lige efter og 24t efter excentriske øvelser, for at opnå den samme størrelse af H refleksen. Dermed blev rekrutteringskurver (stimulus-respons-kurver) forskudt til højre. Denne modulering af stimulus-respons-forholdet kan være forårsaget af præsynaptisk inhibering af Ia-afferente fibres input til motorneuronet fra gruppe III og IV afferente fibre.

Preface

This report is written by student group 1087a from the Department of Health Science and Technology, Aalborg University, Denmark, during the 4^{th} semester of the master education Biomedical Engineering and Informatics. The project was carried out in the period from February the 1^{st} to June the 1^{st} 2011.
In total, the project group has completed the work of a manuscript for publication in a scientific journal as well as a report with worksheets to support the manuscript and document the work carried out during the project period.
The report is addressed to fellow students at the Department of Health Science and Technology (Aalborg University), the project supervisors, and others interested in the research on H reflex changes resulting from exercise and the reproducibility of the H reflex in experimental settings.
In the scientific paper, reference method is based on Harvard Style with the author-date method. Thus, references includes the authors name and year of publication (e.g. Madeleine et al., 1998).
In the report, the reference method is based on Vancouver Style. Thus, references are numbered consecutively in order of appearance in the text. They are identified by Arabic numerals in square brackets (e.g. [8]). References to sections, tables, and figures in the report are noted as e.g. "2.4" where the first number refers to the chapter and the second refers to the section of the chapter.

The project group would like to thank the following persons for their support and contribution to the project:
Associate Professor Janet Taylor, Neuroscience Research Australia, for valuable input to the design of the experimental protocol and interpretation of results.
Research assistant Professor Afshin Samani, Aalborg University, for producing a LabVIEW interface for use in providing subjects with live visual feedback of muscle contraction activity.

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Scientific Paper

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Abstract— **The aim of this study was 1) to test the reproducibility of the H reflex across consecutive days and 2) to investigate the effect on the H reflex immediately after eccentric exercise and during delayed-onset muscle soreness (DOMS) (24h after eccentric exercise).**
H reflexes were recorded from subjects' (n=10) dominant middle trapezius muscle by electrical stimulation of the C3/4 cervical nerve. DOMS was induced by eccentric exercise of the shoulder joint in an experimental setting. H reflexes were obtained in four sessions: "24h before", "Pre", "Post", and "24h after" eccentric exercise. Ratios of maximal H reflex and M wave responses (H_{max}/M_{max}) were compared between sessions. Additionally, ratios of H reflex amplitudes obtained from 75% and 50% of stimulus intensity needed to obtain H_{max} in the session 24h before eccentric exercise (H_{75}/M_{max}, and H_{50}/M_{max}) were also compared between sessions.
Results obtained from sessions before eccentric exercise showed no change in ratios. A decrease in H_{50}/M_{max} ratios was found immediately after eccentric exercise ($P<0.05$). The presence of DOMS 24h after eccentric exercise resulted in a decrease in H_{75}/M_{max} and H_{50}/M_{max} ratios ($P<0.05$).
This study presents evidence that comparison of baseline measures showed acceptable reproducibility of the H reflex with the study's experimental design. Furthermore, stronger stimulus intensity was needed immediately after and 24h after exercise to reach the same magnitude of the H reflex. Hence, recruitment curves (stimulus-response curve) were delineated to the right. This modulation of the stimulus-response relationship may be caused by presynaptic inhibition of Ia afferent fibres' input to the motoneuron by group III and IV afferent fibres.

Keywords—**H reflex, Trapezius, Electrical stimulation, Reproducibility, Delayed-onset muscle soreness**

I. Introduction

WORK -related musculoskeletal disorders (WMSD) pose a significant health care problem in the western world. This is concerning not only because of the health effects on individuals, but also because of the economical aspects, as some studies estimate the cost to be between 0.5% and 2% of the Gross National Product in European countries (Buckle & Devereaux, 1999). A European survey shows that about 23% of workers in European countries reported neck and shoulder pain. Despite comprehensive attention, WMSDs are still difficult to diagnose due to lack of clinical tests.(Schneider & Irastorza, 2010)
The trapezius muscles form very important parts of the neck-shoulder region and are commonly affected by WMSD due to low-level and long-term activity (Mork & Westergaard, 2005; Nimbarte et al., 2010). They extend longitudinally from the occipital bone to the lower thoracic vertebrae and laterally to the spines of the scapulae. The muscles are axially and bilaterally located and they have an important role in supporting the body posture and in movements of the head and shoulders during a number of tasks, e.g. stabilisation of the shoulder joint to allow precise manipulations (Mork & Westergaard, 2005; Nimbarte et al., 2010).
WMSDs are often associated with discomfort and mechanical hyperalgesia in muscles (Madeleine et al., 1998, 2003; Gold et al., 2006; Ylinen et al., 2007). Such hyperalgesia can be induced in a controlled fashion when performing repetitive, eccentric exercises. This type of exercise causes delayed-onset muscle soreness (DOMS) which peaks 24-72h after the exercise (Armstrong, 1984). Several studies (Nie et al., 2005, 2009; Kawczynski et al., 2007; Binderup et al., 2010) have successfully evoked muscular hyperalgesia in the trapezius muscle in healthy subjects with experimentally induced DOMS. This allows for investigation of the underlying mechanisms of DOMS in the trapezius muscle.
In a study by Alexander & Harrison (2001), the Hoffmann reflex (H reflex) was investigated in the trapezius muscle. For this muscle, the supply of the motor and sensory nerves is divided into the accessory nerve and the C3/4 cervical nerve respectively (Grant, 1972; Pu et al., 2008). The separation of the efferent and afferent fibres makes it possible to evoke H reflexes by electrical stimulation of the C3/4 cervical nerve without the influence of the M waves (Alexander & Harrison, 2001). This unique arrangement suggests that study of the trapezius muscle may allow special insight into the actions of Ia muscle fibres' input on the motoneuron pool, e.g. during DOMS(Misiaszek, 2003). Presynaptic inhibition of Ia afferent fibres by group III and IV afferent fibres has been observed during fatigue (Garland & McComas, 1990; Pera et al., 2001). It is possible that the same inhibition is involved in DOMS.
Three studies, all focused on the lower extremities, have found that the H reflex is neither modulated 24h nor 48h after eccentric exercise (Bulbulian & Bowles, 1992; Avela et al., 1999; Racinais et al., 2007). However, these findings may be questioned as recommendations by Brinkworth et al. (2007) and Zehr (2002) on day-to-day recordings of the H reflex, are not fulfilled. Furthermore, reproducibility measures of day-to-day variations of the H reflex in trapezius have not been investigated before.
The purpose of this study was to investigate the reproducibility of the H reflex between days and the effect on the H reflex immediately after eccentric exercise and during DOMS.

II. Methods

A. Subjects

The study was initially conducted on 13 healthy subjects (5 males and 8 females, 12 right-handed and 1 left-handed);

mean age 23.9 years (SD: 1.8); height 171.5cm (SD: 0.1); weight 64.8kg (SD: 9.11); and BMI 21.5kg/m^2 (SD: 2.2). 3 subjects were excluded due to immeasurable reflexes or due to discomfort caused by electrical stimulation. None of the participants reported pain or soreness in the neck-shoulder region before the study and none had a history of previous neck-shoulder disorders. Informed consent was obtained from each subject. All subjects refrained from exercise and maintained normal daily activity during the course of the study. The study was conducted according to the declaration of Helsinki.

B. Experimental Protocol

The study was conducted over three consecutive days separated by 24h and in total consisting of four sessions. Each session consisted of measures for assessing the muscle soreness and measures of neural responses. On day 2, an eccentric exercise procedure in accordance with Binderup et al. (2010) was performed to induce DOMS. Figure 1 shows the study protocol with the parameters measured at each session.

In the present study, the middle dominant trapezius muscle was examined due to limited spread of DOMS in the lower trapezius by the eccentric exercise (Binderup et al., 2010) and immeasurable H reflex in the upper trapezius (Alexander & Harrison, 2001).

B.1 Assessment of Muscle Soreness

The muscle pain intensity in the neck-shoulder region was assessed by using a visual analog scale (VAS) score. The VAS consisted of a 10cm line ranging from 0 (no pain) to 10 (worst pain imaginable). The subjects rated the perceived pain induced by the exercise as sensed during daily-life activity.

Pressure pain threshold (PPT) was measured on the dominant middle trapezius to investigate the effectiveness of the exercise to induce DOMS. The exact point of measure was just above the midpoint between the spine of the scapula and the T3 vertebra. Additionally, PPT was measured on the right tibialis anterior as a control site. The PPT recordings were done using an electronic hand-held pressure algometer (Somedic Algometer type 2, Sweden). The area of the tip was 1cm^2 and was covered with a 2mm thick rubber. The subject was seated against the backrest of a chair while pressure was applied perpendicularly to the skin surface with a constant rate of 30kPa/s. The subject had to press a hand-held button when the perception changed from pressure to pain. For each subject, all PPT measures were performed by the same investigator. Each recording was repeated three times. The mean value of those three values was used as the PPT value. For points with a coefficient of variance equal to 0.2 or more, a fourth recording was made to reduce the intra-individual variance. This procedure is similar to that of the study by Binderup et al. (2010).

B.2 Neural Assessment

The study was following the recommendations of H reflex recordings posed by Brinkworth et al. (2007) and Zehr (2002). Surface electromyograms (sEMG) were recorded from the middle section of the dominant trapezius via a pair of pre-gelled surface electrodes (Ambu A/S, Neuroline, 72001k, Ballerup, Denmark) placed on abraded and ethanol-cleaned skin. Similar to the first PPT measurement spot, the first sEMG electrode was placed close to the midpoint between the spine of the scapula and the T3 vertebra and the second was placed 3cm medially. The reference electrode was placed on the C7 vertebra. Electrode placement was previously described by Taylor et al. (2009). The electrode position was marked on the skin with a permanent marker to ensure that the same spot could be located through all sessions of the experiment. The sEMG signals were filtered with an analogue band pass filter (20-1000Hz) and amplified 500 times. Data were sampled at 2000Hz and recorded on a computer via a laboratory interface (CED 1401, Signal 2.16 software, Cambridge Electronic Devices, Cambridge, UK). sEMG data were recorded both to investigate neural responses to electrical stimuli and as a measure of EMG maximum voluntary contraction (MVC) of shoulder abduction. All recordings of neural responses were done in a quiet environment.

Electrical Stimulation of the Accessory Nerve

Percutaneous electrical stimulation (1ms width, Isolator-11, Axon Instruments) was applied to the accessory nerve with the anode positioned on the mastoid process and the cathode fixed over the accessory nerve. The exact location of the cathode was determined using a custom-build, hand-held electrode to find the site eliciting the largest M wave in the middle trapezius. The area of search was behind the sternomastoid muscle and between the level of the jaw and the upper border of trapezius (upper hashed area in Figure 2A). A self-adhesive Ag/AgCl surface electrode was stuck to the skin over the optimal position for innervating the accessory nerve. The electrode position was marked with a permanent marker on the skin to ensure the same location could be relocated in the following sessions.

Electrical Stimulation of the Cervical Nerve of C3/4

Percutaneous electrical stimulation was also applied to the cervical nerve of C3/4 to record H reflex responses. The anode was positioned just below the midpoint of the clavicle and the cathode was fixed over the cervical nerve of C3/4. Similarly to when eliciting the accessory nerve, the hand-held electrode was used to determine the exact location of the cathode. The cathode was placed where an H reflex could most easily be elicited in the middle trapezius. The search area for the C3/4 cervical nerve was superficially located on the anterior surface of the upper fibres of trapezius above the clavicle, (see lower hashed area in Figure 2(A)).

To condition the reflex with an excitatory input, con-

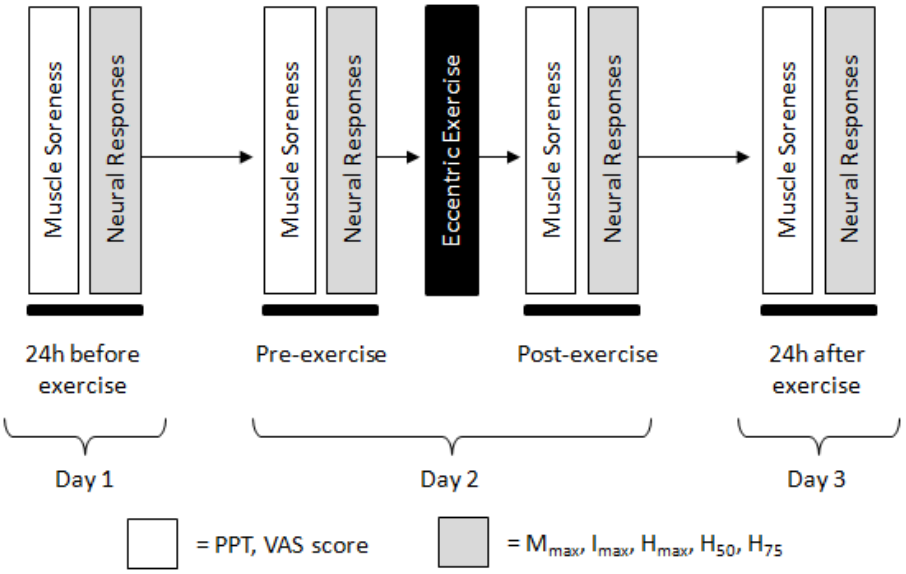

Fig. 1. Schematic Representation of the Study Protocol: the study lasted three days separated by 24h. In total, four sessions were completed, each consisting of measures for assessing muscle soreness and measures of neural responses. On day 2, two sessions were separated by an eccentric exercise procedure. The parameters obtained at each session are shown at the bottom of the figure: M_{max} = maximal peak-to-peak amplitude of M waves, I_{max} = stimulus intensity needed to stimulate M_{max}, H_{max} = maximal peak-to-peak amplitude of H reflexes, H_{75} = reflex peak-to-peak amplitude at the intensity associated with 75% of H_{max} obtained on day 1, and H_{50} = reflex peak-to-peak amplitude at the intensity associated with 50% of H_{max} obtained on day 1.

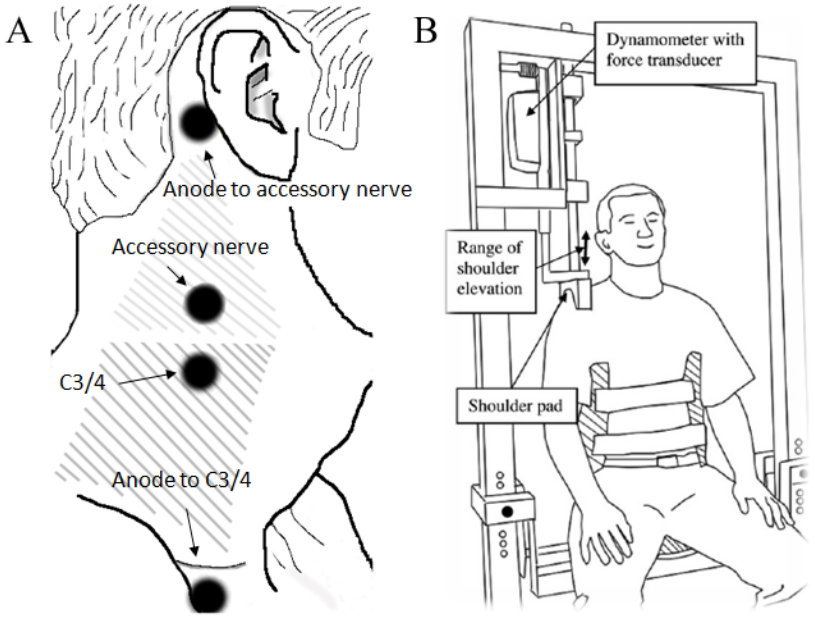

Fig. 2. (A) Placement of Anodes and Cathodes for Electrical Stimulation. (B) Dynamic Shoulder Dynamometer for Performing Exercise: (A) The upper hashed area shows the area of search for the accessory nerve and the lower hashed area shows the area of search for the C3/4 cervical nerve. The anode to the accessory nerve was placed on the mastoid process, the anode to the C3/4 cervical nerve was placed just below the midpoint of the clavicle. (B) The subject was placed in the seat and equipped with a corset. The exercise consisted of bilateral shoulder shrug movement to counter-act the 100% MVC force of the descending shoulder pad. Modulated from Madeleine et al. (2006).

traction of the dominant side was performed by lifting the arm against a horisontal bar located just above the supporting bench. A self-adhesive Ag/AgCl surface electrode was placed to the skin over the nerve. The electrode position was marked with a permanent marker on the skin to ensure the same location could be relocated in the following sessions.
During all electrical stimulations and EMG recordings, the subject was seated on an office chair with the feet on the floor and the dominant arm on a supporting bench with approximately 70° shoulder abduction and 90° of elbow flexion.

Recruitment Curves of the M Waves and the H Reflexes

Recruitment curves of the M waves were obtained by gradually increasing accessory nerve stimulus intensity by ∼0.5mA steps until three consecutive recordings showed no further increase in amplitude despite an increase in stimulus intensity. Recruitment curves of the H reflex were obtained by gradually increasing cervical nerve stimulus intensity by ∼0.2mA steps during muscle contraction. For each intensity, 10 stimulations were delivered and an average was calculated and used in the analysis. The recruitment curve was continued until no further increase in amplitude was detected despite an increase in stimulus intensity.

Trapezius muscle activity was used to provide visual feedback of the subject's level of muscle contraction, in order to ensure a similar level of motoneuron excitability across measures of H reflexes (Zehr, 2002). The level of muscle contraction was displayed on a feedback monitor programmed in LabView (v. 8.2, National Instruments Corp.). EMG MVC was obtained in each session over two trials in which the subject was asked to abduct the dominant arm with as much force as possible against a horizontal bar just above the supporting bench for a period of 3s. A minimum of 1min of rest was given between each EMG MVC. A preset target range of 15±2% MVC was displayed on the feedback monitor. When the target range was reached, a trigger signal was sent to the stimulator, allowing the next electrical stimulus to be delivered to the cervical nerve of C3/4. Electrical pulses were delivered at intervals no faster than every five seconds to avoid the effects of post-activation depression (Brinkworth et al., 2007; Zehr, 2002).

B.3 Exercise Procedure

Before beginning the eccentric exercise, the range of shoulder elevation and MVC force of shoulder shrug were measured using a dynamic shoulder dynamometer (Aalborg University, Aalborg, Denmark). The dynamometer is further described in in Madeleine et al. (2006). To measure the range of shoulder elevation, the subject was asked to lift both shoulders bilaterally as much as possible and then to lower them again as much as possible while position values were saved. The maximum voluntary isometric contraction force of shoulder shrug was recorded unilaterally for the dominant side in a neutral position. The subject was asked to place the shoulders in a neutral, relaxed position. Once the shoulder pad was lowered to the point of contact with the shoulder, the subject performed three MVC force recordings in total by pushing against the contact pad for 3s. The average value of the three recordings were saved as 100% of MVC force. During both the MVC and range of shoulder elevation recordings, the subject was seated in an upright position with back support and no foot support. To protect from lateral bending, the subject was equipped with a corset. The contact point between the dynamometer and the shoulder was approximately 3cm medial from the acromiom.

In accordance with previous studies using the dynamometer (Binderup et al., 2010; Nie et al., 2005, 2009; Kawczynski et al., 2007), the eccentric exercise aimed to induce DOMS in the dominant shoulder. The exercise consisted of 5 bouts of 10 repetitions giving a total of 50 contractions, where the subject acted against the dynamometer moving from the subject's highest to the lowest vertical shoulder position at a force equal to 100% isometric MVC. The contractions had no time restrictions and the subject was encouraged verbally throughout the exercises. The subject relaxed for 2min after every bout. The dynamometer is shown in Figure 2B.

C. Data Analysis

Maximum peak-to-peak amplitudes of the M waves and H reflexes (M_{max} and H_{max}) were based on the recruitment curves from each nerve. Latencies were measured as the first deflection from baseline. Reproducibility of the M wave were tested by comparing intensities (I_{max}) used to stimulate M_{max}. H_{max}/M_{max} ratio was calculated for each subject.
The ascending limb of each H reflex recruitment curve was fitted using a general least square model of a custom three-parameter sigmoid function, as described by Klimstra & Zehr (2008).

From the curve fits, H_{50} and H_{75} were derived (see Figure 3). The stimulus intensities associated with 50% and 75% of H_{max} from the first session (I_{75} and I_{50}) was used as input to the curve fit of the other sessions. With this procedure, the amplitudes of the H reflex responses (H_{75} and H_{50}) were compared on a basis of equal current intensities. Thus, the presence or absence of shifts in the recruitment curve at different stimulus intensities could be investigated. This procedure was similar to a study by Dragert & Zehr (2011).

The Pearson product-moment correlation coefficient (r) was calculated for each fit to determine the goodness of fit between the collected data and the sigmoid curve. For each parameter, group differences between the four sessions ("24h before", "Pre", "Post", and "24h after" exercise) were analysed using a Friedman repeated measures analysis of variance (ANOVA) by ranks (SigmaStat, Aspire Software International v. 2.03). Post-hoc group comparisons between the sessions were performed using a Student-

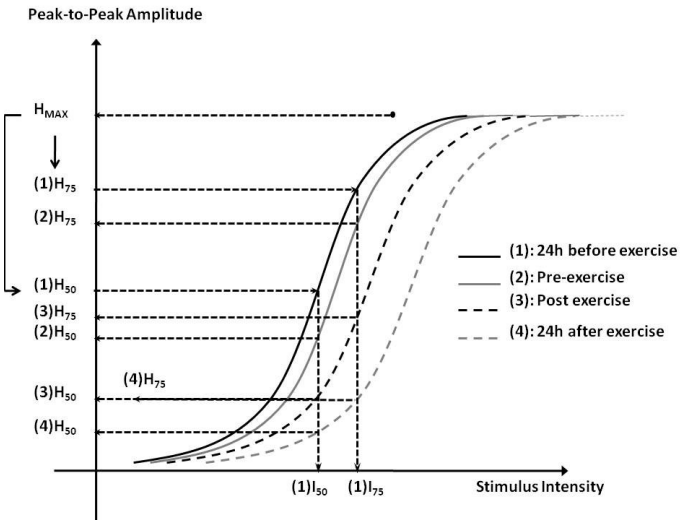

Fig. 3. The Analysis of the H reflex Recruitment Curves: H reflex recruitment curve fits were compared between sessions. The stimulus intensities associated with 50% and 75% of H_{max} from the first session (I_{75} and I_{50}) was used as input to the curve fit of the other sessions. With this procedure, the amplitudes of the H reflex responses (H_{75} and H_{50}) were compared on a basis of equal current intensities.

Newman Keuls test (SNK). A P-value$<$0.05 was considered significant. All values were presented as medians with 1^{st} and 3^{rd} quartiles.

III. Results

A. Muscle Soreness

The VAS score of the session "24h before" was 0.0 (0.0; 0.0), for "Pre" 0.0 (0.0; 0.0), for "Post" 1.5 (1.4; 2.3), and for ťť24h after" 3.0 (2.5; 3.6). Friedman repeated measures ANOVA on ranks were performed on the group data for the four sessions and showed significant differences between the sessions (P<0.001). Post-hoc comparisons showed that the sessions "Post" and "24h after" produced increased VAS scores compared to the sessions "24h before" and "Pre" (P<0.05; SNK).

From measures of the middle trapezius, the PPT values of the session "24h before" was 344 kPa (308; 412), for "Pre" 352 kPa (331; 463), for "Post" 334 kPa (309; 417), and for "24h after" 272 kPa (207; 388). Friedman repeated measures ANOVA on ranks were performed on the group data for the four sessions and showed significant differences between the sessions (P<0.011). Post-hoc comparisons showed that the session "24h after" produced significantly lower PPT values compared with all other sessions (P<0.05; SNK).

From measures of the right tibialis anterior, the PPT values of the session "24h before" was 485 kPa (425; 619), for "Pre 560" kPa (460; 673), for "Post" 585 kPa (550; 628), and for "24h after" 567 kPa (477; 639). Friedman repeated measures ANOVA on ranks were performed on the group data for the four sessions and showed no significant differences between the sessions (P=0.178).

B. Neural Responses to Electrical Stimulation

In Figure 4, an example of a sigmoid fit and average data for each stimulus intensity are shown. The r-values from all curve fits were for session "24h before" 0.971 (0.931; 9.83), for "Pre" 0.967 (0.948; 0.983), for "Post" 0.950 (0.922; 0.966), and for "24h after" 0.960 (0.944; 0.976). Recordings of M wave and H reflex recruitment curves are presented in Table I. Latencies for M waves and H reflexes showed no significant difference between any sessions (P=0.854 and P=0.668 respectively). Furthermore, The M_{max} and intensity to stimulate the M_{max} (I_{max}) were also similar among sessions (P=0.513 and P=0.759 respectively).

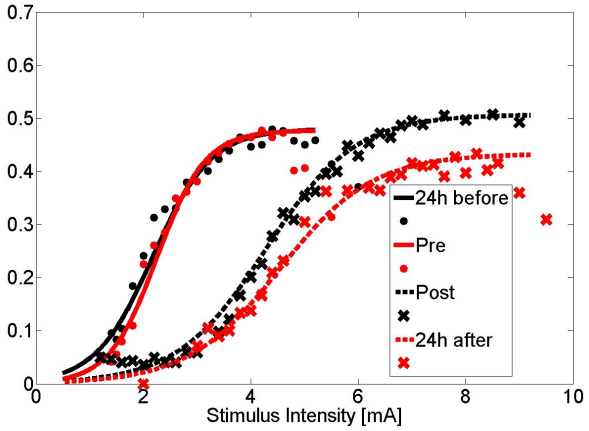

Fig. 4. Example of Curve Fitting: single subject (#5) recruitment curves from all four sessions with a sigmoid function fit. Average measured data values for each stimulus intensity and the sigmoid fit are shown for each session.

For the H_{max}/M_{max} ratios, there was no significant difference between sessions (P=0.472).

For the H_{75}/M_{max} ratios, Friedman repeated measures ANOVA on ranks were performed on the group data for the four sessions and showed significant differences between the sessions (P<0.02). Post-hoc comparisons showed that the session "24h after" produced significantly lower ratios compared with the sessions "24h before" and "Pre" (p<0.05; SNK; Figure 5A).

For the H_{50}/M_{max} ratios, Friedman repeated measures ANOVA on ranks were performed on the group data for the four sessions and showed significant differences between the sessions (P<0.003). Post-hoc comparisons showed that the sessions "24h after" and "Post" produced significantly lower ratios compared with the sessions "24h before" and "Pre" (P<0.05; SNK; Figure 5B).

IV. Discussion

In the present study, H reflex recruitment curves were obtained from the middle trapezius in 10 healthy subjects. This was done by stimulating the cervical nerve of C3/4. To obtain values for normalising the H reflex, stimulation of the accessory nerve was used to elicit M waves. Recruitment curves showed that the H reflexes, like the M waves, increased in amplitude with increasing stimulation

	24h before		Pre		Post		24h after	
	Median	Quartiles	Median	Quartiles	Median	Quartiles	Median	Quartiles
Latency M [ms]	3.0	3.0; 3.0	3.0	3.0; 3.0	3.0	3.0; 3.0	3.0	3.0; 3.0
Latency H [ms]	9.0	8.5; 9.0	9.0	8.5; 9.1	9.0	8.7; 9.0	9.0	8.8; 9.2
M_{max} [mV]	5.73	5.04; 5.98	5.30	4.56; 6.33	4.81	4.56; 5.78	5.23	4.90; 5.70
H_{max} [mV]	1.60	1.27; 2.03	1.58	0.81; 2.00	1.16	0.83; 1.89	1.25	1.10; 2.09
I_{max} [mA]	3.0	3.5; 3.8	3.5	2.8; 5.0	2.8	2.5; 4.3	3.3	2.1; 4.4
H_{75} [mV]	1.23	0.98; 1.66	1.24	0.68; 1.74	0.68	0.36; 0.77	0.77	0.36; 0.98
H_{50} [mV]	0.8	0.63; 1.01	0.77	0.48; 1.13	0.42	0.20; 0.50	0.55	0.18; 0.60
H_{max}/M_{max}	0.30	0.23; 0.37	0.27	0.17; 0.36	0.26	0.15; 0.36	0.24	0.22; 0.38

TABLE I

Results of Neural Responses: The results include recorded latencies of M waves and H reflexes, maximum response values of M waves and H reflexes, intensity needed to obtain the maximum M wave, amplitude values for H_{75} (75% of H_{max}), amplitude values for H_{50} (50% of H_{max}), and the H_{max}/M_{max} ratio.

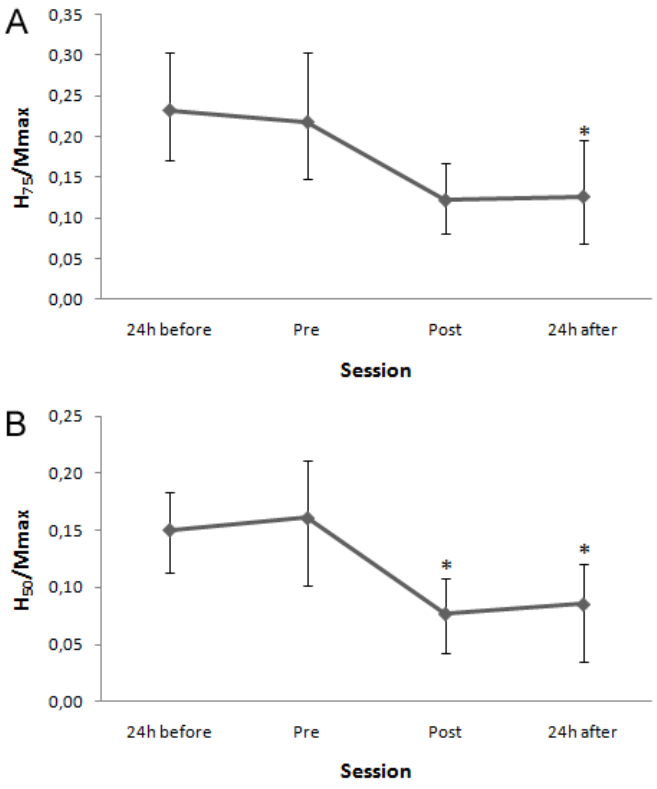

Fig. 5. Ratios of H reflexes and M waves: (A) the H_{75}/M_{max} ratios represented with medians and 1^{st} and 3^{rd} quartiles. (B) the H_{50}/M_{max} ratios represented as medians 1^{st} and 3^{rd} quartiles. (*) indicates a significant difference ($P<0.05$).

intensity and then reached a plateau. By further increasing the stimulus intensity the reflex amplitudes decreased. Since the motor and afferent supply to the trapezius muscle are separated, this antidromic activation probably occurred due to spread of stimulus to the accessory nerve. The recruitment curves were analysed before and after eccentric exercise. No significant differences were observed between the sessions "24h before" and "Pre", indicating the values were reproducible between days. Thus, the results from the study can be considered to be reliable. The analysis of the H reflex recruitment curves showed that after eccentric exercise stronger stimulations were needed to obtain the same size H reflex. No differences were observed in H_{max} before and after exercise at high enough stimulus intensity, delineating a shift of the recruitment curve to the right (Figure 4). The goodness of fit for the generated sigmoid curves were considered to be accurate based on the calculated Pearson product-moment correlation coefficients (r). The r-values obtained in the present study are comparable to the study by Dragert & Zehr (2011) where the same sigmoid function were included (mean r-value 0.95 for the best fit). Soreness parameters, i.e. pain intensity and muscle hyperalgesia, showed that DOMS was successfully induced in the dominant trapezius after the eccentric exercise.

H reflex Variability Between Sessions and Days

Brinkworth et al. (2007) have previously investigated the difficulty in measuring reliable H reflexes between days. Results from that study show day-to-day and trial-to-trial variability in recruitment curves in all trials. However, in trials with 50% MVC the variability were smaller than in trials with relaxed muscles (0% MVC). In the present study, H reflexes were elicited with 15% EMG MVC. Besides making the variability smaller it also made the excitability of motoneurons relatively similar between sessions. To further minimise variability, 10 H reflex responses were averaged for each stimulus intensity. This is also in agreement with recommendations from Brinkworth et al. (2007). Furthermore, the size of the H reflex is a function of three factors: the precision of stimulus delivery, excitability of the entire H reflex arch, and accuracy of recording. Since none of these factors can be fixed, it is evident that the H reflex will vary between subjects and between days (Brinkworth et al., 2007).

Even though no significant differences were observed between the sessions "24h before" and "Pre", parametrical statistical tests on non-normally distributed data increase the

probability of a type II error (Qualls et al., 2010). The post-hoc analysis performed in the present study (SNK) pressumes the data to be normally distributed. This presumption cannot be confirmed for all parameters. However, the lack of significant differences between the sessions before exercise ("24h before" and "Pre") and the presence of a significant difference between sessions after exercise ("Post" and "24h after") compared to before exercise suggest the changes in H reflexes to be more related to a genuine change in excitability of the H reflex circuit and less to a change in electrode placement. This is further supported by no change in the intensity used to stimulate M_{max} between sessions. Thus, the measures of the H reflex were considered comparable between days and sessions.

Immediate Effects of Eccentric Exercise

Muscular fatigue has been associated with reflex inhibition of the motoneuron pool (Garland & McComas, 1990; Woods et al., 1987). The mechanism underlying this inhibition is not completely understood. However, evidence in the spinalised rat suggest that the discharge of capsaicin-sensitive group III and IV muscle afferents during fatigue may decrease spindle support to the motoneurons by presynaptic inhibiton of the Ia afferent fibres (Pettorossi et al., 1999).

The H_{max}/M_{max} ratio is an estimate of the excitability of motoneurons (Palmieri et al., 2004). Bulbulian & Darabos (1986), Avela et al. (1999), and Racinais et al. (2007) reported a significant decrease in the H_{max}/M_{max} ratio immediately after eccentric exercise, i.e. during fatigue, indicating inhibition of the Ia afferent fibres. This is in contrast to the present study, where H_{max}/M_{max} ratios did not show a significant change. However, the present study's method for inducing DOMS differed. In contrast to the more muscle intense eccentric exercise performed with the dynamometer, these studies included more cardio intense exercises which were more inclined to cause an increase in heart rate. As shown by Bulbulian & Darabos (1986), high-intensity exercise and low-intensity exercise reduce the mean H_{max}/M_{max} ratio by 21.5% and 12.8% respectively. This could explain why the H_{max}/M_{max} ratio did not decrease in the present study; although heart rate was not measured during the exercise procedure, frequent breaks of ∼15 seconds between the shoulder pad returning to the top from bottom position in each repetition prevented the heart rate from increasing. Moreover, a shift in the recruitment curve to the right will in most muscles give a reduction in H_{max} because the M wave curve will not be shifted. Thus, more antidromic collision will be present at the new higher intensity for H_{max}. This is not the case for trapezius since the motor and afferent supply is separated and could be an explanation for the lack of difference in H_{max}/M_{max} ratio. Besides, the results on fatigue may have been affected by a delay in time from the end of the eccentric exercise to the beginning of the measurements of neural responses. Thus, the subject may have been in a state of early recovery rather than a state of fatigue. Furthermore, H_{max}/M_{max} ratios are not ideal at reflecting changes in recorded data because the maximal responses are placed on a plateau of the H reflex and M wave recruitment curves. By instead analysing a point on the upslope of a recorded recruitment curve, changes to the stimulus/response relationship would be more evident due to the steepness of the curve (Brinkworth et al., 2007). Therefore, the significant decrease in the H_{50}/M_{max} ratio showed in the present study may have been singled out due to the more sensitive changes in the recruitment curve. Additionally, comparrison between the present study and previous studies can be diffecult due to methodological diffences.

Effect of Delayed-onset Muscle Soreness

DOMS is manifested by mechanical muscle hyperalgesia, occasional resting pain, and altered motor control (Bajaj et al., 2002; Kawczynski et al., 2007; Nie et al., 2005; Samani et al., 2009). Several theories have been put forward to explain how the mechanical muscle hyperalgesia develops with muscle damage and inflammation being the most widely accepted (Graven-Nielsen et al., 2008). Thus, the hyperalgesia could stem from acute damage to the muscle fibres during exercise, causing a mechanical disruption of the ultra structural elements within the muscle fibres such as the Z-line and contractile filaments (Fridén et al., 1983; Fridén & Lieber, 1984). Also, it could stem from an acute inflammation resulting from an immune response to the initial injury, which sensitises the muscle nociceptors and lower their threshold to mechanical stimuli (Smith, 1961). In the present study the PPT was significantly lower 24h after exercise. Correspondingly, the pain intensity showed highest measured values 24h after eccentric exercise. These findings suggests that DOMS was induced in the dominant trapezius muscle. This is in agreement with previous findings by Nie et al. (2005), Binderup et al. (2010), and Kawczynski et al. (2007).

DOMS has been related to ischemia in the muscle, metabolic changes (in e.g. potassium, lactic acid, bradykinin and arachidonic acid concentrations), and appear to reflect vasodilatation within the muscle which activates group III and IV afferents in humans (Rotto & Kaufman, 1988; Sinoway et al., 1993). Presynaptic inhibition of Ia afferents by group III and IV afferents may therefore occur during DOMS. This corresponds with findings from the present study. H/M ratios from the stimulus intensities associated with 50% and 75% of H_{max} in the first session (H_{75}/M_{max} and H_{50}/M_{max}) were decreased significantly in the presence of DOMS compared to sessions before exercise. Since the background EMG was constant in all sessions (i.e. same excitability of the motoneurons), this shift indicates presynaptic inhibitions of Ia afferents.

In the studies by Bulbulian & Bowles (1992) and Avela et al. (1999) no change were seen in H_{max}/M_{max} ratios 24h after eccentric exercise. In these studies, the H reflex was measured when the subjects were relaxed. Since the sensation of DOMS increase with movement (Lieber &

Fridén, 2002), there may have been limited pain at the time of measurement which could explain the lack of difference observed. In the present study the subjects were contracting while the H reflex was evoked. Hence, sensation of pain was present at the time of measurement resulting in an increased firing of group III and IV afferents and thus presynaptical inhibition of Ia afferents.

The sensation of DOMS can be related to the sensation in patients with chronic WMSD (Madeleine et al., 1998; Gold et al., 2006). In previous studies on patients with chronic low back pain (CLBP), an increase in H reflex threshold, i.e. a shift in the recruitment curve to the right, was observed compared with healthy subjects (Ginnaneschi et al., 2007; Mazzocchio et al., 2001). It is proposed by Ginnaneschi et al. (2007) that findings related to the H reflex can contribute to the diagnostic evaluation of CLBP and may be used to monitor the efficacy of treatment. The results from these studies are in agreement with findings from the present study. However, one must bear in mind the difference between experimentally induced pain and chronical pain conditions. To verify the findings of the present study and link them to WMSD, a study on a patient population is needed. These findings may help improve the understanding of the mechanisms involved in WMSD.

Conclusion

The study has presented results indicating that neural responses can be investigated over successive sessions without significant changes in variability when applying the methods described in this study. Therefore, the primary findings of decreased H reflex responses induced by fatigue and especially DOMS, can be related to a genuine change in inhibitory mechanisms of the Ia afferents. This is the first study to show this link between change to H reflex responses caused by DOMS.

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Worksheets

Table of Contents

Chapter 1

Pain in the Neck-shoulder Region

This chapter describes how many people are suffering from neck-shoulder pain and what the costs are. Furthermore, the physiological mechanisms of muscle pain are described and the risk factors regarding neck-shoulder pain are listed.

1.1 Neck-shoulder Pain

Work-related musculoskeletal disorders (WMSD) are defined as work-related disorders due to environmental conditions and performance of the work which expose the worker to several risk factors. WMSDs are involved with pain located around cartilage, tendons, ligaments, nerves and muscles.[1]
Millions of Europeans in all kinds of jobs are suffering from WMSD every year. The main group of people with WMSD have back pain/injuries and upper limb disorders which provide a significant health and cost problem.[1, 2, 3] A study from 2010 reported that 24.7% of the European workers complain of backache and 22.8% of muscular pains[1]. In Figure 1.1, the percentage of workers reporting muscular pain in the neck and shoulders can be seen. In the EU-15 countries, about 23% of the workers reported muscular pain in the neck and shoulders. The range extends from 8.2% in Ireland to 53.5% in Finland. The incidence and prevalence of neck-shoulder disorders are difficult to compare between countries and between studies due to the different covering of the term "neck-shoulder disorder". This includes self reported pain and defined clinical diagnosis. Overall 20-30% of all workers suffers from neck-shoulder disorders.[1, 2]

In general, it is difficult to compare the costs of WMSD between countries due to the differences in insurance policy, the lack of standardised assessment criteria and questions about the validity of the reported data. Furthermore, the costs can be divided into direct and indirect costs. The direct costs are the visible costs such as insurance, medical compensation and administrative costs.

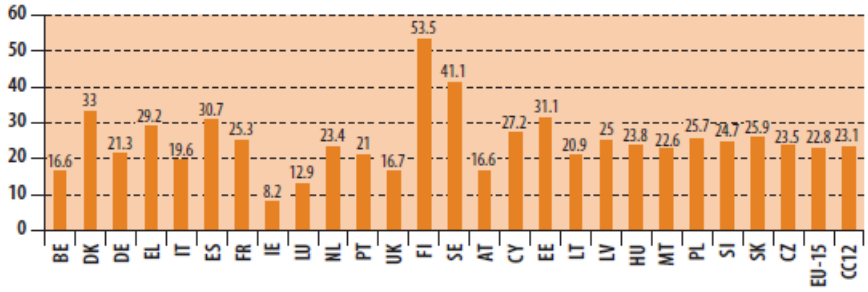


Figure 1.1: **Percentage of the Working Force in European Countries with a History of Muscular Pain in Neck and Shoulders**
Overall 20-30% of all workers suffers from neck-shoulder disorders.[1]

The indirect costs are attributed to sick leave costs, reduced productivity levels, and training of new employees. Nevertheless, the estimated costs of work-related upper-limb musculoskeletal disorders are between 0.5% and 2% of the Gross National Products.[1]

Sensation of Work-related Musculoskeletal Disorders

For the patient, muscle pain from a WMSD is an unpleasant sensory experience associated with discomfort. Muscle pain sensation differs distinctly from that of cutaneous pain because it results in longer lasting, more diffuse, and more poorly localised pain.[4, 5, 6] In clinical practice, manifestations of WMSD are found by manual palpation of the affected muscle[7]. The method for clinical determination of WMSD is in line with experimental findings by Madeleine et al.[8] and Gold et al.[9]. These studies have found significantly lower pressure pain threshold (PPT) values in a population with chronic neck-shoulder pain compared to a control group. This suggests that WMSD is associated with hyperalgesia which is characterised as an increased response to a stimulus which is normally painful.[6]

1.2 The Physiological Mechanisms of Muscle Pain

Pain is sensed by nociceptors which are sensory endings that detects actual or potential tissue damage. The greater the painful stimuli is, the greater is the number of action potentials discharged in the nociceptive fibres. Nociceptors are found in all tissue of the body (except the brain and the liver) and are free nerve endings with a somata (cell body) located in the dorsal root ganglia (in the face located in the nuclei of the trigeminal nerve).[4, 5, 6]
In general, there are two types of nociceptors - group III and IV fibres, which are physiologically differentiated on the basis of conduction velocity. The majority of the nociceptors are the slow-conducting, unmyelinated group IV fibres (< 1 m/s) which are polymodal sensors (i.e. multiple stimulus modalities). These fibres can be activated both by mechanical stimuli, chemical medi-

ations, high-intensity heat, and cold stimuli and can produce a slow lasting feeling of pain over broad areas. On the other hand, group III fibres are myelinated, fast fibres (5-30m/s), which are classified as unimodal nociceptors consisting of thermal, mechanical, or dormant nocisensors. The dormant nocisensors are located in internal organs and respond to e.g. inflammation. However, the division of muscle nociceptors into being either unimodal or polymodal is not a generally accepted classification, as some investigators consider all nociceptors to be polymodal.[4, 5, 6]

Pain is a series of neuronal processes that involve the peripheral nerves, spinal cord and brain. Thus, the perception of pain can be modulated at many levels throughout the pathways and adapt to changes. However, the ability of the nervous system to change in response to dysfuntion evoked by injury or disease can lead to an unreliable relationship between the magnitude of transmission of peripheral stimuli and the perception of pain. This is often the case in chronic pain conditions.[6]

1.3 Risk Factors

Different risk factors contribute to neck-shoulder pain. According to Larsson et al.[2] the identified risk factors are:

- **Gender:** the prevalence of pain in the upper extremities is higher among women than men. This can maybe be explained by woman's jobs which may consist of e.g. more static load on the neck muscle and higher repetitive movements compared to men's jobs.
- **Repetitive movements:** 75% of studies reviewed showed a significant relationship between repeated movements and upper extremity disorders.
- **High force demands:** forceful manipulation with the hand (not directly on the neck muscle) requires high degree of stabilisation in the neck-shoulder region (e.g. heavy lifting).
- **Work posture:** awkward posture is a risk for neck-shoulder disorders. This includes duration and monotony of the posture. Arm lifted over shoulder level is found to be harmful for the neck-shoulder region.
- **Vibration:** vibration is a risk factor for muscle disorders in general. The connection between vibration and shoulder-neck disorders is not clear but some findings suggest that there is a relationship.
- **Computer work:** there is an increased risk for neck-shoulder disorders among computer users due to constrained postures, constant force, and highly repetitive movements. This can be associated with the low activity level in motor units during repetitive and static work in trapezius. Keyboard position and other ergonomic aspects can also be considered as a risk factor.

- **Psychosocial factors:** some evidence suggest that there is a relationship between disorders and high quantitative demands, lack of support from colleagues, low job control, and low influence.

This list of risk factors indicates that if neck-shoulder disorders shall be reduced among workers a physical, psychosocial, and organizational approach of prevention must be combined.[2] In a socio-economic perspective prevention of the disorders are of utmost importance[10].

Chapter 2

The Trapezius Muscle

In this chapter the trapezius muscles are presented. This includes an introduction to the different functions and subgroups of the muscle. Furthermore, a brief description of nerve innervation in the trapezius muscles is included.

2.1 The Anatomical Location

The trapezius muscle is in the group of superficial back muscles. Two trapezius muscles form a trapezoid and are attached to the cervical spine, the skull, and the shoulders. The two muscles can be divided into three parts; the upper trapezius originate from the occipital bone and the ligamentum nuchae and inserts to the lateral part of the clavicle; the middle trapezius originate from the cervical vertebrae (C7) and the thoracic vertebrae (T1-3) and inserts scapula; and the lower trapizius originate from the thoracic vertebrae (T4-12) and inserts the triangular space of the scapula. The three parts of the right trapezius muscle are illustrated in Figure 2.1.[11, 12, 13]

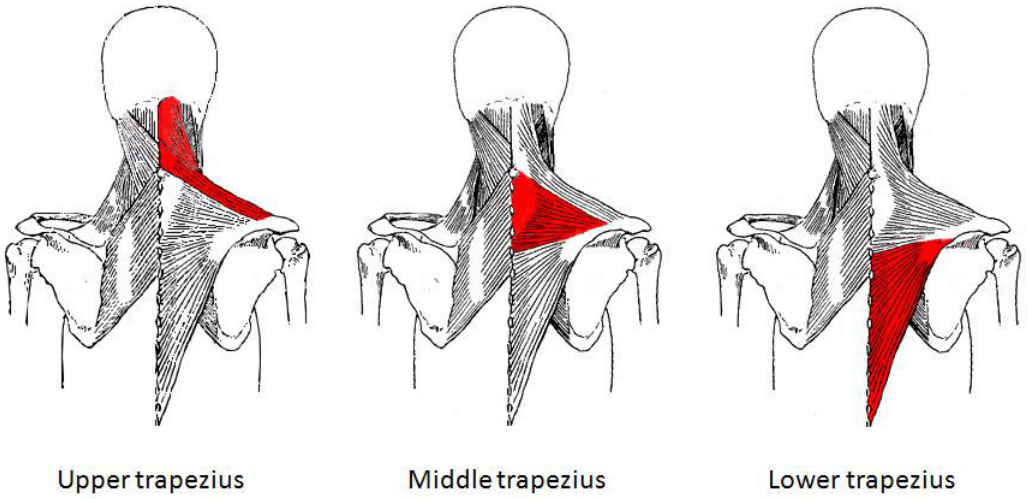


Figure 2.1: **The Three Parts of the Right Trapezius**
Upper, middle, and lower trapezius are marked with the red colour on the sub-figures respectively.[13]

2.2 Functions of the Trapezius Muscles

The trapezius muscles have several functions. They can move the shoulders toward the spine, rotate the shoulder blades inferior and superior, depress and elevate the shoulders, bring the head and neck in a backward direction, rotate and side bend the neck, and assist in breathing.[13, 14, 15]
The trapezius muscles are also important muscles during movements of the scapular. Because of the unstable arrangement of the shoulder the trapezius muscles must provide the necessary base of support when correct and precise hand and arm tasks are performed. The muscles are also important stabilising components in body posture.[12, 16]
The functions of each trapezius part will be described shortly.

Upper Trapezius

The upper trapezius is active when the shoulder is elevated (e.g. elbow is fully extended in a seated position) and in rotation of the glenoid fossa. During elevation, the scapula changes position which is supported by the upper trapezius. In resting position (no elevation) the upper trapezius has a supportive mechanism. When 35° of elevation is performed, the forces acting in the upper trapezius are equally divided in a supportive and a rotational role. Further elevation increases the role of rotation.[13, 14, 15, 17]

Middle Trapezius

The middle trapezius is activated when adduction of the scapular is performed (for instance in a prone position with resistance applied). The muscle potential reaches its maximum at 90° of abduction and decreases at further abduction. In forward flexion the activation of the middle trapezius decreases during early range of movement and increases slightly when the arm is above the head (180° flexion). This indicates that the function of the middle trapezius is to fix the scapula in its plane of motion during abduction.[13, 14, 15, 17]

Lower Trapezius

The lower trapezius is active when the scapular is depressed. Together with the serratus anterior, lower trapezius is a component in the scapular rotation force which are activated in elevation of the arm. Activation of the lower trapezius has also been found in abduction of the shoulder which means that the muscle controls the position of the scapular.[13, 14, 15, 17]

2.3 Nerve Innervation in the Trapezius Muscle

The motor input to the trapezius muscles is mainly transferred by the accessory nerve (cranial nerve XI). This nerve is accessible to superficial, electrical stimulation in the cervical plexus. Motor fibres have also been found in the C2-C4, especially in the ventral rami (C3) and the fourth (C4) cervical nerves.[18, 19] The same two cervical nerves also have an afferent function and can be electrically stimulated by an electrode placed above the clavicle[20]. The accessory, C3 and C4 nerves can be seen in Figure 2.2.

The accessory nerve also provides motor input to the sternocleidomastoid and the C3 and C4 transmit motor signals to levator scapular and diaphragm.[11, 21]

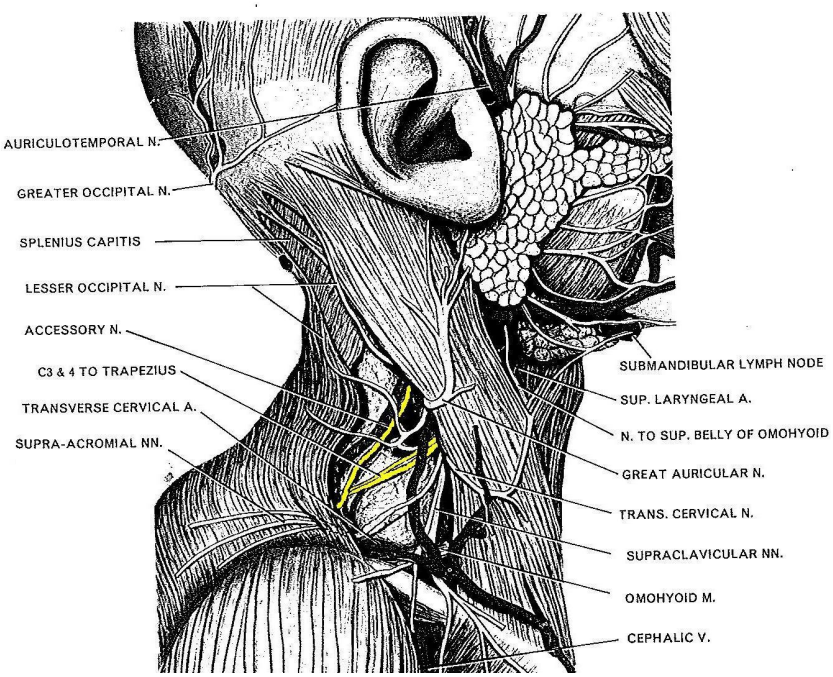


Figure 2.2: **Location of the Nerves in the Cervical Plexus.**
The accessory, C3, and C4 nerves are indicated with yellow on the figure.[19]

Chapter 3

The H Reflex

When studying the excitability of the α-motoneurons, the H reflex (discovered by Paul Hoffmann in 1910) is widely used. The H reflex gives information regarding the characteristics of the connections from Ia sensory fibres to spinal motoneurons in humans[22, 23]. The reflex is induced by electrical stimulation of the Ia fibres in a peripheral nerve. This is analogous to the mechanically induced spinal stretch reflex (tendon tapping). After stimulation, the reflex response is measured in the homonymous muscle by electromyography (EMG).[24]
The pathway of the reflex starts at the point of electrical stimulation of Ia afferent fibres (illustrated in Figure 3.1). This stimulation results in action potentials travelling along afferent fibres until they reach the synapse on the α-motoneurons. When the threshold of the α-motoneuron is passed, an action potential travels along efferent fibres until the neuromuscular junction is reached producing a twitch response in the EMG.[23, 25]

The pathway of the H reflex is nearly similar to the pathway of the monosynaptic reflex. The major difference between the pathways is that the H reflex bypasses the muscle spindles by stimulating Ia afferent fibres at a superficial point.[23] The different anatomical component of the monosynaptic reflex (muscle spindles are included) will be described in the next section.
A lot of factors influence the size of the recorded H reflex. Antidromic collision, background level of EMG, and presynaptic inhibition are some of the factors that modulates the H reflex[23, 25]. Factors that change the behaviour of the H reflex will also be explained in this chapter. Finally, the use of the H reflex in e.g. clinical applications will be explained.

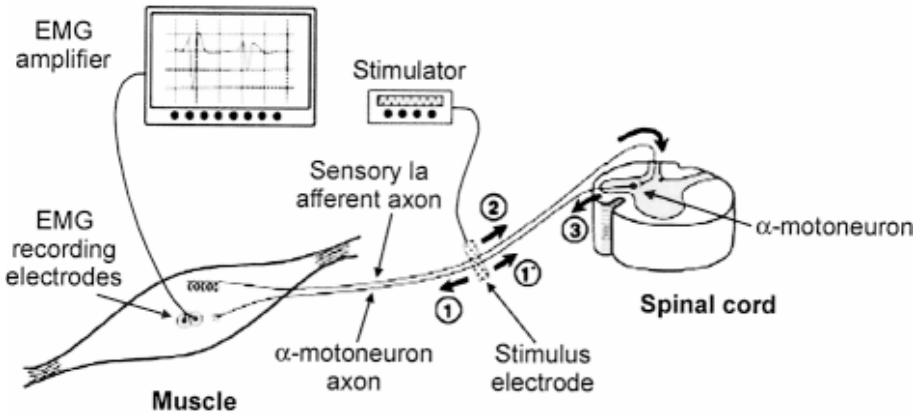


Figure 3.1: **The Hoffmann Reflex (H Reflex) and Muscle Response (M wave) Pathways.** At response (2), a small electric stimulus is delivered to the nerve. This elicits action potentials in the sensory Ia afferents that travel towards the spine. Here, they elicit action potentials in the α-motoneuron axons which leaves the spine and arrives at the target muscle through efferent fibres (response (3)). The muscle twitch is recorded as the H reflex. When the stimulus is increased, it will cause action potentials to activate the α-motoneuron directly. At this point (1), the action potentials can travel directly towards the muscle resulting in a direct muscle response recorded as the wave, or travel towards the spine to collide with the H reflex potential.[23]

3.1 Components of the Reflex Arc

In general, the monosynaptic pathway has three components: the receptor (muscle spindles), the Ia afferent fibre, and the α-motoneuron.[25] The following sections describe the anatomical component which can have influence in the monosynaptic reflex arc.

Muscle Spindles

Muscle spindles are encapsulated sensory receptors located in the fleshy part of the muscle. They have three main components[22]:

- Non-contracting intrafusal muscle fibres.
- Central region of the intrafusal fibres from which the Ia fibres originate (sensory nerve endings).
- Innervation of the polar contractile regions of the intrafusal fibres by the γ-motoneurons (endings).

In Figure 3.2, an illustration of the muscle spindle is shown.

Muscle spindles are used by the central nervous system to get knowledge about the relative position of a segment of the body. Muscle spindles are located parallel to the extrafusal fibres.

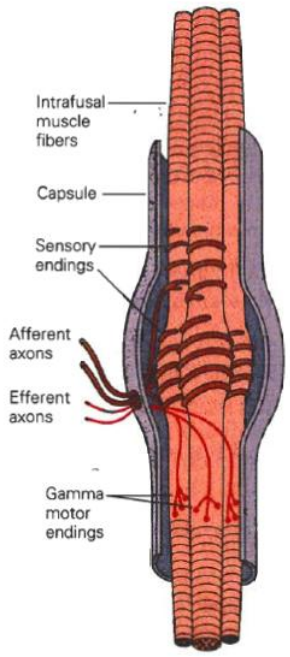


Figure 3.2: **Illustration of a Muscle Spindle**
The muscle spindle consists of intrafusal muscle fibres, sensory endings, and γ-motoneurons. It is ensheathed by a capsule and has afferent and efferent axons.[22]

A change in muscle length will therefore change the length of the muscle spindle. A shortened muscle will stretch the intrafusal fibres and the sensory afferent nerve endings of the spindle (called loading of the spindle) and thereby increase the firing rate of Ia afferent fibres. When the muscle is unstretched the firing rate of the Ia fibres will decrease. Stretch of the muscle is controlled by the α-motoneurons located in the extrafusal muscle fibres (see Figure 3.1).[22] Innervation of the muscle spindle can also occur through γ-motoneurons. Activation of γ-neurons causes shortening of the polar regions of the intrafusal fibres and stretch the non-contractile centre of the fibres. This will again cause the sensory Ia afferent fibres to fire.[22]

Sensory Afferent Fibres

Potentials in sensory afferent nerve endings are transmitted from the muscle spindles to the spine by afferent axons. Muscle sensory fibres are classified according to the diameter (see Table 3.1). A larger diameter means that action potentials can more rapidly be transmitted. In average, Ia fibres have a lower threshold than Ib fibres. Type II fibres are activated by a 2-5 times higher stimulus intensity than type I fibres and group III and IV fibres are activated by a 10-50 times higher stimulation intensity.[22]

Sensory Afferent Fibres			
Type	**Receptor**	**Axon (Diameter)**	**Sensitive to**
Ia	Primary spindle endings	12-20 μm	Muscle length (rate of change)
Ib	Golgi tendon organs	12-20 μm	Muscle tension
II	Secondary spindle endings and Nonspindle endings	6-12 μm	Muscle length and deep pressure
Group III	Free nerve endings	6-12 μm	Pain, chemical stimuli, and temp.
Group IV	Free nerve endings	0.5-2 μm	Pain, chemical stimuli, and temp.

Table 3.1: **Table of Sensory Afferent Fibres**
Sensory fibres are classified according to diameters. Each fibres has a receptor which are sensitive to different events.[22]

The Spine

In the spine, the reflex can be modulated by (1) the α-motoneuron,(2) the interneurons (not included in the monosynaptic pathway), and (3) the presynaptic terminal of the Ia sensory fibres[22]. This can be seen in Figure 3.3.

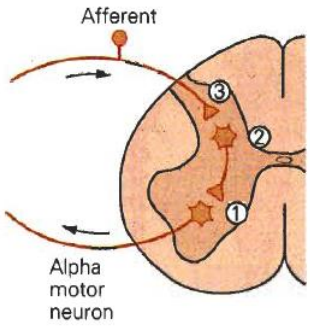


Figure 3.3: **The Reflex Pathway's Point of Modulation**
A reflex can be modulated at three points in the spine. The potential arrives at the sensory afferent fibres and can be modulated at the presynaptic terminals (3). Next, the interneurons at (2) can have a modulatory effect on the potential before finally synapsing with the α-motoneuron at point (1).[22]

The sensory Ia afferent fibres enter the spine though the dorsal root and continues into the gray matter of the spinal cord. In the gray matter the presynaptic terminal is connected to the α-motoneuron. The axon from the α-motoneuron leaves the spinal cord through the ventral root.[22] Many complex connections in the spine can have influence on the excitability of the α-motoneuron and thereby the reflex response in the muscle.[26] These connections will be mentioned later.

Motor Fibres

Motor or efferent fibres can be subdivided into α- and γ-motorneuron fibres. As mentioned before α-motoneuron axon innervate the extrafusal muscle fibres where the γ-fibres innervate intrafusal muscle fibres. The γ-motoneuron fibres are part of the fusimotor system which change the sensitivity of the muscle spindles.[22]

3.2 The H Reflex Response

When stimulating the sensory Ia afferent fibres two responses can be evoked in the electromyogram: the M wave and the H reflex (as shown on Figure 3.4). The H reflex response appears after a short latency period. This is due to the longer travel distance (to the spinal cord, across a synapse and back again). In contrast, the M wave results from a direct stimulation of the muscle and appears when α-motoneuron axons are stimulated.[22]
Responses are recorded by surface electromyography (sEMG) electrodes placed on the target muscle. M waves and H reflexes can be distinguished between due to e.g. latency of the response.[24]

Stimulation of the Sensory Afferent Fibres

To elicit the H reflex a small stimulus amplitude and relatively long (1 ms) stimulus is used[23, 27]. The small stimulus amplitude is used since the diameter of Ia afferents is larger than that of motor axons. Thus, it is possible to evoke an H reflex with a stimulus below motor threshold.[23, 25]
As can be seen on Figure 3.4 a small electrical stimulus elicits a response in sensory Ia afferents fibres only, which is shown as an H reflex on the EMG. As the size of the stimulus increases (Figure 3.4B) α-motoneuron axons are stimulated directly. This results in an M wave on the EMG. Furthermore, the H reflex has decreased in size due to antidromic collision. A further increase in stimuli (3.4C) results in activation of all motor axons. This increases the size of the M wave and blocks all action potentials from orthodromic activity. Thus, the H reflex disappears from the EMG.[23]
The recruitment curves of the M wave and H reflex responses are shown on Figure 3.5. The maximal H reflex activation is an estimate of the number of motoneurons one is capable of activating in a given state. The maximal M wave represents activation of the entire motoneuron pool, equal to maximal muscle activation.[23]

Both uni- and bipolar stimulation can be used when eliciting an H reflex. The most common setup is to use bipolar surface electrodes, placed on the muscle belly and in line with the muscle fibres where the intra-electrode distance is approximately 2cm.[23, 25]

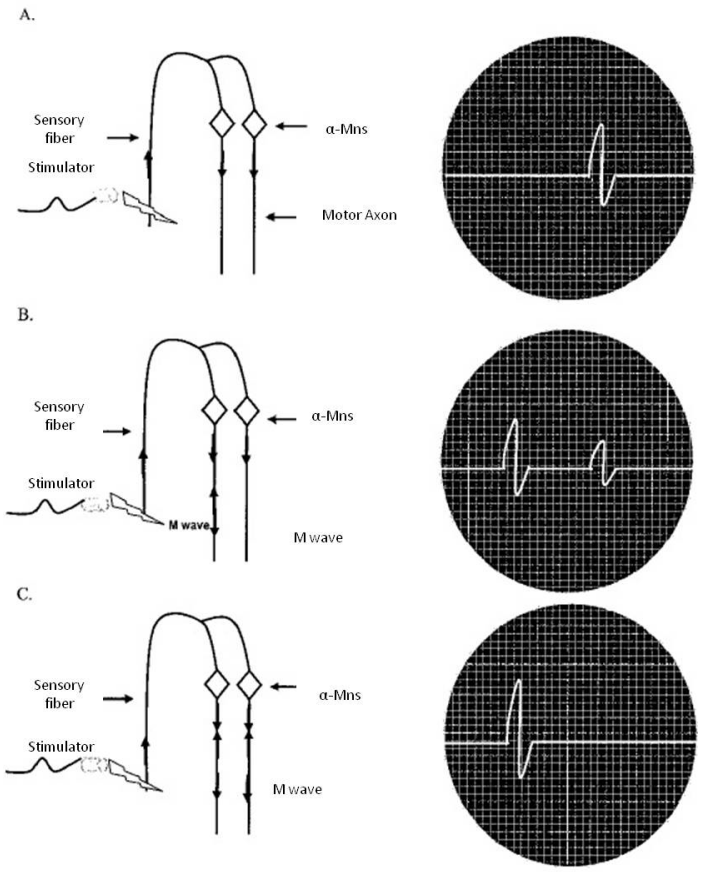


Figure 3.4: **The Events Leading to the Recording of H Reflex and the M wave**
A: electrical stimulus elicits a response only in the sensory Ia afferents. This results in the appearance of the H reflex. B: electrical stimulus also directly activates the α-motoneuron axons. This results in an antidromic impulse towards the spinal cord. This collides with orthodromic impulse and decreases the amplitude of the H reflex. C: Electrical stimulus activates all the motor axons. Antidromic collision blocks all action potentials from orthodromic activity. Hence, the H reflex disappears from the EMG and only the M wave is visible.[23]

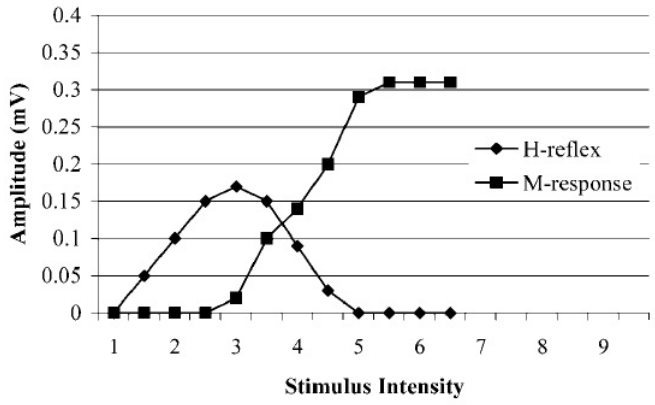


Figure 3.5: **Recruitment Curves of the H Reflex and the M wave**
The stimulation intensity is gradually increased until the maximum H reflex and M wave are reached. The H reflex curve decreases because of the collision by antidromic impulses.[23]

Recordings of H Reflexes

Reflexes can be measured by sEMG electrodes and displayed in an ongoing EMG. The activity of motor units (single α-motoneuron and the corresponding muscle fibres) can be recorded by sEMG electrodes[28]. To distinguish between H reflex deflection and background EMG, background EMG can be recorded before (and/or after) the stimulation. According to the expected reflex latency (of a monosynaptic reflex), the deflection of the H reflex can be found in the EMG. The reflex latency can then be found by a measure of transmission length and speed in nerve fibres of cadavers.[24] An H reflex in the EMG is shown in Figure 3.6.

sEMG electrodes can record potentials on and under the surface of the skin. However, the recorded signals may be affected by other upcoming potentials caused by movement. 50 Hz noise can also affect the EMG signal. A suitable filter can help attenuate such noise.[28]

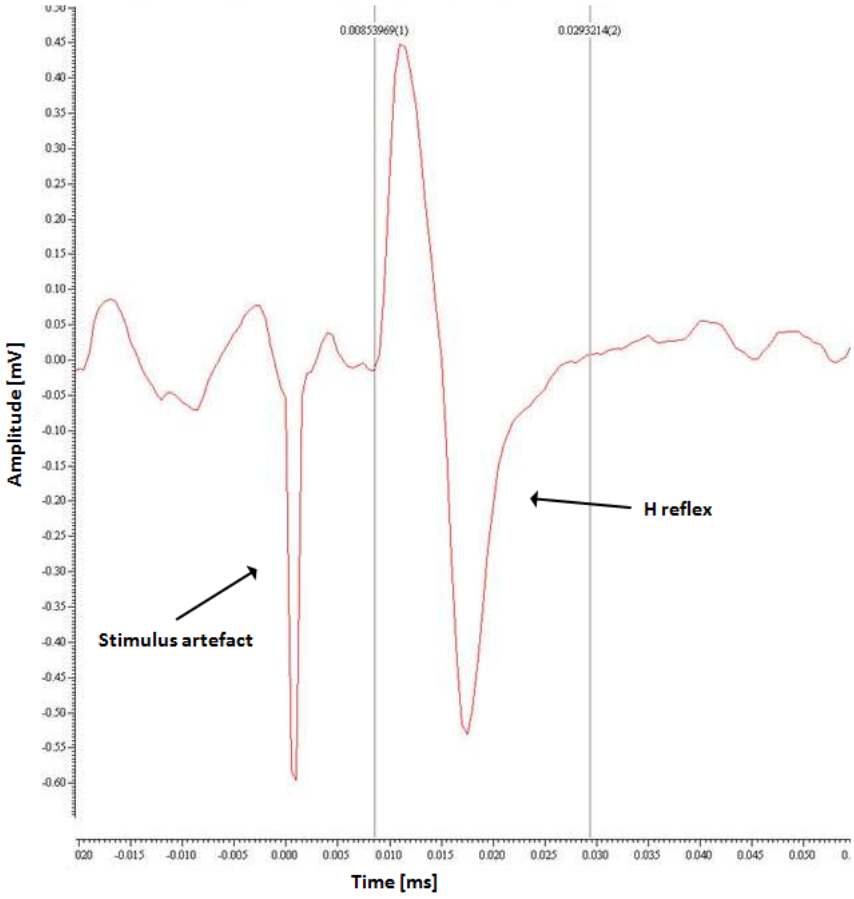


Figure 3.6: **Illustration of an H Reflex in an EMG Recording**
The first potentials (from left) is the stimulus artefact and the second deflection is the H reflex (recorded in the experiment of the project).

3.3 Concerns Regarding the H Reflex

In general, the interpretation of the H reflex is very complicated since changes in amplitude can be affected by four possibilities: (1) presynaptic inhibition, (2) homosynaptic depression, (3) alterations in the excitability of the motoneurons, and (4) variation in the intrinsic properties of the motoneurons (see Figure 3.7).[29]

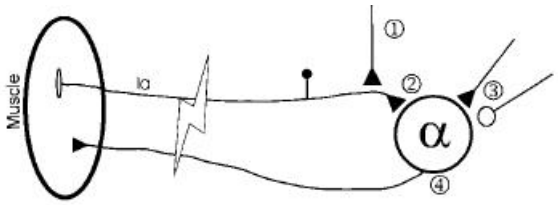


Figure 3.7: **Possible Sources Affecting the Amplitude of the H Reflex**
(1) presynaptic inhibition, (2) homosynaptic depression, (3) motoneuron excitability, and (4) intrinsic properties of the motoneurons are factors that modulates the H reflex.[29]

(1): one of the major drawbacks in the H reflex technique is the presence of presynaptic inhibition of Ia terminals. This is an inhibition of a stimulatory neuron before it synapses by preventing the neuron to generate an excitatory postsynaptic potential. Changes in presynaptic inhibition have been observed during voluntary movements and after cortical stimulation. Presynaptic inhibition is induced by a conditioning volley (vibration or electrical stimulation).[23, 25]
(2): another drawback is the post-activation depression at the synapse Ia afferents motoneruons. This depression is due to a reduced transmitter release from previously activated fibres[29]. This depression requires at least 3s to subside completely[30].
(3): motoneuron excitability can also modulate the amplitude of the H reflex due to excitatory and inhibitatory postsynaptic influence. These mechanisms have been related to voluntary contraction which are explained in the next section.[29]
(4): the last drawback is the change in motoneuron potentials. Change in task or state may change the intrinsic properties of the motoneuron and affect the magnitude of the H reflex.[29]

Change in Motoneuron Excitability by the Environment and Voluntary Contraction

To ensure that the influence on the H reflex is as small as possible, it is very important to keep the subject in the same position throughout the experiment. A lot of factors (e.g. eye closure, head position, joint position or angle etc.) affect the H reflex amplitude. The environment in which the experiment is performed is also important. Noise and other external stress factors could affect the amplitude of the H reflex by changing the motoneuron excitability.[23]

There is a strong connection between background EMG level and the amplitude of the H reflex. Movement in the test muscle that increases or decreases the EMG level will either increase or decrease the reflex amplitude. The reason for this is that the level of depolarisation of motoneurons may rise and fall below the threshold for activation, leading to changes in H reflex amplitude.[29] By doing a voluntary contraction the motoneuron pool is raised to firing threshold. Thus, it is possible to evoke reflexes at a lower stimulus intensity, thereby not seing an M wave on the EMG trace. In a study by Burke et al.[31] different limb muscles were stimulated during relaxation and with a voluntary contraction of the test muscle. This study showed that the reflexes only could be recorded with a small contraction H reflexes could reliably be recorded from the test muscles. Furthermore, a clear separation between the M wave and the H reflex were observed when doing a contraction. No difference in reflex latency was observed between the reflexes recorded during relaxation and the reflexes recorded during a contraction.[31]

Variations of the H Reflex

A further concern regarding the H reflex is the variations in the H reflex from trial-to-trial and from day-to-day. Variations in H reflex recordings are illustrated by the results of Brinkworth et al.[32] (see Figure 3.8). These results indicate that the recruitment curve of the H reflex varies in standardised experimental settings.

As seen in Figure 3.8, recruitment curves vary from trial-to-trail for each subject. The greatest variation was at intensities both including the H reflex and the M responses. During contraction (50% MVC) similarities from trial-to-trial (at the same day) were best.[32]
From day-to-day, larger variations were observed in the study by Brinkworth et al.[32]. Reliability of the H reflex from day-to-day can only be obtained by recording a whole recruitment curve.[32] Due to the complexity of recording the H reflex from trial-to-trial and day-to-day, Brinkworth et al.[32] and Zehr[30] have given following suggestions:

- A whole recruitment curve should be measured because one intensity can give misleading results.
- At least five stimuli, preferably 10, should be delivered at each intensity in order to increase signal-to-noise ratio.
- At least 15 stimuli steps should be used to get a whole and detailed recruitment curve.
- Use recorded background EMG level to keep the same level of motoneuron excitability (e.g. 10% of maximum voluntary activation in the taget muscle).
- Experimental behaviour state such as posture of the subject must be the same for all measurements to get similar levels of motoneuron excitability.

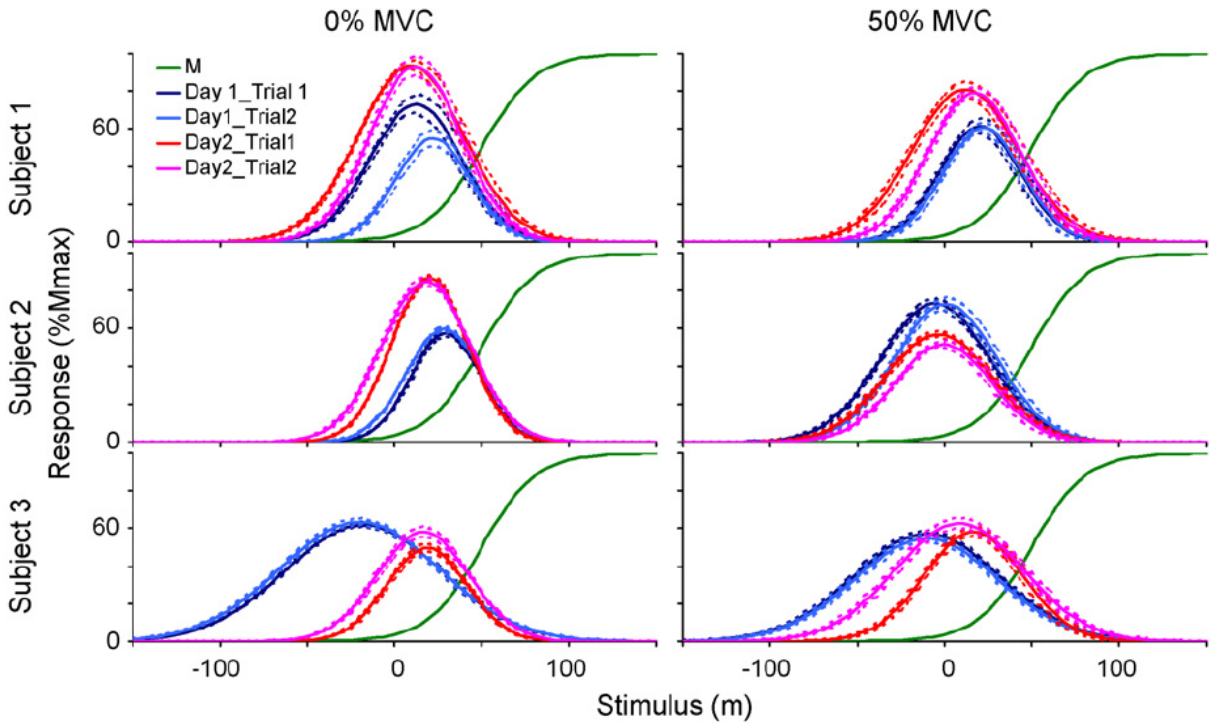


Figure 3.8: **Variations of the H Reflex**
Recordings from trial-to-trial and day-to-day of the H reflex show variations within each subject. The intensity is normalised to M wave measurements. When the subjects contract the targeted muscle 50% of their maximum voluntary contraction (MVC), the variation becomes smaller compared to 0% MVC (relaxed).[32]

- Repetitive stimulation should not be more frequently than 3s due to post activation depression.

Normalisation Procedures

Recordings of the H reflex amplitude can vary among subjects. Examples of causes are variations in skin resistance, different amounts of subcutaneous fat, and locations of the nerve relative to the electrical stimulus. This creates a need to normalise the amplitude for inter-subject comparisons.[29]

H Reflex as a Percentage of M_{max}

The most widely used normalisation technique is to elicit the H reflex as a percentage of the M_{max}. First, the amplitude of the M_{max} is found and then the stimulus intensity is adjusted to produce an H reflex with an amplitude equal to some percentage of the M_{max} amplitude. Most often, a percentage between 10% and 25% of M_{max} is chosen. Theoretically, this technique makes it possible to evaluate the same proportion of the motoneuron pool for every subject.

This is desirable when assessing the motoneuron pool's reaction to different interventions at a consistent point for all subjects.[29]

$\mathbf{H}_{max}/\mathbf{M}_{max}$ Ratio

The H_{max} amplitude is an indirect estimate of the number of motoneurons being recruited and the M_{max} represents the entire motoneuron pool. Therefore, the H_{max}/M_{max} ratio can be interpreted as the proportion of the entire motoneuron pool capable of being recruited in a given state. This normalisation is based on the assumption that the M wave amplitude is a stable value. It is recommended that raw M_{max} values are reported to confirm that no change in M_{max} was detected.[29]
H_{max}/M_{max} ratio is commonly used as a dependent measure when data are being collected on more than one occasion, because of the risk of movement of the stimulating or recording electrodes. However, when reporting the H_{max}/M_{max} ratio, the H reflex is less susceptible to reflect a facilitation or inhibition at higher amplitudes.[29]

3.4 Uses of the H Reflex in Neural Investigations

The H reflex is one of the most studied reflexes in the literature. This is due to the fact that it is easy to elicit in muscles with percutaneous access.[29] In spite of its easy accessibility, the fact that the H reflex is a product of complex interactions makes the correct interpretation hard. Thus, it is hard to use in research or clinical experiments. Still, the H reflex can be used as a tool in neural investigations.[29, 33] In the following its uses as a tool is described.

A potential usage of the H reflex is to investigate the functional organisation of neural pathways. This is done by applying a conditioning stimulus somewhere in the body different from the recording site. If this applied stimulus gives a facilitation or suppression of the H reflex, a clear indication of some kind of connection between the two locations is given. This has been used several times to describe neural connections that exist in humans. However, this only allows an indirect look into the organisation of the sensory-motor system. Another potential usage of the H reflex is to use it as a tool in the investigation of spinal excitability.[29]

As described in Section 3.2, the amplitude of the H reflex is affected by presynaptic inhibition. Accepting that the H reflex is a reflection of the Ia monosynaptic reflex arc, it can be used to monitor changes in the level of presynaptic inhibition affecting the Ia afferent terminals with monosynaptic connections to motoneurons.[29]
A common misunderstanding when using the H reflex as a research tool is that the H reflex is a

measure of the excitability of the motoneuron pool. Since the H reflex is affected by variations in neurotransmitters released from the postsynaptic terminals, this is not a valid assumption. However, it can be used to indicate whether the excitability of the motoneuron pool is constant which it is if the H reflex amplitude remains constant between different conditions.[29]
When using the H reflex as a tool in investigations regarding health and diseases, it is important to investigate how the H reflex differs between patient populations and the normal population. Often described changes include: change in the level of presynaptic inhibition, differential effects of postural constraints, and delays in pre-movement modulation. Looking at the output from these studies gives greater insight into possible motor dysfunction with better potential for therapeutic strategies.[29]

Chapter 4

Experimental Pain in Healthy Humans

Deep tissue pain constitutes a special diagnostic and therapeutic challenge. Human experimental models have been developed and applied to healthy volunteers to investigate different aspects involved in muscle pain, so that new knowledge can be used to improve diagnostic and management strategies. Experimental pain must activate the nociceptive system and assess the evoked sensory and motor responses in a standardised and quantitative way. When inducing experimental pain, healthy subjects become patients with well-defined muscle pain. This allows researchers to assess manifestations and sensory-motor interaction with a known cause-and-effect relationship.[6]
Experimental pain techniques can be divided into exogenous and endogenous methods. Exogenous techniques are external interventions such as electrical stimulation of muscle afferent fibres or injection of pain-producing substances. An example is hypertonic saline which is a commonly used chemical stimulant. When injected into muscle tissue, hypertonic saline reliably causes an acute muscle pain condition with localised and referred characteristics. Endogenous techniques induce muscle pain by natural stimuli like ischemia (e.g. by application of a tourniquet to occlude blood flow) or exercise, causing a widespread deep pain in muscles and other somatic structures. One kind of exercise is the eccentric exercise which induce delayed-onset muscle soreness (DOMS) in the target muscle.[6]
In this chapter, the analysis of experimental pain will focus on DOMS as an endogenous technique for inducing muscle pain.

4.1 Delayed-onset Muscle Soreness

DOMS manifests as a mechanical hyperalgesia and is described by Lieber & Friden[34] as the sensation of muscular discomfort and pain during active contractions that occur in a delayed fashion after strenuous exercise. The muscle soreness is usually described as stiff, tender, or

aching. The sensation usually starts at the muscle tendon junction and then spreads further throughout the muscle[35, 36]. In addition, a study by Nie et al.[37] found that after eccentric exercise, muscle belly sites are more sensitive to pain than the musculotendinous sites. There is usually no spontaneous pain - the symptoms develop during the first 24 and 72 hours, and disappear within 5-7 days, usually without intervention [38, 39]. The affected muscles will be sensitive to palpation, exhibit prolonged strength loss, reduced range of motion and elevated levels of serum creatine kinase.[34]

The onset of DOMS is associated with having performed intense exercise and is more pronounced if the individual is unaccustomed to the exercise. Training may prevent or attenuate the magnitude of muscle injury that occurs after exercise. However, this is only the case if the training consists of the same type of exercise and includes the specific muscle group being tested. Overall, there are three types of muscle actions as shown in Figure 4.1: isometric (a), concentric (b), and eccentric (c).[40] These can be seen in Figure 4.1.

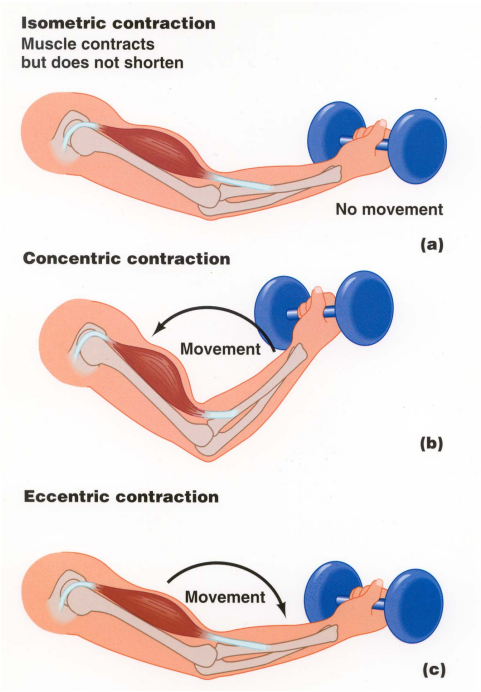


Figure 4.1: **Different Types of Muscle Contraction[41]**
(a) shows an isometric contraction with no change in muscle length, **(b)** shows a concentric contraction where the muscle shortens, and **(c)** shows an eccentric contraction where the muscle elongates.[40]

When performing an isometric contraction, the activated muscle produces tension without a change in muscle length (i.e. static contraction). In contrast, concentric contraction produces tension during muscle shortening. Conversely, during eccentric action the muscle is forced to elongate while producing tension. Eccentric action can generate the highest tension and it has been shown to be the main component in the development of DOMS.[42] Therefore, through ec-

centric exercise in healthy subjects, DOMS can elicit muscle hyperalgesia in a controlled fashion as an endogenous, temporary model of muscle pain.[43, 44]

A number of treatment strategies have been proposed to alleviate the symptoms of DOMS, restoring the maximal function of the muscles faster and/or reducing the magnitude of the initial injury[45]. The strategies have been applied both as a preventative measure and/or therapeutically as a treatment measure. The most common treatment strategies have included cryotherapy, stretching, anti-inflammatory drugs, ultrasound, electrical current techniques, massage, compression, hyperbaric oxygen and exercise. In spite of the many treatment strategies used, only limited success has been reported in research to date. It is interesting to note that although stretching is publicly recommended as an injury prevention measure, the rationale for stretching has yet to be validated by future research.[46]

4.2 Mechanisms Behind the Development of Delayed-onset Muscle Soreness

Different theories have been proposed to explain the mechanisms responsible for the painful sensation associated with DOMS. The following list mentions some of the theories which will be described briefly afterwards:

- The muscle spasm theory.
- The muscle damage theory.
- The inflammation theory.
- The release of chemicals theory.
- The thermal sensitivity theory.

Muscle spasm theory: this theory focus on muscle spasms as the cause of pain development[47]. The muscle spasm theory is based on observations of increased resting muscle activity (EMG) after eccentric exercise[47].
The theory suggests that the increased EMG activity indicates a tonic localised spasm of motor units. This spasm leads to an ischemic compression of local blood vessels along with accumulation of pain substances. A vicious cycle is then created as the pain substances stimulate nociceptors causing further reflex muscle spasms and prolonged ischemic conditions[47, 48]. However, reports on EMG activity in exercised muscles have been inconclusive[39, 46].

The muscle damage theory: this theory focus on muscle damage as the cause of DOMS. A study by Lieber & Friden[49] proposed that damage of the connective tissue that forms sheaths around bundles of muscle fibres was the cause. Additionally, type 2 fibres have a less robust structure than type 1 fibres and studies have shown that eccentric exercise more selectively recruits the weaker type 2 structures.[49]
In support of the muscle damage theory, studies in humans and animals have shown micro-injuries in exercised muscles such as broadening and streaming of Z-bands that mechanically bind neighbouring sarcomeres.[50, 51] In addition, leakage of enzymes from the exercised muscle was shown.[50]

The inflammation theory: this theory is based on an observation of invading inflammatory cells (macrophages) into the muscle[52]. However, a more recent study in humans found no difference in the markers for inflammation between subjects doing eccentric versus concentric contractions. Furthermore, anti-inflammatory drugs have been used to investigate the involvement of inflammation. Unfortunately, the effects of the drugs differed among laboratories. Also, there were more effective results when a drug was administered prophylactically than when it was given therapeutically.[46]

The release of chemicals theory: chemical substances such as bradykinin, serotonin, histamine, and potassium all have the ability to elicit action potentials in group IV fibres which cause pain.[6]

The thermal sensitivity theory: sensitivity to temperature variations (between 38 °C and 48 °C) by group III and IV nerve endings has also been considered to be the cause of pain. Elevation of local temperature during DOMS can affect the nerve endings and cause neural alterations. It is believed that group IV afferent fibres are the primary pain carrier in DOMS because of the dull diffuse pain profile.[34, 39]

No hypothetical mechanism has been sufficient to explain DOMS. Often, a combination of the theories of tissue damage and inflammation is accepted, but further experimental results are needed.[6, 46]

4.3 Methods for Assessing Muscle Soreness

Subjective characteristics of muscle pain are necessary in all clinical and experimental muscle studies. A number of different methods for evaluating the perceived pain location and quality have been used. The following section briefly describes six of these methods of assessment:

- Pressure Pain Threshold (PPT).
- Visual Analogue Scale (VAS) score.
- The McGill Pain Questionaire (MPQ).
- Pain Area Drawing.
- Maximum Voluntary Contraction (MVC).
- Range of Motion (RoM).

Pressure Pain Threshold

PPT is a measure of the pain sensitivity to pressure in the tissues of the investigated area. Using a hand-held algometer, the study by Nie et al.[37] showed that PPT decreased significantly 24h after strenuous eccentric exercise, indicating a peak in DOMS. Also, muscle belly sites were more sensitive to pain than the musculotendinous sites. A study by Ylinen et al.[7] evaluated the repeatability of pressure algometry on neck muscles and concluded that PPT measurements can be used to monitor changes in muscle groups making it suitable for clinical studies. However, when repeated measurements of the PPT were compared against their means, the variation in patients with chronic neck pain showed a two-fold increase compared to that found in previous studies of symptom-free subjects.

Visual Analogue Scale Score

VAS score is used to rate the intensity of pain and compare measures to investigate variations over time, e.g. before and after intervention. Basically, the VAS can be a 10 cm line which ranges from score 0 to 10, where 0 indicates “no pain” and 10 indicates “worst pain imaginable”. Nie et al.[37] showed a significant increase in pain intensity score after DOMS-inducing eccentric exercise from 0 to 48 hours. There was no significant difference in the VAS scores between immediately after exercise and 24h after, but the average pain intensity was higher at 24h compared with 48h.

McGill Pain Questionnaire

MPQ is used to assess the quality of pain. The questionnaire consists primarily of 3 major classes of word descriptors - sensory, affective and evaluative - that are used by subjects to specify

subjective pain experience. The questionnaire was designed to provide quantitative measures of clinical pain that can be treated statistically. Also, the questionnaire is sufficiently sensitive to detect differences among different methods to relieve pain.[53]
In a study by Nie et al.[37], the most chosen word was "tiring" (45,8% of subjects) at 0h, "sore" at (58,3% of subjects) at 24h, and "tender" (33,3% of subjects) at 48h. Finally, there were no significant differences between males and females.

Pain Area Drawing

Using a human body chart consisting of a whole body line diagram, the subject can draw the area of perceived pain. The size of the area can then be calculated and compared to that of other subjects' drawings to gain insight in the spread of the pain and inter-individual differences.
The study by Nie et al.[37] showed a significant increase of pain area after DOMS-inducing exercise and also between the exercise side and the control side. Again, no significant differences between males and females were found. An example of a pain area drawing for evaluating the spread of DOMS can be seen in Figure 4.2.

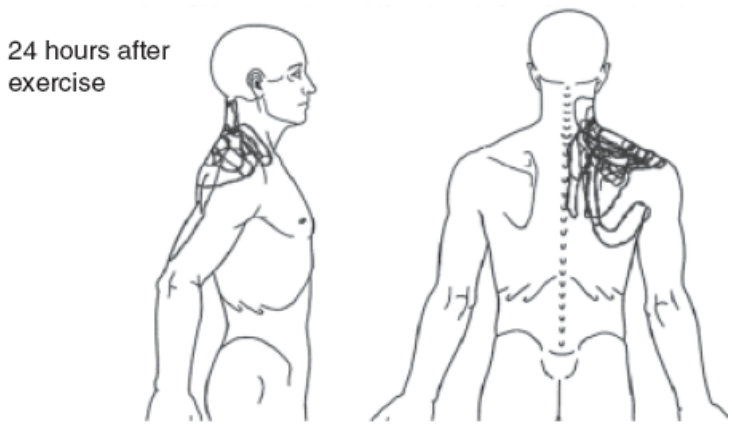


Figure 4.2: **Pain Area Drawing**
The area of delayed onset muscle soreness has been drawed on the body diagram to indicate the spread of muscle soreness developed 24h after exercise (modulated from[14]).

Maximum Voluntary Contraction

MVC has been used as an indicator of muscle soreness. After completing an exercise procedure, an immediate decrease in MVC due to muscle exhaustion will be evident, but the decrease has been shown to consist in some cases as a result of DOMS. As an example, the study by Madeleine et al.[14] showed a decrease in MVC force 24h after exercise compared to before exercise. Alternatively, EMG activity during MVC may be used as an indicator of DOMS.[54]

Range of Motion

The RoM assessment has been used as an indicator of stiffness and muscle soreness. Along with stiffness, a decrease in RoM has linked a reduction in joint flexibility and joint function with post-exercise muscle soreness. However, the decrease in RoM is not always present at the onset of delayed muscle soreness although significantly evident immediately after the exercise.[55]

Chapter 5

Aim of the Project

Based on a synthesis of previous chapters and a review of different articles concerning delayed-onset muscle soreness (DOMS) the aim of the project is stated. This is illustrated in Figure 5.1.

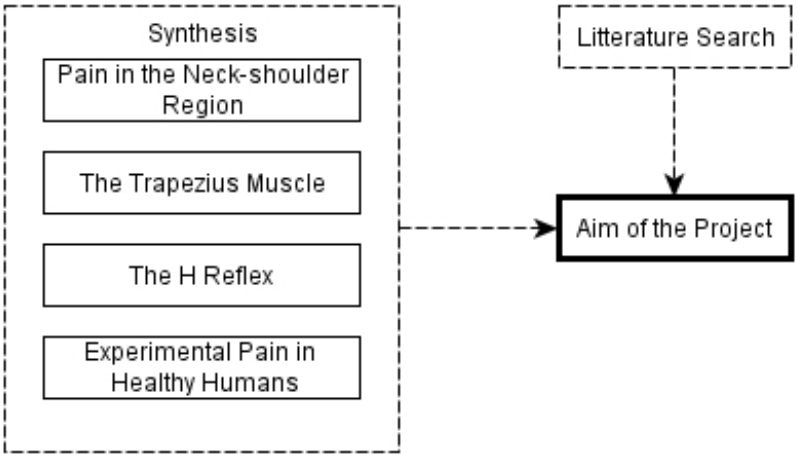


Figure 5.1: **The Background for the Aim of the Project**
Previous chapters are synthesised and a literature search is used to state the aim of the project.

5.1 Literature Search

A literature search concerning DOMS and H reflexes was conducted to clarify previous scientific findings. The search was performed in PubMed in March 2011 using the following combinations of keywords: "Delayed-onset muscle soreness and H reflex", "Delayed-onset muscle soreness and trapezius", "Eccentric exercise and trapezius", and "Eccentric exercise and H reflex". The searches

were limited to articles in English.
The inclusion criteria for articles were a focus on eccentric exercise-induced DOMS-changes in H reflex responses - i.e. in a pre-post design, preferably focusing on the trapezius muscles. Based on the assessment of DOMS (see Chapter 4), the search was limited to a focus on evaluating the modulation of the following soreness parameters: VAS score and PPT. Other parameters from the articles were not included in the table since they were outside the scope of this study. The search resulted in 8 articles.
Table 5.1 summarises the findings of the chosen parameters of the search. The study by Nie et al.[56] was excluded from the table because the study's purpose was to investigate differences between genders and not to investigate the influence of DOMS on the parameters.

As can be seen in Table 5.1, only three studies have investigated the effect of DOMS on the H reflex. None of these are looking at the H reflex in relation to DOMS in the trapezius muscle. Because of DOMS, the VAS score had increased in all studies where the parameter was included[37, 54, 57, 59, 60, 61]. A decrease in PPT due to DOMS is also shown[37, 54, 61, 62]. The VAS score and the PPT parameters must therefore be considered to be good indicators for having induced DOMS - also in the trapezius muscle.

Analysis of the Findings in the Literature Search

Studies by Bulbulian & Bowles[57], Avela et al.[58], and Racinais et al.[59] have investigated changes in H reflex induced by DOMS, but all focused on lower extremities. The H reflex was not modulated in any of the three studies 24h or 48h after eccentric exercise. An explanation of these results may be found in the publication by Brinkworth et al.[32]. They addressed the problem of measuring the H reflex from day-to-day (see Chapter 3). Based on investigations, Brinkworth et al.[32] suggested that a whole recruitment curve must be obtained instead of a single intensity measure from an H reflex. A single measure may therefore not reflect the change in the H reflex. In the publications of Bulbulian & Bowles[57], Avela et al.[58] and Racinais et al.[59], only a single repeated measure of H reflexes (H_{max}) were used to decide the changes in H reflexes. Due to the lack of recruitment curve parameters of the H reflex, the result of all three studies may be misleading. Furthermore, changes in stimuli intensities to elicit H_{max} could also havebeen relevant to compare and report in the three publications, since this value reflects activity in the most excitable Ia afferent fibres and hence could give an indication of a change in motoneuron excitability.
Another point to criticize in the three articles is the missing data from the subject's muscle contraction during H reflex stimulation. Voluntary contraction of the test muscle is an important factor for changing motoneuron excitability and thereby the H reflex amplitude (see also Chapter 3). It is therefore hard to determine the level of contraction during each session of H reflex

Table 5.1: Articles Reviewed

Authors and Year	**Area of DOMS**	**Parameters**	**Results of DOMS**
Bulbulian & Bowles (1992)[57]	Legs and buttocks	VAS and H_{max}/M_{max} ratio	Soreness increased and H_{max}/M_{max} ratio did not change
Avela et al. (1999)[58]	Legs	H_{max}/M_{max} ratio	H_{max}/M_{max} ratio did not change (2 days after)
Nie et al. (2005)[37]	Trapezius	VAS and PPT	VAS score increased and PPT decreased
Racinais et al. (2007)[59]	Plantar flexor muscles	VAS and H_{max}/M_{max} ratio	VAS score increased and H_{max}/M_{max} ratio did not change
Kawczynski et al. (2007)[60]	Trapezius	VAS	VAS score increased
Nie et al. (2009)[61]	Trapezius	VAS and PPT	VAS score increased and PPT decreased
Binderup et al. (2010)[62]	Trapezius	PPT	PPT decreased
Wakefield et al. (2010)[54]	Left upper trapezius	VAS and PPT	VAS score increased and PPT

measurements and be convinced of the standardised contraction method.
Brinkworth et al.[32] also suggest that at least 5 stimuli should be given at each stimulus intensity. This is not fulfilled in the study by Bulbulian & Bowles[57] where only 3 stimuli were used to determine H_{max}. Furthermore, the small number of subjects in the studies by Bulbulian & Bowles[57] (n = 6) and Avela et al.[58] (n = 7) may contribute to the results being insignificant and in the study by Avela et al.[58] neither PPT or VAS score were used to determine the degree of soreness (DOMS).

5.2 Expectations to the H Reflex

As described in Section 1.2, the main component of sensing pain is the group III and IV afferent fibres. Because DOMS is associated with muscle pain findings when stimulating interneurons of group III and IV afferents in humans, DOMS seems to be sensed by firing of these afferent fibres. The fibres are also activated by ischemia in the muscle, metabolic changes (by e.g. potassium, lactic acid, bradykinin and arachidonic acid), and appear to reflect vasodilatation within the muscle.[26, 63] Based on these findings it appears that group III and IV afferent fibres are stimulated and increase in firing rate during DOMS. According to Taylor et al.[63] and Gandevia[26], the firing of group III and IV afferent fibres presynaptically inhibits Ia afferent fibres and reduces descending excitatory drive to α-motoneurons in the spine during fatigue. Studies of the H reflex during fatigue have shown a reduction in reflex amplitude (e.g. Le Pera et al.[64] and Garland & McComas[65]) which suggest that inhibitatory mechanisms take place.

In a study by Matre et al.[66], experimental muscle pain, induced by infusion of hypertonic saline, did not show any change in the H reflex despite an increase in the stretch reflex. This suggests that the effects were peripheral rather than central, and the muscle spindle sensitivity had changed with pain. This result is in line with a study by Leroux et al.[67] where pain relief on the knee were applied by cold application and the H reflex measured before and after. The pain relief did not change the H reflex amplitude suggesting that pain episodes do not affect monosynaptic responses.

5.3 Problem Statement

To summarise, present studies on H reflexes after inducing DOMS suggest that no change in the H reflex amplitude occurs 24h after exercise. However, several drawbacks regarding the methods of these studies question their findings. Studies regarding fatigue suggest a decline in motoneuron excitability, i.e. a decrease in reflex amplitude. Pain studies suggest no change in reflex amplitude. Hence, further investigation of the effect from DOMS on the H reflex needs to be

carried out to further clarify the topic.
As mentioned in Chapter 1, the trapezius muscle is commonly affected by work-related musculoskeletal disorders (WMSD) and is prone to be affected by muscular hyperalgesia. Since DOMS can be induced in a controlled fashion (see Section 4.1) it is possible to investigate the mechanism of pain in the trapezius. According to the literature search, no studies have examined the H reflex in the trapezius muscle during DOMS. Investigations of the H reflex in the trapezius during DOMS may potentially provide further insight into the neural mechanism in WMSD.
This leads to the following problem statement:

How is the H reflex affected by DOMS in the trapezius muscle?

Since the H reflex is effected by several factors (see Section 3.3), it may be difficult to reproduce measures of H reflexes across days. If more studies on the change of H reflex responses across days are to be conducted in the future, it is vital that the day-to-day variability of the reflex is examined. The reproducibility of the H reflex is therefore also investigated in following chapters.

Chapter 6

Experimental Protocol

The purpose of the experiment was:

- *To investigate the effects of experimentally induced delayed-onset muscle soreness (DOMS) on the H reflex.*
- *To examine the reproducibility of H reflex measurements between days.*

DOMS was used as an endogenous model of muscle pain and was induced by repetitive eccentric exercises. Since soreness induced by eccentric exercise usually peaks 24h after the exercise[39], two sessions on two consecutive days were used to measure the effect of DOMS in a pre-post design. In order to have a control measurement for the day-to-day reproducibility of the H reflex, recordings were also done 24h before the eccentric exercise. Each run through of the experimental procedure was therefore executed on three consecutive days consisting of four overall data collecting sessions:

- 24h before exercise: test for reflex reproducibility and obtain a baseline/control measurement for the effect of DOMS.
- Pre-exercise: test for reflex reproducibility and obtain a baseline/control measurement for the effect of DOMS.
- Post-exercise: the effect of muscle fatigue.
- 24h after exercise: the effect of DOMS.

Post-exercise recordings were also included since physiological mechanisms of fatigue have been related hypothetically to physiological mechanisms of DOMS (see Section 5.2). Similarities between post-exercise and 24h after exercise were therefore investigated. To assess the intensity and development of soreness induced in the trapezius muscle, the following control parameters

were measured in each session: visual analogue scale (VAS) score and pressure pain threshold (PPT).
The effect of eccentric exercise on the H reflex was investigated by recordings of H reflexes evoked in the C3/4 cervical nerves. To ensure the same amount of background electromyography (EMG) during H reflex recordings, feedback of EMG activity was provided for the subject. To normalise for changes in the recording electrodes (e.g. position and internal electrode impedance), M waves were evoked in the accessory nerve. Due to stimulus artefact in the upper trapezius[20] and limited spread of induced muscle soreness to the lower trapezius by the used exercise method[62], only responses in the middle trapezius were investigated. Full recruitment curves for both M waves and H reflexes were obtained from the middle trapezius part of the dominant side of the subject in each session. Three peak-to-peak amplitude values and a slope coefficient from the recruitment curve were calculated from H reflex recruitment curves. H reflex peak-to-peak amplitudes were normalised to the maximal M response and compared, like the slope coefficient, within each subject between sessions.

The following experimental details will be described in this chapter in the stated order:

- Subject recruitment (Section 6.1).
- The method to induce DOMS in the trapezius muscles and a description of how DOMS are measured (Section 6.2).
- The method to elicit neural responses in the trapezius muscles (Section 6.3).
- The procedure of the experiment (Section 6.4).
- Settings of the experiment (Section 6.5).
- Data analysis of measured parameters (Section 6.6).

6.1 Subject Information

The following inclusion criteria applied to all subjects:

- Young (18 - 40 years).
- A reliable measure of the H reflex.

The following exclusion criteria applied to all subjects:

- Weight training in the past month.
- Muscle soreness in the neck-shoulder region prior to the study.
- History of previous neck-shoulder disorders.

In regards to the inclusion criteria, the mentioned age group was chosen because of the overall risk of neural deterioration present in older populations[29].

The need for a reliable measure of the H reflex in recruited subjects was necessary as a criterion, as it is not all subjects in whom it is possible to elicit an H reflex in the trapezius muscle by electrical stimulation[20].

Regarding the exclusion criteria, there was a need to exclude all who had participated in regular strenuous weight training of the neck-shoulder region in the past month[46]. This was necessary because trained individuals are more resistant to eccentric exercise-induced muscle soreness than untrained individuals[46]. Likewise, no subjects were allowed to have muscle soreness in the neck-shoulder region prior to the onset of the study to avoid this influencing the baseline measurements ("24h before" exercise and "Pre"-exercise).
Subjects with a history of previous neck-shoulder disorders were excluded due to physiological unknown variations. These exclusion criteria were in line with other studies by Nie et al.[37] and Binderup et al.[62] where DOMS also was induced in trapezius by the same method (see Section 6.2).

Further Restrictions

In order to avoid large inter-subject variations in responses to the experimental protocol, the subjects received note of actions to refrain from during the time of participation:

- Participation in any kind of additional fatiguing physical activity that may induce further soreness in the trapezius muscles, e.g. training.
- Changing their diets.
- Taking any anti-inflammatory drugs.
- Cryotherapy, stretching, massage, compression and tension-relieving exercise in the region of soreness.

To control the intervention of muscle soreness in the neck-shoulder region, subjects were informed to avoid performing additional fatiguing physical activity especially in the neck-shoulder region. Significant changes in diet on the days of testing by e.g. taking protein supplement, was also to be refrained from. It could not be ruled out that a drastic increase in protein-rich intake could decrease the muscle soreness.[46]
Taking anti-inflammatory drugs could affect the inflammatory state present in the muscle when exposed to DOMS and was therefore to be avoided[46].
Cryotherapy, stretching, massage, compression and tension-relieving exercise was avoided because these have all been suggested as treatment strategies for relieving muscle soreness, although their effects have been incohesive[46]. It was important that no subjects relieved the effects of muscle soreness with any of these treatments to avoid inter-subject differences in the develop-

ment of the soreness.

Table 6.1 summaries the information obtained from all subjects before being included in the study. Subject number 1 and 11 were excluded due to immeasurable reflexes and subject number 8 was excluded due to discomfort caused by electrical stimulation. In total 5 Males and 5 females completed the experimental procedure and were used in the data analysis.

Subject	Age	Gender	Dominant Side	Height	Weight	BMI	Comments
1	25	Female	Right	1.74	72	23.78	Excluded
2	26	Male	Right	1.89	79	22.11	
3	26	Male	Right	1.80	72	22.22	
4	24	Male	Right	1.75	76	24.09	
5	19	Female	Right	1.79	64	19.97	
6	25	Female	Right	1.68	68	24.09	
7	24	Female	Right	1.66	49	17.78	
8	24	Female	Right	1.63	51	19.20	Excluded
9	23	Female	Right	1.79	64	19.97	
10	25	Male	Right	1.70	71	24.68	
11	23	Female	Right	1.69	62	21.71	Excluded
12	24	Male	Left	1.73	63	21.05	
13	24	Female	Right	1.69	59	20.66	
Average	24			1.72	64	21.38	
SD	1.80			0.07	9.11	2.21	

Table 6.1: **Subject Data**
In this table data regarding age, gender, dominant side of the body, height, weight, and BMI are presented.

6.2 Inducing Delayed-onset Muscle Soreness in the Trapezius

A dynamic shoulder dynamometer was used to induce DOMS in the subjects' dominant shoulder region. This dynamometer has previously been used by Madeleine et al.[68, 69], Nie et al.[37, 56, 61, 70], Kawczynski et al.[60] and Binderup et al.[62].
Figure 6.1 depicts the dynamometer system which consisted of an actuator, load cell, control unit, cylinder, shoulder contact pad, an adjustable seat fixed on a stainless-steel frame and lastly a corset to prevent uneven low-back loading and lateral bending during the exercise.

In general, the eccentric exercise was performed by resisting the downward movement/pressure

of the dynamometer system by elevating the shoulders. This procedure and how DOMS was evaluated are described in the following.

Initial Adjustments

First, the subject was placed in the seat which was adjusted to ensure a correct location of the shoulder with regard to the shoulder contact pad. The contact point on the shoulder was set to 30mm medial to the acromion (similar to a study by Binderup et al.[62]). Fixation of the subjects' torso to the backrest was done by velcro straps attached to the corset.
The eccentric exercise was defined by measurements of the range of motion (RoM, here the range of shoulder elevation) and the maximum voluntary contraction (MVC) force [68]. RoM for the dynamometer shoulder pad was adjusted by asking the subject to raise his/her shoulders as high as possible without lateral bending. This measure was stored as the top position of the dynamometer shoulder pad and used as the starting point in the beginning of each eccentric exercise. Afterward, the subject lowered the shoulders as much as possible and the value was stored as the bottom position of the dynamometer shoulder pad and used as the stopping point at which each eccentric exercise would end.
The MVC force was measured by asking the subject to first maintain the shoulders in a normal position. The shoulder contact pad was positioned at the point where it reached the shoulder. For 3s, maximal isometric force was applied against the static dynamometer. The maximal value within 3s was computed as MVC and compared to the values from two additional trials. Based on the 3 samples, an average MVC was calculated by the system and stored. The RoM and the MVC force was only used to define the eccentric exercise and not analysed further.

Procedure of the Eccentric Exercise

During each eccentric exercise, the dynamometer was going from the measured top position to the bottom position (RoM) while applying the force of the measured MVC (100% MVC). During this movement, the subject was asked to try to resist or stop the downward force by elevating the shoulders. After the dynamometer reached the bottom position, the shoulder pad automatically moved back to the top position and another exercise was performed.
The total number of eccentric exercises was set to 50 repetitions (in line with a study by Binderup et al.[62]). The 50 repetitions were divided into 5 blocks of 10 repetitions. A relaxation period (2 min) was earned after each block. During the exercises, the subject was encouraged verbally to perform as much as possible.

Assessment of Muscle Soreness

To measure the muscle soreness, a Visual analogue scale (VAS) score and pressure pain threshold (PPT) values were used. The VAS score was recorded using a 10cm line. Furthest to the left (0cm) indicated "no pain" and furthest to the right (10cm) indicated "worst pain imaginable". "24h before", "Pre", "Post", and "24h after" eccetric exercise, the subject was told to indicate on the line the perceived pain (see Section 4.3). During estimation of the VAS score the subject was seated relaxed in a chair in a stationary position.

PPT was measured on the dominant side in relation to electrode recordings of EMG activity (see Section 6.3 for further explanation) using a hand-held algometer. The algometer is shown in Figure 6.2. The area of the probe head was $1cm^2$ and the applied pressure rate was approximated to $30kPa/s$.

The following spots were measured with the algometer:

- Just above the electrodes on the middle trapezius on the dominant side.
- On the muscle belly of the right tibialis anterior muscle.

The right tibialis muscle was included as a control measurement for the site of repeated measure. PPT spots are depicted in Figure 6.3.

During the PPT recordings, the subject was placed in a chair. The hand-held algometer was placed on one of the spots and pressed against the muscle in a perpendicular angle. By pressing a switch, the subject could indicate when the sense of pain occurred and the algometer would display the amount of applied force. The same spot was recorded at least 3 times separated by at least 1min. Based on three recordings a coefficient of variance was calculated (standard deviation divided by mean). If the coefficient of variance was larger than 0.2, a fourth measure was conducted to reduce the intra-individual variation. The mean value of 3 recordings was then used as the PPT value. This method was also applied in a study by Binderup et al.[62].

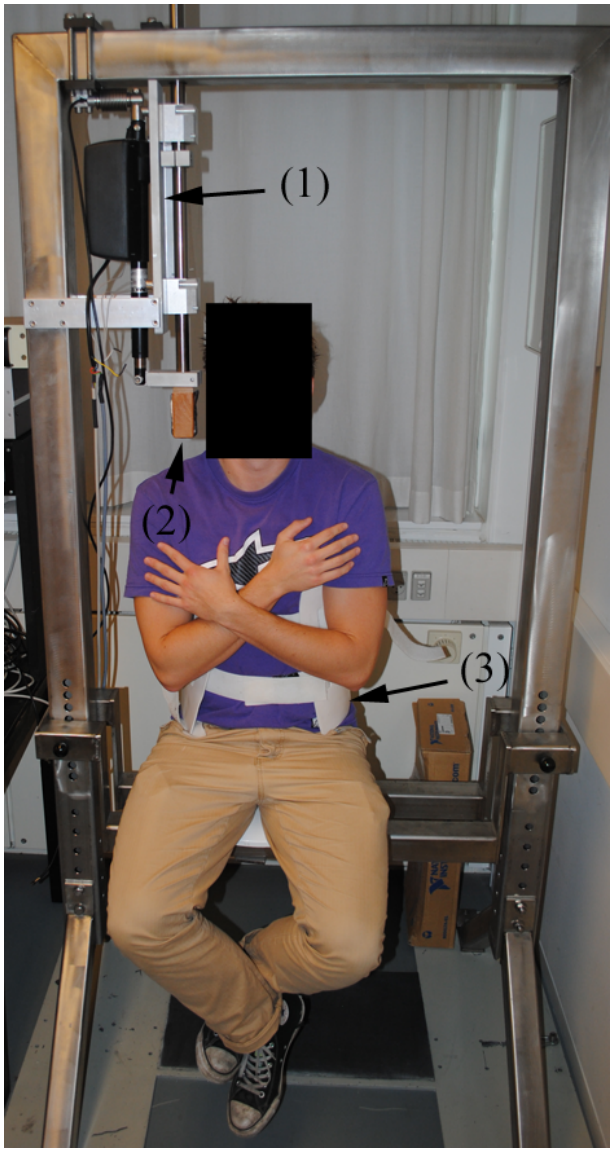


Figure 6.1: **The Dynamic Shoulder Dynamometer System**
The dynamometer was used in the eccentric exercises to induce DOMS in the trapezius muscle. The numbers indicate: (1) dynamometer with force transducer, (2) shoulder pad which can move in the range of shoulder elevation, and (3) plastic corset. Further details on the dynamometer are explained in the text.[68]

Figure 6.2: **Hand-held Algometer**
The force applied through the tip of the algometer was displayed on a screen. The external switch was handed to the subject, who locked the value of the applied force, thus enabling the investigator to note the value of the pressure pain threshold (PPT).

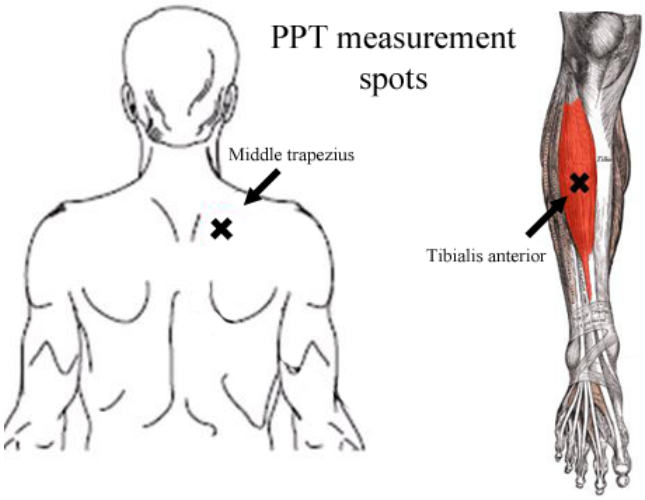


Figure 6.3: **Spots for Measuring Pressure Pain Threshold Values**
PPT was measured on the middle part of the dominant trapezius and on the right tibialis anterior (indicated by a black cross).[16]

6.3 Recording Muscle Activity and Eliciting the H Reflex

This section describes the recordings of the M waves and H reflexes with sEMG-electrodes and the experimental methods used to elicit both responses.

Placement of sEMG Electrodes on the Trapezius Muscle

Muscle activity from the middle trapezius was recorded using sEMG electrodes. Prior to electrode placement, the skin was ethanol-cleaned and abraded to lower resistance. Electrodes for EMG recordings were placed in pairs on the middle trapezius on the dominant side as shown in Figure 6.4. One electrode was placed close to the midpoint between the spine of the scapula and the T3 vertebra, and the other was placed 3cm medially, i.e. inter-electrode distance: 3cm. This is similar to a study by Taylor et al.[16].

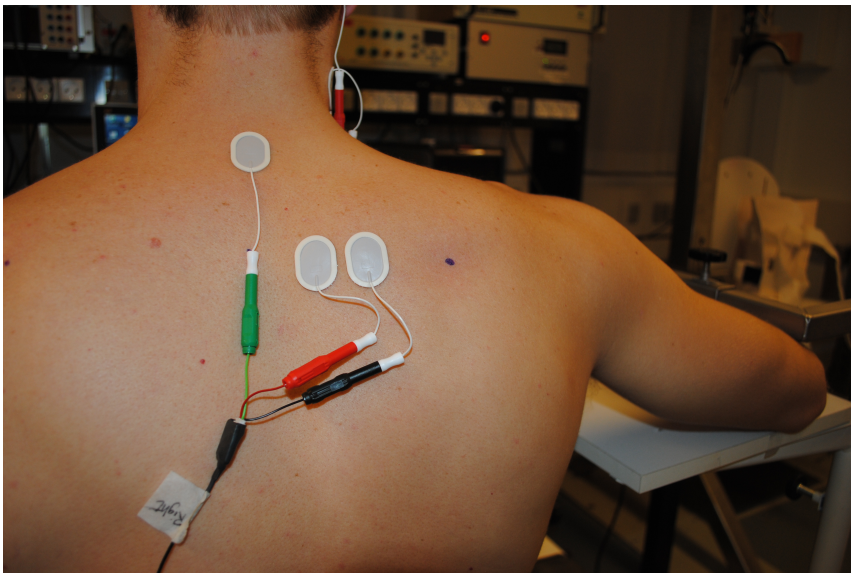


Figure 6.4: **Placement of the sEMG Electrodes on Dominant Middle Trapezius Muscle** The placement of the electrodes on the middle trapezius muscle coincides with the placement used in[16]. The reference electrode is placed on the C7 vertebra. The subject rests the dominant arm on a supporting bench.

Reference electrodes were placed on the C7 vertebra. After placement of sEMG electrodes, the functionality of the channel was verified on-line by tapping the electrodes on the trapezius muscle gently. Likewise, noise in the EMG trace was checked out. To reduce the movement of electrodes during abduction of the shoulder joint, the range of abduction was enclosed by a supporting bench with a horizontal bar fixed just above the resting forearm. The arm of the subject was relaxed in an angle of less than 90° (see Figure 6.6).

Finding the Accessory Nerve

The accessory nerve was found using a hand-held cathode (Figure 6.5A) and the anode was fixed on the mastoid process. When searching for the accessory nerve, electrode gel was used on the cathode. The subject was sitting relaxed in a chair with one arm supported. The area of investigation was behind the sternomastoid muscle and between the level of the jaw and the upper border of trapezius (Figure 6.5B). The method for finding the accessory nerve (and the cervical nerve of C3/4) were similar to that by Alexander & Harrison[20].

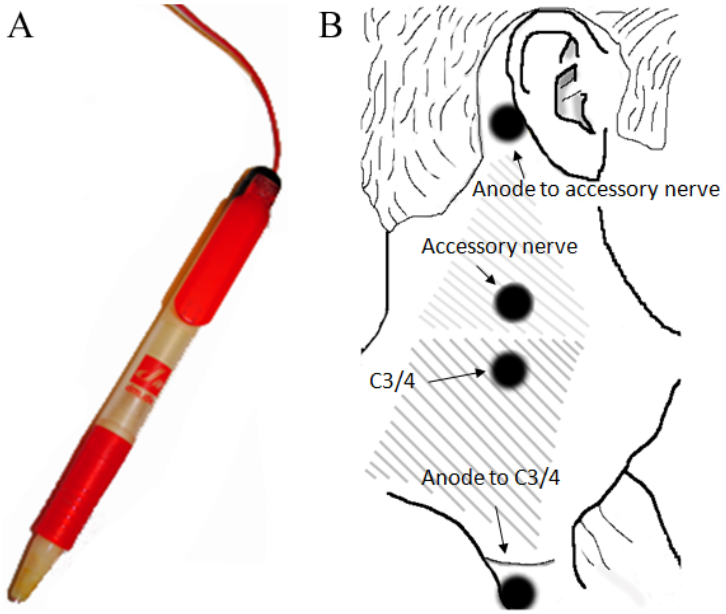


Figure 6.5: **The Hand-held Cathode and the Area of Stimulation**
A: a custom made, hand-held cathode was used to search for the nerves. **B**: the light grey area shows the area of search for the accessory nerve and dark grey area shows the area of search for the cervical nerve of C3/4. On this subject, electrodes have been placed on the optimal stimulation spots in the respective areas.

Stimuli were given manually during the search for the nerve. The intensity of the stimuli was fixed during the searching phase, but could vary between subjects depending on e.g. the excitability of the nerves and skin thickness. The duration of the stimulus was set to 1ms[23]. After each stimulus, the EMG channel was observed to see at which spot an M wave was most easily elicited in the dominant middle trapezius muscle.

Recruitment Curve of the M Wave

A surface electrode was stuck onto the skin (diameter of 1.5cm) over the identified spot. This electrode was used to obtain the recruitment curve, which was done by increasing the stimulus intensity in increments of ∼0.5mA until a plateau of the M wave value was obtained. This was

decided to be where the amplitude of the M wave showed no increase in three consecutive values despite an increase in stimulus intensity.

Finding the Cervical Nerve of C3/4

The cervical nerve of C3/4 was found using a hand-held cathode while the anode was fixed on the right medial clavicle. The searching area for the cervical nerve of C3/4 was superficial on the anterior surface of the upper fibre above the clavicle (see Figure 6.5). Stimuli were given in a manner similar to the procedure of finding the accessory nerve. Instead of M waves, H reflexes were used as a search parameter for finding the right spot. To increase the excitability of the motoneurons, a small abduction of the shoulder joint was used during the stimulations. A sEMG electrode was fixed on the spot were an H reflex was most easily elicited.

Recruitment Curve of the H Reflexes

Stimulation of the cervical nerve of C3/4 and obtainment of a recruitment curve were done nearly in the same way as with the accessory nerve. The difference was that contraction of one arm (further abduction of the shoulder joint) was used to excite motor units in the spine. In order to maintain the same background EMG at every stimulation (i.e. the same excitability of the motoneurons) visual feedback of the EMG signal was provided. This maintained a contraction of 15% EMG MVC. To ensure the background EMG did not change between stimulations, a trigger signal was sent to the stimulator when the RMS EMG was within range of 15% $\pm$ 2% of the EMG MVC and a stimulus were given to the subject.
EMG MVC was determined by the maximum voluntary EMG level in the middle trapezius on the dominant side and based on two recordings of 5s. The maximum recorded value during both recordings was used as the EMG MVC. The subject were seated in a chair with the arm supported (described in Section 6.3). Maximum contraction was exerted by abducting the arm against a horizontal bar as shown on Figure 6.6. EMG MVC data were collected and used as a control parameter for consistent voluntary activation of the middle trapezius.

To obtain a detailed recruitment curve, 10 potentials were collected for every stimulation intensity. To avoid the influence from homosynaptic post-activation depression (see Section 3.3), the stimuli were given with an interval of minimum 5s. Trigger signals from the feedback system were therefore blocked within these 5s.

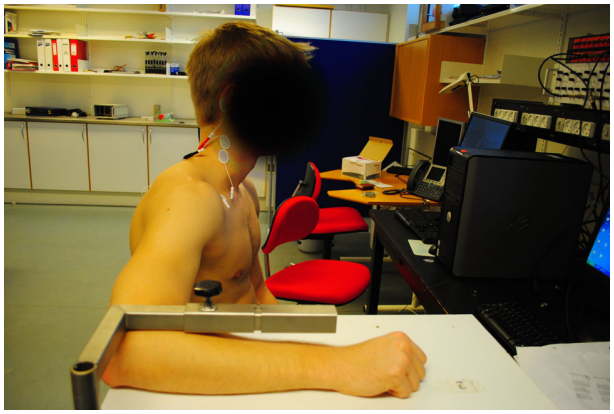


Figure 6.6: **Maximum Voluntary Contraction (MVC) Recording of the EMG Level** EMG MVC was measured while seated upright in a chair and abducting the dominant arm against a horizontal bar. EMG electrodes placed on the dominant middle trapezius muscle recorded the EMG activity.

6.4 Procedure

In this section, the experimental procedure will be described. A detailed description of specific procedures can be seen in Section 6.2 and 6.3. Figure 6.7 shows all the phases of the experiment and when each parameter is collected along with the order and the overall time frame. The same parameters are recorded in each session ("24h before", "Pre", "Post", and "24h after" eccentric exercise) and are illustrated by the dotted boxes in the figure. The explanation and analysis of each parameter can be seen in Section 6.6.

Overall, the experiment are divided into three days - i.e. four sessions each separated by 24h. When the subject arrived the first day ("24h before"), information regarding the experiment was given to the subject and the subject was asked to sign a written consent form. The subject was asked about any neck-shoulder pain or muscle soreness in the trapezius region. Furthermore, name, age, height, weight, and dominant hand were noted. Next, the sEMG electrodes were placed on the dominant middle trapezius. In the meantime, the VAS score value was collected followed by PPT measurements (indicated by the "soreness measures" on the figure). Next, the accessory nerve was found and a recruitment curve of M waves was obtained. EMG MVC measurements were then collected and the cervical nerve of C3/4 was found. this nerve was stimulated and a recruitment curve of H reflexes was obtained. Electrode positions (both recording and stimulation) were used throughout the experiment. A permanent marker was used to mark the electrode positions.
On day 2, the same parameters as on day 1 were first recorded ("Pre"). Afterwards, the subject was placed in the dynamometer to develop DOMS. Here, the subject completed 50 repetitions of the eccentric exercise ("Eccentric Exercise"). After the exercises, VAS, PPT, EMG MVC, and recruitment curve data were collected ("Post").

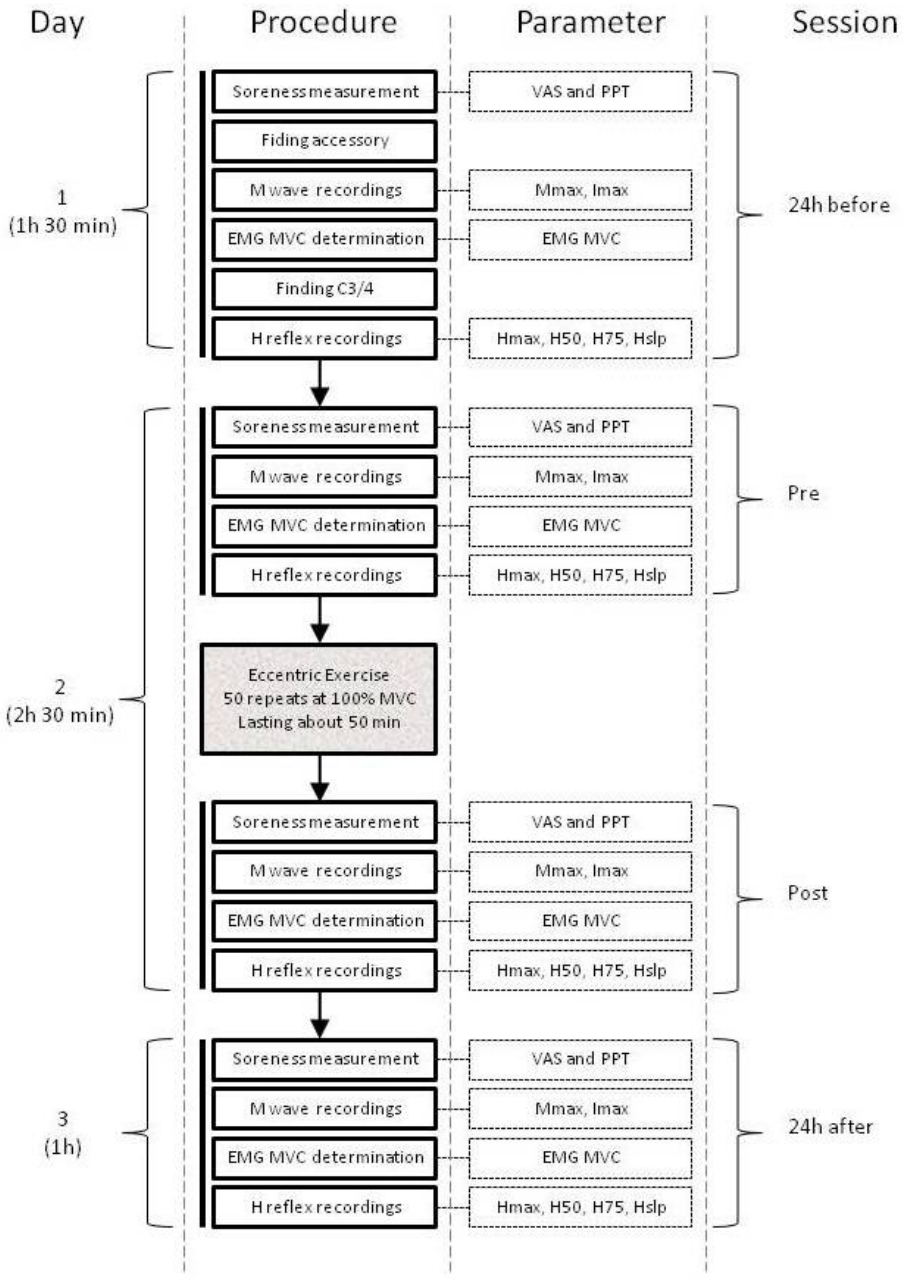


Figure 6.7: **The Procedure of the Experiment**

The components of the experimental protocol are presented on a timeline according to the sequence in which they were executed. All parameters were recorded in each session ("24h before", "Pre", "Post", and "24h after").

On day 3, the DOMS parameters VAS score and PPT were collected ("24h after"). Finally, the last recruitment curves were collected.

6.5 Apparatus

The following materials were used during one execution of the experiment:

- Data acquisition hardware (*CED 1401 puls, Science Park, Cambridge England*).
- 1 EMG amplifier (*Isolated EMG amplifier, EM006-1*).
- 23 self-adhesive sEMG electrodes (*measuring area 9.5 mm^2, Neuroline 720, Ambu, Ballerup, Denmark*).
- 1 stimulator (*Axon Instrument, Isolater-11*).
- 1 hand-held electrode, for finding nerves.
- Shoulder dynamometer (*Aalborg University, Aalborg, Denmark*).
- Electronic hand-held pressure algometer (*Somedic Algometer type 2, Sweden*).
- Signal 2.16 (*Software*).
- Microsoft office Excel 2007 (*Software*).
- SigmaStat 2.03 (*Software*).
- MATLAB R2009a (*Software*).
- LabVIEW 8.2 (*Software*).

In Figure 6.8, the system to record neural responses is illustrated.

The subject was connected to the EMG amplifier by sEMG electrodes (see Section 6.3). Due to hardware limitations and according to the SENIAM standards[71], the EMG signal was amplified 500 times and filtered with a bandwidth of 10-1000Hz. Afterwards the signal was transmitted to the data acquisition hardware (*CED 1401 puls*) where the signal was sampled with 2000Hz and AD converted with 16 bit. Finally, the signal was displayed on the computer screen in Signal 2.16.
A trigger signal was sent (manually or automatically) from the computer with Signal to the stimulator. In the automated settings, the trigger signal was based on the EMG activity level from the recordings of the trapezius muscle by the LabVIEW feedback system. This trigger signal was sent to the Signal computer through the data acquisition hardware followed by Signal sending out another trigger signal to the stimulator. To ensure at least 5s between each stimulus, a time interval of 5s was set in Signal.

The DOMS system consisted of a computer which controlled the settings of the eccentric exercise. On this computer, RoM and force MVC of the dynamometer's shoulder pad were set for each

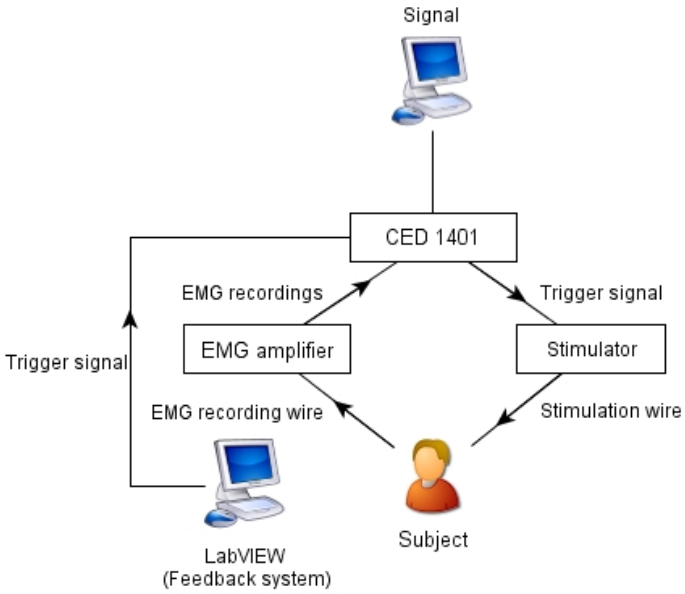


Figure 6.8: **Configuration of the EMG System for Neural Responses**
The EMG system for recording neural responses consisted of PCs with the installed software "Signal" and "LabVIEW". A stimulator was stimulating the subject. Neural responses were amplified and filtered in the EMG amplifier and sampled by the data acquisition hardware "CED 1401".

exercise cycle. As mentioned, PPT measures were done with a hand-held pressure algometer.

The Settings of Signal

Signal 2.16 is a software programme which can analyse signals during real-time recordings. In the experiment, Signal 2.16 was controlling the sampling of the EMG signals, the electrical stimulation of the subject, and the recordings of data.

In Figure 6.9, the settings of the trigger signal to the stimulator are shown. Pulses were set to a duration of 1ms and the digital output from the stimulator were selected according to the analogue out in the "Output" menu.
Each individual pulse state was set in the window "Pulses configuration". During each recording, the trigger pulse was sent after 0 ms indicated by a small pen on channel one in the window. The pulse interval and the recording time were set to 4s (indicated by the bold line in the top of the window).

As mentioned before, triggering of the stimulation was done manually or automatically. In the automatic state, a trigger input was used to start the recordings and stimulation by Signal. This was configured by choosing "Peri-trigger" in the "Sweep mode" drop down menu (see Figure

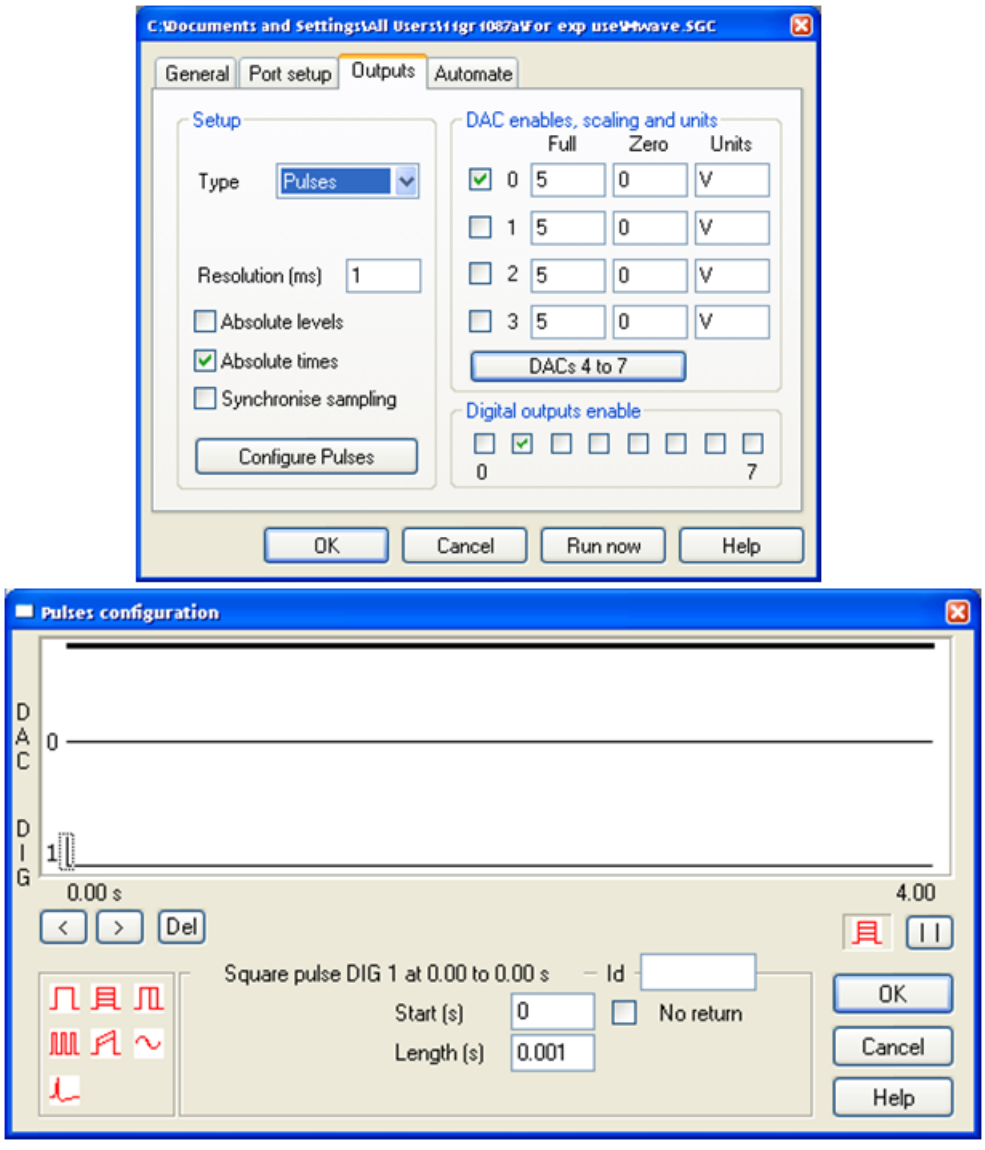


Figure 6.9: **Configuration of the Trigger Signal Sent to the Stimulator**

The upper screenshot shows the setup window for configuring the output signals. Here, it is possible to access the "Pulses configuration" window (lower screenshot), where trigger pulses to the stimulator can be adjusted. As shown, the pulse was configured to start at time 0 with a width of 1ms.

6.10 in the "General" menu). In the "Peri-trigger" menu, the digital trigger input was selected ("Digital input bit 8") and definition of the type of trigger.
During the manual stimulation, the "Basic" was chosen in the "Sweep mode" drop down menu on Figure 6.10. The figure also shows the setting of the frame length (5s), ADC ports/EMG input ports to the EMG amplifiers (5: right middle trapezius), and sample frequency (2000Hz).

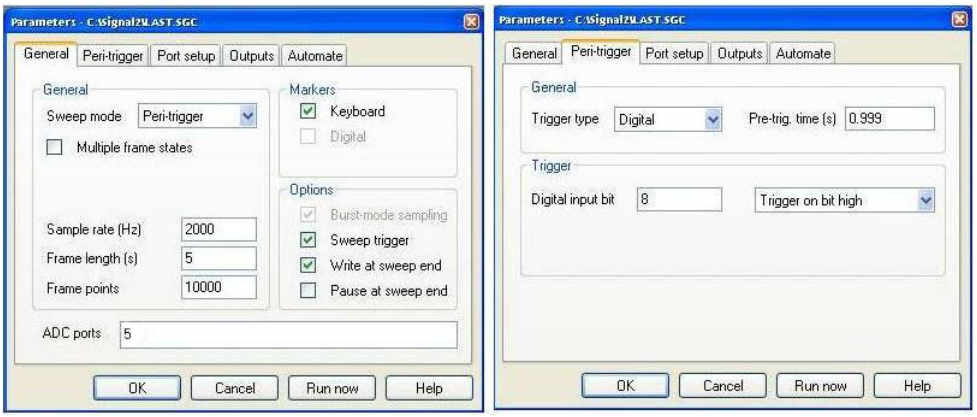


Figure 6.10: **General and Peri-trigger Settings**
In the window to the left, general settings were adjusted (ADC ports, sample rate, frame length). In the window to the right, the peri-trigger was defined (0.999s recordings before triggering).

In Figure 6.11, a screenshot from the recording window is displayed.

The window shows the EMG signal from the right (dominant) middle trapezius in mV. The view of the trace could be changed by vertical and horizontal zooming. Two Cursors were inserted (not shown on the figure) and used to measure amplitude and latency of potentials.

Visual Feedback of Voluntary Contraction Level

An existing interface made in LabVIEW 8.2 was used in the process of recording H reflexes to calculate EMG MVC (maximum voluntary contraction based on the amplitude of EMG activity), display the EMG contraction level to the subject, and to send out a trigger signal when the correct level of background EMG was achieved. In Figure 6.12, a screenshot of the main control panel of the LabVIEW programme is illustrated. The duration of the feedback (Contraction time) and the percentage of EMG MVC (CntrLvl) were the only settings adjusted. The duration was set to 3600s to allow ample feedback time. The contraction level was standardised to control the voluntary activation of motoneuron excitability. The value of 15% of EMG MVC was chosen, as this value proved fairly easy to attain in testing sessions. The window length for both EMG MVC recordings and evaluation of the background EMG was set to 250ms divided into 5x50ms. The mean value of each of these time windows was used to calculate an overall mean value. An

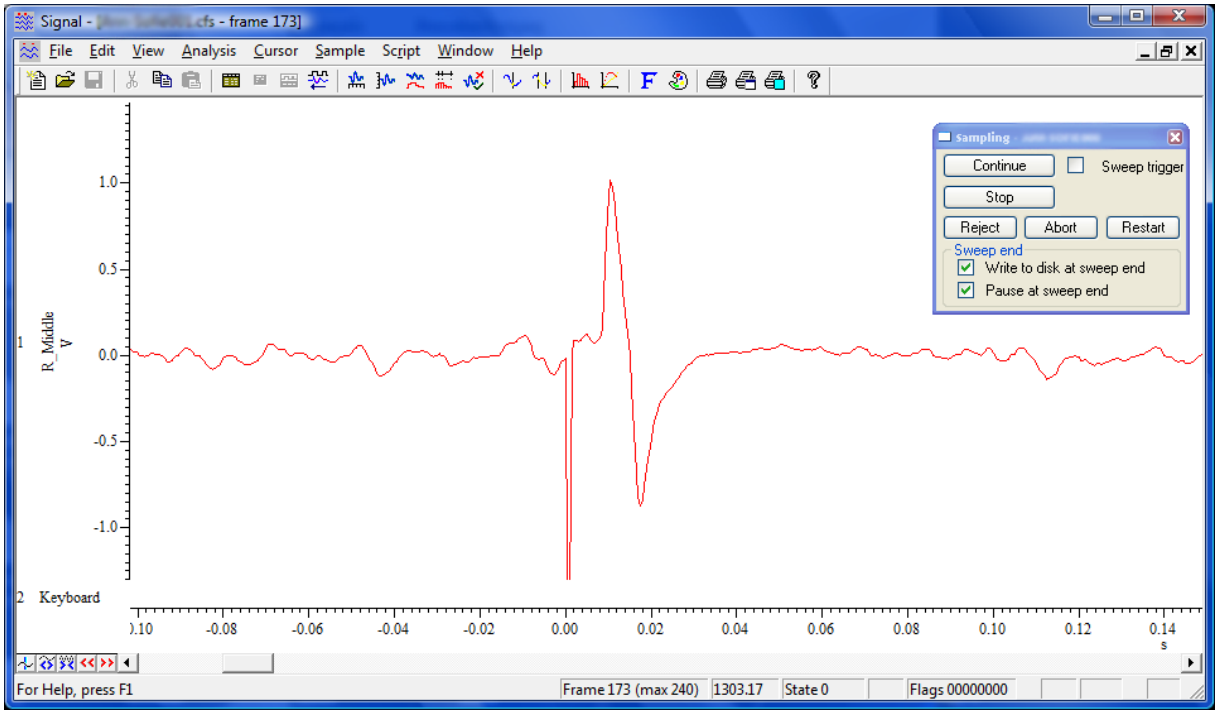


Figure 6.11: **The Recording Window**

The recording from the sEMG electrodes placed on the dominant middle trapezius muscle is displayed. Recording, trigger output, and saving data are controlled in the box "Sampling" at the right side.

example of the evaluation of the background EMG signal is shown in Figure 6.13.

By clicking "Run" in the main control panel (6.12), a new configuration window for the EMG MVC measures was displayed. Figure 6.14 shows an EMG MVC recording in progress. The subject's arm was abducted against the horizontal bar on the supporting bench, thereby resulting in an increased activity measured by the sEMG electrodes on the dominant middle trapezius muscle. The Waveform Chart displays the signal continuously while the bar in the right side of the interface displays the real time amplitude of the signal.

Figure 6.15 shows the interface displayed after the second of two EMG MVC recordings lasting 5s. The maximum amplitude of the EMG signal in both measures was displayed in the "MVCVAL" field. This value was stored and used by LabVIEW to provide feedback.

When "Approve" was pressed in Figure 6.15, the final feedback interface was executed and the subject was provided a range within 15±2% of the EMG MVC. The feedback system is displayed in Figure 6.16. When the contraction was within 15±2% of the MVC (the two arrows), the bar in the right side turned from blue/red (too low/high) to green and a trigger signal was sent from the LabVIEW computer to the Signal computer.

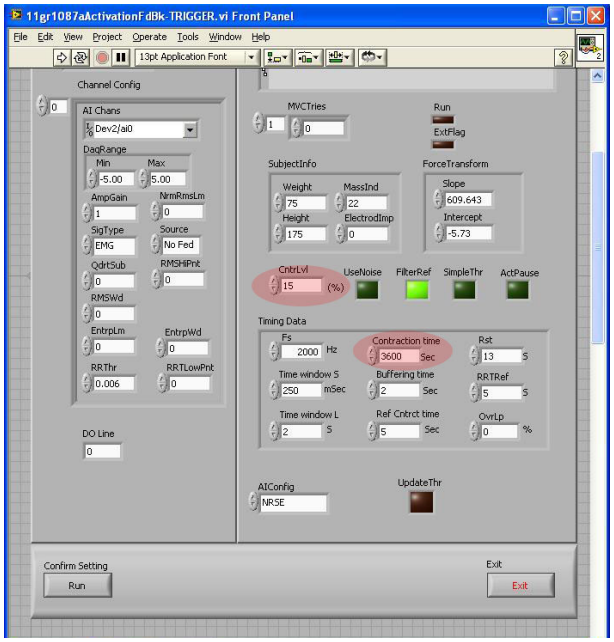


Figure 6.12: **The Control Interface of the Feedback Program**

Relevant settings are limited to contraction level as a percentage of MVC (CntrLvl) and duration of the recording (Contraction time) - both are highlighted on the figure.

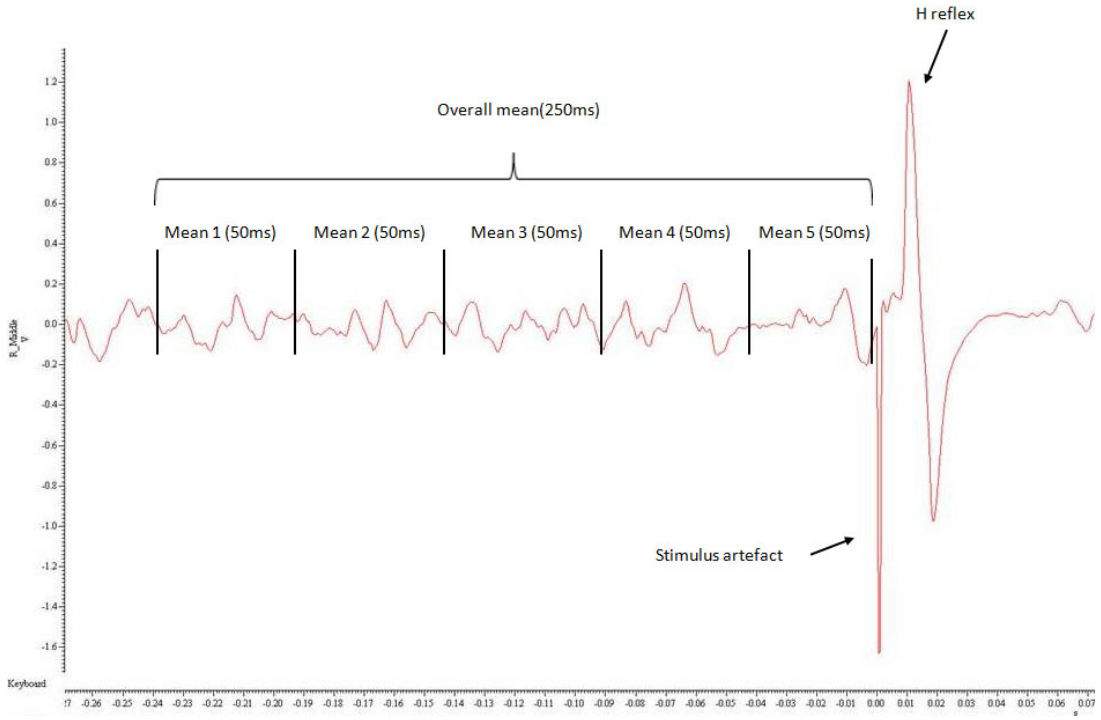


Figure 6.13: **Evaluation of the Background EMG**

Five blocks of each 50ms were analysed and five means were calculated (mean 1-5). Based on these five means, an overall mean was calculated. A trigger signal was sent, as in this example, if the overall mean was in the range of 15±2% of the EMG MVC.

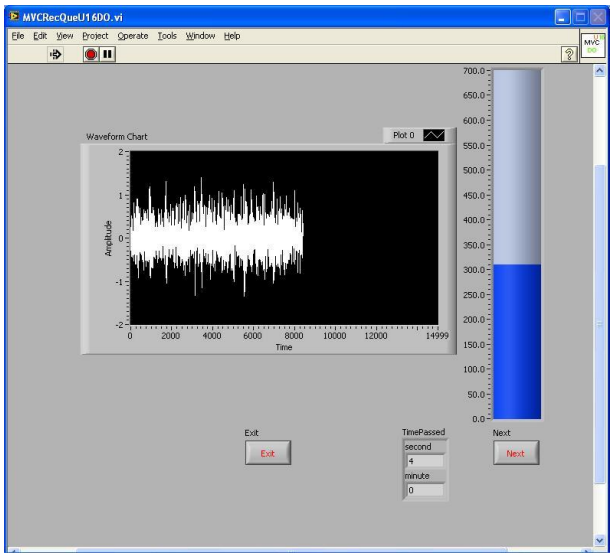


Figure 6.14: **EMG MVC Measuring Interface of the Feedback Program**
The EMG MVC measurement lasts for 5s while continuously displaying the EMG signal (Waveform Chart) and the numerical amplitude of the signal (vertical bar, right side).

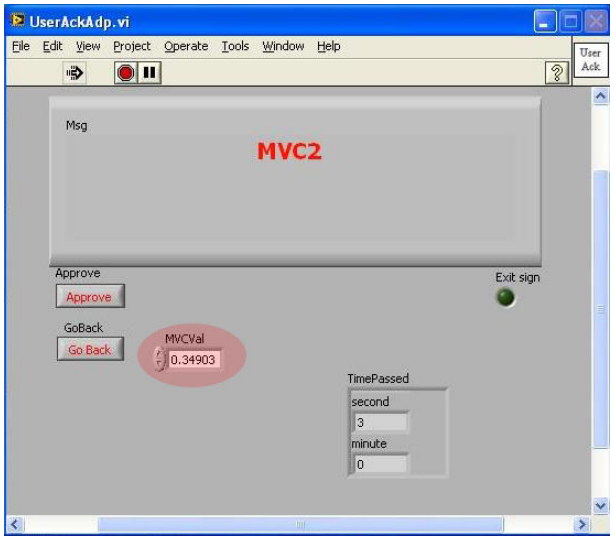


Figure 6.15: **EMG MVC Measurement Interface of the Feedback Program**
The interface shows the maximum amplitude of the EMG signal attained after two MVC measures lasting 5s (in the MVCVal).

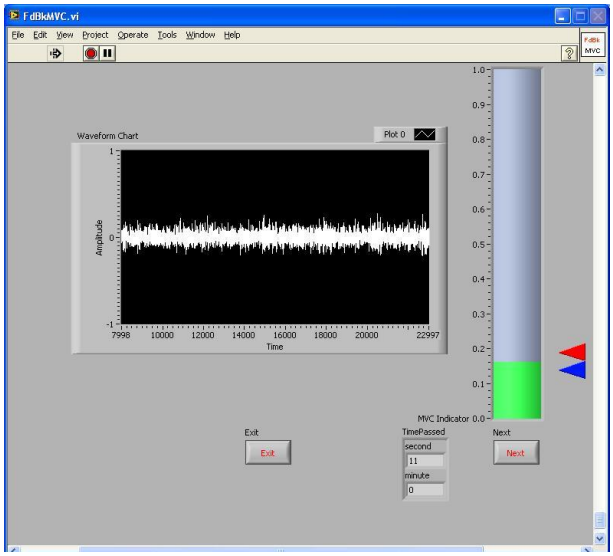


Figure 6.16: **The Feedback Interface**
The interface continuously displays the amplitude of the EMG signal (Waveform Chart) along with the numerical amplitude showed in a bar (MVC Indicator). Whenever the amplitude of the EMG signal is within 15±2% of the EMG MVC recording (red and blue arrows), the bar turns greens and a trigger signal is sent.

Settings of the Dynamometer

The dynamometer used a computer to navigate the different functions needed to complete the setup of the eccentric exercise protocol. From the main menu, the submenus "Range", "Force", and "Training" were accessible. "Range" was used to investigate the shoulders RoM prior to the beginning of the exercises. "Force" was used to measure the subject's MVC force which would determine the driving force of the dynamometer's shoulder pad. Finally, "Training" was used to begin and control the eccentric exercise session in which the shoulder pad would lower from top to bottom position determined by the RoM, and move with the force determined by 100% of the MVC force measurement. Figure 6.17 shows the control device of the dynamometer.

Figure 6.17: **The Device for Controlling the Dynamometer**
Four (black) bottoms control the settings of force, range of the dynamometer, and initiates the training exercise.

Settings of the Pressure Algometer

The hand-held algometer is displayed in Figure 6.2. The algometer was equipped with three buttons, which allowed the user to turn the device on and off, to set the size of the probe head used [cm^2] and to set the rate of applied pressure [kPa/s].

6.6 Data Analysis

This section presents the methods used for analysing the experimental data. Overall, the data analysis was divided into two categories: *Assessment of muscle soreness* and *Assessment of neural responses*. The following parameters were targeted for the data analysis:

Assessment of Muscle Soreness

- Muscle pain intensity (VAS).
- Pressure pain threshold for the middle trapezius and the right tibialis anterior (PPT).

Assessment of Neural Responses

- M wave latency.
- H reflex latency.
- Intensity for stimulating M_{max} (I_{max}).
- The ratio given by the maximal peak-to-peak amplitude of the H reflex and the maximal peak-to-peak amplitude of the M response (H_{max}/M_{max} ratio).
- The ratio given by 75% of the maximal peak-to-peak amplitude of the H reflex and the maximal peak-to-peak amplitude of the M response (H_{75}/M_{max} ratio).
- The ratio given by 50% of the maximal peak-to-peak amplitude of the H reflex and the maximal peak-to-peak amplitude of the M response (H_{50}/M_{max} ratio).
- The slope of a sigmoid curve fitted to the data (H_{slp}).

Parameters as latencies of M waves and H reflexes and the intensity to stimulate M_{max} were used in the analysis of reproducibility between sessions.
All pain and neural parameters were analysed in *Signal*, *Excel*, and *MATLAB* between the four data collection sessions ("24h before", "Pre", "Post", and "24h after" eccentric exercise). The data were further investigated for significant differences by using the statistical data analysis software *SigmaStat*. All parameters were statistically tested by the same test and is presented at the end of this section. In the following, the data analysis of each parameter is explained in details.

Assessment of Muscle Soreness

The VAS score was measured by a ruler from left to right. As described in Section 6.2, the VAS score was calculated in cm (0 = "no pain" and 10 = "worst pain imaginable"). This value was noted in each data collection session and compared. An average value of three PPT measurements were collected in each data collection session for the middle trapezius muscle and the right tibialis anterior muscle. Recorded values in kPa for the same muscle were compared between sessions.

Assessment of Neural Responses

Latency of the M wave and the H reflex were estimated by an average of the data obtained in each session. An example of H reflex latency is illustrated in Figure 6.18. The cursor (*Cursor 1*) was manually placed at the deflection from baseline of the H reflex and the latency from the stimuli artefact (time zero) to the cursor was noted (same procedure for the M wave latency). Latencies were compared between data collecting sessions. The cursors were placed by the same examiner.
All peak-to-peak amplitudes of the H reflexes were normalised to M_{max}. Recruitment curves of M waves and H reflexes were obtained by measurements of the peak-to-peak amplitude at a given stimulus intensity. As illustrated in Figure 6.18, two cursors (*Cursor 1* and *Cursor 2*) were placed around the deflection of the response and the peak-to-peak amplitude were calculated by the software (*Signal*). The first cursor was placed at the beginning of the response and the second cursor was set at the time where the EMG trace was stabilised.

Based on cursor settings, peak-to-peak amplitude values of M waves and H reflexes were plotted as shown in Figure 6.19, **A**. M_{max} was found to be the highest recorded value in each session and the corresponding intensity was determined. Peak-to-peak M_{max} amplitudes for each session was used for normalisation of H reflexes. Frames without an H reflex were discarded and H reflexes at the same stimulus intensity were averaged. This result can be seen in Figure 6.19, **B**. As illustrated on the figure, H_{max} was found to be the highest average value in each session and used for H_{max}/M_{max} calculations.
Furthermore, H reflex average data were used to transform the data points into a sigmoid fit. This fit was used due to the findings by Klimstra & Zehr[72]. The function of the sigmoid fit is defined as:

$$H(s) = \frac{H_{max}}{1 + e^{H_{slp} \cdot (s50 - s)}} \tag{6.1}$$

At a given intensity s, the corresponding peak-to-peak amplitude $H(s)$ was found. $s50$ was the intensity used to evoke the H reflex at 50% of H_{max}. This value was estimated by the linear fit of the upslope values. At the peak-to-peak amplitude value of 50% of H_{max}, the corresponding

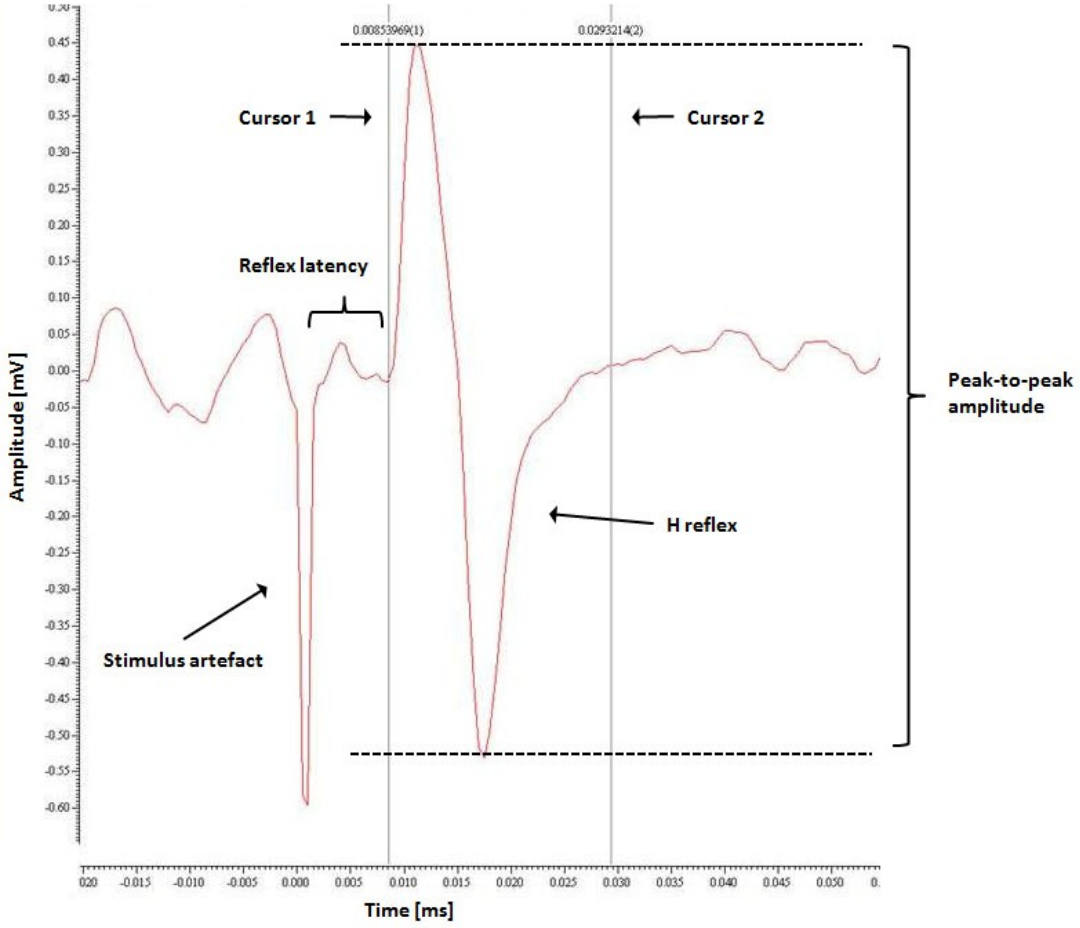


Figure 6.18: **The Analysis done of the Recorded sEMG Signal in Signal**
Cursors were used to estimate the latency of the M wave and the H reflex and to define the area of peak-to-peak amplitude calculation. In this example, data, illustrating an H reflex, are recorded from Subject 5.

stimulus intensity was set to $s50$. The slope parameter H_{slp} of the function was found by parameter estimation. The root mean square error (RMSE) was calculated at varying H_{slp} values and the H_{slp} with the smallest RMSE value was chosen. An example of the sigmoid fit is illustrated in Figure 6.19, **C**. The goodness of fit was determined by the Pearson product-moment correlation coefficient (r).

Based on the sigmoid fit, the H_{75}/M_{max} and the H_{50}/M_{max} parameters were calculated. This is shown in Figure 6.20. As illustrated, 75% and 50% of the peak-to-peak amplitude of H_{max} in the first session (H_{75} and H_{50}) were used to determine the two intensities I_{75} and I_{50}. The H_{max} was chosen to be the maximal average data point. Based on these intensities H_{75} and H_{50} values were calculated for each session and compared.

Statistical Analysis

To test whether there was a significant difference in the parameters between sessions ("24h before", "Pre", "Post", and "24h after" eccentric exercise), the non-parametric Friedman's Two-Way ANOVA by Ranks was performed. This test was selected because of the data not being normally distributed (see Figure 6.21) and because of the experimental design of repeated measure[73].

Friedman's test compares blocks of data with each parameter based on the variance. The null hypothesis was tested by using the probability distributions of the parameters. If at least two of the distributions were different in location the null hypothesis was rejected.[74]
The level of significance (α) was chosen to be 0.05. The following null-hypothesis (H_0) and alternative hypothesis (H_A) were examined by the Friedman's test for each parameter:

H_0: There is no difference between the four sessions.
H_A: There is a difference between the four sessions.

If the null hypothesis was rejected by the Friedman test, a post-hoc group comparison analysis was done to identify the sessions that differed significantly from each other. This was done by a Student Newman-Keuls test which estimates the level of significance based on ordered means [75].

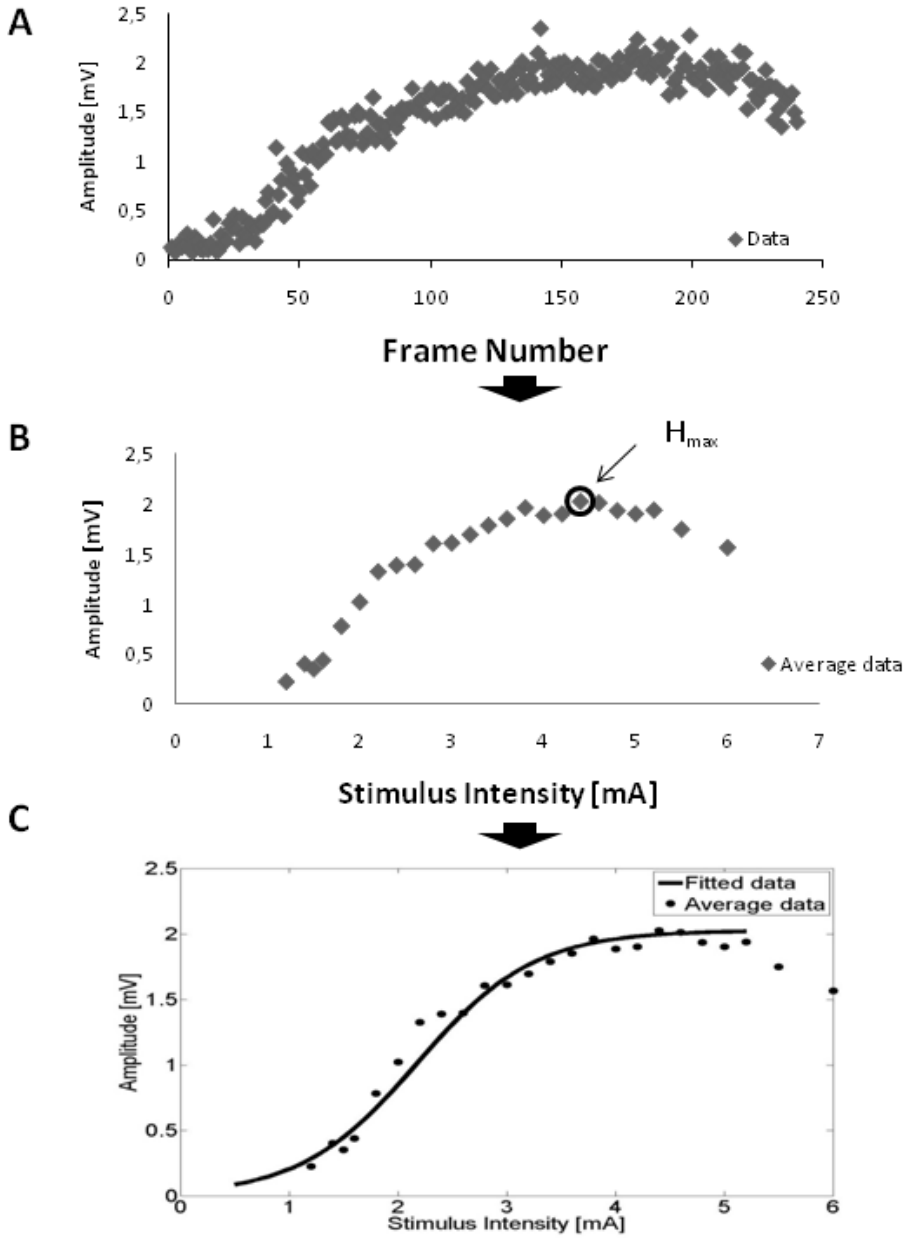


Figure 6.19: **Process of the Data Analysis Regarding H Reflex Recordings**
A: peak-to-peak amplitude were found by the cursor function in Signal and the recruitment curve was plotted. **B**: H reflexes at the same intensity were average together and the H_{max} was determined. **C**: a sigmoid function was fitted to the data and used for further analysis. The illustrated data is obtained from Subject 5.

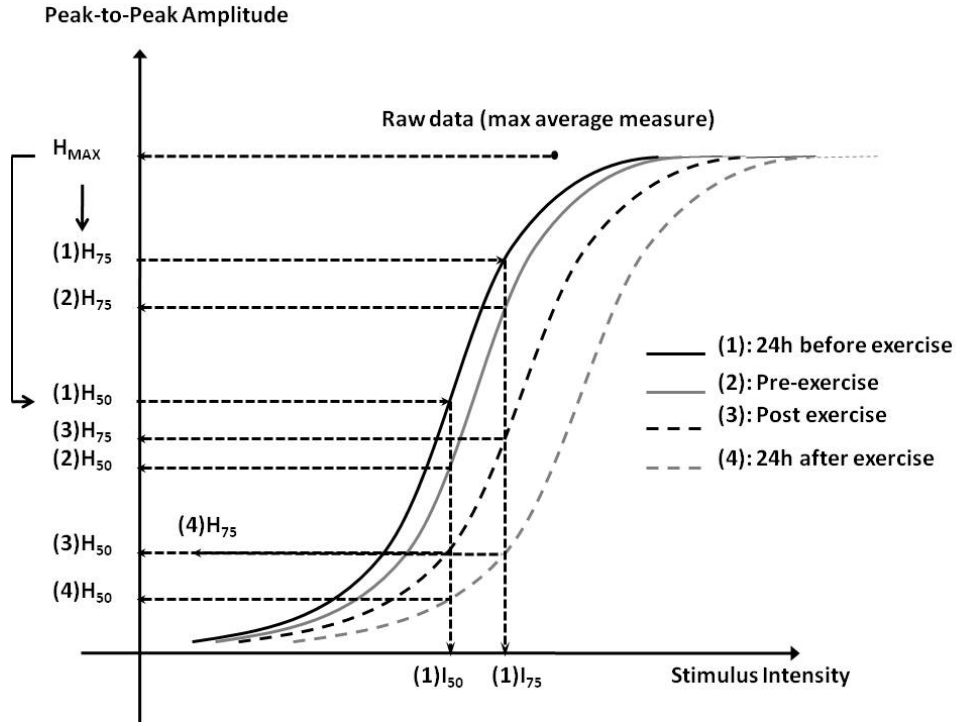


Figure 6.20: **Drawn Illustration of the H Reflex Analysis on the Fitted Data.**
I_{75} and I_{50} were found by 75% and 50% of the peak-to-peak amplitude value of average H_{max} on the first session (1). Based on these intensities H_{75} and H_{50} values for the other sessions (2, 3, and 4) were calculated.

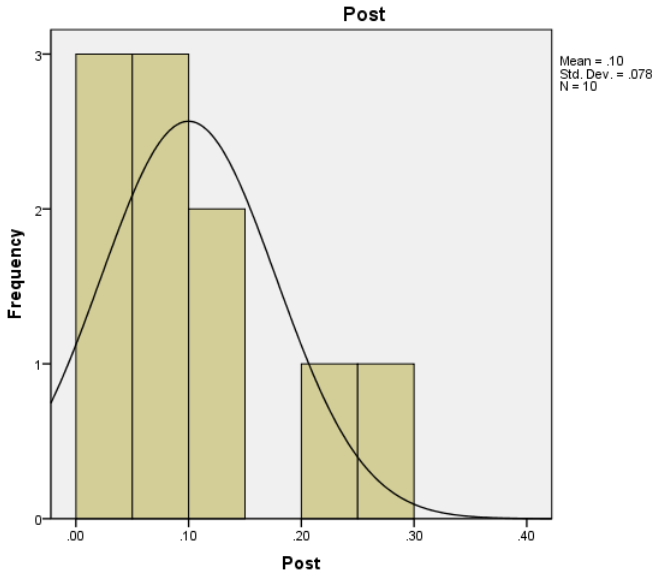


Figure 6.21: **Histogram based on Parameter**
Shown in the histogram the data is not normally distributed.

Chapter 7

Results

This chapter reports the results of all the experimental data. These both includes data from assessment of muscle soreness and from assessment of neural responses. The data are represented with median values and 1^{st} and 3^{rd} quartiles. Lastly, the statistical results are presented.

7.1 Results from Assessment of Muscle Soreness

The following subsections reports the results from assessment of muscle soreness, i.e. muscle pain intensity (VAS score) and pressure pain threshold (PPT) results.

Muscle Pain Intensity

VAS scores were used to evaluate the development of the intensity of muscle pain for subjects in all sessions. Table 7.1 and Figure 7.1(a) presents the results.

VAS Score (0 - 10)				
Subject	**24h before**	**Pre**	**Post**	**24h after**
2	0.0	0.0	1.5	3.0
3	0.0	0.0	4.0	7.5
4	0.0	0.0	2.0	1.5
5	0.0	0.0	1.0	3.5
6	0.0	0.0	1.5	2.5
7	0.0	0.0	1.0	4.0
9	0.0	0.0	1.5	2.5
10	0.0	0.0	3.0	3.0
12	0.0	0.0	1.0	1.0
13	0.0	0.0	3.0	7.5
Median	0.0	0.0	1.5	3.0
1^{st} quartile	0.0	0.0	1.4	2.5
3^{rd} quartile	0.0	0.0	2.3	3.6

Table 7.1: **Visual Analogue Scale (VAS) Score Data**
All VAS values were recorded in each session. The scale was based on a 10cm line, where 0 indicated "no pain" and 10 indicated "worst pain imaginable".

In Table 7.1 and Figure 7.1(a) it can be seen that the VAS score increased after the eccentric exercise and peaked 24h after.

Pressure Pain Threshold Results

Table 7.2, Figure 7.1(b), and Figure 7.1(c) shows the obtained results from PPT measures of all subjects in each session. Overall, the PPTs for the middle trapezius muscle were lower than those for the tibialis anterior muscle.

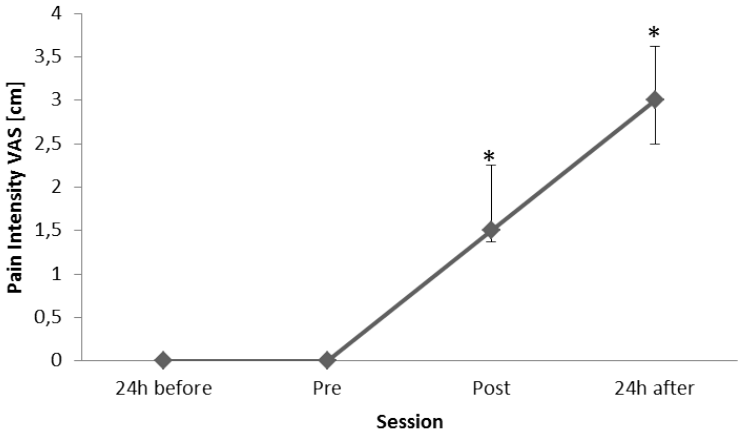
Measurement Site: Middle Trapezius				
Subject	**24h before** [kPa]	**Pre** [kPa]	**Post** [kPa]	**24h after** [kPa]
2	350	445	338	290
3	385	517	465	401
4	571	768	806	742
5	292	321	401	254
6	493	351	307	383
7	215	293	234	135
9	304	354	310	220
10	330	334	314	167
12	546	544	584	538
13	339	316	328	249
Median	344	352	334	272
1^{st} quartile	301	331	309	207
3^{rd} quartile	412	463	417	388

Measurement Site: Tibialis Anterior				
2	348	717	632	654
3	608	531	616	580
4	480	880	1452	974
5	450	431	542	469
6	651	588	552	634
7	309	295	343	312
9	685	658	627	480
10	490	469	553	555
12	809	821	946	889
13	386	404	393	411
Median	485	560	585	567
1^{st} quartile	425	460	550	477
3^{rd} quartile	619	673	628	639

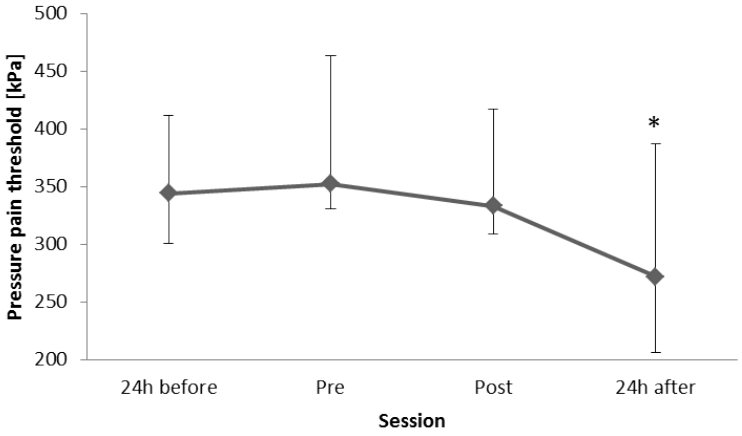
Table 7.2: **Pressure Pain Threshold (PPT) Data**

All PPT values were recorded on both the dominant middle trapezius and the right tibialis anterior for all subjects in all sessions.

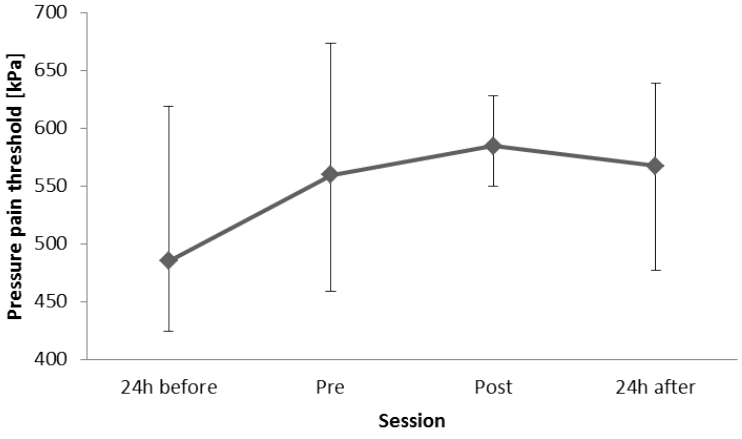
Figure 7.1(b) shows that the PPT values measured from trapezius decreased 24h after exercise. Figure 7.1(c) shows that the PPT values measured from tibialis anterior were lowest 24h before exercise and afterwards stabilises.



(a) Muscle Pain Intensity (VAS)



(b) Pressure Pain Threshold (PPT) From Trapezius



(c) Pressure Pain Threshold (PPT) From Tibialis Anterior

Figure 7.1: Muscle pain intensity (VAS), and pressure pain thresholds for trapezius and tibialis anterior are presented as median values for each session along with 1^{st} and 3^{rd} quartile ranges. (*) indicates a significant difference ($P<0.05$).

7.2 Results from Assessment of Neural Responses

The following results focus on recordings of the EMG activity and the derived parameters for assessing the neural responses. First, the following subsections report neural response latencies and maximum M wave and H reflex responses as well as the intensity used to evoke maximal M waves in each session and the H_{max}/M_{max} ratios. Afterwards, results of parameters based on mathematically fitted data will be reported. These parameters include Pearson product-moment correlation coefficient (r) values for data fits for each subject followed by data on fitted amplitude ratios (H_{75}/M_{max} and H_{50}/M_{max} ratio values). Fitting of the data was performed as described in Section 6.6.

M Wave Latencies, Maximum Amplitudes, and Maximum Intensities

For all subjects, the latencies of the M waves were obtained directly from the recorded signals and the results can be seen in Table 7.3.

Latencies of M Waves				
Subject	**24h before**	**Pre**	**Post**	**24h after**
	[ms]	[ms]	[ms]	[ms]
2	3.5	3.5	3.5	3.5
3	3.0	3.0	3.0	3.0
4	3.0	3.0	3.0	3.0
5	3.0	3.0	3.0	3.0
6	3.0	3.0	3.0	3.0
7	3.0	3.0	3.0	3.0
9	3.0	3.0	3.0	3.0
10	3.0	3.0	3.0	3.0
12	3.0	3.0	3.0	3.0
13	3.0	3.0	3.0	3.0
Median	3.0	3.0	3.0	3.0
1^{st} quartile	3.0	3.0	3.0	3.0
3^{rd} quartile	3.0	3.0	3.0	3.0

Table 7.3: **Latencies of M Waves**
The table lists the latencies of the M waves for each subject in all sessions.

The maximum responses of the M wave are reported in Table 7.4.

M_{max} Responses				
Subject	**24h before**	**Pre**	**Post**	**24h after**
	[mV]	[mV]	[mV]	[mV]
2	6.00	6.85	6.78	7.69
3	5.07	4.43	4.54	4.79
4	6.06	5.19	4.96	5.57
5	5.03	4.59	4.67	4.82
6	4.22	4.56	4.08	5.13
7	5.90	6.00	5.84	7.06
9	4.11	4.22	4.60	4.90
10	5.71	6.05	5.82	5.74
12	6.39	6.42	4.14	4.91
13	5.75	5.41	5.66	5.32
Median	5.73	5.30	4.81	5.23
1^{st} quartile	5.04	4.56	4.56	4.90
3^{rd} quartile	5.98	6.33	5.78	5.70

Table 7.4: **Maximum M Responses**
The table lists the maximum responses of the M waves for each subject in all sessions derived from their recruitment curves.

In Table 7.5, intensities used for stimulating M_{max} are given (I_{max}).

Stimulation Intensities				
Subject	**24h before** [mA]	**Pre** [mA]	**Post** [mA]	**24h after** [mA]
2	4.0	3.5	2.0	2.0
3	4.5	2.5	2.5	6.0
4	3.0	2.0	3.0	4.0
5	3.0	3.5	2.5	2.0
6	3.0	3.5	3.5	2.5
7	2.5	5.0	2.5	2.0
9	5.5	5.0	5.5	5.0
10	3.0	5.5	6.0	4.5
12	3.0	5.0	4.5	3.5
13	1.5	1.5	2.0	3.0
Median	3.0	3.5	2.8	3.3
1^{st} quartile	3.5	2.8	2.5	2.1
3^{rd} quartile	3.8	5.0	4.3	4.4

Table 7.5: **Intensity for Stimulating M_{max} in Each Session**
The table lists the intensities used for stimulating M_{max} for each subject in all sessions.

H Reflex Latencies and Maximum Amplitudes

For all subjects, the latencies of the H reflexes were obtained directly from the recorded signals and the results can be seen in Table 7.6.

Latencies of H Reflexes				
Subject	**24h before**	**Pre**	**Post**	**24h after**
	[ms]	[ms]	[ms]	[ms]
2	9.1	9.0	8.8	8.9
3	9.5	9.5	9.5	9.5
4	9.0	9.0	9.0	9.5
5	9.0	9.5	9.0	9.0
6	8.5	8.5	8.5	8.5
7	8.5	8.5	9.0	9.0
9	8.0	8.0	8.0	8.0
10	9.0	9.0	9.0	9.0
12	10.0	10.0	10.0	10.0
13	9.0	9.0	9.0	9.0
Median	9.0	9.0	9.0	9.0
1^{st} quartile	8.5	8.5	8.7	8.8
3^{rd} quartile	9.0	9.1	9.0	9.2

Table 7.6: **Latencies of H Reflexes**

The table lists the latencies of the H reflexes for each subject in all sessions.

The maximum responses of the H reflex are reported in Table 7.7. It can be seen that H_{max} was smaller after exercise compared to before.

H_{max} Reflexes				
Subject	**24h before** [mV]	**Pre** [mV]	**Post** [mV]	**24h after** [mV]
2	0.96	0.76	0.79	1.01
3	0.72	0.46	0.47	0.32
4	1.30	1.53	1.36	1.22
5	1.31	0.69	1.28	1.17
6	2.03	2.17	2.07	2.22
7	2.65	3.23	2.29	2.69
9	1.26	0.95	0.94	1.07
10	2.02	1.93	2.40	2.10
12	1.90	1.63	1.04	2.07
13	2.14	2.02	0.77	1.28
Median	1.60	1.58	1.16	1.25
1^{st} quartile	1.27	0.81	0.83	1.10
3^{rd} quartile	2.03	2.00	1.89	2.09

Table 7.7: **Maximum H Reflexes**
The table lists the maximum H reflex responses for each subject in all sessions derived from their recruitment curves.

$\mathbf{H}_{max}/\mathbf{M}_{max}$ Ratio Values

The H_{max}/M_{max} ratios are shown in Table 7.8 and in Figure 7.4(a). A small decrease in the ratio was seen after exercise.

H_{max}/M_{max} Ratio Values				
Subject	**24h before** [mV]	**Pre** [mV]	**Post** [mV]	**24h after** [mV]
2	0.16	0.11	0.12	0.13
3	0.14	0.10	0.10	0.07
4	0.21	0.29	0.27	0.22
5	0.26	0.15	0.27	0.24
6	0.48	0.48	0.51	0.43
7	0.45	0.50	0.39	0.38
9	0.31	0.22	0.21	0.22
10	0.35	0.32	0.41	0.37
12	0.30	0.25	0.25	0.42
13	0.37	0.37	0.14	0.24
Median	0.30	0.27	0.26	0.24
1^{st}	0.23	0.17	0.15	0.22
3^{rd}	0.37	0.36	0.36	0.38

Table 7.8: **Derived $\mathbf{H}_{max}/\mathbf{M}_{max}$ Ratio Values**
The H_{max}/M_{max} ratios are reported for each subject in all sessions.

EMG Activity of the Middle Trapezius Muscle During Maximum Voluntary Contraction of Shoulder Abduction Muscles

The EMG values are shown in Table 7.9 and Figure 7.2. All values in the Table were the maximum EMG activity measured from two measures, each lasting 5s.

EMG Activity During Maximum Voluntary Contraction				
Subject	**24h before** [mV]	**Pre** [mV]	**Post** [mV]	**24h after** [mV]
2	0.45	0.47	0.43	0.56
3	0.22	0.22	0.33	0.24
4	0.42	0.50	0.53	0.49
5	0.22	0.17	0.20	0.18
6	0.29	0.42	0.32	0.42
7	0.59	0.69	0.56	0.63
9	0.30	0.23	0.21	0.27
10	0.33	0.36	0.54	0.43
12	0.55	0.54	0.32	0.49
13	0.30	0.28	0.27	0.28
Median	0.31	0.39	0.33	0.42
1^{st} quartile	0.29	0.24	0.28	0.27
3^{rd} quartile	0.44	0.49	0.50	0.49

Table 7.9: **EMG Activity During MVC of Shoulder Abduction**
The EMG MVC values are reported for each subject in all sessions.

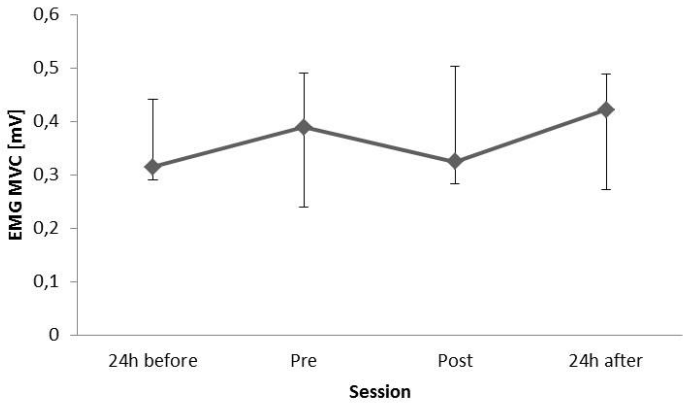


Figure 7.2: **EMG Activity During MVC of Shoulder Abduction**
The changes in EMG MVC values are presented.

Fitted Data

In Figure 7.3, fitted recruitment curves (H reflexes) obtained from subject 5 are shown. Both the raw data (dots) and the fit (sigmoid function) are illustrated in the figure. Based on these fits, H_{75} and H_{50} and the slope of the function (H_{Slp}) were calculated and tested for significant differences.

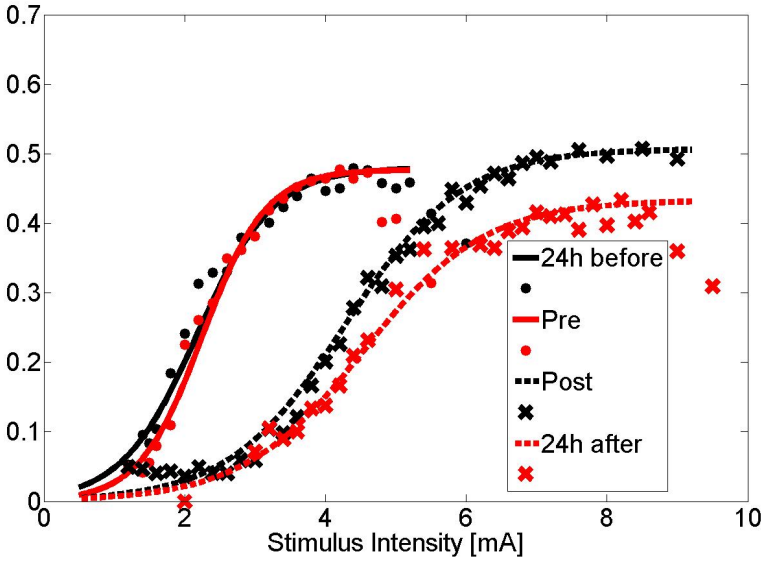


Figure 7.3: **Fitted H Reflex Recruitment Curves**
An example of fitted data in each session from Subject 5. The data are normalised to M_{max}.

Similar fits were estimated from all other subjects and analysed. In Table 7.10, the Pearson product-moment correlation coefficient (r) for each fit are presented.

Pearsons Product-moment Correlation for Fitted Data				
Subject	**24h before**	**Pre**	**Post**	**24h after**
2	0.94	0.94	0.95	0.97
3	0.97	0.87	0.88	0.94
4	0.97	0.96	0.95	0.93
5	0.99	0.98	0.99	0.99
6	0.99	0.99	0.99	0.99
7	0.91	0.90	0.92	0.91
9	0.99	0.96	0.97	0.98
10	0.92	0.98	0.94	0.95
12	0.93	0.97	0.95	0.95
13	0.98	0.99	0.84	0.98
Median	0.97	0.97	0.95	0.96
1^{st} quartile	0.93	0.95	0.92	0.94
3^{rd} quartile	0.98	0.98	0.97	0.98

Table 7.10: **r coefficient for Fitted Data**

The r coefficients for each subject in all sessions are reported.

Table 7.11 and Figure 7.4(b) shows the H_{75}/M_{max} ratios. A decrease after exercise can be seen, 24h after exercise the ratios were lowest.

H_{75}/M_{max} Ratio Values				
Subject	**24h before**	**Pre**	**Post**	**24h after**
2	0.12	0.09	0.09	0.10
3	0.11	0.06	0.05	0.01
4	0.16	0.17	0.15	0.14
5	0.20	0.14	0.16	0.18
6	0.38	0.39	0.08	0.06
7	0.36	0.44	0.38	0.39
9	0.24	0.19	0.17	0.11
10	0.31	0.30	0.39	0.20
12	0.23	0.25	0.06	0.21
13	0.29	0.31	0.08	0.04
Median	0.23	0.22	0.12	0.13
1^{st} quartile	0.17	0.15	0.08	0.07
3^{rd} quartile	0.30	0.30	0.17	0.20

Table 7.11: **H_{75}/M_{max} Ratio Values**
The H_{75}/M_{max} ratios are reported for each subject in all sessions.

Table 7.12 and Figure 7.4(c) shows the H_{50}/M_{max} ratios. The H_{50}/M_{max} ratio decreased after exercise and 24h after exercise the ratios were lowest.

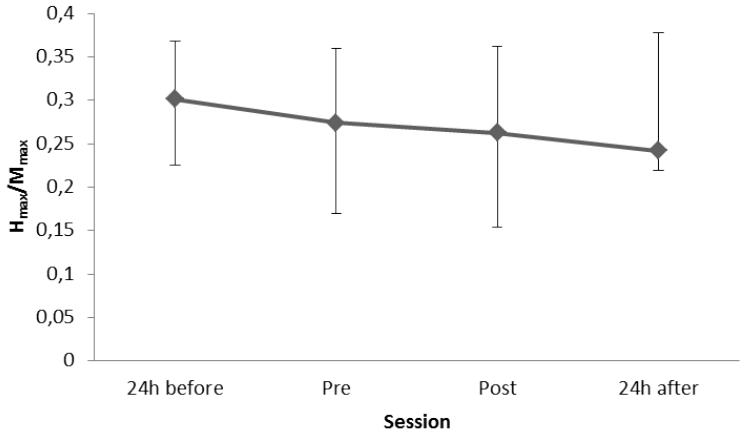
H_{50}/M_{max} Ratio Values				
Subject	**24h before**	**Pre**	**Post**	**24h after**
2	0.08	0.06	0.06	0.07
3	0.07	0.04	0.03	0.01
4	0.11	0.11	0.10	0.10
5	0.13	0.10	0.10	0.13
6	0.24	0.22	0.04	0.03
7	0.22	0.31	0.21	0.31
9	0.15	0.13	0.11	0.06
10	0.18	0.19	0.26	0.10
12	0.15	0.24	0.04	0.15
13	0.19	0.19	0.06	0.018
Median	0.15	0.16	0.08	0.09
1^{st} quartile	0.11	0.10	0.04	0.04
3^{rd} quartile	0.18	0.21	0.11	0.12

Table 7.12: **$\mathbf{H_{50}/M_{max}}$ Ratio Values**
The H_{50}/M_{max} ratios are reported for each subject in all sessions.

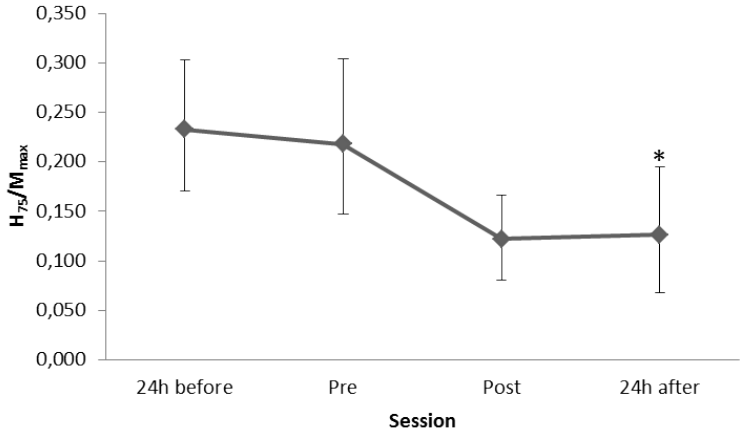
Table 7.13 shows slope parameters for curves fitted to the data (H_{slp}).

H_{slp} Values				
Subject	**24h before**	**Pre**	**Post**	**24h after**
2	1.28	1.29	1.36	1.10
3	1.77	0.98	0.67	0.96
4	1.59	1.29	1.16	1.06
5	2.56	3.89	2.00	2.17
6	1.86	2.23	1.21	1.16
7	1.40	2.00	3.67	3.39
9	2.30	2.43	2.47	1.90
10	0.93	1.11	0.55	1.23
12	2.77	2.49	1.39	1.45
13	2.95	3.36	1.66	2.24
Median	1.82	1.88	1.38	1.34
1^{st} quartile	1.45	1.29	1.17	1.12
3^{rd} quartile	2.50	2.48	1.92	2.10

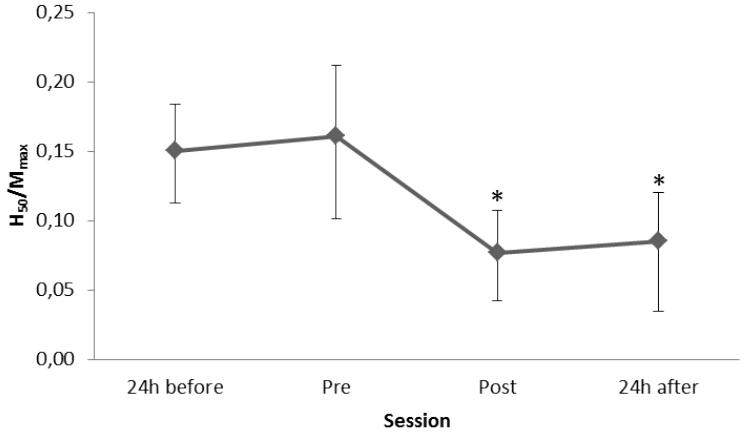
Table 7.13: **Slope parameter values of curves fitted to the data (H_{slp})**
The H_{slp} values are reported for each subject in all sessions.



(a) H_{max}/M_{max} Ratios



(b) H_{75}/M_{max} Ratios



(c) H_{50}/M_{max} Ratios

Figure 7.4: The changes in H_{max}/M_{max}, H_{75}/M_{max}, and H_{50}/M_{max} ratios are presented as median values for each session along with 1^{st} and 3^{rd} quartile ranges. (*) indicates a significant difference ($P<0.05$).

7.3 Statistical Results

In this section, the statistical results are presented. A Related-Samples Friedman's Two-Way Analysis of Variance (ANOVA) by Ranks test was conducted on each of the parameters to test for any significant difference between the four sessions ("24h before", "Pre", "Post", and "24h after" eccentric exercise). The significance level (α) was set at 0.05. In Table 7.14 the results from the Friedman tests are presented.

Friedman Test Results		
Parameter	**Sig. (*P-value*)**	**Decision**
VAS score	$<$0.001	**Reject the null hypothesis**
PPT values from trapezius	0.011	**Reject the null hypothesis**
PPT values from tibialis anterior	0.178	Retain the null hypothesis
EMG MVC value	0.772	Retain the null hypothesis
Latency for M wave	0.854	Retain the null hypothesis
M_{max}	0.513	Retain the null hypothesis
M_{max} stimulation Intensity	0.759	Retain the null hypothesis
Latency for H reflex	0.668	Retain the null hypothesis
H_{max}/M_{max} ratio	0.472	Retain the null hypothesis
H_{75}/M_{max} ratio	0.020	**Reject the null hypothesis**
H_{50}/M_{max} ratio	0.003	**Reject the null hypothesis**
Derived slope parameter (H_{slp})	0.086	Retain the null hypothesis

Table 7.14: **Statistical Results From the Friedman Test on the Parameters**
The parameter tested, P-value, and decision are shown.

Post-hoc Analysis

In order to identify which sessions that significantly differed from each other, a post-hoc Student-Newman-Keuls test was performed on the parameters in which the null hypotheses were rejected by the Friedman test. In the Tables 7.15, 7.16, 7.17, and 7.18 the results from the post-hoc analysis are presented.

Muscle Pain Intensity (VAS)	
Samples	**P<0.05**
24h Before - Pre	No
24h Before - Post	**Yes**
24h Before - 24h After	**Yes**
Pre - Post	**Yes**
Pre - 24h After	**Yes**
Post - 24h After	No

Table 7.15: **Post-hoc Test Results From the Visual Analogue scale (VAS) Score**
Post-hoc analysis with the Student-Newman-Keuls method.

PPT Values From Trapezius	
Samples	**P<0.05**
24h Before - Pre	No
24h Before - Post	No
24h Before - 24h After	**Yes**
Pre - Post	No
Pre - 24h After	**Yes**
Post - 24h After	**Yes**

Table 7.16: **Post-hoc Test Results From the Pressure Pain Threshold (PPT) Values From Trapezius**
Post-hoc analysis with the Student-Newman-Keuls method.

H_{75}/M_{max} ratios	
Samples	**P<0.05**
24h Before - Pre	No
24h Before - Post	No
24h Before - 24h After	**Yes**
Pre - Post	No
Pre - 24h After	**Yes**
Post - 24h After	No

Table 7.17: **Post-hoc Test Results From the H_{75}/M_{max} Ratios**
Post-hoc analysis with the Student-Newman-Keuls method.

H_{50}/M_{max} ratios	
Samples	**P<0.05**
24h Before - Pre	No
24h Before - Post	**Yes**
24h Before - 24h After	**Yes**
Pre - Post	**Yes**
Pre - 24h After	**Yes**
Post - 24h After	No

Table 7.18: **Post-hoc Test Results From the H_{50}/M_{max} Ratios**
Post-hoc analysis with the Student-Newman-Keuls method.

Chapter 8

Discussion

In this study, M waves and H reflexes were found in 10 out of 13 subjects. Latencies for each response were similar across all sessions and similar to latencies observed in the study by Alexander & Harrison[20]. Recordings of recruitment curves showed that both M waves and H reflexes got larger with increasing stimulation intensity before reaching a plateau. Furthermore, 2 subjects exhibited no antidromic effect in the H reflex recruitment curve, as indicated by no decrease in H reflex amplitude due to a dominating influence of M waves. As indicated by similar values of EMG MVC during recordings of H reflex responses, similar levels of muscle activation for the dominant middle trapezius was found in all four sessions ("24h before", "Pre", "Post", and "24h after" eccentric exercise). The muscle pain intensities (VAS score) and the pressure pain thresholds (PPTs) showed consistency between the first two sessions. This confirms that subjects refrained from physical activity which would have caused muscle soreness between sessions. Thus, any changes to neural responses were caused by the experimental design. Furthermore, none of the subjects had any perception of pain in the neck-shoulder region at the beginning of the experiment and did not develop discomfort before the exercise (total VAS score of zero for the first two sessions). The goodness of fit is also considered equal in each session based on Pearson product moment correlation coefficients.

In this chapter, the results of the study are discussed and future perspectives are suggested. First, results regarding the reproducibility of the study and the effect of the eccentric exercise are discussed. Next, improvements of the experimental design are suggested and perspectives related to work-related musculoskeletal disorders (WMSD) are commented. Finally, the most important findings are highlighted in a conclusion.

8.1 H Reflex Variability Between Sessions and Days

The present study has confirmed that all parameters obtained in the study are reproducible between days. None of the parameters showed any significant change from Session 1 ("24h before" exercise) to Session 2 ("Pre"-exercise).

A measure of the reproducibility of the M wave and H reflex was obtained by comparison of the results from the first two sessions. Since no significant variations were observed in M_{max} between the first two sessions, placement of recording electrodes and stimulation of the accessory nerve were sufficiently reproducible. H reflex ratios were also reproducible which suggests that stimulation of the C3/4 cervical nerve were reached in a sufficiently similar way for each session.

As described in Section 3.3, Brinkworth et al.[32] have previously investigated the difficulty in measuring the H reflex between days. Results from that study show day-to-day variability in recruitment curves in all trials (see Figure 3.8). However, in trials with 50% MVC the variability were smaller than in trials with relaxed muscles (0% MVC). In the present study, H reflexes were elicited with 15% EMG MVC. Besides of making the variability smaller it also made the excitability of motoneurons relatively similar between sessions. Another approach to standardise the background EMG could be to make it a fixed value based on the first session. Hereby, the same absolute level of EMG activity would have been reached in all sessions. However, it is possible that the MVC changed with DOMS and the subjects would have to recruit more of the muscle to reach the same level, i.e. recruit more motorneurons.

To further minimise variability, 10 H reflex responses were averaged for each stimulus intensity. This is in line with the recommendations from Brinkworth et al.[32]. No change in the intensity used to stimulate M_{max} were observed between sessions. This suggest the findings regarding the H reflex are related to a genuine change in excitability of the H reflex circuit.[32]

Since the neural parameters did not change significantly between sessions before exercise, the findings suggest that the experimental parameters are reproducible so that changes to neural responses can be attributed to the effects of the performed eccentric exercise procedure. Still, as concluded by Brinkworth et al.[32] the size of the H reflex is a function of three factors: the precision of stimulus delivery, excitability of the entire H reflex arch, and accuracy of recording. Since none of these factors can be fixed, it is self-evident that the H reflex will vary between subjects and between days.

8.2 The Effect of Eccentric Exercise

In this section, the results will be divided between a comparison of Session 3 ("Post" exercise) with Session 1 and 2 ("baseline" measures) and a comparison of Session 4 ("24h after" exercise) with Session 1 and 2 ("baseline" measures). The results from Session 3 will express the changes

occurring as a response to a state of muscle fatigue, whereas the results from Session 4 will express changes due to a state of delayed-onset muscle soreness (DOMS). Through the section, each parameter will be analysed in subsections regarding fatigue and DOMS respectively.

Fatigue

A significant modulation of the ratio derived by 50% of the maximal peak-to-peak amplitude of the H reflex from Session 1 and the maximal peak-to-peak amplitude of the M response (H_{50}/M_{max}) was detected in the "Post" session. None of the other ratios (H_{max}/M_{max} and H_{75}/M_{max}) were found to be significantly different from control sessions ("24h before" exercise and "Pre"-exercise). The fact that significant differences were limited to the H_{50}/M_{max} ratio and not present in the other two ratios, might be explained by the position of H_{50} on the upslope of the recruitment curve. Compared to H_{max} and H_{75}, the H_{50} value is more susceptible to changes due to larger variation in upslope position on the middle part of the recruitment curve for each increase in stimulation intensity (see Figure 6.20 in Section 6.6). Effectively, smaller variations in peak-to-peak amplitude of the H reflexes are more detectable on the steep upslope curve than closer to the plateau of the maximum values of the H reflex recruitment curve. However, other studies primarily investigating the changes to the H reflex as a result of muscle fatigue (Bulbulian & Bowles[57], Avela et al.[58], and Racinais et al.[59]) have found significant changes in the H_{max}/M_{max} ratio. Overall, their exercise procedures differ from the one used in the present study, which may be the cause of differences in outcome. Bulbulian & Bowles[57] used downhill running to induce muscle soreness, Avela et al.[58] used a marathon run and Racinais et al.[59] used walking backwards downhill at 1m/s. In contrast to the eccentric exercises performed in the dynamometer, these types of exercises include more muscle groups working in synergy with different types of contractions making it more difficult to reject other influencing mechanisms. Also, these exercises are more intense, i.e. increasing the heart rate of the participants. As shown by Bulbulian & Darabos[76], high-intensity exercise and low-intensity exercise reduce the mean H_{max}/M_{max} ratio by 21.5% and 12.8% respectively. This could be an explanation for the lack of difference seen in H_{max}/M_{max} ratio in the present study. Although heart rate was not measured during the exercise procedure, the frequent brakes of ∼15s between the shoulder pad reaching the bottom of the shoulder elevation position and returning to the top position to start a new, prevented the heart rate from increasing and thus the exercise was considered relatively low in intensity for cardiac load, although it was high in load for muscle intensity. Another reason for the lack of difference could be that the post exercise measurements were done 5-10min after the eccentric exercise. Thus, the recovery process may have begun and the measurement may not have been an exclusive fatigue measure.

The fatiguing effect of training was not reflected in the PPTs measured on the dominant middle trapezius muscle, as there was no significant difference compared with baseline measures from

Session 1 and Session 2. Similarly, control measures of the tibialis anterior muscle showed no significantly different values when comparing across sessions. The studies by Binderup et al.[62] and Nie et al.[37] used similar methods to induce DOMS and both studies only found a significant difference when comparing PPT values from 24h before exercise with values 24h or more after exercise. Thus, the results of the present study were in line with these findings of no effect on PPT measures immediately after the exercise. However, a state of muscle pain was overall detected by the measures of pain intensity (VAS score). The VAS score for fatigue induced by the exercise was indicated by an average of 1.5cm (0cm = "no pain", 10cm = "worst pain imaginable"). An increase of the pain intensity is also in line with findings by Nie et al.[37]. However, the total VAS score after exercise in the study by Nie et al. was ∼6.2cm. Compared to the present study, the higher ratings of the pain intensity may be explained by the pain being more dominating during active movements, as Nie et al.[37] obtained the VAS score during active contraction. In the present study, the subject was relaxed in a sitting position with no movement. Furthermore, an increase in pain intensity may also result from the mechanical contact between the shoulder pad and the subject's shoulder area. Sometimes this contact area on the subjects was bruised by the exercise (still present at least 24h after exercise). The increase in VAS may therefore, in part, be an expression of skin damage from pressure rather than only deep tissue muscle soreness.
PPT measurements by Binderup et al.[62] are in line with the findings from the present study immediately after exercise, i.e. no significant difference were observed (see Figure 8.1).

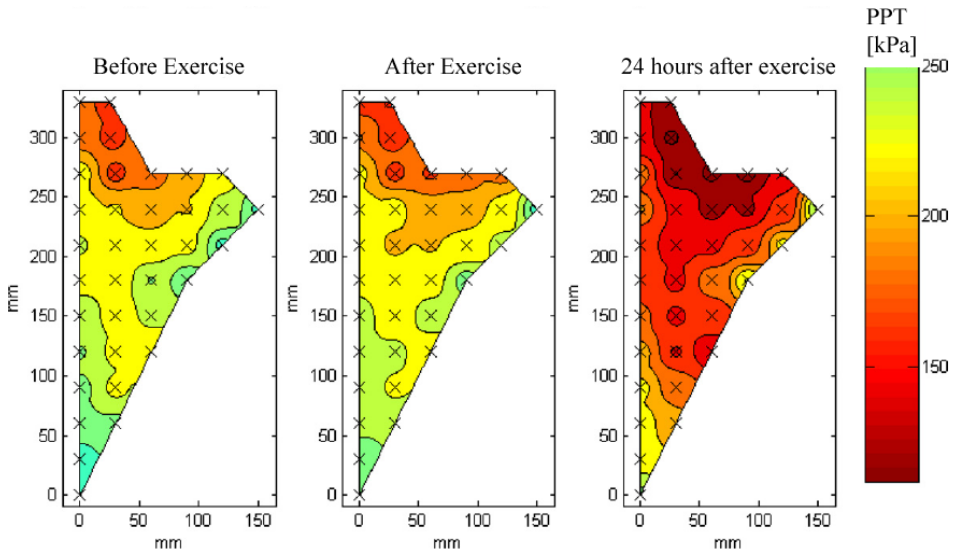


Figure 8.1: **Pressure Pain Threshold (PPT) Maps**
Absolute pressure pain threshold maps from the trapezius muscle before (left), immediately after (middle), and 24h after (right) eccentric exercise (n=10). Modulated from Binderup et al.[62].

Delayed-onset Muscle Soreness

24h after exercise, the H/M ratios from 75% and 50% of the maximal peak-to-peak amplitude of the H reflex (H_{75}/M_{max} and H_{50}/M_{max}) were decreased significantly compared to sessions before exercise. Thus, after the eccentric exercise it takes stronger stimulation to get the same size of H reflex, i.e. the recruitment curves shifted to the right. Since the background EMG is relatively constant in all sessions (i.e. same excitability of the motoneurons) this shift indicates presynaptic inhibitions of Ia afferents. This inhibition has previously been described by Taylor et al.[63] and Gandevia[26]. According to those studies, the inhibition may be related to increased firing of group III and IV afferents. Since the shift is also observed immediately after exercise, it may not be exclusively related to DOMS as fatigue or recovery effects may carry over.
Like in the "Post" session, the H_{max}/M_{max} ratio did not change in the presence of DOMS. This is similar to findings in the studies by Bulbulian & Bowles[57], Avela et al.[58], and Racinais et al.[59] where the H_{max}/M_{max} ratio did not change due to the effect of DOMS. As with the results of Session 3 on fatigue, the significant differences in H_{75} and H_{50} between sessions can be attributed to these values being more sensitive to changes due to their positions on the upslope of the recruitment curve, whereas the peak-to-peak amplitude of the H_{max} was less susceptible to change on the plateau of the recruitment curve.
Pain intensity and PPT values both showed an increase in muscle soreness 24h after exercise which was similar to the finding of DOMS in the shoulder region by Nie et al.[37], Binderup et al.[62], and Kawczynski et al.[60]. However, the eccentric exercise was not specifically targeted at the middle trapezius, as the shoulder shrug movement did not include an abduction motion of the arms. Although other muscles contribute, the main muscle agonists in the shoulder shrug motion are levator scapulae and the upper trapezius muscle[77]. As seen in Figure 8.1, the upper trapezius was more sensitive to pressure than the other parts in a study by Binderup et al.[62], who used the same method to induce DOMS as in the present study.
Ultimately, the change in pressure pain sensitivity is an expression of a state of hyperalgesia in the targeted muscles. As described in Section 4.2, there are several theories on how this state of hyperalgesia develops in the muscles with muscle damage and inflammation being the most widely accepted[6]. Thus, the hyperalgesia could stem from acute damage to the muscle fibres during exercise, causing a mechanical disruption of the ultra structural elements within the muscle fibres such as the Z-line and contractile filaments[78, 79]. Also, it could stem from an acute inflammation resulting from an immune response to the initial injury, which sensitizes the muscle nociceptors and lower their threshold to mechanical stimuli[52].
Finally, results from PPT measurements on the tibialis anterior muscle indicated that the increase in muscle soreness was limited to active muscles and did not have a central effect on the central nervous system.

8.3 Future Perspectives

The findings from this study indicate a change in presynaptic inhibition after inducing DOMS in the trapezius muscle. To further confirm these findings, a study on a patient population is neccessary. It could then be investigated if the results found in this study can be related to WMSD. If so, the H reflex results may help create a better understanding of the neural mechanisms involved in WMSD.
Before using the experimental design in a study on a patient population, further improvement of the scientific method can be considered.

In terms of reproducibility, the statistics showed that there was no significant difference when comparing parameters between the two baseline sessions (Session 1 and 2). However, the fact that M_{max} values varied between trials and sessions, indicated that changes did occur in some scale, as the M_{max} should be a constant value of the maximum motoneuron firing at a given stimulation spot. Using surface electrodes to electrically evoke neural responses causes variance due to differences in electrodes and the electrical propagation through dermal layers. Other electrode technologies may have secured a much more selective and consistent response from electrical stimulus such as the placement of needle electrodes or implanting cuff electrodes. However, these electrode technologies would have significantly increased the time requirements and the expenses associated with the experiment.

Another point of improvement inherent in the experimental design is the method by which both stimulus and recording electrodes were placed and replaced between conditions. Using a permanent marker to mark the location of the electrode centres on the skin may not have been optimal. Although the marks were not removed by washing, it was difficult to replicate the exact same electrode placement (incl. orientation) and even the smallest change in especially stimulus electrode placement could potentially affect the evoked reflex responses. Other electrode technologies, as mentioned, may reduce the variability to electrode placement.

External influences could also have been limited to improve the experimental design. In an experiment where a difference in position, back posture and head orientation could potentially affect the output, more could have been done to standardise the measurement conditions. For instance, the subject could have been equipped with a head restraint to keep the position of the head as stable and straight as possible. The abduction movement of the dominant arm during H reflex response recordings also introduces some variation. The subject was told to try and keep the elbow in line with the back side of the supporting bench when raising the arm to touch the horizontal bar. Instead, the horizontal bar could have been lowered in a fixed position to

always touch the arm on the supporting bench, so that the shoulder abduction movement could have been generated by an isometric contraction requiring no movement. Limiting the range of motion was the reason the supporting bench was introduced in the first place.

8.4 Conclusion

In the present study, H reflex recruitment curves were obtained in 10 out of 13 subjects and analysed before and after an exercise intervention causing delayed-onset muscle soreness. Reproducibility measures of the H reflex showed no significant changes before intervention. Recordings of H reflexes in this study must therefore be considered reliable.
Soreness was induced in the middle trapezius 24h after intervention with success. H reflex recruitment curves recorded after ("Post") and 24h after intervention showed a significant decrease in peak-to-peak amplitudes of the H reflex. This suggest that DOMS increases firing of nociceptors which inhibit motoneuron excitability and the magnitude of the H reflex. This mechanism may be related to findings during fatigue.
To relate findings of this study to physiological mechanism occurring in work-related musculoskeletal disorders, further studies on a patient population must be carried out.

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Appendix A

Deltagerinformation til forsøgspersoner

Tak fordi du har vist interesse for at deltage i vores forskningsprojekt! Projektets forsøg udføres ved Center for Sanse-Motorisk interaktion, Aalborg Universitet, i lokale A2-104.
Før du beslutter, om du vil deltage i forsøget, skal du fuldt ud forstå, hvad forsøget går ud på og hvorfor vi gennemfører forsøget. Vi vil derfor bede dig om at læse denne deltagerinformation grundigt. Du vil desuden altid være velkommen til at stille yderligere spørgsmål til forsøget. Hvis du beslutter dig for at deltage i forsøget, vil vi bede dig om at underskrive en samtykkeerklæring. Det er frivilligt at deltage i forsøget. Du kan når som helst og uden at give en grund trække dit samtykke tilbage.

Formålet med projektet

Formålet med projektet er at øge forståelsen af, hvordan kroppen fysiologisk kompenserer for muskelømhed i nakke-skulder-regionen. Det er en viden, som er relevant for forebyggelsen af arbejdsskader og kronisk smerteudvikling.

Forsøgsprotokol

Forsøget handler om at undersøge, hvordan muskelømhed spiller ind på h-refleksen i nakke-skulder-regionen (trapezius-musklen). Forsøget vil være opdelt i tre dage med i alt fem dele - se figur A.1 herunder:

Bortset fra træningsøvelserne ved dag 2, består alle delene af de samme fem målinger.
For at kvantificere graden af ømhed, vil vi hver gang spørge dig om din smerte i nakke-skulder-regionen på en skala fra 0-10, hvor 0 er ingen smerte overhovedet og 10 er den værst tænkelige smerte. Herefter bruger vi et håndholdt algometer til at måle din smertegrænse i fht. tryk. Disse

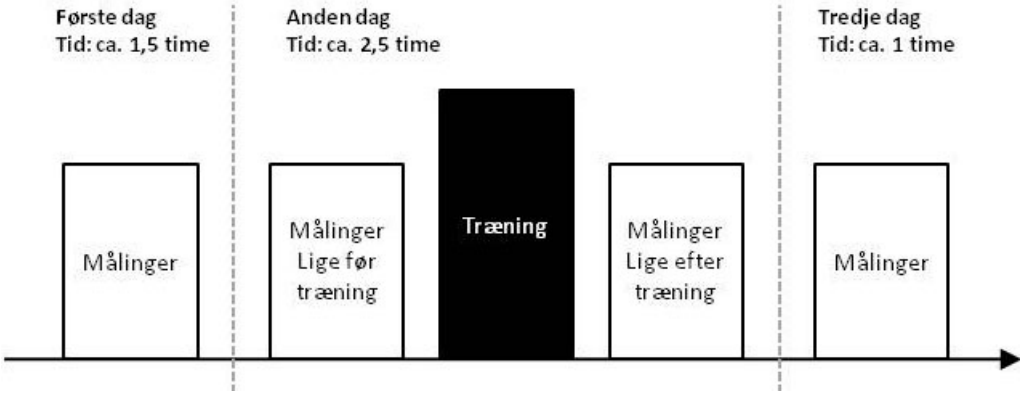

Figure A.1: **Forsøgsoversigt**
Forsøget forløber over tre dage med i alt fem dele.

måles tre gange i trapezius-musklen og tre gange på tibialis anterior (TA)-musklen på underbenet. Vi placerer EMG-elektroder på den midterste del af trapezius-musklen, for herefter at kunne måle M- og H-bølger (responser) fra denne muskel. Det er nødvendigt at have den øverste del af ryggen bar, så elektroderne kan blive placeret og målingerne kan optages. Kvindelige deltagere vil få udleveret en kittel til at dække sig foran. Elektrodeplaceringerne bliver markeret med en prik fra en markeringstusch for at kunne finde disse igen dagen efter. Det er vigtigt at disse **ikke vaskes af** imellem sessionerne.
Vi bruger svage elektriske stimuleringer til at udløse reflekserne. Vi opsamler en række datapunkter til en graf, så vi starter med at stimulere ved lav intensitet og langsomt øge intensiteten til størrelsen af refleksen ikke bliver større. H-refleks-målingerne opsamles samtidig med muskelkontraktion, så din arm vil under disse stimuleringer være hævet i fht. en procentdel af din maksimale muskelkontraktion (MVC). Du skal forsøge at holde en konstant kontraktion under stimuleringerne og vil blive hjulpet af en feedback-brugergrænseflade.

Følgende data vil altså blive opsamlet i nævnt rækkefølge.

- VAS-score: 0-10.
- Smertegrænsen ved tryk: 3 gange på trapezius + 3 gange på TA.
- M-bølge: Datapunkter til en graf.
- MVC-måling: To gange skal du løfte armen så kraftigt du kan.
- H-bølge: Datapunkter til en graf.

Responsen fra H-refleksen er forsøgets primære parameter og denne er ekstremt påvirkelig overfor mange faktorer såsom position i stol, kropspositur, elektrodeplacering, blikkets fokus og støj for at nævne enkelte faktorer. Det er derfor vigtigt at forsøget udføres så præcist som muligt. Vi vil også bede dig om **ikke at dyrke motion og vægttræning**, med ømhed til følge, op til forsøgsperioden samt under forsøgsperioden. Ømhed kan indtræde 1-7 dage fra træning og kan

påvirke vores ømhedsmålinger og refleksresponserne og dette skal undgås. Derudover vil vi bede dig spise normalt og undgå smertestillende medicin.
Træningsøvelserne på dag 2 vil tage omkring en time, hvor du bliver placeret i et dynamometer, hvor du skal forsøge at modvirke en nedadgående kraft fra et stempel indenfor skulderens bevægelsesområde. Øvelsen er designet til at skabe muskelømhed, så det forventes at det bliver fysisk anstrengende og at du får varmen. Øvelserne består af 50 gentagelser med 2 min pause efter hver 10 gentagelser. Under øvelsen vil du have et plastik-korset på, for at beskytte dig mod at bøje din krop skævt. Øvelserne tager ca. en time.
De nerver, som vi stimulerer omkring siden af halsen, kan være svære at finde, så forsøgets længde kan godt variere, men overordnet forventer vi, at forsøget varer: Dag 1: 1,5 time, dag 2: 2,5 timer og dag 3: 1 time, hvis alt går planmæssigt. Det er vigtigt at forsøget eksekveres med ca. 24 timers mellemrum.
Du vil blive kompenseret med 500 kr. for at deltage i forsøget.

Tusind tak fordi du vil hjælpe os! Mvh Lars, Steffen og Brian

Appendix B

Informeret samtykkeerklæring

Projektets titel: Investigation of the H Reflex Behaviour in the Trapezius Muscle after Inducing Delayed-Onset Muscle Soreness.
Studerende: Lars Tønners Nørgaard, Steffen Vangsgård og Brian Korsholm Flaskager.
Forsøgsansvarlig: Pascal Madeleine

Undertegnede erklærer:

- at jeg er indforstået med deltagelse i ovennævnte forsøg.
- at jeg er blevet grundigt informeret om forsøget, både mundtligt og skriftligt, og jeg ved nok om formål, metode, fordele og ulemper ved forsøget.
- at jeg har modtaget en kopi af deltagerinformationen og samtykkeerklæringen.
- at jeg er sund og rask.
- at jeg er blevet informeret om at min deltagelse er frivillig og at jeg når som helst kan trække mit tilsagn om deltagelse tilbage, uden skal kunne give en forklaring på dette.
- at jeg har haft mulighed for at stille yderligere spørgsmål og har haft tid til at overveje min deltagelse.

Jeg giver hermed mit samtykke til at deltage som forsøgsperson i eksperimentet.

Aalborg, den

Underskrift:

Bevidnelse:

Hermed bekræfter jeg, den ansvarlige forsker, at jeg har forklaret formålet med undersøgelsen samt de risici og/eller den evt. værdi, som er forbundet med deltagelse i forsøget. Desuden bekræfter jeg at forsøgspersonen har læst den skriftlige information og erklæret sig villig til at deltage i den beskrevne undersøgelse.

Aalborg, den

Underskrift:
Informerende forsker