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Diplomarbeit

EMG-signal processing for neuro-excitability tests using Matlab

Eine Arbeit zur Erlangung des akademischen Grades
Diplom-Ingenieur

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Fakultät für Elektrotechnik und Informationstechnik

ausgeführt von

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Kurzfassung

Einleitung: Elektromyographie (EMG) wird vielseitig eingesetzt in Bereichen wie etwa Prothetik, Rehabilitation, Sportanalyse oder Forschung. Aufgrund der vielseitigen Verwendung gibt es auch eine große Nachfrage an individuellen Analyseprogrammen. Das Ziel dieser Arbeit war es, in Matlab ein anwenderunterstützendes Programm für die Signalanalyse verschiedener elektromyographischer Untersuchungen zu entwickeln und die Validität mithilfe von bereits vorhandenen Ergebnissen zu überprüfen. Dieses Programm versucht sowohl auf die verschiedenen analytischen Anforderungen von stimulierten und willkürlichen Kontraktionen einzugehen, als auch dem Anwender dabei zu unterstützen einen besseren Überblick über die Daten zu erhalten.

Methodik: Diese Diplomarbeit basiert auf einem Praktikum am Institut der Myologie in Paris. Die EMG-Signale von nicht-dystrophischen Myotonie-Patienten und gesunden Probanden wurden analysiert. Sechs verschiedene Tests (maximale M-Welle, 5Hz Stimulation, Refraktoritäts-Test, Supernormalitäts-Test, maximale willkürliche Kontraktion, Fatigue) wurden durchgeführt und dabei das EMG-Signal mit Laplace-Elektroden gemessen. Die Analyse der Daten erfolgte vollständig mit Matlab. Dabei wurden alle Daten gefiltert, nach ihrer Qualität beurteilt und die untersuchungsspezifischen Parameter berechnet. Um die Validität zu überprüfen wurden die Ergebnisse, mit bereits publizierten Ergebnissen desselben Datensatzes, verglichen.

Ergebnisse: Alle Bearbeitungsschritte die sich nicht auf die Signal-Qualität beziehen, wurden automatisiert. Die Qualitätsklassifizierung wurde durch Entscheidungsvorschläge vom Programm unterstützt. Mit nur einer Ausnahme bestanden alle stimulierten Kontraktionen der Kontrollgruppe die

Qualitätskontrolle. Bei den Patienten konnten jedoch nur 38% der maximalen M-Wellen und 42% der 5Hz Stimulationen verwendet werden. Die restlichen EMG Signale mit schlechter Qualität wurden in 4 verschiedene Fehlerklassen unterteilt. Aufgrund der schlechten Qualität der maximalen M-Wellen und der doppelten Stimulationstests, konnten weder der Refraktoritäts-Test noch der Supernormalitäts-Test ausgewertet werden. Der Vergleich der Ergebnisse mit bereits vorhandenen Werten zeigte die Validität des Programms. Sowohl die Kraftwerte als auch die Effektivwerte und die mittleren Frequenzen der Patienten waren generell niedriger als jene der gesunden Probanden. Patienten mit Myotonia Congenita und Paramyotonia Congenita scheinen unterschiedliche EMG-Eigenschaften aufzuweisen. Spezifische Aussagen können, wegen zu kleinen Gruppengrößen und inkonsistenten Ergebnissen, leider nicht getroffen werden.

Zusammenfassung: Durch die Analyse der EMGs gesunder Probanden konnte gezeigt werden, dass es generell möglich ist EMG Signale halb automatisch zu analysieren. Die für diesen Zweck entwickelten Programme können für jegliche M-Wellen-, 5Hz Stimulations-, maximale Kontraktions- und Fatigue-Tests verwendet werden. Allerdings werden gewisse Grundanforderungen, wie etwa gute Signalqualität und ausreichende Gruppengrößen vorausgesetzt. Diese können aber vor allem für Patientenstudien mit sehr seltenen Krankheiten ein Problem darstellen.

Schlüsselwörter: Elektromyographie, EMG, nicht-dystrophische Myotonie, neuromuskuläre Erregbarkeit

Abstract

Introduction: Electromyography (EMG) is a standard practice in various fields such as prosthetics, rehabilitation, sport analysis or research. Due to its diversity of application, there is a high demand for individual signal processing solutions to fit the specific requirements. The task was to develop a semi-automatic signal processing interface in Matlab for different electromyographic tests, to validate its results by comparison with values in the literature and to apply it to control subject and patient data. The application was customized for the specific testing protocol comprising stimulation evoked as well as voluntary contractions and should give the user a general overview over the data.

Methods: This thesis is based on an internship at the Myology Institute in Paris. The EMG signals of non-dystrophic myotonia patients as well as healthy control subjects were analysed. The signals were recorded during 6 different neuromuscular excitability tests (maximum M response, 5Hz stimulation, refractory, supernormality, maximum voluntary contraction, fatigue), using laplacian electrodes. Matlab was used for further analysis of the data. The signals were filtered, their quality classified and test specific parameters were calculated. The results were compared with already published results of the same dataset in order to gain information about validity.

Results: All non signal-quality related processing steps were automatized and the quality classification decisions were guided by the developed program. All but one stimulation evoked control EMG file passed the signal quality assessment, whereas only 38% of the patients compound muscle action potential and 42% of the patients 5Hz tests were valid. The remaining poor quality signals were classified into 4 different error classes. Due to

the combination of the patients poor quality CMAP and double stimulation recordings, neither the supernormality nor the refractory test could be processed. The control group results were all comparable with the literature and previous calculations. The patients force as well as root mean square and mean power frequency values were generally lower than those of the control group. Myotonia congenita and paramyotonia congenita patients tend to have different EMG behaviour but no general statements could be made due to small group numbers and mostly inconsistent results on the different days of examination.

Conclusion: The application of the algorithms on healthy control data showed that it is generally possible to semi-automatically process EMG data. The adaptable user interfaces created for this testing protocol are applicable on any compound muscle action potential, 5Hz, refractory, supernormality, maximum voluntary contraction or fatigue recordings. However, basic requirements such as adequate signal quality and subgroup numbers still have to be fulfilled. For patient data, this might be especially difficult as for some rare diseases it is simply not possible to obtain more participants or disease specific characteristics complicate the recording of EMG signals.

Keywords: Electromyography, EMG, non-dystrophic myotonia, neuromuscular excitability

Contents

1 Introduction

1.1 Motivation

The interest in medical application of electromyographic (EMG) signals has been rising continuously since Galvani discovered the direct connection between muscle and electricity in 1790 [1]. The first analog EMG system was developed in Copenhagen and commercially available in 1950 [2]. Since then, the technology for EMG signal recordings has developed tremendously and there is a wide range of easily applicable recording systems available.

The variety of EMG recording systems, types of application and possible parameters, makes EMG signals one of the most versatile but at the same time one of the most difficult among physiological signals to process and interpret. Hence, the need for systematic and objective processing systems is high. Big companies like Delsys [3] or Motion Lab Systems [4] already offer ready-to-use signal processing software. However, these programs are costly, rather inflexible in terms of which parameters can be calculated and mostly need to be used with company specific electrodes. Therefore, research centers are often in need of individual solutions, which perfectly fit the experimental protocol but at the same time are able to process large amounts of data. While the individual analysis of EMG signals might be appropriate for a small data set, it becomes more and more troublesome with a growing number of signals. Therefore, an automatic solution adapted to the individual situation of the problem is needed.

This thesis is based on an internship at the Myology Institute of the Pitié-

Salpêtrière Hospital in Paris. The goal of the internship was to standardize the processing of a vast amount of EMG signals, recorded during a predefined neuromuscular excitability testing procedure. The resulting process should be objective and applicable to EMG signals from healthy controls as well as from patients suffering from neuromuscular diseases. EMG recordings of ten healthy controls and twelve patients with non-dystrophic myotonia (NDM) have been processed using the developed software. All together 150 control and 1188 patient signals were evaluated. The control subjects were analysed to ensure the programs validity and reliability while the patients data were processed in order to show treatment response and to compare the values with healthy subjects.

1.2 Research Questions

The goal of this work was to develop an analysis protocol and to automatize the analysis steps for the voluntary as well as the stimulation evoked EMG signals of the given test protocol. However, due to the quality of the EMG signals and the small size of the abductor digitori minimi (ADM) muscle, it was still necessary to manually check every step after applying the algorithm. The aim was to minimize the manual corrections after the algorithm. At the end, these parameters were used to evaluate patients response to treatment and to compare them with the values obtained in healthy subjects.

Formulation of the Research Questions:

- How can a dataset of more than 1000 EMG signals derived from 6 different tests be processed adequately?
  - Which filtering steps are necessary?
  - How can the signal quality be assessed?
  - How can the Laplace channel be selection?
  - Which parameter need to be calculated?
- How can the analysis protocol be validated?

  - Apply on data from healthy individuals and compare with previously published results for algorithm validation
  - Apply on patients data and compare with healthy individuals
- How can the EMG Signals be used to differentiate between healthy individuals and NDM patients?

1.3 Approach

The analysis for the nerve excitability study data can be separated into voluntary and stimulated EMG signals. These two groups were analysed separately, bearing in mind different goals.

For the voluntary EMG signals the fatigue behaviour of healthy individuals and patients with NDM was of interest. Therefore the time and frequency domain was analysed calculating standard parameters that are generally used to assess fatigue during isometric contractions. Likewise, the stimulated signals were analysed in time and frequency domain. However, with preliminary mean signal calculation and focusing on different parameters.

The results were validated using two different approaches. On the one hand, they were checked through the application of the process on data from healthy subjects and comparison with results of previous studies, which have used the same dataset as basis for their calculations. On the other hand, a comparison between healthy and patients data was made as a consequence of missing pre-existing data for the patients.

2

Theoretical Background

2.1 Abductor Digiti Minimi

The Abductor Digiti Minimi (ADM) is a small skeletal muscle located at the palm of the hand. Its main function is to move the little finger away from the other fingers. It is quite popular for neuromuscular studies because it can be individually excited through the ulnar nerve without exciting any other muscles, which reduces the possibility of cross talk. Furthermore, there is only a small amount of intermediate fat between muscle and electrode. However, due to its small size of only a few centimeters, electrode positioning is not easy and signal quality is therefore strongly affected by small movements of the nerve junction during contraction.

2.2 Physiology of Myoelectric Signals

Each human movement is controlled by a complex interplay of muscles, nerves and brain. Thereby, it is important to regulate the force output depending on the movement. This is achieved through various mechanism, which will be described more closely in this section.

A group of muscle fibers is innervated by one single nerve fiber. This combination of nerve fiber and innervated muscle fibers is called motor unit (MU) and is the smallest functional unit of a muscle. Depending on the size and task, the number of MUs per muscle may vary between 100 and more

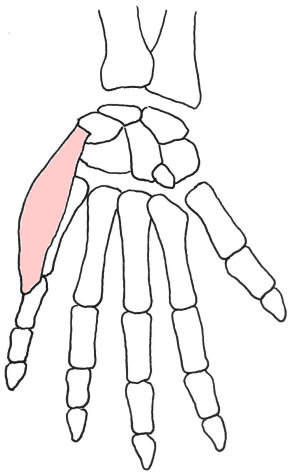

Figure 1: Sketch of the anatomical location of the abductor digiti minimi in the hand

than 1000 [5].

Based on contraction speed and fatigability, three different motor unit types have been identified [6]:

- fast-twitch, fatigable (FF) - high force and fast contraction but fatigues within seconds
- fast-twitch, fatigue-resistant (FR) - fast contraction speed but more fatigue resistnant
- slow twitch (S) - low force, slow contraction speed but highest fatigue resistance

During histochemical testing it has been discovered that FF MUs consist of type IIa muscle fibers, FR MUs of type IIb muscle fibers and slow twitch MUs of type I muscle fibers [6]. All motor unit types are randomly distributed over the cross section of a muscle, but their ratio may change depending on the muscle function.

The termination of nerve fibers on the muscle is defined as the end-plate region or neuromuscular junctions. When an action potential travels along an axon and reaches the neuromuscular junction, all innervated muscles fibers

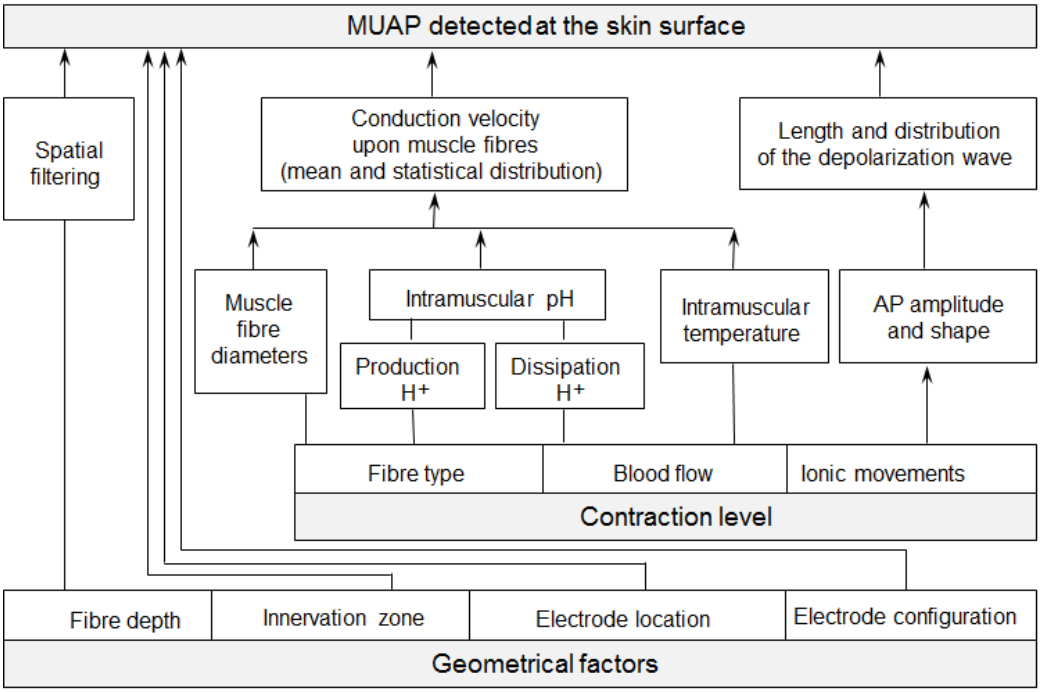

Figure 2: Summary of factors influencing the EMG signal; Graph adapted from [7]

of this MU contract almost simultaneously. The force output is controlled by the central and peripheral nerve system through modulating the number of active MU and the firing rate of the motor neurons. [7]

The electrical discharge of a single motor unit is called motor unit action potential (MUAP) and can be recorded with needle electrodes or high spatial resolution surface electrodes such as Laplacian electrodes. The spatially filtered summation of several MUAPs recorded on the skin surface, is called surface electromyography (EMG). Its shape depends on various physiological and experimental factors summarized by Merletti et al. [7] displayed in Figure 2. On the one hand, the signal is influenced by geometrical factors such as the fiber location leading to spatial filtering, innervation zone and electrode placement, which are categorized as geometrical factors. These factors

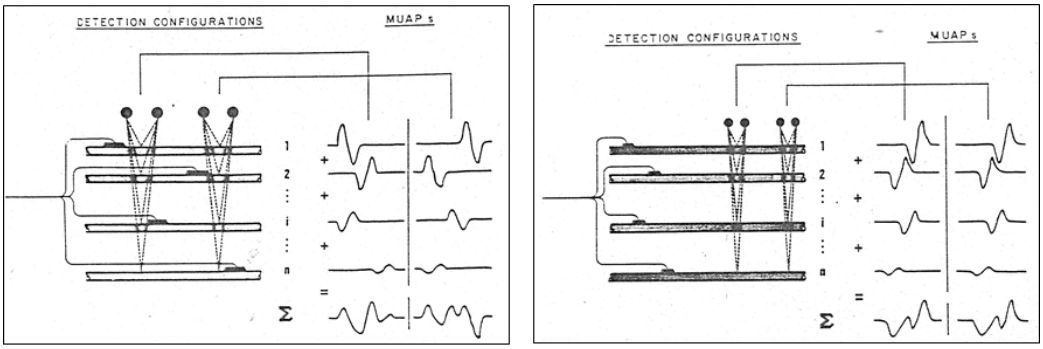

Figure 3: Influence of the electrode position on the recorded EMG signal; left: electrodes placed directly over the nerve junction; right: electrodes placed away from nerve junction; Figure taken from "Muscles Alive" by Basmajian and De Luca [8]

are considered as time invariant. On the other hand, the more physiological factors such as the muscle fiber diameter, intra-muscular temperature, and intra- and extra-cellular pH control the force level. Together with the action potential amplitude and duration they represent the time-variant part of the EMG signal.

Of these factors, the main influencing component for the reproducibility of an EMG signal is the electrode placement. In Figure 3 two detection configurations for single MUAPs are compared. When comparing two signals detected close to each other on top of the innervation zone (left image) and further away from the innervation zone (right image) in Figure 3, it can be observed that the shape of the EMG signal is highly variable directly over the innervation zone and more similar when detected further away from the innervation zone. Basmajin and De Luca [8] therefore suggest that the electrode should always be placed halfway between the innervation zone and the distal tendon. Additionally, muscle fibers are anisotropic [9], therefore it is also important to position the electrodes along the muscle fiber direction. [10]

The neuromuscular origin of the recorded signals relevant for the given test

setup can be divided in two groups:

- Voluntary muscle contraction
- Electrically evoked muscle contraction

They have distinct characteristics and need to be processed separately. In the next two sections, their origins and what has to be considered for their analysis are summarised.

2.2.1 Voluntary Muscle Contraction

A body can be seen as a volume conductor. Surface electrodes detect potential differences between two points on this volume conductor. The collected signal is the weighted sum of all MUAPs in the detection area as illustrated in Figure 4. The contribution of each MUAP is defined by its distance to the detecting electrode and the filtering properties of the tissue in between. Fatty tissue and skin tissue behave like a lowpass filter with decreasing bandwidth and gain with increasing distance. [8]

During voluntary contraction MUs are not activated simultaniously. The sum over all MUAPs results in an stochastic signal where the probability density function has almost Gaussian properties. [7]

2.2.2 Electrically Evoked Muscle Contraction

During a contraction evoked by artificial nerve stimulation, all muscle fibers innervated by the stimulated nerves are excited at the same time. The resulting signal is called compound muscle action potential (CMAP) or M-wave. Figure 5 displays the model for stimulated EMG generation. It shows that if all MUs are stimulated at the same time, the output signal resulting from the summation of several MUAPs has a characteristic shape.

The stimulation artefact, resulting from the nerve stimulation, may become a major problem for signal processing, especially when stimulation and detection sites are close to each other. The type of stimulation (rectangular,

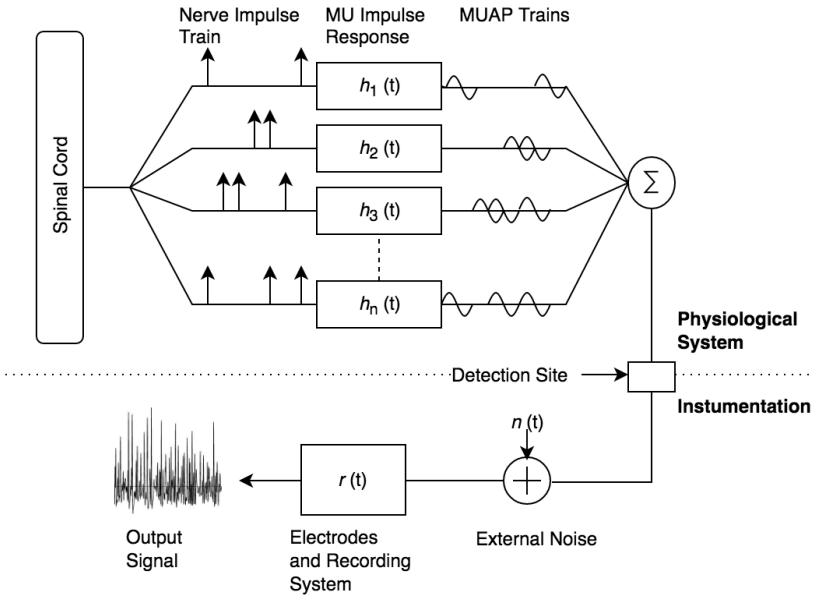

Figure 4: schematic representation of voluntary EMG signal model generation adapted from [8]

gaussian, monophasic, biphasic,..) has been shown to have minimal influence in the response shape. [11]

An example CMAP derived from a healthy individual is displayed in Figure 5 as output signal. In healthy individuals, this stimulation response is relatively similar. Its characteristic time domain properties are the positive peak amplitude, peak to peak amplitude, positive peak latency and duration. Its amplitude gradually increases with increasing stimulation intensity until the maximum M-response is reached. At this point a higher stimulation intensity does not lead to an increase in M-wave amplitude anymore because all MUs are excited. In this condition the M-response is not stochastic but rather deterministic and the associated signal processing is different to voluntary contractions.

Remark on convention: It is important to note that in this thesis all positive CMAP peaks are displayed upwards and negative CMAP peaks down-

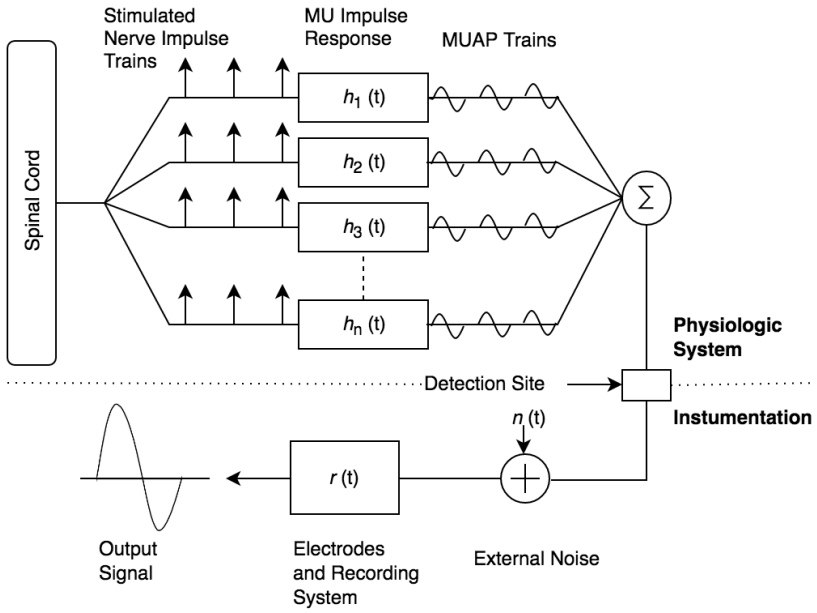

Figure 5: schematic representation of stimulated EMG signal model generation adapted from [7]

wards not following neurophysiological conventions where it is often practice to display it the other way around.

2.3 The Concept of Fatigue

Fatigue is commonly used as a term to describe the decrease in physical performance, generally characterized as the inability to sustain a certain effort or fulfill a task. This quantitative approach suggests that fatigue has a rather sudden onset or "break point" and indicates that there is no fatigue before this point. However, it has been shown that there are already many neurophysiological phenomena going on even before the subjective onset of fatigue [12] [13] [14]. These changes may occur slowly or rather fast depending on the type of MU, exercise and the load level.

A distinction is made between peripheral fatigue and central fatigue [15]

[16]. The former, summarises effects such as [15]:

- Neuromuscular transmission
- Excitation-contraction coupling
- Availability of metabolic substrates
- Performance of the contractile apparatus
- Blood flow

Central fatigue on the other hand, describes factors such as:

- Excitation of the motor cortex
- Excitatory drive of CNS to the motoneuron
- Motor neuron excitability

Many of these neurophysiological changes are detectable within surface EMG measurements. To reduce the complexity of these influences on the EMG, most fatigue tests have been conducted using isometric contractions. However, they are not representative for most contractions in daily life, where mainly dynamic movements are utilized.

2.3.1 Peripheral Fatigue

Peripheral fatigue encompasses all neuromuscular and metabolic factors originating from the muscle itself. Within the muscle, physiological changes may occur on a cellular or more general on a muscular level. It has been shown that accumulation of metabolic products and alterations in transmitter release are directly linked with fatiguing processes [17] [18] [19].

2.3.2 Central Fatigue

While the influence of peripheral fatigue is generally accepted, the existence of central fatigue is still being debated. It was first introduced in the early 1900s by Mosso [20] followed by others [21] [22] [12] [23]. The findings to underpin the theory of central fatigue are mostly based on measuring the interpolation twitch, which is the additional force produced by a supramax-

imal electrical stimulation, delivered during a voluntary contraction. The measurement of this phenomenon and the exercise induced increase of the stimulated amplitude is generally seen as evidence for central fatigue [24] [25] [26]. However, this method has been strongly criticised by de Haan et al. [27] or Herzog [28]. Recently, Contessa et al. [29] has indicated that it is possible to explain all fatigue behaviours through peripheral fatigue alone, strongly questioning the existence and necessity of central fatigue.

2.4 Noise Sources in EMG

The most common noise sources are summarised below [30] [10]:

a) **Ambient Noise**

The human body constantly acts like an antenna, collecting electromagnetic radiation from all surrounding sources such as radios, power lines, light bulbs or any other electrical device. Thereby the power line has the greatest impact and depending on the country, its frequency can be 50Hz (Europe) or 60Hz (North America) and its amplitude can be as big as 3x the EMG signal [30]. The ambient noise is usually filtered offline, using different kind of filters e.g. notch filter or frequency interpolation [30].

b) **Motion Artifact**

Motion artefacts are generated by movement of the cable connecting electrodes and amplifier or at the interface between electrode and skin. Its frequency ranges between 1-10Hz. To reduce this kind of noise recessed electrodes with a conductive gel layer between skin surface and electrode can be used. The second type of motion artefact originates from potential differences between skin layers. Treating the skin with sandpaper [31] or using a puncture electrode technique [32] has shown to reduce the skin impedance.

c) **Inherent Noise**

All electronic parts of the recording system are known to generate electrical noise (inherent noise). Its frequency can range from 0Hz to several 1000Hz. It can not be eliminated but only be reduced by using high quality equipment.

d) **Internal Noise**

Various factors such as the muscle fiber per unit, depth and location of active fibers and amount of intermediate tissue influence the quality of the EMG signal. Therefore, the signal to noise ratio depends largely on the distance between the MUAP sources and the recording electrodes.

e) **Cross Talk**

Recorded EMG signal from unwanted neighboring muscles is called cross talk. It can be minimized by decreasing the electrode size and inter-electrode distance.

2.5 High Spatial Resolution Electromyography

Until recent years, in choosing the type of electrode there has always been a trade-off between spatial selectivity and reproducibility. While it is possible to gain detailed information about specific selected muscle fibers by using needle electrodes, it is also an invasive and painful method. Its high selectivity also causes the problem of reduced reproducibility because a slightly different position of the needle can already cause changes in the EMG signal.

The sEMG signal recorded with conventional electrodes in monopolar or bipolar configuration has been successfully used for monitoring the skeletal muscle activation, the muscle activation onset or fatigue behaviour of the

muscle in general. [33] However, it gives little or no information about single motor units and their individual behaviour.

Therefore high spatial resolution (HSR) electrodes have been developed. With these electrodes, it has become possible to decompose the signal into its individual MUAP trains or to gain information about the location of the tendons, end-plates and the length of the muscle fibre through the amplitude of the signal. [33] They usually consist of one- or two- dimensional electrode arrays with small inter-electrode spacing of a few mm. Different kinds of spatial filters have been developed and tested for their ability to detect single MUs. They are based on the fact that the volume conductor between electrode and active motor unit acts as a spatial lowpass filter [34]. Hence, the closer the motor unit is located to the electrode, the higher are its spatial frequencies and therefore increases its distribution to the resulting potential distribution.

Mathematically, spatial filtering can be expressed as followed, described by Merletti and Parker [14]:

A potential distribution $\phi(x, z)$ is moving along the fiber direction, which is equal to the z axis and of infinite length. For simplicity we assume $t_0 = 0$, $x_0 = 0$, and $z_0 = 0$. In case of a single point detection electrode we get:

$$V_0 = [\phi(x, z) * (\delta(x)\delta(z))]_{z=0}^{x=0} \tag{2.1}$$

where $\delta(.)$ is the Dirac distribution. For a generic point $(x_\mathrm{i}, z_\mathrm{i})$ we get

$$V_\mathrm{i} = [\phi(x, z) * (\delta(x + x_\mathrm{i})\delta(z + z_\mathrm{i}))]_{z=0}^{x=0} \tag{2.2}$$

The linear summation of several different points results in

$$\sum_{\mathrm{i}=0}^{M} a_\mathrm{i} V_\mathrm{i} = \sum_{\mathrm{i}=0}^{M} [\phi(x, z) * (a_\mathrm{i}\delta(x + x_\mathrm{i})\delta(z + z_\mathrm{i}))]_{z=0}^{x=0} = \left[\phi(x, z) * \sum_{\mathrm{i}=0}^{M} (a_\mathrm{i}\delta(x + x_\mathrm{i})\delta(z + z_\mathrm{i}))\right]_{z=0}^{x=0} \tag{2.3}$$

where the number of electrodes is $M+1$ and $a_{\mathrm{i}}(\mathrm{i} = 0, ..., M)$ are the weights assigned to the linear combination. By adding the temporal variable t to Eq. 2.3 we get

$$\sum_{\mathrm{i}=0}^{M} a_{\mathrm{i}} V_{\mathrm{i}}(t) = \left[\phi(x,z) * \sum_{\mathrm{i}=0}^{M} a_{\mathrm{i}}\delta(x+x_{\mathrm{i}})\delta(z+z_{\mathrm{i}})\right]_{z=-vt}^{x=0} = \left[\phi(x,z) * h(x,z)\right]_{z=-vt}^{x=0} \tag{2.4}$$

where $h(x,z)$ is equal to a two-dimensional spatial impulse response

$$h(x,z) = \sum_{\mathrm{i}=0}^{M} a_{\mathrm{i}}\delta(x+x_{\mathrm{i}})\delta(z+z_{\mathrm{i}}) \tag{2.5}$$

The two dimensional Fourier transform of Eq. 2.5 is given by

$$H(f_{\mathrm{x}}, f_{\mathrm{y}}) = \sum_{\mathrm{i}=0}^{M} a_{\mathrm{i}} e^{j2\pi f_{\mathrm{x}} x_{\mathrm{i}}} e^{j2\pi f_{\mathrm{x}} z_{\mathrm{i}}} \tag{2.6}$$

To design spatial filters with certain characteristics, different weights a can be selected. The basic condition, which is usually met by all spatial filters, is

$$H(0,0) = \sum_{\mathrm{i}=0}^{M} a_{\mathrm{i}} = 0 \tag{2.7}$$

which ensures the rejection of DC components in both spatial directions and thereby the absence of common mode signals.

Some common filter masks are displayed below:

$$M_{\mathrm{BipT}} = \begin{bmatrix} 1 & -1 \end{bmatrix} or M_{\mathrm{BipL}} = \begin{bmatrix} 1 \\ -1 \end{bmatrix} \tag{2.8}$$

$$M_{\mathrm{DDT}} = \begin{bmatrix} -1 & 2 & -1 \end{bmatrix} or M_{\mathrm{DDL}} = \begin{bmatrix} -1 \\ 2 \\ -1 \end{bmatrix} \tag{2.9}$$

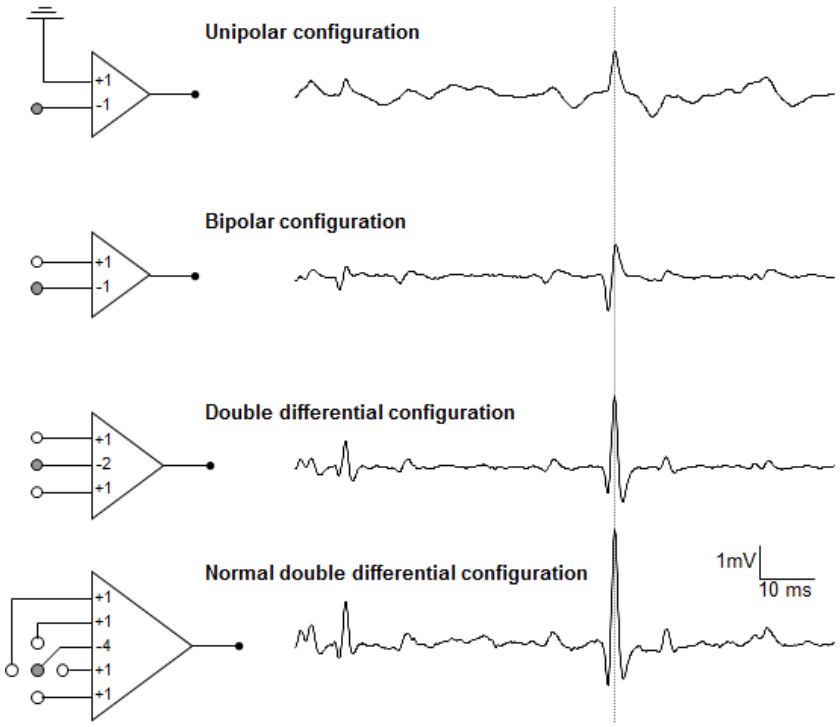

Figure 6: EMG signals recorded with monopolar, bipolar, double differential or normal double differential (Laplacian) electrode configuration; Figure obtained from Hogrel, unpublished

$$M_{\text{Lap}} = \begin{bmatrix} 0 & -1 & 0 \\ -1 & 4 & -1 \\ 0 & -1 & 0 \end{bmatrix} \tag{2.10}$$

Various studies have been conducted, trying to find the best electrode configuration and weighing coefficients. An example EMG signal, recorded from the m. abductor pollicis brevis at maximum voluntary contraction, for all four electrode configurations, can be seen in Figure 6. Simulations as well as studies with real measurements have identified the normal double differential (NDD) configuration, or also called laplacian configuration, as the preferred

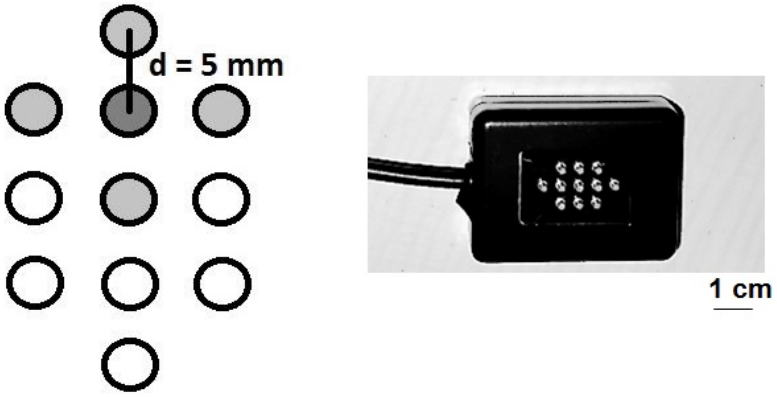

Figure 7: left: schematic representation of the laplacian electrode; four light grey monopolar electrodes are combined with the dark grey electrode to form one laplace channel; inter-electrode spacing is 5mm; right: picture of the original laplacian electrode

high spatial electrode configuration. [35][36][37]

Figure 7 shows the Laplace electrode setup. Eleven monopolar electrodes, with an inter-electrode spacing of 5mm, simultaneously record EMG signals. The spatial filter maske described in Equation 2.10 is applied. The electrodes marked as light grey correspond to a weighing factor of -1 and dark grey to a weighing factor of 4. This filter mask can be applied three times within one laplacian electrode, resulting in three laplacian channels.

2.6 Signal Processing in EMG

In general there are two different approaches to process EMG signals: Time Domain and Frequency Domain. Whereas with frequency domain, usually the Fourier transform is meant. These two methods have been used for many decades now and there is a wide variety of applications for bio-signals such as EMG, EEG or ECG but also other signals for example audio signals etc.

While the time domain analysis can give a first impression for EMG signals, its results have to be interpreted with caution. Especially absolute values of amplitude, root mean square (RMS) etc. might be influenced by noise, which can make them incomparable between subjects or test repetitions. Normalization is one possibility to overcome this problem. Typical normalization methods include [38]:

- Maximum (peak) activation levels during maximum voluntary contractions (MVC)
- Peak or mean activation levels obtained during the task under investigation
- Activation levels during sub-maximal isometric contractions
- Peak to peak amplitude of the maximum evoked M-wave (M-max)

Another possibility is to move from the time domain to the frequency domain. While the standard function for bio-signals is the Fourier transform, there are also other mathematical functions such as the Fourier Series, Z-transform, or wavelet transform that can be applied. Especially the wavelet transform has gained interest in the past years and new ways of analysing signals have been developed. For the wavelet transform the signal is compared to a so called mother wavelet and the wavelets coefficients are calculated.

2.7 Electromyography for Diagnostic Purposes

Surface Electromyography is a thoroughly researched procedure, which has found a large number of applications in various fields such as prosthetics, rehabilitation and research. Three major clinical reasons for the use of sEMG have been identified by Hogrel in 2005 [39]: 1) physiopathological insight, 2) diagnosis and 3) the followup of patients. Various research studies have shown that sEMG has the ability to give physiological insight and has diagnostic capacitz for various neuromuscular pathologies [40] [41] [42] [43]. Nevertheless, its diagnostic purpose in everyday clinical practice is still poorly

accepted [44] [39], because of its complex interpretation and possible pitfalls. Therefore, the following issues should be considered before EMG testing [45]:

- noise/interference
- room temperature
- patient cooperation (e.g. MVC tests)
- patient relaxation

Especially the influence of the room temperature is often overlooked when working with neuropathy patients, even though cooling might have a severe impact on the electromyographical output. On the other hand, heat does not influence the EMG of normal subjects and should therefore be preferred.

2.8 Non-Dystrophic Myotonia

For this thesis, the EMG signals of Non-Dystrophic Myotonia (NDM) patients were processed. NDM is a rare skeletal muscle disorder that is characterized by an increased electrical excitability of the muscle fiber membrane. Worldwide 1 out of 100 000 people are affected [46]. While the worldwide prevalence is rather low, Baumann et al. [47] has shown that the prevalence can vary with geographic location. He conduced an epidemiologic and genetic study in northern Finland and discovered that in his study area MC appeared in 7.3 out of 100 000 people, which is higher than the worldwide prevalence.

The general symptoms include delayed relaxation or stiffening of the muscle (myotonia) after voluntary contraction and may be increased after sudden and strong contractions. [48] [49] The muscles usually appear hypertrophic, which is contrary to most other muscular diseases. [48] The two main causes are mutations in voltage-gated skeletal muscle sodium (SCN4A) and chloride (CLCN1) channels. [48] [49] However, the type of symptoms and severity of the disease can differ depending on the type of NDM. It is classified according to the location of the mutation in the gene. The two major disorder

classifications are described below.

a) **Chloride Channel Disorder**

The mutation in the chloride channel gene (CLCN1) on chromosome 7q is collectively referred to as myotonia congenita (MC) and can be inherited recessively or dominantly. Depending on the mechanism of inheritance MC is separated into autosomal-recessive myotonia congenita (Becker's disease) and autosomal-dominant myotonia congenita (Thomsen's disease). Thomsen's disease is a rare and mild form of MC.

MC causes the permanent reduction of the resting chloride conductance of muscle fiber membranes, which is necessary for fast re-polarisation of the muscle fibre membranes. Due to the dysfunction of the chloride channel, the muscle fiber membrane stays depolarised. Myotonia congenita may appear in early childhood, but is generally not progressive. [50] A warm up phenomenon can be observed in patients with MC. This means that the symptoms decrease with prolonged activity.

b) **Sodium Channel Disorder**

The second major form of NDM is based on a mutation in the sodium channel gene (SCN4A) on chromosome 17q. It is autosomal-dominantly inherited and can be separated in paramyotonia congenita (PC) and sodium channel myotonias (SCM). In patients with PC, the mutation causes an activation defect of sodium channels leading to a long lasting depolarisation of the muscle fibre membrane. Myotonia occurs during exercise and worsens with continuous activity[51].

Neuromuscular diseases such as MC and PC are usually diagnosed through genetic testing. However, since gene tests can be time consuming, expensive and may have a false negative rate of 20% [52], electrophyosiological tests have been tested for their ability to diagnose neuromuscular diseases. For this purpose two tests have been developed: Short Exercise Test (SET) [53] and

		PC	**MC**
needle EMG	**myotonic discharges**	Abundant	Abundant
CMAP after SET	**post-exercise myotonic potentials (PEMP)**	Yes	Yes or no
	Amplitude change after first trial	Increase or decrease	Transient decrease
	Amplitude change after second or third trial	Gradual decrease	No
CMAP after LET	**Immediate change of amplitude**	Decrease	No or slight decrease
	late change of amplitude	Decrease	No
Cold induced changes		Yes	No

Table 1: Electrophysiological pattern, adapted from Fournier et al. [55] [59]

Long Exercise Test (LET) [54]. During the SET, CMAPs are recorded before (baseline) and every 10s after (post exercise) a 10s fatigue exercise. Whereas for the LET, CMAPs are recorded at baseline and every minute after a 5 min exercise period. Various research groups have detected electromyographic similarities and differences between MC and PC patients, applying these two, or similar test procedures [55] [56] [57] [58]. Their findings are summarised in Table 1.

Even though the understanding of neuromuscular diseases has increased during the last decades, there are still some unresolved questions. Current issues, which need to be addressed are the correlation between genotype and phenotype, treatment effectiveness and why some treatments lose their effectiveness over time [52].

3 Methodology

3.1 Problem Description

Two sets of EMG signals were analysed during the course of this internship. One derived from a healthy control group and one from patients with non-dystrophic myotonia. The testing protocol was similar for both groups, albeit using slightly different recording electrodes.

The control group has already been analyzed manually. Part of the data has been previously published to show differences between myotonia dystrophy type 1 and control [60]. The results from this study were used as reference values for the automatic processing in Matlab.

The patient study was conducted at the Myology Institute, Pitié Salpetrière Hospital in Paris. Its aim was to gain insight into the neuromuscular excitability of non-dystropic myotonia patients and compare them with healthy subjects. Therefore, the EMG from 12 patients with myotonia congenita or paramyotonia was recorded. This neuromuscular excitability study was part of a bigger clinical study, testing the influence of the drug Mexiletine in NDM patients. The patients were separated in two blinded groups and for 18-22 days they received either the drug or a placebo. After a washout period of 4-8 days they would again receive either the drug or placebo. The study design is shown in Figure 8.

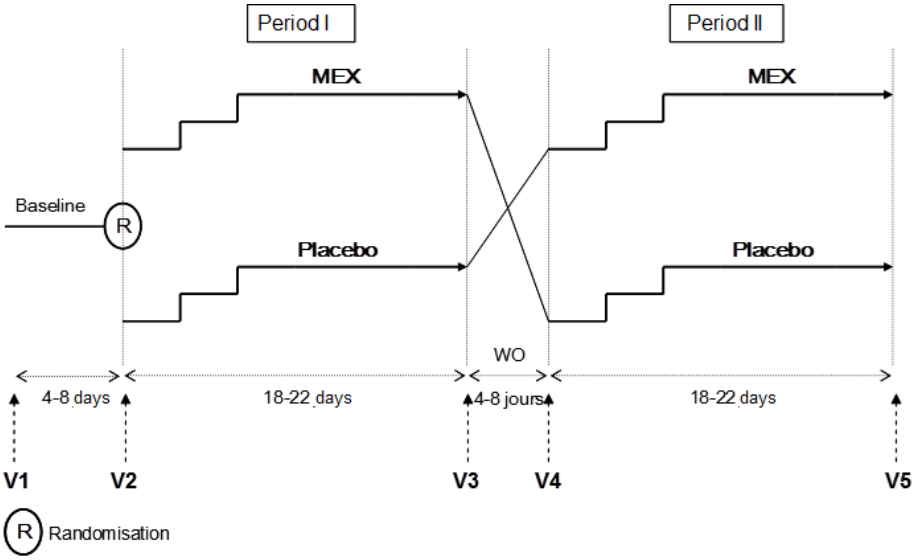

Figure 8: Study design of the Mexiletine Study, EMG measurements were conducted on visit 1, 3 and 5

3.2 Control Group

The EMG signals from 10 healthy control subjects have been previously recorded and processed manually using LabView. This data set was used as a control for the automated calculations in Matlab. The group consists of 5 male and 5 female and recordings were done on the dominant hand.

3.3 Patient Group

Surface EMG signals from 12 patients (7 male and 5 female) diagnosed with either myotonia congenita (MC) or paramyotonia congenita (PC) were recorded (see Table 2). The diagnosis was made on the basis of a genetic test and the symptoms had to be severe enough to justify treatment. This is the case when at least two segments (upper limb, lower limb or face) are affected and the patients are affected on at least 3 out of 7 days. The pa-

tients were aged between 21 and 59 years. The data was collected from the dominant hand of the patients, out of which eleven were right-handed and one left-handed.

Patient	Age	Sex	handedness	measured hand	Type of NDM
1	48	M	R	R	MC
2	50	M	R	R	MC
3	53	F	R	R	PC
4	39	M	R	R	PC
5	59	M	R	R	PC
6	49	M	R	R	PC
7	38	F	R	R	PC
8	24	M	R	R	PC
9	49	F	R	R	MC
10	21	M	R	R	PC
11	43	F	R	R	PC
12	35	F	L	L	PC

Table 2: age, gender, measured hand and type of NDM for each patient

3.4 Equipment

3.4.1 Stimulation device

For the stimulation the DS5 (Isolated Bipolar Constant Current Stimulator) from Digitimer Lgt was used. [61] Its technical specifications are summarized in Table 3. In combination, the Ambu BlueSensor electrodes were utilized to apply the stimulation.

Output	Bipolar constant current proportional to the input voltage
Output range	+-10; +-25; +-50 mA for a full scale input
Input ranges	+-1; +-2.5; +-5; +-10 V full scale
Safety limits	50mJ/300mJ pulse energy
	50uA average "idle" current
	10mA average pulse current
	50mA peak current
	1s/5s maximum pulse duration

Table 3: Technical specification of the DS5 stimulator [61]

3.4.2 Electrodes

The electrodes used for the patient study were secondary laplacian electrodes. They consist of 11 individual monopolar electrodes, from which the laplacian configuration can be calculated. Therefore, the sum with laplacian weighing factors is calculated over the 5 monopolar electrodes marked in grey in Figure 7 on page 17. Usually this is done by the electrodes itself, resulting in only three EMG channels. However, for the patient study the 11 monopolar EMG signals were recorded and the laplacian signal calculated later during digital signal processing. This gives the possibility to compare the output of different electrode configurations as for example bipolar, double differential or laplacian as we can see in Figure 6. The laplacian configuration was used for further processing steps in order to be able to compare the results with the existing data of healthy subjects.

The EMG signals for the controls were recorded using built in laplacian electrodes which automatically calculate the laplacian signals with an amplification factor of 100. Further, it was possible to add an additional amplification factor of 2, or 5 afterwards. This factor could change from subject to subject and was not written down for all controls. Therefore, the RMS and amplitude values of only 5 controls could be used because of the missing information about the amplification factor. However, this was only a problem

for the control group as a different set of electrodes was used for the patients.

3.4.3 Amplifier

The Biosignal Amplifier from g.tec [62] was used for the monopolar signal amplification. It has the possibility to simultaneously collect multi-modal signals such as EEG, EMG, EOG or ECG. It has up to 16 bipolar/real differential input channels and its analog output ranges between +/- 5V. It is compatible with a variety of user-specific systems.

3.4.4 Force Transducer

For the recording of the force, a load cell was used. The recorded electrical signal is directly proportional to the applied force. It has a sensitivity of 1g and a range of 0-10kg.

3.5 Experimental Protocol

EMG signals were recorded from the ADM of the right hand using high spatial resolution electrodes. The developed strength of the ADM was recorded using the force transducer. Stimulations were performed on the ulnar nerve at the wrist level. During the fatigue test the subjects adjusted their force output through visual feedback of the recording system. The measuring setup is displayed in Figure 9.

For the patients the following tests were repeated on three different test days:

- Finding maximal M-wave pre fatigue
- Refractory Test pre fatigue
- Supernormality Test pre fatigue
- 5Hz stimulation pre fatigue

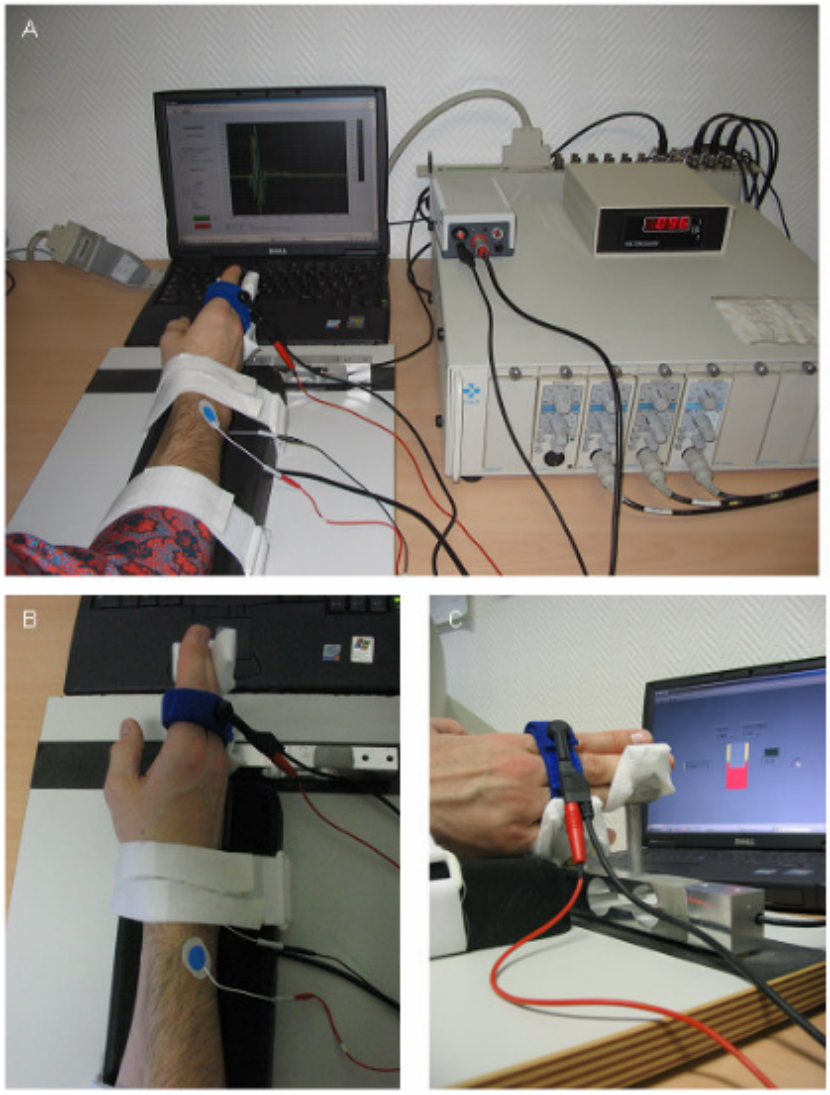

Figure 9: Measurement setup for the control group measurements; A: position of the test person in front of the force feedback interface; B: wrist and finger fixation; C: hand placed on the force transducer measuring the output force

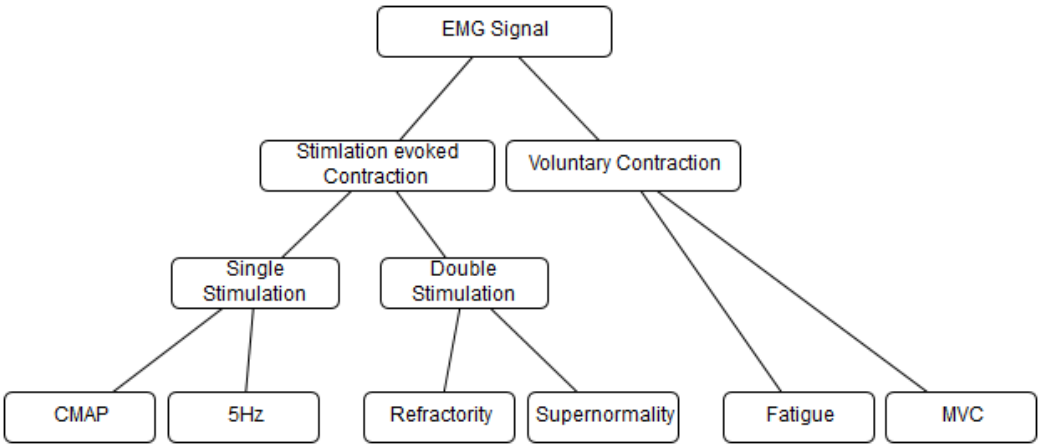

Figure 10: Subdivision of the EMG signals derived during the neuromuscular excitability study

- MVC pre fatigue
- Fatigue Test
- Stimulation at maximal M-wave Intensity post fatigue
- Refractory Test post fatigue
- Supernormality Test post fatigue
- 5Hz stimulation post fatigue
- MVC post fatigue

The control group conducted the same test protocol without the 5Hz, refractory and supernormality test.

For better understanding and also because of similarities during processing, these individual tests can be summarized in subgroups as seen in Figure 10. During CMAP and 5Hz test a single stimulation is performed, whereas refractory and supernormality are double stimulation tests. Single as well as double stimulation are both stimulation evoked contractions, while Fatigue and MVC test are based on voluntary contraction.

The individual tests and its measurement parameters are described more closely in the next few sections.

3.5.1 CMAP

During nerve stimulation, two major types of responses can be observed, H-reflex and M-wave. The former was first discovered by Hoffman in 1910 [63] and occurs when a sub-maximal stimulus activates a peripheral sensory nerve. The activation of efferent Ia fibers triggers a signal at the motor neuron in the spinal cord, which leads to a small contraction of the muscle fibers called the H-reflex. If the stimulation intensity increases, the H-reflex decreases and the direct motor response (M-wave) increases.

The goal of the maximum M-wave test is to increase stimulation intensity until maximum amplitude of the M-response is achieved and no H-reflex occurs.

For this test, a single biphasic stimulation with a duration of 0.5ms was used.

3.5.2 5Hz Test

The repetitive stimulation test is based on the fact that repeated stimulation may lead to a decrease in amplitude for patients with myotonia. [64] Similar to the CMAP test, a single biphasic stimulation with a duration of 0.5ms was applied. A stimulation frequency of 5Hz within an time interval of 1 second resulting in 5 successive stimuli.

3.5.3 Refractory Test

The refractory period is a time window, following a stimulation, during which a muscle or nerve fiber cannot be excited. In humans, this time period may vary between 2.2 and 4.6ms [65]. For this test, two stimuli were applied 2.6ms apart, to see if it is possible to trigger a response. It was conducted using a biphasic double shock where the first stimulation was at 120% of the maximum intensity detected during the maximum M-wave test and the second stimulation at 70%.

3.5.4 Supernormality Test

Contrary to the refractory period, the excitation of a response is facilitated during the supernormality period. [66] This phenomenon is due to an after-potential phenomenon [67] and has its maximum at around 7ms after the stimulation. For this purpose, a double stimulation with an inter-stimulus interval (ISI) of 7ms was applied. Again, a biphasic double shock with the same intensity as for the refractory test was used.

3.5.5 MVC

This test is generally applied to detect the maximum voluntary contraction strength of an individual. The person is asked to perform a maximal contraction of a predefined muscle group. The strength results of this test may be used for further tests such as for example fatigue testing or the EMG signal may be used for normalization. However this test strongly relies on the participation of the test subjects. It therefore has some limitations when it comes to children or patients who might be afraid of pain when contracting maximally.

3.5.6 Fatigue

The fatigue test was performed for 45 seconds at 60% of the maximum voluntary contraction. The force level was targeted through a visual feedback system.

3.6 Signal Processing

3.6.1 Spatial Filtering - Laplace

For this study, spatial filtering was applied. In Figure 11, four different electrode configurations (monopolar, single differential/bipolar, double dif-

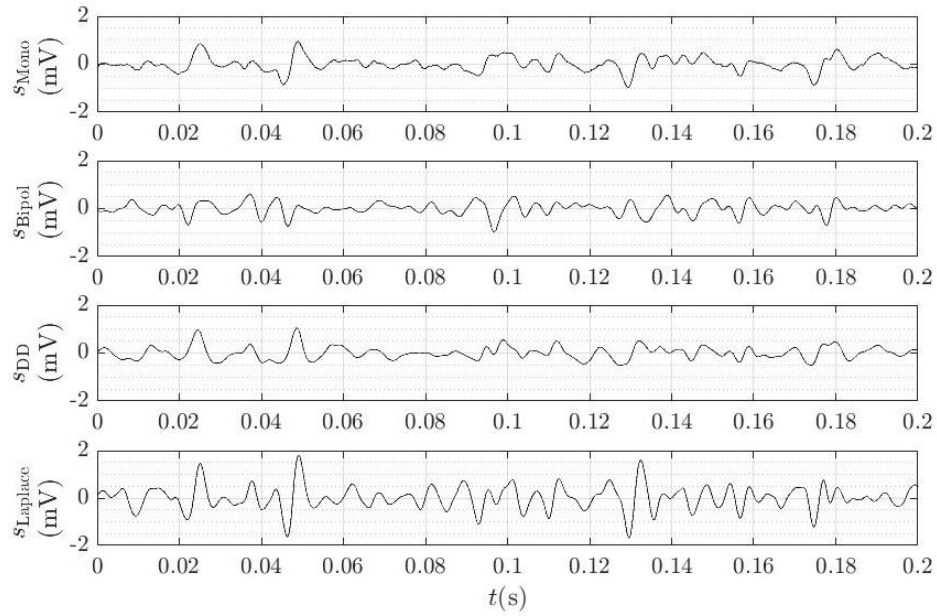

Figure 11: EMG signal during MVC of a patient with non dystrophic myotonia in monopolar, bipolar, double differential (DD) and laplacian configuration

ferential, Laplace) are displayed. RMS in Laplacian electrode configuration is considerably higher than during the other three configurations. The Laplace configuration was chosen for further calculations in order to be able to compare the results with already existing results of healthy subjects.

3.6.2 Stimulation Evoked EMG Signals - Single Stimulation

For the maximum M-response as well as the 5Hz stimulation test, several repetitions were made. While the 5Hz test was conducted automatically five times in one second, the amount of repetitions made during the maximum M-response test could vary between 5 and 18 times. Thereby, the examiner tried to record at least 5 valid repetitions.

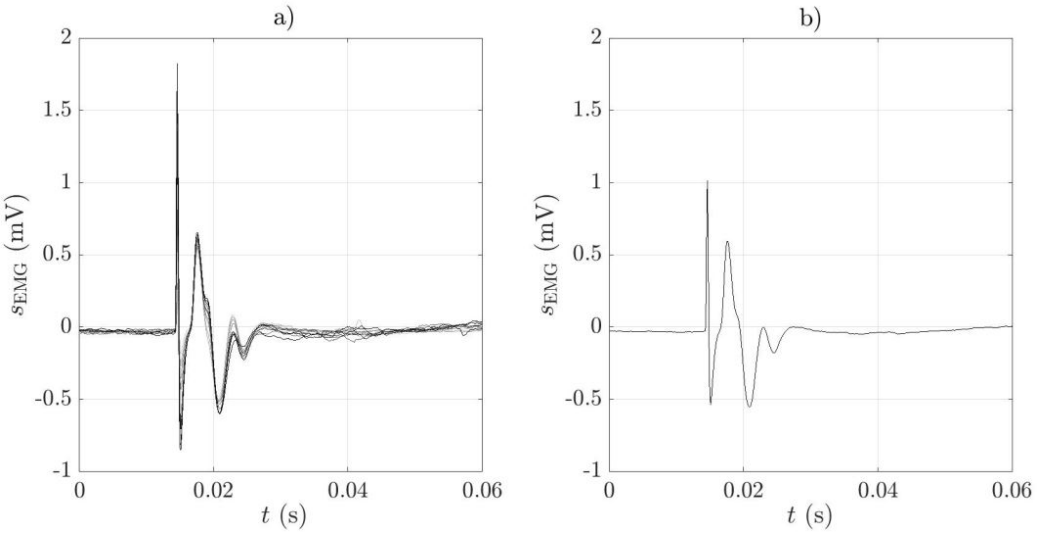

Figure 12: a) shows the individual EMG signals from various iterations that were selected for further calculations; b) shows the mean signal calculated from the signals shown in a)

The mean signal is only calculated for the maximum M-wave test. For the 5Hz test at least 4 out of the 5 recordings had to be valid and were analysed individually.

3.6.2.1 Calculation of mean Signal

Once the Laplace signal was calculated for each stimulation, the signals were checked for their similarity. Therefore, the correlation between the individual signals was calculated. Only if 3 or more signals have a correlation coefficient above 0.9, they are included for further processing steps. From the remaining signals the mean of all repetitions is calculated. The formation of a mean signal helps to filter random noise from the signal. [55]

3.6.2.2 Manual Signal Quality Validation

After selecting only similar recordings, signals similar to the example in Figure 12b) are the result. However, this is only the case if the signal quality of the recordings is sufficient. The following major error classes can be identified:

1) The M-response shape of healthy individuals depends on the recording location. Usually the electrode is placed between the nerve junction and the distal tendon, resulting in an M-response as seen in Figure 12. If the electrode is placed on the other side of the nerve junction towards the proximal tendon, it leads to the inversion of the polarization [68]. The resulting EMG is displayed in Figure 13a.

2) Another problem occurs when the signal is recorded on top of the nerve junction. It may lead to the decrease of the response amplitude and distorted signals [68] displayed in Figure 13b.

3) One major noise for stimulated signals is the baseline drift after stimulation. It is characterised through a signal offset directly after the stimulation and may take up to a few milliseconds to return to the original baseline as it can be seen in Figure 13c. The signal distortion appears similar to the shape changes described by McGill et al. [69] and might therefore be due to the saturation of the amplifier. The baseline drift is so severe that the signal becomes unusable for further processing. This amplifier problem can be avoided by increasing the distance between stimulation and recording electrodes, decreasing the amplifier's high-pass cutoff [69] or by blocking the stimulation peak with simple circuits [70].

4) One last problem was the complete absence of stimulation artefact as well as the stimulation response. Usually, the recording is triggered automatically by the device 20ms before the actual stimulation. However, it is

possible that the recording was triggered but no stimulation was made, resulting in an "empty" recording. (Figure 13d)

The signal quality validation is done manually as well as automatically by visual control of the signal. The mean signals are classified as one of the problem classes described above or as valid response signal. While the absence of stimulation artefact as well as the stimulation response is considered as wrong signal, the absence of a response signal with present stimulation artefact is treated as valid response for patients with PC as the electrical silence is considered a symptom for cold temperatures.

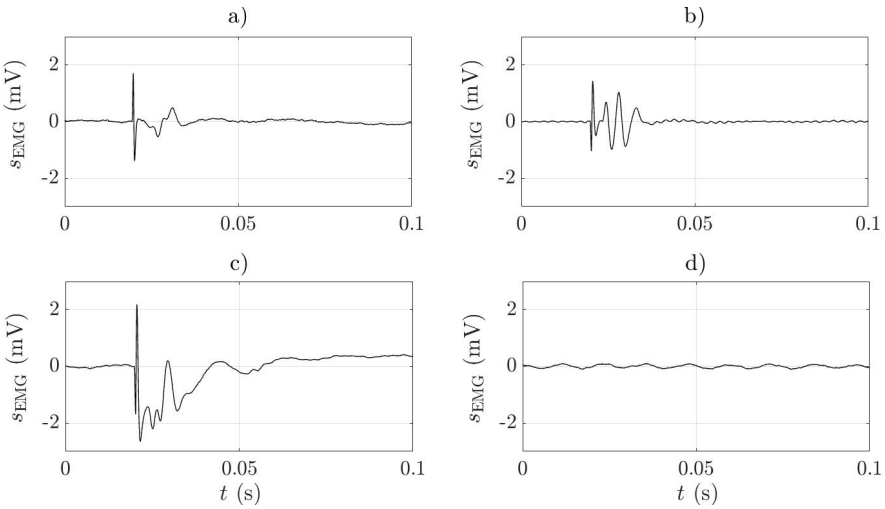

Figure 13: add caption

3.6.2.3 Parameters Calculation

Classical parameters of interest of the CMAP derived from the time domain include:

- Total amplitude
- Response latency
- Area under the positive curve (pp surface)
- Area under the negative curve (np surface)

- Duration of the positive peak (duration pp)

The amplitude is simply defined as the voltage difference between the maximum positive peak and maximum negative peak and represents the sum of all potential amplitudes. The latency is commonly defined as the time between stimulation and response onset while the duration is the time between departure from the baseline till return to the baseline. The duration of the positive peak only takes into account the duration of the first positive peak. Where there is underlying pathology the nerve conduction velocity may vary, which leads to a reduction of the amplitude and longer durations. [71]

An alternative way of looking at the CMAP has been proposed by Mahbub and Rabbani in 2007 [72]. Neurological disorders often result in a delay in fiber stimulation and hence in a prolonged or distorted CMAP signal. This should not only be clearly visible in the time domain but also notable in the frequency domain. Therefore, instead of processing the EMG signals in the time domain, they transformed the signals to the frequency domain and compared various different frequency parameters for their ability to discriminate between different neurological diseases. They were able to identify 10 parameters, showing highly significant differences and 17 with significant differences using a basic t-test. The following 13 parameters were selected and calculated for the control and patient groups:

- peak amplitude (A)
- peak frequency (f)
- frequency width at 10% of maximum peak Amplitude ($\Delta f_{10\%}$)
- frequency width at 50% of maximum peak Amplitude ($\Delta f_{50\%}$)
- frequency width at 50% of maximum peak Amplitude ($\Delta f_{90\%}$)
- area under the curve from 0 to 1 kHz (a_{0-1})
- area under the curve from 1 to 5 kHz (a_{1-5})
- $R_{10/90} = \frac{\Delta f_{10\%}}{\Delta f_{90\%}}$
- $R_{10/50} = \frac{\Delta f_{10\%}}{\Delta f_{50\%}}$

- $R_{10/\mathrm{p}} = \frac{\Delta f_{10\%}}{f}$
- $R_{50/\mathrm{p}} = \frac{\Delta f_{50\%}}{f}$
- $R_{90/\mathrm{p}} = \frac{\Delta f_{90\%}}{f}$
- $R_{\mathrm{a}} = \frac{a_{0-1}}{a_{1-5}}$

For the 5 Hz test the development of the amplitude over time was of interest. Therefore, the linear regression for the total amplitude was calculated to see if there is a potential decease.

$$y = kx + d \tag{3.1}$$

Where k is defined as the slope of the regression and d as the intercept.

3.6.2.4 Channel Selection

After the signals were already classified for their quality. It was assumed that only signals with adequate signal quality had been included for parameter calculations. Out of the remaining channels, the channel with the highest CMAP amplitude was selected for further calculations.

3.6.3 Stimulation Evoked EMG Signals - Double Stimulation

The double stimulation signals (refractory and supernormality test) are treated similarly to the single stimulation tests in terms of filtering, mean signal calculation and signal quality classification. Afterwards, it is necessary to remove the response of the first stimulation from the signal, by means of maximum M-Wave subtraction.

Therefore, the mean signal of all recorded CMAPs as well as of the refractory and supernormality tests are calculated, and subsequently the mean maximum M-response is subtracted from the mean double stimulation signal. This approach is schematically drawn in Figure 14.

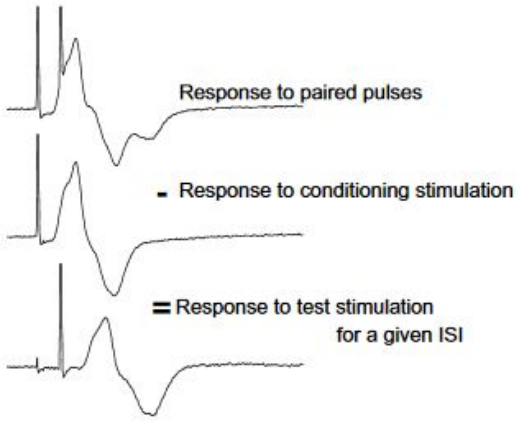

Figure 14: Double stimulation response minus maximum M-response results in single response of the second stimulation; Figure adopted with permission of Böerio et al. [73]

3.6.4 Voluntary EMG Signals

3.6.4.1 Filtering/Noise Cancellation

Filtering plays a major role in signal analysis. Applied correctly, it can help to emphasise wanted features from the surrounding noise and make them visible. Misapplied however, it may also produce more noise than it removes, leading to false conclusions. To avoid this problem it is important to know common noise sources, which were summarised in Figure 13 on page 34, and to record EMG signals with as little noise as possible in order to keep filtering to a minimum.

Two kinds of filters were applied on the voluntary EMG Signals described in the following two sections:

a) **Bandpass Filtering**

To remove movement artefacts of low frequency and interferences as well as the high frequent stimulation artefact, a bandpass filter is applied. Specification in the literature about the lowpass cutoff frequency range

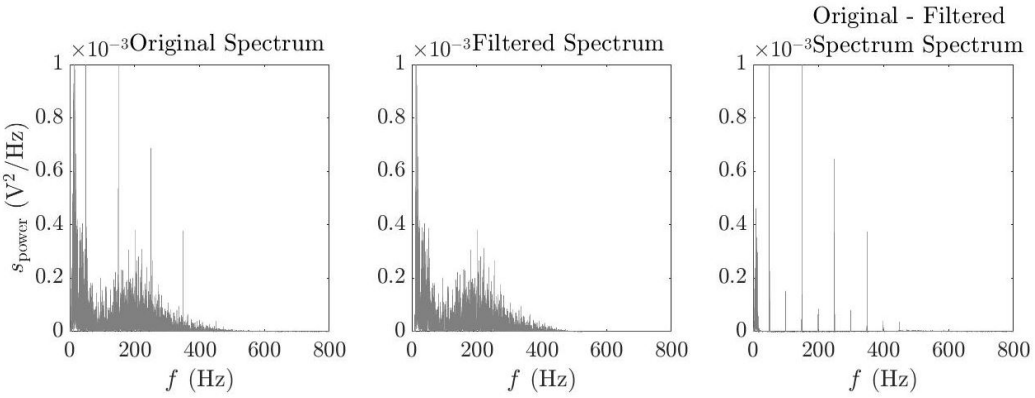

Figure 15: Example Frequency Spectrum of a control subject; left image: it shows original frequency response containing noise and peaks at 50Hz and its harmonics; middle image: filtered power spectrum; right image: filtered spectrum was subtracted from the original spectrum to show only the filtered frequencies

between 5-20Hz. [74] [75] These standards however, are mostly based on laboratory practice and not empirical studies. An exact cutoff frequency is therefore not defined. For this study a band-pass filter between 10-500Hz was applied, using a 4^{th} order butterworth filter. The frequency spectrum of an unfiltered and bandpass filtered stimulated EMG signal is displayed in Figure 15.

b) **Rejection Filtering**

Several ways to remove the 50Hz power line interference (PLI) are proposed in the literature. In 2001, Mewett et al. [76] compared a simple notch filter with Regression-Subtraction and Spectrum Interpolation. He found that the notch filter cannot discriminate between hum and EMG component and therefore distorts the signal. Even though Regression-Subtraction works very well under ideal conditions, it is not able to remove harmonics, which are present in our data. Additionally a reference signal, recorded from the muscle at rest, is necessary for this method but

such a reference signal is not available for this dataset. The second presented alternative to the simple notch filter is the Spectrum Interpolation. It is also not an ideal method, since it does not distinguish between periodic interference and the EMG signal as well. However, in contrast to the notch filter, it does not introduce a phase distortion to the signal. It is therefore a promising method for 50Hz filtering and is used for further calculations. Figure 15 shows example frequency spectra before and after rejection filtering.

3.6.4.2 Channel Selection

As the ADM is a rather small muscle, the standardizion of electrode position is considerably difficult. The goal is to have at least two Laplace channels located in between the innervation zone and tendon to be able to calculate all the parameters and also conduction velocity (CV). The selection of the channel with the best electrode placement is based on two parameters: RMS and Mean Power Frequency (MPF). These two parameters show a contrary behaviour in relation with the location between innervation zone (IZ) and tendon. Mesin et al. [77] have shown that the narrow window at which RMS, MPF and CV is reliable, is located in between the IZ and tendon. For this region the RMS is maximal, whereas CV and MPF are minimal. This relation is displayed in Figure 16. The selection was therefore based on maximizing the RMS and minimizing the MPF.

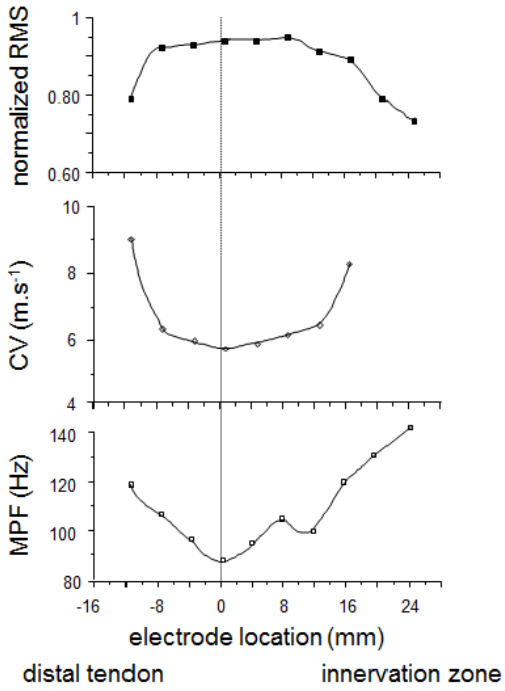

Figure 16: RMS, CV and MPF are displayed for different electrode locations between tendon and innervation zone; at the optimum position is reached when RMS is maximal while CV and MPF are minimal; Figure optained from Hogrel, unpublished

3.6.4.3 Parameter Calculations

As mentioned above, the parameters of interest for the MVC test include:

- Maximum Force
- Root mean square (RMS)
- Mean power frequency (MPF)
- Conduction velocity (CV)

These parameters give information about the EMG signal in time as well as frequency domain.

The RMS level of a vector X is described as:

$$X_{\mathrm{RMS}} = \sqrt{\frac{1}{N}\sum_{\mathrm{i}=1}^{N} \mid X_N \mid^2} \tag{3.2}$$

where N is equal to the sample number of the vector X.

For the frequency domain, the MPF indicates the center of the power distribution and is given as:

$$f_{\mathrm{MPF}} = \sum_{\mathrm{i}=1}^{f_c} f_{\mathrm{i}} P_{\mathrm{i}} \Big/ \sum_{\mathrm{i}=0}^{f_c} P_{\mathrm{i}} \tag{3.3}$$

where f_{i} is the frequency variable, and P_{i} is the i^{th} line in the power spectrum. f_c indicates the cutoff frequency due to the Nyquist theorem.

The conduction velocity v_{CV} is calculated from two EMG channels as followed:

$$v_{\mathrm{CV}} = \frac{d}{t} \tag{3.4}$$

where d is the electrode distance in mm and t the time delay between the channels in ms. The electrode distance for the Laplace electrodes is 5mm and the delay is given by the time difference between the maximum peaks of two channels.

Furthermore the coefficient of variation (VC) c_{VC} for the force can be calculated. It gives information about how well the test person was able to uphold a constant force level. It is calculated as followed:

$$c_{\mathrm{VC}} = \frac{\sigma}{\mu} \tag{3.5}$$

where σ is the standard deviation of the signal and μ the mean value.

Muscle fatigue is a complex phenomenon, which is detectable in various parameters. Numerous research groups [13] [78] [79] have shown that during a fatiguing task the RMS and the average rectified value (ARV) show an initial increase and when approaching the mechanical failure they start to

decrease. More importantly, it has been shown that CV and MPF are reduced significantly over time compared to the beginning [13] [78] [79]. However, while subjective fatigue measures increase linearly with load, MPF is only affected during high load fatigue tasks [79]. Therefore, it is important to ensure an adequate load level when measuring MPF.

To indicate the increase or decrease of the various parameters, different variables may be calculated. One possibility is to compare the mean values gained during the first two seconds with the values calculated from the last two seconds. These values can be used to calculate the ratio r between the mean μ of the first two seconds and the last two seconds of the task as shown in the following formula:

$$r = \frac{\mu_{\text{last 2s}}}{\mu_{\text{first 2s}}} \tag{3.6}$$

Furthermore it is possible to calculate a regression model (e.g. linear regression). Though the outcome always depends on the chosen regression model and the decreasing behaviour of the variable. Therefore Merletti et al. [13] has introduced the area ratio, which is an regression free outcome measure. The area ratio AR is given as:

$$AR = \frac{B}{B + A} \tag{3.7}$$

where A is the area under the curve (grey in Figure 17) and B is the difference between the reference area and the area under the curve (white in Figure 17). It varies between 0 and 1 for decreasing values and is negative for increasing patterns.

3.7 Statistics

For this thesis, the comparison of pre-post observations within individual groups was conducted using a paired t-test. It allows the investigation of the

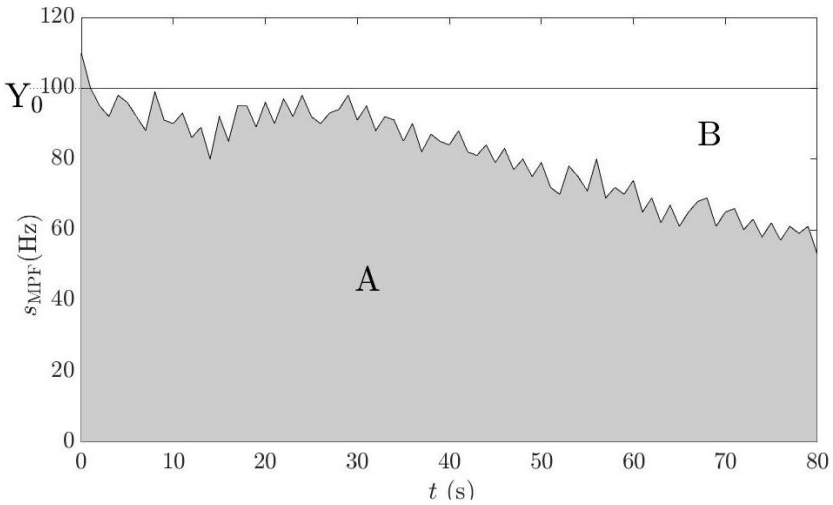

Figure 17: Area Ratio

difference between two populations average and is often used in medicine to find differences before and after an intervention or treatment.

Four assumptions need to be met [80]:

- matched pairs
- normal distribution
- equal variance of two samples
- independent cases

The following steps were conducted [80]:

1) Two hypotheses are defined: the null hypothesis, which assumes that the mean of two paired samples are equal and the alternative hypothesis, which assumes that the means of two paired samples are not equal

2) The level of significance or α level was set to 5%. It defines the probability at which the null-hypothesis is rejected even though it is true.

3) Parameter calculation:

$$t = \frac{\bar{d}}{\sqrt{s^2/n}} \tag{3.8}$$

where $\bar{d}$ is the mean difference between two samples, s^2 is the sample variance, n is the sample size and t is a paired t-test with n-1 degrees of freedom.

4) Null hypothesis is rejected or confirmed depending on the parameter results. If the derived P value is less than the chosen level of significance, the Null Hypothesis is rejected.

4 Results

4.1 Matlab Implementation

Figure 18 shows individual processing steps, which were implemented for the two EMG signal types (voluntary/stimulation evoked). All steps marked as dark grey are completely automatised and all steps marked in light grey are supervised by the user.

During a preliminary Data import/filtering step, the eleven monopolar EMG recordings were transformed into three laplacian channels and filtered if necessary. The signals recorded during electric stimulation were checked individually for their signal quality and classified in the error classes described in section 3.6.2.2. Afterwards, the test specific parameters of interest were calculated and stored. For the EMG signals derived from voluntary contraction, the quality assessment and parameter calculation was combined in one single step because the parameter represent a major factor for the decision process. Basic statistics (mean, standard error (SE), t-test) were applied on the output variables and the results are displayed in the following sections.

The following figures show the interfaces used for the EMG signal processing. Customized graphical user interfaces (GUI) were designed for each test to meet the individual requirements.

The CMAP processing is generally conducted automatically for the whole set of patients or controls. This can be achieved through the left side of the user interface, circled orange in Figure 19. Selections need to be made for the

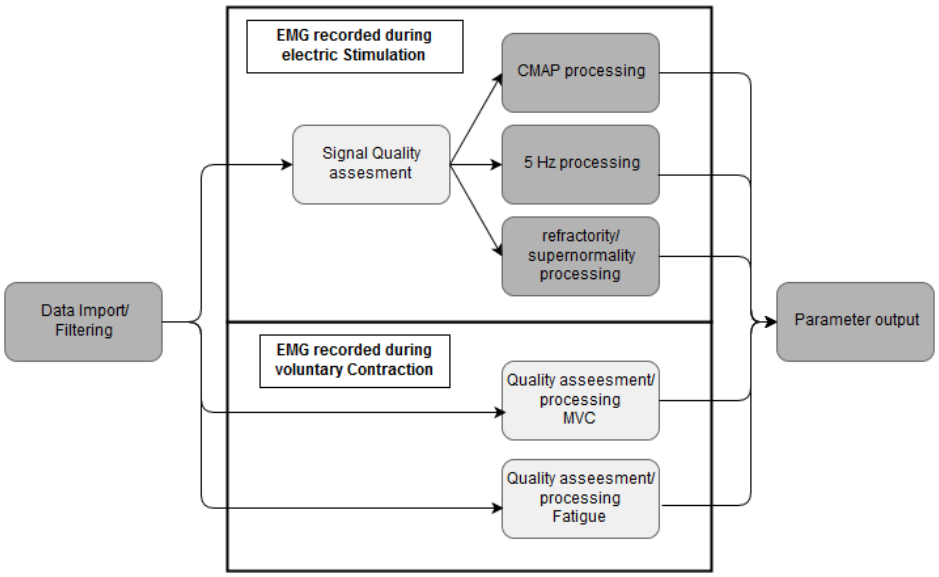

Figure 18: schematic representation of the processing steps; grey - automatic steps, white - supervised steps

mean signal calculation (top left listbox) and if the CMAP should be filtered or unfiltered (bottom left listbox). In case an individual CMAP should be processed, the right side of the GUI, circled in blue, can be used. Again the CMAP file and the type of mean signal calculation are chosen. Afterwards, the signal channels and the parameters of interest are displayed in the graphs and the table.

Figure 20 shows the interface, which was developed for MVC processing. Since the MVC test was conducted twice before and twice after the fatigue test, channels need to be selected for both of them simultaneously. Suggestions for the channel selection are given in the table on the bottom, circled in green. Changes can be made by altering these suggestions directly in the table. The information needed for the decision can be found in the table on the bottom (RMS, MVC, etc.), circled in red, or in the graphs on the top. The table on the left contains all RMS values displayed in the graphs on the

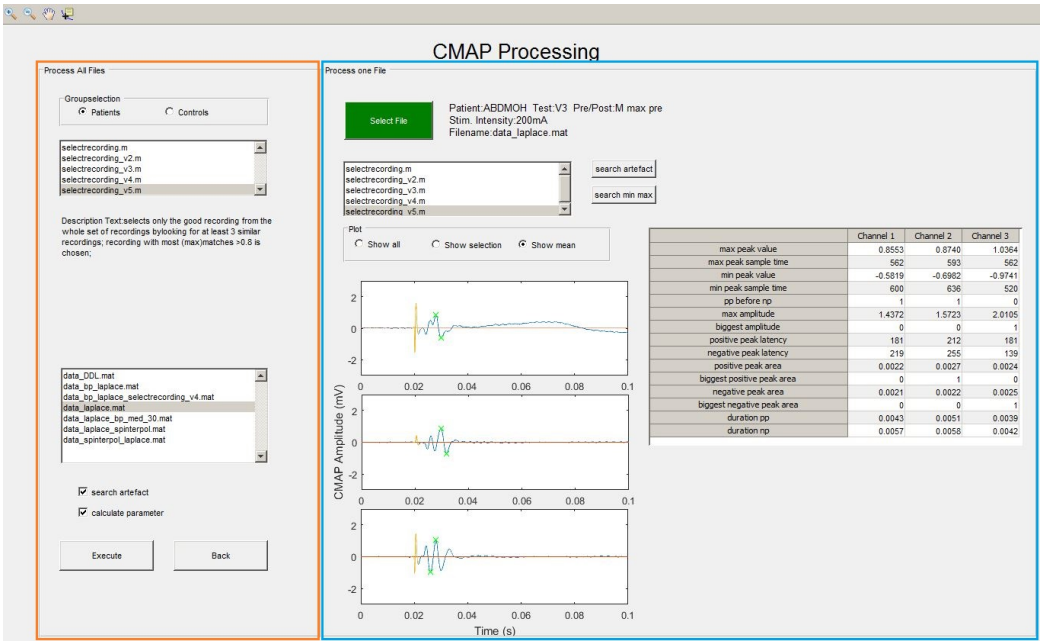

Figure 19: Matlab user interface developed for the CMAP processing

right.

For the Fatigue test, the channel selection is based on the information gained from the tables and graphs circled red in Figure 21. The tables contain the exact force, RMS, MPF and CV values calculated from the first and the last two seconds, while the figures show the development of these variables over time. The selection is done via the two radio button boxes circled in green. Individual choices can be made for the channel from which RMS and MPF are calculated, and the two channels used for the CV calculation. The remaining six graphs show the actual EMG signal of all three laplacian channels over the whole recording period and additionally for a short time window. They help to detect movement artefacts and help to see if the signals are actually EMG signals or just noise. The big table on the left upper side displays all variables used for the force, RMS, MPF and CV graphs.

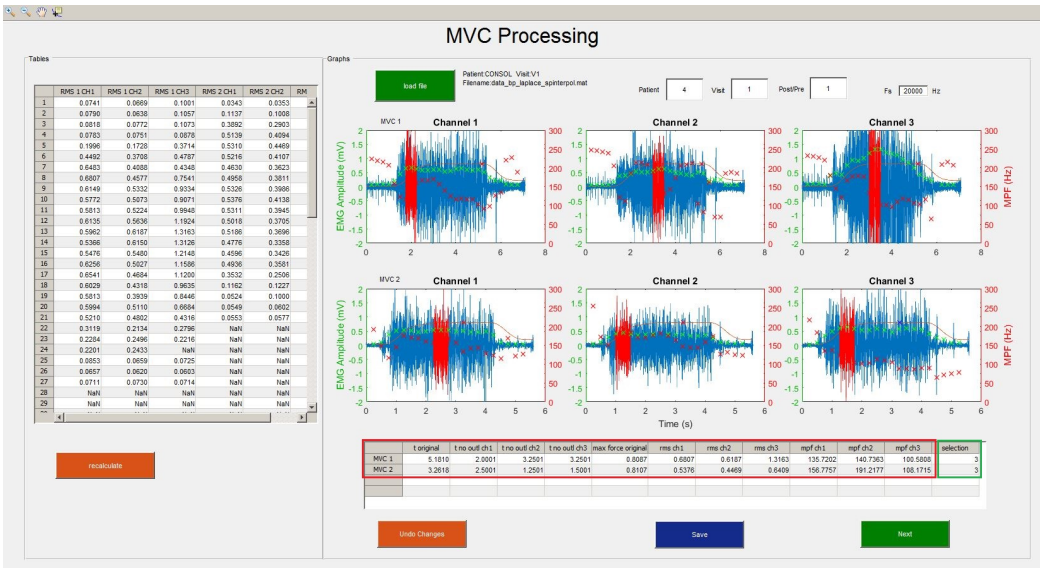

Figure 20: Matlab user interface developed for the MVC processing

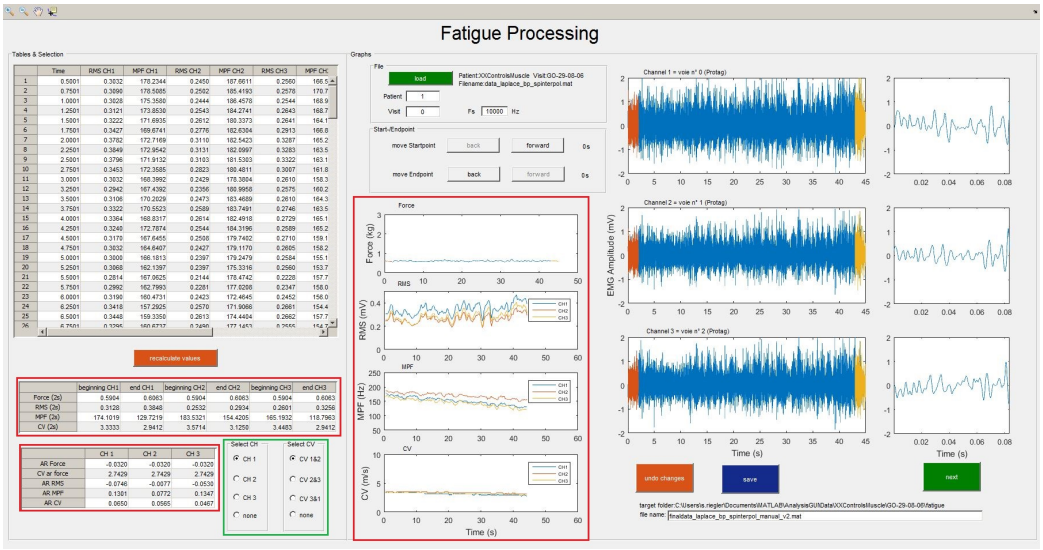

Figure 21: Matlab user interface developed for the Fatigue processing

4.2 Signal Quality

4.2.1 Control

The signal quality of the controls was generally high. Except for one control subject during the maximum M response pre-fatigue, all files were included.

4.2.2 NDM Patients

In the following two sections manual and automated signal quality classification is compared. This classification was only necessary for patient data as the dataset from the healthy subjects was already free from most of the described error classes. The healthy dataset simply had to be checked for error class a and b. The selection was made by choosing one channel with the highest amplitude and positive first peak.

4.2.2.1 CMAP

The signal quality of the maximum M-response test was analysed first. All together 216 maximum M-wave files (12 patients x 3 test days x pre/post fatigue x 3 Laplace electrode channels = 216) were checked. The results are summarised in Table 4. From this table, it can be seen that only 83 out of 216 files show "normal" responses. However, what is not indicated in this Table is that 35 out of these 83 "normal" responses were responses that had a stimulation artefact but no actual measurable muscle response so that at the end there are only 48 measurements with actual M-wave responses.

There are two main reasons for a measurement to have no mean signal. Firstly, it is possible that there was no recording for this patients test and secondly, what happened more frequently, was that none of the recordings had a correlation coefficient above 0.9 with other repetitions. Consequently, in both cases no mean signal could be calculated.

	n	normal response	error class a	error class b	error class c	error class d	no mean signal
MC	54	28 (4)	9	4	3	1	9
PC	162	55(31)	21	2	50	3	31
all	216	83(35)	30	6	53	4	40

Table 4: results of manual max M-wave signal quality classification

Overall only two patients (P2, P9) could be identified which had at least one good quality channel for each pre/post fatigue recording and all three test days. These patients are two out of the three MC patients in the study. Only these two patients were used for further calculations with the double stimulation data. Unfortunately, non of the PC patients signal quality was satisfactory for all test days.

4.2.2.2 5Hz

The repeated stimulation signal quality is summarized in Table 5. Again, 40 out of 93 overall normal responses did not have an actual M-response. They did not show any response to the stimulation.

	n	normal response	error class a	error class b	error class c	error class d	no valid recordings
MC	54	29 (4)	10	1	6	0	8
PC	162	64(36)	24	4	48	12	10
all	216	93(40)	34	5	54	12	18

Table 5: results of manual 5Hz signal quality classification

4.3 Double Stimulation - Signal Subtraction

During double stimulation, two bursts are created shortly after each other. The stimulation response of the first stimulation mostly covers up the stim-

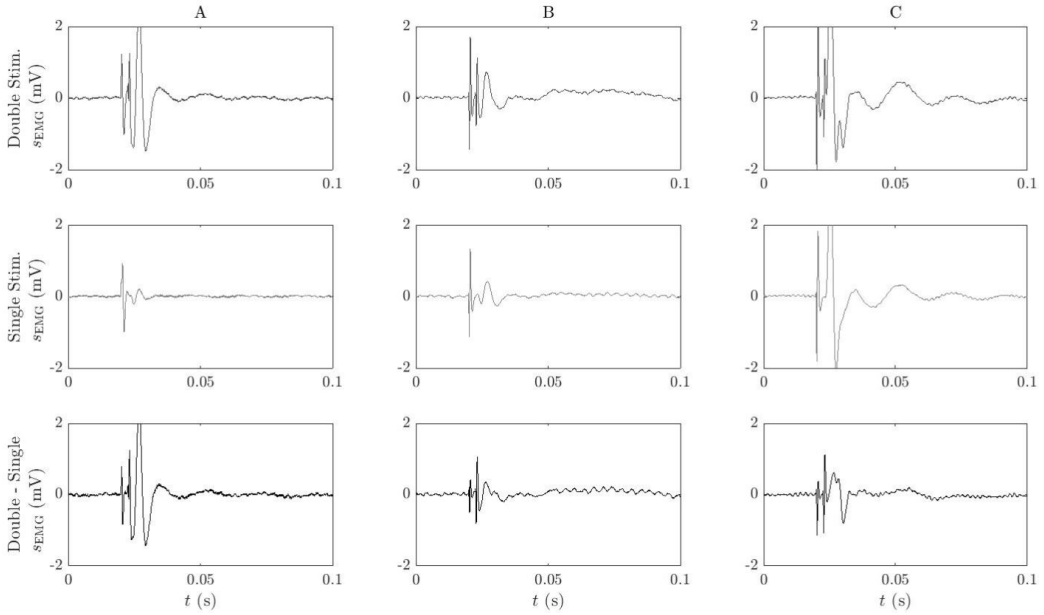

Figure 22: Each column A, B and C displays one example for the refractory test where the first row shows the original double stimulation signal, the second row the original single stimulation signal and the bottom row the final signal for the refractory test (double stimulation - single stimulation)

ulation and response of the second one. Therefore, the stimulation of the previous CMAP recording is used to subtract it from the double stimulation signal. This way the first response is supposed to be canceled out leaving behind only the second stimulation response.

All double stimulation tests were performed only with patients. As a result of the bad signal quality during the maximum M-response test, the double stimulation signals of only two patients with results for each testing day were considered. Hence, only 12 EMG signal files for refractory as well as supernormality were processed. However, out of the remaining data it was not possible to gain any adequate final signals. Figure 22 and 23 display three examples for the refractory and supernormality test. As we can see in the bottom row, which shows the final signal of the double stimulation tests,

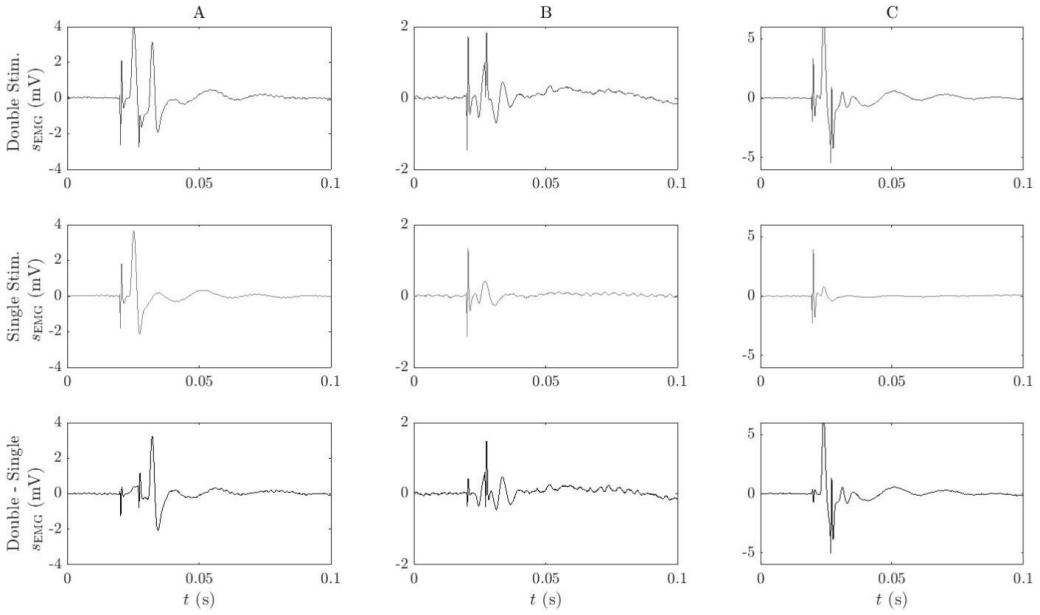

Figure 23: Each column A, B and C displays one example for the supernormality test where the first row shows the original double stimulation signal, the second row the original single stimulation signal and the bottom row the final signal for the supernormality test (double stimulation - single stimulation)

it was not possible to properly remove the first stimulation from the original double stimulation signal.

4.4 Parameter

4.4.1 Control

Table 6 and 7 contain the time and frequency parameter results obtained from the CMAP test. The data of one control subject was excluded for the calculations because of its poor signal quality. Additionally, the CV could only be calculated if at least two channels were valid, which was not always

the case, resulting in the reduced sample number for CV.

Of all maximum M-response parameter, only the post-fatigue peak frequency f in Table 7 is decreased significantly in comparison with pre-fatigue.

	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
total amplitude (mV)	9	3.60	0.62	10	3.07	0.57
pp surface (µVs)	9	2.96	0.62	10	2.99	0.74
np surface (µVs)	9	2.94	0.60	10	3.48	1.01
duration pp (ms)	9	2.58	0.14	10	2.96	0.26
CV (m/s)	6	3.14	0.10	8	2.94	0.02

Table 6: CMAP: time domain parameter results for the control group

	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
A **(V^2s/Hz)**	9	52.67	9.38	10	51.58	11.84
f **(Hz)**	9	328.89*	28.11	10	228.00*	33.76
$\Delta f_{10\%}$ **(Hz)**	6	873.17	123.58	5	976.80	135.85
$\Delta f_{50\%}$ **(Hz)**	9	448.00	30.14	10	389.30	36.47
$\Delta f_{90\%}$ **(Hz)**	9	163.78	16.60	10	130.20	16.69
a_{0-1} **(V^2s)**	9	618.59	105.76	10	538.09	110.31
a_{1-5} **(V^2s)**	9	58.96	16.13	10	51.12	11.55
$R_{10/90}$	6	6.67	1.69	5	10.27	3.11
$R_{10/50}$	6	2.06	0.18	5	2.42	0.20
$R_{10/\mathrm{p}}$	6	3.72	1.43	5	6.08	2.29
$R_{50/\mathrm{p}}$	9	1.54	0.29	10	2.08	0.39
$R_{90/\mathrm{p}}$	9	0.51	0.04	10	0.63	0.06
R_{a}	9	14.88	3.38	10	16.17	5.24

Table 7: CMAP: frequency domain parameter results for the control group; * level of significance $p < 0.05$

The results for the MVC test are summarised in Table 8. Only the subjects with known amplification factor could be included for its RMS calculations.

None of the parameters had significant differences between pre and post fatigue.

	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
Force (daN)	10	2.61	0.39	10	2.66	0.45
RMS (mV)	5	0.88	0.09	5	0.84	0.11
MPF (Hz)	10	162.58	12.56	10	148.58	12.36
CV (m/s)	10	3.81	0.23	10	3.83	0.22

Table 8: MVC: parameter results for the control group

The parameters calculated for the fatigue test are summarised in Table 9. Again it was not possible to calculate the CV for all controls, as it was not possible to identify two channels with good quality. RMS as well as MPF and CV significantly decrease during the fatigue task.

	n	**mean first 2s**	**SE first 2s**	**n**	**mean last 2s**	**SE last 2s**
Force (daN)	10	1.76	0.26	10	1.73	0.26
RMS (mV)	5	0.52*	0.07	5	0.34*	0.04
MPF (Hz)	10	187.74*	9.05	10	134.00*	7.38
CV (m/s)	10	4.03*	0.23	10	3.37*	0.30
	n		**mean**		**SE**	
r_{Force}	10		0.9951		0.0407	
r_{RMS}	10		0.8111		0.0958	
r_{MPF}	10		0.7160		0.0241	
r_{CV}	10		0.8398		0.0578	
$\mathbf{VC}_{\text{Force}}$ **(%)**	10		4.28		1.1810	
AR_{Force}	10		-0.0007		0.0254	
AR_{RMS}	10		0.1164		0.0674	
AR_{MPF}	10		0.1516		0.0236	
AR_{CV}	10		0.0780		0.0267	

Table 9: Fatigue: parameter results for the control group; * level of significance $p < 0.05$

V2 MC						
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
total amplitude (mV)	2	4.01	1.78	2	1.72	0.50
pp area (µVs)	2	4.03	2.00	1	1.16	0.00
np area (µVs)	2	2.51	1.48	1	1.52	0.00
duration pp (ms)	2	3.03	0.23	1	2.00	0.00
CV (m/s)	2	3.23	0.77	1	1.22	0.00
V2 PC						
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
total amplitude (mV)	5	2.29	0.76	4	2.26	1.22
pp area (µVs)	4	2.19	1.02	2	5.18	0.79
np area (µVs)	4	2.63	1.66	2	4.34	2.69
duration pp (ms)	4	2.29	0.73	2	4.20	0.70
CV (m/s)	4	2.59	0.18	2	4.40	1.99

Table 10: CMAP: time domain parameter results for patients; split in subgroups MC and PC

4.4.2 NMD Patients

The patients results are summarized in the following tables. Overall, there are 3 MC patients and 9 PC patients. Their results are displayed separately, as literature suggests that they may show differing electrophysiological behaviour [55] [59].

Table 10 contains the time frequency parameters for the patient group. In contrast to the control group it was not possible to obtain at least one good channel for every patient on every test day. Especially for PC patients it was not possible to find many good quality recordings. Because of the low sample numbers, no t-test was applied.

In Table 11 the frequency parameter of the maximum M-response for MC and PC patients are summarized. No statistical test was applied because of the low sample number but some trends can be observed. All Patients have

a higher peak amplitude but lower peak frequency than the control group. MC patients show the highest pre fatigue peak amplitude and a decreased post fatigue peak amplitude for all three test days. The frequency width is decreased for MC as well as PC patients in comparison with the control group. The surface between 0-1kHz is highest for MC patients which is in relation with its high peak amplitude. The ratio between 0-1KHz and 1-5kHz is generally higher for patients than control group. This indicates higher surface at 0-1kHz or smaller surface at 1-5kHz.

In Table 12, MVC parameter results are summarised. Overall they all show lower RMS values than the control group but in general, results for MC patients are more similar to the controls than those from PC patients. It was almost not possible to select two channels for CV calculations. Therefore, there are only very few results for patients which do not allow any statements about differences between groups.

Fatigue parameters for MC and PC patients are displayed in Table 13. PC patients results are generally lower than MC patients values. They also do not show an increase of RMS or decrease of MPF. VC is overall higher than for the control group.

The slope and intercept of the 5Hz test are summarized in Table 14. The linear regression was only calculated when at least 4 out of 5 stimulations were good quality recordings. For the MC patients it was possible to calculate the linear regression in 78% of the cases. For the PC patients it was only possible for 26% of the patients. Slope and intercept vary too much from day to day for MC as well as PC patients, in order to be able to say anything about group differences.

V2 MC						
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
A **(V^2s/Hz)**	2	113.59	44.38	2	53.91	15.04
f **(Hz)**	2	80.00	0.00	2	120.00	40.00
$f_{10\%}$ **(Hz)**	1	422.00	0.00	0	NaN	NaN
$f_{50\%}$ **(Hz)**	2	227.00	41.00	2	196.00	13.00
$f_{90\%}$ **(Hz)**	2	69.50	5.50	2	52.00	19.00
a_{0-1} **(V^2s)**	2	735.38	321.78	2	325.67	83.58
a_{1-5} **(V^2s)**	2	25.65	7.41	2	6.98	1.52
$R_{10/90}$	1	6.59	0.00	0	NaN	NaN
$R_{10/50}$	1	1.57	0.00	0	NaN	NaN
$R_{10/\mathrm{p}}$	1	5.28	0.00	0	NaN	NaN
$R_{50/\mathrm{p}}$	2	2.84	0.51	2	1.80	0.49
$R_{90/\mathrm{p}}$	2	0.87	0.07	2	0.43	0.02
R_{a}	2	27.33	4.66	2	51.73	23.26
V2 PC						
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
A **(V^2s/Hz)**	5	88.35	25.88	4	98.33	48.97
f **(Hz)**	5	96.00	9.80	4	60.00	20.00
$f_{10\%}$ **(Hz)**	1	458.00	0.00	2	261.50	77.50
$f_{50\%}$ **(Hz)**	4	143.25	13.53	3	125.00	20.30
$f_{90\%}$ **(Hz)**	5	45.20	8.22	3	29.67	0.88
a_{0-1} **(V^2s)**	5	446.70	143.21	4	437.62	220.53
a_{1-5} **(V^2s)**	5	26.26	10.28	4	28.63	8.72
$R_{10/90}$	1	11.74	0.00	2	9.02	3.09
$R_{10/50}$	1	2.65	0.00	2	2.46	0.60
$R_{10/\mathrm{p}}$	1	5.73	0.00	2	3.27	0.97
$R_{50/\mathrm{p}}$	4	1.51	0.27	3	1.56	0.25
$R_{90/\mathrm{p}}$	5	0.49	0.10	3	0.37	0.01
R_{a}	5	27.65	13.56	4	17.11	9.03

Table 11: CMAP: frequency domain parameter results for patients; split in subgroup MC and PC

V2 MC						
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
Force (daN)	2	2.12	0.79	3	1.79	0.33
RMS (mV)	2	0.38	0.13	3	0.27	0.06
MPF (Hz)	2	134.29	4.14	3	144.27	9.49
CV (m/s)	0	NaN	0.00	0	NaN	NaN
V2 PC						
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
Force (daN)	7	1.38	0.22	6	1.82	0.54
RMS (mV)	7	0.34	0.05	6	0.39	0.10
MPF (Hz)	7	104.08	9.52	6	113.29	12.44
CV (m/s)	2	3.06	0.28	2	2.18	0.57

Table 12: MVC: parameter results for patients; split in subgroups MC and PC

MC						
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
slope V1 (mV/s)	3	-0.03	0.28	2	0.34	0.38
intercept V1 (mV)	3	2.33	0.50	2	1.21	0.45
slope V2 (mV/s)	3	-0.19	0.19	2	-0.83	1.21
intercept V2 (mV)	3	2.56	1.08	2	4.29	3.40
slope V3 (mV/s)	1	0.44	0.00	3	-0.04	0.30
intercept V3 (mV)	1	6.21	0.00	3	1.47	0.08
PC						
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
slope V1 (mV/s)	1	-0.01	0.00	2	-0.15	0.13
intercept V1 (mV)	1	0.34	0.00	2	0.79	0.31
slope V2 (mV/s)	5	0.64	0.22	4	0.50	0.35
intercept V2 (mV)	5	1.75	0.13	4	1.54	0.63
slope V3 (mV/s)	1	-0.13	0.00	1	0.15	0.00
intercept V3 (mV)	1	0.32	0.00	1	2.05	0.00

Table 14: 5Hz: slope and intercept of total CMAP amplitude for patients; split in subgroups MC and PC

V2 MC						
	n	**mean first 2s**	**SE first 2 s**	**n**	**mean last 2s**	**SE last 2 s**
Force (daN)	2	1.43	0.46	2	0.91	0.06
RMS (mV)	2	0.35	0.13	2	0.21	0.09
MPF (Hz)	2	156.08	16.02	2	99.69	16.67
CV (m/s)	0	NaN	2.98	2	5.30	1.85
		n	**mean**		**SE**	
r_{Force}		2	0.69		0.17	
r_{RMS}		2	0.59		0.03	
r_{MPF}		2	0.63		0.04	
r_{CV}		2	0.46		0.04	
$\mathbf{VC}_{\text{Force}}$ **(%)**		2	17.66		17.66	
AR_{Force}		2	0.15		0.15	
AR_{RMS}		2	0.28		0.28	
AR_{MPF}		2	0.23		0.23	
AR_{CV}		1	0.31		0.31	
V2 PC						
	n	**mean first 2s**	**SE first 2 s**		**mean last 2s**	**SE last 2 s**
Force (daN)	7	0.88	0.15	7	0.76	0.16
RMS (mV)	7	0.20	0.05	7	0.12	0.05
MPF (Hz)	7	118.08	15.24	7	114.48	22.51
CV (m/s)	4	1.73	1.52	4	1.11	0.86
		n	**mean**		**SE**	
r_{Force}		7	0.81		0.07	
r_{RMS}		7	0.66		0.13	
r_{MPF}		7	1.00		0.19	
r_{CV}		2	0.90		0.32	
$\mathbf{VC}_{\text{Force}}$ **(%)**		7	13.83		13.83	
AR_{Force}		7	0.10		0.10	
AR_{RMS}		7	0.24		0.24	
AR_{MPF}		7	0.00		0.00	
AR_{CV}		4	0.04		0.04	

Table 13: Fatigue: parameter results for patients; split in subgroups MC and PC

CMAP	pre difference (%)	post difference (%)
total amplitude (mV)	-0.6	-20.1
pp surface (µVs)	-17.3	-33.4
duration pp (ms)	-1.1	-4.2
CV (m/s)	-0.9	-0.7

MVC	pre difference (%)	post difference (%)
Force (daN)	4.4	10.8
RMS (mV)	17.3	33.3
MPF (Hz)	-4.1	-17.1
CV (m/s)	12.1	10.1

Fatigue	pre difference (%)	post difference (%)
Force (daN)	17.3	18.5
RMS (mV)	30.0	13.3
MPF (Hz)	-4.6	-5.0
CV (m/s)	2.3	6.6

Table 15: Difference between published mean results and new calculations pre and post fatigue; Values <0 indicate a decrease in comparison to published results and values >0 indicate an increase

4.5 Program Validation

The processing steps were validated by comparing the control group parameter results with the previously published results [60]. The major limitation for this step was that only 5 out of the 10 control subjects were identical as in the publication. The other 5 subjects have not been included in the publication but were used as controls for this thesis. Some variations might therefore be due to this discrepancy in control subject inclusion. Additionally, the channel choice also influences the parameter results.

Control Subject 1			
CMAP	**mean post 1**	**mean post 2**	**difference (%)**
total amplitude (mV)	0.768	0.841	9.6
pp surface (µVs)	0.746	0.715	-4.2
duration pp (ms)	2.400	2.300	-4.2
CV (m/s)	3.330	3.333	0.1
MVC	**mean post 1**	**mean post 2**	**difference (%)**
Force (daN)	2.059	2.042	-0.8
RMS (mV)	0.185	0.198	6.8
MPF (Hz)	85.690	86.221	0.6
CV (m/s)	4.160	3.571	-14.1
Fatigue	**mean last 2s 1**	**mean last 2s 2**	**difference (%)**
Force (daN)	1.040	1.045	0.5
RMS (mV)	0.053	0.055	3.8
MPF (Hz)	152.000	150.799	-0.8
CV (m/s)	3.030	2.941	-2.9

Table 16: Comparison of individual values for control subject 1; column 1 contains the published values from [60] and column 2 contains the automatically calculated values

The new CMAP results in Table 15 are generally lower than the original values calculated by Boerio [60]. Five out of the eight parameter show only 1-4% difference. However, the parameter total amplitude post fatigue and peak to peak surface pre and post fatigue show up to 30% differences.

The comparison of MVC and Fatigue test shows that the RMS values has the biggest discrepancy in both tests but it has to be noted that only five out of the ten subjects were included for new calculations because of the missing amplification factor.

For better comparison, an individual control subject is given in Table 16. Parameters calculated from absolute values like the RMS indicate bigger differences than frequency parameter like the MPF. The highest discrepancy is between the CVs during the MVC test.

5 Discussion

5.1 Signal Quality

The signal quality between control subjects and patients differs significantly. This discrepancy may have two major reasons. Firstly, two different kinds of electrodes were used. Technically they were both laplacian electrodes. However, the electrodes used for the controls calculated the laplacian signal before the AD-conversion on-site, while for the patients, 11 monopolar signals were recorded analogously and the 3 laplacian signals were calculated digitally. In theory, analog or digital calculation of the signal should not make a difference. However measuring and storing the 11 monopolar signals separately increases the possibility of noise influencing the individual channels uniquely and therefore may lead to distorted laplacian signals. Additionally, after careful analysis of the patients' individual monopolar channels, it was found that for some patients, during the voluntary contraction tests (MVC and Fatigue), part of the monopolar electrodes did not record any signal. This was probably due to bad contact between the electrode and the skin and should have been checked before the recording. Hence, the laplacian signals of these patients are not valid. One example of this error was selected and displayed in Figure 24. Figure 24b) shows the monopolar electrodes Ch4, Ch7 and Ch10, where no EMG signal was recorded. Sometimes, this affects only one of the three laplacian channels leaving the others for calculations, but it might as well affect all of them. For the control group, the monopolar signals are not available, hence they cannot be checked. However, consider-

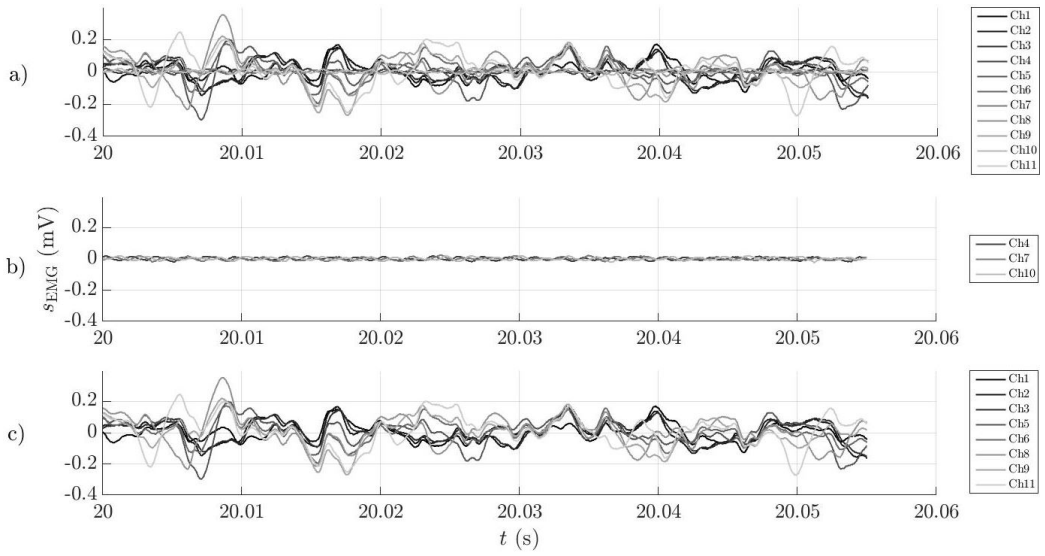

Figure 24: control subject 12 - Fatigue test

ing the good quality of these recordings, it can be said that contact problems were not an issue with these electrodes.

Secondly, and more difficult to determine, the lower quality of the EMG signals might be a symptom of the patient and therefore not actually a source of error. A voluntary contraction EMG signal of a control subject is considered as a "bad signal", that is when RMS, MPF or CV are too high or too low. However, for patients it is not always clear if these changes are due to noise or patient symptoms. For the stimulation evoked tests, the missing response after a stimulation was considered as a symptom for PC patients. This was the case for more than 50% of all stimulation evoked EMG recordings when diagnosed with PC and about only 10% for MC patients (Table 4 and 5). It is clearly visible that MC patients were more likely to have actual CMAP responses than PC patients. The latter group was also more prone to show baseline fluctuation after stimulation (error class c).

5.2 Channel Selection

The channel selection is the most sensitive step, and the reason for most differences between the reference values and my recalculated results of the control group. Previously, the selection was based on the observers subjective decision and was therefore completely dependant on his or her experience and attentiveness. Especially, when no documentation of the decision finding process is available, the decision becomes incomprehensible, seems to be arbitrary and is very unlikely to be reproducible. This is a serious problem considering that the results can be completely different depending on which channel was selected. Originally, this step was planned to be completely automated. For a perfectly noise free recording, this might even be possible. However, especially the patients EMG was very noisy and it is not easy to differentiate between patients normal response and errors due to noise and artefacts. Therefore, the channel selection for the stimulation evoked tests was used, based on the results of the previously described manual error classification and afterwards automatically selecting the channel with the biggest amplitude. For the voluntary contractions, no preliminary quality assessment was performed. Signal quality assessment and channel selection were combined in one processing step and the decision was made with the aid of a computer.

For the voluntarily evoked EMG signals, the selection is guided by two variables: the RMS and MPF. At the optimum position, according to literature [81] [77], the RMS should be at its maximum and the MPF at its minimum. The developed Matlab program gives recommendations for the selection, based on the RMS and MPF, which can be accepted or discarded according to the user. If there are no artefacts affecting the signal, it should not be necessary to alter the computed selection. Yet, even with these two guidelines, the same channel was not always selected in comparison with the reference results of the control group. The patients signals constitute a particular problem, as their EMG was generally more affected by noise and it is

difficult to differentiate between real patients EMG and noise.

5.3 Program Validation

Due to missing documentation, only 5 out of the 10 control subjects could be matched with complete certainty with the original results. The other subjects are missing information about the EMG gain factor and could not be directly linked to any existing results. Therefore, the RMS of the voluntary contractions could only be calculated for 5 controls. For the other frequency based parameters and the stimulation evoked tests, the missing gain factor did not present a limitation. Finally, it cannot be said with complete certainty that the same 10 healthy control subjects were included in this calculations as in the previous publication [60].

Through the comparison of the new parameter results in Table 6,8 and 9 with previously published data [60] in Table 15, it can be seen that the biggest discrepancies are within the RMS values with up to 33%. There are three major possibilities for these differences: (1) filtering, (2) subject mismatch and (3) different channel selection. Since the comparison of one individual subject in Table 16 shows only small differences of up to 7% for the RMS parameter, it is very likely that the gap originates from the difference in subject inclusion.

The differing CMAP surface results might be due to calculation differences. No documentation about the exact calculations for the original values were available, though the surface unit is stated as (mV/s) in the paper [60]. However, if it is a surface, the unit should be (mVs) or perhaps (µVs). The question therefore arises, was the surface calculated as indicated by the unit or did the differences have another origin.

5.4 Parameter

5.4.1 Control

5.4.1.1 CMAP

No significant differences could be found for the time domain parameter of the control group pre and post the fatigue test.

The CMAP frequency parameters were based on a paper by Mahbub and Rabbani [72]. The underlying idea is, that controls have a standard CMAP response while patients with different neuromuscular diseases show kinks in their CMAP response. These kinks are due to excitability changes of muscle or nerve fibers. The actual results however, are not comparable with the results of Mahbub and Rabbani because of the different recording systems. Additionally the boundaries for the surface calculation were changed from the original 0-2kHz/2-5kHz to 0-1kHz/1-5kHz, which seems more plausible considering that a normal EMG is expected to be between 10-500Hz and everything above is usually only noise. All frequency parameters, except the peak frequency, remained constant (no significant differences) before and after the fatiguing task (Table 7). The peak frequency is significantly different in pre and post fatigue in controls, showing a shift towards lower frequencies, while the actual peak amplitude is unchanged. There are no literature results available to confirm these findings.

5.4.1.2 MVC

Again, no significant differences between pre and post fatigue could be found. Only the MPF indicates a non-significant decrease after the fatigue test which is contrary to what can be found in [60]. The discrepancy could be due to different subjects and channel selection.

5.4.1.3 Fatigue

For the fatigue results, significant differences in RMS, MPF and CV between the first two seconds and the last two seconds are comparable to previous results [60]. The coefficient of variance VC_{force} for the force is low and indicates that the force was held constant which is normal for healthy individuals.

5.4.2 NDM Patients

The EMG signals of 12 patients with non-dystrophic myotonia were processed. Originally the intent was to process the EMG signal of all patients, combine them and compare them with healthy controls. Best case scenario would have been if it were possible to find differences for those patients who received medication and those with placebo treatment during visit 2 and visit 3. The examination protocol was chosen because it has been tested successfully with controls as well as patients with myotonic dystrophy type 1 [60]. However, a few limitations have been identified:

- (1) different electrodes for controls and patients
- (2) MC and PC patients have to be processed separately (small subject number)
- (3) influence of room temperature

These three factors significantly increased the complexity of the problem. Not only did the choice of electrodes deteriorate the recorded EMG signals, but factors 2 and 3 additionally complicate the interpretation of the results. Due to the splitting of the group into MC and PC patients (2), the maximum group sizes were only 3 (MC) and 9 (PC). In combination with the bad EMG signals, due to the electrodes used (1), this often resulted in groups containing only one or two valid measurements (e.g. Table 14). However, the bad EMG recordings are not the only reason for the small subject numbers. Often, the reason for missing responses was because the patients simply did not show any measurable response to electrical stimulation. This electrical

silence affected mostly PC patients and is the main reason for missing EMG recordings. This phenomenon might be due to temperature influences (3). PC is known to be temperature sensitive [82]. The cooling of the muscle leads to paralysis and muscle weakness. While the attempt has been made to keep the room temperature constant at 23°C, small fluctuations can never be avoided. Also, the individual influence of small changes in temperature on a patient are unknown. The same temperature might have more or less influence depending on the patient. Without closer information about the exact room and skin temperature, the influences can only be estimated. To avoid this uncertainty it would have been possible to increase the room temperature until all influences would have been eliminated.

With these limitations in mind, the parameters of the CMAP, 5Hz, MVC and Fatigue tests are discussed in the following sections.

5.4.2.1 CMAP

The CMAP results, displayed in Table 10, indicate that PC and MC patients might have a different electromyographic behaviour. While the control group shows a constant total amplitude before and after the fatigue test (see Table 6), the amplitude seems to decrease for MC patients and is constant but generally lower for PC patients compared to healthy controls. Additionally, surface, duration and CV seems to decrease for MC patients while it increases for PC patients. However, these pattern are not constant over all three visits (see Appendix A.2 Table 18) and might therefore be influenced by changes due to the Mexiletine medication.

The frequency parameter of the CMAP response in Table 11 indicates the difference between PC and MC patients even more drastically. Peak frequency (p_f) as well as peak amplitude (p_a) are generally lower for patients than for controls. While p_a deceases in MC, it stays rather constant for PC. However, the inconsistent changes for different testing days V1-3 (see Appendix A.2 Table 19), low patient numbers and high standard error prohibit

any further interpretation of the results.

5.4.2.2 MVC

The maximal force, RMS as well as MPF seems to be decreased for all patients in comparison with the healthy control group (Table 12). Again, any indication for decreasing or increasing parameters before and after fatigue are not consistent over all three visits V1-3 (see Appendix A.3 Table 20). The increase of force might be due to force potentiation. This is a phenomenon where the force level is increased after a fatiguing task [83]. The divers behaviour of the other parameters might again be due to medication influences.

5.4.2.3 Fatigue

The fatigue behaviour of MC patients is similar to control subjects. The main difference can be seen in the maintenance of the force. While controls were mostly able to uphold the 60% MVC with a low coefficient of variance, MC patients seem to have troubles maintaining the force till the end of the measurement and are also not able to keep up a constant force level, in combination resulting in a higher coefficient of variance. The PC patients on the other hand, are quite different from control and MC patients. Their force level is generally lower but does not show any significant decrease over time like MC patients. However, their coefficient of variance is comparably high like those for MC patients. In this case, the increase of coefficient of variance is solely due to the incapability to keep a constant force and not influenced by a general decrease of the force.

RMS, MPF and CV are generally low for PC patients and do not always show a significant decrease as in the control group. For all 3 visits, the CV is extremely low, which could either be characteristic for PC patients or due to inclusion of invalid results. The latter explanation is more likely since the CV for PC patients is drastically higher in all other tests conducted. Further,

the PC patients conspicuously low RMS in combination with low MPF raises the question if any of the recordings are actual EMGs or only recorded noise.

5.4.2.4 5Hz

The 5Hz test results are shown in Table 14. For visit 1 and visit 3, it was not possible to find more than one or two valid EMG recordings for PC patients. For these, only V2 will be taken into account in the discussion. During V2 slope and intercept stay rather constant before and after the fatiguing task. MC patients behave inversely on V1 and V2 (V3 is not taken into account because of low subject number).

5.4.2.5 Double Stimulation

Unfortunately, the signal quality did not allow any closer analysis of the double stimulation tests. It was not possible to remove the first stimulation which is an essential step for the double stimulation signal processing. The examples in Figure 22 and 23 show that the subjects often did not have the same maximum M-wave and first stimulation, which made it impossible to cancel out the first stimulation during the double stimulation tests.

6 Conclusion

For this thesis, the EMG signals, recorded during a neuromuscular excitability study, were processed. The protocol included the measurement of stimulation evoked as well as voluntary contractions. The signals were gathered from healthy subjects and NDM patients before the internship.

For better understanding of the signal and to ensure the signal quality, a user interface for each test was created. Depending on the test, the interface allowed the selection of the channel, signal quality classification and adaptation of the parameter calculation time point. Mean signals as well as the 50Hz rejection and 10-500Hz bandpass filter were applied beforehand.

The signal quality for the control group, using on-site laplacian electrodes, was excellent, simplifying all processing steps. The only problem encountered was the missing documentation about the EMG gain factor for half of the control population. For those 5 subjects, it was therefore not possible to calculate any absolute amplitude parameter for the MVC and Fatigue test. In general, results match previous calculations and literature values (see Section 4.5). Absolute value parameter like the RMS may show bigger differences because of (1) filtering, (2) subject inclusion and (3) channel selection. Consequently, the processing software developed in Matlab was therefore seen as validated and subsequently applied to patient data.

The signal processing for NDM patients was made more difficult through various factors (see Section 5.4.2). Taking into account of these three factors, the parameter interpretability and comparability with the control group was sharply decreased. At the end, the resulting group numbers were too small

to apply any statistical tests and presumed parameter trends were rarely constant over all three visits with large standard errors, indicating that the results were probably not due to patient symptoms but rather blurred by noise.

The application of the algorithms on healthy control subjects showed that it is generally possible to automatically, or for most parts at least semi-automatically, process EMG data. The validation through comparison with previously published data has shown that similar results are obtained. However, one of the keystones to enable this automatic process is signal quality. Yet, for EMG signals, this is often what is hardest to achieve. Therefore, it was not possible to completely automatically process the data, but rather develop a semi-automatic supervised processing environment where the program pre-evaluates the signal but they are still checked and can be adapted by the experienced user. With this, it was possible to calculate all parameters of interest and at the same time make sure of the signal quality.

Unfortunately, due to the bad signal quality and low group number of the patients, it was not possible to make reliable comparisons between healthy and patient groups.

For future studies with NDM patients, but also other neuromuscular diseases, it would be important to pay careful attention to the following points:

- check signal quality before testing
- check for disease specific external influences (e.g. room temperature)
- document all relevant data (gain factors, exact time of recording, etc.)
- ensure adequate subgroup numbers

The developed user interfaces may be used for the processing of EMG signals from different studies, which use the same laplacian electrodes. They are applicable to MVC, CMAP and Fatigue tests.

Bibliography

[1] L. Galvani, *Commentary on the effects of electricity on muscular motion.* No. 10, Burndy Library, 1953.

[2] J. Ladegaard, "Story of electromyography equipment," *Muscle & nerve*, vol. 25, no. S11, pp. S128–S133, 2002.

[3] "Delsys Software - System Integration." `http://www.delsys.com/products/software/`. Accessed: 2017-05-10.

[4] "Motion Lab Systems: EMG Analysis Software." `https://www.motion-labs.com/software_emg_analysis.html`. Accessed: 2017-05-10.

[5] E. Henneman and L. M. Mendell, "Functional organization of motoneuron pool and its inputs," *Comprehensive Physiology*, 2011.

[6] R. Burke, "Motor units: anatomy, physiology, and functional organization," *Comprehensive Physiology*, 2011.

[7] R. Merletti, M. Knaflitz, C. J. De Luca, *et al.*, "Electrically evoked myoelectric signals," *Critical Review in Biomedical Engineering*, vol. 19, no. 4, pp. 293–340, 1992.

[8] J. V. Basmajian and C. De Luca, *Muscles Alive: Their Functions Revealed by Electromyography*, vol. 5. Williams and Wilkins, 1978.

[9] R. Aaron, M. Huang, and C. Shiffman, "Anisotropy of human muscle via non-invasive impedance measurements," *Physics in medicine and biology*, vol. 42, no. 7, p. 1245, 1997.

[10] C. J. De Luca, "Surface electromyography: Detection and recording," *DelSys Incorporated*, vol. 10, p. 2011, 2002.

[11] F. Mandrile, D. Farina, M. Pozzo, and R. Merletti, "Stimulation artifact in surface emg signal: effect of the stimulation waveform, detection system, and current amplitude using hybrid stimulation technique," *Neural Systems and Rehabilitation Engineering, IEEE Transactions on*, vol. 11, no. 4, pp. 407–415, 2003.

[12] B. Bigland-Ritchie and J. Woods, "Changes in muscle contractile properties and neural control during human muscular fatigue," *Muscle & nerve*, vol. 7, no. 9, pp. 691–699, 1984.

[13] R. Merletti, L. L. Conte, and C. Orizio, "Indices of muscle fatigue," *Journal of Electromyography and Kinesiology*, vol. 1, no. 1, pp. 20–33, 1991.

[14] R. Merletti and P. A. Parker, *Electromyography: physiology, engineering, and non-invasive applications*, vol. 11. John Wiley & Sons, 2004.

[15] S. Boyas and A. Guével, "Neuromuscular fatigue in healthy muscle: underlying factors and adaptation mechanisms," *Annals of physical and rehabilitation medicine*, vol. 54, no. 2, pp. 88–108, 2011.

[16] B. Bigland-Ritchie, "Emg and fatigue of human voluntary and stimulated contractions," *Human muscle fatigue: physiological mechanisms*, pp. 130–156, 1981.

[17] B. Ahlborg, J. Bergström, L.-G. Ekelund, and E. Hultman, "Muscle glycogen and muscle electrolytes during prolonged physical exercise1," *Acta Physiologica Scandinavica*, vol. 70, no. 2, pp. 129–142, 1967.

[18] L. Hermansen, E. Hultman, and B. Saltin, "Muscle glycogen during prolonged severe exercise," *Acta Physiologica Scandinavica*, vol. 71, no. 2-3, pp. 129–139, 1967.

[19] B. Pernow and B. Saltin, "Availability of substrates and capacity for prolonged heavy exercise in man.," *Journal of Applied Physiology*, vol. 31, no. 3, pp. 416–422, 1971.

[20] M. A, *Fatigue*. London: Swan Sonnenschein, 1904.

[21] F. Bellemare and B. Bigland-Ritchie, "Central components of diaphragmatic fatigue assessed by phrenic nerve stimulation," *Journal of Applied Physiology*, vol. 62, no. 3, pp. 1307–1316, 1987.

[22] B. Bigland and O. Lippold, "Motor unit activity in the voluntary contraction of human muscle," *The Journal of Physiology*, vol. 125, no. 2, p. 322, 1954.

[23] C. Reid, "The mechanism of voluntary muscular fatigue," *Quarterly journal of experimental physiology*, vol. 19, no. 1, pp. 17–42, 1928.

[24] M. Berchicci, F. Menotti, A. Macaluso, and F. Di Russo, "The neurophysiology of central and peripheral fatigue during sub-maximal lower limb isometric contractions," *Frontiers in human neuroscience*, vol. 7, p. 135, 2013.

[25] A. Shield and S. Zhou, "Assessing voluntary muscle activation with the twitch interpolation technique," *Sports Medicine*, vol. 34, no. 4, pp. 253–267, 2004.

[26] J. L. Taylor, "Point: Counterpoint: The interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle," *Journal of Applied Physiology*, vol. 107, no. 1, pp. 354–355, 2009.

[27] A. de Haan, K. Gerrits, and C. de Ruiter, "Counterpoint: the interpolated twitch does not provide a valid measure of the voluntary activation of muscle," *Journal of Applied Physiology*, vol. 107, no. 1, pp. 355–357, 2009.

[28] W. Herzog, "Twitch interpolation represents muscle activation in a qualitative manner only.," *Journal of applied physiology (Bethesda, Md.: 1985)*, vol. 107, no. 1, p. 360, 2009.

[29] P. Contessa, A. Puleo, and C. J. De Luca, "Is the notion of central fatigue based on a solid foundation?," *Journal of neurophysiology*, vol. 115, no. 2, pp. 967–977, 2016.

[30] R. H. Chowdhury, M. B. Reaz, M. A. B. M. Ali, A. A. Bakar, K. Chellappan, and T. G. Chang, "Surface electromyography signal processing and classification techniques," *Sensors*, vol. 13, no. 9, pp. 12431–12466, 2013.

[31] H. Tam and J. G. Webster, "Minimizing electrode motion artifact by skin abrasion," *IEEE Transactions on Biomedical Engineering*, vol. 2, no. BME-24, pp. 134–139, 1977.

[32] D. P. Burbank and J. G. Webster, "Reducing skin potential motion artefact by skin abrasion," *Medical and Biological Engineering and Computing*, vol. 16, no. 1, pp. 31–38, 1978.

[33] M. C. Garcia and T. Vieira, "Surface electromyography: Why, when and how to use it," *Revista Portuguesa de Cardiologia*, vol. 4, no. 01, pp. 17–28, 2011.

[34] L. H. Lindström and R. I. Magnusson, "Interpretation of myoelectric power spectra: a model and its applications," *Proceedings of the IEEE*, vol. 65, no. 5, pp. 653–662, 1977.

[35] H. Huang, P. Zhou, G. Li, and T. Kuiken, "Spatial filtering improves emg classification accuracy following targeted muscle reinnervation," *Annals of biomedical engineering*, vol. 37, no. 9, pp. 1849–1857, 2009.

[36] G. Rau and C. Disselhorst-Klug, "Principles of high-spatial-resolution surface emg (hsr-emg): single motor unit detection and application in

the diagnosis of neuromuscular disorders," *Journal of Electromyography and Kinesiology*, vol. 7, no. 4, pp. 233–239, 1997.

[37] H. Reucher, G. Rau, and J. Silny, "Spatial filtering of noninvasive multielectrode emg: Part i-introduction to measuring technique and applications," *Biomedical Engineering, IEEE Transactions on*, no. 2, pp. 98–105, 1987.

[38] M. Halaki and K. Ginn, "Normalization of emg signals: To normalize or not to normalize and what to normalize to?," 2012.

[39] J.-Y. Hogrel, "Clinical applications of surface electromyography in neuromuscular disorders," *Neurophysiologie Clinique/Clinical Neurophysiology*, vol. 35, no. 2, pp. 59–71, 2005.

[40] M. Muro, A. Nagata, K. Murakami, and T. Moritani, "Surface emg power spetral analysis of neuro-muscular disorders during isometric and isotonic contractions.," *American Journal of Physical Medicine & Rehabilitation*, vol. 61, no. 5, pp. 244–254, 1982.

[41] M. Meyer, P. Hilfiker, and A. Gygi, "Surface emg for diagnosis of neuromuscular diseases," *Computer-aided electromyography and expert systems. Amsterdam: Elsevier*, pp. 181–8, 1989.

[42] J. Van der Hoeven, M. Zwarts, and T. Van Weerden, "Muscle fiber conduction velocity in amyotrophic lateral sclerosis and traumatic lesions of the plexus brachialis," *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, vol. 89, no. 5, pp. 304–310, 1993.

[43] M. Frascarelli, L. Rocchi, and I. Feola, "Emg computerized analysis of localized fatigue in duchenne muscular dystrophy," *Muscle & nerve*, vol. 11, no. 7, pp. 757–761, 1988.

[44] C. Krarup and M. Moldovan, "Nerve conduction and excitability studies in peripheral nerve disorders," *Current opinion in neurology*, vol. 22, no. 5, pp. 460–466, 2009.

[45] L. Gutmann, "Pearls and pitfalls in the use of electromyography and nerve conduction studies," in *Seminars in neurology*, vol. 23, pp. 077–082, Copyright© 2002 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel.:+ 1 (212) 584-4662, 2003.

[46] A. E. Emery, "Population frequencies of inherited neuromuscular diseases—a world survey," *Neuromuscular disorders*, vol. 1, no. 1, pp. 19–29, 1991.

[47] P. Baumann, V. V. Myllylä, and J. Leisti, "Myotonia congenita in northern finland: an epidemiological and genetic study.," *Journal of medical genetics*, vol. 35, no. 4, pp. 293–296, 1998.

[48] K. Jurkat-Rott, H. Lerche, and F. Lehmann-Horn, "Muskuläre kanalopathien," *Der Nervenarzt*, vol. 82, no. 4, pp. 511–521, 2011.

[49] E. Matthews, D. Fialho, S. Tan, S. Venance, S. Cannon, D. Sternberg, B. Fontaine, A. Amato, R. Barohn, R. Griggs, *et al.*, "The non-dystrophic myotonias: molecular pathogenesis, diagnosis and treatment," *Brain*, vol. 133, no. 1, pp. 9–22, 2010.

[50] S. C. Cannon, "Pathomechanisms in channelopathies of skeletal muscle and brain," *Annual Review of Neuroscience*, vol. 29, pp. 387–415, 2006.

[51] A. Cherian, N. N. Baheti, and A. Kuruvilla, "Muscle channelopathies and electrophysiological approach," *Annals of Indian Academy of Neurology*, vol. 11, no. 1, p. 20, 2008.

[52] J. C. Cleland and E. L. Logigian, "Clinical evaluation of membrane excitability in muscle channel disorders: potential applications in clinical trials," *Neurotherapeutics*, vol. 4, no. 2, pp. 205–215, 2007.

[53] E. W. Streib, S. F. Sun, and T. Yarkowsky, "Transient paresis in myotonic syndromes: a simplified electrophysiologic approach," *Muscle & Nerve*, vol. 5, no. 9, pp. 719–723, 1982.

[54] P. G. McManis, E. H. Lambert, and J. R. Daube, "The exercise test in periodic paralysis," *Muscle & nerve*, vol. 9, no. 8, pp. 704–710, 1986.

[55] E. Fournier, M. Arzel, D. Sternberg, S. Vicart, P. Laforet, B. Eymard, J.-C. Willer, N. Tabti, and B. Fontaine, "Electromyography guides toward subgroups of mutations in muscle channelopathies," *Annals of neurology*, vol. 56, no. 5, pp. 650–661, 2004.

[56] M. K. Hehir and E. L. Logigian, "Electrodiagnosis of myotonic disorders," *Physical medicine and rehabilitation clinics of North America*, vol. 24, no. 1, pp. 209–220, 2013.

[57] S. Subramony, C. Malhotra, and S. Mishra, "Distinguishing paramyotonia congenita and myotonia congenita by electromyography," *Muscle & nerve*, vol. 6, no. 5, pp. 374–379, 1983.

[58] V. K. Nielsen, M. L. Friis, and T. Johnsen, "Electromyographic distinction between paramyotonia congenita and myotonia congenita effect of cold," *Neurology*, vol. 32, no. 8, pp. 827–827, 1982.

[59] E. Fournier, K. Viala, H. Gervais, D. Sternberg, M. Arzel-Hézode, P. Laforêt, B. Eymard, N. Tabti, J.-C. Willer, C. Vial, *et al.*, "Cold extends electromyography distinction between ion channel mutations causing myotonia," *Annals of neurology*, vol. 60, no. 3, pp. 356–365, 2006.

[60] D. Boerio, J.-P. Lefaucheur, G. Bassez, and J.-Y. Hogrel, "Central and peripheral components of exercise-related fatigability in myotonic dystrophy type 1," *Acta Neurologica Scandinavica*, vol. 125, no. 1, pp. 38–46, 2012.

[61] Digitimer Ltd, "Datasheet, ds5 - isolated bipolar constant current stimulator." http://digitimer.com/wp-content/uploads/2015/01/ds5.pdf. Accessed: 2017-05-10.

[62] g.tec medical engineering GmbH, "g.bsamp, biosignal amplifier." http://www.gtec.at/Products/Hardware-and-Accessories/g.BSamp-Specs-Features. Accessed: 2017-05-10.

[63] P. Hoffmann, "Beitrag zur kenntnis der menschlichen reflexe mit besonderer berucksichtigung der elektrischen erscheinungen," *Arch Anat Physiol*, vol. 1, pp. 223–246, 1910.

[64] M. J. Aminoff, R. B. Lay, S. Satya-Murti, and A. I. Faden, "The declining electrical response of muscle to repetitive nerve stimulation in myotonia," *Neurology*, vol. 27, no. 9, pp. 812–812, 1977.

[65] T. W. Farmer, F. Buchthal, and P. Rosenfalck, "Refractory period of human muscle after the passage of a propagated action potential," *Electroencephalography and clinical neurophysiology*, vol. 12, no. 2, pp. 455–466, 1960.

[66] M. C. Kiernan, I. Mogyoros, and D. Burke, "Differences in the recovery of excitability in sensory and motor axons of human median nerve," *Brain*, vol. 119, no. 4, pp. 1099–1105, 1996.

[67] A. Blight and S. Someya, "Depolarizing afterpotentials in myelinated axons of mammalian spinal cord," *Neuroscience*, vol. 15, no. 1, pp. 1–12, 1985.

[68] B. G. Lapatki, J. P. Van Dijk, I. E. Jonas, M. J. Zwarts, and D. F. Stegeman, "A thin, flexible multielectrode grid for high-density surface emg," *Journal of Applied Physiology*, vol. 96, no. 1, pp. 327–336, 2004.

[69] K. C. McGill, K. L. Cummins, L. J. Dorfman, B. B. Berlizot, K. Luetkemeyer, D. G. Nishimura, and B. Widrow, "On the nature and elimination of stimulus artifact in nerve signals evoked and recorded using surface electrodes," *Biomedical Engineering, IEEE Transactions on*, no. 2, pp. 129–137, 1982.

[70] V. O. Andersen and F. Buchthal, "Low noise alternating current amplifier and compensator to reduce stimulus artefact," *Medical and biological Engineering*, vol. 8, no. 5, pp. 501–508, 1970.

[71] L. D. Weiss, J. M. Weiss, and J. K. Silver, *Easy EMG: a guide to performing nerve conduction studies and electromyography*. Elsevier Health Sciences, 2015.

[72] Z. B. Mahbub and K. Rabbani, "Frequency domain analysis to identify neurological disorders from evoked emg responses," *Journal of biological physics*, vol. 33, no. 2, pp. 99–108, 2007.

[73] D. Boërio, J.-Y. Hogrel, G. Bassez, and J.-P. Lefaucheur, "Neuromuscular excitability properties in myotonic dystrophy type 1," *Clinical Neurophysiology*, vol. 118, no. 11, pp. 2375–2382, 2007.

[74] R. Merletti and P. Di Torino, "Standards for reporting emg data," *Journal of Electromyography and Kinesiology*, vol. 9, no. 1, pp. 3–4, 1999.

[75] D. Stegeman and H. Hermens, "Standards for surface electromyography: the european project (seniam," in *In: Hermens HJ, Rau G., Disselhorst-Klug C., Freriks B.(Eds.), Surface Electromyography Application Areas and Parameters. Proceedings of the Third General SENIAM Workshop on surface electromyography*, Citeseer, 1998.

[76] D. T. Mewett, H. Nazeran, and K. J. Reynolds, "Removing power line noise from recorded emg," in *Engineering in Medicine and Biology So-*

ciety, 2001. Proceedings of the 23rd Annual International Conference of the IEEE, vol. 3, pp. 2190–2193, IEEE, 2001.

[77] L. Mesin, R. Merletti, and A. Rainoldi, "Surface emg: the issue of electrode location," *Journal of Electromyography and Kinesiology*, vol. 19, no. 5, pp. 719–726, 2009.

[78] L. Arendt-Nielsen and T. Sinkjær, "Quantification of human dynamic muscle fatigue by electromyography and kinematic profiles," *Journal of Electromyography and Kinesiology*, vol. 1, no. 1, pp. 1–8, 1991.

[79] T. Öberg, L. Sandsjö, and R. Kadefors, "Subjective and objective evaluation of shoulder muscle fatigue," *Ergonomics*, vol. 37, no. 8, pp. 1323–1333, 1994.

[80] "Paired Sample T-Test." `http://www.statisticssolutions.com/manova-analysis-paired-sample-t-test/`. Accessed: 2017-05-10.

[81] J.-Y. Hogrel, J. Duchêne, and J.-F. Marini, "Variability of some semg parameter estimates with electrode location," *Journal of Electromyography and Kinesiology*, vol. 8, no. 5, pp. 305–315, 1998.

[82] B. Mohammadi, N. Mitrovic, F. Lehmann-Horn, R. Dengler, and J. Bufler, "Mechanisms of cold sensitivity of paramyotonia congenita mutation r1448h and overlap syndrome mutation m1360v," *The Journal of physiology*, vol. 547, no. 3, pp. 691–698, 2003.

[83] D. Lorenz, "Postactivation potentiation: An introduction," *International journal of sports physical therapy*, vol. 6, no. 3, p. 234, 2011.

Appendices

A

NDM Patients - Tables

A.1 5Hz

	MC					
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
slope V1 (mV/s)	3	-0.03	0.28	2	0.34	0.38
intercept V1 (mV)	3	2.33	0.50	2	1.21	0.45
slope V2 (mV/s)	3	-0.19	0.19	2	-0.83	1.21
intercept V2 (mV)	3	2.56	1.08	2	4.29	3.40
slope V3 (mV/s)	1	0.44	0.00	3	-0.04	0.30
intercept V3 (mV)	1	6.21	0.00	3	1.47	0.08
	PC					
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
slope V1 (mV/s)	1	-0.01	0.00	2	-0.15	0.13
intercept V1 (mV)	1	0.34	0.00	2	0.79	0.31
slope V2 (mV/s)	5	0.64	0.22	4	0.50	0.35
intercept V2 (mV)	5	1.75	0.13	4	1.54	0.63
slope V3 (mV/s)	1	-0.13	0.00	1	0.15	0.00
intercept V3 (mV)	1	0.32	0.00	1	2.05	0.00

Table 17: *5Hz:* parameter results for patients; split in subgroups MC and PC

A.2 Maximum M-response

	V1 MC						V1 PC					
	n	mean pre	SE pre	n	mean post	SE post	n	mean pre	SE pre	n	mean post	SE post
total amplitude (mV)	2	4.45	0.13	2	1.26	0.70	1	0.82	0.00	1	0.74	0.00
pp area (μVs)	1	3.67	0.00	2	1.63	1.41	1	0.82	0.00	1	0.32	0.00
np area (μVs)	1	1.67	0.00	2	0.13	0.02	1	0.14	0.00	1	0.40	0.00
duration pp (ms)	1	2.15	0.00	2	2.18	1.53	1	2.60	0.00	1	1.20	0.00
CV (m/s)	2	2.10	1.23	0	NaN	NaN	1	2.20	0.00	0	NaN	NaN

	V2 MC						V2 PC					
	n	mean pre	SE pre	n	mean post	SE post	n	mean pre	SE pre	n	mean post	SE post
total amplitude (mV)	2	4.01	1.78	2	1.72	0.50	5	2.28	0.76	4	2.26	1.22
pp area (μVs)	2	4.03	2.00	1	1.16	0.00	4	2.19	1.02	2	5.18	0.79
np area (μVs)	2	2.51	1.48	1	1.52	0.00	4	2.63	1.66	2	4.34	2.69
duration pp (ms)	2	3.03	0.23	1	2.00	0.00	4	2.29	0.73	2	4.20	0.70
CV (m/s)	2	3.23	0.77	2	33.94	32.72	4	2.59	0.18	2	4.40	1.99

	V3 MC						V3 PC					
	n	mean pre	SE pre	n	mean post	SE post	n	mean pre	SE pre	n	mean post	SE post
total amplitude (mV)	3	3.28	0.99	3	1.21	0.25	3	2.70	1.10	2	2.01	0.35
pp area (μVs)	2	3.50	2.37	3	0.87	0.27	3	2.78	1.29	2	1.03	0.03
np area (μVs)	2	2.52	1.49	3	0.50	0.23	3	1.34	0.78	2	0.59	0.08
duration pp (ms)	2	2.65	0.50	3	2.00	0.46	3	2.53	0.69	2	1.35	0.70
CV (m/s)	2	3.13	1.88	3	3.06	1.05	3	2.72	1.37	0	NaN	NaN

Table 18: *maximum M-response:* time domain results for patients; split in subgroups MC and PC

	V1 MC					V1 PC				
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**	**n**	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
A	2	137.93	29.60	2	58.61	41.29	1	28.39	0.00	1	28.57	0.00
f	2	160.00	80.00	2	100.00	20.00	1	80.00	0.00	1	80.00	0.00
$\Delta f_{10\%}$	0	NaN	NaN	0	NaN	NaN	0	NaN	NaN	0	NaN	NaN
$\Delta f_{50\%}$	2	243.00	77.00	2	160.00	35.00	1	149.00	0.00	0	NaN	NaN
$\Delta f_{90\%}$	2	49.50	15.50	2	47.00	25.00	1	58.00	0.00	1	66.00	0.00
a_{0-1}	2	880.74	21.72	2	262.91	162.95	1	166.21	0.00	1	139.86	0.00
a_{1-5}	2	26.70	9.20	2	18.40	10.63	1	6.62	0.00	1	10.45	0.00
$R_{10/90}$	0	NaN	NaN	0	NaN	NaN	0	NaN	NaN	0	NaN	NaN
$R_{10/50}$	0	NaN	NaN	0	NaN	NaN	0	NaN	NaN	0	NaN	NaN
$R_{10/p}$	0	NaN	NaN	0	NaN	NaN	0	NaN	NaN	0	NaN	NaN
$R_{50/p}$	2	1.70	0.37	2	1.59	0.03	1	1.86	0.00	0	NaN	NaN
$R_{90/p}$	2	0.35	0.08	2	0.44	0.16	1	0.73	0.00	1	0.83	0.00
R_{a}	2	37.12	11.98	2	29.13	25.69	1	25.10	0.00	1	13.39	0.00
	V2 MC						**V2 PC**					
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**	**n**	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
A	2	113.59	44.38	2	53.91	15.04	5	88.35	25.88	4	98.33	48.97
f	2	80.00	0.00	2	120.00	40.00	5	96.00	9.80	4	60.00	20.00
$\Delta f_{10\%}$	1	422.00	0.00	0	NaN	NaN	1	458.00	0.00	2	261.50	77.50
$\Delta f_{50\%}$	2	227.00	41.00	2	196.00	13.00	4	143.25	13.53	3	125.00	20.30
$\Delta f_{90\%}$	2	69.50	5.50	2	52.00	19.00	5	45.20	8.22	3	29.67	0.88
a_{0-1}	2	735.38	321.78	2	325.67	83.58	5	446.70	143.21	4	437.62	220.53
a_{1-5}	2	25.65	7.41	2	6.98	1.52	5	26.26	10.28	4	28.63	8.72
$R_{10/90}$	1	6.59	0.00	0	NaN	NaN	1	11.74	0.00	2	9.02	3.09
$R_{10/50}$	1	1.57	0.00	0	NaN	NaN	1	2.65	0.00	2	2.46	0.60
$R_{10/p}$	1	5.28	0.00	0	NaN	NaN	1	5.73	0.00	2	3.27	0.97
$R_{50/p}$	2	2.84	0.51	2	1.80	0.49	4	1.51	0.27	3	1.56	0.25
$R_{90/p}$	2	0.87	0.07	2	0.43	0.02	5	0.49	0.10	3	0.37	0.01
R_{a}	2	27.33	4.66	2	51.73	23.26	5	27.65	13.56	4	17.11	9.03
	V3 MC						**V3 PC**					
	n	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**	**n**	**mean pre**	**SE pre**	**n**	**mean post**	**SE post**
A	3	118.94	40.12	3	31.13	6.87	3	90.52	38.33	2	95.24	40.68
f	3	120.00	23.09	3	146.67	13.33	3	146.67	13.33	2	120.00	40.00
$\Delta f_{10\%}$	1	408.00	0.00	2	474.00	1.00	2	347.50	1.50	0	NaN	NaN
$\Delta f_{50\%}$	3	164.67	8.76	3	260.00	8.02	3	176.00	15.04	2	129.00	29.00
$\Delta f_{90\%}$	3	34.00	3.21	3	88.67	21.96	3	57.67	9.94	2	36.50	17.50
a_{0-1}	3	628.14	177.35	3	223.34	38.28	3	494.61	178.56	2	427.73	84.09
a_{1-5}	3	17.21	2.40	3	15.99	7.81	3	24.64	3.69	2	24.27	10.71
$R_{10/90}$	1	10.20	0.00	2	7.14	3.15	2	6.21	1.72	0	NaN	NaN
$R_{10/50}$	1	2.52	0.00	2	1.80	0.07	2	1.82	0.03	0	NaN	NaN
$R_{10/p}$	1	2.55	0.00	2	3.46	0.49	2	2.53	0.35	0	NaN	NaN
$R_{50/p}$	3	1.51	0.38	3	1.80	0.16	3	1.23	0.20	2	1.12	0.13
$R_{90/p}$	3	0.30	0.03	3	0.59	0.11	3	0.41	0.11	2	0.29	0.05
R_{a}	3	39.10	12.13	3	22.89	9.18	3	19.14	5.13	2	19.98	5.35

Table 19: *maximum M-response:* frequency domain parameter results for patients; split in subgroups MC and PC

A.3 MVC

	V1 MC						V1 PC					
	n	mean pre	SE pre	n	mean post	SE post	n	mean pre	SE pre	n	mean post	SE post
Force (aN)	1	1.34	0.00	2	1.83	0.57	6	0.88	0.15	7	1.18	0.26
RMS (mV)	1	1.12	0.00	2	0.57	0.44	6	0.15	0.02	7	0.31	0.08
MPF (Hz)	1	176.47	0.00	2	146.71	32.57	6	120.63	14.61	7	127.30	12.02
CV (m/s)	1	2.37	0.00	0	NaN	NaN	3	2.48	0.11	5	2.59	0.35
	V2 MC						V2 PC					
	n	mean pre	SE pre	n	mean post	SE post	n	mean pre	SE pre	n	mean post	SE post
Force (aN)	2	2.12	0.79	3	1.79	0.33	7	1.38	0.22	6	1.82	0.54
RMS (mV)	2	0.38	0.13	3	0.27	0.06	7	0.34	0.05	6	0.39	0.10
MPF (Hz)	2	134.29	4.14	3	144.27	9.49	7	104.08	9.52	6	113.29	12.44
CV (m/s)	1	5.32	0.00	0	NaN	NaN	2	3.06	0.28	2	2.18	0.57
	V3 MC						V3 PC					
	n	mean pre	SE pre	n	mean post	SE post	n	mean pre	SE pre	n	mean post	SE post
Force (aN)	2	2.13	0.50	3	2.33	0.35	7	1.26	0.19	6	1.17	0.19
RMS (mV)	2	0.47	0.23	3	0.31	0.04	7	0.20	0.09	6	0.35	0.12
MPF (Hz)	2	133.58	36.03	3	123.79	18.09	7	95.44	16.71	6	122.75	21.02
CV (m/s)	0	NaN	NaN	1	3.91	0.00	1	3.03	0.00	5	3.81	0.60

Table 20: *MVC:* parameter results for patients; split in subgroups MC and PC

A.4 Fatigue

	V1 MC						**V1 PC**					
	n	**mean first 2s**	**SE first 2s**	**n**	**mean last 2s**	**SE last 2s**	**n**	**mean first 2s**	**SE first 2s**	**n**	**mean last 2s**	**SE last 2s**
Force (aN)	1	0.90	0.00	1	0.82	0.00	7	0.45	0.09	7	0.40	0.13
RMS (mV)	1	0.71	0.00	1	0.48	0.00	7	0.15	0.05	7	0.11	0.04
MPF (Hz)	1	175.59	0.00	1	131.13	0.00	7	130.85	19.13	7	115.07	13.76
CV (m/s)	1	3.33	0.00	1	3.23	0.00	4	1.82	0.83	4	1.49	0.77
	n	**mean**			**SE**		**n**	**mean**			**SE**	
r_{Force}	1	0.92			0.00		7	0.86			0.25	
r_{RMS}	1	0.67			0.00		7	0.79			0.11	
r_{MPF}	1	0.75			0.00		7	0.90			0.07	
r_{CV}	1	0.97			0.00		3	0.77			0.10	
VC$_{\text{Force}}$ **(%)**	1	6.40			0.00		7	33.31			11.33	
AR_{Force}	1	0.02			0.00		7	0.01			0.12	
AR_{RMS}	1	0.15			0.00		7	0.16			0.11	
AR_{MPF}	1	0.22			0.00		7	0.08			0.03	
AR_{CV}	1	0.25			0.00		3	-0.03			0.16	
	V2 MC						**V2 PC**					
	n	**mean first 2s**	**SE first 2s**	**n**	**mean last 2s**	**SE last 2s**	**n**	**mean first 2s**	**SE first 2s**	**n**	**mean last 2s**	**SE last 2s**
Force (aN)	2	1.43	0.46	2	0.91	0.06	7	0.88	0.15	7	0.76	0.16
RMS (mV)	2	0.35	0.13	2	0.21	0.09	7	0.20	0.05	7	0.12	0.05
MPF (Hz)	2	156.08	16.02	2	99.69	16.67	7	118.08	15.24	7	114.48	22.51
CV (m/s)	2	11.31	2.98	2	5.30	1.85	4	1.73	1.52	4	1.11	0.86
	n	**mean**			**SE**		**n**	**mean**			**SE**	
r_{Force}	2	0.69			0.17		7	0.81			0.07	
r_{RMS}	2	0.59			0.03		7	0.66			0.13	
r_{MPF}	2	0.63			0.04		7	1.00			0.19	
r_{CV}	2	0.46			0.04		2	0.90			0.32	
VC$_{\text{Force}}$ **(%)**	2	17.66			8.00		7	13.83			4.17	
AR_{Force}	2	0.15			0.07		7	0.10			0.04	
AR_{RMS}	2	0.28			0.06		7	0.24			0.12	
AR_{MPF}	2	0.23			0.06		7	0.00			0.09	
AR_{CV}	1	0.31			0.00		4	0.04			0.04	
	V3 MC						**V3 PC**					
	n	**mean first 2s**	**SE first 2s**	**n**	**mean last 2s**	**SE last 2s**	**n**	**mean first 2s**	**SE first 2s**	**n**	**mean last 2s**	**SE last 2s**
Force (aN)	3	1.74	0.42	3	1.23	0.22	9	0.95	0.16	9	0.83	0.13
RMS (mV)	3	0.22	0.10	3	0.12	0.05	9	0.14	0.04	9	0.08	0.02
MPF (Hz)	3	155.83	25.88	3	129.43	22.73	9	127.53	19.64	9	135.80	24.92
CV (m/s)	2	3.13	0.10	2	2.65	0.57	3	2.86	1.61	3	1.98	1.05
	n	**mean**			**SE**		**n**	**mean**			**SE**	
r_{Force}	3	0.83			0.26		9	0.91			0.05	
r_{RMS}	3	0.63			0.14		9	0.73			0.13	
r_{MPF}	3	0.83			0.01		9	1.07			0.09	
r_{CV}	2	0.84			0.16		2	0.80			0.38	
VC$_{\text{Force}}$ **(%)**	3	26.28			11.11		9	10.08			2.52	
AR_{Force}	3	0.15			0.18		9	0.03			0.04	
AR_{RMS}	3	0.13			0.14		9	0.24			0.08	
AR_{MPF}	3	0.21			0.04		9	0.00			0.05	
AR_{CV}	1	0.35			0.00		3	0.00			0.11	

Table 21: *Fatigue:* parameter results for patients; split in subgroups MC and PC