Rui Dinis Teodoro Candeias

Licenciatura em Engenharia Biomédica

Evaluation of motor neuron excitability by CMAP scanning with modulated current

Dissertação para obtenção do Grau de Mestre em
Engenharia Biomédica

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FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Setembro 2014

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À minha família

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I would like to express my thanks to my supervisor Prof. Gamboa, my coordinator Tiago and Neuza of PLUX, for all the help and support in this work.

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Abstract

It is important to have better evaluation and understanding of the motor neuron physiology, with the goal to early and objectively diagnose and treat patients with neurodegenerative pathologies. The Compound Muscle Action Potential (CMAP) scan is a non-invasive diagnosis technique for neurodegenerative pathologies, such as ALS, and enables a quick analysis of the muscle action potentials in response to motor nerve stimulation. This work aims to study the influence of different pulse modulated waveforms in peripheral nerve excitability by CMAP scan technique on healthy subjects.

A total of 13 healthy subjects were submitted to the same test. The stimuli were applied in the medium nerve on the right wrist and electromyography signal collected on the Abductor Pollicis Brevis (APB) muscle surface on the right thumb. Stimulation was performed with an increasing intensities range from 4 to 30 mA, with varying steps, 3 stimuli per step. The procedure was repeated 4 times per subject, each repetition using a different single pulse stimulation waveform: monophasic square, monophasic triangular, monophasic quadratic and biphasic square. Results were retrieved from the averaging of the stimuli on each current intensity step. The square pulse needs less current intensity to generate the same response amplitude regarding the other waves and presents a more steep curve slope and this effect is gradually decreasing for the triangular and quadratic pulse, respectively, being the difference even more evident regarding the biphasic pulse. The control of the waveform stimulation pulse allows varying the stimulus-response curve slope.

Keywords: Compound Muscle Action Potential scan, Peripheral Nerve Stimulation, surface Electromyography, Amyotrophic Lateral Sclerosis.

Resumo

É importante haver uma melhor avaliação e compreensão da fisiologia neuromotora, com o objectivo de diagnosticar precocemente e objectivamente pacientes com doenças neurodegenerativas. O CMAP Scan é uma técnica de diagnóstico não-invasiva para doenças neurodegenerativas, como a ELA, e permite uma análise rápida dos potenciais de acção do músculo em resposta à estimulação neuromotora. Este trabalho pretende estudar a influência de diferentes tipos de onda com pulso de corrente modelado na excitabilidade do nervo periférico através da técnica de CMAP scan.

No estudo efectuado em pessoas saudáveis, um total de 13 sujeitos foram submetidos ao mesmo teste. Os estímulos foram aplicados no nervo mediano do pulso direito e o sinal de electromiografia recolhido na superfície muscular do APB do polegar. A estimulação foi efectuada com um intervalo crescente de intensidades dos 4 aos 30 mA, distribuídos em vários passos, 3 estímulos aplicados por passo. O procedimento foi repetido 4 vezes por sujeito, cada repetição efectuada usando um tipo diferente de pulso de onda: formas de onda quadrada, triangular e quadráticas monofásicas e uma forma de onda quadrada mas bifásica. Os resultados foram recolhidos efectuando a media de todos os estímulos em cada incremento de corrente. A onda quadrada monofásica necessita de menor intensidade de corrente do estímulo para gerar a mesma amplitude de resposta em relação às outras ondas e apresenta um declive da curva mais acentuado e este efeito é gradualmente decrescente para as ondas triangular e quadrática, respectivamente, sendo a diferença ainda mais evidente comparativamente à onda bifásica. O controlo da forma do pulso de onda permite variar a inclinação da curva representativa da resposta-estímulo.

Palavras-chave: CMAP scan, PNS, sEMG, ALS.

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Acronyms

ALS – Amyotrophic Lateral Sclerosis

CMAP – Compound Muscle Action Potential

sEMG – surface Electromyography

ES – Electrical Stimulation

PNS – Peripheral Nerve Stimulation

TENS – Transcutaneous Electrical Nerve Stimulation

PeNS – Peripheral Nervous system

SI – Stimulus Intensity

MU – Motor Unit

LMN – Lower Motor Neuron

UMN – Upper Motor Neuron

AP – Action Potential

MUAP – Motor Unit Action Potential

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1. Introduction

1.1. Motivation

In the last years, there is a growing scientific and clinical interest on objective evaluation of the motor capability and better understanding of the motor neuron physiology, with the goal to early and objectively diagnose and treat patients with neurodegenerative pathologies.

Amyotrophic Lateral Sclerosis (ALS), also known as Motor Neuron Disease or commonly Lou Gehrig's disease, is one of the major neurodegenerative diseases, characterized by being a progressive incurable motor neuron disorder and also fatal. Population-based studies in Europe estimate that the occurrence of ALS is 2-16 per 100 000 person-year. Patients with ALS are diagnosed when there is already extensive motor neuron degeneration, since no definitive diagnostic test or biomarker for ALS is available at the moment, and neurologists only rely on clinical indicators for diagnosis [1].

The Compound Muscle Action Potential (CMAP) scan is a non-invasive diagnosis technique for neurodegenerative pathologies, such as ALS. It enables a quick analysis of the muscle action potentials in response to motor nerve stimulation, by electrical stimulation applied on the surface of the motor nerve and response evaluation by surface EMG at muscle level. It can be used as a tool for a better understanding of the neuromuscular excitability, allowing the study and development of diagnosis protocols for patients with neurodegenerative disorders [3].

This work aims to study the influence of pulse modulated waveforms in peripheral nerve stimulation, through CMAP scan technique.

1.2. State of the Art

Electrical Stimulation (ES) is the activation of the nerve/muscle, applying artificial stimulation through an electronic device directly on the nerve or muscle. Nerve response is evaluated through EMG, with electrodes placed on the surface of the muscle on study. Varying the intensity of the stimuli applied, it is possible to obtain a graphical representation of the evoked action potential of the muscle, in a sigmoid graphic form, that corresponds to the stimulus-response curve and composes the CMAP scan [9].

The CMAP scan technique has been studied as a non-invasive diagnostic and monitoring tool to neurodegenerative disorders, since it gives information about reinnervation processes, number of functional motor units and neuromuscular activity. To be used as a clinical tool, stimulation parameters must be standardized and quantified to enable uniform collection and comparison of data [4].

Several studies have been made recently, in order to verify the potentiality of this technique, investigating the influence of different parameters in the quality of the CMAP scan.

Maathuis et al.[4] studied its reproducibility on healthy patients in several parameters, like the maximum CMAP, S5 (the stimulus intensity that elicited 5% of the maximum CMAP), S50 (stimulus intensity that elicited 50% of the maximum CMAP), S95 (stimulus intensity that elicited 95% of the maximum CMAP), SI range (S95 – S5) and step percentage (steps are clear visible jumps in CMAP amplitude within consecutive stimuli). It was concluded that both inter-observer reproducibility and different-day reproducibility were good for all tested parameters, with evidence that this technique is suitable to detect physiological alterations in the considered parameters [4].

Henderson et al.[9] examined the differences of the stimulus-intensity curve and the variability of the CMAP scan between healthy and ALS subjects. It was showed that there is a significant difference on the CMAP scan, regarding CMAP variability and step number and size, as ALS patients present more and larger steps on the stimulus-response curve than healthy controls, as it is sown in figure 1. The presence of several steps indicates loss of motor units and reinnervation. A CMAP decrement was defined as difference greater than 10% between the first and fifth CMAP negative peak amplitude [9].

The influence of stimulus duration on nerve excitability is well known, and shorter stimulus duration results in higher stimulus intensity needed to elicit the same CMAP amplitude. The effect of other parameters on the properties of the CMAP scan, such as stimulus frequency and total number of stimuli, are not yet fully known, especially on the number and size of steps. Maathuis et al.[3] pretended to define the optimal stimulus protocol settings for frequency, pulse duration and stimuli number, taking into consideration subject discomfort, movement artefacts and recording duration. Obtained results showed that stimulus duration and number of stimuli required further standardization, in order to guarantee that data from different studies can be compared. Optimal value for stimulus duration, despite its influence on the excitable variables, is yet arbitrary for the CMAP scan, but shorter stimulus duration will increase the resolution of the curve. Based on practical issues, stimulation pulse duration of 0.1ms is recommended. Stimulus

frequency has no influence on the CMAP variables in healthy controls, although high frequency augments the chance of decrements on the CMAP, so low frequency stimulation is advised. On the referred study, 2 Hz stimulation appeared to present better results concerning recording time and reducing movement artefacts and decrements. Experiments regarding stimuli number recommended that around 500 stimuli give enough detail in the stimulus-response curve, without excessive recording time (which increases movement risk and patient discomfort) [3].

A study has shown that decrement in motor response, due to repetitive nerve stimulation, in ALS patients is different between median and ulnar nerves, as muscle wasting preferentially affects the thenar muscles rather than the hypothenar muscles in these patients. The greater CMAP decrement in the median nerve was related to preferential involvement of the Abductor Pollicis Brevis (APB) in the pathophysiology of ALS [10].

During another work, Maathuis[3] also noticed that downwards recording direction was better tolerated by patients. Fixation of the thumb is highly advised, since it shortens the decrement size and limits the change in muscle fibre conduction velocity, enhancing the CMAP scan [3].

Mamede de Carvalho et al.[11] evaluated clinical neurophysiological methods of diagnosis to measure disease progress in ALS. Review of the CMAP technique acknowledged that CMAP amplitude showed the combined effects of denervation, muscle atrophy, compensatory reinnervation and also constitutes an indirect measure of the number of innervated fibres. CMAP amplitude has significant correlation with muscle strength, motor unit number estimation (MUNE) and functional disability in ALS. It was concluded that MUNE, M-Wave amplitude and Neurophysiological Index are reliable and sensitive to be used in clinical trials in ALS patients [11].

Maathuis et al.[21] refers that LMN disease progression electrophysiological features should be evaluated considering three pathophysiological aspects of the disease progression (axonal/MU loss, reinnervation and remaining number of functioning muscle fibres). All these aspects can be assessed in the CMAP scan [21].

Since denervation and reinnervation may be present at the same time in a single muscle affected by MND, these phenomenon need to be evaluated in combination in order to assess their effect on the remaining number of functioning muscle fibres, which the CMAP scan is suited for. When collateral reinnervation increases MU size, it is visible in new or larger steps in the CMAP scan and it also results in an increased step percentage and mean step size. The maximum amplitude gives essential information about the remaining functional muscle fibres, since it is a measure of the total number of muscle fibres that respond to the stimulus. Studies have found evidence that MUs are not subject to reinnervation the same way, but without assessing all MUs in the muscle. Maathuis et al.[21], using CMAP scan technique (first technique that can provide indication of the Motor Unit potential of all large and also reinnervated MUs, without the disadvantage of sampling bias), confirmed that some MUs are influenced by reinnervation much more than others. Different effects of reinnervation and motor unit loss are not limited to a specific muscle [21].

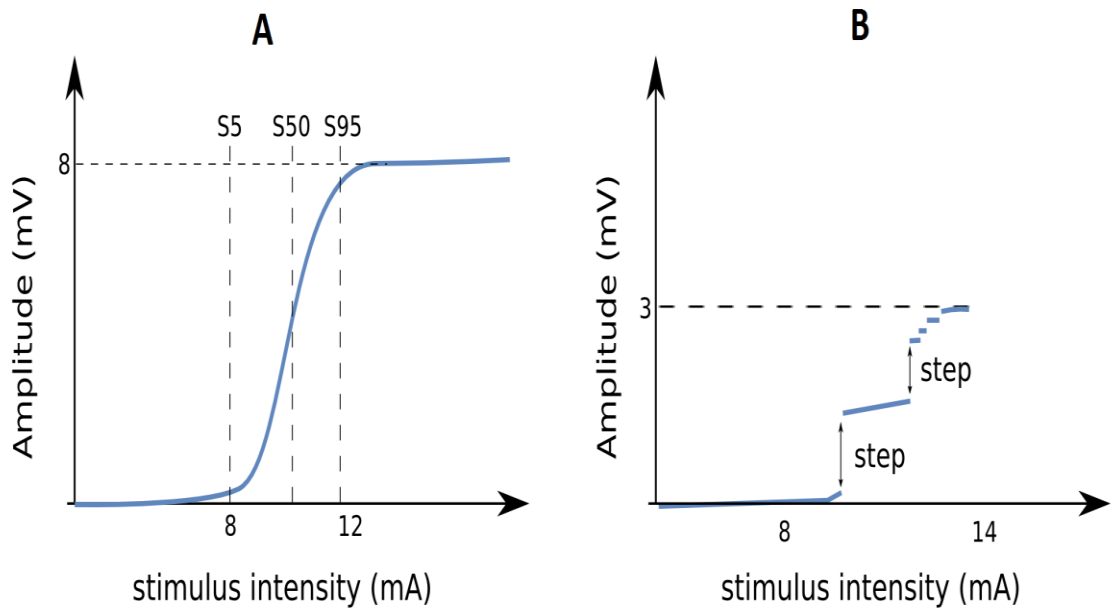

Figure1.1A: CMAP scan of a healthy individual, with the stimulus-response curve obtained with 500 stimuli. The horizontal line indicates the CMAP maximum amplitude while the vertical lines refer to S5,S50 and S95. These indicators correspond to the stimulus intensity that elicited 5%, 50% and 95% of the maximum CMAP, respectively. Figure 1.1B: CMAP scan of an ALS patient with 76 years old, six months after being diagnosed. Differences in the CMAP scan between the healthy and the ALS patient are visible because of several steps observed in the ALS CMAP scan and also the decrement in maximum amplitude. Adapted from [3].

1.3. Objective

The main goal of this work was to study the influence of modulated waveforms in the excitability of the peripheral nerve, through the CMAP scan technique.

An electrical stimulation protocol was developed, and biosignals from subjects were acquired, analysed and processed, in order to extract features that allowed the analysis of the influence of different waveforms in the stimulation of the peripheral nerve.

1.4. Thesis overview

To begin the preparation of the thesis, an introductory study to biosignals acquisition tools and processing was necessary. In order to process the acquired signals and compute the CMAP scans, Python language was used. An extensive research was conducted regarding the state of the art on the used methodologies and also literature revision concerning the main theoretical concepts.

An electrical stimulation protocol was defined, evaluating the parameters for optimal stimulation, and using different waveforms to study its influence on reflex response of the nervous system. Electrical stimulation using different types of waveforms was applied in the median nerve on the wrist and muscular response on the APB muscle of the thumb evaluated. Acquisition of biosignals was performed, as subjects electromyography signals were recorded, while being submitted to electrostimulation.

In order to extract the desired features and enable the proposed study, data processing algorithms were designed and developed in Python. Obtained results were analysed and influence of the parameters in study discussed. Writing of a scientific paper for a conference was effectuated.

The following figure presents a schematic of the work plan executed for this thesis.

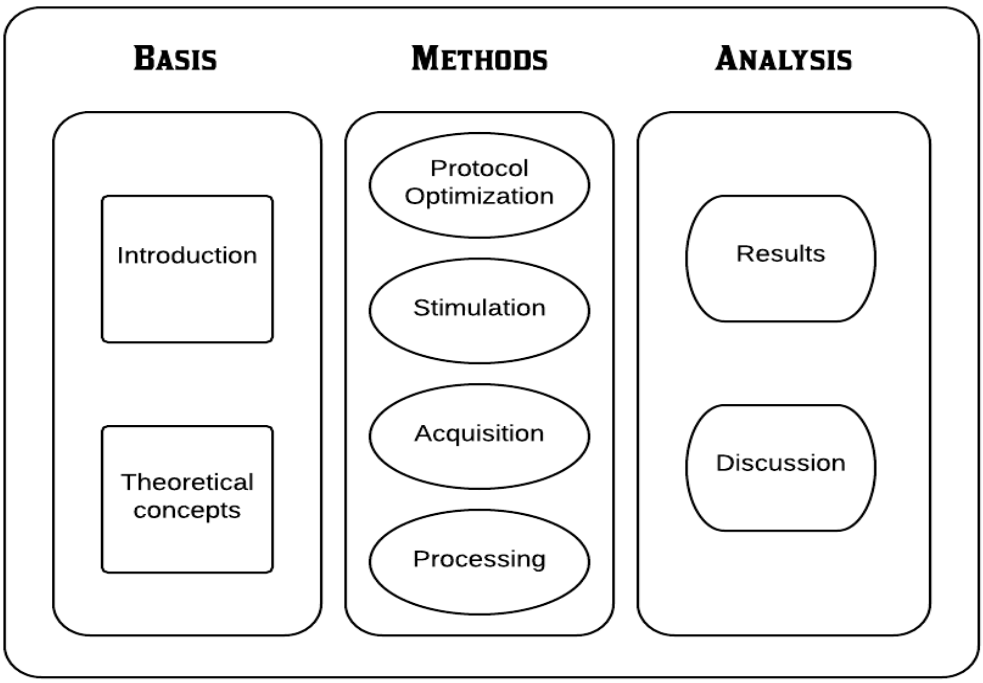

Figure 1.2: Schematic thesis overview, where it is shown the organization of the different chapters

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2. Theoretical Background

In this chapter an exposition of the main theoretical concepts will be presented. Surface EMG, electrical nerve stimulation and the CMAP scan technique will be approached. A brief description of the peripheral nervous system, motor units, concepts of electrical stimulation and amyotrophic lateral sclerosis will also be discussed.

2.1. **Eletromyography (EMG)**

EMG stands for Electromyography, which is the recording of the electrical activity created on the muscle excitable cell membrane during muscular contraction. The obtained signal is the spatial and temporal algebraic sum of all the detected signals within a certain area and represents voltage as a function of time. It is an important method to analyse muscular functioning, expressing in real time, muscular activation during movement and its intensity and duration [22]. It reflects neuromuscular activity and propagation of action potentials along muscle fibres, as the nervous system controls muscle activity. Besides that, it depends of the muscle anatomical and physiological properties and also acquires noise travelling through different tissues, making it a complicated signal to analyse. The signals can be analysed to detect clinical abnormalities, activation level, recruitment order or to analyse the movement biomechanics. From EMG it is possible to determine whether a particular muscle is responding appropriately to stimulation and whether a muscle remains inactive when not stimulated. One of the reasons for the interest in EMG signal analysis is in clinical diagnosis for neurodegenerative pathologies and biomedical applications as rehabilitation of motor disability [12].

The signals can be acquired attaching surface electrodes to the skin over the target muscle or by needle electrodes inserted invasively into the muscles tissue. Surface EMG measures overall action potentials of the muscle fibres under the skin along the entire recorded area underneath the electrodes, while needle electrodes measure action potentials from a small number of fibres and may not be representative of the entire muscle involved. In surface EMG two electrodes are placed on the skin over the muscle region to be analysed and the difference of potential between them is acquired and amplified [14].

In this work surface EMG, which is a non-invasive EMG technique, will be used. It will enable the analysis of motor unit properties that are difficult to measure with invasive technology, like muscle fibre conduction velocity, and provides more information related to the number of detectable motor units (which is a limitation of invasive methods). The sEMG signal can be spread into motor unit action potentials and gives information about neuromuscular activity and membrane fibre properties [13]. On the following figure it is shown an example of an EMG signal.

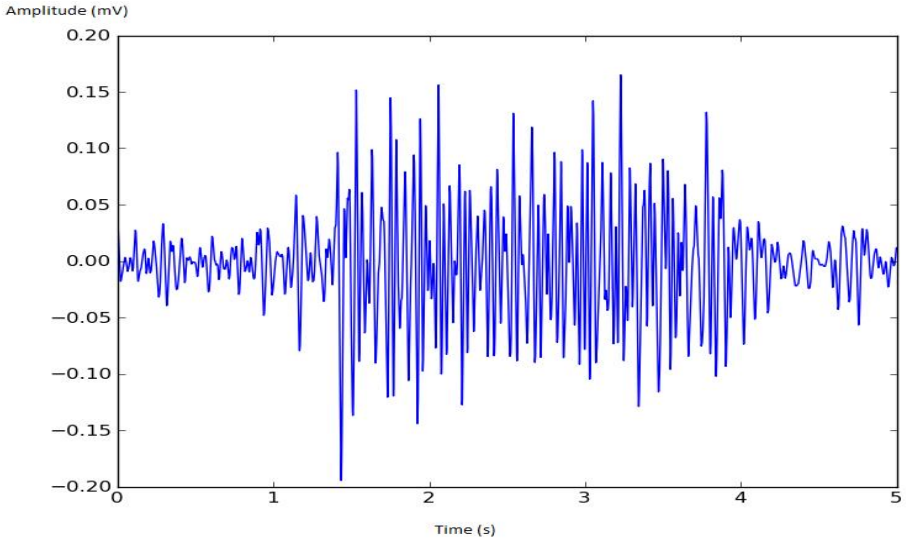

Figure 2.1: Representation of an EMG signal

Electrodes placement is important in order to acquire EMG signal, since it can occur interference due to neighbour muscles electrical activity, also referred as cross-talk. Other properties like the signal-to-noise ratio and the common mode rejection ratio can influence the quality of the EMG signal, as higher ratios correspond to better signal quality [14].

Sampling frequency is very important to correctly acquire EMG digital signal and therefore, according to Nyqüist Theorem, at least the double of the highest frequency in EMG signal should be used as the sampling frequency. sEMG may have frequencies of up to 500 Hz (these values are affected by motor unit and contraction, electrodes sizes and distance between them) and the minimum sampling rate value should be 1 KHz [23].

2.2 **Electrical Nerve Stimulation**

Peripheral Nerve Stimulation (PNS) consists in stimulating the peripheral nervous system, applying electrical current to activate a specific nerve. Electrical Stimulation (ES) on the selected nerve can be performed transcutaneously (transcutaneous electrical nerve stimulation – TENS), percutaneously with temporary electrodes (percutaneous electrical nerve stimulation - PENS) and with surgically or percutaneously implanted electrode [8].

TENS is an external neuromodulation modality, in which electrical current is delivered through intact skin along the path of the underlying chosen nerve. Usually, it is utilised as a non-invasive neuromodulation approach together with other physical therapy modalities and constitutes an alternative to more invasive methods. In contemporary medical practice, it is considered the most common application of peripheral neuromodulation. Electrical stimulation of peripheral nerve is an established modality in treatment of chronic pain and there have been recent reviews showing evidence in application of TENS for treatment of neuropathic and cancer pain [8].

Activation of neuromuscular tissue by electrical stimulation requires a minimum of two electrodes in order to produce a current flow. Electrodes are normally arranged in a monopolar or bipolar configuration. On bipolar configuration an electrode, usually named the active electrode, is placed near the peripheral nerve to be stimulated [15].

Stimulation is delivered as a waveform of electrical current pulses, which is characterized by pulse frequency, amplitude and duration. Suitable electrical stimuli can elicit action potentials in the innervating axons of the nerve, and the strength of the resultant muscle contraction can be controlled by modulating the stimulus parameters [15].

Also referred as transcutaneous systems, surface systems use electrodes that are connected with flexible leads to a stimulator. These electrodes are placed on the skin over the nerve or over the “motor points” of muscle to be activated. Advantages of surface systems are non-invasive and simple technologically, making them easily applied, reversible, relatively inexpensive, and good for utilisation in clinical and therapeutic applications [15].

The waveform is a graphical representation over time of a signal, as it reflects its shape. In electrical stimulation, the waveform represents the variation over time of the applied current or voltage on the muscle or nerve. It can be monophasic or biphasic, and also have different shapes like sine, square, among others, like it is shown on figure 2.2. In this study it is intended to evaluate the influence of different waveforms and therefore monophasic and biphasic as also different shaped waveforms like square, triangular and quadratic will be used.

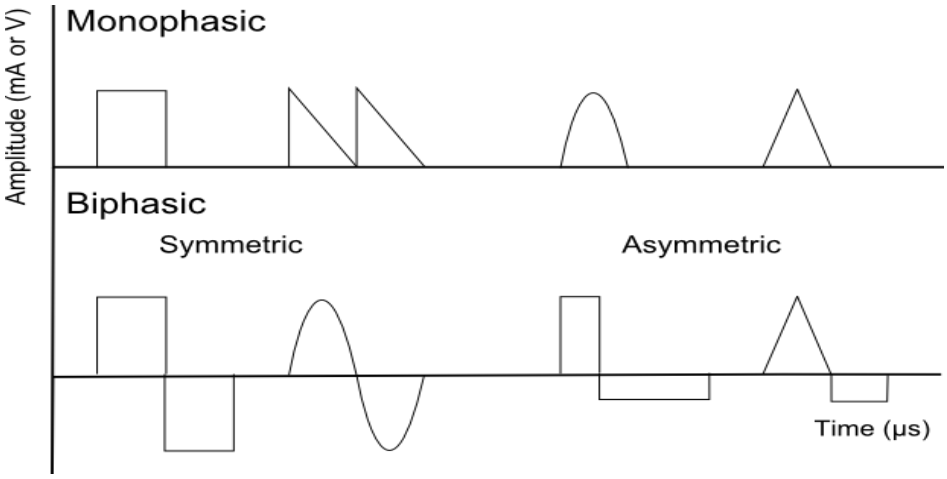

Figure 2.2: Examples of different types of waveforms. The monophasic square and the biphasic square types are some of the waveforms used in this work and are represented on the left.

2.3 Peripheral Nervous System, Motor Unit and Action Potential

The nervous system can be divided into Central Nervous System (CNS) and Peripheral Nervous System (PeNS).

Central components are nerves entirely contained within the brain and spinal cord. Central nervous system influences muscle activity through two sets of neurons: Upper Motor Neuron (UMN) and Lower Motor Neuron (LMN). They can be classified according to the muscle fibre type they innervate: alpha motor neurons, which innervate extrafusal muscle fibres and are responsible for muscle contraction, and gamma motor neurons, which innervate intrafusal muscle fibres. Peripheral components are nerves originated in the brain or spinal cord and ended peripherally, as well as cranial and spinal nerves. The Peripheral Nervous System includes motor, sensory, sympathetic and parasympathetic neurons, with the majority of nerves comprising a mixture of these types of neurons. Motor nerves are originated in the anterior horn of the spinal cord [6,7].

Efferent pathways, responsible for sending messages from the centre to the periphery, include somatic motor nerves that innervate skeletal muscles and the autonomic nervous system, with sympathetic and parasympathetic divisions that coordinate smooth muscle, cardiac muscle and glandular activity. Afferent pathways, which send messages from the periphery towards the centre, are responsible for a set of sensory modalities such as touch, position, vibration and pain. Motor neurons are efferent nerves which carry signals from the spinal cord to the muscle to produce movement. They can be classified in Upper Motor Neurons (UMN) and Lower Motor Neurons (LMN). UMNs carry impulses for voluntary muscle activity from the motor cortex or

brainstem to a specific nerve while LMNs carry the information from UMNs to muscle fibres. The alpha-motor neurons are the largest neurons in the spinal cord, with myelinated axons that exit the spinal cord through the ventral roots and travel in peripheral nerves to innervate muscles. Each muscle fibre is innervated by only one motor neuron but one motor neuron innervates many muscle fibres, all of the same fibre type, as its axon branches in the muscle. One motor neuron along with all the skeletal muscle fibres it innervates is named Motor Unit (MU), like it is shown on figure 2.3. Muscle fibres in different muscles are grouped into basic types specialised for different functions and there are two major types of muscle fibres: Type I - high level of vascularization, slow contraction and high fatigue resistance; Type II – low level of vascularization, rapid and strong contraction. As physiological properties of motor neurons and the muscle fibres they innervate are related, Motor Units cluster into basic types based on properties of twitch speed, amount of force produced and fatigability. For most movements, MUs are recruited in orderly sequence, based on the size of the motor neuron. According to this, MUs that produce the smallest amount of force will be the first to begin firing, and MUs that produce greater force will be progressively recruited, as the muscle makes progressively stronger contractions. Motor neurons innervating different muscles can be activated with great precision by different sources that together determine the degree of activation and the timing of the motor neurons of a given muscle [6,7].

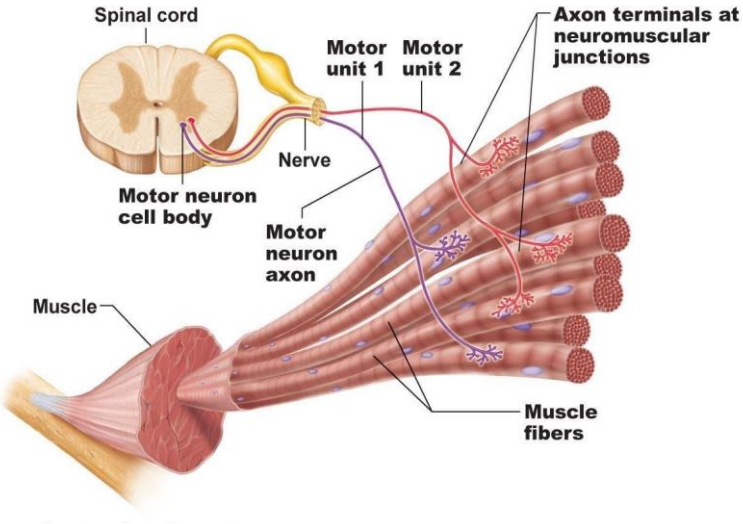

Figure 2.3: Motor Unit schematic. A Motor Unit is composed by a motor neuron from the spinal cord that innervates the neuromuscular junctions of different muscle fibres.

Motor Units are the basic component of muscular strength and contraction. To initiate the generation of muscle contraction, the CNS sends an electrical signal to a motor neuron, which spreads along muscle fibres, initiating a cascade of electrophysiological and electrochemical processes, giving rise to de-polarization and re-polarization events known as action potentials or nervous impulses, which can be electrically measurable. On figure 2.4 it is show the cell membrane potential variation that occurs during an action potential. Transmitting information via the nervous system is based on the propagation of action potentials along a nervous fibre, by means of diffusion, and the intensity of muscle contraction is controlled by the regularity of action

potentials. When a motor neuron receives an excitable signal, an action potential reaches muscles fibres innervated by it through one terminal branch of the axon. The combination of these APs along all the muscle fibres of a single MU is called the Motor Unit Action Potential (MUAP). If the nerve impulse arrives more often, the intensity of muscle contraction is greater. Muscle strength is associated to mechanical summation: as a result of higher stimulation regularity, the generated muscle force increases [14].

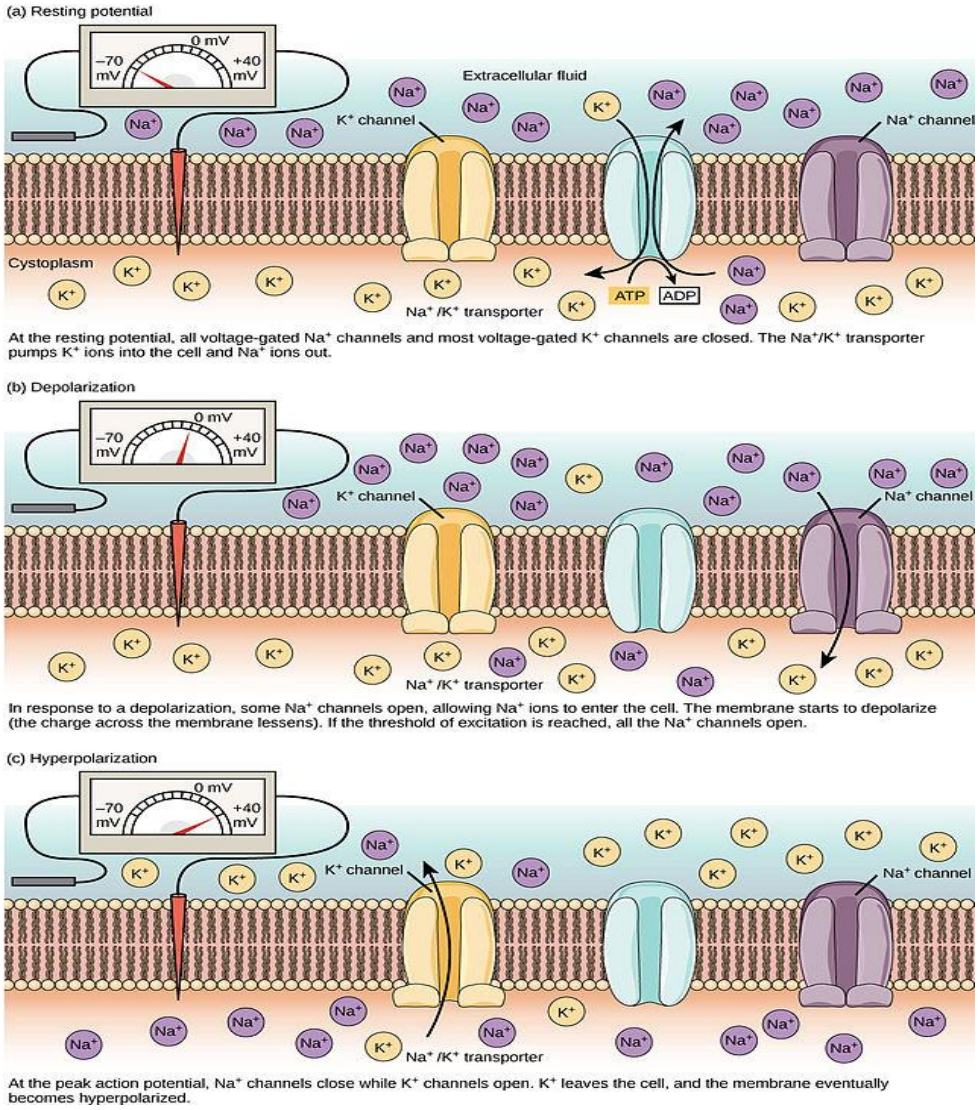

Figure 2.4: Cell membrane potential variation occurred during an action potential. The variation is due to the ionic flux of sodium and potassium through the membrane cell, which is different when the cell is resting (a), when occurs the depolarization (b), and at peak potential (c), which is followed by a period of hyperpolarization.

The potential is influenced by the ionic concentration gradients and the permeability of the cell membrane to certain ionic compounds. Cellular environment has high concentration of potassium (K+) and low concentration of sodium (Na+), and in resting position, will balance this

difference by being more permeable to the flux of potassium, which passes through the membrane to the cell exterior, in favour of the concentration gradient, generating a negative cell electrical potential. For the nerves and fibres of the smooth muscle, the value of the resting potential is about -70mV. The action potential translates on an electrical potential wave that goes through the membrane and revert its potential from -70 to +30 mV, in approximately 1ms. The membrane action potential value which generates an action potential is known as excitability threshold. The nervous impulse is initiated by the membrane depolarization due to chemical unbalance or by a perturbation like an electrical impulse. Depolarization above excitability threshold provokes the activation of the sodium channels in the cell membrane, which allows passing of sodium by diffusion to the cell interior, in favour of the concentration gradient, which rapidly revert the cell membrane negative polarity. It also provokes consequent activation of the potassium channels (and inactivation of the sodium channels), which allows the influx of potassium ions, and the potential inside the cell decreases [29]. After this phase, there is a hyperpolarization period, which is a change in a cell's membrane potential that makes it more negative and inhibits action potentials by increasing the stimulus required to move the membrane potential to the action potential threshold. Hyperpolarization is important in the transmission of information as it assures the signal is propagated in one direction, since it prevents any stimulus already sent up an axon from triggering another action potential in the opposite direction [30].

Functional Electrical Stimulation (FES) applications for motor function operate under the principle that electrical stimulation generally activates nerve rather than muscle, because the threshold charge for producing muscle fibre action potentials is much greater that the threshold for producing neurons action potentials. Electrical current pulses applied to nerves are able to produce Action Potentials (AP). The active electrode creates a localized electric field that depolarizes cell membranes of neighbour neurons. When the depolarization achieves a certain threshold, produces an action potential which is propagated in both directions away from the stimulus region. APs propagating proximally in the peripheral nerves axons will be annihilated at the cell body, and APs propagating distally will be transmitted across the neuromuscular junction causing the contraction of muscle fibres. A single motor unit, with sufficient stimulus, will induce all the skeletal muscle fibres it innervates to contract [16].

Information transmission on the nervous system is due to the propagation of the action potentials over the length of the nervous fibre. According to the propagation on different nervous fibres, the velocity of the nervous impulse varies also and normally the propagation velocity is proportional to the diameter of the nervous fibre. The higher the propagation velocity, the higher the length of the nervous fibre depolarized in each period [31].

The median nerve is a major peripheral nerve of the upper limb. It innervates flexor muscles in the anterior compartment of the forearm and also innervates some of the muscles in the hand via two branches. The recurrent branch of the median nerve innervates the thenar muscles – muscles associated with movements of the thumb. The palmar digital branch innervates the lateral two lumbricals – perform flexion at the metacarpophalangeal joints of the index and middle fingers. The abductor pollicis brevis is a thenar muscle in the hand responsible for the abduction function of the thumb [17].

2.4 Compound Muscle Action Potential Scan Technique

The Compound Muscle Action Potential (CMAP) scan can be used as a diagnostic and monitoring tool for neurodegenerative disorders, as it permits visualization and quantification of disease progression in a muscle with Motor Neuron Disease, such as ALS, like referred by Maathuis et al. [21]. The CMAP scan is a non-invasive electrodiagnostic technique, which records the electrical activity of a muscle in response to repetitive transcutaneous stimuli of motor nerve. Stimulus is applied by an electrostimulator positioned on the surface of the motor nerve in analysis and his response evaluated by surface EMG at the muscle to observe. Each Motor Unit (MU) of muscles has a different Stimulus Intensity (SI) at which it is activated, which means that MUs have different thresholds. If stimulus intensity is gradually increased, from subthreshold to supramaximal values, it will successively activate all of the MUs in the muscle. Making the plot of the CMAP amplitudes versus the stimuli intensities, results in a stimulus-response sigmoid curve, thus obtaining the CMAP scan [32].

The CMAP is recorded using a differential amplifier. The three electrodes connected to the amplifier are usually called active, reference, and ground electrodes. For CMAP recordings the active electrode is placed over the muscle belly, whereas the reference electrode is at the tendon or at other locations off the muscle. Conversely, it is also assumed that the CMAP is composed of signal recorded by the active electrode, as a nearfield potential, generated by muscle fibres that are immediately under the electrode [24].

If made with enough stimuli and therefore a high resolution, the stimulus-response curve provides information not available through conventional methods. For instance, it enables identification and quantification of steps, which are clearly visible size differences in the CMAP amplitudes between consecutive stimuli. These amplitude differences increase with stimulus intensity, which are originated by the firing of large and newly recruited motor units. Henderson et al. showed that patients with ALS had significant differences in the steps and CMAP variability in comparison with healthy controls [9].

In the following figure we can observe the stimulus-response graphics. Through the amplitude of the responses, originated by each stimulus (example on figure 2.5.A with stimulus of 8mA), it is possible to compose the CMAP scan. On figure 2.5.B we can see a CMAP scan obtained with current modulated stimulus with a monophasic waveform. The graphical representation of the different stimuli-responses amplitudes will result in a sigmoid stimulus-response curve.

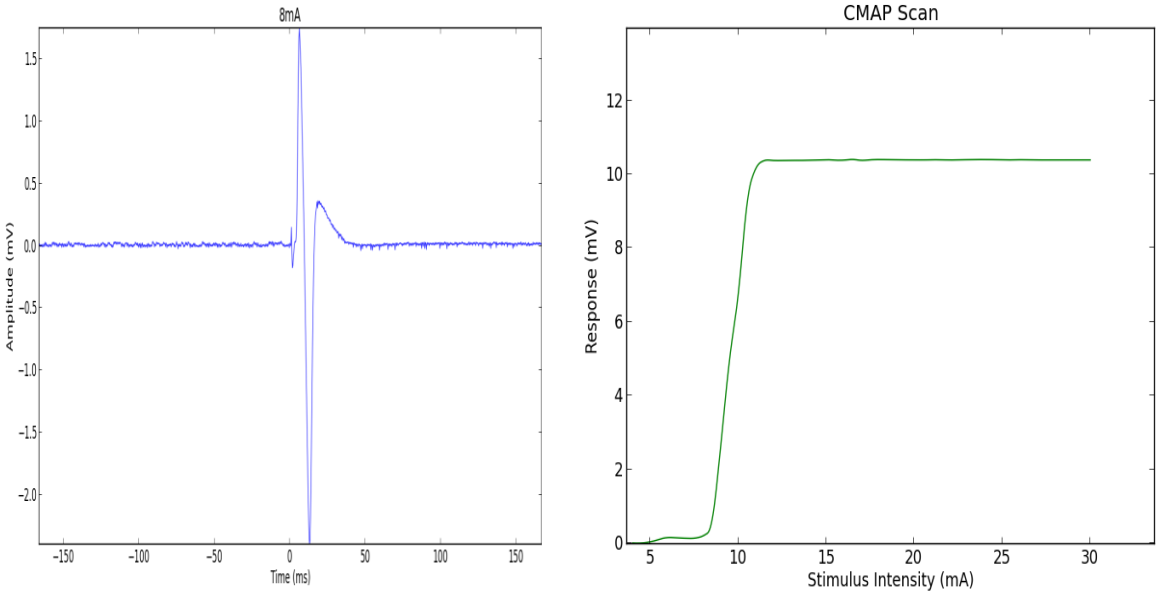

Figure 2.5.A: Response with stimulus of 8mA. The electrical stimulus marked at 0ms provokes the contraction of the thumb, whose response amplitude is above represented, with a stimulation intensity of 8mA. Figure 2.5.B: Representation of a CMAP scan. When stimulus intensity is gradually increased from subthreshold to supramaximal values, all the MUs in the muscle are recruited and the plot of the CMAP amplitude versus stimulus intensity results in the CMAP scan.

The CMAP scan also provides information on nerve excitability since an increase in SI corresponds to an increase in the recorded CMAP, depending on the excitability of individual Motor Units. The excitability parameters of the CMAP scan are the stimulus intensity that elicits 5%, 50% and 95% of the maximum CMAP - S5, S50 and S95 - and the range between S5 and S95 [33].

In Lower Motor Neuron degeneration, as in amyotrophic lateral sclerosis, occurs loss of Motor Units, subsequent reinnervation and, eventually, muscle fibre loss. Because of collateral reinnervation, the symptoms of motor neuron diseases (MND) like muscle weakness develop late during the disease and are not aligned with the actual disease progression measured considering loss of motor neurons. Before muscle strength decreasing is noticed, up to half of the MUs may be lost [21].

Maathuis refers that LMN disease progression electrophysiological features should be evaluated considering three pathophysiological aspects of the disease progression - axonal/MU loss, reinnervation and remaining number of functioning muscle fibres. All these aspects can be assessed in the CMAP scan [21].

Hence, various properties of the curve may provide clinically relevant information concerning reinnervation processes, MU number, MU size and stability, and axonal excitability, which can be available through quick and visual assessment of MU potentials. Therefore, this technique can be a valuable tool for monitoring disease progression or the speed and quality of nerve recovery in motor neuron disease and demyelinating diseases. To be established as a clinical

tool, the effect of the stimulus settings on the CMAP scan and its quantification must be defined, to enable standardized collection and comparison of CMAP scan data [3,4].

2.5 **Amyotrophic Lateral Sclerosis**

ALS is a fatal neurodegenerative disease of the human motor system. Its clinical features indicate degeneration of motor neurons at all levels, with destruction of layer V pyramidal neurons from the motor cortex to the anterior horn of the spinal cord. Those features include both Upper Motor Neuron and Lower Motor Neuron physical degeneration signs in multiple neuronal regions: bulbar, cervical, thoracic and lumbar [2].

ALS is a devastating disorder with yet uncertain pathogenesis, rapid progression and fatal, as 50% of the patients die in less than 3 years of symptom onset and about 20% survives 5 to 10 years after symptom onset. Survival in patients with ALS is dependent on several factors, which comprise clinical presentation (phenotype), rate of disease progression, early presence of respiratory failure and nutritional status of the patients [1,2].

Many causal and pathogenic theories have been proposed for ALS over the years, but it still remains poorly understood in terms of causal hypothesis. Some factors can increase the risk of developing ALS such as environmental factors, toxic risk factors, family history, tabacco, neurotoxins, consanguinity and genetic mutations with the copper/zinc superoxide dismutase considered the major one. Recent studies also targeted glutamate-induced excitoxicity, dysregulation of intracellular calcium, autophagy, structural abnormalities of mitochondria, dysfunction of the sodium/potassium ion pump, axonal transport defects and protein aggregation as other additional pathogenic hypotheses [1,2].

There are some prognostic indicators, such as increased age of onset, bulbar onset, low forced vital capacity and short time from first symptom to presentation of disease. The symptoms can be different concerning the neurological regions affected but some common features observed are rapidly progressive weakness, muscle atrophy and spasticity, difficulties in breathing (dyspnea), swallowing (dysphagia) and speaking (dysarthria). All these can greatly diminish the life quality of patients, as they tend to lose the ability to control voluntary movements [1].

Several functional rating scales have been developed in the last years, being ALS Functional Rating Scale the most used in clinical trials at present. The variability in clinical findings early in the course of the disorder and the lack of a biological marker make absolute diagnosis of ALS very difficult. One of the most important criteria for the diagnosis of ALS is the El Escorial criteria, which has been widely accepted and revised, and provides a structured approach to the assessment of people suspected of having the disease. ALS is diagnosed based on clinical and electromyographic data and also by excluding other possible diseases with similar physiological signs, since there are no specific tests or biological markers to have a sure confirmation. To make the diagnosis of ALS it is necessary: i) evidence of LMN degeneration, ii) evidence of

UMN degeneration, iii) progressive spread of symptoms within a region or to other regions; along with: iv) absence of electrophysiological or pathological evidence of other disease processes, v) absence of neuroimaging evidence of other diseases. When ALS is considered, patients should have electrophysiological studies performed to confirm LMN dysfunction in clinically uninvolved regions and exclude other pathophysiological processes [5].

In terms of medicine, just an inhibitor of glutamate neurotransmitter (Riluzole) has been licensed as disease-modifying for ALS, which extends the life of the patients by 3 to 6 months. Since this disorder is incurable, patients are administered with drugs that can relieve some symptoms and help cope with pain [2].

2.7 **Electrostimulation concepts**

Current is applied through the use of stimulation electrodes and the selection of the electrode depends of the application desired. This selection has to consider the electrode type, its dimensions and the anatomical positioning required. The electrodes can be self-adhesive, metal or conductive rubber. The self-adhesive electrodes were the ones used in this work, since they are easy to apply and don't need fixation, while ensuring good electrical contact along all contact surface of the electrode. The type and location of the electrodes influence the dimensions of the selected electrodes and larger electrodes result in a lower current density by area. The electrode positioning defines the larger current density local and the specific positions depend on the stimulation objective. For instance, if it is intended to stimulate an innervated muscle, the electrode positioning must assure that the nerve responsible for the muscle contraction is on the current pathway (over the nervous trunk or the motor point of the muscle or on a muscle termination). One of the most common electrode configurations used is the bipolar configuration, in which the current floats alternatively on both ways and uses two equal sized electrodes positioned over each of the terminations of the nerve or muscle to stimulate [26,27].

Electric current is defined as the quantity of electrical charges that flow across a conductive surface on a certain period of time. There are two kinds of current: continuous or alternate and pulsed or constant. The pulsed current is not continuous through time and allows defining a frequency and a pulse width associated. Usually, the pulsed current utilizes 1ms duration pulse or less, applied to frequencies that could reach 100 Hz. An action potential is provoked by each current pulse, if intensity stimulus is above the excitability threshold. Current pulses with shorter stimulus duration are less uncomfortable and have higher discrimination between sensorial and motor stimulation. The electric current polarity is due to charge unbalance. The current flux can be unidirectional (constant polarity/continuous current) or bidirectional (alternate polarity/alternate current). The stimulation intensity amplitude can be presented in miliAmpere

(mA) or Volts (V), which expresses the stimulation intensity that activates the fibres and the consequent response amplitude.[26]

The pulse duration or pulse width is the duration of the output waveform pulse at 50% of maximum amplitude (usually represented in microseconds (μs)). This parameter is important as it influences the current amplitude necessary to originate an action potential. If the stimulation time is higher than a few hundreds of microseconds there is an event of accommodation to the pulse waveform, which implies that higher current intensity is needed to elicit the action potential. The pulse time instant is also to consider as during the refractory period pulses will not elicit an action potential, unless with high intensity stimulus [25].

As referred, MUs are constituted by a motor neuron and all the muscle fibres it innervates. The number of muscle fibres a motor neuron can activate depends on the muscle specificity. For a small muscle, which requires more accurate movement control, a motor neuron activates a small number of muscle fibres, while on a larger muscle, a motor neuron can be associated with more than a thousand muscle fibres. On voluntary muscle contraction, motor neuron activation is triggered in an asynchronous way and contraction force is generated by the number of recruited motor units and the frequency of the nervous impulses action potentials, which means fibre force varies with stimulus frequency for intensities above motor threshold. The electrical stimulation mimics the events that originate muscle voluntary contraction. However, the muscle activation pattern in electric stimulus induced contraction is different from muscle activation in the voluntary contraction: electrical stimulation activates various neuron motors simultaneously while in voluntary activation they are triggered asynchronously, electrical stimulation does not activate the motor neurons by the same recruiting order as in voluntary contraction and in neuromuscular stimulation sensorial nerves are necessarily stimulated [28].

3

3. Methods

In this chapter it will be explained the biosignals collection from the group of subjects, the acquisition protocol and its optimization. It will also be exposed the signal processing applied to analyse the data acquired to evaluate the influence of the parameters in study.

3.1 Protocol optimization

The protocol optimization had several stages and the first one was the conception of the different types of waveforms to be used. On a first approach, 8 waveforms were generated and tested with different number of stimuli per current step, with varied current incremental steps and stimulation intensity range. Several tests were made with each waveform, varying the referred parameters, in order to obtain a protocol test that would allow obtaining a graphic with enough resolution of the stimuli-response curve but without excessive test duration, in order to avoid making the test uncomfortable for the subjects. Some waveforms, like the exponential, were removed due to reduction of time and stimulation intensity range (given this type of waveform required more stimuli intensity to generate the same response comparatively to the other waveforms) and also due to charge equalization issues (discussed in the charge equalization section). Also other biphasic waveforms were tested like triangular, quadratic or exponential but were not included on the test because of test duration issues, stimulation intensity required and also due to the inability in acquisition of an analysable signal elicited by these types of stimuli on

some subjects. Number of stimuli, incremental steps and stimulation intensity range were tested and reduced to prevent excessive test duration but at the same time to allow obtaining a graphic with enough resolution of the stimuli-response curve for the study.

Electrode positioning was also subject to testing, with different positioning of stimulation electrodes and acquisition electrodes experimented, in order to allow collecting a good signal with high stimulus-response amplitude and definition of the CMAP scan curve. A short protocol was developed in order to test the response obtained with the different positioning, without having to perform the whole test, making it simpler for tests regarding the optimal positioning.

Besides the acquisition and processing scripts, another script was made, which had the functions that generated the different types of waveforms according to determined amplitude and regarding the pulse-width time. It encompassed also the function responsible for the charge equalization between all waveforms, which calculated the pulse-width time necessary for a determined waveform to generate the same amplitude of the other waveforms, in order to equalize the charge.

The current charge difference from each waveform was taken into account in the data analysis. The stimulation charge was computed accordingly to the stimulation intensity and waveform, based on the following formula:

$$(1) \quad Q = \int_{t1}^{t2} I \, dt$$

Where Q represents the charge value, *I* represent the current intensity and the range from *t1* to *t2* is the stimulus time interval.

Given as reference the pulse-width time of the monophasic square waveform, which is the common waveform used, and according to a given amplitude range, the function calculates the value of the pulse-width time of the other waveforms in a way that elicits the same response amplitude of the reference waveform, maintaining the charge value. One issue with some waveforms, like the exponential, given the area differences between the reference waveform, was that would need to reduce the reference pulse-width and his own be longer than supposed, in order to be comparable and to accomplish charge equalization. Illustrative graphics of the selected waveforms to perform the study with the equalized charge are shown next on figure 3.1, which allow to compare the different waveforms pulse-width, which are generated on a window with the same number of points.

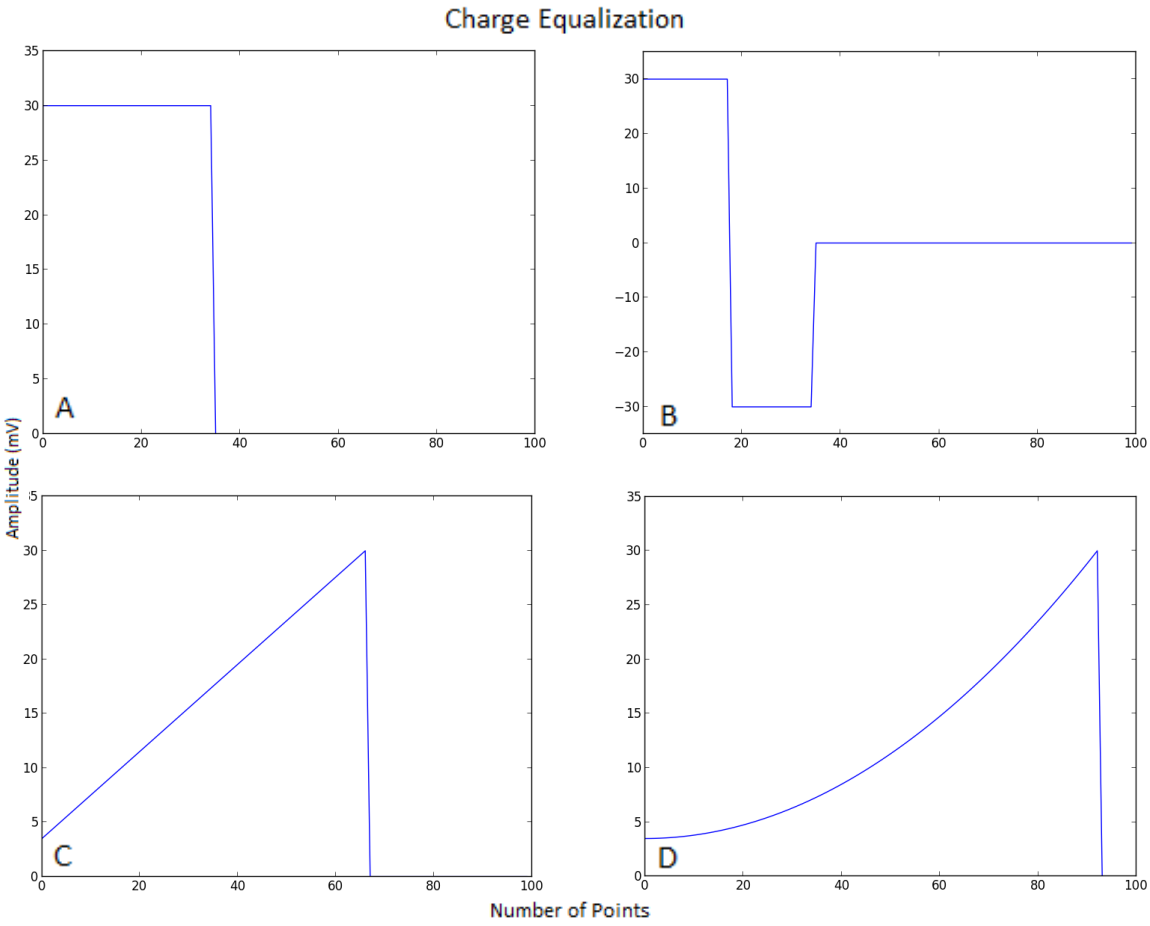

Figure 3.1: Charge equalization A- Monophasic Square B -Biphasic square C- Monophasic Triangular D- Monophasic Quadratic. In this figure it is shown the differences in pulse-width time regarding the different waveform types needed for the charge value to be equal among them, maintaining the same response amplitude.

3.2 Subjects

To perform the study of the different waveforms influence on the motor neuron excitability, a total of 13 healthy subjects were submitted to the same test and evaluated. This group was composed of 7 males and 6 females, with a mean age of 26 years (standard deviation of 3.63), ages comprehended between 20 to 36 years old. None of the subjects that performed this test had any clinical history on neurologic disorders.

Other subjects were tested but on some of them the valid acquisition of the electromyography signal was not successful given the non-invasive nature of the stimulation and acquisition method, which made more difficult the correct positioning of the stimulation electrodes over the

nerve and also of signal acquisition. On some other patients it was not possible to obtain the stimulus-response curve of the biphasic waves, since stimulation with this pulse did not elicit any response.

During the execution of the test, the subjects were straight seated, motionless, relaxed and with the right forearm in supination position with the palm of the hand facing posteriorly and making a 45 degree angle with the forearm. Thumb fixation was necessary in order to minimize movement artefacts and also limit the change in muscle fibre conduction velocity. For this purpose, a fixation support for the hand, elastic bands and a glove with lateral supporting bars along the thumb were used, as shown in figure 3.2.

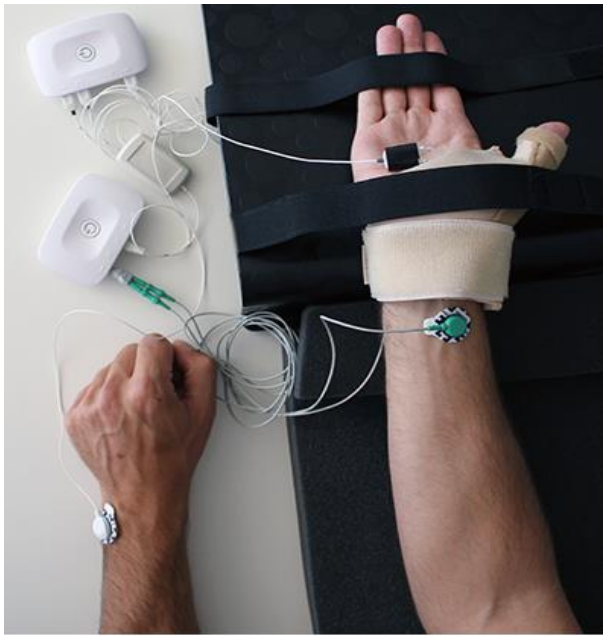

Figure 3.2: Hand fixation schematic. In order to avoid thumb movement during the test, the hand was fixated with elastic bands to a support. It was also used a glove with lateral support bars along the thumb.

3.3 Stimulation and acquisition

The test consisted on applying electrical stimulation on the medium nerve located on the wrist and surface electromyography signal acquisition of the stimuli-response collected on the Abductor Pollicis Brevis (APB) muscle surface of the thumb. The stimulation electrodes were placed on the median nerve on the wrist area (with the positive electrode on bottom and negative on top) of the right hand. The acquisition electrodes were placed on the muscle surface of the APB and on the proximal phalange of the right thumb, according to SENIAM standards [18], and the ground electrode was placed on the opposite wrist ulnar styloid process, as represented in figure 3.3.

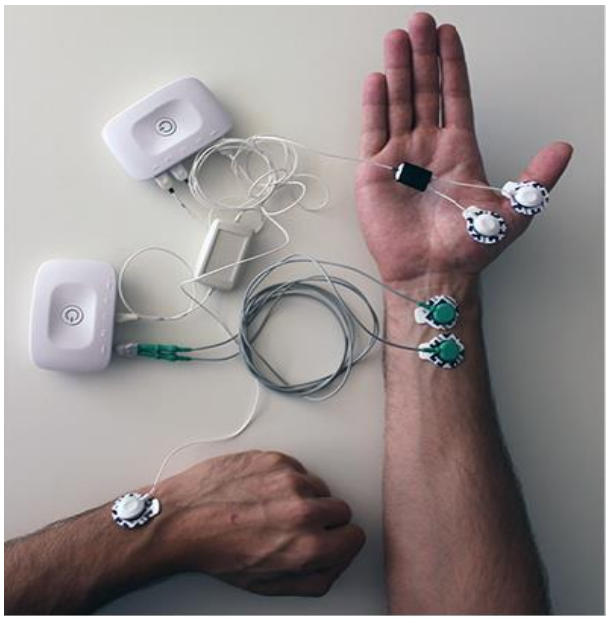

Figure 3.3: Electrode positioning. Stimulation electrodes are placed on the right wrist (anode on top). Ground electrode is placed on the opposite wrist. Acquisition electrodes are placed on the muscle surface of the thumb.

The EMG signal was acquired with a 3000Hz sampling frequency, 12bits of resolution and amplified with a gain of 201. For EMG acquisition, a combined wireless, miniaturized and synchronized unit was used [19]. This device has eight analog input channels with 12-bit of resolution, sampling frequency until 5 KHz, an external channel to be used as reference ground electrode for electrophysiology measures and a digital port for external synchronism, which connects with the electrostimulation unit by a synchronization cable. The electrostimulation device allows a stimulation intensity range up to 100mA [20]. The equipment used in this work was developed by PLUX [19,20] and shown on figure 3.4. Self-adhesive pre-gelled Ag/AgCl electrodes were used for EMG acquisition and peripheral nerve stimulation.

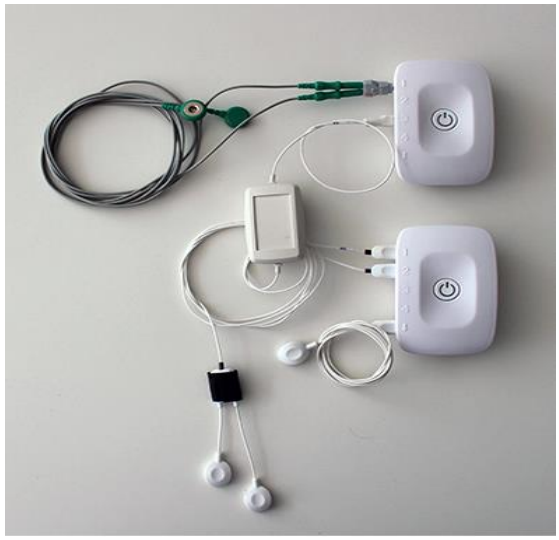

Figure 3.4: Electrostimulator and EMG acquisition units. The electrostimulation unit on top connected with the acquisition device with the EMG sensor on bottom.

The signal acquisition method is illustrated in the following flowchart.

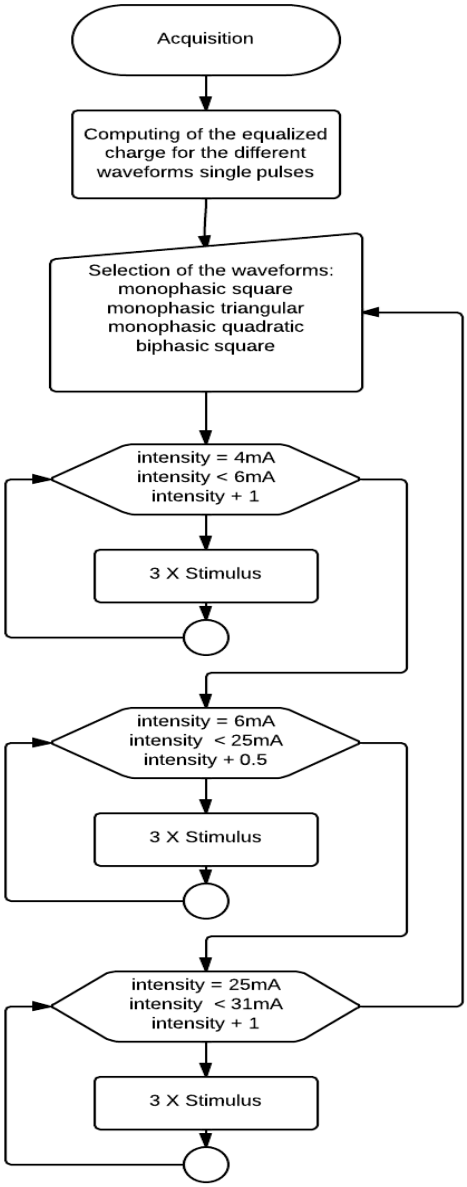

Figure 3.5: Illustrative flowchart of the steps of the stimulation protocol used in the biosignals acquisition.

The stimulation protocol was performed with increasing intensities, which ranged from 4 to 30 mA, with varying current increment steps and several stimuli by step. The procedure was repeated 4 times per subject, each repetition using a different single pulse stimulation waveform. A standard square waveform pulse was applied in test 1, a triangular waveform pulse in test 2 and a quadratic waveform pulse in test 3. In all these 3 protocols, monophasic single pulses were used with the same intensities. A 4^{th} protocol was tested with a biphasic single pulse square waveform, with the same intensities of the other tests using monophasic waveforms.

3.4 Processing

After acquisition step, the collected biosignals were processed and several features were gathered, in order to evaluate the obtained response. To achieve that, processing scripts were developed using Python, for the data analysis of the acquired signals and automated extraction of the selected features. An illustrative schematic is presented in figure 3.6, and main steps described:

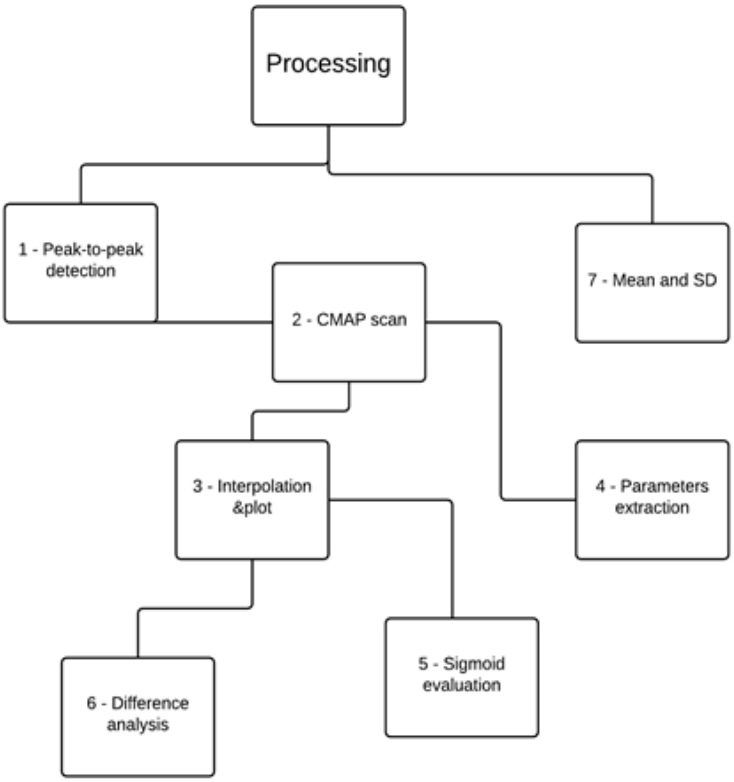

Figure 3.6: Diagram of the different steps of the processing

The processing comprehended the following steps:

1. Detection of the peak-to-peak amplitude of the stimulus response M-wave
2. CMAP scan composition
3. Interpolation and plotting of the CMAP scan
4. Extraction of S5, S50, S95 and stimulus-response amplitude elicited by these parameters
5. Detection of the beginning, final and slope of the resulting sigmoid
6. Analysis of the differences in the computed parameters regarding each waveform
7. Calculus of the mean and standard deviation of the computed parameters

1-Peak-to-peak detection

Detection of the response after stimuli application, through derivate and arithmetic signal changes. It is evaluated if a stimulus generates a disproportionate response or if there is an abnormal movement or event on the stimulation moment and these exceptional transitions are removed from the signal. This procedure is effectuated for all the stimuli and each subject and visually validated. The maximum and amplitude of the peaks are detected (example on figure 3.7).

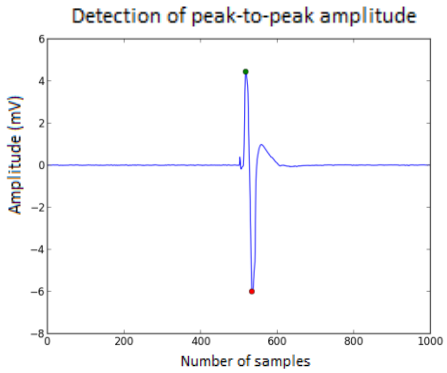

Figure 3.7: Detection of absolute amplitudes of the response generated

2- CMAP scan

Movement artefact removals were made through considering the mean baseline of the signal before stimulation and recalculating the signal between the stimulation point and the actual contraction of the thumb. After signal conversion and unwanted stimuli transitions and artefacts removed, the stimuli signal averaging of each current step is done. Values of maximum, minimum and absolute amplitude of the peaks for each current increment are collected in order to plot the CMAP scan graphic (example on figure 3.8).

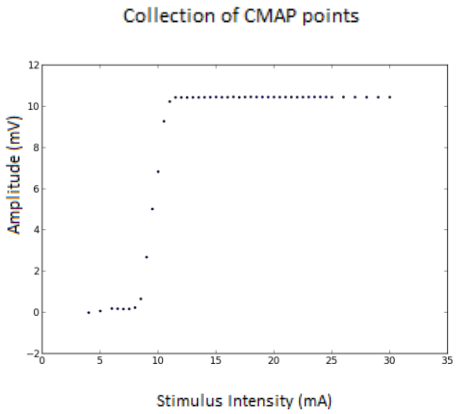

Figure 3.8: Collection of the points necessary to generate the CMAP scan

3- Interpolation & plot

Given the reduction of the number of stimuli applied in order to maintain an acceptable test duration (since the test was made with four different types of waveforms instead of just one) an interpolation was made to better fit the graphic curve to the given points and enhance the CMAP scan obtained. After that, the final CMAP scan plot with each stimulus, for all amperage range, is generated (example on figure 3.9).

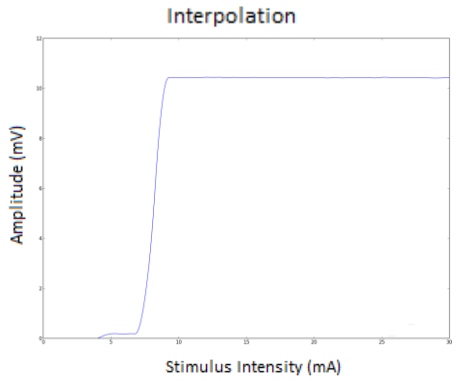

Figure 3.9: Interpolation effectuated to generate the CMAP scan

4- Parameters extraction

Maximum CMAP amplitude is obtained by averaging of the stabilization threshold after reaching the maximum stimulation amplitude on the sigmoid curve. A normalization step is effectuated in order to extract the values of the stimulation parameters that elicit 5%, 50% and 95% of the maximum response amplitude (S5, S50 and S95) as well as the response amplitude correspondent to these parameters (example on figure 3.10).

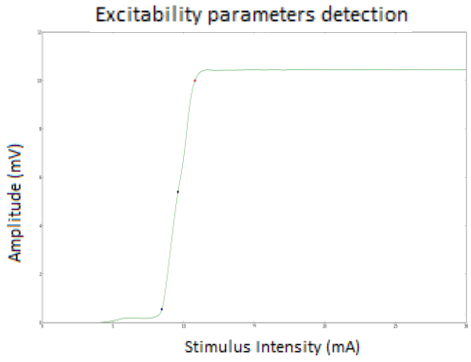

Figure 3.10: Detection of the excitability parameters S5, S50 and S95

5- Sigmoid evaluation

The variations on the steepness of the sigmoid curve for the different types of waveform are also calculated, as the beginning and final of the curve are detected and slope of the sigmoid calculated. All these parameters and values are saved on a file, as all the points that constitute the CMAP scan.

6- Difference analysis

The analysis of the differences in the computed parameters regarding each waveform is done. The intensity differences values between each waveform to produce the same event and steepness relations are computed.

7- Mean & SD

For all the parameters and values computed, the median and standard deviation values were calculated and saved on file for posterior analysis.

The processing routine generates the plot of the different CMAPs and the CMAP scan and calculates the parameters of interest like the excitability parameters, their response amplitude and characteristics of the sigmoid curve, saving them on a file. These steps are repeated for the different types of waveforms. A CMAP Scan generated with a monophasic triangular waveform with the excitability parameters identified can be seen on figure 3.11.

This procedure was repeated for all subjects and all data had posterior visual validation by two M.D. specialists.

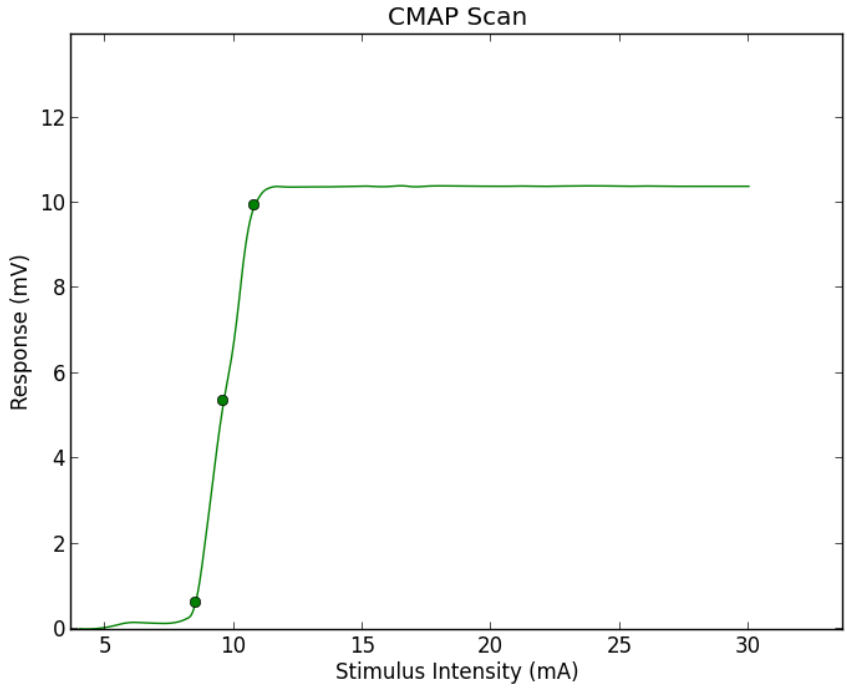

Figure 3.11: CMAP scan representation. This CMAP scan was obtained with the monophasic triangular pulse and the excitability parameters (S5, S50, S95) are signalled on the sigmoid.

4

4. Results

Peripheral nerve stimulation is influenced by external variables that are hard to control, like the adipose tissue layer that the electrical current has to pass, the distance between the stimulation electrodes and the nerve to be stimulated, among others. Taking this into consideration, the chosen analysis parameters were the ones that allowed a more objective assessment of the considered effects inter subjects.

Each subject was analysed regarding the CMAP amplitudes, excitability parameters (S5, S50, S95 - regarding stimulus current intensity (mA) and absolute response amplitude (mV)), sigmoid slope and current intensity differences of the CMAP scan between each different waveform.

Given certain stimulation intensity it is noticeable the differences in the response amplitude generated by the different kind of waveforms tested. In figure 4.1 it is observable the differences in the response amplitude in the CMAP elicited by the same current intensity, which in this case is 10,5mA, between the different waveform types. The monophasic square waveform reaches almost the maximum amplitude for this intensity stimulus, and the response elicited by the same stimulus is lower to the monophasic triangular waveform and the lowest to the monophasic quadratic waveform, which would need a higher stimulus intensity to reach the same amplitude. The difference is even higher regarding the biphasic square waveform, since this current intensity value does not even provoke a response to the stimulus generated using this type of pulse.

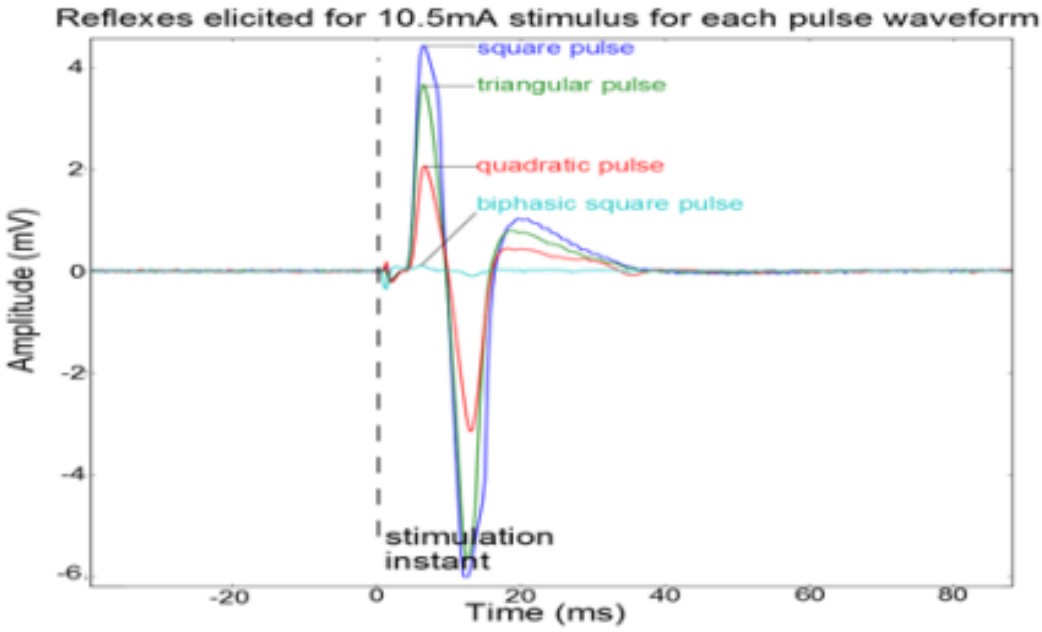

Figure 4.1: CMAP acquired in a fixed intensity step for each waveform (10.5mA), where it is observable the differences in the waves' response amplitude, generated with the same intensity stimulation.

In figure 4.2 we can observe the CMAP scan originated by stimulation intensity values from subthreshold to supramaximal threshold, which means the recruitment of all motor units of the muscle fibres.

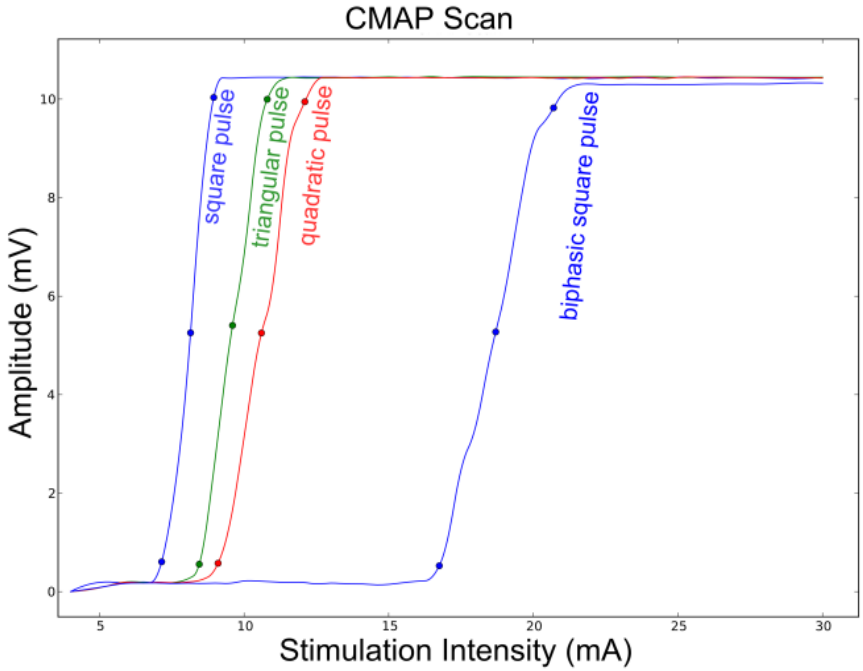

Figure 4.2: CMAP scan, where it can be seen the sigmoid generated by all the stimulation thresholds regarding all the waveforms. It should be noted the differences between the waveforms in the current intensity stimulation to generate the same response amplitude to the stimulus.

The CMAP scan of all the waveform types used in the study are represented and it is observable the differences in the stimulation intensity range needed to provoke the same stimulus response amplitude, regarding the different pulses, with very significant differences to the biphasic waveform in comparison with the monophasic pulses. Also to be noted the differences in the beginning and final of the sigmoid as well as in the steepness of the curve when comparing the different kinds of waveform.

Table 1 presents the CMAP scan S95´s stimulus-response amplitude (the amplitude of the response of the stimulus that elicited 95% of the maximum CMAP amplitude) of the different subjects evaluated.

Amplitude (mV)	S95 wave 1	S95 wave 2	S95 wave 3	S95 wave 4
Subject 1	4,59	4,46	4,43	5,39
Subject 2	10,03	9,99	9,95	9,82
Subject 3	6,25	6,20	6,63	6,37
Subject 4	5,11	4,97	4,91	5,22
Subject 5	9,91	9,88	9,94	9,93
Subject 6	5,91	5,90	6,05	6,06
Subject 7	6,03	6,05	5,97	5,99
Subject 8	7,35	7,36	7,27	6,31
Subject 9	9,67	9,45	9,41	9,17
Subject 10	7,01	7,02	7,03	6,73
Subject 11	7,86	8,07	8,06	8,06
Subject 12	4,65	4,63	4,71	4,58
Subject 13	5,75	5,78	5,72	5,95

Table 1 – S95 response amplitudes. Wave 1 corresponds to the monophasic square pulse, wave 2 to monophasic triangular pulse, wave 3 to monophasic quadratic pulse and wave 4 to the biphasic square pulse.

This table presents the value of the amplitude elicited by the excitability parameter S95, which corresponds to the amplitude of the response caused by a stimulus intensity that would generate 95% of the maximum CMAP amplitude value. Although it presents stimulus response amplitude variations inter subjects, the amplitude value obtained for a given subject, regarding the different waveform types, remains approximately constant.

Table 2 presents the mean CMAP scan sigmoid slope differences between the different types of waveforms used in the subjects' stimulation, using the monophasic square waveform as reference.

Slope differences	Mean	SD
Square-Triangular	81%	11%
Square-Quadratic	67%	11%
Square-Biphasic	44%	19%

Table 2 – Waveforms slope differences

As it could be observed on figure 5 one of the differences regarding the different kinds of waveforms is the steepness of the sigmoid curve generated. The monophasic square waveform will be used as reference in the comparisons made with the other types of pulses as it is the standard waveform type used in these studies and it presents the lower stimulation intensity range needed to achieve the maximum CMAP amplitude and also the curve with the higher steepness. The monophasic triangular waveform presents a slope approximately of 4/5 in comparison with the monophasic square waveform and this value is reduced to 2/3 relatively to the monophasic quadratic waveform. This difference is again more significant when comparing the monophasic and biphasic square waveforms, as the biphasic pulse presents approximately half of the sigmoid slope regarding the monophasic pulse.

Table 3 and 4 present the waveforms current intensity differences and the data shown allows a better assessment of the current intensity differences between each type of pulse.

In table 3 it is shown the current intensity values of the excitability parameter S5, stimulus intensity that elicited response amplitude that corresponds to 5% of the maximum CMAP amplitude, for the different waveforms and all subjects.

S5 (mA)	wave 1	wave 2	wave 3	wave 4
Subject 1	5,10	6,10	6,45	8,10
Subject 2	7,10	8,35	9,05	16,75
Subject 3	4,45	5,35	6,10	8,25
Subject 4	4,85	5,10	5,55	8,35
Subject 5	7,80	8,70	9,55	16,55
Subject 6	5,10	6,40	7,15	10,70
Subject 7	6,05	7,20	8,10	10,65
Subject 8	5,30	7,05	8,25	10,10
Subject 9	7,70	9,10	9,90	14,15
Subject 10	8,80	10,55	11,60	17,70
Subject 11	4,15	4,40	4,45	5,45
Subject 12	6,20	7,80	8,35	9,95
Subject 13	7,00	8,50	9,50	13,00

Table 3 – S5 current intensities. Wave 1 corresponds to the monophasic square pulse, wave 2 to monophasic triangular pulse, wave 3 to monophasic quadratic pulse and wave 4 to the biphasic square pulse.

As referred and as we can see by table 3, the monophasic square waveform needs the lowest current intensity value to reach the excitability parameters and obtain determined response amplitude. This value increases for the monophasic triangular and quadratic waveforms, respectively. The increase in the stimulation threshold to provoke the same event for the biphasic square waveform is very significant in comparison with the monophasic waveforms.

In table 4 it is shown the mean arithmetic differences in the stimulation current intensity values for each excitability parameter (S5, S50, S95) between each type of waveform.

Intensity differences (mA)	S5 Mean (SD)	S50 Mean (SD)	S95 Mean (SD)
Triangular-Square	1,22 (0,57)	1,60 (0,63)	1,92 (0,89)
Quadratic-Square	1,82 (0,70)	2,44 (0,50)	3,01 (0,81)
Biphasic-Square	5,39 (2,41)	7,48 (2,27)	8,62 (2,79)

Table 4 – Waveforms current intensity differences

Taking into reference the monophasic square waveform, we can analyse on table 4 the differences in the stimulation intensities for the different excitability parameters and between the different waveform types. Once again, there is an increase in the stimulation intensity from the monophasic square waveform to the monophasic triangular waveform, which is higher when comparing the monophasic quadratic waveform with the monophasic square pulse. The increase in the stimulation threshold to reach the same response for the different excitability parameters relatively to the biphasic square waveform is very significant in comparison with the monophasic waveforms.

In table 5 it is shown again data that allow once more observing differences regarding the excitability thresholds of the different waveform pulses type. It is observable through this table the differences between the different kinds of waveforms regarding moment of maximum amplitude, stimulus intensity and response amplitude.

S 50	mA				mV			
	wave1	wave2	wave3	wave4	wave1	wave2	wave3	wave4
Subject 1	8,15	9,60	10,60	18,70	5,30	5,39	5,24	5,27
Subject 2	5,55	7,05	8,40	11,60	3,45	3,43	3,59	3,37
Subject 3	5,70	6,55	7,30	12,30	2,75	2,71	2,59	2,87
Subject 4	8,80	10,05	10,80	21,60	5,35	5,27	5,31	5,33
Subject 5	5,90	6,95	7,55	11,40	2,25	2,22	2,17	2,77
Subject 6	5,75	7,50	8,40	12,20	2,90	3,02	3,04	3,03
Subject 7	7,30	8,60	9,50	14,65	3,18	3,16	3,04	3,12
Subject 8	6,45	7,90	9,25	10,95	3,77	3,90	3,74	3,32
Subject 9	8,70	10,30	11,35	16,10	5,01	4,90	4,75	4,77
Subject 10	10,05	12,30	13,45	19,10	3,73	3,62	3,64	3,52
Subject 11	5,10	6,30	7,10	10,00	4,22	4,34	4,32	4,27
Subject 12	7,35	9,05	10,00	14,20	2,48	2,49	2,42	2,40
Subject 13	8,61	12,07	11,44	17,88	3,06	3,04	3,01	3,16

Table 5 – S50 expressed in current intensity and response amplitude. Wave 1 corresponds to the monophasic square pulse, wave 2 to monophasic triangular pulse, wave 3 to monophasic quadratic pulse and wave 4 to the biphasic square pulse.

Next it is presented, as an example, a CMAP scan graphic with waveforms in a medium phase of testing, which still has implemented three biphasic waveforms.

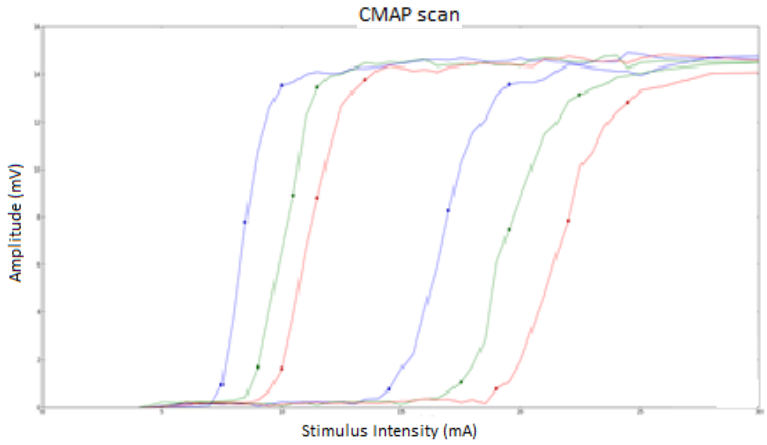

Figure 4.3: CMAP scan representation with 3 types of monophasic and biphasic waveforms. It is particularly visible the differences in stimulation intensity between monophasic and biphasic waveform types and it is noted an order of appearance in the graphic of the waveforms regarding the type.

On the next figure is illustrated an example of a subject which did not present response to the biphasic waveform stimulation.

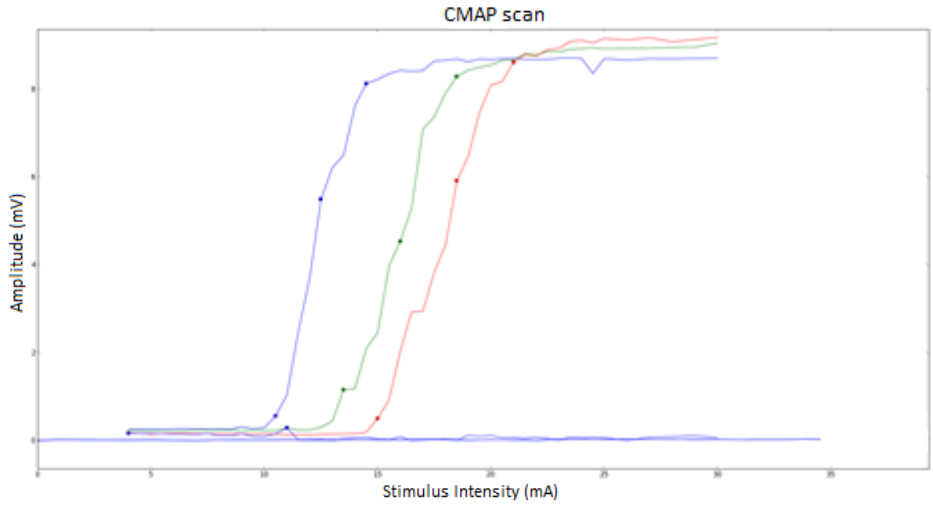

Figure 4.4: CMAP scan representation with invalid acquisition of the biphasic waveform. As it is observable, the biphasic pulse does not elicit any response on this subject in all intensity range.

5

5. Discussion

In this chapter it will be analysed and discussed the obtained results in the study with the healthy patients and the application to the diagnosis and monitoring of neurodegenerative pathologies.

5.1 Results analysis

The stimulus-response peak-to-peak maximum amplitude remained constant between different waveforms (Table 1). This fact is common to every subject, as expected given the charge equalization effectuated. However, there were significant differences among the subject's maximum amplitude absolute. This behaviour was expected because, as it was mentioned before, nerve stimulation is affected by external variables, like the adipose tissue layer, among others, that are intrinsic to each subject.

The results show that the square pulse needs less current intensity to generate the same response amplitude as the other waves (Table 1 and Figure 5), and it is also the one that presents a more steep curve slope (Table 2). This means that, for the square wave, the time interval between the beginning and final of the stimulation is shorter than for the other waves and the stimulation threshold is lower. As we can verify by the tables, this effect is gradually decreasing for the triangular and quadratic pulse, respectively.

The quadratic wave, among the monophasic waves group, represents the stimulation pulse that needs a larger current intensity value and range to elicit the same response amplitude in comparison with the other waves. This fact consequently translates on an inferior sigmoid slope.

This is due to the nervous fibre sensibility to charge transfer rate, since in the used setup all waves have charge equalization, meaning that the variable cause is only the waveform, which has different charge transfer rates.

Concerning the biphasic square pulse it is possible to verify that it has a very distinct behaviour from the monophasic pulses, with activation intensities of the response levels S5 and S95 quite superior, with higher stimulation current intensities needed and higher time to reach from subthreshold to supramaximal stimulation value and consequently a rather inferior sigmoid slope. This fact indicates that, possibly, only one of the flanks of the biphasic waveform is activating the nerve fibres (corroborated by comparing to the same waveform but monophasic pulse, since the slope is approximately half and stimulation intensity needed to provoke the same response approximately double).

The monophasic waveforms have a more linear behaviour, while the biphasic waveform presents a more unstable behaviour with greater variations.

The analysis of the effect of the waveform on the peripheral nerve stimulation permits to reveal new effects in the context of the nerves' excitability. Also the control of this parameter allows varying the stimulus-response curve slope. These facts can open new doors on the context of the CMAP scan applied to ALS diagnosis or other neurodegenerative disorders.

5.2 Application on the diagnostic of neurodegenerative pathologies like ALS

Given the results observed on the previous study effectuated with healthy subjects, it was intended to make the correlation with neurodegenerative pathologies, verifying if the variations observed when performing the stimulation with different waveform types showed the same results relatively to patients with ALS.

To accomplish that, acquisitions in patients with ALS and other neurodegenerative pathologies were made in Hospital Santa Maria (Lisbon, Portugal), with the collaboration of Professor Mamede de Carvalho (Department of Neurology of Hospital de Santa Maria and Laboratory of Electromyography, Centro de Estudos Egas Moniz, Faculty of Medicine, Institute of Molecular Medicine, Lisbon, Portugal). Due to technical issues, constraints with the patients and also their availability, to the moment of the elaboration of this document, only 2 valid acquisitions on patients with ALS were available to discuss. Due to the advanced condition of the neurodegenerative disease of the patients in question, it was not observable a response elicited by the stimuli, since they presented very reduced response amplitude, and after raw data analysis it was concluded that the none of the waveforms elicited a valid CMAP. Next is presented a stimulus-response representation of a control and one of the patients with ALS, with

30 mA intensity stimulus, where it is visible the response to the stimulus presented by the control and the simple electrical artefact, given by the negative spike presented by the patient.

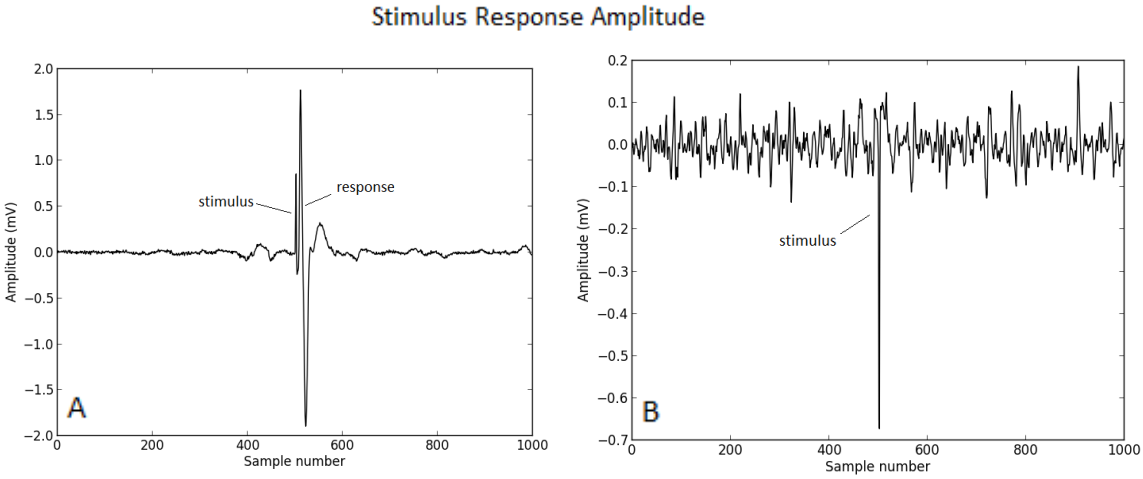

Figure 6.1: A – Amplitude elicited by a stimulus of 20 mA on control subject. B – Amplitude elicited by the same stimulus on ALS patient. Due to progressive state of disease there is no response amplitude to the stimulus, being only visible electrical artefact on the stimulus moment (sample number 500) contrary to the control patient that presents a visible response after the stimulus moment.

To test other pathologies like Progressive Muscular Atrophy and demyelinated pathologies, the stimulation protocol was altered, with different stimuli intensity range and current increment steps.

Given the results observed on the previous study effectuated with healthy subjects relatively to the biphasic square waveform and discussed before, the acquisition protocol used on the patients only contemplated the monophasic pulse types.

Due to patients' sensibility and motor limitations, the stimulation protocol had small alterations and fixation support was not used, just the glove with lateral support, optimizing the protocol application time.

To further investigate the effects of modulated current on neuron motor excitability and assess its effects in patients, more acquisitions on patients of Hospital Santa Maria with ALS, and other neurodegenerative pathologies like Progressive Muscular Atrophy and demyelinated pathologies are being made and respective results will be assessed in the future (it is intended to see if the parameters in study present a similar behaviour on the patients regarding the observed on the healthy subjects and if some of the waveform types will allow a better assessment of the patients' obtained data.

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Appendix

Here will be presented a publication made on the following of the work accomplished during this master thesis and accepted in conference BIOSIGNALS - BIOSTEC 2015.

Evaluation of motor neuron excitability by CMAP scanning with modulated current

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Keywords: Compound Muscle Action Potential scan, Peripheral Nerve Stimulation, surface Electromyography.

Abstract
This work aims to study the influence of different pulse modulated waveforms in peripheral nerve excitability by CMAP scan technique on healthy subjects.
A total of 13 healthy subjects were submitted to the same test. The stimuli were applied in the medium nerve on the right wrist and electromyography signal collected on the muscle surface on the right thumb. Stimulation was performed with an increasing intensities range from 4 to 30 mA, with varying steps, 3 stimuli per step. The procedure was repeated 4 times per subject, each repetition using a different single pulse stimulation waveform: monophasic square, monophasic triangular, monophasic quadratic and biphasic square. Results were retrieved from the averaging of the stimuli on each current intensity step.
The square pulse needs less current intensity to generate the same response amplitude regarding the other waves and presents a more steep curve slope and this effect is gradually decreasing for the triangular and quadratic pulse, respectively, being the difference even more evident regarding the biphasic pulse. The control of the waveform stimulation pulse allows varying the stimulus-response curve slope.

1 INTRODUCTION

The Compound Muscle Action Potential (CMAP) scan is a non-invasive diagnosis technique for neurodegenerative pathologies, such as Amyotrophic Lateral Sclerosis (ALS). It allows a quick analysis of the muscle action potentials in response to motor nerve stimulation, by electrical stimulation applied on the surface of the motor nerve and stimulus response evaluation by surface Electromyography (sEMG) at muscle level. Each Motor Unit (MU) of muscles has a different Stimulus Intensity (SI) at which it is activated, meaning that MUs have different thresholds. Varying the intensity of the stimuli applied, gradually increasing from subthreshold to supramaximal values, will sequentially activate all MUs in the muscle. This way, it is possible to obtain a graphical representation of the evoked action potentials amplitude in the muscle versus the stimulation intensity. This record will show a sigmoid tendency which is called the CMAP scan. (Maathuis et al. 2011,2012).

Henderson et al. (2006) examined the variability of the CMAP scan between healthy and ALS subjects and it showed there is a significant difference in relation to CMAP scan evolution, number of steps and size, since ALS patients present more and larger steps on the stimulus-response curve than healthy controls (steps are clear visible jumps in CMAP amplitude within consecutive stimuli), as it can be seen in figure 1.

The CMAP scan can be used as a monitoring tool for neurodegenerative disorders, as it permits visualization and quantification of disease progression in a muscle with Motor Neuron Disease, such as ALS, as referred by Maathuis et al. (2012). Several studies have been made, investigating the influence of different parameters in the quality of the CMAP scan. Maathuis et al. (2011) studied CMAP scan reproducibility on healthy patients in several excitability parameters, like the maximum CMAP, S5, S50, S95 (the stimulus intensity that elicited 5%, 50% and 95% of the maximum CMAP, respectively), SI range (the difference between S95 and S5) and results were good for tested parameters.

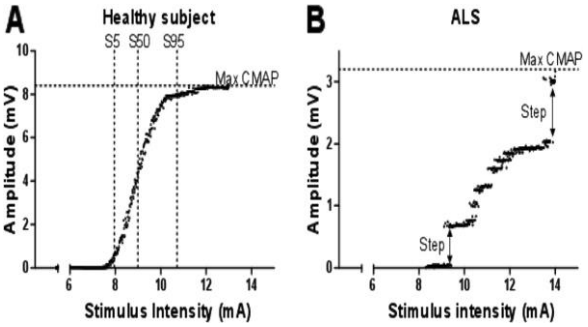

Figure 1A: CMAP scan from healthy individual, with the stimulus-response curve obtained with 500 stimuli. The horizontal line indicates the CMAP maximum amplitude while the vertical lines refer to S5, S50 and S95 (that correspond to the stimulus intensity that elicited 5%, 50% and 95% of the maximum CMAP, respectively). Figure 1B: CMAP scan of an ALS patient. Differences in the CMAP scan between the healthy and the ALS patient are visible because of steps observed in the ALS CMAP scan and the decrement in maximum amplitude. Adapted from Maathuis et al. (2012).

Shorter stimulus duration implicates higher stimulus intensity required to elicit the same CMAP amplitude. The effect of other parameters on the CMAP scan, like stimulus frequency and total number of stimuli, are not yet fully established. Maathuis et al. (2012) attempted to define optimal stimulus protocol settings for these parameters, considering subject discomfort, movement artefacts and recording duration. Optimal value for stimulus duration is yet arbitrary, but shorter stimulus duration will increase the resolution of the curve. Stimulus frequency has no influence on the CMAP variables in healthy controls, although high frequency augments the chance of decrements on the CMAP, so low frequency stimulation is advised. Experiences regarding stimuli number recommended that around 500 stimuli give enough detail in the stimulus-intensity response curve, without excessive duration time. Fixation of the thumb is highly advised, since it shortens the decrement size and limits muscle fibre conduction velocity changes, improving the CMAP scan.

An untested parameter of the CMAP scan is the waveform of the pulse used in the electrical stimulation of the nerve. In electrical stimulation, the waveform represents the variation over time of the applied current or voltage on the muscle or nerve. This work aims to study the influence of different pulse modulated waveforms in peripheral nerve excitability, by CMAP scan technique, on healthy subjects. The different types of waveforms tested were monophasic square, triangular and quadratic waves and also a biphasic square wave.

2 METHODS

2.1 Subjects

In this study a total of 13 healthy subjects were submitted to the same test. This group was composed of 7 males and 6 females, mean age of 26.00 (3.63) years, range from 20 to 36 years, without clinical history on neurologic disorders.

The stimuli were applied in the medium nerve on the right wrist and electromyography signal collected on the Abductor Pollicis Brevis (APB) muscle surface on the right thumb. The acquisition electrodes were placed according to SENIAM standards and the ground electrode was placed on the left wrist ulnar styloid process. During the test, the subjects were seated, motionless and relaxed, with thumb fixation to minimize movement artefacts (figure 2C).

2.2 Stimulation and acquisition

Stimulation was performed with increasing intensities range from 4 to 30 mA. Different number of stimuli and current increment steps were tested in order to have a stimulation protocol that would allow obtaining a curve with enough resolution and not excessive test duration. The acquisition protocol is illustrated in the flowchart (figure 3).

The procedure was repeated 4 times per subject, each repetition using a different single pulse stimulation waveform. A monophasic square wave was applied in test 1, a triangular wave in test 2 and a quadratic wave in test 3. In all these 3 protocols, monophasic single pulses were used with the same intensities. A 4th protocol was tested with a biphasic single pulse square wave, with the same intensities of the other tests.

The current charge difference from each waveform was taken into consideration. The stimulation charge was computed accordingly to the stimulation intensity and waveform, based on the following formula:

$$(1) \quad Q = \int_{t1}^{t2} I \, dt$$

Where I represent the current intensity and $t1$ to $t2$ is the stimulus time range. In each current intensity step, the charge of the different waveform types have been equalized, maintaining the amplitude and varying the pulse-width time. Given a reference pulse-width time of the monophasic square waveform (the commonly used waveform), and

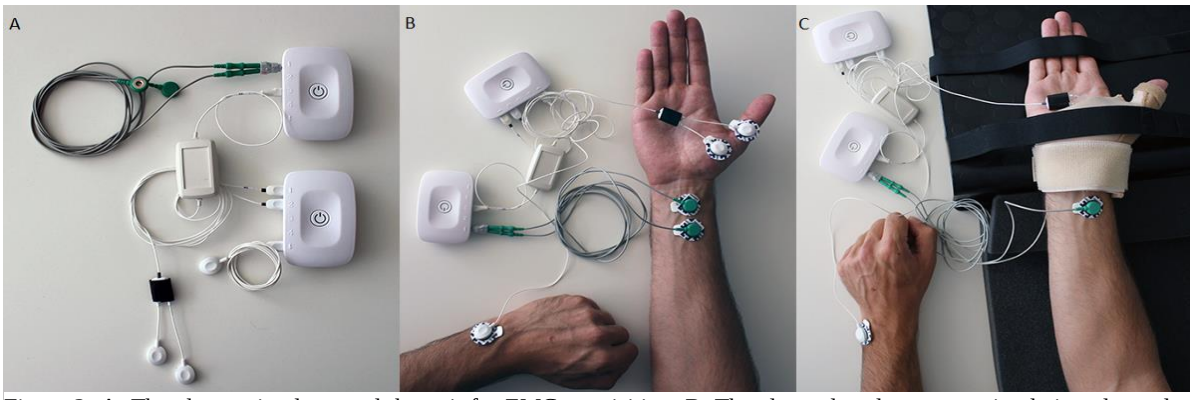

Figure 2: A. The electrostimulator and the unit for EMG acquisition. B. The electrodes placement: stimulation electrodes on the median nerve, sEMG sensor on the thumb and ground electrode on the wrist. C. Hand fixation for the test.

according to the current amplitude, it is calculated the value of the pulse-width necessary for the other waveforms to elicit the response amplitude of the reference waveform, in order to equalize the charge.

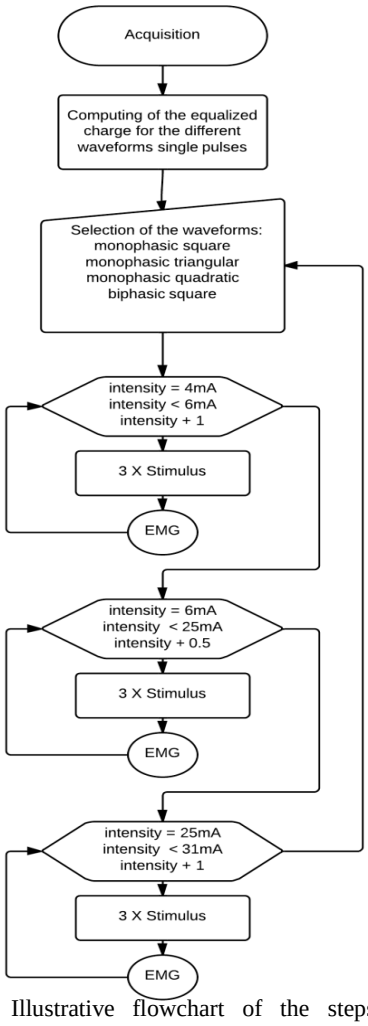

Figure 3: Illustrative flowchart of the steps of the stimulation protocol used in the biosignals acquisition

The EMG signal was acquired with a 3000Hz sampling frequency, amplified with a gain of 201 and 12bits of resolution. A combined wireless, miniaturized and synchronized units were used for EMG acquisition and nerve stimulation (figure 2A), developed by PLUX (2014). Self-adhesive pre-gelled Ag/AgCl electrodes were used for EMG acquisition and peripheral nerve stimulation (figure 2B).

2.3 Processing

After acquisition, the collected biosignals were processed and features were extracted, according to the following steps:

1. Detection of the peak-to-peak amplitude of the stimulus response M-wave
2. CMAP scan composition via interpolation
3. Extraction of S5, S50, S95, SI range and stimulus-response amplitude elicited by these parameters
4. Detection of the beginning, final and slope of the resulting sigmoid
5. Calculus of the mean and standard deviation of the computed parameters
6. Analysis of the differences in the computed parameters regarding each waveform

These steps were repeated for each waveform type and all subjects. For the data analysis of the acquired signals, processing scripts were developed utilising Python. All data had posterior visual validation by two M.D. specialists.

3 RESULTS

Peripheral nerve stimulation is influenciated by external variables that are hard to control, like the adipose tissue layer that electrical current has to pass, the distance between the stimulation electrodes and the nerve to be stimulated, among others. Taking this into consideration, the chosen analysis parameters were the ones that allowed a more objective assessment of the considered effects inter subjects.

Each subject was analysed regarding the amplitudes, excitability parameters (S5, S50, S95 - regarding stimulus current intensity (mA) and absolute response amplitude (mV)), sigmoid slope and current intensity differences of the CMAP scan between each different waveform.

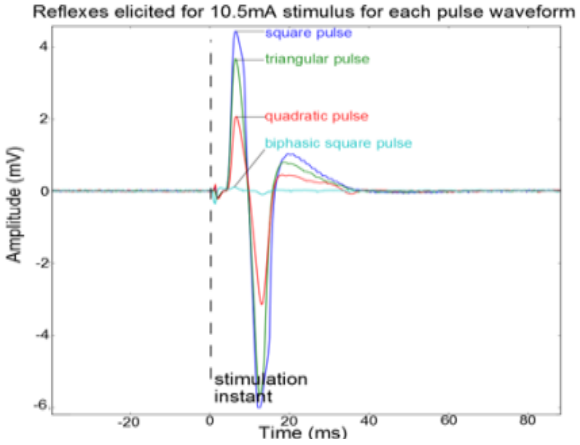

Figure 4: CMAP acquired in a fixed intensity step for each waveform (10.5mA), where we can observe the differences in the waves' amplitude, with the same intensity stimulation.

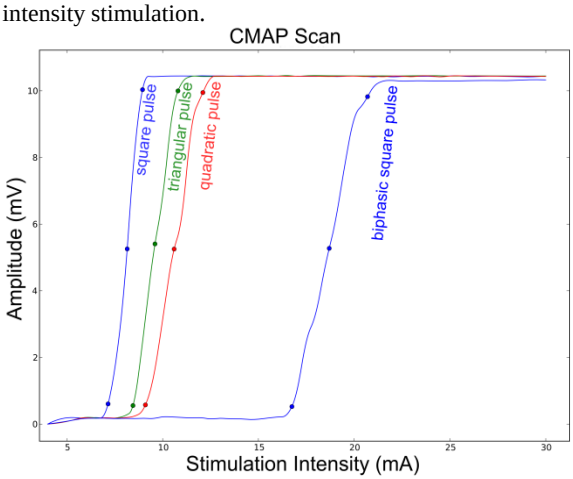

Figure 5: CMAP scan, where it can be seen the sigmoid generated by all the stimulation thresholds regarding all the waveforms. It should be noted the differences between the waveforms in the current intensities stimulation to generate the same response amplitude to the stimulus.

Table 1 presents the CMAP scan S95´s stimulus-response amplitude (the amplitude of the response of the stimulus that elicited 95% of the maximum CMAP amplitude) of the different subjects evaluated.

Amplitude (mV)	S95 wave 1	S95 wave 2	S95 wave 3	S95 wave 4
Subject 1	4,59	4,46	4,43	5,39
Subject 2	10,03	9,99	9,95	9,82
Subject 3	6,25	6,20	6,63	6,37
Subject 4	5,11	4,97	4,91	5,22
Subject 5	9,91	9,88	9,94	9,93
Subject 6	5,91	5,90	6,05	6,06
Subject 7	6,03	6,05	5,97	5,99
Subject 8	7,35	7,36	7,27	6,31
Subject 9	9,67	9,45	9,41	9,17
Subject 10	7,01	7,02	7,03	6,73
Subject 11	7,86	8,07	8,06	8,06
Subject 12	4,65	4,63	4,71	4,58
Subject 13	5,75	5,78	5,72	5,95

Table 1 – S95 response amplitudes. Wave 1 corresponds to the monophasic square pulse, wave 2 to monophasic triangular pulse, wave 3 to monophasic quadratic pulse and wave 4 to the biphasic square pulse.

Table 2 presents the mean CMAP scan sigmoid slope differences between the different types of waveforms used in the subjects' stimulation.

Slope differences	Mean	SD
Square-Triangular	81%	11%
Square-Quadratic	67%	11%
Square-Biphasic	44%	19%

Table 2 – Waveforms slope differences

Table 3 and 4 present the waveforms current intensity differences. In Table 3 it is shown the mean intensity differences regarding the stimulation parameters S5, S50 and S95 between each type of waveform. In Table 4 it is shown the stimulation threshold for S5 (the current intensity stimulation that elicited 5% of the CMAP scan maximum amplitude) for the different subjects and waveforms.

Intensity differences (mA)	S5 Mean (SD)	S50 Mean (SD)	S95 Mean (SD)
Triangular-Square	1,22 (0,57)	1,60 (0,63)	1,92 (0,89)
Quadratic-Square	1,82 (0,70)	2,44 (0,50)	3,01 (0,81)
Biphasic-Square	5,39 (2,41)	7,48 (2,27)	8,62 (2,79)

Table 3 – Waveforms current intensity differences

S5 (mA)	wave 1	wave 2	wave 3	wave 4
Subject 1	5,10	6,10	6,45	8,10
Subject 2	7,10	8,35	9,05	16,75
Subject 3	4,45	5,35	6,10	8,25
Subject 4	4,85	5,10	5,55	8,35
Subject 5	7,80	8,70	9,55	16,55
Subject 6	5,10	6,40	7,15	10,70
Subject 7	6,05	7,20	8,10	10,65
Subject 8	5,30	7,05	8,25	10,10
Subject 9	7,70	9,10	9,90	14,15
Subject 10	8,80	10,55	11,60	17,70
Subject 11	4,15	4,40	4,45	5,45
Subject 12	6,20	7,80	8,35	9,95
Subject 13	7,00	8,50	9,50	13,00

Table 4 – S5 current intensities. Wave 1 corresponds to the monophasic square pulse, wave 2 to monophasic triangular pulse, wave 3 to monophasic quadratic pulse and wave 4 to the biphasic square pulse.

4 DISCUSSION

The results show that the square pulse, besides needing less current intensity to generate the same response amplitude as the other waves (tables 1 and 3 and figures 4 and 5), it is also the one that presents a more steep curve slope (Table 2). This means that, for the square wave, the time interval between the beginning and final of the stimulation is shorter than for the other waves and the stimulation threshold is lower. As we can verify by the tables (1-4), this effect is gradually decreasing for the triangular and quadratic pulse, respectively.

The quadratic wave, among the monophasic waves group, represents the stimulation pulse that needs a larger current intensity value and range to elicit the same response amplitude in comparison with the other waves (tables 1, 3 and 4 and figures 3 and 4). This fact consequently translates on an inferior sigmoid slope (table 2).

This is due to the nervous fibre sensibility to charge transfer rate, since in the used setup all waves have charge equalization, meaning that the variable cause is only the waveform, which has different charge transfer rates.

Concerning the biphasic square pulse it is possible to verify that it has a very distinct behaviour from the monophasic pulses, with activation intensities of the response levels S5 and S95 quite superior, with higher stimulation current intensities needed and higher time to reach from subthreshold to supramaximal stimulation values, and consequently a rather inferior sigmoid slope. This fact indicates that, possibly, only one of the flanks of the biphasic waveform is activating the nerve fibres (comparing the same waveform but monophasic pulse, since the slope is approximately half and stimulation intensity needed approximately double).

The monophasic waveforms have a more linear behaviour, while the biphasic waveform presents a more unstable behaviour with greater variations.

The analysis of the effect of the waveform on the peripheral nerve stimulation permits to reveal new effects in the context of the nerves' excitability. Also the control of this parameter allows varying the stimulus-response curve slope.

To further investigate the effects of modulated current on motor neuron excitability, acquisitions on patients with ALS and other neurodegenerative pathologies are going to be made to see the effects of different waveforms on the context of the CMAP scan applied to motor neuron disease diagnosis.

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