Vienna University of Technology

Medical University of Vienna

Diploma Thesis

Influencing Neuron Recruitment By Means Of Sub-Threshold Ramp Prepulses

Carried out at the Center for Medical Physics and Biomedical Engineering
for the purpose of obtaining the degree of Master of Science (Dipl.-Ing.).
Submitted at TU Wien, Faculty of Mechanical and Industrial Engineering by

Peter Repczuk, BSc

Under the Supervision of

ao. Univ.-Prof. Dipl.-Ing. DDr. Winfried Mayr

Institute of Mechanics and Mechatronics, Vienna University of Technology

Center for Medical Physics and Biomedical Engineering, Medical University of Vienna

January 11, 2021

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.

TU Bibliothek Your knowledge hub WIEN

Affidavit

I declare in lieu of oath, that I wrote this thesis and performed the associated research myself, using only literature cited in this volume. If text passages from sources are used literally, they are marked as such.
I confirm that this work is original and has not been submitted elsewhere for any examination, nor is it currently under consideration for a thesis elsewhere.

Vienna, January 10th 2021 *Peter Repczuk*

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

Acknowledgements

First and foremost I would like to express my gratitude to my parents, Rosi and Herbert, who made it possible for me to enjoy great education, to pursue my dreams and who supported me at any time along the way.

I would like to especially thank my thesis advisor, Professor Winfried Mayr, for his guidance, help and support during my research; for his input, expertise and feedback and his calm, optimistic nature during the whole process.

Christoph Kast and Dr. Jose Luis Vargas Luna shall not go unmentioned. I wish to thank them for their guidance and valuable input, as well as an open ear at all times.

I also wish to thank my colleague Johannes Proksch, with whom I shared many a discussion on a long day in our lab. Thank you for great input, help with the equipment and immensely important support concerning my programming skills.

Appreciation is also extended to all friends and colleagues who participated as subjects in my study.

Naturally, I would like to express my deepest and warmest thanks to Isabella, who supported me on every step of the way. Thank you for reading and rereading my work and tremendously improving its verbal quality. Thank you for your honest feedback. And thank you for all the little things that make our home office more Home than office.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

Contents

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

Abstract

Selective recruitment of nerve fibers in functional electrical stimulation has been the focus of attention for numerous research projects over the last decades. The feasibility of applying sub-threshold prepulses immediately prior to a stimulating pulse is supported by multiple evidence in the literature. There is, however, a range of inconsistent findings concerning shape, configuration, polarity and subsequent effects of prepulses. In my thesis, I investigate the influence of ramp shaped sub-threshold depolarizing and hyperpolarizing prepulses (DPP/HPP) on the recruitment order of motor neurons and different fiber types. I examine the influence on membrane excitability and threshold levels. Conditioning ramps with durations of *50ms* and *100ms* and a slope of *0.1A/s* were delivered together with rectangular test pulses of *0.5ms* and *1ms* phase width. Stimulation was administered to the common peroneal nerve on the lateral side of the lower extremity and the tibial nerve in the popliteal fossa. Stimulus response curves for the electrophysiological responses (motor wave, Hoffmann reflex) of the tibialis anterior and the soleus muscles were recorded.

The data from the electromyogram show that, under the specific conditions of the experiments, depolarizing prepulses of a certain intensity and in biphasic settings tend to reduce a membrane's excitability and raise the excitation thresholds for achieving 10%, 50% and 90% of a full recruitment. Hyperpolarizing prepulses of equal magnitude but in a monophasic configuration consequently reduce threshold intensity levels. Motor response is affected to a larger extent than H-reflex behaviour. DPPs (biphasic) cause a net broadening of the H-reflex curve, whereas HPPs (monophasic) yield an opposite effect. Selectivity in motor unit recruitment can be achieved by conditioning ramp prepulses with intensities below the excitation threshold. This thesis provides insight into the dependence of the conditioning effects on pre-pulse intensity, alongside polarity and configuration. Excitation threshold levels can deliberately be increased or decreased by the use of appropriate compound pulses. Ramp prepulses allow for more specific access to various nerves and fiber groups and seem to be a suitable tool to achieve a more differentiated activation.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

1 Introduction

Electrical stimulation has been both a well-established diagnostic tool as well as a method to evoke and partially control neural or motor activity from outside the body. By application of rectangular electrical pulses of different pulse length, intensity and frequency, nervous or muscular tissue can be excited to either perform or prevent a certain action or provide the experimentalist or researcher with information about a persons condition, if not the human organism as a whole. Using different pulse forms than a simple rectangular curve might affect excitable cells in a differentiated way. Triangular or ramp-shaped stimulation pulses have been hypothesized to have an effect on sensory, motor and even pain tolerances since the last century (Balogun, 1991). Studies have since shown that ramp-shaped prepulses below the activation threshold can significantly affect said threshold (Luna et al., 2018) or even the recruitment order of motor neurons (Hennings et al., 2004). This diploma thesis further investigates the possible influence on nerve fiber recruitment of ramp-shaped sub-threshold prepulses in combination with and contrast to classical electrical stimulation. In the first two chapters, an introduction to information pathways within organisms as well as the necessary theoretical background will be provided.

The human body uses two different systems to transmit information from one part to another: the endocrine and the nervous system. In the endocrine system communication is mediated by hormones, which can reach every part of the body via the blood stream. Hence, target and non-target areas are supplied by the respective gland's message promiscuously (Baxter & Funder, 1979). The regulating function of any hormone can only be fulfilled if a corresponding so-called receptor is present and ready for binding. While the endocrine system has a slow transmission paired with a prolonged effect (Baxter & Funder, 1979), the second information-transmitting system, the nervous system, reaches high transmission velocities (from 1 up to $100\frac{m}{s}$) (Russell, 1980) but the impact usually whithers significantly faster than for a hormone, if not immediately. Messages are hereby conveyed by propagating changes in the electrical trans-membrane potential of nerve fibers. Via these neurons, signals are transmitted throughout the body, exchanged between the brain, spinal chord and periphery, enabling us to sense, to act, to react (Kaniusas, 2012).

Information-conveying activities can also be evoked artificially, for instance by means of electrical stimulation. It is a well-established and important diagnostic and therapeutic tool of easy applicability and high reproducibility, suited for many an application. Since the invention of the transistor in the middle of the last century this method has gained importance due to the possibility of building simple, practical or even portable or implantable devices. The advance of scientific and clinical research has enabled the usage of electrical stimulation in a variety of fields, such as rehabilitation and assistance in physical therapy, treatment of patients with spinal chord injuries, denervated muscles or incurable conditions like cerebral palsy (Postans & Granat, 2007) (Kern, Rossini, et al., 2004). Especially the use of different kinds of surface electrodes gives electrical stimulation the tremendous advantages of an easy application and non-invasiveness. These advantages outweigh the drawback of the indiscriminant neural activation evoked by an extracellular stimulation (Rattay, 1990). leading to signals far different from naturally occurring neural firing (Hennings, 2004).

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

Functional electrical stimulation (FES) is aimed at bringing about a certain response of the body, e.g. supporting gait during walking (M, 1984) or improving grasping function (Pfurtschneller et al., 2005). The promiscuous activation of nerve fibers, however, imposes certain limitations on the applicability. Since there are three hundred fifty thousand axons innervating solely the human upper limb (of which only ten percent are motor neurons) (Gesslbauer et al., 2017), the external stimulation of a muscle is always too rough, delicate movements are almost impossible to stir effectively (Hennings, 2004). These movements include but are not limited to grasping or subsequently writing, eating, drinking etc. Another problematic complication is the rapid fatiguing of muscles activated by FES. Whereas remarkable results can be achieved in rehabilitation technology e.g. supporting gait (Postans & Granat, 2007), an application cannot be sustained indefinitely, since this artificially evoked neural activity causes the involved muscles to fatigue rapidly (M, 1984). These and other limitations of FES, however, are not impassable for today's technological advances in the field of biomedical engineering. Selective electrical stimulation might be one solution to the presented problems and a way to overcome them by controlling the externally triggered neural activity (Hennings, 2004), by inducing action potentials in specified nerve fibers and in a controlled manner.

1.1 Inverse Recruitment Order

Whereas action potentials elicited by electrical stimulation and those that occur naturally are basically indistinguishable, artificially evoked neuronal activity per se does not share too many similarities with its natural counterpart; resemblance, if any can be observed at all, is minimal (Hennings, 2004), (N. Rijkhoff & Sinkjaer, n.d.). This missing conformity is caused by the recruitment order of motor neurons involved in the respective process. The various motor neurons are in consequence responsible for the activation of different muscle fibers. Nervous- and muscle cells are therefore considered to work together as one motor unit. An important role for a muscle's efficient performance of movement hereby falls to the order of activation of the motor units. Different types of muscle cells are activated in a certain sequence, whereas due to their respective attributes the fibers can roughly be categorized as type I (large/ fast) and type II (small/ slow) muscle fibers (Ørtenblad et al., 2018).

Physiologically, the first cells to be excited are the slower fibers, i.e. those with a smaller cross-sectional area (McNeal, 1976). Only at higher intensities the larger type I fibers which fatigue more rapidly are activated to support the initiated action. This is referred to as physiologic recruitment order (Kugelberg & Skoglund, 1946). Contrarily, in electrically induced excitation, the large-diameter muscle fibers are activated before the slower twitching ones (Feiereisen et al., 1997), requiring higher amounts of energy. As a consequence muscles activated by electrical stimulation fatigue more rapidly. This defiance of the pre-set activation sequence is called inverse recruitment order.

Another aspect to inverse recruitment is the source of an action potential. A natural, physiologic excitation of a neuron has its origin at the axon hillock, from where the signal travels entirely orthodromically (i.e. directed towards the synaptic knobs) along the axon (N. Rijkhoff & Sinkjaer, n.d.). Figure 1 illustrates origin and path of an electric signal in a simple axon model. Following the signal transmission via this pre-defined path, neurons and subsequently motor units are recruited in the physiologic order. The situation is dif-

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

ferent though for electrical stimulation, where action potentials can be elicited anywhere in an axon, solely limited to the section passing near to the stimulating electrodes (McNeal, 1976). The stimulation is essentially the introduction of an electric current through tissue, causing a change in the extra-cellular electrical potential. If the introduced current exceeds a specific threshold level, an action potential will be initiated within the nerve fiber, mimicking the physiological process. Herein lies the reason for inverse recruitment: The different activation thresholds of various neurons, which can be associated with a number of factors. One would for example assume that nerve fibers closer to the stimulating electrodes would be activated first, i.e. at lower stimulus intensities, which has been found by W. M. Grill and Mortimer, 1995. This poses a problem especially with the application of surface electrodes, generally activating superficial cells before more profound fibers. Further factors influencing the threshold intensities of nerve fibers include their type (sensory or motor) (Panizza et al., 1998) and, foremost, their diameter (Rattay, 1990).

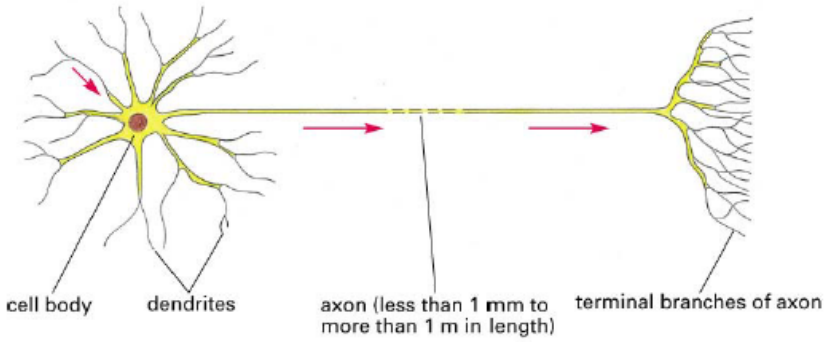

Figure 1 – Illustration of a neuron, consisting of the cell body with the nucleus, the dendrites, receiving signals from other cells; axon, transmitting the signal orthodromically and the terminal branches, establishing connections to subsequent receiving cells like other neurons or muscle cells. Arrows indicate the direction of the signal transmission. Source: Alberts, 2002

Especially the diameter as a threshold-determining factor is of interest, since it is the defining criterion for inverse recruitment. Considering the model of a myelinated nerve, the expression

$$f = \frac{\partial^2 \Phi_e}{\partial x^2} \tag{1}$$

$$f = \frac{\partial^2 \Phi_e}{\partial x^2} \propto x_{int} \propto D_{fiber} \tag{2}$$

known as the activation function has been found to be the essential factor that determines the excitability of a nerve fiber (hence its name) (Rattay, 1990).

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

The second-order differential quotients of various extra-cellular potentials explain the recruitment of larger before smaller nerve fibers when stimulated electrically (Nilsson & Berthold, 1988). Hence, the activation function explains inverse recruitment order. Since nerve fibers larger in diameter are associated with larger motor neurons (and therefore muscle fibers), inverse recruitment subsequently explains the more rapid fatigue of muscles when activated by means of electrial stimulation.

Φ_e ..... extra-cellular potential
x_{int} ..... internodal distance
D_{fiber} ..... fiber diameter

1.2 Selective Nerve Fiber Recruitment

To overcome the obstacle in applicability posed by inverse recruitment has been the aim of studies and research projects for several years. Various methods show promising results for selective activation of motor neurons and, therefore, motor units. Experimental setups have been tested with one or two stimulating electrodes. They usually rely on the blocking of undesired activation to achieve a more efficient motor unit recruitment. In the present thesis I use different setups for the two experiments. Once only one stimulating and one return electrode are used, whereas in the second setup two electrodes are applied for biphasic stimulation. Different approaches to a more specific recruitment include anodal blocking (Kuffler & Williams, 1953), slowly rising waveforms (Kugelberg & Skoglund, 1946) and sub-threshold prepulses (Deurloo et al., 2001; Eickhoff & Jarvis, 2020; Hennings et al., 2004). The latter is also used in this thesis. The introduced blocking techniques all make use of the very fact which causes the complication of inverse recruitment in the first place. The lower activation thresholds of larger fibers or such closer to the stimulating electrodes are exploited in order to selectively activate the desired smaller and/or more profound fibers. Any condition causing larger fibers to be depolarized earlier can be considered to hold true for fibers close to the stimulating electrode, regardless of diameter.

1.2.1 Anodal Blocking

Due to the respective charge injection the membranes of electrically stimulated nerve fibers becomes depolarized under cathodes and hyperpolarized under anodes. Stimulating at specific intensities can cause a strong enough hyperpolarization at the anode that action potentials generated at the negative electrode may be blocked (Hennings, 2004), hence the naming of the phenomenon anodal blocking. Kuffler and Vaughan-Williams (1953) were the first to use anodal block for selective electrical stimulation, inducing the block in frog motor nerves by using rectangular pulses. Their method, however, is not applicable to mammalian neurons, due to the abrupt cessation of current which comes with rectangular pulses. It can cause re-excitation of the blocked large neurons, and thereby rendering the process obsolete (anodal break excitation, Hennings, 2004). Other waveforms have been proposed to circumvent this obstacle (Accornero et al., 1977; Fang & Mortimer, 1991; N. J. M. Rijkhoff et al., 1995), but have not been shown to have an effect with surface electrodes yet.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

1.2.2 Slowly Rising Waveforms

Kugelberg and Skoglund (1946) demonstrated that the same order of activation observed during voluntary movement can be obtained by stimulation with slowly rising waveforms. Their observations, however, were left without explanation, since their studies pre-dated the work of Hodgkin and Huxley about the processes leading to an action potential (Hodgkin & Huxley, 1952) and no biophysical explanation for the findings of Kugelberg and Skolgund has been given since.

1.2.3 Sub-threshold Prepulses

Herein lies the main interest of this Master thesis concerning the possibility of selective nerve fiber recruitment. The idea behind the use of sub-threshold, i.e. non-activating prepulses is to change the conditions for the stimulated membrane or, more accurately, the excitability of the cell. As large nerve fibers normally have lower excitation thresholds (McNeal, 1976), they are also more responsive to sub-threshold prepulses (STPPs). Changes in their excitability should increase their activation threshold significantly more then that of their relatives with smaller diameters (Hennings et al., 2004). From these considerations comes the reasoning that STPPs may be used to achieve physiologic recruitment, which is why they have been targeted in various studies.

1.3 Aim of the Master Thesis

After my first experience with FES during my master's studies I found the rapid fatigue of activated muscles to be the most problematic side effect. However, my opinion changed when conducting measurements with an amputee who usually wears a prosthesis from his right elbow downward. A specific setting of the stimulation parameters enabled him to feel his fingers for the first time after a decade. This encounter and the knowledge of comparable clinical phenomena inspired me to try and work on ways for more specific stimulation of nerves and fiber groups, to enable a more efficient, more natural communication with the human body.

After some theoretical research I found that sub-threshold prepulses, applied directly before the stimulating test pulse, might be a feasible way forward. Grill and Mortimer (1995) already investigated selectivity and showed effects of de- and hyperpolarizing prepulses on activation thresholds. In an effort to generalize their findings, Eickhoff and Jarvis (2020) compared these different forms under consideration of electrode configuration, including mono- and bipolar electrodes in their experiments and were able to manipulate the activation threshold intensities of rat common peroneal nerves. When depolarizing prepulses were first investigated with biphasic configuration in 2018, their application enhanced the activation of nerve fibers in comparison to stimulation without prepulses, with the most significant effect occurring when pre- and stimulating pulse have the same polarization (Luna et al., 2018).

This Master thesis follows this direction of research, specifically testing for the possible effect of ramp prepulses on motor neuron recruitment order, as well as selectivity and excitability. As a primary indicator to determine the impact of the specifically generated pulse forms used for stimulation I considered the variation in excitation thresholds and

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

necessary simulation intensities to elicit certain percentages of the maximum muscle response, represented by the m-wave of EMG readings. The externally triggered activation of motor neurons is achieved via transcutaneous electrical stimulation of the common peroneal nerve and the tibial nerve in healthy human subjects. The work of Hennings et al., 2004 is hereby of the utmost significance for my experiments, since in the course of several studies this researcher also investigated the possibility of affecting the recruitment order using ramp prepulses (Hennings et al., 2004). However, the prepulses used by Hennings are set to a duration of up to 500 ms and are only applied in biphasic setting with cathodic leading phase. In my thesis I propose to achieve notable effects with pre-pulse durations not surpassing 100 ms, thereby reducing the risk of tissue damage inflicted by surface electrode stimulation.

As another indication for the applicability of the custom-generated pulses used in my experiments I consider the occurrences of H-Reflexes. Dean and Collins, 2009 showed that the relationship between M-Wave amplitude and torque was not linear and that the torque contributions of motor units recruited at M-wave and H-reflex latencies do not sum linearly. To gain a holistic understanding of the effects of different wave forms (e.g. with or without sub-threshold ramp prepulses) one must consider indirectly activated motor units as well. It stands to reason that H-wave contribution in the EMG recording is an important indicator for successful differentiating recruitment, due to the anatomic differences between the respective fibers (Gesslbauer et al., 2017). Signals derived from monosynaptic reflexes have also been reported as possible measures of motor control (Pierrot-Deseilligny & Mazevet, 2000).

A third focus of my attention lies on the differences in onset time and duration for responses to stimulation with or without a conditioning pre-pulse. In the inverse recruitment order inherent to electrical stimulation, large motor neurons, associated with fast-twitching fibers are the first to be recruited. By investigating latency periods of responses to different pulse forms, information about the nature of the respective fibers recruited by the stimulating current should be obtainable. Different latency periods for different pulse forms thereby provide additional knowledge concerning desirable effects on recruitment behavior. Hence, the recruitment order could be changed by application of ramp-shaped sub.threshold prepulses.

In the following main body of my thesis I illustrate the conditions of an excitable membrane and its resting potential, as well as the biophysical processes that lead to the generation and propagation of action potentials. The empirical work was performed in two experiment series, with different stimulation sites. In chapter 3 I describe the experimental setup and methods used in the experiments for my thesis, including the virtual instruments used for stimulation and recording, as well as the procedures of analysis, before presenting and discussing the results of my work. Lastly, I conclude my thesis.

2 Underlying Physical and Physiological Principles

2.1 Biophysical Background

Communication within the nervous networks of the human body occurs by conducting electical signals in a unique, bioelectrical way. By changing the electric states of neuronal

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

membranes, i.e. axon membranes, information is conveyed as a propagating spike in the transmembrane potential.
Note that termini *transmembrane potential*, *resting potential* and - first and foremost - *action potential* are universally used and agreed upon in science and clinics. For the sake of completeness, however, I find it necessary to clarify that from a technical point of view concerning the underlying physics, what is meant by the word *potential* here is actually a potential difference, namely between the inside potential Φ_i and the outside Φ_e of the cell membrane, yielding per definition a voltage. Also, since the membrane potential is defined as the difference of potentials from intracellular space to extracellular space

$$V_{membrane} := \Phi_i - \Phi_e \tag{3}$$

whereas

$$\Phi_{i,rest} < \Phi_{e,rest} \tag{4}$$

the (resting) membrane potential is actually a negative voltage. Resulting from this comes the problematic convention, that a reduction of the potential often refers to a decrease in the absolute potential difference, which, since the resting voltage is negative, would strictly speaking mean an increase in transmembrane potential. However, in order to be conform with the underlying literature and scientific conventions, I will use the well-established terminology in this thesis as well. Due to both a personal and professional preference I will use the better-suited and also commonly used term *depolarization* for describing a shift of the membrane potential towards the positive voltage scale and *hyperpolarization* for the corresponding opposite.

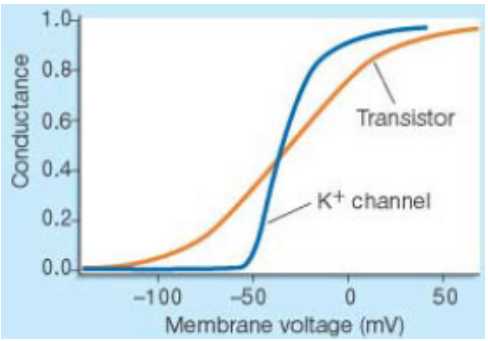

Figure 2 – Sensitivity of a voltage-gated ion channel (here: $K^+ - channel$) compared to a transistor. The ion flow through the membrane channel is a lot more sensitive to the voltage applied, i.e. across the membrane. Source: Alberts, 2002

Cause for the different potentials inside and outside of the cell and the resulting voltage across the membrane are the different concentrations of various ions present in and around the cell. Constituing membrane potentials are mainly the ion sorts Sodium (Na^+), Potassium (K^+) and Chlorine (Cl^-) (Rattay, 1990). Since the ion concentrations of intra- and

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

extracellular space need to differ significantly in order to achieve the desired voltage, concentration gradients of the contributing ions are the consequence. Following the gradient, Na (e.g.) diffuses into the cell via ion channels. In order to maintain the resting membrane potential, an ion pump integrated into the membrane, the sodium-potassium-pump, constantly transports Na^+-ions out of and K^+-ions into the cell (Hille, 1984). Since this is a form of active transport across the membrane, energy in form of ATP is consumed to keep the process running. An approximation of the transmembrane voltage is given by the so-called NERNST-EQUATION, which describes it depending on the different concentrations c_1 and c_2:

$$V_{membrane} = \frac{R \cdot T}{Z \cdot F} \cdot ln[\frac{c_2}{c_1}] \qquad (5)$$

R..... Universal Gas constant
T..... Temperature in Kelvin
F..... Faraday constant
Z..... charge number

Assuming a constant electrical gradient over the membrane in which the same movement of ions under gradients in the ion channels as in the solution, one obtains the GOLDMAN-EQUATION for the membrane voltage:

$$V_{membrane} = \frac{R \cdot T}{F} \cdot ln[\frac{P_K[K]_e + P_{Na}[Na]_e + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_e}] \qquad (6)$$

(Rattay, 1990) with terms P_X describing the permeability and the subscripts i and e marking intra- and extracellular space. Due to the respective concentrations maintained by the sodium-potassium-pump, the Goldman-Equation yields a value for the voltage at the resting state of

$$V_{rest} = -70mV \qquad (7)$$

which is commonly referred to as the resting potential. In this resting state, the membrane is generally considered to be excitable and is therefore ready to receive and/or transmit a signal in the form of an action potential (AP) (Rattay, 1990).

An AP is a positive spike in the transmembrane potential difference (depolarization), elevating the voltage value to

$$V_{AP} \approx +40mV. \qquad (8)$$

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

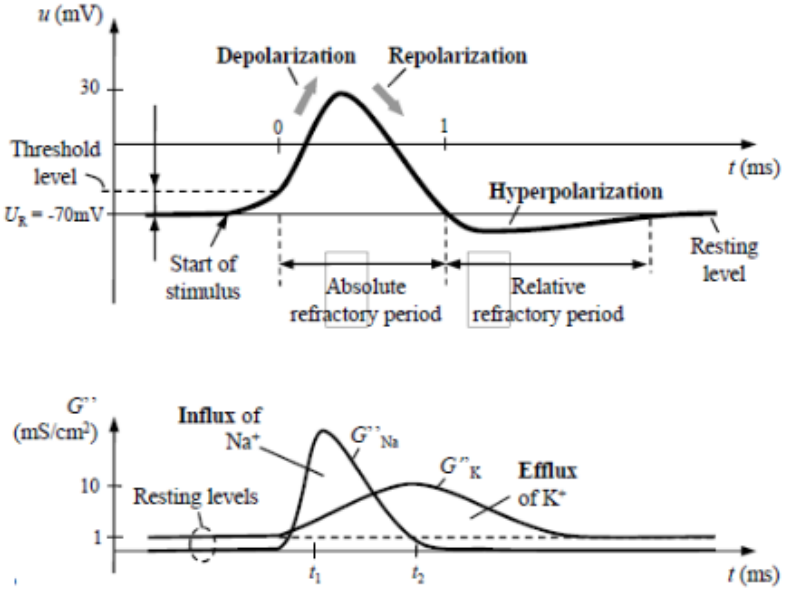

Figure 3 – Temporal progression of action potentials. Top: curve of the transmembrane voltage; bottom: specific conductance for sodium and potassium ions. Note that the elevated sodium conductance gives rise to an influx of ions, whereas the slightly delayed rise of potassium conductivity causes a net efflux of ions. Both currents are driven by respective gradients across the membrane. Source: Kaniusas, 2012

As the resting transmembrane voltage is generated by the different ion concentrations and the associated charge separation, a different charge distribution causes the membrane to be depolarized to the level of the AP. To provide the possibility of ion movement necessary for this change of distribution the membrane is equipped with in-built ion channels. In sensory cells these channels may be triggered by various receptors (like mechanoreceptors or temperature sensors) (Kaniusas, 2012). In most signal-transmitting neurons, however, the ion channels are generally voltage-gated (Hille, 1984), causing them to be opened when a specific membrane voltage is prevalent (see section 2.2 and figure 2). Figure 3 depicts the temporal progression of the AP: membrane depolarization, re-polarization and the period of hyperpolarization, all associated with ion flows.

In the event of an AP the sodium channels are the first to be opened, allowing sodium ions to follow the electrical and concentration gradients into the cell. Once the sodium influx has caused a depolarization of the membrane, the subsequent opening of the potassium channels allows them to exit the cellular body. This is necessary since a new electro-chemical gradient has been formed through the redistribution of ions. The membrane will afterwards seek to re-establish the resting state, by letting the sodium-potassium-pump do its work. The action potential is over (Rattay, 1990).

A spike of this nature propagates according to physiological mechanisms along the axon (Palay et al., 1968) in any direction in which it is currently excitable. The positive voltage spike is followed by a refractory period in which the membrane is shortly hyperpolarized. This hyperpolarization renders the cell temporarily inexcitable in the area, enabling a unidirectional signal transmission
(Wyart et al., 2002).

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

The shape of the AP as portrayed in Fig. 3 is, however, only a sort of macroscopic appearance. The given explanation is incomplete. For an AP not just to be elicited but also to be able to propagate, all ion channels in a certain area have to contribute. The change in polarization can travel from one membrane location to another, like in the case of saltatory conduction from one node of Ranvier to the next. Lots of currents linked with the different ion channels cause a manifold of little spikes in the voltage, which sum up to a measurable signal. What we consider to be an AP is actually the envelope of all the spikes contributing to the signal. Mathematically speaking, an action potential can be considered the integral over many contributions to the firing activity of an excited cell by the individual ion channels as described by Rattay, 1990.

2.2 All-Or-Nothing-Law: The Activation Threshold

Due to their voltage-controlled nature the ion channels in the axon membrane will not open at any arbitrary signal. For an excitable cell to actually fire, i.e. to generate an action potential, the transmembrane voltage has to be elevated to a certain minimum value. This is referred to as the activation threshold, and it amounts to approximately

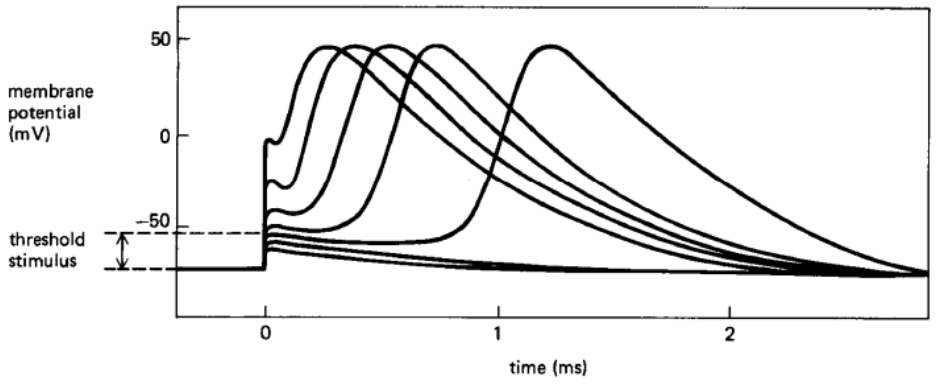

Figure 4 – The All-or-nothing property of action potentials, illustrated by a set of superimposed curves. Depicted are responses to stimuli of different strengths. A stimulus below the excitation threshold produces no action potential. Note that all stimuli above the ET produce APs of the same (standard) magnitude. Source: Alberts, 2002

$$V_{threshold} \approx 50mV \tag{9}$$

(Kaniusas, 2012). To reiterate, I am referring to this step of the process as a rise of the membrane potential, simply due to its definition and the consequential negative value at resting state; the membrane voltage's absolute value decreases in this step.
If, however, the described voltage change is too little, the membrane will not be excited, no action potential will be evoked (Rattay, 1990). A comparison of effects of different stimuli is illustrated in figures 4 and 5.The process leading to an action potential hence follows an all-or-nothing law, there are no APs with a smaller amplitude. A stronger

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

stimulus causes the same value of depolarization in an axon membrane as a light one might. While the (positive) amplitude in the voltage always remains the same, signal strength is encoded via the frequency of subsequent action potentials.

2.3 Functional Electrical Stimulation

Depending on the sensory receptors of an excitable cell, there are various ways for the activation threshold to be reached and an AP to originate. Temperature-sensitive, mechano receptors or olfactory sensory cells have the possibility of exciting the membrane and transmitting a signal. The neuromuscular system though is exclusive in the possibility for voluntary acivation - a healthy human being can decide to lift their arm or pull a leg towards the chest but have no control over e.g. the movement of the small intestine, the constriction of blood vessels or the heart rhythm. In this respect it is important to state that the human body has three types of muscle cells:

- the smooth muscular tissue, which lines the walls of organs such as the gastrointestinal system or arteries
- myocardial tissue, explicitly present in the heart and enabling its unique sequence of excitation and relaxation
- striated muscular tissue, constituting the skeletal muscles which we can control voluntarily.

By the introduction of electric charge into the tissue, action potentials may be elicited in an unphysiologic way. Electrical Stimulation creates the possibility to directly activate motor units in order to perform a distinctive movement. Stimulation hereby occurs via a set of electrodes which can be invasive (e.g. implanted electrodes or cuffs around nervous fibers) or non-invasive (surface electrodes). Especially the latter allows for easy applicability and high reproducability. Hence, FES is by now an integral part of the medical diagnostic and treatment landscape as described in previous sections. Depending on the case, FES can be an alternative to (long-term) medication, surgery (Pfurtschneller et al., 2005), or can even restore some motor function to denervated muscles (Kern, Salmons, et al., 2004), as for example in spinal cord injury patients.

Rectangular impulses are usually used in electrical stimulation to elicit some neurologic and/ or motoric response. These impulses consist of the parameters *phase width* (i.e.duration), *stimulus intensity* (i.e. amplitude) and, if not a single pulse but a train or sequence is applied, the *frequency* (sometimes encoded via a pause). Figure 5 shows the course of the membrane voltage following a rectangular supra-threshold (AP, red) or sub-threshold stimulus (green). Finding the optimal parameters and tailoring the setting for a specific task, movement or condition, has been subject to many investigations. Naturally, stimulating pulses are not at all limited to the rectangular shape. For distinct applications or experiments, other pulse forms might be more favorable, like triangular pulses, exponentially rising/falling or even Gaussian wave forms (Eickhoff & Jarvis, 2020). In this thesis, ramp pulses are in the focus of my considerations, either isolated or in a compound pulse with a rectangular stimulus attached.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

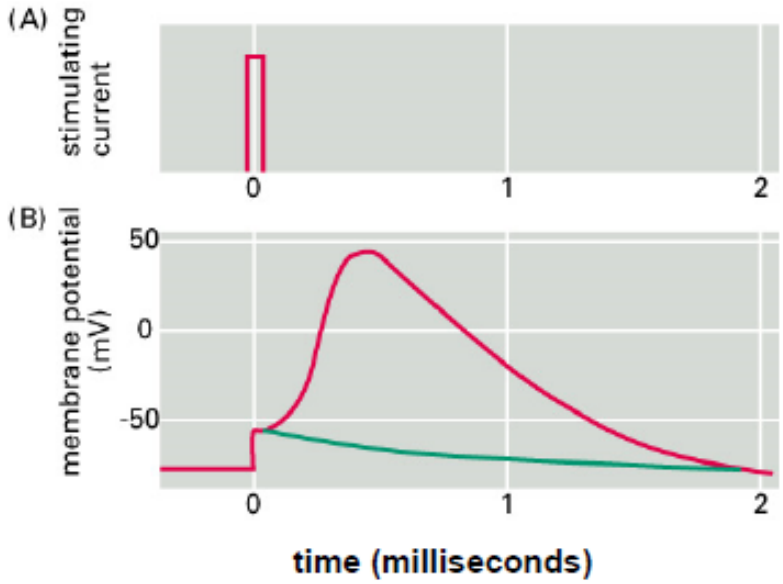

Figure 5 – AP (B, red curve) triggered by current pulse (A). The green curve in (B) shows the course of the membrane voltage without activation, slowly returning to the resting value due to the delayed opening of voltage-gated potassium channels. Source: Alberts, 2002

A last important aspect is the electrode configuration. Activation can be achieved by different kinds of pulses, mainly differentiated according to their sign. Cathodic pulses (negative sign) introduce a negative potential and may have a depolarizing effect, whereas anodic pulses (positive sign) act in an opposite way. Stimulation with pulses oriented in only one direction, positive or negative, is called *monophasic*. It usually is applied via a single active electrode and a (larger) counter electrode on the opposite side.
If positive and negative pulses are combined, the stimulation is *biphasic*. An active electrode and a (proximal but separated)) return electrode are used to stimulate in biphasic mode. Biphasic stimuli can be charge-balanced, so no net charge is transferred to the stimulated tissue. However, the working principle of FES application via surface electrodes remains the same, with the introduction of electrical charge eliciting electrical activity in an excitable cell.

2.4 Principles of Electrical Stimulation

During the research for this thesis I became aware that a simple, intuitive explanation of the functional principle of extracellular electrical stimulation was hardly included in scientific reports, instruction manuals or teaching materials. Without going into too much detail I would like to offer an intuitive and purely physical alternative for the understanding of extracellular membrane stimulation, solely relying on the activation threshold and simple laws of electro statics.

Certain conditions must be met for the voltage-gated ion channels to open and allow the flow of currents leading to an excitation of the cell. Namely, the determining condition is the rise of the transmembrane voltage to its threshold level. There are, however, different ways to affect a voltage. Since per definition a voltage is a potential difference, to change its value is to change that of one or both contributing potentials. For instance, in the

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

resting state of an excitable membrane, the potential outside the cell has to be higher then inside, causing the net negative voltage (due to the definitions given in 2.1). During electrical stimulation an electric charge is introduced into the encompassing tissue.

Naturally there are many mechanisms taking place when a cell's surroundings are subjected to such a change. Considering solely and stubbornly the potential difference (calculated from inside to outside), the introduction of a cathodic (i.e. negative) charge to the extra-cellular space decreases the electric potential at this specific site. This reduction inevitably affects the transmembrane voltage. Since the subtrahend in reduced, the difference has to *increase*. Note that in this particular case, the absolute value still *decreases*. Regardless of the numeric point of view, this arithmetic consideration leads to a transmembrane voltage closer to or even surpassing the activation threshold, exciting the cell and eliciting an AP near the site of stimulation.

One has to keep in mind, however, that the presented argument is of a highly simplified nature, serving only the purpose of developing an intuitive approach to electrical stimulation. A severe simplification made here can be found in most (usually superficial) discussions and explanations concerning action potentials. The electric field is usually only considered to be orthogonally oriented towards a nerve fiber. For the excitation of a cell, however, this component probably will not be enough to reach activation. A more holistic and mathematically accurate discussion was for instance given by Rattay in 1990, but will not be included in this thesis.

2.5 Electrical Activity: M-Wave and H-Reflex

The excitation of an axon membrane or a muscle fiber is, as discussed, basically a positive spike in the voltage. As such, the electrical signal produced by muscular activity can be derived by surface electrodes and used for further investigation. Sampling electrical signals from muscles is a process called electromyography (EMG), the resulting plot is named electromyogram.

M-Wave

An EMG of voluntary muscle contractions would present several oscillating signals from the numerous fibers contributing to a muscle's contaction. Muscle fibers activated by FES, however, produce a synchronized burst, clearly distinguishable in the EMG signal. This kind of response with its characteristic shape is known as the motor wave, or *M-Wave* (Dean & Collins, 2009). M-waves are only detectable in an EMG signal once the stimulation intensity reaches the respective excitation threshold (Feiereisen et al., 1997) and increase in their strength with increasing stimulation intensity (Crone et al., 1999) until a certain maximum is reached. The signal strength of a motor response, given e.g. by the peak-to-peak voltage of the m-wave, is directly proportional to the number of motor units activated (Palmieri et al., 2004). Therefore the EMG signal allows for deductions concerning the efficacy of electrical stimulation with respect to muscle activation.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

H-Reflex

An EMG of electrically evoked muscle activity does not only show m-waves but can contain signals from secondary activation as well. Paul Hoffmann first described a phenomenon that was later named after him in 1910 (Hoffmann, 1910). The so-called H-reflex is basically the electrical manifestation of a muscular reflex. The spinal stretch reflex is usually induced mechanically, when the spindle afferences in a muscle are activated due to a mechanical stretch. The spindle cells cause an electric signal to travel through afferent nerve fibers to the spinal cord, were the signal is monosynaptically transmitted to the efferent alpha motor neurons, causing a muscle twitch (Schieppati, 1987).

The main difference between the described spinal stretch reflex and the H-reflex is that the latter is induced solely electrically (Hoffmann, 1910), effectively bypassing the muscle spindle. In an EMG the H-reflex can be observed at stimulation intensities below the actual excitation threshold of the motor units when a mixed nerve (i.e. containing both afferent and efferent fibers) is stimulated. This early occurrence is related to properties of the afferent fibers, e.g. their larger diameter. Such intrinsic characteristics cause these fibers to be primarily activated at low levels of stimulation (Latash, 1998).

With increasing stimulation intensity the amplitude of the H-reflex increases as well, until reaching a maximum value and declining again. At higher input currents, not only the afferent fibers are activated, but the efferent motor neurons as well. This even leads to antidromically (i.e. against the physiologic direction of signal transmission) traveling excitations. When these collide with the orthodromic signals from the spinal cord, they may partly or completely cancel each other out (Palmieri et al., 2004), a phenomenon called antidromic collision. As the reverse-traveling signal increases in strength, the overall H-reflex amplitude in the EMG decreases, until it finally disappears completely. Figures 12 and 13 show the different appearances of both responses to higher and lower stimulation intensities.

The two responses m-wave and H-reflex can be differentiated due to their distinctive latency times, that is the period of time between the stimulation and the detection of a signal. Since the signal pathway for the reflex is persistently longer (afferent part towards the spinal cord + efferent part towards neuromuscular junction) the H-reflex is visible considerably later than the primary response represented by the m-wave (Buschbacher, 1999). Note that the latency times vary for different muscles, depending on their distance to the upper motor neurons. As the early emergence of H-reflex signals is connected to preferential recruitment of afferent fibers, I consider the H-reflex to be a reliable indicator of specific nerve fiber group recruitment in a mixed nerve.

Recruitment Curve

Once the excitation threshold for motor units is reached, an increase of the level of stimulation corresponds to a stronger muscle response and therefore higher m-wave amplitudes. As the m-wave amplitude is proportional to the number of motor neurons recruited, the analysis of the raw EMG readout allows for connections between the applied stimuli and the portion of muscle recruitment. The obtained results yield a so-called *recruitment curve*, or *stimulus-response-curve* (SRC), which clearly depicts the excitation threshold, the maximum muscle response M_{max} and a characteristic, almost sigma-shaped course of

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

the EMG signal. SRCs are the primary measure of qualitative data control I chose for the analysis of my experiment data. Figure 6 depicts an example for a motor unit recruitment curve. A full SRC also depicts the H-reflex contribution to neuromuscular recruitment.

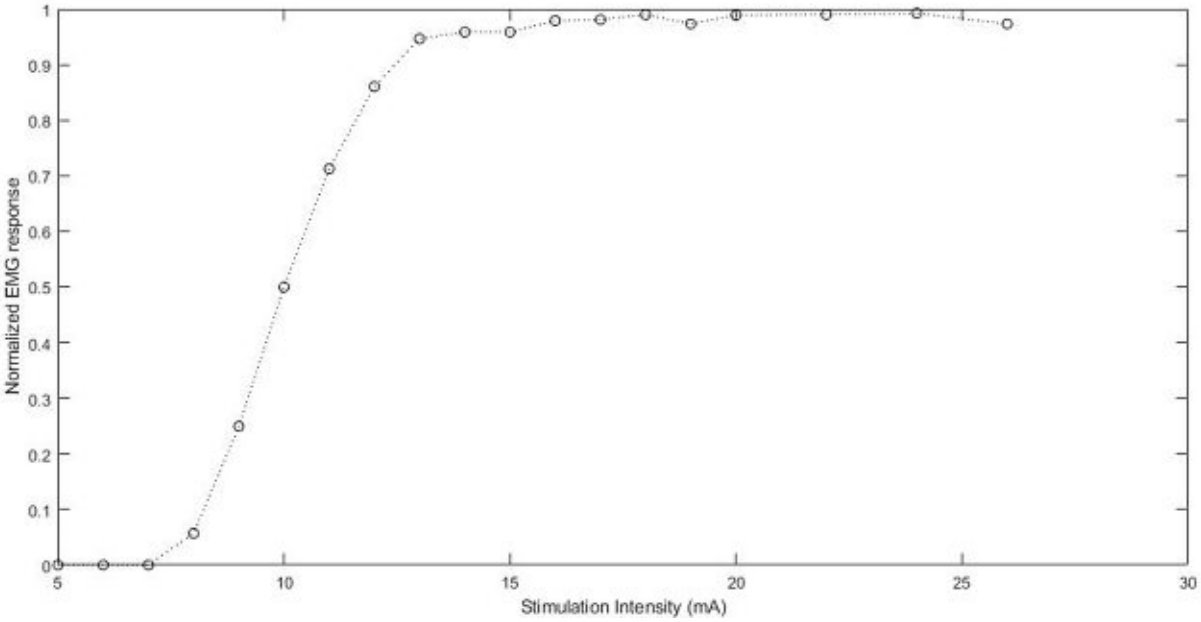

Figure 6 – Image of a typical stimulus response curve (SRC), derived from EMG data of the Tibialis Anterior muscle during experiment A. The peak-to-peak values are normalized to M_{max}. The curve shows only data from the motor response.

3 Materials and Methods

Nomenclature

In order to provide the reader with the possibility to follow the following description, the presentation of the results and subsequent discussion, I include at this point a short summary/ repetition of the terminology that will be used repeatedly in the following sections. Fig. 7 illustrates a compound pulse as used in experiment B.

- RPP..... ramp pre-pule; same sign as the (leading) phase
- CP..... compound pulse; consisting of RPP and stimulating test pulse
- M_{max}, H_{max}..... maximum motor recruitment value for M-wave and H-Reflex
- I_X..... stimulation intensity to achieve **X** % of maximum recruitment
- $\triangle I_X$..... difference between said intensity levels with and without RPP
- $FWHM$ ..... full width at half maximum (only for H-Reflex response curves)
- σ..... standard deviation

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.

3.1 Subjects

A total of eight volunteers (five males, three females) in the age between 22 and 26 years participated in the experiments for my thesis. Four volunteers participated in preliminary measurements to determine parameters for the main experiments (see section *Experimental Setup*). Of these four, only one contributed in the final experiment included in this thesis.

3.2 Experimental Setup

The main interest of the present thesis is to investigate possible recruitment of specific fiber types and groups in a mixed nerve. This should be accomplished by the use of transcutaneous electrical stimulation. To achieve this goal, I am interested in the feasibility of a specific pulse form in comparison to the "standard" rectangular stimulating pulse. Therefore, I am using a specific *compound pulse* (CP) for my experiments, consisting of a sub-threshold ramp pre-pulse (RPP) and a stimulating rectangular test pulse. The idea behind the usage of the RPP is to block certain neurons, primarily those which would react earlier to a stimulus, i.e. have a lower excitation threshold.These neurons are rendered inactive via a sub-threshold pulse, which only slowly reaches its amplitude. The subsequent stimulating test pulse is supposed to activate only that portion of the (mixed) nerves that was not affected by the RPP. To summarize, the basic idea is to change the excitability of the fibers in the stimulated nerve and thereby affect the recruitment order.

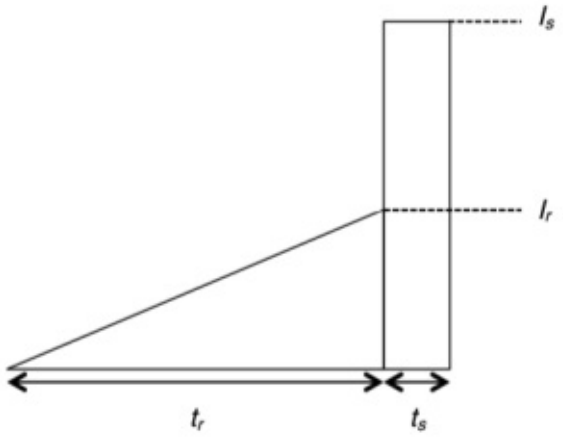

Figure 7 – Basic form of the compound pulses used for stimulation in the experiments of this thesis. The parameters indicated in the figure are the duration of the ramp pulse (t_r), the amplitude of the ramp pulse (I_r), the phase duration of the stimulating rectangular pulse (t_r) and the corresponding intensity (I_r).

To determine the effects of stimulation with compound pulses in a reliable way, a series of measurements was conducted, separated into two main experiments. In the first experiment, only one muscle, namely the tibialis anterior, and its motor response are under consideration. In the second and main experiment, both the tibialis anterior and the soleus muscle are under investigation. The topic of interest is their full SRC, containing

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

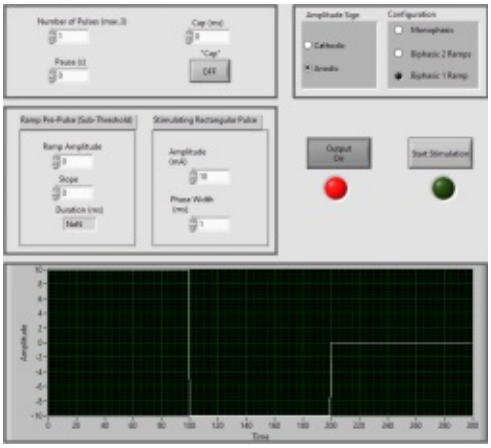

Figure 8 – Picture from the custom written LabView VI's interface, showing the adjustable parameters. Stimulation is set to a stimulus of 1ms duration, with an amplitude of 10 mA.

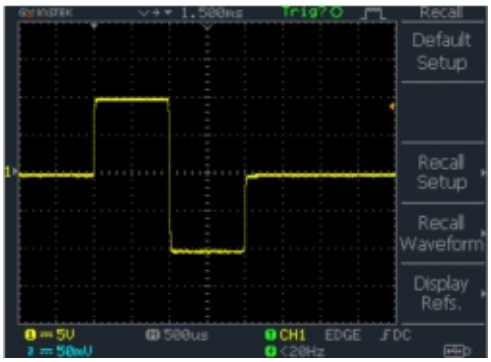

Figure 9 – Picture from the oscilloscope screen, showing a biphasic rectangular pulse of 1 ms duration, with an amplitude of 10 mA, measured over a 1kΩ resistance.

m-wave and H-reflex contributions.

A common challenge in FES is to find the optimal electrode position and configuration, as well as appropriate parameters for stimulation. In order to choose reliable stimulation sites, not only was a profound literature review conducted, but also a series of pre-measurements on a smaller number of subjects was performed. The stimulation protocols chosen for the main experiments are intensity modulated, meaning that a constant pulse width of the rectangular test pulse was combined with varying stimulation intensities.

Custom written LabView programs were developed in the course of this thesis to achieve appropriate pulse forms for the desired experiments. The correct functionality of the program was, as it was revised and optimized, tested and controlled thoroughly via an oscilloscope. Figures 8 and 9 show an exemplary biphasic pulse generated and measured. For these tests the stimuli were applied to both a simple 1 $k\Omega$ resistor and a "dummy", consisting of a resistance in series with a capacitor parallel to a resistance. Only after all safety measures have been confirmed and all concerns eradicated a final measurement protocol was chosen (Merrill et al., 2004) and the actual experiments were performed.

3.2.1 Experiment A: Tibialis Anterior SRC

Under consideration is solely the tibialis anterior (TA) muscle. All EMG-recordings collected, analyzed and presented in this experiment are from this particular muscle from the participating subjects.
The surface electrodes used for stimulation were placed as follows: The distal electrode was placed lateral to the patella, with the top front corner above the fibula head. Figure 10 (left) shows the experimental setup. This placement results in the common peroneal nerve passing approximately below the electrode. The second electrode was placed approximately ten centimeters proximal to the first one, also on the lateral side of the leg. In biphasic stimulation mode the proximal electrode is the active electrode (red); in a

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

monophasic configuration the polarities are reversed. By stimulation of the common peroneal nerve (CPN) an excitation of the TA can easily be accomplished, once the difficult task of electrode placement has been fulfilled successfully. In lower ranges of stimulating intensity it is possible to solely recruit the desired muscle. EMG-electrodes were placed on the bulk of the TA muscle for recording, with the third electrode on the ankle bone as a reference, as recommended by Barbero et al., 2012. The subjects were sitting down with light inclination and the knee at an ankle of approximately 90 degrees.

3.2.2 Experiment B: Soleus and Tibialis Anterior Full SRC

In the second experiment two muscles are stimulated and observed simultaneously. The soleus muscle of the lower calf and, again, the tibialis anterior are the subjects of interest in the series of measurements performed. Both of these muscles are innervated by branches of the tibial nerve. Hence, by stimulation of the nerve at a position proximal to the bifurcation location a simultaneous stimulation of motor neurons associated with each muscle respectively is possible. Since an elicitation of the H-Reflex is commonly performed and recorded on the soleus muscle, parallel stimulation was used as an aid to better detect the H-Reflex in the tibialis anterior. As a stimulation site the poplietal fossa was chosen in accordance with recommendations from the literature (Hopkins et al., 2000; Palmieri et al., 2002).

In a monophasic setting, the cathode was placed slightly proximal to the poplietal fold and the corresponding anode on the opposite side of the leg, proximal to the patella. The electrode position was revised and optimized for each subject via application of test stimuli under simultaneous EMG observation. The recording electrodes for the soleus muscle were placed approximately 2 cm distal and 2 cm lateral to the medial gastrocnemius (Barbero et al., 2012). Early evaluation of test measurements was included into the protocol design. These preliminary measurements revealed that under the chosen electrode positioning anodic monophasic pulses are best suited to activate the tibial nerve. Tibialis EMG electrodes were positioned over the muscle belly, as described for experiment A above. All subjects were sitting in a slightly reclined position with the leg under investigation relaxed at a knee ankle of approximately 90 degrees. The electrode placement can be seen in figure 10 (right).

3.2.3 Stimulation: Equipment and Software

Regulation of the electrical stimulation was performed digitally in a custom-written program environment. I designed a so-called Virtual Instrument (VI) with National Instrument's LabView, which enabled the generation of different customized pulse forms applied in the experiments. Adjustable parameters in the program include slope and amplitude of the ramp pre-pulse (the corresponding duration is calculated and displayed by the VI), duration and amplitude of the rectangular stimulating test pulse, the number of subsequent pulses and a pause between them. I also included the possibility to choose between charge-balanced (i.e. biphasic) and monophasic stimulation, with a third option that was used in the main measurements. Here, a biphasic stimulus is generated but not charge-balanced, i.e. the RPP has only its single occurance and is followed by a biphasic rectangular pulse. The sign of the stimulus intensity, cathodic (negative) or anodic (positive), can be decided upon as well. In biphasic mode, the chosen sign refers to the first

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

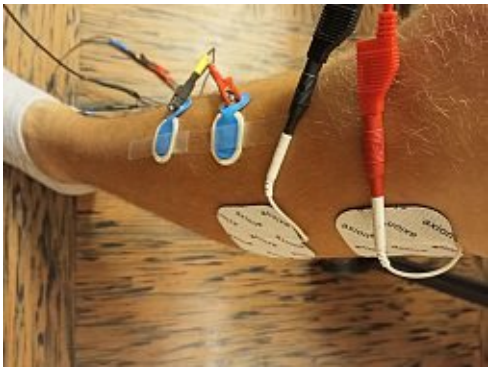
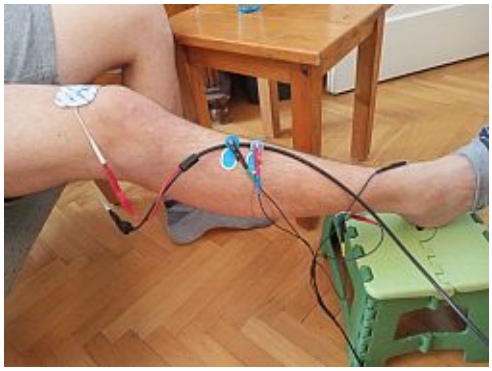

Figure 10 – Experimental setup and electrode placements

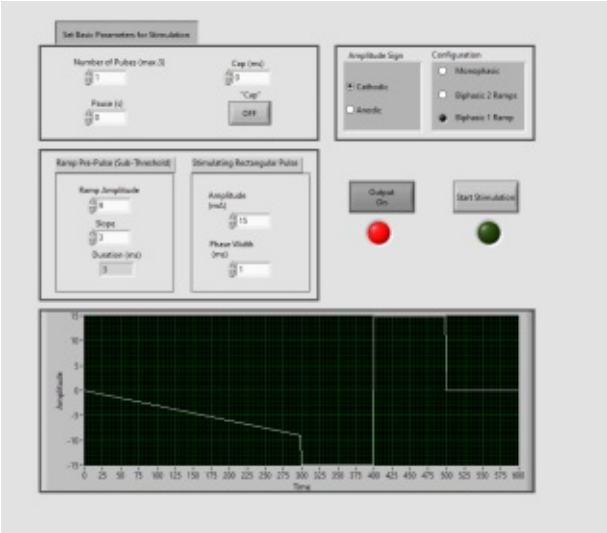

Figure 11 – Screenshot of the custom written LabView VI's interface. The parameters are set to generate a compound pulse with an arbitrary ramp pre-pulse (parameters chosen randomly for illustration).

phase.

From the operating system running the VI the dynamic data is converted into a signal voltage by a USB-NI 6261 Data Card (max. voltage ± 10V) and subsequently amplified by a Constant Current or Voltage Linear Isolated Stimulator (STIMISOLA) from Biopac Systems, Inc (max. Output: voltage-controlled mode, VC: ± 200V; current-controlled mode, CC: ±100mA with a 100 Ω resistor, ±10mA with $1k\Omega$). Standard commercial self-adhesive surface gel electrodes were use to apply the voltage from the stimulator to the subject.

Data Acquisition

To record EMG signals, a set of Ambu® BlueSensor N ECG electrodes were used, placed on the respective muscle belly and connected to a USB-connected EMG measurement device developed at the Medical University of Vienna, with a board based on a Texas Instruments ADC of the ADS1299-x device family, specifically designed to measure electrophysiological signals. EMG data has been sampled by a frequency of 4kHz and amplified

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

by a gain factor of 6, recording was performed by a suitable LabView (National Instruments, USA) VI and a dedicated driver library.

3.3 Stimulation Protocol

Electrical stimulation was administered through a current-controlled stimulation system (STIMISOLA, BIOPAC Systems, Inc.). A sub-threshold stimulation intensity was manually set before the beginning of each measurement. At no point the stimulating current surpassed the pain threshold of the subjects.

Experiment A

The characteristics of the rectangular stimulating test pulse were as follows:

- biphasic rectangular pulse
- cathodic phase leading
- phase width $t_s = 500\mu s$
- $5mA \leq I_s \leq 50mA$

The minimum stimulation intensity was set to 5mA. The intensity was increased by 1mA until 20mA were reached; thereafter the amplitude was elevated by 2mA steps to the maximum stimulation intensity.
Two different compound pulses were applied, differing in amplitude and duration of their RPPs. The RPPs were designed not to elicit any action potentials by themselves and had the same sign as the leading phase of the test pulse. The slope was kept at a constant 0.1 $\frac{A}{s}$. The form of the CP is visible e.g. in Fig. 11.

Short Pulse

- $t_r = 50ms$
- $I_r = 5mA$

Long Pulse:

- $t_r = 100ms$
- $I_r = 10mA$

Experiment B

Monophasic rectangular pulses were applied either as sole rectangular stimulating test pulse or in combination with a 100ms long RPP. Larger stimulation intensities were necessary in order to achieve measurable recruitment, since the tibial nerve's fibers are deeper within the surrounding tissue (Luna et al., 2018). Therefore the electrical field is stronger attenuated and thus higher stimulation intensities are required. The longer phase width was chosen since it favors the elicitation of H-reflex responses (Pierrot-Deseilligny & Mazevet, 2000).

- phase width $t_s = 1ms$
- $15mA \leq I_s \leq 90mA$
- $t_r = 100ms$
- $I_r = 10mA$

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

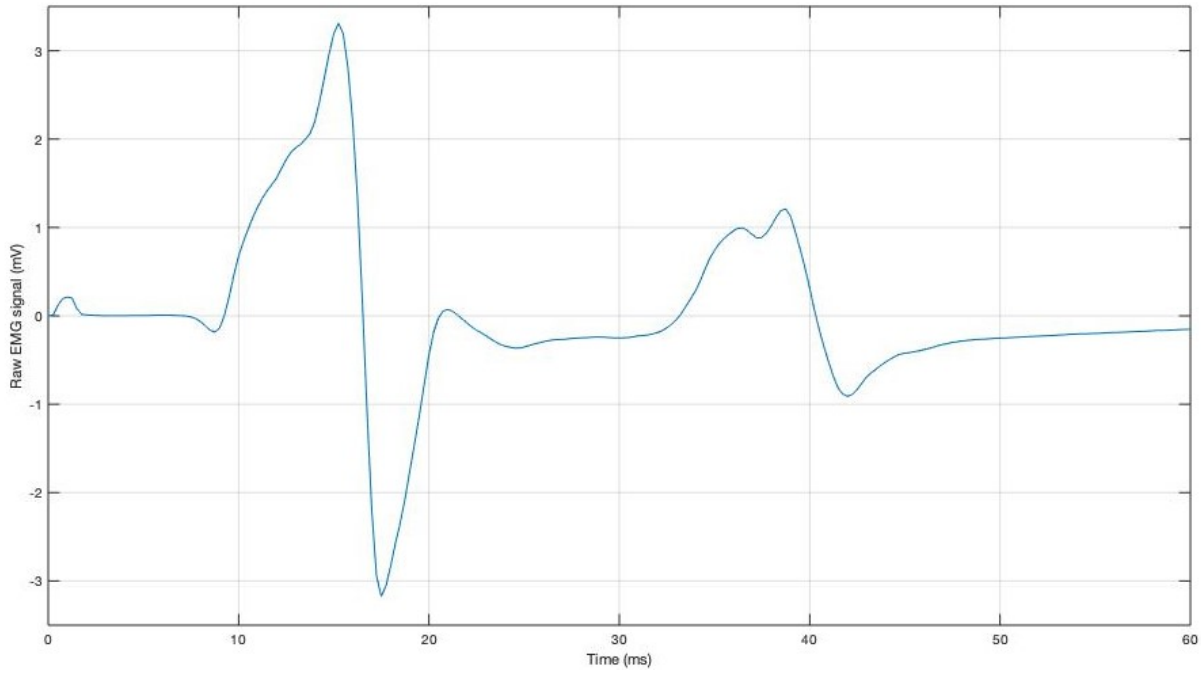

Figure 12 – Raw EMG readings, showing the stimulation artifact (left), the motor wave (center) and the H-reflex (right). Note the longer latency of the H-reflex, caused by the longer signaling pathway, and the smaller amplitude. The appearance of the H-reflex with a delay of about 30ms enables a clear separation form the M-wave and, hence, a more detailed analysis of the measured signal. EMG data from soleus muscle.

"Subject Zero'

The pulse form for experiment B was altered to a biphasic test pulse with a leading cathodic phase in one subject. This way the different effects of mono- and biphasic stimulation could be investigated and the comparability between experiments A and B was ensured. Site and levels of stimulation were according to the protocol of experiment B. In order not to confuse the data and results of this particular experiment with those of the others, I dubbed it *Subject Zero*.

3.4 Data Analysis

The EMG datasets were imported and post-processed in MATLAB (The MathWorks, Inc., USA). Analysis was performed with custom-written programs. The raw EMG value was detected for each stimulation intensity. The corresponding strength of the muscle recruitment was assessed by peak-to-peak (P2P) analysis, which yielded the final SRC when plotted over the stimulation intensity. Fig. 14 illustrates how the course of the positive peak values of EMG responses (both M-wave and H-reflex components) resembles an actual stimulus response curve.

Subsequently all responses per subject were collected as one set of data and normalized to the maximum stimulation intensity M_{Max}. Stimulation intensity thresholds for 10%, 50% and 90% of maximum recruitment were detected. As an indicator for the magnitude of the prepulses' effect the different threshold levels were subtracted from each other, always subtracting the CP intensities from the test pulse intensities (without a RPP): .

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

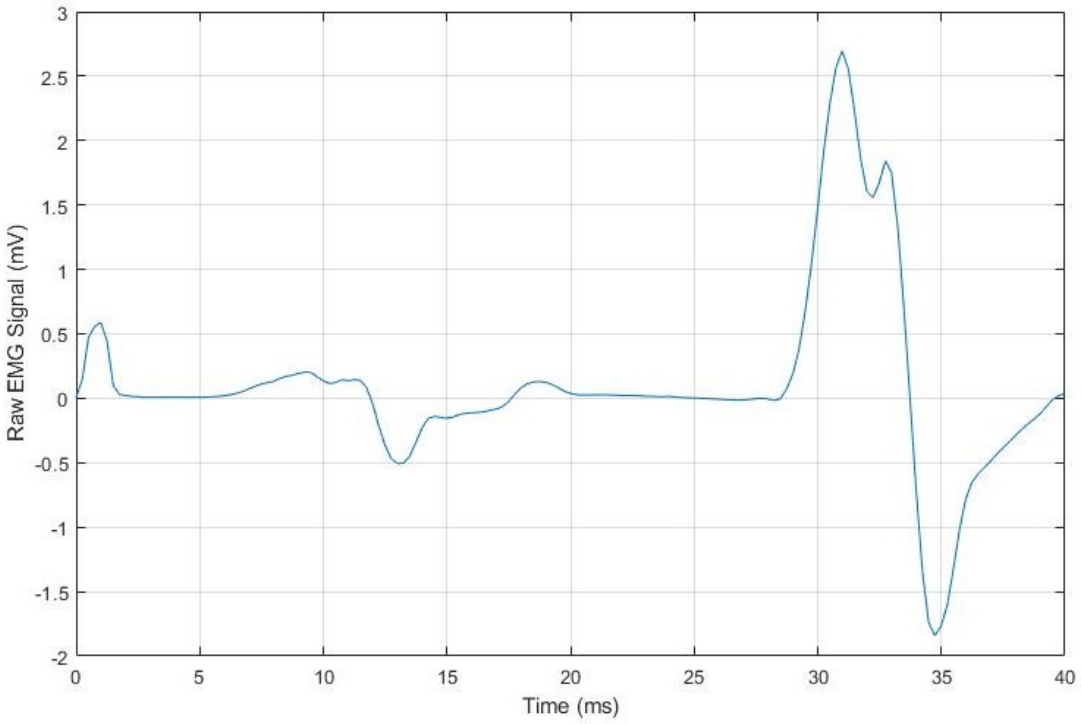

Figure 13 – Raw EMG readings, showing the stimulation artefact (left), the motor wave (center) and the H-reflex (right). Note that in this image the H-reflex amplitude is considerably larger than that of the M-wave. Thus the stimulation intensity can be considered quite low at this point. EMG data from TA muscle

$$\triangle I_X = I_X^{test\ pulse} - I_X^{CP} \tag{10}$$

$$\triangle(\triangle I_X) = |\triangle I_X^H| - |\triangle I_X^M| \tag{11}$$

A one-sample t-test was performed on the collective intensity differences to study the effect of the prepulses. .

The M-response behaviour in experiment B was investigated in a similar fashion as in experiment A. Additionally, H-Reflex results were collected per subject and normalized with respect to the maximum reflex amplitude H_{max}. Intensities for the different response levels were detected and differences calculated the same way as for the M-wave results. In addition, the full width at half maximum (FWHM) was determined for each muscle and data set, followed by calculation of the differences. Same as before, CP-results were subtracted from test pulse results. A statistical analysis consisting of a t-test was performed for the total of all sets. Each muscle was investigated isolated from the other one.

In a final step the differences in the behavior of afferent versus efferent fibers, the absolute values of the differences in M-Wave contribution were subtracted from the those of the differences in H-reflex contribution. Using absolutes in this step is essential to determine which part of the responses, H or M, has been submitted to changes of a higher magnitude (see eq. 10). The final results of this assessment were also statistically analyzed. Generally, a value of $p<0.05$ was considered statistically significant.

To additionally determine the effects of RPPs on differentiating between fiber types, in-

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

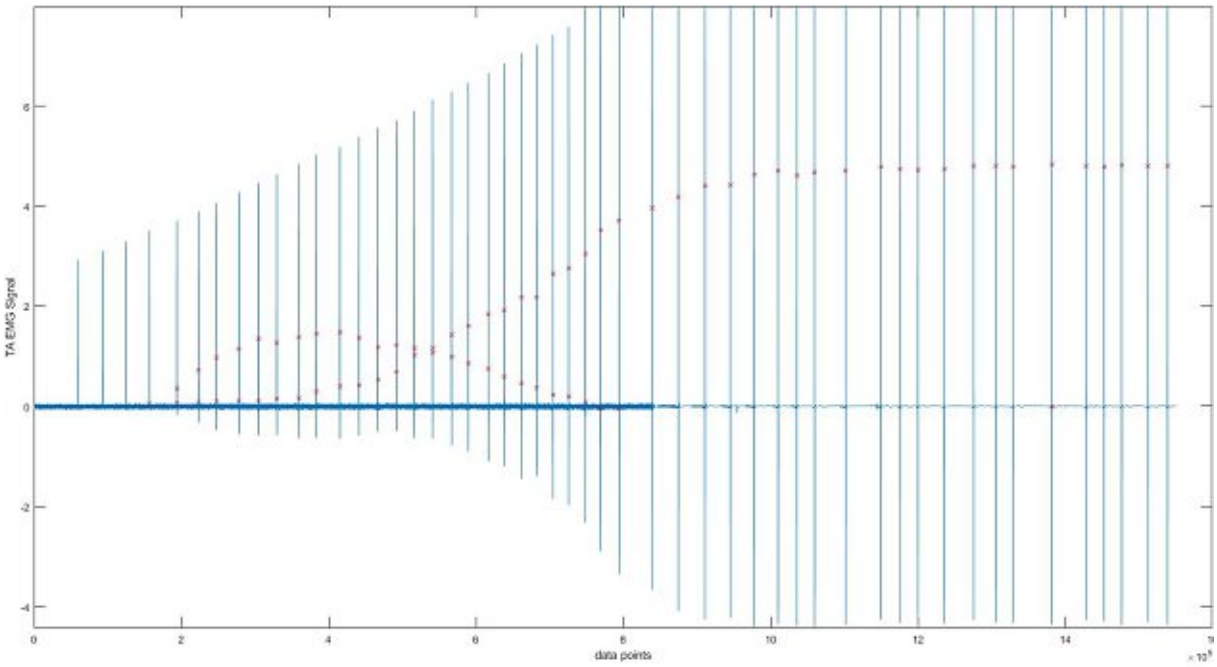

Figure 14 – Raw EMG responses and stimulation artefacts (blue) with peaks of single responses marked by red crosses (mV) plotted over data points). Note that the course of the red markers closely resembles an actual SRC. Recording from the TA muscle.

dividual EMG response signals containing M-wave and H-reflex shares were analyzed. Onset, peak time and duration for both responses were calculated. A signal from test pulse stimulation was compared to one of a CP stimulation. I compared responses following several criteria:

- Signals elicited by stimulation currents of equal strength
- Signals of the same magnitude
- Signals of approximately equal percentage of maximum recruitment (for 10, 50 and 90%).

Two corresponding signals were plotted in superposition. Offset between the curves was corrected and the time scales were synchronized, with the first flank of a stimulating pulse set to *t=0ms*. Hence, RPPs were considered to be at negative times. For biphasic stimulation, the first flank, i.e. the cathodic phase was set to zero. Acquired time variables were compared between the two respective EMG signals. Figures 15, 16 and 19 depict such comparative EMG plots.

A total of ten valid datasets for experiment A and and five datasets for experiment B were acquired from the eight participating subjects. The discrepancy arises primarily from technical difficulties. Still, due to the respective setups, a data set from experiment B contains twice the amount of data than for experiment A. On a single subject, the setup for experiment B was tested with the stimulation protocol of experiment in order to assess the transferability of the results.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

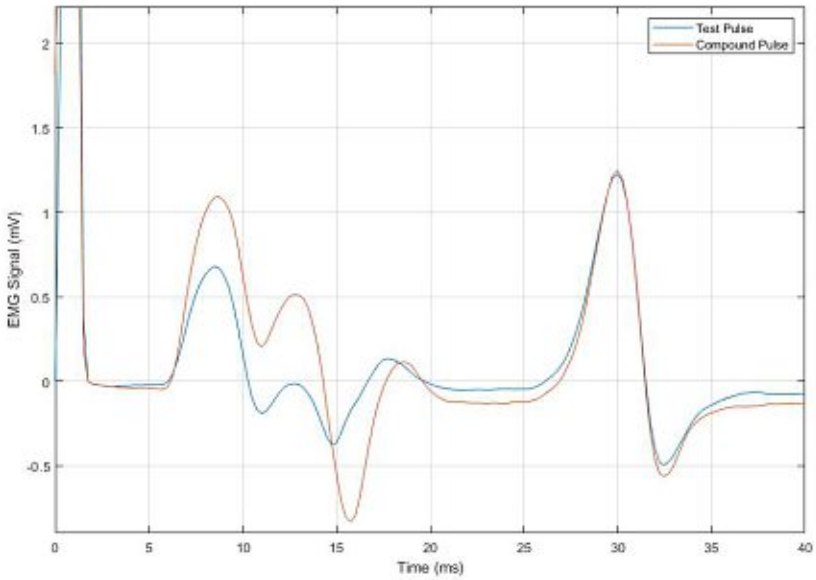

Figure 15 – Superposition of EMG responses for the tibialis anterior muscle. Responses represent 10% of the full recruitment. Monophasic configuration. Orange graph represents reading from CP stimulation.

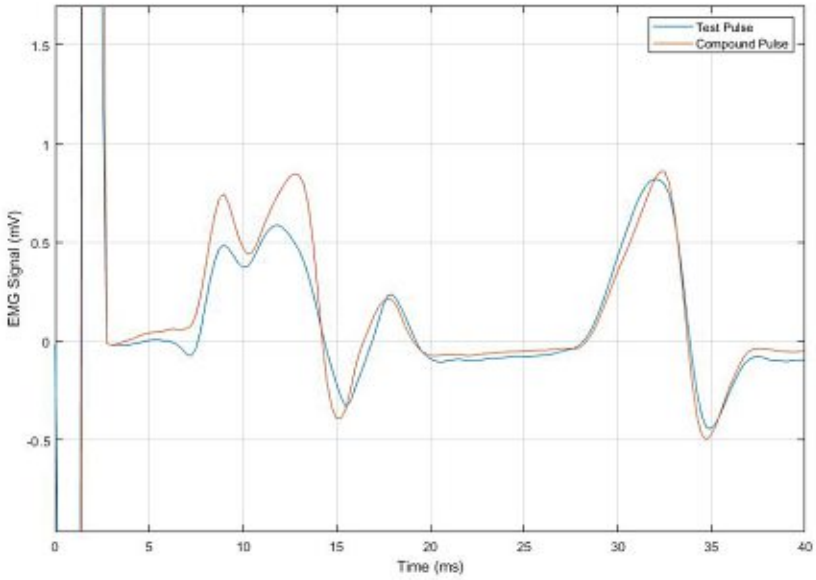

Figure 16 – Superposition of EMG responses for the tibialis anterior muscle. Responses represent 10% of the full recruitment. Biphasic configuration. Orange graph represents reading from CP stimulation.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.

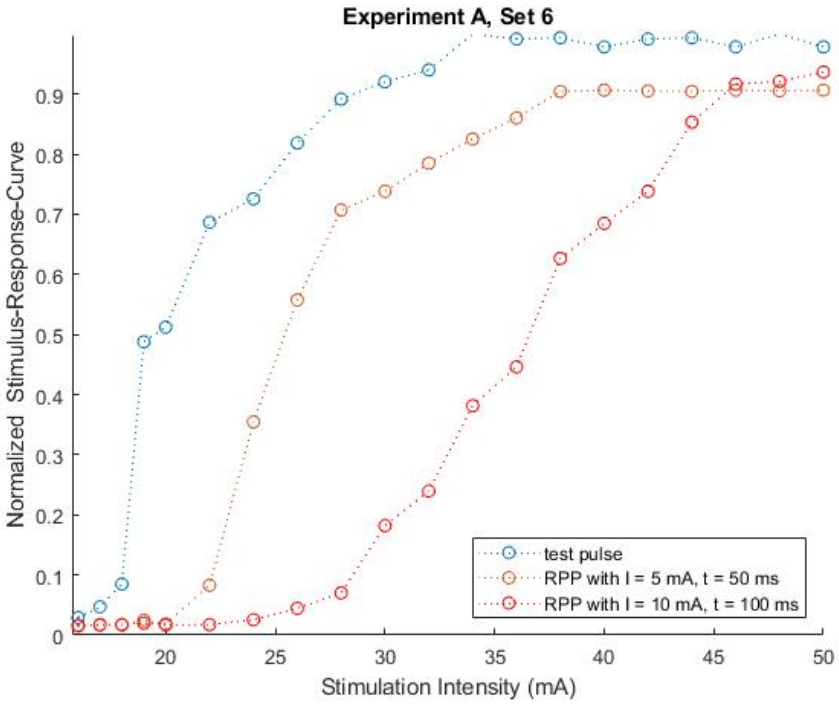

Figure 17 – Exemplary full SRC of a data set belonging to experiment A. Note that the SRC.s of responses elicited by compound pulses are shifted to the right, representing higher respective recruitment thresholds. EMG data normalized with M_{max}.

4 Results

4.1 Experiment A

The desired effect of manipulating threshold intensities for motor unit recruitment is observable in nine of the ten valid data sets for this experiment. Figure 17 clearly demonstrates the shift in stimulation intensity thresholds towards higher levels for compound pulses. The test pulse reaches the excitation threshold and the intensity level for a 10 % recruitment before the long ramp CP (i.e. lower $I_{10\%}$) for all measurements. $I_{90\%}$ is found to be lower for the test pulse than the CP with the long RPP in seven cases, whereas in half of the total cases, the test pulse's SRC reaches the 90% recruitment limit first. In contrast, the other half of the dataset shows that the test pulse reaches 90% of M_{max} after the short CP. In two cases even, it reached this threshold after the long one as well.

The responses are normalized with the maximum of all data in one set. Hence, it is possible for single response curves not to reach full or even 90% of the maximum recruitment. This is the case for the test pulse in two instances, where it neither elicits full activation nor even 90%. Naturally, analysis under these conditions does not yield a numerical value for the respective stimulation intensity (e.g. for I_{90}), thus effectively reducing the sample with respect to the considered level of recruitment. As an effect, statistical significance becomes harder to achieve with the available data.

A similar sample reduction is caused due to the long-ramped CP not reaching 90% of the full activation in three subjects. The sample for the CP with the 50ms-RPP is smaller by default, since for two subjects the experiment was only performed with the longer RPP,

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

	$\triangle I_{10\%}$	$\triangle I_{50\%}$	$\triangle I_{90\%}$
mean	-6.4391 mA (*)	-9.534 mA (*)	-7.1753 mA
σ	4.0223 mA	6.5676 mA	8.7180
n	10	10	6

Table 1 – Results for the CP with 100ms RPP. Differences in the stimulation intensities necessary to recruit a certain percentage of M_{max}: mean value, standard deviation and sample number n. The Asterisk marks statistical significance.

	$\triangle I_{10\%}$	$\triangle I_{50\%}$	$\triangle I_{90\%}$
mean	-2.867 mA	-3.9038 mA (*)	-4.4760 mA
σ	3.5728 mA	4.0349 mA	8.3337
n	8	8	7

Table 2 – Results for the CP with 50ms RPP. The Asterisk marks statistical significance.

causing the missing SRC for the short RPP of these sets' data.

In one subject, the response elicited by the CP with the longer ramp surpasses the 90% M_{max}-limit before the test pulse. In the measurements of two subjects, the long ramp CP does not reach 90% of the full recruitment within the applied interval of the stimulation intensity.

The short-ramped CP generally shows the expected behavior of a shift in the thresholds, causing the respective SRCs to run between those of the test pulse and the longer RPP CP. In three instances, however, this prediction is not or only partly met. For one subject the shorter CP is the first to recruit 10% of the full activation, whereas in another one it reaches 90% of M_{max} at the lowest intensities by comparison. Lastly, the response curves of one subject show the CP with the 50ms RPP to elicit recruitment proportions on all investigated levels prior to both the test pulse and the long ramp CP.
The sample sizes are therefore effectively reduced to n=6 for I_{90} (long CP) / n=8 for I_{10} and I_{50}, n=7 (short CP).

To quantify the effect of the RPPs, their respective stimulation intensities for different levels of recruitment (I_{10}, I_{50}, I_{90}) are subtracted from the corresponding intensities for the test pulse. Concerning the compound pulses with 100ms RPPs, the differences in the 10% and 50% intensities are found to be statistically significant ($p<0.001$). For the short-ramped pulses, the t-test shows significance only for the 50% recruiting intensities ($p<0.03$). The smaller sample size has to be considered when regarding these numbers. Results for Experiment A are shown in tables 1 and 2 for the long and short RPP compound pulses, respectively.

4.2 Experiment B

The results for the two muscles investigated in experiment B, namely the tibialis anterior and the soleus muscle, are considered separately for each data set.
The number of valid data sets is smaller for this experiment due to various reasons. Technical difficulties in recording EMG responses occurred in the two channel measurement

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

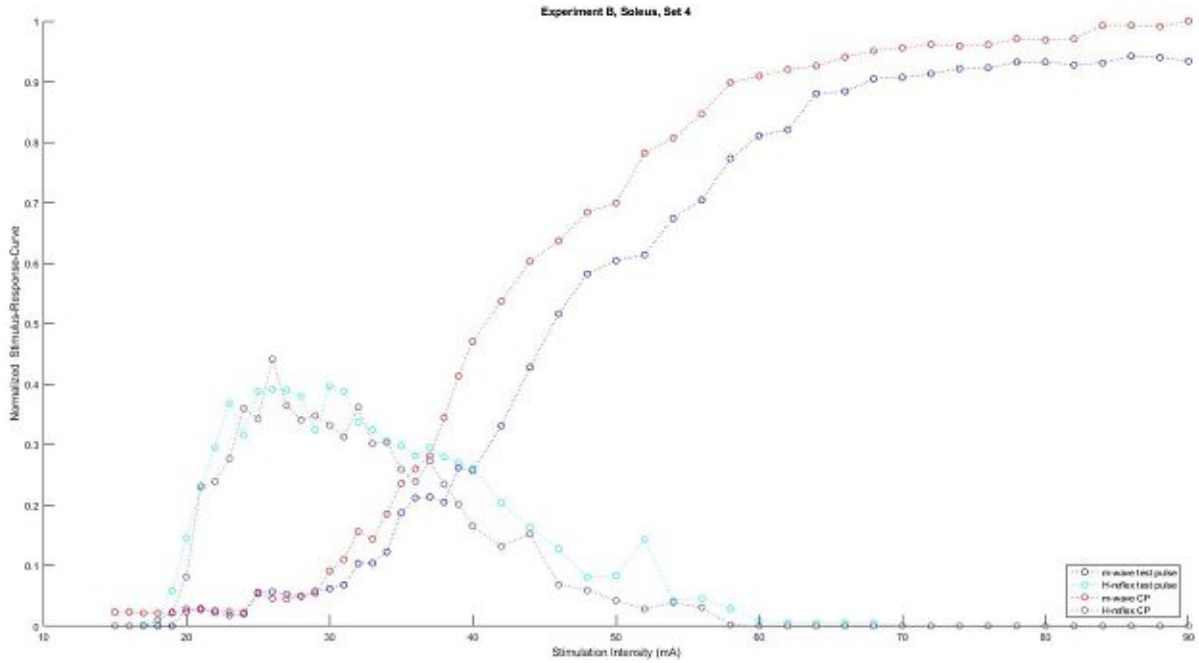

Figure 18 – Full SRCs for soleus muscle (data set 4); Small observable shift towards lower intensities of the curve corresponding to compound pulse stimulation.

setup used. In two instances, probably due to ineffective electrode placement, sufficient excitation of motor neurons could not be achieved. One measurement had to be terminated preliminarily because of problems with the used equipment. Lastly, in one case the interval of the stimulation intensities was chosen to small, so that no full recruitment could be elicited and thus measured. The definite sample numbers for the various results are noted in the respective tables; the maximum count being at n=5 valid data sets. Naturally the net reduction of usable samples for the statistical analysis influences the possibility of achieving significant results dramatically.

The most prominent observation is that experiment B presents an opposite effect to experiment A. The data show a clear tendency of the intensity levels required to elicit a certain recruitment (given as a percentage of M_{max} and H_{max}, respectively) to be shifted towards lower numbers. This represents a decrease in the excitation thresholds for both M-wave and H-reflex responses. Figure 18 shows an exemplary response curve of the acquired data, figure 19 a time-synchronized superposition of two EMG signals from the same data set. Both responses are elicited by a current of the same intensity levels, with (orange) and without (blue) an RPP. The difference in the motor response and H-reflex signal is clearly visible.

In addition to stimulation levels required to elicit a certain percentage of maximum stimulation (i.e. I_{10}, I_{50} and I_{90}), the FWHM values for the H-reflex curves are included as a measure for the specific effect on afferent fibers. Differences in these values (calculated, as all deviations used, test pulse minus CP response) are listed in the respective tables alongside the other results from the H-Reflex evaluation.
All subjects report an unpleasant, needle-like sensation when stimulated with a RPP. However, the applied currents are still below the subjects' respective thresholds for pain sensation.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

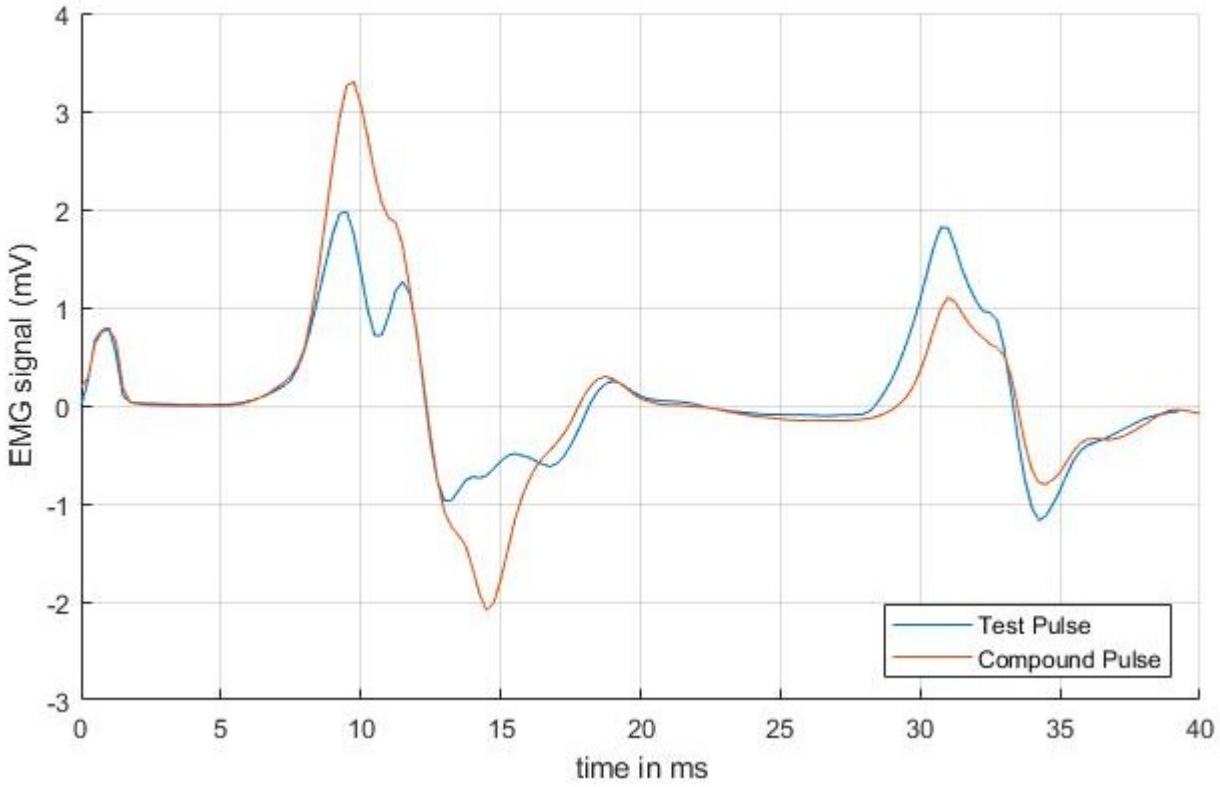

Figure 19 – Exemplary filtered EMG signal, showing M-waves and H-reflexes from the soleus muscle (data set 4). Responses from stimulation with (orange) and without (blue) RPP. The stimulation intensity was the same.

4.2.1 Soleus Muscle

Other than with the data from experiment A, the sign of all mean differences in recruitment threshold intensities is positive. This indicates the tendency of the RPP to alter the aforementioned thresholds, or more explicitly to decrease them. The behaviors of M-wave and H-Reflex are consistent in this respect. The mean measurable shift in thresholds, though, is remarkably higher for M-waves than the reflex components of the responses. To support this observation, it is important to note that the difference in the 10% recruitment level of M-waves between the test pulse and the CP is statistically significant, even with the small sample number of this thesis. Table 3 summarizes the results of experiment B for the soleus muscle.

The data shows a large variance in the measurements that were acquired for the H-Reflex contribution to the SRCs. Regarding the differences in the thresholds of the two lower investigated levels (normalized with H_{max}), it has to be noted that the standard deviation is larger than the mean value for both intensities. Solely two results presented here do not follow this behavior: The deviation of I_{90} between test- and compound pulse shows statistical significance, with a mean deviation of 5.8675 mA. Secondly, $\triangle FWHM$ also has a standard deviation smaller than the mean (in absolutes). The mean deviation is found to be positive, indicating a narrowing effect of the RPP. Statistical significance is not provided by the data, especially since a smaller sample number (n=4) yields the considered data.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.

	$\triangle I_{10\%}$	$\triangle I_{50\%}$	$\triangle I_{90\%}$	$\triangle$FWHM
$\boxed{M-Wave}$				
mean	3.75 mA (*)	8.6150 mA	9.2950 mA	-
σ	1.8239 mA	6.0939 mA	7.6330 mA	-
n	5	5	5	-
$\boxed{H-Reflex}$				
mean	0.0975 mA	1.3350 mA	5.8675 mA (*)	2.7167 mA
σ	2.5242 mA	4.9495 mA	3.0452mA	1.33449 mA
n	5	5	5	4

Table 3 – Results of experiment B measurements from soleus muscle. Statistical Significance is indicated by the asterisk.

	$\triangle(\triangle I_{10\%})$	$\triangle(\triangle I_{50\%})$	$\triangle(\triangle I_{90\%})$
mean	-1.8775mA	-4-7300 mA	-3.4275 mA
σ	1.6542 mA	5.4642 mA	8.7816

Table 4 – Deviations of differences between H-Reflex and M-wave behavio. Calculation: $|\triangle I_X^H| - |\triangle I_X^M|$. Statistical Significance is indicated by the asterisk. Data from soleus muscle.

As a final measure, the total difference between the respective threshold level differences of M-wave and H-reflex data is listed. On all three levels, the mean total difference, calculated according to eq. 11, show a negative sign. This implies that the M-wave behavior was affected by RPPs to a larger extent. The results are not of statistical significance; in fact, only the total difference for the 10% threshold yields a mean value larger (in absolute numbers) than the corresponding standard deviation.

4.2.2 Tibialis Anterior Muscle

All results for the experiment B measurements acquired from tibialis anterior are listed in Table 5. The accumulated data for the second investigated muscle show the same tendency of the RPPs to lower the considered excitation thresholds, with the largest absolute shift in the M-wave data being the mean of 4.7950 mA for $\triangle I_{50}$. The results for this recruitment level are the only ones showing a standard deviation smaller than the mean (3.2987 mA). Still, the conditions for statistical significance are not met; neither in this subset nor in any of the results for the tibialis anterior measurements. No clear trend for increasing or decreasing magnitude of the deviations with respect to the recruitment level can be observed in the data.

The H-reflex measures yield contradicting results. For the lower two difference values ($\triangle I_{10\%}$ and $\triangle I_{50\%}$), the means show negative signs, implying a rise in the corresponding threshold intensities. The difference between the 90%-thresholds is the only one to assume a similar behavior as the additional data from M-waves and the whole soleus measurements. $\triangle I_{50\%}$ is the only one to present a standard deviation smaller (2.2203 mA) than the corresponding mean (5.0700 mA). However, these numbers are based on but three valid data sets, in comparison to the five data sets yielding the other data. Statistical significance is not achieved.
The FWHM deviations exhibit a behavior similar to those calculated from the soleus

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

	$\triangle I_{10\%}$	$\triangle I_{50\%}$	$\triangle I_{90\%}$	$\triangle$FWHM
$\boxed{M-Wave}$				
mean	1.605 mA	4.7950 mA	1.1725 mA	-
σ	1.7936 mA	3.2987 mA	3.9386 mA	-
n	5	5	5	-
$\boxed{H-Reflex}$				
mean	-1.1325 mA	-0.3025 mA	5.0700 mA	2.92330 mA
σ	1.770 mA	3.7735 mA	2.2203 mA	1.3417 mA
n	5	5	3	4

Table 5 – Results of experiment B measurements from tibialis anterior muscle. No statistically significant differences in recruitment thresholds were observed.

	$\triangle(\triangle I_{10\%})$	$\triangle(\triangle I_{50\%})$	$\triangle(\triangle I_{90\%})$
mean	-0.4325 mA	-2.1325 mA	2.8500 mA
σ	1.7446 mA	6.8724 mA	1.8243 mA

Table 6 – Deviations of differences between H-Reflex and M-wave contributions. Calculation: $|\triangle I_X^H| - |\triangle I_X^M|$. Results from TA muscle.

muscle EMG data. The standard deviation is well under the absolute of the mean value, the sign is once again positive. Consequently, a definite effect of the RPP (i.e. causing a narrowing of the H-reflex range) is implied, however not proven to be statistically significant yet again.

The final assessment of the acquired data yields no significant results. The total differences between behavioral deviations of H-reflex an M-wave responses mostly show results consistent with the complementary outcome from the soleus muscle. Only $\triangle(\triangle I_{90\%})$ assumes a positive value and a standard deviation smaller than the mean. A positive sign in this mean value suggests that the H-reflex behavior has been influenced to a larger extent than that of the M-wave.

4.2.3 Subject Zero

Well within the expectations is the outcome of the single case study termed *Zero*. The change in the experimental procedure is reflected accordingly in the results, which are listed in Tables 8, 7 and 9. Data from both M-wave and H-reflex responses all assume negative mean values, corresponding to an elevation of the respective percentage recruitment thresholds. Figure 22 shows a comparison of two EMG responses (10% recruitment for the test pulse). The effect of the RPP on M-wave and H-reflex is prominent. Similarly, the difference in FWHM values is negative, suggesting a broadening of the H-Reflex recruitment curve. The opposition to the complementary results from experiment B is well in line with the predictions for this configuration.
The difference in 90% excitation thresholds for the TA muscle has a positive sign, but the smallest magnitude of all responses.
No statistical test has been performed due to the mere supplemental nature of the experiment.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

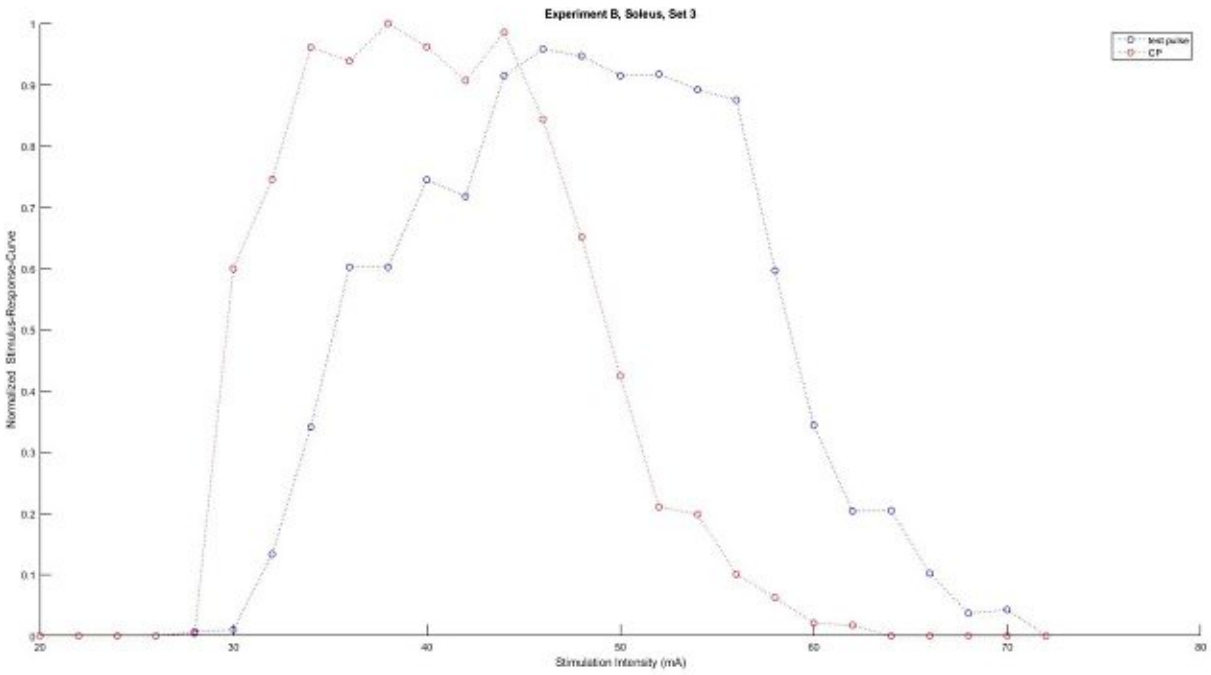

Figure 20 – Comparison of two H-Reflex response curves, normalized with H_{max}. Note that the data corresponding to activation by the CP show a significantly narrower curve but higher amplitude than the SRC of the test pulse. Data from soleus muscle.

	$\triangle I_{10\%}$	$\triangle I_{50\%}$	$\triangle I_{90\%}$
Soleus	-1.9500 mA	-9.3100 mA	-17.403 mA
Tibialis anterior	-5.5200 mA	-7.3100 mA	-28.1100 mA

Table 7 – Results of experiment B, Subject Zero measurements. M-wave behavior of SRCs. Data from a single case study.

The final analysis of Subject Zero data yields no surprises but shows similar behavior as found in experiment B. With the exception of $\triangle(\triangle I_{10\%})$ of the soleus data, which is slightly positive (0.52 mA), all other calculated results are in compliance with those of experiment B. In comparison with the noted exception, the other deviations are of (partly exceptional) larger amount (see Table 9 for more details).

Figures 25, 27, 26 and 28 nicely illustrate how the same stimulation intensity causes different motor responses for mono- and biphasic configuration. Also the effects of the RPPs are presented in an intuitively accessible manner. Conformational changes of the individual responses are clearly visible in the figures. These variations in shape are present in the responses from all valid datasets, with different magnitudes of manifestation. Analysis of individual, time-synchronized response curves show slight deviations between 0.9 and 6 ms for the motor response. Generally, HPPs cause earlier M-wave onset and a prolonged m-wave course. As with all other results from experiment B, DPPs in biphasic configuration evoke opposite effects.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.

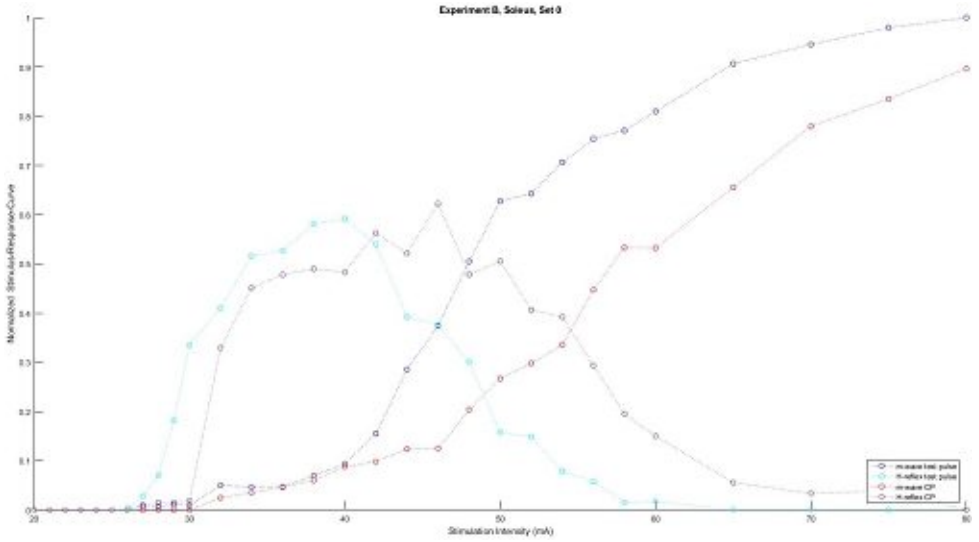

Figure 21 – Full stimulus-response-curves for subject zero single case study. The effects of the RPP on both M-wave and H-reflex behavior are clearly visible. Data from soleus muscle.

	$\triangle I_{10\%}$	$\triangle I_{50\%}$	$\triangle I_{90\%}$	$\triangle$FWHM
Soleus	-2.4700 mA	-2.0400 mA	-4.6400 mA	-5.8800 mA
Tibialis anterior	-2.1700 mA	-2.0200 mA	0.9900 mA	-7.4100 mA

Table 8 – Results of experiment B, Subject Zero measurements. H-Reflex behavior of SRCs. Data from a single case study.

5 Discussion

In my thesis I have presented evidence that ramp pulses, which by themselves do not elicit any action potentials, can influence the behavior of nerve cells under subsequent electrical stimulation. Effects are clearly detectable for a variety of configurations and parameter settings. Diverging results cannot be completely avoided due to the experimental setting: With respect to the stimulation site, stimulation intensities needed to elicit any or a specific response differ significantly. Furthermore, different subjects may react slightly different to certain stimulating currents, if only because of the impossibility of exactly identical electrode placement. Even within a single measurement, imprecision might occur. Causes may be as simple as an involuntary or voluntary movement of the leg that is currently recorded.

The position of the leg during stimulation has a large effect on excitation threshold values. This is clearly visible in the SRC of one subject (see figure 23): After the (normalized) recruitment curve reaches its maximum value, it shortly declines to a local extremum. Afterwards the response reaches saturation again, yielding a sort of flat u-shaped curve. This decrease and re-increase of the motor response coincide with the subject straightening their leg and settling it into a new resting position. This variation of the extremity's position effectively changes the conditions for the stimulation. Variation of both amplitude and also time parameters corresponding to changes in joint position are well-established in the literature (Farina et al., 2001; Frigon et al., 2007). However, whereas the amplitude changes can be explained by the varying anatomical and experimental conditions, the differing response times are to the best of my knowledge not understood as of yet.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

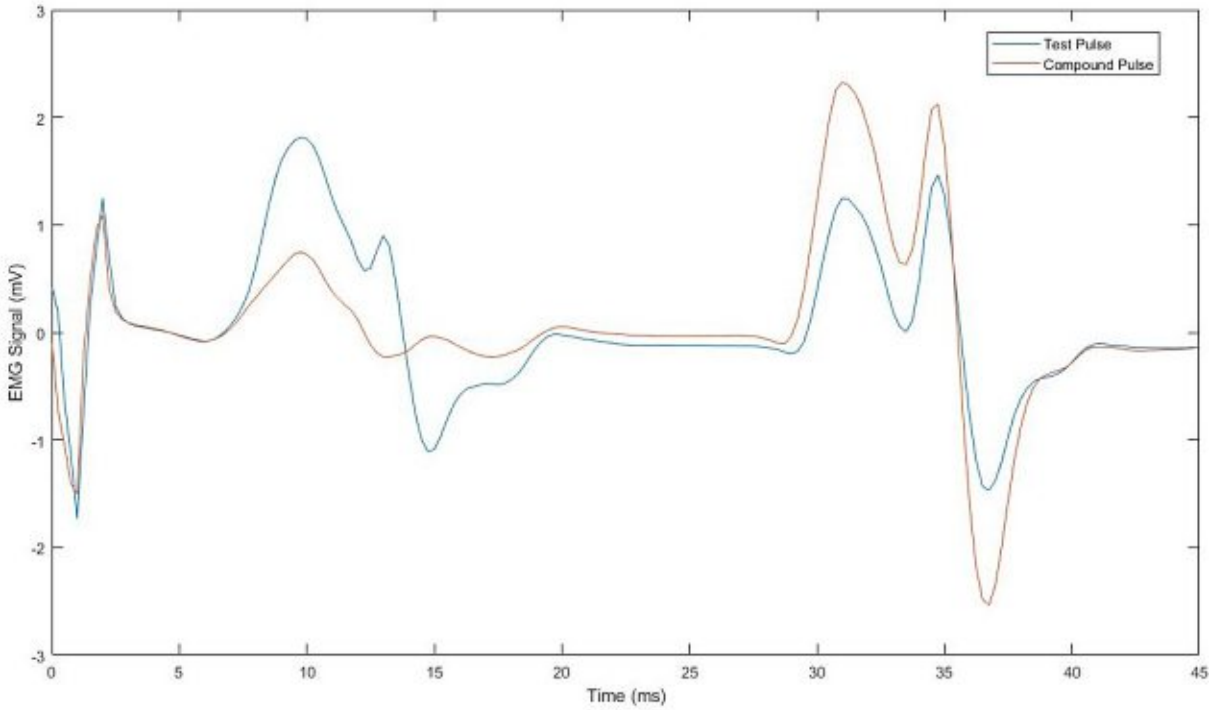

Figure 22 – Exemplary time-synchronized EMG signals from Subject Zero experiment, soleus muscle. M-waves and H-reflexes were elicited by a stimulating pulse with (orange) and without (blue) RPP, at a stimulation intensity of 46 mA.

	$\triangle(\triangle I_{10\%})$	$\triangle(\triangle I_{50\%})$	$\triangle(\triangle I_{90\%})$
Soleus	0.52 mA	-7.2700 mA	-12.79 mA
Tibialis anterior	-3.3500 mA	-5.2900 mA	-27.1900 mA

Table 9 – Comparison of RPP effect on H-reflex versus M-wave. Normalization with M_{max}. Data from a single case study.

For future investigation of motor unit behavior under FES, I would recommend for the subject to remain in a lying position. Thereby both voluntary and involuntary movement and their effects on the readings can be reduced to a minimum. Nevertheless, the flexion of the knee at an angle of approximately 90 degrees enables recruitment at significantly lower intensity levels than with a stretched leg. Considering this circumstance, the phenomenon might be exploited to gain knowledge about positions of limbs or angles of joints by measuring responses to electrical stimulation. With an appropriate feedback this mechanism might find application as proprioception enhancement, for example in patients suffering from denervation or amputees. This, however, would have to be investigated more profoundly in a different setting.

In any case, the uncertainty of stimulation intensity values rapidly becomes clear during the data analysis process. Following these considerations, I find numeric values for stimulation intensities not to be very informative, let alone a sound measure. Hence, I use the differences between stimulation levels required to recruit a certain proportion of the maximum number of motor neurons to quantify the effects of different ramp prepulses. These differences are more reliable, since they can be compared between subjects and measurements. By comparing the respective intensities for different configurations per

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

	DPP	HPP
monophasic	↑	↓
biphasic	↓ (weak pre-pulse)	↑
	↑ (strong pre-pulse)	

Table 10 – Change of excitation thresholds caused by sub-threshold prepulses. To the best of my knowledge the effect reversal for strong DPP in biphasic mode has not been demonstrated for any other configuration.

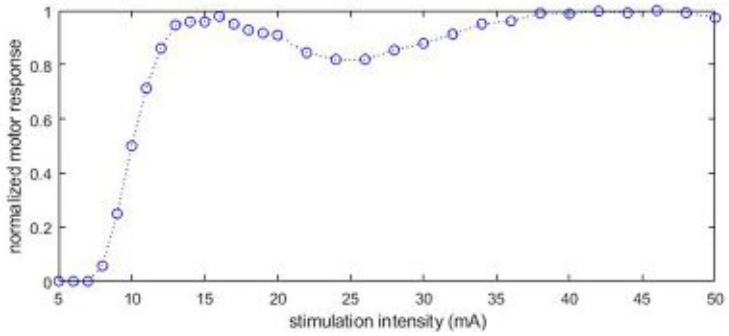

Figure 23 – SRC of a subject who moved their leg during stimulation. Note the recline in motor response before it reaches a maximum again at a higher intensity. EMG data from tibialis anterior muscle

subject, I am able to provide qualitative statements about the effects of sub-threshold RPPs on excitation manipulation and recruitment order. Simultaneously I gain information about the type of the recruited fibers and fiber groups.

Various studies report different findings concerning the effects of pre-pulse polarity, intensity and configuration (Eickhoff & Jarvis, 2020; W. M. Grill & Mortimer, 1995; Hennings et al., 2004; Luna et al., 2018). Some of these discrepancies can be explained by the differing experimental setups and methods used. W. M. Grill and Mortimer, 1997 use models of mammalian peripheral myelinated axons and experimental measurements on cat sciatic nerve for their investigation of DPP effects. Hennings et al., 2004 and Luna et al., 2018 perform measurements with TENS on the upper and lower limb, respectively. While the first administer RPPs, the latter use rectangular sub-threshold prepulses. Both mono- and biphasic stimulation is tested, with varying results. Eickhoff and Jarvis, 2020 report electrode configuration as one of the primary parameters for the effects of sub-threshold prepulses. Their data is derived from invasive experiments on rat's common peroneal nerve (CPN). Interestingly, my results are consistent with some findings of each of these researchers. The different setups of the two experiments in my thesis favor the comparability with the literature.

In experiment A, DPPs in a biphasic setting (leading phase cathodic) commonly increase the threshold intensities for all of the considered recruitment proportions. More explicitly, the sensitivity of motor neurons is reduced by the conditioning effect of the ramps. Whereas a sub-threshold depolarization has recently been reported to increase

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

the excitability of axon membranes (Eickhoff & Jarvis, 2020; Luna et al., 2018) in bipolar stimulation, my findings are in line with those of Hennings et al., 2004 and Vastani et al., 2013. These researchers report that "strong" DPPs raise excitation thresholds (i.e. reduce the excitability) of nerve cells. The RPPs in this report have durations of up to *500 ms*, whereas I was able to detect the same effect with much shorter impulses (*50, 100 ms*). The data from experiment A also suggests an additive character of the reduction in excitability (see Tables 1 and 2, as well as Figure 17). A longer ramp duration, which directly corresponds to a higher pre-pulse intensity, causes a larger shift in the excitation levels. Eickhoff and Jarvis, 2020 also apply ramp prepulses, but the durations in their experiments are with *10ms* well under those used in my thesis. The corresponding pre-pulse intensity is thereby limited to 20% of the following stimulation pulse.

Comparison of these results suggests that the actual amount of injected charge (in this case mediated by the ramp duration) directly influences the direction of the shift of threshold levels. The dichotomy of DPP effects, depending on the magnitude of the pre-pulse intensity, as suggested by Hennings et al., 2004, can be explained by the effect of sub-threshold stimulation on sodium channel gates. The state model presented by Armstrong, 2006 describes how the states of a sodium channel's different domains can influence its overall state, i.e. open or inactive. Figure 24 illustrates the architecture of sodium channels. Luna et al., 2018 argues that this model for sodium channel inactivation might provide an explanation for the DPP influence on threshold levels.

Considering only sodium channels to be significant for the effects demonstrated by the experiment might appear restricted. However, in case of a *severe nerve fiber depolarization*, the inactivation of sodium channels is of the utmost importance for accommodation to sub-threshold prepulses (Baker & Bostock, 1989). Since RPPs represent a more natural approach to accommodation due to the slow increase of current (Vastani et al., 2013), higher intensities than with rectangular pulses can be reached. The criterion of a "severe" conditioning depolarization appears to be met. Thus, the focus of the following considerations will be on sodium channel behavior.

According to Armstrong's model, whether a channel gate is active or inactive depends on the subset of activated or inactivated domains. The apparently opposite effects of high and low current sub-threshold prepulses can be understood as a consequence of the different current strengths influencing the channel domains. A higher amount of injected charge could simultaneously affect all four domains, i.e. causing the channel to transition to a fully inactivated state. Naturally this reduces the excitability, thus effectively elevating threshold intensities. Another aspect is that, since the electric field is strongly attenuated in the cutaneous and subcutaneous tissue, the DPP strength can only have this inhibitory effect on fibers closer to the electrode or with a larger fiber diameter. The model of Armstrong, 2006, applied to sub-threshold ramp prepulses, therefore also enables and explains enhanced differentiating between fiber types in electrical stimulation. Smaller pre-pulse intensities on the other hand might only alter the extracellular potential enough to activate some of the domains, but not cause a full inactivation. Rather a state of higher excitability is brought about, with the consequence of a lower excitation threshold for a pulse following a weak DPP (Luna et al., 2018). The argument for specific recruitment remains unchanged.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

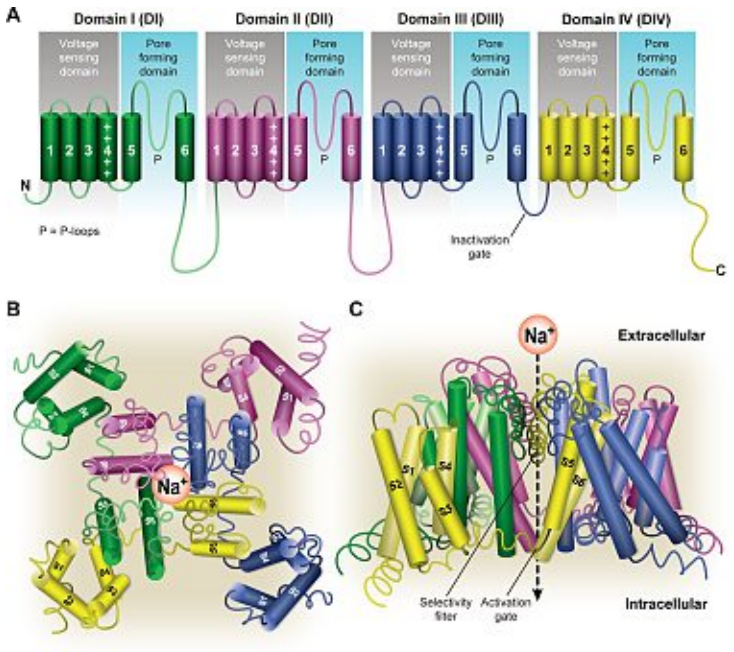

Figure 24 – Architecture of a voltage-gated sodium channel. A: Topology of the human Na_V-channel sub-units. Each protein consists of four domains, which contain six transmembrane segments. S1-S4 form the voltage-sensing domain, S5 and S6 form the channel pore (together with the pore loops). B: Open-channel conformation, extracellular view (Na_V of a marine bacteria). C: Same open-channel conformation channel, side view. Source: de Lera Ruiz and Kraus, 2015

Another aspect in this regard is the maximum recruitment. The data show a tendency for maximum activation to decrease when a RPP is administered prior to the stimulating pulse. This further strengthens the hypothesis of distinction between fiber groups in motor neuron recruitment due to different respective characteristics. Since the M-response represents the number of motor units activated (Palmieri et al., 2004), it can be assumed that a certain number is blocked by the RPP. As argued above, nerve fibers will not be affected equally by the sub-threshold conditioning.

The conditions for FES in experiment B are significantly different to those of lateral side CPN stimulation. The neurons and fibers of the tibial nerve are situated deeper within the surrounding tissue. This causes electrical signals applied via surface electrodes to be weakened to a larger extent. Hence, stronger stimulating currents are required to evoke any kind of recruitment. The respective numerical intensities are not listed in my thesis, as they are not representative for and comparable between subjects and muscles. It is, however, thinkable that the magnitude of the RPP is not high enough to achieve a relevant or recognisable effect. Data from the Subject Zero single case study dismiss this concern. As the DPP of 10mA strength still shows the same effect as in experiment A, it is save to assume that a HPP of the same magnitude will also affect the excitable cells in a reliable way, consistent with established models.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

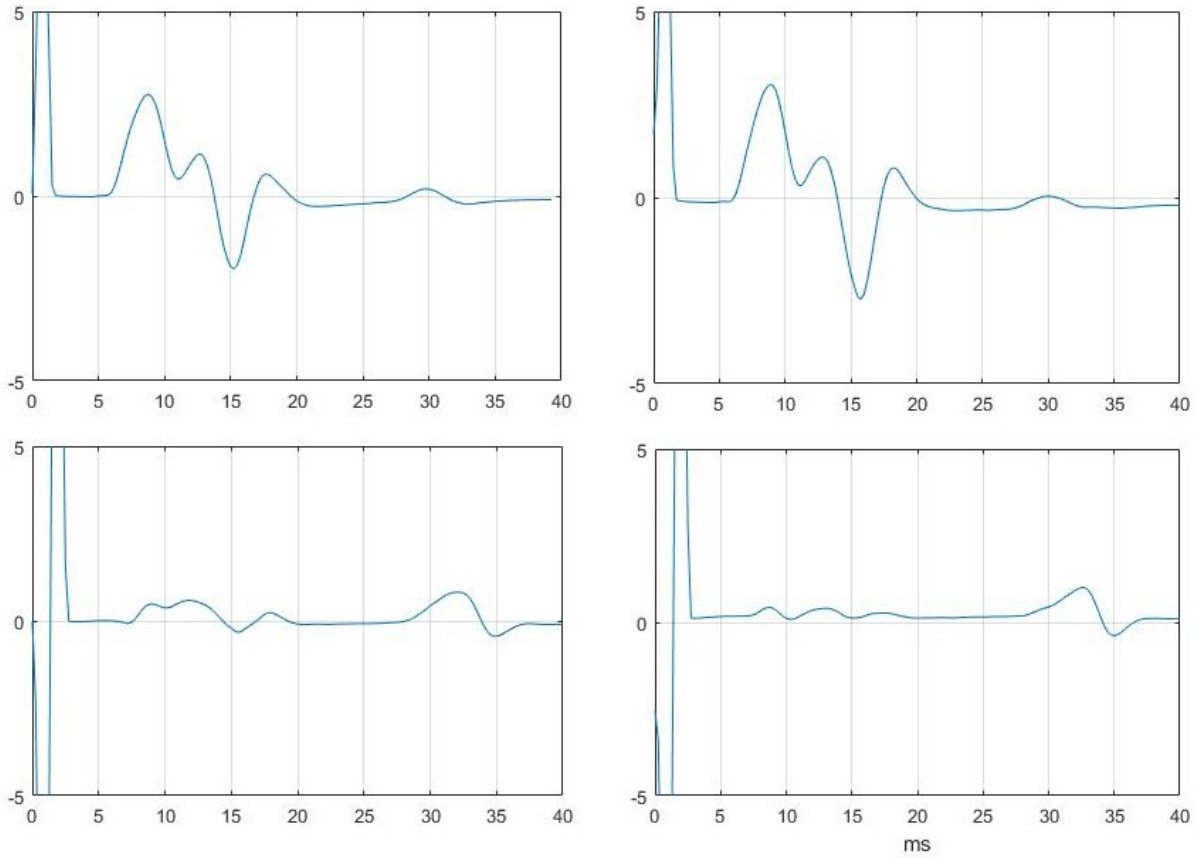

Figure 25 – Comparison of exemplary EMG signals. Left: no RPP. Right: RPP.
Top row: anodic monophasic stimulation (Experiment B).
Bottom row: cathodic biphasic stimulation (Subject Zero).
All recordings from TA muscle, stimulating current I = 40mA.

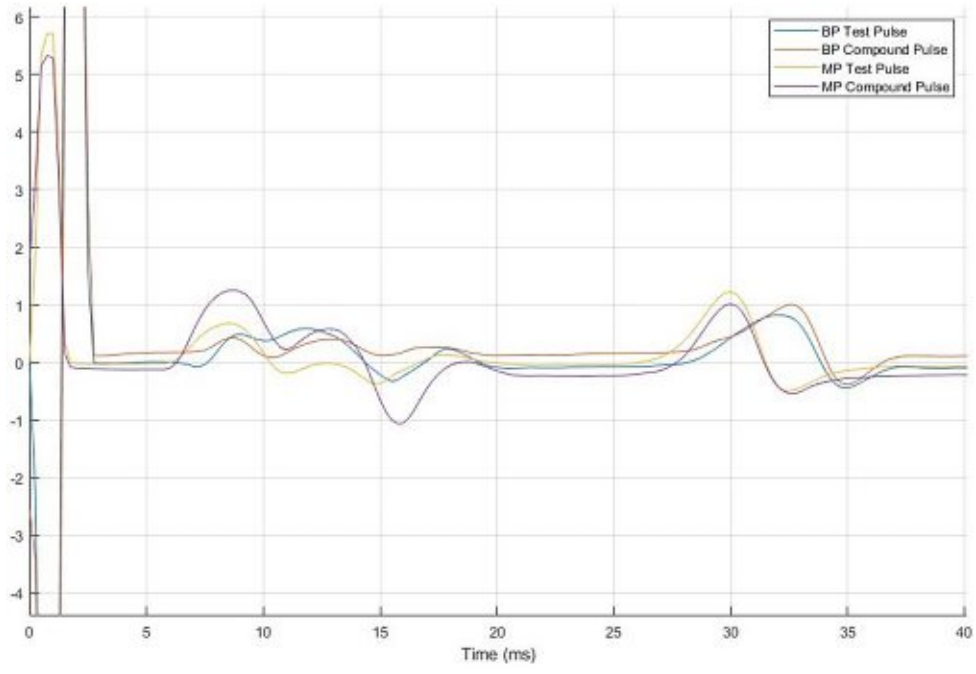

Figure 26 – Time-synchronized superposition of the EMG responses described in Fig. 25.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.

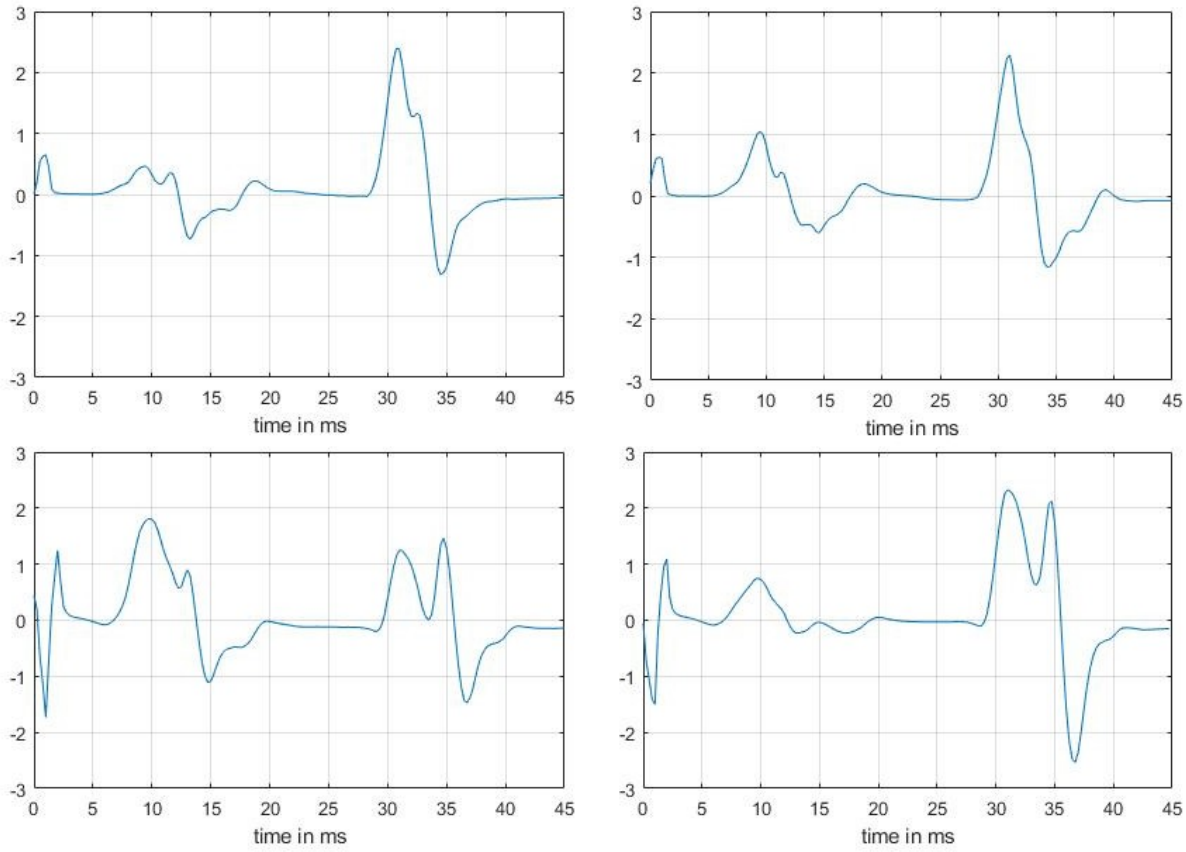

Figure 27 – EMG signals in the same pattern as Fig.25.
All recordings from soleus muscle, stimulating current I = 46mA.

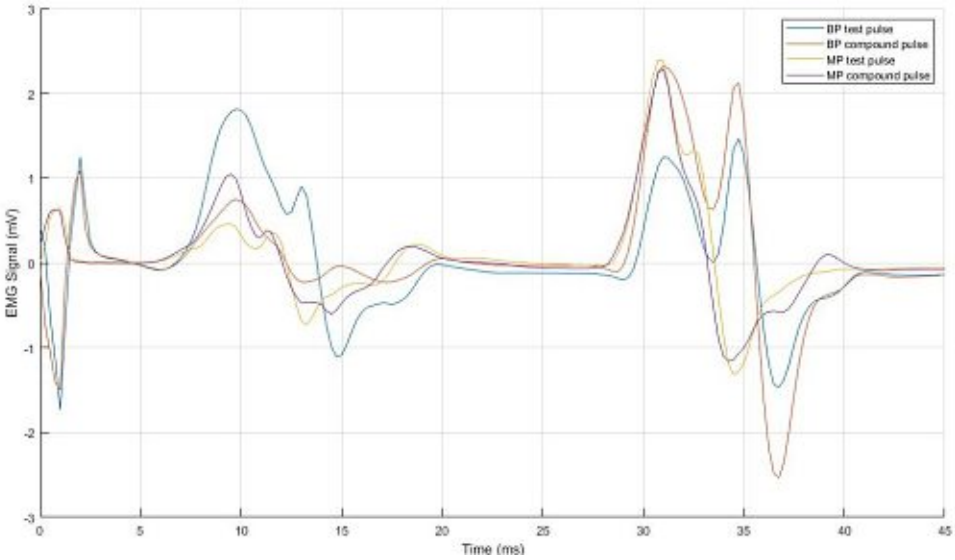

Figure 28 – Time-synchronized superposition of EMG responses described in 27.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.

The reliability of the electrode placement is another aspect causing increasing difficulties with the experimental setup. In one subject, the excitation threshold is absurdly high, which is without a doubt a consequence of insufficient electrode placement revision and supervision of the EMG recordings during stimulation. The conditions in general but also especially for early measurement were not optimal in this experimental setup. Nevertheless, I am able to demonstrate effects consistent with the literature and the specific hypotheses of my thesis. The statistical significance, however, suffers and the sample number is to small to compensate for problems with the experimental procedures.

The aforementioned larger intensities required to elicit motor recruitment and, especially, full recruitment are also partly resembled in the mean deviations of the tibialis anterior data. Their magnitude can, on the one hand, be attributed to high variations in the respective threshold levels, corresponding to the difficult circumstances for effective stimulation. On the other hand, the results from recordings in this experimental sub-set are not very robust.

Excitation thresholds for recruitment of the different proportions established in my thesis are affected by sub-threshold ramp prepulses. This statement is supported by the data from experiment B as well. There are, however, some deviations from the results of experiment A. The most prominent difference is the sign of the intensity level shifts. With a sole exception, the shifts are mathematically positive, meaning that stimulation with compound pulses requires a weaker stimulation current than without a prior ramp pulse; the excitability is elevated. These results are not unexpected. Experiment B uses monophasic stimulation with an explicit anodic phase. Previous studies investigating sub-threshold prepulses in monophasic configuration report an equal effect of such hyperpolarizing prepulses, regardless of pulse form (Baker & Bostock, 1989; Eickhoff & Jarvis, 2020; W. Grill & Mortimer, n.d.; Hennings, 2004; Luna et al., 2018; Vastani et al., 2013).

My findings confirm that HPPs applied before a monophasic stimulating rectangular pulse lower the excitation thresholds for recruitment of 10%, 50% and 90% of maximum motor response. The results of experiment B are therefore in good agreement with the literature, most prominently the original work of W. M. Grill and Mortimer, 1995. In their research they report that HPPs cause the membrane to be easier to excite. A HPP hereby represents a positive charge injection into the extracellular space at the site of stimulation. As a consequence the extracellular potential is increased, causing the transmembrane voltage to further decrease (the amount of the potential difference of course increases). The membrane is hyperpolarized.

Why this causes a decrease of the excitation threshold is not entirely clear. A possible explanation might be that the cellular mechanisms start to try and reduce the absolute potential difference by themselves. Figuratively speaking, a stimulation pulse following hyperpolarization would therefore not start a process of depolarization, but rather enhance it. Ionic flows that are already being initiated and in progress are supported and amplified by the stimulating pulse. In total, this represents a reduction of the excitation threshold, caused by the HPP. My results and the provided SRCs illustrate the according behavior. However, the data of my thesis does not allow for a conclusion regarding the underlying mechanisms of accommodation to RPPs.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

It is noteworthy that a DPP of 10 mA intensity and 100 ms ramp duration is strong enough to reverse the effect of DPPs in biphasic stimulation mode (see experiment A and e.g. Hennings et al., 2004). A HPP of exactly the same magnitude but in a monophasic configuration still evokes the exact effect as proposed by the literature, namely a reduction of the respective excitation thresholds. The question arises whether a bifurcation of effects depending on the strength of the sub-threshold pre-pulse, as demonstrated and argued for biphasic DPPs above, can possibly occur in a monophasic setting as well. If so, one has to wonder at which pre-pulse intensity, since the 10 mA clearly suffice to cause the effect reversal for a biphasic stimulus.

Of course it is conceivable that the pre-pulse at the experiment B stimulation site is nevertheless too weak in order to show effect reversal. Indeed the higher amounts of stimulating currents are presented above. These higher requirements could easily apply to the pre-pulse intensities as well. The results of the Subject Zero experiment, however, clearly demonstrate the effect reversal for the DPP in biphasic mode when stimulating the tibial nerve in the poplietal fossa. The same effect as in experiment A, i.e. a threshold elevation by conditioning the membrane via depolarizing RPPs, is present and persistent for an intensity of equal magnitude but opposite sign compared to experiment B.

Comparing the results of experiments A and B should therefore be allowed. Hence, I would argue that the effect of prepulses in monophasic settings always remains the same, independent of their amplitude. The data suggest that the described effect reversal of conditioning prepulses does not appear in monophasic configurations as a rule. With the behavior of the DPPs in biphasic mode exemplary explained by Armstrong's ion channel model, the lack of a similar effect for monophasic HPPs remains an unresolved issue. It is, however, beyond the scope of this thesis to provide a satisfactory explanation for the presented discrepancy. The results of my experiments do not allow any deductions concerning the underlying mechanisms.

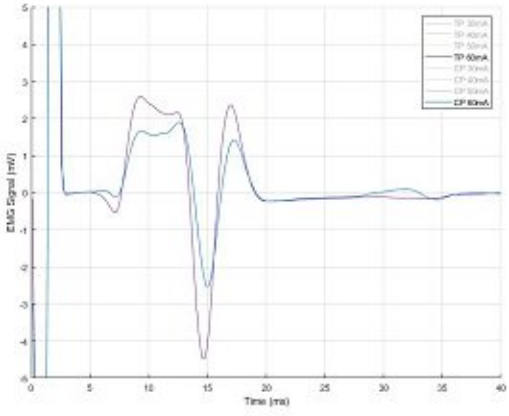

Figure 29 – Responses to stimulation with (blue) and without (purple) depolarizing RPP in biphasic setting. I = 60mA.

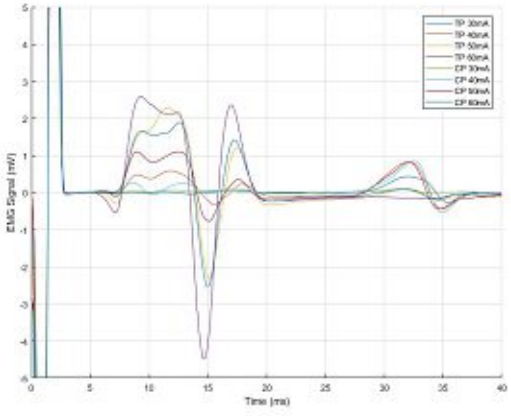

Figure 30 – Comparison of EMG responses to different stimulation intensities with and without RPP. I = { 30, 40, 50, 60} mA

The comparison of both experiments' results holds another noteworthy outcome. Not

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

only are the shifts in thresholds of opposite signs, but also the magnitude of the shifts are smaller in the second experiment. Interestingly, these deviations are not present for the soleus muscle, but only for the TA. Contrarily, the threshold shifts for the soleus muscle are comparable to those for the TA in experiment A, concerning their numerical amounts. Even though the excitation thresholds *per se* differ significantly in their amount for both experiments, the differences between stimulation with and without pre-pulse are of equal range and value. This implies that the 10 mA / 100 ms RPP is able to evoke the desired effect of threshold manipulation in an effective manner, even though the electrical field is substantially weakened by the cutaneous and subcutaneous tissue.

These results once more illustrate the importance of choosing the correct stimulation site. Whereas the fossa poplieta has the advantage of enabling the simultaneous stimulation of both muscles, the change in recruitment thresholds for the TA is much more prominent when the stimulation is applied on the lateral side of the leg as in experiment A (Fig. 10). Note, however, that the different configurations might also play a role in this respect. The Subject Zero results cannot fully resolve the problematic deviations, as they do not show such a clear differentiation between the two muscles. Generally, the $\triangle I_X$ are larger for the single case study.

The visual inspection of full stimulus-response curves motivates me to include another measure in my considerations. The conditioning RPPs have a certain influence on H-reflex behavior, more so than the described shift in thresholds. Since the applied HPPs lower the recruitment thresholds, it is reasonable to assume that the complete inverse U-shaped curve of the H-reflex recruitment will be shifted towards lower stimulation intensities, that is toward the left in an SRC graph. This effect can indeed be observed, for example in Fig. 20. In addition, H-reflex behavior seems to be influenced in terms of the intensity interval in which it can be evoked and recorded as well. This is reflected in a narrowing of the H-reflex curve corresponding to the CP stimulation.

To quantify the effect, I determine the full width at half maximum for each individual response curve. "Maximum" hereby refers to the maximum H-reflex response H_{max}. The results, as listed in Tables 3 and 5, support the impression given by visual examination. The FHWM changed in response to the application of the HPP by 2.7167±1.3345 mA and 2.9233±1.3417 mA in the soleus and TA muscle, respectively. H-reflexes for both muscles are affected by the pre-pulse in a similar magnitude. This fact seems to be in contradiction to the results from motor unit recruitment, for which the TA sensitivity to the RPP seems to be lower than that of soleus. Indeed, the H-reflex responses in general are similar in both muscles concerning their magnitude; especially the 90% differences differ only by an amount of approximately 0.8 mA (mean). This is especially interesting concerning selectivity in nerve fiber stimulation, since the H-reflex behavior reflects that of afferent Ia neurons.

The decrease of the H-reflex amplitude is caused by antidromic collision. Since the excitability of nerve fibers is raised by the HPP and lower stimulating currents suffice to evoke action potentials following their designated pathway, it stands to reason that the same is true for antidromic signal transmission. To be specific, the results of experiment B suggest that action potentials transmitted by efferent nerve fibers towards the upper motor neurons (i.e. towards the spinal cord) are also elicited at lower intensity levels when

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

a sub-threshold HPP is applied prior to the actual stimulation. The conditioning effect not only acts on othodromic, but also on antidromic signal transmission. This explains the narrowing of the H-reflex recruitment curve.

For the sake of completeness one has to consider the possible influence of DPPs, for example in biphasic stimulation mode. Since reverse effects for this kind of stimulation have been shown above, one would expect a similarly reverse effect, i.e. a sort of broadening of the H-reflex curve, with a configuration as in experiment A. Subject Zero data indeed show this broadening of the curve, with notably higher values than in experiment B. The results also differ for the two muscles by almost 1.6 mA. Keep in mind, though, that this data is only from one subject and solely serves the purpose of gaining a more complete understanding of the effects at play.

Finally, I would like to consider changes in the temporal progression and individual curve shape of motor response. M-waves and H-reflexes have very characteristic appearances in EMG recordings. When applying conditioning prepulses, their respective effects are bound to manifest in these individual recordings (see e.g., Figures 15, 19 or 27). Several conformational and temporal effects can be attributed to RPP application.

The detailed analysis of stimulus-response curves provides a good understanding of the overall effects of RPPs and makes it possible to quantify said effect. An additional investigation of individual response signals, however, allows more qualitative conclusions concerning the actual recruitment, which leads to the macroscopically demonstrated effects in the first place.

Figure 15 clearly reveals the distinguishing effect of prepulses. Larger fibers seen to be masked by the conditioning. While the most obvious change is the increase in M-wave amplitude, it can be observed that not all components of the response curve are simply amplified by a constant factor. Instead, different regions of the M-wave become more pronounced and experience slight shifts in their temporal position. This behavior can only be explained by assuming that some neuronal fibers are affected to a larger extent than others. Comparing individual curves with and without depolarizing RPPs can support this hypothesis, as e.g., figures 29 and 30. Herein the change in the waves becomes even clearer, as responses to several stimulation intensities (30mA, 40mA, 50mA and 60mA) are superimposed. Such deviations in the temporal course of EMG signals can be attributed to a selective effect of the depolarizing RPP. This conclusion becomes yet more convincing when the influence of the RPP on the H-reflex is taken into consideration as well.

I have demonstrated that motor units corresponding to M-wave response are influenced by RPPs more strongly than their H-reflex counterparts. Considering motor unit recruitments individually clearly supports this finding, as figure 15 shows a large variation in M-wave, but almost none in H-reflex behavior. While the measurements constituting these specific graphs refer to a comparison of similar response strengths, figure 19 exhibits equal reactions to RPPs. In this case, the stimulation intensity was the same for test- and compound pulse. The H-reflex appears more responsive than in the other case, but still notably less than the direct motor response. This observation remains the same, therefore it stands to reason that the conclusion about differentiation in activation also holds true.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

A definite conclusion about the mechanisms behind the more specific recruitment can only be drawn from changes in the characteristic time variables of motor response signals. First and foremost this refers to the latency period, followed by the temporal position of the signal peak. Visual examination of experiment B data suggests that the decrease of excitation thresholds corresponds to accelerated recruitment of motor units. These could otherwise only contribute to motor response at higher intensities. In fact, M-wave peaks occur earlier, while the latency period becomes insignificantly shorter, if it is affected at all. Higher resolution of the EMG readings may be necessary for better analysis, although significant deviations should be in the easily detectable millisecond-range. A change in the latency period is not to be expected for a HPP, though, since the fast responding neurons are also the first to be affected by the conditioning effect, i.e. the heightened excitability. Hyperpolarizing RPPs are no suitable tool to achieve physiologic motor unit recruitment. They can, however, be exploited to gain higher sensitivity of excitable tissue.

The data obtained from the single-case study paints a different picture, figuratively speaking. As the exemplary graphs in figure 16 demonstrate, the depolarizing RPP causes a differentiation in the fiber type recruitment. The peaks in the M-wave are more prominent and clearly distinguishable, more so than for the test pulse. This reflects the differentiated recruitment of neurons with slightly varying response times. It is safe to assume that the blocking effect of a DPP (within these specific experimental conditions) does not affect all motor units equally. Rather, I would argue that larger neurons and such closer to the stimulating electrode are influenced prior to others, explaining the results gained from this experiment. Hence, depolarizing RPPs promise to be an effective measure to not just manipulate excitation thresholds but also achieve a motor recruitment order that is even closer to the physiologic one.

Naturally, systemic errors play an important role in *in-vivo* experiments as those included in my thesis. Offset and offset-wandering in the recordings pose large problems in the process of data analysis. The measurements collected via EMG are in many respects too imprecise to be interpreted correctly and allow for sound deductions. Conditions for stimulation and electriophysiologic measurements are always subject to fluctuations. In addition, long ramp pulses affect the electrical conditions in and around the considered tissue, e.g. the skin-electrode-impedance. Since several interfering effects are taken into account, examination of my data still provides truly conclusive results.

Nevertheless, an important limitation of the presented study has not been discussed yet. I use prepulses with a duration of 100 ms. While the ramp shape of the prepulses in my thesis is a natural approach towards accommodation and enables higher intensities to be reached, the long phase duration poses a problem for the applicability. Since pauses between individual compound pulses also have to be considered, the prepulse length strongly limits the possible frequency of a pulse train. In addition, charge balance has to be considered for application of a stimulation protocol with prepulses, further limiting the frequency. Otherwise, only individual pulses with long pauses can be applied, as I do in my thesis. While I can show the efficacy of sub-threshold RPPs this way, the protocol and pulse duration have to be adapted in order to provide applicability in clinical practice.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

6 Conclusion

Achieving physiologic motor unit recruitment is one of the main goals of today's research in FES. The addition of conditioning sub-threshold prepulses to an electrical stimulation pulse has been investigated multiple times. My thesis strongly supports the measure of such compound pulses. To be exact, the results of my experiments support the application of slowly rising ramp prepulses in transcutaneous electrical neuromuscular stimulation to influence the recruitment behavior of motor neurons in a deliberate way.

The results of my thesis prove that neuron recruitment in electrical stimulation can be influenced by means of sub-threshold ramp prepulses. Different neurons do not only react differently to supra-threshold stimuli. They also accommodate more or less than others to a sub-threshold conditioning. Specific behavior can be attributed to the characteristics and conditions of motor units, including but not limited to their position with respect to the stimulating electrode, fiber diameter and type. These can be exploited in order to vary the recruitment behavior of a mixed nerve. More precisely, specific fiber types or groups can be recruited. This can come at the cost of unpleasant sensations, however. For long ramp prepulses, a needle-like feeling in the area of the stimulating electrode can be expected.

Sub-threshold ramp prepulses can be used to manipulate the excitation thresholds of axon membranes. For a biphasic stimulation protocol with a leading cathodic phase, depolarizing prepulses of 50ms and 100ms duration and a slope of 0.1 A/s reduce membrane excitability. Differences in latency periods and peak positions show that type I fibers accommodate more quickly to the conditioning effects. Inverse recruitment in electrical stimulation can therefore be avoided by administering a sub-threshold depolarizing RPP immediately prior to a biphasic stimulating pulse. These findings are in good agreement with the literature (Hennings et al., 2005; Vastani et al., 2013).

In a monophasic configuration setup, the application of a hyperpolarizing RPP of 100ms duration and 10mA amplitude causes an increase in membrane excitability. This confirms numerous findings from previous studies, most recently e.g. the work of Eickhoff and Jarvis, 2020. However, to the best of my knowledge monophasic stimuli with durations of 100ms have never been tested in TENS for their effect on membrane excitability. This is understandable, since stimulation of this sort comes with the risk of skin damage. Hence, appropriate inter-pulse pauses should be included in stimulation protocols to provide a phase of regeneration.

Both M-wave and H-reflex behavior are affected by application of RPPs. The influence on motor unit recruitment is notably stronger on all levels of stimulation tested. This statement holds true for both mono- and biphasic stimulation with hyper- and depolarizing RPPs, respectively.

I have presented evidence that by considerate selection of the stimulation parameters duration, intensity, and polarity for a ramp-shaped pre-pulse, excitation thresholds and recruitment order of motor neurons can be influenced in various ways. A next step in this line of research would be to include different slopes of ramp pulses into the experiments and considerations. The relation of RPP amplitudes with measurable effects and

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

excitation thresholds without conditioning would be important for the design of effective protocols with ramp prepulses. Continuing investigation of the use of conditioning ramp prepulses promises to enable direct influence on the recruitment order via electrical stimulation and enhanced efficacy. Mechanisms behind the influence on neuron recruitment, however, remain unclear to a large extent at the moment. The results of this thesis do not allow for conclusive explanation of the underlying physiologic principles. Hence, to conclude the series of open questions, I would like to quote the great physicist Richard Feynman:

If you thought that science was certain - well, that's an error on your part.

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

List of Figures

List of Tables

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

References

Accornero, N., Bini, G., Lenzi, G. L., & Manfredi, M. (1977). Selective activation of peripheral nerve fibre groups of different diameter by triangular shaped stimulus pulses. *The Journal of Physiology*, *273*(3), 539–560. https://doi.org/10.1113/jphysiol.1977.sp012109

Alberts, B. (2002). *Molecular biology of the cell*. Garland Science.

Armstrong, C. M. (2006). Na channel inactivation from open and closed states. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 17991–17996. https://doi.org/10.1073/pnas.0607603103

Baker, M., & Bostock, H. (1989). Depolarization changes the mechanism of accommodation in rat and human motor axons. *The Journal of Physiology*, *411*(1), 545–561. https://doi.org/10.1113/jphysiol.1989.sp017589

Balogun, J. A. (1991). Effects of ramp time on sensory, motor and tolerance thresholds during exogenous electrical stimulation. *The Journal of sports medicine and physical fitness*, *31*, 521–526.

Barbero, M., Merletti, R., & Rainoldi, A. (2012). *Atlas of muscle innervation zones*. Springer Milan. https://doi.org/10.1007/978-88-470-2463-2

Baxter, J. D., & Funder, J. W. (1979). Hormone receptors. *New England Journal of Medicine*, *301*(21), 1149–1161. https://doi.org/10.1056/nejm197911223012104

Buschbacher, R. M. (1999). NORMAL RANGE FOR h-REFLEX RECORDING FROM THE CALF MUSCLES1. *American Journal of Physical Medicine & Rehabilitation*, *78*(Supplement), S75–S79. https://doi.org/10.1097/00002060-199911001-00014

Crone, C., Johnsen, L. L., Hultborn, H., & Ørsnes, G. B. (1999). Amplitude of the maximum motor response (m max) in human muscles typically decreases during the course of an experiment. *Experimental Brain Research*, *124*(2), 265–270. https://doi.org/10.1007/s002210050621

Dean, J., & Collins, D. (2009). Nonlinear twitch torque summation by motor units activated at m-wave and h-reflex latencies. *Wiley InterScience*.

de Lera Ruiz, M., & Kraus, R. L. (2015). Voltage-gated sodium channels: Structure, function, pharmacology, and clinical indications [PMID: 25927480]. *Journal of Medicinal Chemistry*, *58*(18), 7093–7118. https://doi.org/10.1021/jm501981g

Deurloo, K. E. I., Holsheimer, 1., & Bergveld, P. (2001). The effect of subthreshold prepulses on the recruitment order in a nerve trunk analyzed in a simple and a realistic volume conductor model. *Biological Cybernetics*, *85*(4), 281–291. https://doi.org/10.1007/s004220100253

Eickhoff, S., & Jarvis, J. (2020). An investigation of neural stimulation efficiency with gaussian waveforms. *IEEE Transactions on Neural Systems and Rehabilitation Engineering, Vol 28*.

Eickhoff, S., & Jarvis, J. C. (2020). The effect of sub-threshold pre-pulses on neural activation depends on electrode configuration. *IEEE Transactions on Biomedical Engineering*, *67*(9), 2552–2559. https://doi.org/10.1109/tbme.2020.2964071

Fang, Z.-P., & Mortimer, J. (1991). A method to effect physiological recruitment order in electrically activated muscle. *IEEE Transactions on Biomedical Engineering*, *38*(2), 175–179. https://doi.org/10.1109/10.76384

Farina, D., Merletti, R., Nazzaro, M., & Caruso, I. (2001). Effect of joint angle on emg variables in leg and thigh muscles. *IEEE Engineering in Medicine and Biology Magazine*, *20*(6), 62–71. https://doi.org/10.1109/51.982277

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

Feiereisen, P., Duchateau, J., & Hainaut, K. (1997). Motor unit recruitment order during voluntary and electrically induced contractions in the tibialis anterior. *Experimental Brain Research*, *114*(1), 117–123. https://doi.org/10.1007/pl00005610

Frigon, A., Carroll, T. J., Jones, K. E., Zehr, E. P., & Collins, D. F. (2007). Ankle position and voluntary contraction alter maximal m waves in soleus and tibialis anterior. *Muscle nerve*, *35*, 756–766. https://doi.org/10.1002/mus.20747

Gesslbauer, B., Hruby, L., Roche, A., Farina, D., Blumer, R., & Aszmann, O. (2017). Axonal components of nerves innervating th human arm. *Ann Neurol 2017*.

Grill, W. M., & Mortimer, J. T. (1995). Stimulus waveforms for selective neural stimulation. *Engineering in Medicineand Biology (1995)*.

Grill, W. M., & Mortimer, J. T. (1997). Inversion of the current-distance relationship by transient depolarization. *IEEE transactions on bio-medical engineering*, *44*, 1–9. https://doi.org/10.1109/10.553708

Grill, W., & Mortimer, J. (n.d.). Selective activation of distant nerve fibers. *Proceedings of the 15th Annual International Conference of the IEEE Engineering in Medicine and Biology Societ*. https://doi.org/10.1109/iembs.1993.979119

Hennings, K. (2004). *Selective electrical stimulation of peripheral nerve fibers:accommodation based methods* (Doctoral dissertation). Laboratory for Experimental Pain Research, Center for Sensory-Motor Interaction (SMI), Aalborg University.

Hennings, K., Arendt-Nielsen, L., & Andersen, O. K. (2004). Orderly activation of human motor neurons using electrical ramp prepulses. *Clinical Neurophysiology 116 (2005)*.

Hennings, K., Arendt-Nielsen, L., & Andersen, O. K. (2005). Breakdown of accomodation in nerve: A possible role for persistent sodium current. *Theoretical Biology and Medical Modelling*.

Hille, B. (1984). Ionic channels of excitable membranes.

Hodgkin, A. L., & Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *The Journal of Physiology*, *117*(4), 500–544. https://doi.org/10.1113/jphysiol.1952.sp004764

Hoffmann, P. (1910). Arch. anat. physiol.

Hopkins, J. T., Ingersoll, C. D., Cordova, M. L., & Edwards, J. E. (2000). Intrasession and intersession reliability of the soleus h-reflex in supine and standing positions. *Electromyography and clinical neurophysiology*, *40*, 89–94.

Kaniusas, E. (2012). *Biomedical signals and sensors i: Linking physiological phenomena and biosignals*. Springer-Verlag Berlin Heidelberg.

Kern, H., Rossini, K., Carraro, U., Mayr, W., Vogelauer, M., Hoellwarth, U., & Hofer, C. (2004). Muscle biopsies show that FES of denervated muscles reverses human muscle degeneration from permanent spinal motoneuron lesion. *The Journal of Rehabilitation Research and Development*, *42*(3sup1), 43. https://doi.org/10.1682/jrrd.2004.05.0061

Kern, H., Salmons, S., Mayr, W., Rossini, K., & Carraro, U. (2004). Recovery of long-term denervated human muscles induced by electrical stimulation. *Muscle & Nerve*, *31*(1), 98–101. https://doi.org/10.1002/mus.20149

Kuffler, S. W., & Williams, E. M. V. (1953). Small-nerve junctional potentials. the distribution of small motor nerves to frog skeletal muscle, and the membrane characteristics of the fibres they innervate∗. *The Journal of Physiology*, *121*(2), 289–317. https://doi.org/10.1113/jphysiol.1953.sp004948

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

Kugelberg, E., & Skoglund, C. R. (1946). NATURAL AND ARTIFICIAL ACTIVATION OF MOTOR UNITS—a COMPARISON. *Journal of Neurophysiology*, *9*(5), 399–412. https://doi.org/10.1152/jn.1946.9.5.399

Latash, M. (1998). *Neurophysiological basis of human movement*. Human Kinetics, Champaign, IL.

Luna, J. L. V., Mayr, W., & Cortés-Ramirez, J.-A. (2018). Sub-threshold depolarizing pre-pulses can enhance the efficiency of biphasic stimuli in transcutaneous neuromuscular electrical stimulation. *Medical & Biological Engineering & Computing*, *56*(12), 2213–2219. https://doi.org/10.1007/s11517-018-1851-y

M, S. (1984). External control of the neuromuscular system. *IEEE Trans.Biomed.Eng.*

McNeal, D. R. (1976). Analysis of a model for excitation of myelinated nerve. *IEEE Trans.Biomed.Eng (1976).*

Merrill, D. R., Bikson, M., & Jefferys, J. G. (2004). Electrical stimulation of excitable tissue: Design ofefficacious and safe protocols. *Journal of Neuroscience Methods 141 (2005).*

Nilsson, I., & Berthold, C. H. (1988). Axon classes and internodal growth in the ventral spinal root l7 of adult and developing cats. *J.Anat. 1988;156:71-96.*

Ørtenblad, N., Nielsen, J., Boushel, R., Söderlund, K., Saltin, B., & Holmberg, H.-C. (2018). The muscle fiber profiles, mitochondrial content, and enzyme activities of the exceptionally well-trained arm and leg muscles of elite cross-country skiers. *Frontiers in Physiology*, *9*. https://doi.org/10.3389/fphys.2018.01031

Palay, S. L., Sotelo, C., Peters, A., & Orkand, P. M. (1968). THE AXON HILLOCK AND THE INITIAL SEGMENT. *The Journal of Cell Biology*, *38*(1), 193–201. https://doi.org/10.1083/jcb.38.1.193

Palmieri, R. M., Hoffman, M. A., & Ingersoll, C. D. (2002). INTERSESSION RELIABILITY FOR h-REFLEX MEASUREMENTS ARISING FROM THE SOLEUS, PERONEAL, AND TIBIALIS ANTERIOR MUSCULATURE. *International Journal of Neuroscience*, *112*(7), 841–850. https://doi.org/10.1080/00207450290025851

Palmieri, R. M., Ingersoll, C. D., & Hoffman, M. A. (2004). The hoffmann reflex: Methodologic considerations and applications for use in sports medicine and athletic training research. *Journal of athletic training*, *39*, 268–277.

Panizza, M., Nilsson, J., Roth, B. J., Grill, S. E., Demirci, M., & Hallett, M. (1998). Differences between the time constant of sensoryand motor peripheral nerve fibers: Further studies and considerations. *Muscle Nerve 1998.*

Pfurtschneller, J., Rupp, R., Müller, G., Fabsits, E., Korlsek, G., Gerner, H. J., & Pfurtschneller, G. (2005). Funktionelle elektrostimulation anstatt operation: Verbesserung der greiffunktion mittels fes bei einem patienten mit tetraplegie c5. *Unfallchirurg.*

Pierrot-Deseilligny, E., & Mazevet, D. (2000). The monosynaptic reflex: A tool to investigate motor control in humans. interest and limits. *Neurophysiologie Clinique/Clinical Neurophysiology*, *30*(2), 67–80. https://doi.org/10.1016/s0987-7053(00)00062-9

Postans, N. J., & Granat, M. H. (2007). Effect of functional electrical stimulation, applied during walking, on gait in spastic cerebral palsy. *Developmental Medicine & Child Neurology*, *47*(1), 46–52. https://doi.org/10.1111/j.1469-8749.2005.tb01039.x

Rattay, F. (1990). *Electrical nerve stimulation: Theory, experiments and applications.* Springer, Vienna.

Rijkhoff, N. J. M., Holsheimer, J., Debruyne, F. M. J., & Wijkstra, H. (1995). Modelling selective activation of small myelinated nerve fibres using a monopolar point

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN

electrode. *Medical & Biological Engineering & Computing*, *33*(6), 762–768. https://doi.org/10.1007/bf02523007

Rijkhoff, N., & Sinkjaer, T. (n.d.). Orderly recruitment of motoneurons in an acute rabbit model. *Proceedings of the 20th Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Vol.20 Biomedical Engineering Towards the Year 2000 and Beyond (Cat. No.98CH36286)*. https://doi.org/10.1109/iembs.1998.744976

Russell, N. J. (1980). Axonal conduction velocity changes following muscle tenotomy or deafferentation during development in the rat. *The Journal of Physiology*, *298*(1), 347–360. https://doi.org/10.1113/jphysiol.1980.sp013085

Schieppati, M. (1987). The hoffmann reflex: A means of assessing spinal reflex excitability and its descending control in man. *Progress in Neurobiology*, *28*(4), 345–376. https://doi.org/10.1016/0301-0082(87)90007-4

Vastani, N., Seifert, B., Spahn, D. R., & Maurer, K. (2013). Preconditioning depolarizing ramp currents enhance the effect of sodium channel blockers in primary sensory afferents. *Neuromodulation : journal of the International Neuromodulation Society*, *16*, 336–344. https://doi.org/10.1111/ner.12031

Wyart, C., Ybert, C., Bourdieu, L., Herr, C., Prinz, C., & Chatenay, D. (2002). Constrained synaptic connectivity in functional mammalian neuronal networks grown on patterned surfaces. *Journal of Neuroscience Methods*, *117*(2), 123–131. https://doi.org/10.1016/s0165-0270(02)00077-8

Die approbierte gedruckte Originalversion dieser Diplomarbeit ist an der TU Wien Bibliothek verfügbar
The approved original version of this thesis is available in print at TU Wien Bibliothek.
TU Bibliothek Your knowledge hub WIEN