Faculdade de Engenharia da Universidade do Porto

Tese de Programa Doutoral em Engenharia Biomédica

Carolina Júlia Félix Vila-Chã

Thesis submitted to the Faculty of Engineering in fulfillment of the requirements
for the degree of Doctor of Philosophy in Biomedical Engineering

Supervisor: Prof. Dr. Miguel Velhote Correia

Co-supervisor: Prof. Dr. Dario Farina

Porto, November 2011

Grant

The Fundação para a Ciência e Tecnologia (FCT) of Portugal supported the candidate with a Ph.D. Grant (Grant ID - SFRH / BD / 31796 / 2006).

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA

Preface

This thesis is based on the experimental work carried out during the period September 2007 - December 2010 in the laboratory of Neural Engineering and Neurophysiology of Movement of the Center for Sensory-Motor Interaction - Aalborg University – Denmark.

From this work resulted three original manuscripts which have been published (or accepted for) in international peer-reviewed journals. The papers upon which this thesis is based are as follows:

- **Study I:** Vila-Chã C, Falla D, Farina D. Motor unit behavior during submaximal contractions following six weeks of either endurance or strength training program. J Appl Physiol. 2010 Nov;109 (5):1455-66.
- **Study II:** Vila-Chã C, Falla D, Correia M, Farina D. Adjustments in Motor Unit Properties during Fatiguing Contractions Following Training. Med Sci Sports Exerc. Sep 7, 2011. (Epub ahead of print)
- **Study III:** Vila-Chã C, Falla D, Correia M, Farina D. Changes in H-reflex and V-wave following short-term endurance and strength training. J Appl Physiol. Oct 13, 2011 (Epub ahead of print)

Some of the findings reported in this thesis have been presented in abstract form at the following international conferences:

- Vila-Chã C, Falla D, Correia M, Farina D. (2010). Effects of short-term endurance and strength training on motor unit conduction velocity (oral presentation). XVIII Congress of the International Society of Electrophysiology and Kinesiology, Aalborg, Denmark, June- 16-19
- Vila-Chã C, Falla D, Correia M, Farina D. (2009). Differences in motor unit behavior following endurance and strength training. (poster presentation). Neuroscience. Chicago, USA, October 17-21

Acknowledgements

This dissertation would not have been possible without the scientific contribution and emotional support of several people whom I would like to express my sincere gratitude, in particular to:

- My supervisor, Professor Miguel Velhote Correia for encouraging me in this doctorate project and for helping me to reach the necessary conditions for a long training period in the Sensory-Motor Interaction (SMI) at Aalborg University - Denmark. I am also very thankful for his meaningful scientific contributions for this thesis as well as for his support and encouragement during the moments of doubts.
- My co-supervisor, Professor Dario Farina and Professor Deborah Falla, for promptly receiving me in their research group and for offer all the laboratory conditions to develop the Ph.D project. The accomplishment of this work would have been barely possible without their innumerous, valuable and inspiring discussions throughout these years. Their contributions in experimental design and implementation as well as for their constructive support in the elaboration of the manuscripts, greatly improve the quality of this work. I am also very grateful for giving me the opportunity to participate in other research projects contributing to my scientific development.
- My dear colleagues at SMI that have made just unforgettable the 3 years that I lived in Denmark. I would like to thank them for the cultural exchange, for the "*cappuccino* morning" on Saturdays, for the cultural dinners on the weekends and for having made the distance less hard of supporting. Special thanks to a singular person that picked me up from the train station when I arrived to Aalborg for the first time; thank you very much Silvia Muceli, for your friendship, for your technical support, for being always interested in helping me (even when you were awfully busy). I also thank Francesco Negro, not only for his friendship but also for making so easy and enthusiastic the technical problems that, at first glance, seemed so complex to me.

- My family, without their support I would never dare to delve into such scientific experience. I am incredibly grateful for their wonderful support and constant encouragement. Above all, I thank to my husband Nuno for his love and unconditional support throughout the period that we were 3000km apart from each other, delaying our life plans.
- The FCT, for awarding the grant that enabled my research (SFRH / BD / 31796 / 2006) and allowed the international cooperation.
- The Polytechnic Institute of Bragança for endowing me an off-duty time so that I could fully benefit from the grant awarded by FCT.

Abstract

Over the last decades, it has been shown that the human neuromuscular system is highly adaptive and can be modified in response to different motor training programs. Depending on the demands of the motor training, the adaptations seem to involve distinct structural and functional changes across the motor cortex, spinal cord and skeletal muscle. The technological development observed in the last years, increased the use of electrophysiological techniques to assess the neuromuscular adaptations to motor training. Nonetheless, the current evidences on the neuromuscular adaptations to different motor training are inconsistent and incomplete, in particular regarding endurance and strength training. This is mainly due to lack of studies based on a rigorous consideration of the limitations of the available techniques. Therefore, the main goal of this dissertation is to give new insights on the adaptations of the neuromuscular system by systematically investigating the changes in its central and peripheral properties in response to endurance and strength training. For this purpose, recent developed techniques for recording and processing electromiographycal (EMG) signals were applied. The first study (STUDY I) investigated if 6 weeks of either endurance or strength training alters the motor unit behavior and if such changes were accompanied by alterations in muscle fiber properties. Intramuscular and multichannel surface EMG recordings were used to investigate the motor unit discharge rates and motor unit conduction velocity (MUCV) of the vastus medialis obliquus and vastus lateralis during submaximal isometric contractions. The results demonstrated that endurance training increased endurance capacity and was accompanied by a decrease of the motor unit discharge rates. In contrast, strength training enhanced maximum force output and was accompanied by an increase of the motor unit discharge rates. By the end of 6 weeks of training, both training programs elicited increases in the motor unit conduction velocity, revealing electrophysiological adaptations of the muscle fiber membrane properties in similar directions. However, in the first 3 weeks of training, when changes in motor unit discharge rates were most marked, changes in MUCV were not observed. These findings reveal different time courses of some of the neural and peripheral adaptations in response to different motor training programs. The observed changes may contribute for distinct neuromuscular fatigue profiles among endurance and strength-trained athletes. Therefore, the aim of the second study (STUDY II) was to investigate the effects of 6 weeks of endurance and a strength training program on acute responses of the muscle fiber membrane properties and discharges rates of low threshold motor units of the vastus medialis obliquus and vastus lateralis muscles during prolonged submaximal isometric contractions. The conduction velocity of the individual motor units was estimated from the averaged multichannel EMG surface potentials by a spike triggered average technique. It was shown that motor unit discharge rate declines over the duration of the sustained contraction and their trend was not significantly affected by training. Conversely, the rate of decline of motor unit conduction velocity during sustained contractions was reduced following six weeks of both endurance and strength training, however a greater reduction is observed following endurance training. These alterations likely contribute to longer times to task failure following endurance training. The third study (STUDY III) intended to clarify the mechanisms involved in the opposite adjustments of the motor unit discharge rate observed in the study I. The results revealed that following 3 weeks of endurance training the excitability in the H-reflex pathway increased but the V-wave amplitude remained unchanged. In contrast, following strength training the V-wave amplitude increased whereas subtle changes were observed in the H-reflex pathway. These results suggest that the elements of the H-reflex pathway are strongly involved in chronic adjustments in response to endurance training, contributing to enhance resistance to fatigue. Conversely, following strength training, it is more likely that increased descending neural drive during MVC and/or modulation in afferents other than Ia afferents contributed to increased motoneuron excitability and maximal voluntary contraction.

The present work revealed for the first time that endurance and strength training induces opposite adjustments in the motor unit behavior. Moreover, the distinct adjustments in the spinal cord output, seems to result from changes in different neural mechanisms located at supraspinal and/or spinal level. The neural adjustments following endurance training seems to result from changes at spinal level whereas the adjustments following strength training are likely due to changes at supraspinal level. These adaptations occurred following a short period of training, while no changes in the contractile and electrophysiological properties of the muscle fibers were detectable. Changes at peripheral level occurred only following a longer period of training.

Resumo

Ao longo das últimas décadas, tem sido demonstrado que o sistema neuromuscular do ser humano é extramente adaptativo, podendo as suas propriedades serem alteradas em resposta a diferentes programas de treino motor. Dependendo das exigências do treino motor, as adaptações parecem implicar diferentes estruturas e funções a nível do córtex cerebral, medula espinal e sistema músculo-esquelético. O desenvolvimento tecnológico, observado nos últimos anos, favoreceu a aplicação de diversas técnicas electrofisiológicas para aceder às propriedades centrais e periféricas do sistema neuromuscular. Contudo, as actuais evidências sobre as adaptações neuromusculares impostas por diferentes tipos de treino são inconsistentes e incompletas, particularmente no âmbito do treino de resistência aeróbia e força muscular. Este facto prende-se essencialmente com a falta de estudos baseados numa rigorosa ponderação das limitações associadas às técnicas electrofisiológicas actualmente existentes. Tendo por base estes pressupostos, o principal objectivo desta tese centrou-se em providenciar novos conhecimentos sobre adaptações do sistema neuromuscular ao treino de resistência aeróbia e força muscular através de aplicação de técnicas electrofisiológicas avançadas, recentemente desenvolvidas. Para este efeito foram desenvolvidos três estudos principais. O primeiro estudo (STUDY I) teve como objectivo compreender se o treino de resistência aeróbia ou de treino de força alteram o comportamento das unidades motoras (propriedades centrais) e se tais alterações são também acompanhadas por modificações das propriedades electrofisiológicas das fibras musculares (propriedades periféricas). Para este efeito foram utilizados técnicas de electromiografia multicanal de profundidade e de superfície para investigar a frequência de disparo e a velocidade de propagação dos potenciais de acção das unidades motoras dos músculos vasto medial e vasto lateral, durante contracções isométricas submáximas. Os resultados demonstraram que o treino de resistência aeróbia aumentou a capacidade de resistência à fadiga, que foi acompanhado por uma diminuição da frequência de disparo das unidades motoras. Contrariamente, o treino de força aumentou a capacidade de força e aumento a frequência de disparo das unidades motoras. No final de seis semanas de ambos os tipos de treino foi possível observar um incremento da velocidade de

condução das unidades motoras, revelando adaptações electrofisiológicas das fibras musculares. Contudo, nas três primeiras semanas, período em que as modificações do comportamento das unidades motoras foram mais evidentes, não se observaram alterações na velocidade de propagação dos potenciais de acção. As alterações observadas podem contribuir para as diferenças nos perfis de fadiga neuromuscular existente entre os atletas de provas de resistência aeróbia e os atletas de provas de força muscular. Assim, o objectivo do segundo estudo (STUDY II) foi investigar os efeitos de seis semanas de treino (resistência aeróbia ou força muscular) na resposta aguda: (i) das propriedades electrofisiológicas das membranas das fibras musculares e (ii) da frequência de disparo das unidades motoras de baixo limiar de recrutamento dos músculos vasto medial e vasto lateral, durante contracções isométricas submáximas prolongadas. A velocidade de condução das unidades motoras foi estimada a partir dos valores médios dos potenciais de acção registados com sistemas de electromiografia multicanal utilizando a técnica de média desencadeada por impulsos ("spike triggered average") com base no sinal intramuscular. Foi demonstrado que durante contracções isométricas prolongadas, a frequência de disparo das unidades motoras declina ao longo do tempo e esta tendência não é alterada por nenhum dos tipos de treino utilizado no estudo. Também se verificou, que a velocidade de condução das unidades motoras declina durante contracções isométricas prolongadas. No entanto o treino alterou a taxa de declínio. Após seis semanas, ambos os tipos de treino, reduziram a taxa de declínio da velocidade de condução das unidades motoras, sendo essa redução significativamente maior para o grupo do treino de resistência aeróbia. Estas alterações parecem contribuir para retardar o aparecimento do momento de exaustão. O objectivo do terceiro estudo (STUDY III) foi clarificar os mecanismos neurais potencialmente envolvidos nas adaptações opostas do comportamento das unidades motoras identificadas no estudo I. Os resultados revelaram que, após três semanas de treino de resistência aeróbia a excitabilidade do circuito do reflexo de Hoffman (reflexo H) aumentou significativamente mas a amplitude da resposta volitiva ("*V-wave*") permaneceu inalterada. Em contraste, o treino de força muscular aumentou a resposta volitiva e apenas alterações subtis do reflexo H foram observadas. Estes resultados sugerem que elementos do circuito do reflexo H estão fortemente envolvidos nas adaptações crónicas ao treino de resistência aeróbia contribuindo para um aumento da resistência à fadiga. Contrariamente, o treino de força parece aumentar o *input* neural descendente e/ou a modelação da informação nervosa aferente, excluindo a Ia aferente. Estas alterações parecem contribuir para um aumento da excitabilidade do motoneurónio e, consequentemente, da força máxima voluntária.

A presente tese demonstrou, pela primeira vez que, o treino de resistência aeróbia e de força muscular induzem alterações opostas do comportamento das unidades motoras. Estas alterações resultam de ajustamentos em diferentes mecanismos neurais situados ao nível da medula espinal e do cérebro. As adaptações neurais observadas após treino de resistência

aeróbia parecem resultar de ajustamentos neurais essencialmente ao nível da medula espinal enquanto após treino de força muscular devem-se fundamentalmente a alterações a nível supra-espinal. Estas adaptações ocorreram após um pequeno período de treino, na ausência de alterações das propriedades contracteis e electrofisiológicas das fibras musculares. Alterações periféricas do sistema neuromuscular só foram observadas após seis semanas de treino. Isto revela que as propriedades centrais e periféricas do sistema neuromuscular do ser humano apresentam diferentes períodos temporais de adaptação ao treino motor.

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Abbreviations

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AHP - After hyperpolarization
5HT - Serotonin
ARV - Averaged rectified value
ATP - Adenosine triphosphate
ATPase - Adenosine triphosphate enzyme
CNS - Central nervous system
CSA - Cross sectional area
EMG - Electromyography
fMRI - Functional magnetic resonance imaging
H-reflex - Hoffman reflex
MEP - Motor evoked potential
MHC - Myosin heavy chain
MUAP - Motor unit action potential
MUCV - Motor unit conduction velocity
MVC - Maximal voluntary contraction
M-wave - Motor wave
P_0 - Peak force
pps - Pulses per second
RFD - Rate of force development
TMS - Transcranial magnetic stimulation
V_0 - Shortening velocity
V-wave - Volitional wave

Introduction

The complexity of motor actions that humans can achieve reflects the complexity of their motor system (Enoka 2008; Latash 1998). This characteristic results from a sophisticated interaction between biological structures that integrates the motor system of the animals and in particular of mammalians. Although the diversity of movement depends on both skeletal muscle and nervous system properties, it is the central nervous system (CNS) that plans and coordinates the purposeful motor action (Ghez and Krakauer 2000). The muscles, independently of their architecture and composition, act in response to the neural commands to produce the required movement (Bawa 2002; Kernell 2006).

Optimization of a purposeful motor output can be achieved with practice, reflecting the extraordinary ability of the motor system to adapt and refine the motor output towards high efficiency. Work over recent decades has shown that not only muscle tissue, but also the nervous system changes in response to a variety of different motor experiences, including strength and endurance training (Adkins et al. 2006; Duchateau et al. 2006). Moreover, such adaptations persist in the absence of continued use or training, indicating that the motor system is able to "memorize" the adaptations (Bawa 2002; Enoka 2008; Nielsen 2004).

Many different mechanisms and structures from the cortex, spinal cord and muscle are involved in this process and, moreover, they seem to be distinct depending on the training type (Adkins et al. 2006; Wolpaw 2010). For specific types of training, such as skill training, it is known that there is functional and structural plasticity of the central nervous system (Perez et al. 2005; Wolpaw 2007).However, for other types of motor training, such as endurance and strength training, much more attention was given to muscle adaptation and cardio-respiratory responses. Nonetheless it is increasingly recognized that many of the adaptations induced by both training types that result in increased motor performance, may also reside at the nervous system (Carroll et al. 2011; Gardiner 2011). The evidences suggest that the effects of strength and endurance training on motor performance also reflect supraspinal and spinal adjustments, which ultimately influence the neural drive to the muscles, i.e. the behavior of motor units

(Adkins et al. 2006; Gardiner 2001; Hakkinen et al. 2003). Nonetheless, as we move from the muscle to the central nervous system, information becomes less abundant and controversial, in particular regarding endurance training (Gardiner 2011).

Endurance and strength training, in addition to being commonly used in the rehabilitation and exercise training fields, also represent a good model to investigate the response of the neuromuscular system to physical activity. Because the physiological stimuli imposed by endurance and strength training are divergent in nature (Hakkinen et al. 2003; Sale et al. 1990), it may be expected that they induce divergent changes in the neuromuscular system, which may contribute to our understanding of the spectrum of neuromuscular adaptations to training. Accessing such information is of great interest for applied fields such as rehabilitation, exercise training and applied physiology. In both rehabilitation and exercise training the objective is often to induce long–lasting changes in motor behavior, either to restore correct motor patterns or to increase motor performance (Falla et al. 2007; Nielsen and Cohen 2008; Zehr 2006). Thus, an adequate exercise intervention requires an understanding of how and to which extent the neuromuscular system can be changed in response to repeated exposure to motor training (Farina et al. 2004c; Zehr 2006).

The changes in the neuromuscular system can be investigated by applying a variety of techniques that ranges from imaging to electrophysiology. The advantage of the electrophysiological techniques, such as electromyography (EMG), is that they offer the possibility of investigating simultaneously the motor output from the spinal cord as well the electrophysiological properties of the muscle fibers (Basmajian and De Luca 1985; Merletti et al. 2001a). Moreover, when combined with electrical stimulation of peripheral nerves changes in spinal circuitry can be estimated (Pierrot-Deseilligny and Burke 2005).

In recent years, the use of electrophysiological techniques to investigate the potential neuromuscular adaptations to motor training has increased. Nonetheless, the current evidence on neuromuscular adaptations to different motor training is incomplete and inconsistent (Carroll et al. 2011; Kernell 2006), in particular regarding endurance and strength training. This is mainly due to lack of studies based on a rigorous consideration of the limitations of the available techniques (Carroll et al. 2011). In addition, the available studies have employed subjects with different physical fitness levels and applied different experimental methodologies, which also contribute to the observed discrepancies between studies. A full understanding of the neuromuscular adaptations to training and their time-course would not only contribute to explaining increased motor performance but, more importantly, would help design motor training programs and technologies to apply in the treatment of specific pathologies of the neuromuscular system.

Objectives of the Ph.D. project

The main objective of this dissertation was to give new insights on the adaptations of the neuromuscular system by systematically investigating the changes in its central and peripheral properties in response to distinct motor training types (i.e. endurance and strength training). For this purpose, recent developed techniques for recording and processing EMG signals were used and particular caution was taken in the experimental design in order to allow a direct comparison between the neural changes elicited by both training programs.

The specific objectives of this Ph.D. project were:

I. To determine how and to which extent endurance and strength training induces changes in the motor unit behavior (central changes) and in the electrophysiological properties of the muscle fibers (peripheral changes).
II. To investigate the time course of changes in both central and peripheral properties in response to endurance and strength training, over the training period.
III. To determine the influence of both training types on the acute adjustments of muscle fiber membrane properties and discharge rates of low threshold motor units during fatiguing isometric contractions.
IV. To investigate the effects of endurance and strength training on the input to the spinal cord by examining the Hoffman (H-) reflex and Volitional (V-) wave responses during voluntary contractions.

These objectives were investigated in three research studies:

Study I: "Motor unit behavior during *submaximal contractions following six weeks of either endurance or strength training program*". J Appl Physiol. 2010 Nov;109 (5):1455-66.

The research was structured to firstly understand if divergent motor training programs (such as endurance and strength training), alters the neural command strategies to the lower limb muscles and if such changes were accompanied by alterations in the muscle fiber properties. We also intended to investigate the time course of both central and peripheral adaptations to training and their possible interactions. Thus, the purpose of study I was to investigate and compare changes in motor unit behavior of synergistic knee extensor muscles following 6 weeks of either strength or endurance training programs commonly used in conditioning and rehabilitation. It was hypothesized that these two training programs would induce different adjustments in motor unit discharge rates. In addition, muscle fiber conduction velocity was measured for individual motor units as an indicator of changes in muscle properties following the training programs. The motor unit discharge rates and motor unit conduction velocity were estimated from both vastus medialis obliquus and vastus lateralis muscles during isometric knee extension at 10% and 30% of the maximum voluntary contraction (MVC). To assess such information intramuscular and surface multichannel EMG techniques were used. All variables were measured in the control, strength and endurance groups, before (week 0), during (week 3), and after 6 weeks.

Study II: *"Adjustments in Motor Unit Properties during Fatiguing Contractions Following Trainin*g". Med Sci Sports Exerc. Sep 7, 2011. (Epub ahead of print)

This study was designed to investigate if the neuromuscular adaptations observed in the previous study affect the acute peripheral and/or central mechanisms responses during fatiguing contractions, which may contribute for distinct neuromuscular fatigue profiles among endurance and strength-trained athletes. Therefore, the aim of this study was to investigate the effects of 6 weeks of endurance and a strength training program on acute responses of the muscle fiber membrane properties and discharges rates of low threshold motor units of the vastus medialis obliquus and lateralis muscles during prolonged isometric contractions. Motor unit discharge rates and EMG amplitude of the vastus medialis obliquus and lateralis muscles and biceps femoris were measured during sustained isometric knee extensions at 10% and 30% MVC before and immediately following 6 weeks of training. The conduction velocity of the individual motor units was estimated from the averaged multichannel EMG surface potentials by a spike triggered average technique.

Study III: *"Changes in H-reflex and V-wave following short-term endurance and strength training". J Appl Physiol*. 2011 Oct 13. (Epub ahead of print)

Following up the evidence encountered in the preceding research, this study intended to explore the adjustments in the spinal cord inputs that may have contributed to changes in the motor unit behavior observed following endurance and strength training. For this purpose we applied protocols of electrical stimulation to analyze the evoked Volitional (V-) wave, Hoffman (H-) reflex and Motor (M-) wave recruitment curves, before and after 3 weeks of training. The MVC and time-to-task-failure (isometric contraction at 40% MVC) of the plantar flexors were also measured in all experimental sessions.

Thesis outline

The present thesis is divided in two parts:

- **Part I** – Encompasses three chapters:

  Chapter 1 – provides a background on the basic properties and organization of the neuromuscular system, including basic aspects of the motor unit physiology, muscle control and synaptic input.

  Chapter 2 – reviews the literature on the neuromuscular adaptations to different motor training, in particular to endurance and strength training. Current evidence on the changes in muscle fiber properties, spinal motor output and neural mechanisms involved in such alterations are discussed.

  Chapter 3 – describes in detail the electrophysiological techniques that have been used to extract information on both central and peripheral properties of the neuromuscular system, in particular those applied in the Ph.D. project. The advantages and limitations of these methods are also discussed.

- **Part II -** Encompasses four chapters:

  Chapter 4, 5 and 6 – presents each the original studies developed within the PhD project that have been published or accepted for publication.

  Chapter 7 – provide the general discussion of the main outcomes from the reported studies.

Main contributions

The scientific literature on neuromuscular adaptations to motor training, have produced inconsistent and incomplete results, largely due to methodological issues. This Ph. D. project was designed to systematically investigate the neuromuscular adaptations elicited by divergent motor training programs (i.e. endurance *vs.* strength training), which are frequently used in sports and rehabilitation fields. For this purpose different electrophysiological techniques were combined and applied to assess both central (neural) and peripheral (muscular) adaptations to motor training.

The studies resulting from this Ph.D. project were the first to concurrently investigate the neuromuscular adaptations evoked by two distinct types of motor training by applying the same experimental procedures, including the same advanced EMG techniques and training groups with similar anthropometrical fitness level characteristics. This allowed a direct comparison of results, contributing for a better understanding of the neuromuscular adaptations to divergent motor training programs. The novel finding of the present work was that, at submaximal force levels, these two distinct training interventions induced divergent adaptations in the motor unit discharge rates, but similar changes in the electrophysiological properties of the muscle fibers. The results revealed that endurance training increases resistance to fatigue and is accompanied by decreased motor unit discharge rates. In contrast, strength training improves maximum force output and is accompanied by increased motor unit discharge rates. Such adjustments in the spinal cord output, seems to result from changes in different neural mechanisms located at supraspinal or spinal level. Based on the V-wave and H-reflex measures, the neural adjustments following endurance training seems to result from changes at spinal level whereas the adjustments following strength training are likely due to changes at supraspinal level.

PART I

Literature Review

CHAPTER

1

Basics of movement control and muscle contraction

The complexity of motor actions that humans can achieve reflects the complexity of their motor system (Enoka 2008; Latash 1998). From intention to execution of a motor action, several structures of the central nervous system (CNS) are involved, following a hierarchal functional organization (Ghez and Krakauer 2000). The lowest level of this hierarchal organization is the spinal cord, where all motor commands converge on motoneurons (Loeb and Ghez 2000) (Fig. 1-1). They transform the internal actions of the brain into motor behavior, translating patterns of neuronal activity into commands for skeletal muscle contraction that may vary considerable with respect to the magnitude of force and speed production.

Together, the motoneurons and their muscles fibers forms the neuromuscular system which can be considered the interface between CNS and muscles (Kernell 2006). The diversity in properties of this system (both motoneurons and muscle fibers properties) enables the same muscle to be used across a variety of motor tasks (e.g. posture maintenance, high-precision movements, rhythmic submaximal contractions, or powerful maximal contractions), contributing to the high repertoire of motor behaviors that mammalians can produce (Gardiner 2011).

The purpose of this chapter is to describe the basic properties of the neuromuscular system involved in voluntary movement production and its variability. Some of the items discussed are needed as background knowledge for later chapters. This includes basic aspects of motor unit physiology, muscle control and central nervous system organization of motor function.

1.1 Motor unit

The term *motor unit* was first introduced by Liddell and Sherrington in 1925 to designate the basic unit of motor function (Ghez and Krakauer 2000). It is through this basic functional unit the central nervous system controls muscle force and movement by varying its activity (Enoka 2008; Ghez and Krakauer 2000). Motor unit refers to a single motoneuron and an ensemble of muscle fibers innervated by its axonal branches (Fig. 1-1). Each motoneuron innervates on average 300 muscle fibers, but the range extents from tens to thousands (Enoka and Fuglevand 2001). The ensemble of muscle fibers belonging to the same motor unit is called muscle unit.

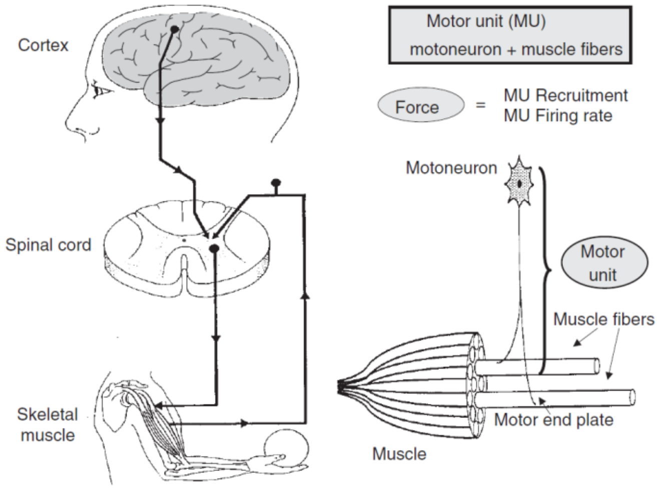

Figure 1-1 – Schematic representation of basic movement control mechanisms, motor unit and its components [from Moritani et al. (2004)].

When a motoneuron is activated it causes all its muscles fibers to contract. Therefore, the muscle units are the smallest fraction of muscle that the central nervous system can activate (Kernell 2003). Within a muscle, the fibers belonging to a given muscle unit are distributed more or less uniformly through an extensive "territory" that is also shared with fibers from many other units (Burke and Tsairis 1973). This territory is restricted to a portion of the muscle that may, as much, represent 1/5 of the total volume of the muscle (Burke 1978; Burke et al. 1974).

A voluntary movement may involve few or even all muscle units of one or several muscles. Indeed, for almost all kinds of motor behavior, several muscles are used together, in particular when strong contractions are produced (Kernell 2006). Nonetheless, each muscle is typically controlled by different groups of motor neurons referred as a motor unit pool (Burke et al. 1977). The motor unit population that forms the motor pool is heterogeneous with respect to the properties of both motoneurons and the muscles fibers that they innervate (Burke 1975; Kernell 2003). Different types of motor tasks require different mechanical, metabolic and circulatory features (Kernell 2003). Thus within the same muscle, there are muscles fibers with different physiological and histochemical properties (Kernell 2003) and typically, all muscle fibers belonging to the same muscle unit have similar properties (Burke et al. 1971; Henneman and Olson 1965; Wuerker et al. 1965). According to the functional demands, the nervous system activates the appropriate combination of muscle units in order to evoke the correct force and contraction speed in all the muscles concerned (Kernell et al. 1999). This is possible due to particular properties of the motoneurons that are closely linked to those of their target muscle units (Gardiner 2011; Wuerker et al. 1965; Zajac and Faden 1985).

1.1.1 Motoneuron properties

The motoneurons obey commands from the CNS and assist in deciding which motor units will be recruited and, to what extent they will be recruited (Gardiner 2011). These nerve cells possess different properties regarding to their morphology, excitability and distribution of input. Several features of the neuronal function depend on motoneurons morphology (Kernell 2006). Among these features, the motoneuron size has received the greatest attention (Enoka 2008). Henneman and colleagues were the first to demonstrate consistently that the excitability of the motoneurons varied inversely with their size (Henneman 1957; Henneman et al. 1965a; b). These observations led to the formulation of the "size principle", which states that motoneurons are recruited by order of their size (Henneman et al. 1965b). They speculate on the mechanisms underlining this principle, proposing that motoneuron excitability was related to size-associated differences in neuronal input resistance (Henneman et al. 1965b).

The subsequent studies on electrical properties of the motoneurons demonstrated that although the amount of current needed for eliciting a single action potential (rheobase current) depends on threshold depolarization (depolarization amplitude required to reach the threshold for spike generation) and non-linear properties of the membrane, the input resistance plays the major role (Gardiner 2011; Gustafsson and Pinter 1984; Kernell et al. 1999). The higher the resistance, the less current is needed for depolarizing the cell to the threshold for action potential generation (Kernell 2006). Thus, small motoneurons with less surface area have higher input resistance and produce larger voltage drop for a given synaptic input than large ones, allowing them to reach threshold at a lower level of synaptic input (Gustafsson and Pinter 1984; Kernell 1966). This translates into lower rheobase current, indicating, therefore, that small motoneurons are more susceptible to recruitment than large motoneurons cells (Gustafsson and Pinter 1984; Kernell 1966; Kernell and Zwaagstra 1981). Another relevant characteristic of the small motoneurons is their long afterhyperpolarization duration (prolonged hyperpolarization that follows the action potential, AHP) compared to larger cells (Gardiner and Kernell 1990; Gustafsson and Pinter 1984). This electrophysiological property plays an important role in the rhythmic discharge patterns of the motoneurons in response to sustained excitation (Kernell 2006). Moreover, the variation in the AHP duration is highly correlated with variation in contractile properties across motor units (Eccles et al. 1958; Gardiner and Kernell 1990; Gossen et al. 2003).

1.1.2 Muscle fibers properties

The muscle fibers within a same muscle may differ considerably with respect to their mechanical and biochemical properties, including power output, velocity of shortening, adenosine triphosphate (ATP) consumption and resistance to fatigue (Bottinelli and Reggiani 2000; Burke et al. 1973). Different physiological (contractile properties), histochemical [e.g. myofibrillar ATP enzymes (ATPase) activity] and molecular [e.g. myosin heavy chain isoforms (MHC)] criteria have been applied to define groups of muscle fibers (Bottinelli et al. 1994; Brooke and Kaiser 1974; Burke et al. 1971). Despite a continuous variation of the muscle fibers properties across a wide range, the human muscle fibers have been classified into three major types, independently of the classification scheme. The terms to define the muscle fiber types may vary between classification systems. Nonetheless the dominant terminology has been type I or slow-twitch and type II or fast-twitch that is then subdivided into IIa and IIx (Kernell 2006; 2003; Wilmore et al. 2008).

The results of combined physiological and histochemical studies shows that type I fibers tend to be particularly suited to produce prolonged low-intensity activities, since that consumes much less ATP and are highly resistant to fatigue due to their mostly oxidative metabolism (Bottinelli et al. 1994; Brooke and Kaiser 1974; Westerblad et al. 2010). Nonetheless, they are unable to produce very powerful movements, because of their low capacity of developing power (Bottinelli and Reggiani 2000; Kernell 2006; Widrick et al. 1996). On the other extreme, IIx fibers are able to develop high power at high velocity, which makes them suitable for fast and powerful movements (Bottinelli et al. 1996; Widrick et al. 1996). However, their low aerobic metabolism and high anaerobic power indicates that fast fibers are only able to support short bursts of activity (Bottinelli and Reggiani 2000; Kernell 2006). The fiber type IIa have an intermediate optimal velocity and power which are more suitable for a wide range of movements at intermediate speeds (Bottinelli and Reggiani 2000).

Apart from the histochemical and mechanical properties, the muscle fiber types also present differences in their electrophysiological properties. When a motoneuron is activated it generates an end-plate potential in the muscle fibers innervated by its axonal branches, resulting in an action potential (Loeb and Ghez 2000). Each action potential compromises sodium (Na^+) influx during the depolarization phase and potassium (K^+) efflux for the repolarization phase, leading to transient changes in the membrane potential. Once triggered at neuromuscular junction, the potential produces local currents of its own that depolarizes the region adjacent to it causing the propagation of the action potential along the length of the muscle fiber (Fig. 1-2).

The velocity with which an action potential propagates along the muscle fiber membrane depends on the fiber diameter and might range between 2 – 6m/s (Blijham et al. 2004). The larger the fiber diameter, the faster the action potential propagates. This is because a large fiber offers less resistance to local current allowing more ions to flow and bringing adjacent regions of the membrane to threshold faster. The propagation of the action potentials is referred as to conduction velocity and it can be measured with multi-channel electromyography (Arendt-Nielsen and Zwarts 1989).

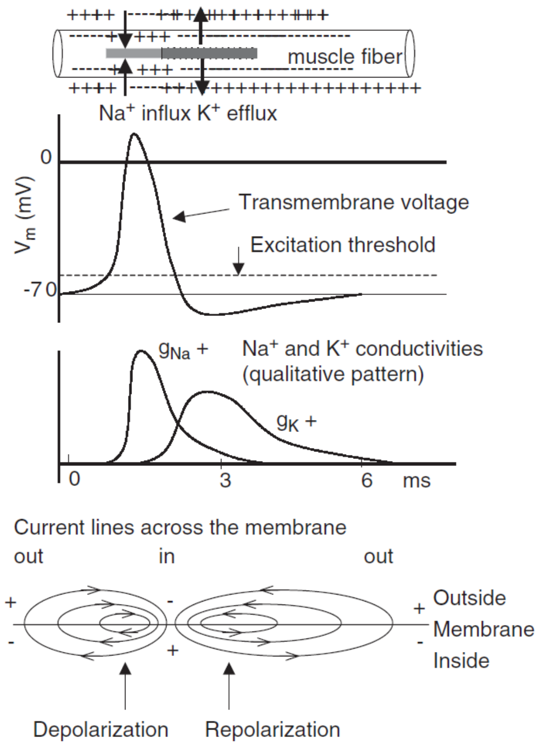

Figure 1-2 - Representation of the generation and propagation of an action potential in an excitable cell. The influx of Na^+ followed by the efflux of K^+ creates a quick voltage change called action potential. The cell depolarizes the membrane potential becomes less negative and repolarizes as it returns to resting membrane potential (-70mV). One action potential triggers another in the plasma membrane just distal to it until the end of the length of the membrane. The local circuit currents spread the depolarization to the left into the unexcited membrane [adapted from Moritani et al. (2004)].

The conduction velocity is an important physiological parameter since it reflects the electrophysiological properties of the muscle fibers and it provides information on the alterations of the peripheral properties neuromuscular system to disease (van der Hoeven 1995; Zwarts et al. 2000), fatigue (Bigland-Ritchie et al. 1981; Farina et al. 2002a) or exercise (Pozzo et al. 2004; Rainoldi et al. 2008).

Several studies observed that muscle fiber conduction velocity is positively correlated with fiber composition (Linssen et al. 1991; Sadoyama et al. 1988) and muscle force level (Arendt-Nielsen et al. 1984; Broman et al. 1985b), reflecting the linear association between muscle fiber diameter and conduction velocity (Kupa et al. 1995). In addition, other studies also reported a strong association between changes in the muscle fiber conduction velocity and in the contractile properties of the motor units (Andreassen and Arendt-Nielsen 1987; Farina et al. 2002a). These findings raised the possibility of estimating muscle fiber composition, and thus motor unit recruitment strategies, from conduction velocity estimates (Gerdle et al. 1991; Kupa et al. 1995; Wakeling et al. 2001).

Muscle fiber conduction velocity can also be used to monitor muscle fatigue. During fatigue the accumulation of intracellular lactate and hydrogen ions increases, which reduces the membrane excitability and impairs the velocity of propagation of the action potentials along the muscle fibers (Allen et al. 2008; Arendt-Nielsen and Mills 1988). Such alterations may perturb excitation-contraction coupling leading to force and motor control impairments (Allen et al. 2008). By measuring muscle fiber conduction velocity, relevant information can be obtained on pathological fatigue diseases (Zwarts et al. 2000) or on the neuromuscular fatigue profiles among athletes (Rainoldi et al. 2008). The alterations of the peripheral neuromuscular system in response to motor training can also be investigated by assessing to conduction velocity. This has been explored in study II and presented in the paper “Adjustments in motor unit properties during fatiguing contractions following training” (part II of the thesis).

However the conduction velocity is affected by other physiological parameters, such as muscle temperature (Farina et al. 2005; Gray et al. 2006) and motor unit discharge rates (Farina 2011; Nishizono et al. 1989) or/and non-physiological factors associated with conduction velocity detection and estimation methods. For example, increase of either muscle temperature or mean discharge rates lead to an increment of the muscle fiber conduction velocity (Farina et al. 2005; McGill and Lateva 2011), which may mask the results. The non-physiological factors are discussed in chapter 3.

1.1.3 Motor unit types

According to the *size principle,* variations in size-related motoneurons properties such as input resistance should be systematically linked to the type of muscle fibers innervated by the respective motoneuron, as well to variations in synaptic input (Henneman and Olson 1965; Henneman et al. 1965a; b; McPhedran et al. 1965; Wuerker et al. 1965). Thus, the essence of the motoneuron pool represents a spectrum of sizes and thresholds that are linked to a spectrum of forces and energy expenditure that can be triggered off peripherally (Henneman et al. 1965b). Both experimental human and animals studies have consistently demonstrated that the units of a muscle express a continuous variation in maximum force, isometric twitch speed and endurance rather than a discrete distribution (Kernell et al. 1999; Reinking et al. 1975; Van Cutsem et al. 1997). Nonetheless, a scheme classification for motor units is useful either for discussing motor units within an organizational framework or for characterizing changes in the muscle properties (Burke 1978; Duchateau and Enoka 2011).

Classically, the motor units have been categorized into three main physiological types based on contraction time, fatigability, and the “sag” property: slow contracting fatigue resistant (type S); fast contracting fatigue resistant (Type FR) and fast contracting fast to fatigue (type FF) (Burke et al. 1974; Burke et al. 1973; Burke and Tsairis 1974). In addition some studies reported lower conduction velocity of the action potentials in the type S motor units than in the type FF with type FR showing an intermediate response (Andreassen and Arendt-Nielsen 1987). The main physiological, histochemical and electrical properties of the three major motor unit types are summarized in table 1-1.

Table 1-1– Summary of the motor unit types characteristics [from Moritani et al. (2004)].

Motor unit Type	**Histochemical and metabolic properties**	**Mechanical properties**	**Electrical properties**	**Others**
type S	Oxidative	Slow twitch, small forces, fatigue resistant, smaller fiber diameter and MU size	Lower nerve and muscle fiber conduction velocity	Small motoneurons, recruited at low force levels
Type FR	Oxidative glycolytic	Fast twitch, fatigue resistant	Intermediate nerve and muscle fiber conduction velocity	Intermediate motoneurons size, recruited at moderate force levels
type FF	Glycolytic	Fast twitch, large forces fatigable, larger fiber diameter and MU size	High nerve and muscle fiber conduction velocity	Large motoneurons, recruited at high force levels

1.2 Motor unit pool and gradation of the muscle force

The ability of a muscle to contract over a wide range of force arises from the control of populations of non-overlapping motor units via two mechanisms: (1) number of motor units that are activated (recruitment gradation of force) and (2) rate at which the motoneurons discharge action potentials (rate gradation) (Kernell 2006).

The balance between both mechanisms might differ across muscles (De Luca et al. 1982a) and even between different tasks for the same motoneuron pool (Mottram et al. 2005). But, in general, to produce weak contractions, recruitment and rate gradation occurs concurrently; an additional increment in force will be produced by recruiting new motor units and, simultaneously, increasing the discharge rate of those already recruited. To produce a moderately strong contraction all motor units would be recruited and further increments will be due to increase in discharge rates (rate gradation) (Kernell 2006; 2003) (Fig. 1-3).

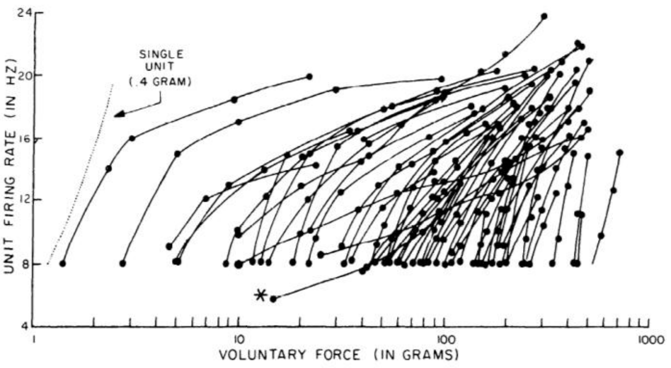

Figure 1-3- Discharge rate behavior of 60 motor units during a progressive increment of the isometric voluntary contraction of the extensor digitorum communis muscle. The motor units are gradually recruited and their discharge rate is increasing progressively [from Monster and Chan (1977)].

1.2.1 Recruitment gradation

The recruitment of a motoneuron depends on two factors: (1) its intrinsic threshold — cells with high input resistance have lower thresholds, thus require less input to generate action potentials and to be recruited — and (2) its relative share of the synaptic input — due to uneven distribution of the synaptic input across the motor unit pool, a motoneuron that receives larger synaptic input will tend to be recruited before cells with smaller inputs (Heckman et al. 2009; Kernell and Hultborn 1990). The range of intrinsic threshold is large (~10-fold) and is mainly determined by differences in input resistance as predicted by Henneman in 1965 (Heckman et al. 2009). According to the *size principle*, small motoneurons tend to innervate slow twitch, low force but highly fatigue resistant muscle units and they need less synaptic input to be recruited. Gradually larger motoneurons innervate faster, stronger and more fatigable units requiring progressive increment in the synaptic input to be activated.

The size-ordered recruitment serves two main important purposes (Kernell 2006; Loeb and Ghez 2000). First it minimizes the development of fatigue by allowing the most fatigue-resistant muscles fibers to be used most of the time and just recruiting more powerful but fatigable fibers when is necessary to achieve higher forces (Henneman and Olson 1965; Henneman et al. 1965b). Moreover, the first recruited units are the most energetically efficient (Kushmerick 1995). Second, it ensures the gradation of force because the more resistant to fatigue units exert the least force and further increments in force are approximately proportional to the level of force at which each individual motor unit is recruited (Henneman and Olson 1965; Henneman et al. 1965b).

In general, the recruitment order according the size principle seems to be preserved either during slow and ballistic isometric contractions (Desmedt and Godaux 1977a; b) and during anisometric contractions (Kossev and Christova 1998; Sogaard et al. 1996; Thomas et al. 1987). Nonetheless, exceptions may occur, in particular in multifunctional muscles (Denier van der Gon et al. 1985) or when changes in the sensory feedback are transmitted to the motoneuron pool which may alter the distribution of the synaptic input (Duchateau and Enoka 2011). Thus, different motor tasks may involve different motor unit recruitment thresholds. For example, the motor unit recruitment thresholds during ballistic isometric contractions (Desmedt and Godaux 1977a) and dynamic contractions (Kossev and Christova 1998; Sogaard et al. 1996) are lower than during slow-ramp isometric contractions.

1.2.2 Rate gradation

In each muscle unit, force can be graded by varying the discharge rate of the motoneuron (Kernell et al. 1999). The relation between force and discharge rate follows a sigmoid curve (referred as to a tension-frequency curve) and an effective gradation of force takes place within a restricted range of discharge rates covering the middle range of the curve (Heckman and Enoka 2004; Kernell 2006). The range occurs at lower rates for units with slow twitches than for those with faster twitches (Kernell et al. 1999). The minimum rates are around 5 to 8 pulses per second (pps) for low-threshold motor units and around 10-15 pps for high-threshold motor units (Heckman and Enoka 2004; Van Cutsem et al. 1997). This indicates that the intrinsic mechanisms controlling motoneuron discharge rate are also matching the motor unit mechanical properties, providing an optimal increase in force (Sawczuk et al. 1995).

Once a motoneuron is activated, it generates action potentials at a rate approximately proportional to the amount of synaptic input that it receives [frequency-current (*f-I*) slope also referred as rate gain](Kernell 2006; Schwindt 1973). However, the rate gain is dependent on the relative distribution of the synaptic input across the motoneuron pool (Heckman and Enoka 2004). Thus, motoneurons receiving a higher proportion of the synaptic input would not only tend to be recruited earlier but also have higher rate gains. This can be strongly modulated by metabotropic inputs (Heckman et al. 2009), by changing for example the AHP duration (please see following section).

The duration of the AHP is a major mechanism controlling the minimum rate of a maintained discharge rate and its time-course and size are important for the steepness of the *f-I* relation (Heckman et al. 2009; Kernell 1995). Although the AHP might not be the only mechanism controlling the minimum discharge rates, it has been suggested that the modulation of the AHP properties, and thus of the motor unit discharge rates, allows the nervous system to rapidly meet the demands of a particular task (Duchateau and Enoka 2011). For instance, during ballistic contractions the initial discharge rate is very high and frequently double discharges (doublets) occur at the onset of the contraction (Desmedt and Godaux 1977a; b). This phenomenon is also observed in the initial phase of concentric contractions (Kossev and Christova 1998). Such alteration in motor unit behavior may represent a mechanism to generate extra force at the onset of a contraction perhaps to overcome inertia (Bawa and

Calancie 1983; Desmedt and Godaux 1977a), but the phenomenon is not necessarily correlated with a physiological need for strong contractions (Garland and Griffin 1999).

1.3 Synaptic input distribution

In natural conditions motoneurons receive synaptic inputs from many sources such as the cortex, brain stem and peripheral sensory receptors, causing their intrinsic properties to fluctuate (Burke 1978; Heckman and Enoka 2004). The integrative effect of these inputs, together with the intrinsic motoneuron properties, determines the generation of action potentials propagated along the peripheral motor nerve to the neuromuscular junctions of the muscle fibers, resulting in mechanical output (Gardiner 2011; Heckman and Enoka 2004). Nevertheless, the relative distribution of the input from different sources vary across motoneuron pools (Kernell and Hultborn 1990). For instance, it has been shown that the synaptic input from the Ia afferents, originating from muscle spindles, is larger in small motoneurons innervating slow-twitch motor units that in large motoneurons innervating fast-twitch motor units (Binder et al. 2002; Eccles et al. 1957). The reciprocal la-inhibition input generated by the Ia afferents of the antagonist muscles and recurrent inhibition input from the Renshaw cells appeared to be distributed uniformly within the motoneuron pool (Binder et al. 2002; Pierrot-Deseilligny and Burke 2005). In contrast the synaptic input from the skin nerves containing feedback from cutaneous receptors is greater in the largest motoneurons (Burke 1978; Enoka 2008). In addition, stimulating the rubrospinal tract produces predominantly depolarizing synaptic input currents in large motoneurons and predominantly hyperpolarizing currents in small motoneurons (Binder et al. 2002; Burke et al. 1970). A non-uniform distribution of synaptic effects to low- and high-threshold motor units it may eventually cause reversals on the recruitment order if it modifies the range of recruitment thresholds established by differences in the intrinsic current thresholds of the motoneurons (Binder et al. 2002; Kernell 2006). Even if no recruitment reversal takes place, such distribution influences recruitment in terms of its gain (Kernell 2006). For instance, if a certain synaptic system increases the excitability of intrinsically less excitable motoneurons, the differences in recruitment threshold within the pool decreases and thus recruitment gain increases (threshold compression) (Kernell and Hultborn 1990). A compression of the recruitment would allow all motor units to be recruited while firing at relatively low discharge rates, which may be advantageous to counteract fatigue (Kernell 2006). The rate gain may increase due to modulation of metabotropic inputs, in particular by serotonin (5HT), which reduces AHP conductance and thus increases the *f-I* slope (rate gain) (Heckman et al. 2009; Kernell 2006).

Although it is not known how all these different patterns of synaptic inputs are used in motor behavior, it seems clear that the nervous system has alternative mechanisms available for differential control of particular sets of motor units (Burke 1978; Kernell 2006). Moreover these mechanisms might be changed with training experience, revealing high plasticity of the nervous system (Gardiner 2011; Kernell 2006).

CHAPTER

2

Adaptations of the neuromuscular system to training

Because the neuromuscular system represents the interface between the central nervous system and the muscles, it offers remarkable possibilities for investigating the sites and mechanisms of adaptation as well as for determining how they account for motor behavioral changes (Adkins et al. 2006; Wolpaw 2010). Different models can be used to study neuromuscular adaptation in humans, including aging, disuse (spinal cord injuries, bed rest, space flight weightlessness or joint immobilization) or use (voluntary training or chronic electrical stimulation) (Kernell 2006). Knowledge on the neuromuscular adaptation to training is of great interest for both rehabilitation and exercise training fields (Zehr 2006). Both fields aim to induce long–lasting changes in motor behavior either to restore correct motor patterns or to increase motor performance (Falla et al. 2007; Nielsen and Cohen 2008; Zehr 2006). Thus, a proper training intervention requires an understanding of how and to which extent the neuromuscular system can accommodate to distinct motor behavior experiences.

The work of the recent decades has shown that not only muscle tissue, but also the nervous system, changes in response to a variety of different motor experiences, including strength and endurance training (Adkins et al. 2006; Duchateau et al. 2006). Activity-dependent neural adaptations involve many different mechanisms and structures across cortex and spinal cord (Adkins et al. 2006; Wolpaw 2010). Moreover, depending on the type of motor behavior experience, the mechanisms and structures involved in the neural adaptations might be distinct (Adkins et al. 2006).

Strength and endurance training represents a good experiment to investigate the response of the neuromuscular system to physical activity since the physiological stimuli imposed by them are divergent in nature, representing the extremes of the motor training (Hakkinen et al. 2003; Sale et al. 1990). Moreover, they are commonly applied in the rehabilitation and exercise training fields. However, due to the characteristics and aims of these types of motor training, much more attention was given to muscle adaptation and cardio-respiratory responses. For a long time now it has been demonstrated that both endurance and strength training induce distinct anatomical and physiological adaptations contributing for specific motor performance (Fitts 2003; Folland and Williams 2007; Gabriel et al. 2006; Hawley 2002). Nonetheless, it is increasingly recognized that many of the adaptations induced by both training types, resulting in increased motor performance, may also reside at the nervous system (Carroll et al. 2011; Gardiner 2011). But, as we move from the muscle to the central nervous system, information becomes less abundant and controversial, in particular regarding to endurance training.

In the following sections, current evidences are revised regarding neural and muscle fibers adaptations to endurance and strength training. Muscle adaptations are briefly reviewed, since they are not significantly relevant for the main subject of this thesis.

2.1 Muscle adaptations

Endurance and strength training typically induce different anatomical and physiological adjustments (Aagaard et al. 2001; Costill 1970; Costill et al. 1979; Seynnes et al. 2007). It has been extensively documented that strength training enhances maximum force, which is accompanied by increased muscle cross-sectional area (CSA) and pennation angle (Aagaard et al. 2001; Costill et al. 1979; Fitts 2003; Seynnes et al. 2007) whereas, endurance training improves resistance to fatigue and is associated with reduction in muscle fiber CSA, mitochondrial biogenesis and angiogenesis (Baldwin and Haddad 2001; Costill 1970; Gibala et al. 2006; Hoppeler and Fluck 2003; Malisoux et al. 2007). These structural alterations take several weeks (at minimum 5-8 weeks) to occur and contribute significantly for specific improvements in motor performance (i.e., maximum force or endurance capacity) (Folland and Williams 2007; Hawley 2002). In recent decades, it has been shown that changes in the MHC isoforms (Fitts 2003; Fluck and Hoppeler 2003), Na^+-K^+ pump activity (Green et al. 2004; Nielsen and Clausen 2000) and Ca^{2+} sensitivity (Fluck and Hoppeler 2003; Green et al. 2003) occur earlier than changes in the whole muscle morphology.

Although most of the molecular and histochemical adaptations to endurance and strength training are distinct, some are common. For example, is has been shown that both endurance and strength training increase the concentration of Na^+-K^+ ATPase pump in the trained muscle (Green et al. 1999; Madsen et al. 1994; McKenna et al. 1993). The Na^+-K^+ ATPase pump is an integral membrane protein that is determinant for the regulation of the Na^+-K^+ distribution and maintenance of membrane excitability during muscle contractions (McKenna 1995; Nielsen and Clausen 2000). Increases in Na^+-K^+ ATPase pump content could have potential beneficial effects in protecting muscle contractibility against fatigue during repetitive activity, such as those involved in endurance training (Clausen 2003; McKenna et al. 2008). Moreover, it would enable the sarcolemma to conduct repetitive action potentials at high frequencies, as it is required by strength training exercises (Clausen 1996; Green et al. 1999). Nonetheless, the time course of such adaptations may vary across motor training experiences (Clausen 2003; Green et al. 1999; Green et al. 2003). Changes in the Na^+-K^+ ATPase pump (content and/or activity) affect the conduction velocity of action potentials in the muscle fibers, which can be measured with specific electromyographic systems (Farina et al. 2002a; Merletti et al. 2003). Changes in conduction velocity elicited by endurance and strength training were investigated in studies I and II.

Like many other proteins of the muscle cell, the proteins involved in the sarcoplasmic reticulum Ca^{2+} handling can also be affected by motor training (Green et al. 2003; Majerczak et al. 2008; Ortenblad et al. 2000). For instance, it has been shown that 5 to 10 weeks of prolonged endurance training induced a down regulation in Ca^{2+} release and uptake (Green et al. 2003; Majerczak et al. 2008). On the other hand, high-intensity resistance training seems to increase Ca^{2+} ATPase activity (Green et al. 1998) and single-fiber Ca^{2+} sensitivity (Malisoux et al. 2006). These adaptations seem to affect the contractile properties of the muscle fibers, in particular the rate of force development (Ortenblad et al. 2000). Nevertheless, changes in MHC composition of the muscle fibers seem to be the major determinant of their functional properties (Bottinelli and Reggiani 2000). The MHC isoform expression can be altered by training (Malisoux et al. 2007). It is generally accepted that following several weeks of endurance-type training a sequential transition from fast to slow muscle fibers occurs (MHC IIx → MHC-IIa → MHC-I) (Malisoux et al. 2007; Trappe et al. 2006). Following strength training a sequential transition from MHC IIx to MHC-IIa can be observed and the fraction of type I fibers seems to be not affected (Fitts 2003; Malisoux et al. 2007). Further, strength training is known to increase peak force (P_0) of both type I and II muscle fibers, without decreasing velocity (V_0) and P_0/CSA, whereas, endurance training can reduce P_0 and increase V_0 (Fitts 2003; Malisoux et al. 2007).

From the metabolic point of view, it is generally accepted that endurance exercise training leads to an increase of the mitochondrial density and increases the use of lipids as a substrate, both with regard to relative and absolute exercise intensities (Hoppeler and Fluck 2003; Wilmore et al. 2008). These adaptations are also accompanied by increased maximal oxygen uptake capacity (Wilmore et al. 2008). On the other hand, the strength training primarily stresses the anaerobic metabolism and, depending on the training intensity, it may result in elevated glycolytic, phosphocreatine and mitochondrial activities (Costill et al. 1979; Wilmore et al. 2008).

2.2 Neural adaptations

The first indications of neural adaptation to training came from indirect evidences based on the time-course of the adaptations to strength training. Early studies reported a larger disproportionality increase in muscle force than in muscle size, particularly during the initial stages of the strength training programs (Maughan 1984; Moritani and deVries 1979; Sale 1988). Further studies suggested that neural factors such increased agonist muscle activation (Aagaard et al. 2002a; Moritani and deVries 1979; Sale 1988) and decreased antagonist activation (Carolan and Cafarelli 1992) would be main contributors for the initial gains in force. Second evidence comes from the cross-education effect, revealing that the training of one limb induces an increment of the muscle force in the contralateral untrained limb (Lee and Carroll 2007; Zhou 2000). Yet, these effects have not been demonstrated consistently, either for the agonist (Holtermann et al. 2005; Rich and Cafarelli 2000), antagonist muscle activation (Aagaard et al. 2000; Hakkinen et al. 2003) or cross-training effects (Davies et al. 1988; Garfinkel and Cafarelli 1992). The controversial results might reflect the differences between training paradigms and experimental procedures applied across studies as well methodological issues. In the last decades, researchers have been making an effort to assess neural adaptations in response to specific training paradigms and recent neurophysiological methods have been developed and applied.

Intramuscular recording of single motor unit activity is one of the few neurophysiological techniques that can provide unequivocal information on the motoneuron behavior during voluntary contractions (Carroll et al. 2011). However, assessing the effects of a longitudinal intervention on motor unit behavior is challenging, mainly due to the inability to record the same motor units before and after a training period (Carroll et al. 2011; Duchateau et al. 2006). This makes it difficult to clearly demonstrate the effects of a training intervention. Thus, the comparison of the motor unit behavior pre and post training normally requires a reasonable sample size of motor units and a substantial number of measurements to adequately characterize the changes with training. Moreover, despite of the recent advances in signal processing that allows a quasi-complete identification of all motor units with high accuracy (i.e, depending on the characteristics of the EMG signal; cf. Chapter 3, section 3.2.2), the nature of the experiments are time consuming. As a consequence, there are only few studies that have adequately compared the motor unit behavior before and after training (Duchateau et al. 2006).

Although investigating motor unit behavior provides relevant information on the output from the spinal cord (which may reveal changes of the nervous system in response to training) such information does not identify the mechanisms and sites of the neural adaptations. The motor unit behavior results from the integration of thousands of synaptic inputs arising from many sources. Thus, training-induced adaptations might occur either at supraspinal and/or spinal levels, involving different structures and mechanisms (Carroll et al. 2001; 2002; Enoka 2008). Figure 2-1 shows some of the potential sites where the neural adaptations may occur.

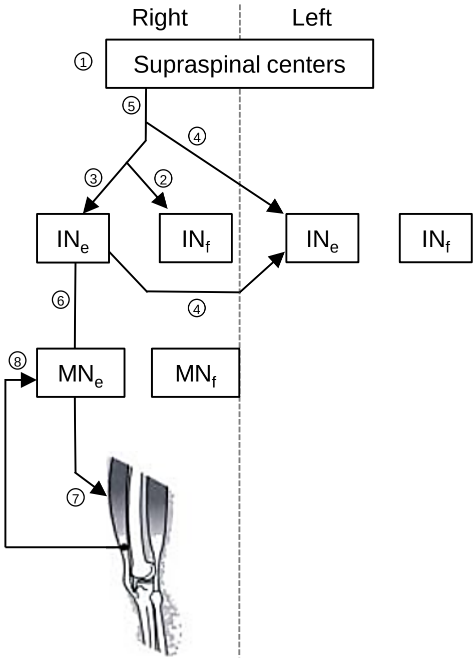

Figure 2-1 – Potential sites where the neural adaptations to training may occur: (1) enhanced output from the supraspinal centers; (2) reduced co-activation of the antagonist muscles; (3) greater activation of agonist and synergist muscles; (4) enhanced coupling among spinal interneurons (IN) that produces cross-education; (5) changes in descending drive that influence the bilateral deficit; (6) shared input to motoneurons that increases motor unit synchronization; (7) greater muscle activation and (8) heightened excitability of motoneurons as indicated by reflex potentiation and motoneuron plasticity. $\mathrm{IN_e}$ – interneurons that project to motoneurons innervating extensor muscles; $\mathrm{IN_f}$ – interneurons that project to motoneurons innervating flexor muscles; $\mathrm{MN_e}$ – motoneurons innervating extensor muscles; $\mathrm{MN_f}$ – motoneurons innervating flexor muscles [from Semmler and Enoka (2000)].

The information available on the potential sites and mechanisms of the neural adaptations that might alter the spinal cord output has been provided by different neurophysiological techniques including transcranial magnetic stimulation (TMS), electrical stimulation of peripheral nerves and more recently functional magnetic resonance imaging (fMRI). Despite of this, it has been challenging to identify the sites and mechanisms that underlie the neural adaptations (Enoka 2008). The main results on neural adaptations to strength and endurance training are discussed below.

2.2.1 Changes in the motor unit behavior

The few longitudinal studies, investigating the effects of training on the motor unit behavior of humans, have applied strength training paradigms (Duchateau et al. 2006). Most of the studies aimed to investigate the effects of strength training on: (i) the mean discharge rates either during maximal or submaximal contractions (Christie and Kamen 2010; Kamen and Knight 2004; Patten et al. 2001; Pucci et al. 2006; Rich and Cafarelli 2000); (ii) the interspike variability (Cracraft 1975; Kornatz et al. 2005; Van Cutsem et al. 1998) and (ii) the synchronization of the motor unit activity (Christou et al. 2007; Griffin et al. 2009; Kidgell et al. 2006).

The studies have reported that following strength training, of either upper or lower limbs, the increase in maximal voluntary contraction (MVC) was accompanied by an increase in the maximal motor unit discharge rates (Christie and Kamen 2010; Kamen and Knight 2004; Patten et al. 2001). It was been shown that following training the maximal discharge rates might increase between 23 and 49% for the elder and between 7 and 11% for younger subjects (Christie and Kamen 2010; Kamen and Knight 2004; Patten et al. 2001). With young subjects, Van Cutsem et al. (1998), showed that larger increases in maximal discharge rate (up to 29%) can be reached following particular strength training protocols involving ballistic contractions. However, the results are not unanimous; for example Pucci et al. (2006) did not find significant changes in the maximal discharge rates following a period of 3 weeks of isometric training of the knee extensors. Controversial results have also been reported when analysing the motor unit discharge rates during submaximal contractions. For example, Kamen and Knigth (2004) observed significant increments in discharge rates of the vastus lateralis after 1 week of strength training, but the values returned to baseline following 6 weeks of training, while Rich and Cafarelli (2000) did not observe changes in motor unit discharge rates of the vastus lateralis after 8 weeks of training.

Experimental evidence suggests that more subtle changes in the motor unit activity, such as in increased incidence of brief interspike intervals (doublets), may contribute to enhanced rate of force development following training. Van Cutsem et al. (1998) showed that the increment in rate of force development of the ankle dorsiflexors observed after 12 weeks of ballistic training were accompanied by increased incidence of doublets. Changes in the interspike intervals observed following strength training (decreased interspike intervals variability) have been also associated with increased force steadiness during submaximal contractions (Griffin et al. 2009), although the results are not consistent (e. g. , Cracraft 1975; Kornatz et al. 2005). The only study on changes in motor unit behavior following one kind of local endurance training, reported that 6 weeks of dynamic low-resistance training of the dorsiflexors of medium duration decreased the motor unit interspike intervals variability, while 6 weeks of high resistance increased their discharge variability (Cracraft 1975).

Another mechanism that has raised much interest is the motor unit synchronization. Such interest has been motivated by an original study from (Milner-Brown et al. 1975), who reported a 128% increase in motor unit synchronization following 6 weeks of isometric training of a hand muscle. However, the changes in motor unit synchronization were indirectly estimated from surface EMG signals, which have been considered an unreliable procedure for such estimation (Yue et al. 1995). The most direct methods (cross-correlation of individual discharge times from pairs of concurrently active motor units) reveal that the increase in strength following strength training were not accompanied by significant increments in motor unit synchronization (Christou et al. 2007; Griffin et al. 2009; Kidgell et al. 2006). The results suggest that this feature is not important to enhance muscle strength (Kidgell et al. 2006). Therefore, it appears that increased discharge rates and changes in interspike intervals are more relevant to improve motor performance, in particular muscle strength and steadiness (Carroll et al. 2011).

2.2.2 Sites and mechanisms of the neural adaptations

The neural adaptations to training might occur at either of two main levels: (1) supraspinal levels that include corticospinal neurons, subcortical neurons and inhibitory and excitatory intracortical interneurons; (2) spinal level that compromises the intrinsic motoneuron properties and inhibitory and excitatory neural circuits (Duchateau et al. 2006).

Most of the studies investigating the changes at supraspinal level involved skill and strength training. The TMS results consistently indicate that skill training evokes changes within the primary motor cortex and increases the excitability of the corticospinal pathway [measured as the size of a motor evoked potential (MEP) elicited TMS] (Jensen et al. 2005; Perez et al. 2004; Remple et al. 2001). However, regarding to strength training effects, the results are not revealing a clear pattern of adaptation in excitability of the corticospinal pathway. Following a period of strength training, the size of MEPs, assessed during weak isometric contractions, has been shown to increase in the plantar flexors (Beck et al. 2007; Griffin and Cafarelli 2007), be unchanged in finger muscles (Carroll et al. 2009; Carroll et al. 2002) or trend lower in arm muscles (Jensen et al. 2005). Nonetheless, during strong contractions the MEP size has been shown to be significantly lower following strength training of the finger and wrist muscles (Carroll et al. 2009; Carroll et al. 2002). In addition, the size of TMS responses seems to be context-specific. For example, Beck et al. (2007) showed that, following 4 weeks of ballistic ankle strength training, the MEP size of the tibialis anterior only increased during the performance of the trained task, while no changes were observed during the execution of other non-trained tasks or at rest. Thus, contradictory results on the changes within motor cortex may be due to differences in motor tasks which were performed by the subjects while neurophysiologic measures were recorded (Beck et al. 2007). Another confounding factor may be related with the task complexity and skill learning involved in the correct execution of the strength training exercises. It has been advocated that learning is a prerequisite or factor in modulating neural adaptations, suggesting that movement repetition *per si* does not lead to functional changes within the motor cortex if any degree of skill acquisition is involved (Adkins et al. 2006; Jensen et al. 2005). Based on this, it has been suggested that the neuromuscular adaptations to strength training mainly result from changes in the functional properties of the spinal cord rather than from changes within motor cortex (Duchateau et al. 2006).

The training adaptations within the spinal cord are frequently evaluated by testing electrically evoked reflexes, such as Hoffman (H-) reflex. The H-reflex measures are usually combined with Volitional (V-) wave measures (cf. chapter 3). Although these evoked responses are affected by common neural mechanisms, during voluntary contractions, the H-reflex is more sensitive to altered presynaptic inhibition and motoneuron excitability (Ekblom 2010; Hultborn et al. 1987; Misiaszek 2003; Nordlund et al. 2002; Pierrot-Deseilligny and Mazevet 2000), whereas the V-wave is more sensitive to changes in supraspinal input to the motor neuron pool (Aagaard et al. 2002b; Duclay and Martin 2005; Upton et al. 1971). Thus, combined measures of the H-reflex and V-wave may provide a better understanding of the neural adaptations elicited by specific motor training programs.

Reflex investigations have been performed in few longitudinal strength training studies. Some studies have reported increases in the V-wave, without changes of the H-reflex (Del Balso and Cafarelli 2007; Ekblom 2010; Fimland et al. 2009a; Fimland et al. 2009b), while others observed increases in both V-wave and H-reflex responses (Aagaard et al. 2002b). The different results may be attributed to diverse training protocols and/or methodological approaches (Carroll et al. 2011; Del Balso and Cafarelli 2007).

For example, Aagaard et al. (2002b) demonstrated a significant increase in evoked H-reflex (measured at 90% MVC with a stimulus intensity adjusted to produce a Motor (M-) wave response amplitude of ~20% maximal M-wave (M_{max}) and V-wave responses following 14 weeks of dynamic strength training of the quadriceps and plantar flexors. The authors suggested that adaptations to strength training may compromise both supraspinal and spinal mechanisms, including increased descending drive from the corticospinal pathway, increased motoneuron excitability and/or reduced pre-synaptic inhibition. On the other hand, Del Balso and Cafarelli (2007) have shown that after 4 weeks of isometric strength training, the increments in the MVC and rate of force development of the plantar flexors were accompanied by significant increase of the ratio V/M_{max} without changes in the H_{slp}/M_{slp} (slope method to analyze the H and M recruitment curves) measured at 10% MVC. Also Ekblom (2010), reported that following 5 weeks of predominant eccentric exercise of the plantar flexors evoked an increment of the V/M_{max} while no changes in the H-reflex amplitude (measure based in the ratio H_{max}/M_{max}). Accordingly, the authors suggested that elements of the H reflex pathway may not be directly involved in chronic adjustments in response to isometric strength training (Del Balso and Cafarelli 2007; Ekblom 2010). Because changes in the ratio V/M_{max}

were found, it was suggested that the changes in force output might be due to increase of the descending volitional drive to the muscle (Del Balso and Cafarelli 2007; Ekblom 2010).

Lagerquist et al. (2006) also observed no changes in the H_{max} after isometric strength training during tonic contractions at 10% of the maximal EMG. However, significant increase of the spinal excitability was observed at low stimulus strength (H-reflexes from the ascending limb of the recruitment curve, with a corresponding M-wave of 5%M_{max}) (Lagerquist et al. 2006). Similarly, Dragert and Zehr (2011) observed increased excitability at H-reflex threshold (obtained at lower stimulus intensity) but no changes of the H-reflex were observed in other points of the ascending limb of the recruitment curve.

It is possible that the motor unit types in the motoneuron pools recruited by H-reflexes of low intensity (e.g., at 5% M_{max}) or during maximal H-reflex are different. Low intensities would presumably recruit the smallest spinal motoneurons whereas higher stimulus (maximal H-reflex) would recruit high-threshold motoneurons (Dragert and Zehr 2011; Lagerquist et al. 2006). Different levels of tonic contraction would also involve different types of motoneurons, which can contribute for the different results observed between studies. For example, Aagaard et al. (2002b) used tonic contractions of 90% MVC while De Balso and Cafarelli (2007), measured the H-reflex at 10% MVC of the plantar flexors.

The only longitudinal study on the effects of endurance training on the size of the H-reflex revealed that 75% of the subjects increased both spinal excitability and maximal aerobic capacity while for the remaining participants these variables were barely modified or even decreased (Perot et al. 1991). The heterogeneity of the results were likely due to differences in the subject's fitness level and/or their involvement in other sport activities (Perot et al. 1991). Additional information is provided by cross-sectional studies, revealing that athletes performing aerobic sports (distance runners and swimmers) have higher H_{max}/M_{max} ratios than athletes from anaerobic sports (i.e. sprinters and volleyball players) and in athletes in general compared to in sedentary people (Kyrolainen and Komi 1994; Maffiuletti et al. 2001; Ogawa et al. 2009). However such cross-sectional comparisons are difficult to interpret since the H-reflex excitability may be affected by anatomical or genetic differences and not by the training effects (Aagaard et al. 2002b).

The specific neural adaptations evoked by specific training experiences contributes certainly for the optimization of the motor performance, however such association has been difficult to clearly demonstrate (Nielsen and Cohen 2008). Understanding the extent to which the nervous system can adapt to specific motor training programs is of extreme importance not only in the exercise field but also in the rehabilitation field (Zehr 2006). However, based on the current literature, it is difficult to compare and interpret training-induced neural adjustments since studies have employed subjects with different physical fitness levels and applied different experimental methodologies, which contributes to the observed discrepancies between studies.

CHAPTER

3

Electromyography (EMG) as a tool to assess neuromuscular adaptations

The adaptations of the neuromuscular system in response to environmental conditions or motor training can be investigated by applying a variety of techniques raging from imaging to electrophysiological techniques. The advantage of electrophysiological techniques is that they can concurrently provide relevant information on both central and peripheral properties of the neuromuscular system. The activation of the muscle fibers by the nervous system generates an electrical field that can be measured with electrodes, generating the electromyographic signal (Basmajian and De Luca 1985). The EMG signals offer the possibility of investigating the motor output from the spinal cord as well the electrophysiological properties of the muscle fibres (Basmajian and De Luca 1985; Merletti et al. 2001a). Moreover when combined with electrical stimulation of peripheral nerves, changes in the spinal circuitry can be estimated (Pierrot-Deseilligny and Burke 2005). The possibility of accessing such information, even if indirectly, is fascinating and different techniques and devices have been developed over the last decades. Depending on the type of information intended to extract, different methods can be applied. Although it is easier to obtain signals from the surface of the muscle, the extraction of information is complex due to the influence of many factors on the EMG signal features (Merletti and Farina 2004).

This chapter is devoted to the description of the methods used to extract information on both central and peripheral properties. The advantages and limitations of the main methods, in particular those applied in the thesis, are explored in the following subsections. In addition the basic concepts of generation and detection of the EMG signal and the main factors influencing this process will be described. Lastly, the methods using electrical stimulation to peripheral nerves to extract information on spinal cord excitability will be discussed.

3.1 EMG signal generation and detection

When a motoneuron is activated it generates an end-plate potential in the muscle fibres innervated by its axonal branches, resulting in an action potential that propagates along the muscle fibres (Loeb and Ghez 2000). The depolarization of the muscle fibres membrane (sarcolemma) generates a current field in the vicinity of the muscle fibres that can be recorded either by intramuscular or by non-invasive (surface) detection systems (Basmajian and De Luca 1985; Merletti and Farina 2009; Merletti et al. 2003) (Fig. 3-1).

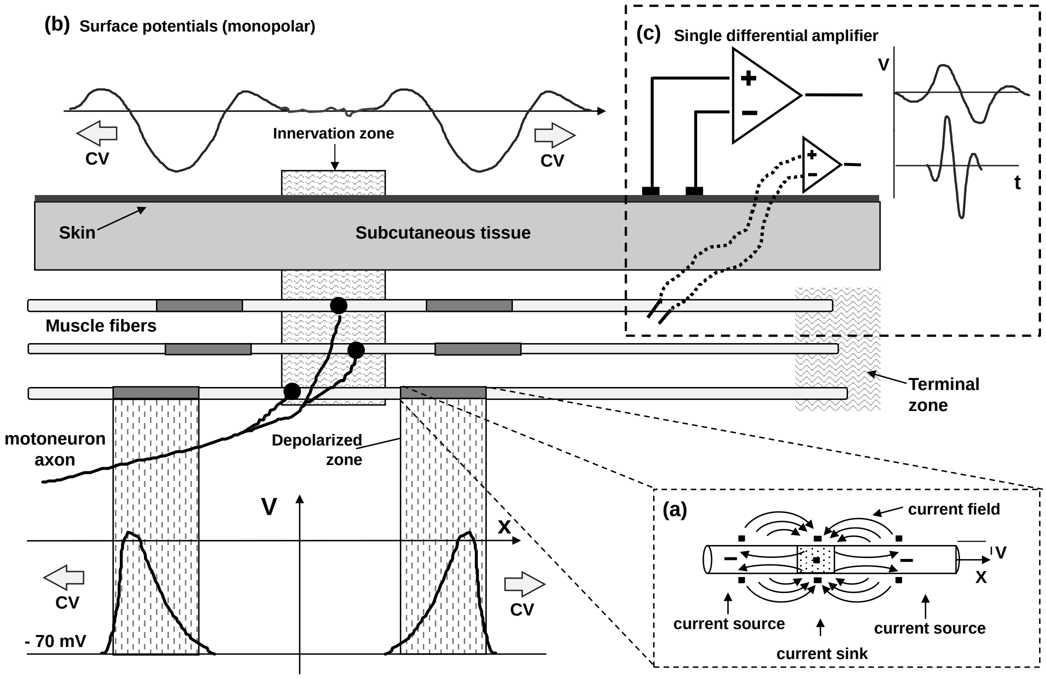

Figure 3-1 - Schematic diagram of the EMG generation mechanism. In the scheme is represented a motor unit with 3 fibers and its motor unit action potential (MUAP). (a) Each generator (muscle fiber) can be represented as a current tripole (two sources and sink), travelling from the neuromuscular junctions to the tendons (terminal zone), that generates a transmembrane voltage V along x (action potential). (b) The extracellular action potentials of the fibers of a motor unit add up to form the superficial MUAP that propagates in both directions from the innervation zone. (c) An electrode pair placed between the innervation and tendon zones will detected an electric signal, whose wave shape and form is majorly determined by the biological tissues between the signal sources and the recording sensors [adapted from Merletti et al. (2001a)].

Because the muscle fibres are not excited individually, the detected signal represents the net summation of individual action potentials, which is designated by motor unit action potential (MUAP) (Basmajian and De Luca 1985; De Luca 1979; Stashuk 2001). If in the surrounding space, muscle fibres belonging to other motor units are active, their MUAPs can be also detected by the same electrode (Basmajian and De Luca 1985). As the level of muscle contraction increases the number of motor units active and the number of MUAPs in each train per second also increases (Stashuk 2001). Thus, due to the property of superposition of electrical fields, the EMG signals recorded during voluntary contraction correspond to the spatial and temporal superposition of the potential trains of the active motor units, as described by the model proposed by De Luca (1979) (Fig. 3-2).

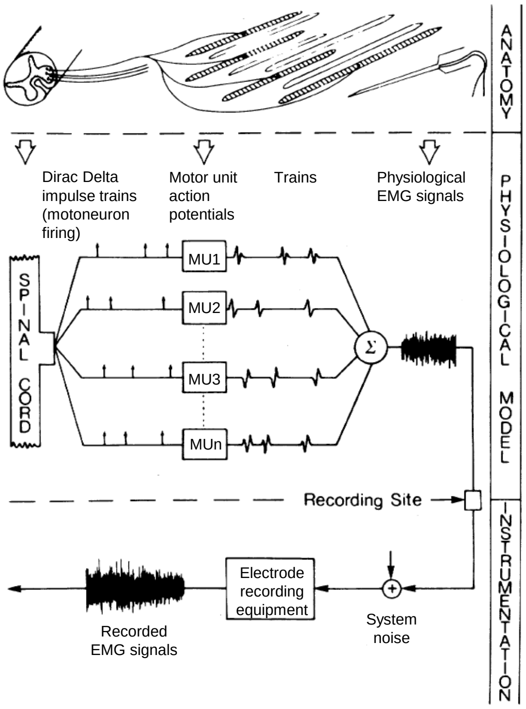

Figure 3-2 - Schematic representation of the model for the generation of EMG signals. The inputs to the filter bank are Delta trains which reflect the central nervous system activation strategy and impulse responses are the motor unit action potentials. The physiological EMG signals correspond to the sum of all trains of motor unit action potentials. Nonetheless the recorded signal is affected by several factors, including instrumentation properties and volume conductor [adapted from De Luca (1979)].

Theoretically the EMG signals would correspond to the sum all trains of MUAPs, nonetheless when the EMG signal is detected, an electrical noise is introduced (Basmajian and De Luca 1985). Moreover the signal is also affected by the transfer function of the recording electrodes (Basmajian and De Luca 1985; De Luca 1979) and the biological tissues between the signal sources and the recording signals (referred as volume conductor) that acts as spatial low-pass filters and it can attenuate the amplitude and high-frequency content of the distant action potentials (Farina et al. 2004e; Stashuk 2001).

The effect of the volume conductor on the recorded EMG signals depends on the type of detection techniques used. While for intramuscular recordings the effect of the volume conductor is relatively small due to the closeness of the recording electrodes to the source, in the case of surface recordings the EMG signal is strongly affected by the characteristics of volume conductor (Basmajian and De Luca 1985; Farina et al. 2004e). The low-pass filtering of the volume conductor reduces the bandwidth of signal detected at the surface, which enlarges the time duration of the action potentials (Basmajian and De Luca 1985). This increases the number of action potential superimpositions and difficults the separation of individual MUAPs from the interference surface signals, even at low contraction levels (Basmajian and De Luca 1985; Farina et al. 2004e; Pozzo et al. 2003)(Fig. 3-3). Typically the bandwidth for intramuscular signals is between 1 to 5KHz, while for surface signals the frequency content is below 300 to 400 Hz. Thus, depending on the type of information to be extracted from the EMG signals, different detection systems may be applied.

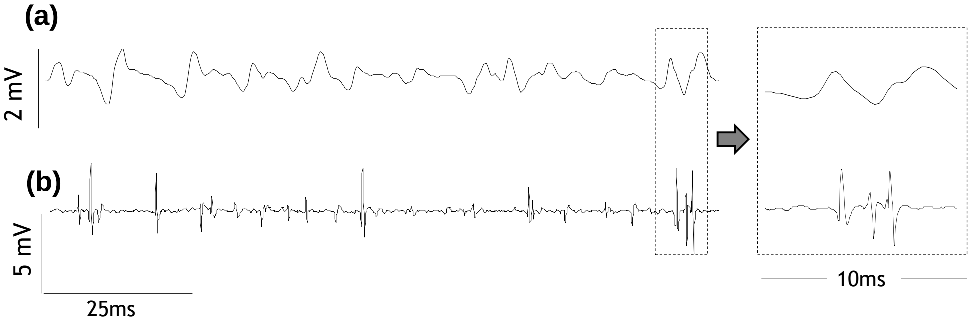

Figure 3-3 - Example of EMG signals recorded with surface (a) and intramuscular (b) electrodes in a single differential configuration. Both signals were collected simultaneously from the vastus medialis obliquus during an isometric contraction at 10% of maximum voluntary contraction. In the right panel it is possible to identify the action potentials belonging to three distinct motor units in the intramuscular but not in the surface recordings. Figure plotted with data from study I.

3.1.1 Intramuscular and surface EMG recording techniques

The electric signals generated by motor units can be detected with different types of intramuscular and surface electrodes (Basmajian and De Luca 1985; Farina et al. 2004e; Stalberg and Falck 1997) (Fig. 3-4 and 3-5). Depending upon the technique, few up to several motor units can be study; the smaller the detection area is, the smallest the uptake area. The recorded area is determined mostly by electrode type and its recording surface size (Stalberg and Falck 1997). For example, due to their high spatial selectivity, intramuscular electrodes are typically used to investigate individual MUAPs, whereas large surface electrodes are used to analyze the global activation of the muscle.

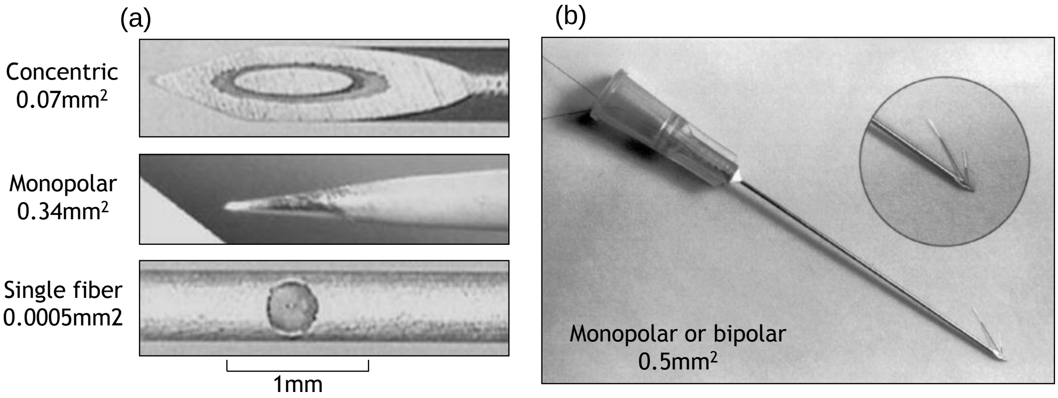

Figure 3-4 - The figure shows the types of intramuscular electrodes commonly used in clinical and research field. (a) Needle electrodes with different shapes and sizes of the detection area. (b) A pair of wire electrodes made of Teflon coated stainless steel with a detection area of 0.05mm^2, used for the study I and II.

Although needle electrodes are commonly used in clinical neurophysiology (Stalberg and Falck 1997), the wire electrodes are preferred in studies in which the EMG signals are recorded over long periods or during movement (Merletti and Farina 2009). The wire electrodes are made of flexible non-oxidizing wires with insulation and have of small diameter. The advantage of these electrodes is that (once removed the needle used to introduce the wires) they can be hardly felt in the muscle, allowing the subject to perform strong contractions without pain (Basmajian and De Luca 1985). Nonetheless, following needle removal, their position in the muscle cannot be adjusted as the needle electrodes and thus is difficult to have several recordings within a single system (Merletti and Farina 2009).

Surface electrodes have the advantage of being painless, has no risk of infection or small hemorrhages and larger area of activity can be pickup (Disselhorst-Klug et al. 2000). The resulting detection area depends on the characteristics of the electrode configuration and on anatomical factors. Classic surface detection systems are composed by two large electrodes arranged in a single differential configuration (with and additional reference electrode, placed on an electrically inactive area). Several versions are commercially available differing in shape and size of the detection surface areas (circular with 9-30 mm^2 or parallel-bars – 1 mm x 10 mm) and inter-electrode distances up to 35mm. (Fig. 3-5 a). The spatial resolution of this type of system is very poor and thus the standard surface EMG signal reflects the compound activity of a higher number of motor units (Rau et al. 1997). Thereby, only global muscle properties can be extracted from the macro-features of EMG signals (Farina et al. 2004c; Merletti and Farina 2004).

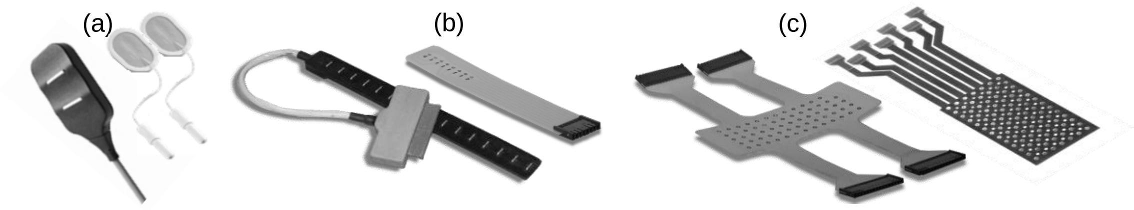

Figure 3-5 - Type of surface electrodes used to detect surface EMG signal. (a) The classic system compromises 2 electrodes that vary in size and shape of the surface area and inter-electrode distance. (b)Linear electrodes arrays are typically used to identify innervation zones and to estimate conduction velocity. They may contain 4 to 16 electrodes in the same array and the inter-electrode distance may vary between 2.5 mm and 10mm. The matrix or grids may be constituted up to 100 electrodes and the inter-electrode distances ranges in the order of a few millimeters.

High spatial resolution can be achieved by the spatial arrangement of small bar or pin electrodes with small inter-electrode distance (in the order of mm) (Fig. 3-5 b and c) in combination with spatial filter processing (Rau et al. 1997; Reucher et al. 1987; Reucher et al. 1987b). The surface electrodes can be arranged in one- and two-dimensional arrays (also referred as arrays, matrixes or grids) and they serve different purposes (Fig. 3-5 b and c). Figures should appear below their reference

The first systems were developed by Masuda, Merletti and De Luca (Broman et al. 1985; Masuda et al. 1985; 1983; Merletti and De Luca 1989), who applied linear array electrodes along the muscle fiber direction. With this type of systems is possible to follow the action potential from the generation at the motor end-plate to the extinction at the tendons, providing information on motor unit anatomical properties (i.e., length and innervation zones) (Masuda et al. 1985; Rau et al. 1997; Zwarts and Stegeman 2003) and on the conduction velocity of the action potentials along the muscle fibers (Broman et al. 1985; Farina et al. 2000).

In the last decades, more complex systems with electrodes located both longitudinally and transversally with respect to the muscle fibers have been developed to determine motor unit territory and size (Roeleveld et al. 1998; Slawnych et al. 1997; Sun et al. 1999) and to discriminate trains of MUAPs from individual motor units (De Luca et al. 2006; Disselhorst-Klug et al. 2000; Holobar et al. 2009).

Apart from electrode arrangement, spatial filters are applied to improve selectivity by enhancing the signals of the most superficial motor units and suppressing the contributions of more distantly located traveling sources (Pozzo et al. 2003). Different one- and two-dimensional spatial filters have been applied displaying different spatial selectivity which may affect the estimation of the MUAPs features and its conduction velocity (Farina et al. 2003a; Farina et al. 2003b). Currently, two-dimensional spatial filters are preferable since it provides the best selectivity (Farina et al. 2003b).

Although detection systems with the high spatial resolution provide the possibility to assess a variety of information on the neuromuscular system, they are also sensitive to parameters involved in the generation and detection of the EMG signal (Farina et al. 2004c; Merletti and Farina 2004).

3.1.2 Factors influencing the EMG features

A number of simulation and experimental studies have investigated the effects of the anatomical characteristics, physical properties of the volume conductor and of the detection system on the surface EMG signals (Dimitrov et al. 2003; Farina et al. 2002b; Farina and Merletti 2001; Keenan et al. 2006; Merletti et al. 1999b; Rainoldi et al. 2004). Many physiological and non-physiological parameters have been identified and recently a systematic work on this field has been made by several research groups, in particular by Merletti and Farina's groups. The factors that influence the surface EMG are summarized in the table 3-1.

Table 3-1 – Factors that influence the surface EMG [from Farina et al. (2004c)]

Factors that influence the surface EMG		
Non-physiological	**Anatomical**	• Shape of the volume conductor • Thickness of the subcutaneous tissues layers • Distribution of the motor unit territories in the muscle • Size of the motor unit territories • Distribution and number of fibers in the motor unit territories • Length of the fibers • Spread of the end-plates and tendon junctions within and among motor units • Presence of more than one pennation angle
	Detection system	• Skin-electrode contact (impedance, noise) • Spatial filter for signal detection • Inter-electrode distance • Electrode size and shape • Inclination of the detection system relative to muscle fiber orientation • Location of the electrodes over the muscle
	Geometrical	• Muscle fiber shortening • Shift of the muscle relative to the detection system
	Physical	• Conductivities of the tissues • Amount of cross-talk from nearby muscles
Physiological	**Fiber membrane properties**	• Average muscle fiber conduction velocity • Distribution of motor unit conduction velocities • Distribution of conduction velocities of the muscle fibers within the motor units • Shape of the intracellular action potentials
	Motor unit properties	• Number of recruited motor units • Distribution of motor unit discharge rates • Statistics and coefficient of variation for discharge rate • Motor unit synchronization

According to Farina et al. (2004a), the effects of some of the referred factors are not intuitive and might differ with experimental conditions. However, some of these can be reduced significantly by constraining experimental setups to isometric contractions (Merletti and Farina 2004) and by appropriate placement of the electrodes (Farina et al. 2004c; Merletti and Farina 2004). For example, it has been shown that EMG variables are highly affected by electrode location with respect to innervation zones and tendons (Farina and Merletti 2001; Merletti et al. 1999a; Merletti et al. 1999b; Rainoldi et al. 2004). When the electrodes are placed over regions surrounding the innervation zones an error on the estimated variables will occur because the action potentials are travelling in opposite directions. This decreases the EMG amplitude and shifts the signal spectrum and conduction velocity towards higher values (Fig 3-6 b,c,d). Similar effects can be observed if the electrodes are located over the tendon regions because of the action potential extinction (Farina et al. 2002b; Farina et al. 2001a; Rainoldi et al. 2004). Thus the best locations for the electrodes positioning are the areas between the innervation zone ad tendon terminations where the EMG variables estimates are less affected by signal generation and extension as well by slight electrode displacements (Farina and Merletti 2001; Rainoldi et al. 2004). The proper electrode placement can be established by preliminary use of linear electrode arrays in order to identify the innervation zones and tendon regions (Masuda et al. 1985; Merletti et al. 2001b).

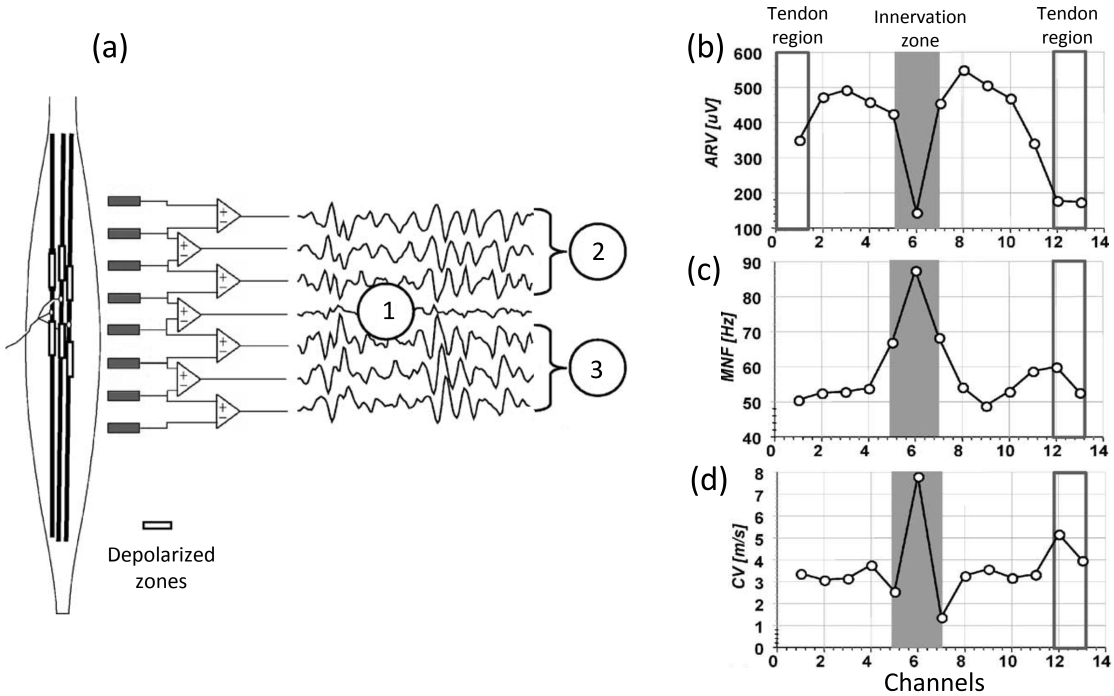

Figure 3-6 - Example of the EMG signal recorded with linear electrode array, in single differential configuration during a strong contraction of the vastus medialis muscle (a). The EMG amplitude is minimal over the innervation zone (1) and the propagation of the action potentials can be observed in opposite directions towards tendon terminations (2 and 3). Right side of the figure shows the variations of (b) amplitude (averaged rectified value), (c) spectrum (median power frequency) and (d) conduction velocity estimated along the muscle length [adapted from Rainoldi et al. (2004)].

Other factors, such as cross-talk, are non-intuitive. The cross-talk refers to a signal recorded over a muscle that is generated in a nearby muscle and that propagates through the volume conductor reaching the recording electrodes (De Luca and Merletti 1988; Farina et al. 2004c). It has been suggested that detection systems with high spatial resolution would reduce the contribution of this phenomena to the EMG signals (Disselhorst-Klug et al. 1997; Reucher et al. 1987). However, because the cross-talk seems to result majorly from the signals generated at the extinction the potentials at the tendon regions (Dimitrova et al. 2002), this non-propagating component of the signal, seems to be similarly detected by different detection systems and spatial filtering (Dimitrova et al. 2002; Farina et al. 2004d). The phenomena is considered complex and despite efforts this issue is not yet satisfactorily solved (Farina et al. 2002b; Farina et al. 2004c; Merletti et al. 2010).

3.2 Extraction of properties of the neuromuscular system from EMG signals

3.2.1 Global EMG variables

Global EMG variables describe macro-features of the signal, including zero crossing rate, spike properties, amplitude and frequency content (Merletti and Farina 2004). Classically, these EMG features have been computed from the interference surface EMG signals recorded with two electrodes in single differential mode. The amplitude and frequency content are the most used to infer on neuromuscular properties under different experimental conditions.

3.2.1.1 Amplitude

Because the surface EMG signals comprise the sum of the electrical contributions of the active motor units (both recruitment and discharge rates contributions), the amplitude of the signal is often used to quantify the magnitude of the motor unit activity and thus to estimate output from the spinal cord (Basmajian and De Luca 1985; Farina et al. 2004c). In the recent decades, it has been shown that EMG amplitude generally underestimates the neural activation sent to the muscles due to overlapping of positive and negative phases of the motor unit action potentials (Day and Hulliger 2001; Keenan et al. 2005). The cancellation may result in a reduction of the amplitude up to 62% at maximal activation (Keenan et al. 2005). In addition, during fatiguing contractions the duration of the action potentials increases, resulting in higher amplitude cancellation (up to~85%) (Keenan et al. 2005). This may confound interpretation of changes in surface EMG signals, such as estimation of the muscle force fluctuations and in explaining the failure of EMG amplitude to reach maximal levels at task failure during submaximal fatiguing contractions (Farina et al. 2004c; Keenan et al. 2005). In addition, the surface detection of the action potentials of the low-threshold motor units are more affected by cancellation (Keenan et al. 2006), which means that surface EMG amplitude is relatively insensitive to changes in the activity of this type of motor units (Farina et al. 2010). The effects of amplitude cancellation can be substantially reduced if the absolute EMG amplitude is normalized to a value obtained in a reference maximal contraction (Keenan et al. 2005; Yang and Winter 1984). Although the EMG amplitude is a valuable feature, still it is a crude index of the neural drive that is relative insensitive to small adjustments in motor unit activity in both fatiguing and non-fatiguing conditions (Farina et al. 2010).

3.2.1.2 Spectral analysis

The spectral analysis has been frequently applied to investigate muscle fatigue (Bigland-Ritchie et al. 1981; Bonato 2001; Komi and Tesch 1979; Merletti et al. 1990) or to infer changes in motor unit recruitment (Gerdle et al. 1991; von Tscharner et al. 2003; Wakeling et al. 2001). Different technical approaches have been applied ranging from the classic periodogram (Merletti and Lo Conte 1997) to more advanced methods, such as Cohen's class time-frequency distributions (Bonato et al. 2001; Ebenbichler et al. 2002) and wavelet analysis (Sparto et al. 2000; von Tscharner et al. 2003).

The rationale for these applications is originally coming from mathematical models developed by Lindstrom et al. (1970, 1977) and Stulen and DeLuca (1981), which have shown that mean and median frequency are linearly related to the average conduction velocity of the action potentials on the muscle fibers. Although these models contain several simplified assumptions (e.g., tissue isotropy; identical shape of the action potentials among single muscle fibers; homogeneity of the muscle fibers), their predictions have been confirmed by empirical experiments. During fatiguing isometric contractions, it has been shown that the decline in conduction velocity result in a proportional shift of the EMG power spectrum to lower frequencies (Arendt-Nielsen and Mills 1985; Naeije and Zorn 1982; Sadoyama et al. 1983). In addition, several studies observed that conduction velocity is positively correlated with fibre composition (Linssen et al. 1991; Sadoyama et al. 1988) and muscle force level (Arendt-Nielsen et al. 1984; Broman et al. 1985b). It is argued that these associations reflect a linear association between muscle fiber diameter and conduction velocity (Kupa et al. 1995). These findings raised the possibility of estimating muscle fiber composition, and thus motor unit recruitment strategies, from the spectral properties of the EMG signal (Gerdle et al. 1991; Kupa et al. 1995; Wakeling et al. 2001). However, the variations in power spectrum do not always follow the variations in conduction velocity (Falla et al. 2006; Farina et al. 2006; Kupa et al. 1995; Zwarts et al. 1987). Factors such as, recruitment and de-recruitment of motor units, changes in their discharge rates and distance between the active fibers and recording electrodes are some of the factors that may mask the association between conduction velocity and EMG spectral properties (Farina 2008; Farina et al. 2004c; van Boxtel and Schomaker 1984). For example, the power spectra of the action potentials of motor units with identical fiber membrane properties, located in different parts of the muscle may differ considerably due to the volume conductor (Farina 2008; Farina et al. 2004c). In addition, the power spectrum of the EMG signals is also dependent of anatomical properties of the muscle fibre such as length, position of the end-plate and fiber inclination (Dimitrov and Dimitrova 1998). Thus, these indicate that spectral properties of the EMG signals are affected by physiological parameters others than conduction velocity (Farina et al. 2004c; Farina et al. 2006; Zwarts et al. 1987). Moreover, these factors are intrinsic to the properties of the EMG signals and thereby they do not depend on the method used to estimate the power spectrum (Farina 2008). For that reason, a direct measure of the conduction velocity would be preferable.

3.2.2 Motor unit control strategies

The investigation of discharge rate patterns of single motor units provides relevant information on the motor output from the spinal cord, and this gives an insight into CNS control strategies (De Luca et al. 1982b; Erim et al. 1996). Such information can be achieved by decomposing EMG signals into their motor unit action potentials constituents (De Luca et al. 1982a; b; LeFever and De Luca 1982). The decomposition has been used to describe the process of identification and classification of individual MUAPs from a set of superimposed MUAPs trains belonging to concurrently active motor units (Fig. 3-7).

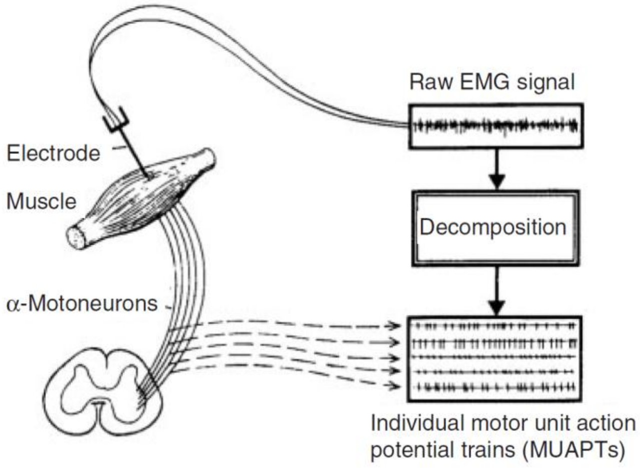

Figure 3-7 – Schematic representation of source, detection and decomposition of five superimposed motor-unit action potential trains that contribute to a detected intramuscular EMG signal [from De Luca et al. (1982)].

The classic approaches are based on the intramuscular EMG signals and they were firstly developed by De Luca and co-workers in the early 80’s (De Luca et al. 1982a; b; LeFever and De Luca 1982; LeFever et al. 1982). Since then, several researchers have been developing algorithms in order to provide a more complete and reliable decomposition of the intramuscular EMG signals (McGill et al. 2005; Nawab et al. 2008; Parsaei and Stashuk 2011; Stashuk 2001). These methods are typically implemented by employing digital signal processing and pattern recognition techniques (Parsaei and Stashuk 2011; Stashuk 2001). They involve complex rule-based algorithms that are able to: (i) identify action potentials using template matching and probability of firing statistics, (ii) resolve superimpositions of MUAPs and (iii) allocate the action potentials to motor units that created them (De Luca and Adam 1999) (for review see Parsaei and Stashuk 2011).

The accuracy that can be achieved in decomposing a particular signal depends both on the complexity of the signal and on the capability of the algorithm (McGill and Marateb 2011). If the EMG signal is too complex, or if the MUAPs are too similar, it might not be possible to accurately decompose the signal (McGill and Marateb 2011; Merletti and Farina 2009). The complexity, and therefore decomposability of the detected signals, is affected by factors, such as type and positioning of electrode and intensity of the muscle contraction (De Luca and Adam 1999; Stashuk 2001). For example, during electrode positioning is important to ensure that during a minimal contraction is possible to detect MUAPs of maximum amplitude and sharpness. This guarantees that the electrode is close to active muscle fibers and the detected MUAPs will be more distinct between them as well with respect to noise (Stashuk 2001). Moreover, controlling and reducing artifacts, such as electrode movement can also improve decomposition accuracy (Parsaei and Stashuk 2011). In general, signals detected during strong contractions are very complex (large number of active motor units) and thereby more difficult or even impossible to decompose (Merletti and Farina 2009). The performance of the decomposition algorithm can also be improved by filtering the signal in a signal pre-processing stage. Usually band-pass filters or low-pass differentiating filters are used to improve the stability of the baseline by reducing the baseline noise and accentuate the differences between MUAPs (Parsaei and Stashuk 2011). In addition, it reduces the amplitude of many similar shaped MUAPs of motor units that do not have fibers close to the electrode (Trontelj et al. 2004).

Despite recent advances, the correct determination of the discharge rate pattern and instant of recruitment or de-recruitment typically requires some degree of operator interaction in particular to verify and resolve superimpositions or to determine discharge rate pattern validity (Stashuk 2001). This might be very time-consuming, depending on complexity and quality of the signal as well as on the accuracy and ability of the decomposition method to resolve superimposed MUAPs (Merletti and Farina 2009). Moreover, the number of identified motor units is limited (normally less than 12), which is restricted to a very small portion of the muscle (Nawab et al. 2008; Trontelj et al. 2004).

An effort has been done during the recent years to improve accuracy and to achieve complete decomposition of the intramuscular signals. Example of this is the interactive computer program for decomposing single and multichannel intramuscular signals developed by McGill and collaborators (McGill et al. 2005; McGill and Marateb 2011). This program was designed to meet the demands of the decomposition process by including advanced algorithms for template matching, resolving superimpositions and waveform averaging as well as an useful interface for manually editing and verify the decomposition. This program proved to be highly accurate in decomposing intramuscular signals generated at low to moderate force levels (McGill et al. 2005; McGill and Marateb 2011). Nonetheless, depending on the complexity of the EMG signals, the referred automatic procedures may not achieve complete decomposition or incorrectly identify the time of occurrence of some individual action potentials. For this reason, it is important to have graphical commands that allow the user to correct the mistakes or fulfill the gaps in the discharge rate pattern. This graphical interface should provide relevant information to help the user in the manual decomposition, which includes the presentation of the EMG segment under decomposition; the templates of the identified motor unit spikes, the discharge rate patterns of the identified MUAP train and a close-up of the signals for resolving superimpositions (McGill et al. 2005) (Fig. 3-8).

In the present work, the information on the motor unit discharge rate patterns were extracted from the intramuscular EMG signals with a decomposition algorithm recently developed by McGill et al., (2005). This algorithm starts by reading the initial 2s of signal and based in statistical procedures automatically creates templates for all spikes that occur at least three times with a high degree of similarity. Then, over the same segment, the program attempts to automatically classify the remaining spikes by using template matching. Subsequently, the automatic decomposition should be manually inspected in order to complete the decomposition process and to verify the results. In the presence of a complex signal this process may take longer due to increased gaps or mistakes committed by the automatic decomposition. In this situation, the accuracy is subjectively assessed by inspecting (i) the residual signal following subtraction of the identified MUAPs from the signal and (ii) the discharge rate patterns. When the residual signal is very low, this indicates a good fit between the identified templates and the signal. Conversely, a large residual indicates an incomplete or incorrect decomposition. In addition, during steady contractions, the motor units usually discharge at regular inter-spike intervals. Thus when identified a regular discharge rate pattern it indicates with confidence that the decomposition is correct (McGill et al., 2005). The discharge rate patterns can be evaluated by observing the spike trains or the instantaneous firing rates following smoothing with a 2 Hz zero-phase-shift low pass filter. Therefore, when small residual is achieved and regular discharge rate pattern is observed, these indicate a complete and accurate decomposition (McGill et al., 2005). The decomposition algorithm will proceed in the same way in the subsequent selected EMG segments and slow changes in the spike shape are tracked by periodically re-averaging the templates by means of median averaging.

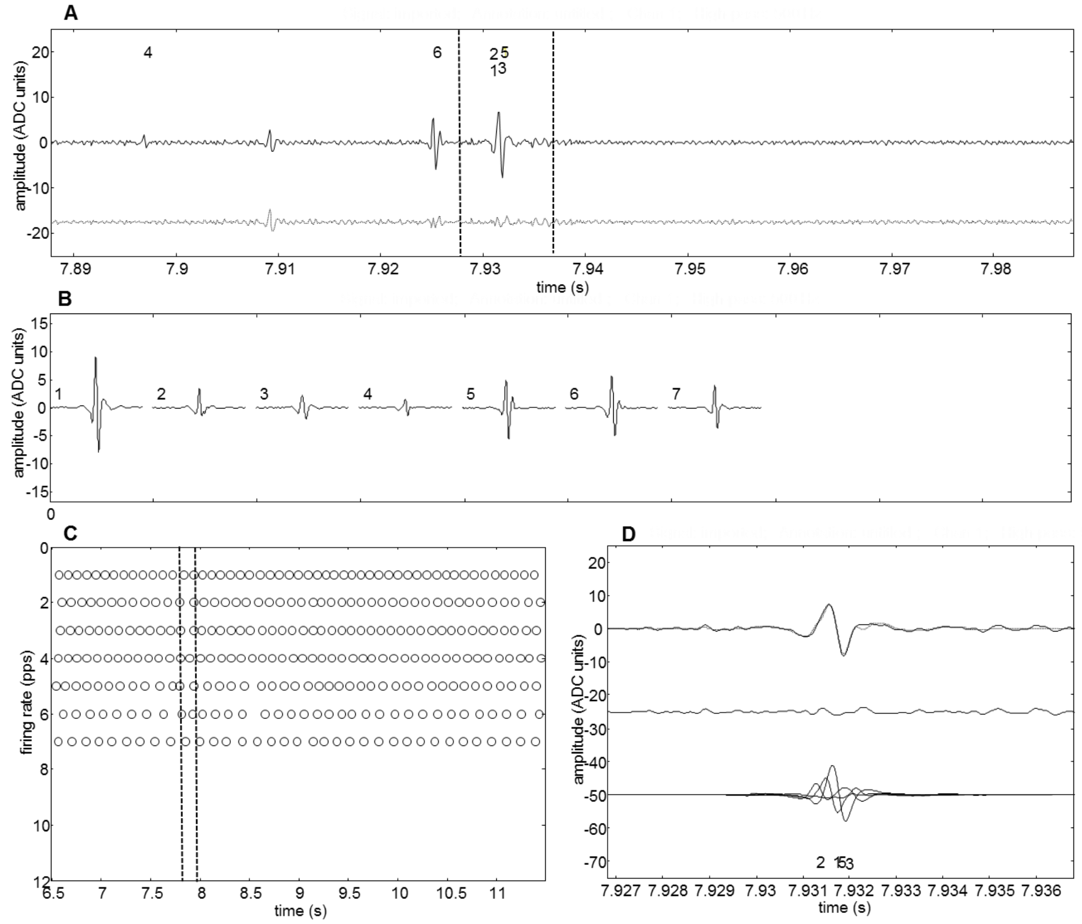

Figure 3-8 – Graphical interface of the interactive algorithm (EMGLAB) developed by McGill et al. (2005). (A) Signal panel showing a segment of the EMG signal (top trace) and the residual signal following subtraction of the identified MUAPs from the signal (bottom trace). The numbers indicate the identified motor unit discharges and the vertical lines indicate the interval of segment displayed in the close-up panel. (B) This panel shows the templates of the identified motor units. (C) Discharge panel, displaying the time occurrence of the discharges of each motor unit. The vertical lines refer to the time interval identified in A and zoomed in D. (D) Close-up panel showing a superimposition at an expanded scale (top solid line). The bottom traces are the templates of several motor units that solve the superimposition. The sum of the templates is represented by the top traced line. The middle line shows the residual signal. Figure plotted with data from study I (intramuscular EMG signal from the vastus medialis obliquus during an isometric contraction at 30% of the maximal voluntary force).

When several motor units are discharging within a time interval lower than 4 ms, the MUAPs sum together and generate a superimposition. Depending on the complexity of the superimposition (i.e. number of motor units and precise timing of discharge), the algorithm may be unable to identify all motor units involved. In such cases the superimposition needs to be solved manually. The interactive algorithm proposed by McGill et al., (2005), provides a close-up panel which displays the superimpositions at an expanded scale that permits the user to adjust different templates in order to find the best fit (Fig. 3-9).

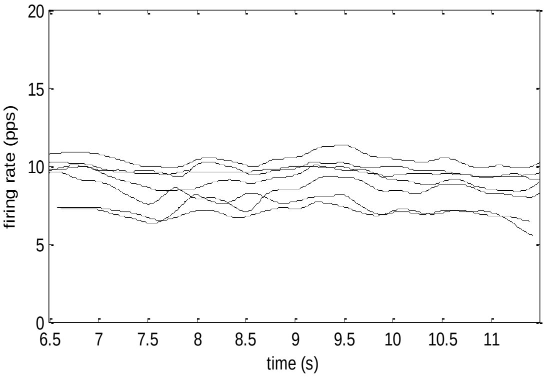

Figure 3-9 – Discharge panel displaying the discharge rate patterns plotted as instantaneous discharge rates (smoothed with a 2 Hz zero-phase-shift low pass filter). The figure shows the same data displayed in the Fig. 3-8.

Despite of recent advances the fully decomposition still relies on manual interaction with the interactive algorithms. Notwithstanding the limitations, discrimination of MUAPs from intramuscular signals with decomposition algorithms still is the best method to assess motor unit behavior. Important information can be extracted from the decomposed signals. Once the signal has been correctly decomposed a time series of all discharges of each detected motor unit is obtained, revealing information on recruitment and de-recruitment thresholds, discharge rate decay, inter-spike interval variability, synchronization and common drive (De Luca and Adam 1999; Merletti and Farina 2009). Moreover when combined with other measures such as force and surface EMG signals, information on contractile and muscle fiber membrane properties can be assessed by means of spike triggered averaging methods (Merletti and Farina 2009).

In the last years it has been shown that discrimination of single MUAPs from surface EMG signals is also possible if high-density surface EMG detection systems and advanced computational methods are applied (Farina et al. 2004b). Several algorithms have been proposed to decompose the surface EMG signals in their MUAPs constituents, showing promising results in particular during isometric contractions at low force levels (Disselhorst-Klug et al. 2000; Holobar et al. 2009; Kleine et al. 2007). However, the capacity of surface EMG recordings to discriminate MUAPs under different conditions as can be attained with intramuscular recordings is still debatable (for review see Farina et al. 2010). At the moment, the discrimination of MUAPs from intramuscular signals still presents fewer limitations than from surface signals and, moreover, they have been used to validate surface decomposition algorithms.

3.2.3 Conduction velocity estimation

Muscle fiber conduction velocity can be estimated either from intramuscular or surface EMG signals recordings (Arendt-Nielsen and Zwarts 1989; Zwarts 1989). The invasive methods are typically used in the clinical field and they involve stimulation of the muscle with a needle electrode (Arendt-Nielsen and Zwarts 1989; Troni et al. 1983). Following identification of the muscle fiber twitch a needle uptake electrode is inserted at knowing distance and the muscle fiber conduction velocity is computed from the distance and the spike latencies electrical stimulation (Arendt-Nielsen and Zwarts 1989; Troni et al. 1983). However, this method is time-consuming, painful and inherently limited to controlled experimental conditions (Troni et al. 1983). Thus, for the study of the conduction velocity during voluntary contractions, non-invasive methods are preferable (Merletti et al. 2003). Moreover, these methods provide valuable information on modifications of the peripheral properties of the neuromuscular system in response to fatigue (Farina et al. 2002a; Naeije and Zorn 1982), exercise (Pozzo et al. 2004; Rainoldi et al. 2008) or pathology (Zwarts and Stegeman 2003).

The conduction velocity can be directly estimated by computing the delay between two or more surface EMG signals detected along the muscle fiber direction (Arendt-Nielsen and Zwarts 1989; Farina and Merletti 2004b; Naeije and Zorn 1982; Zwarts 1989). Based on these signals it is possible to estimate either single motor unit (Farina et al. 2002a; Huppertz et al. 1997; Schulte et al. 2003) or global (Farina et al. 2000; Naeije and Zorn 1982; Zwarts 1989) conduction velocity.

Several approaches have been suggested and applied to estimate both motor unit and global conduction velocity, including cross-correlation function (Naeije and Zorn 1982; Zwarts and Stegeman 2003), estimation of a delay between any reference point (e.g. zero crossing, valley or peak) in two signal waveforms (Gydikov 1981; Hogrel and Duchene 2002) and maximum-likelihood techniques (Farina et al. 2001b; Schulte et al. 2003). All of them are based on the assumption that the EMG signals are in fact traveling along the muscle fibres and detected in this direction of propagation (Broman et al. 1985; Farina and Merletti 2004b). Under this condition, it is assumed that the distance between electrodes divided by the delay between the two signals provides a correct conduction velocity estimation (Arabadzhiev et al. 2004). Ideally the surface EMG signals detected along the muscle fiber direction would correspond to delayed versions of the same waveform (Farina and Mesin 2005). However, this is not the case during experimental conditions (Arabadzhiev et al. 2004; Farina and Mesin 2005).

Many factors contribute to changes in the shape of the detected signals, including a non-perfect alignment of the electrodes with respect to fiber direction, inhomogeneity of the volume conductor, the end-plate and end-of-fiber components, additive noise and sampling (Farina and Merletti 2004b). Thus, from the mathematical point of view the definition of delay between signals with different shapes is not trivial leading to different definitions of delay that may result in different conduction velocity estimates (Farina and Mesin 2005). Each definition represents an estimation method which may present different sensitivity to different factors (Farina and Merletti 2004b; Schulte et al. 2003). As a result, each method may perform differently depending on the conditions analyzed, with each having advantages and limitations that are more or less relevant depending on the application (Farina and Merletti 2004b). Thereby, as mentioned by Farina and Merletti (2004b), despite basic requirements for non-invasive conduction velocity estimation being rather simple, several limitations remain and many factors influence the results which one has to be aware of (Farina and Merletti 2004b; Farina et al. 2004f; Schulte et al. 2003).

3.2.3.1 Factors influencing the conduction velocity estimation

Early studies revealed that estimation of the conduction velocity from surface and intramuscular EMG signals were clearly correlated, but resulted in systematically higher conduction velocity values (~1m/s) with the surface than with the intramuscular approach (Sollie et al. 1985; van der Hoeven 1995; Zwarts 1989). It was suggested that a combination of physiological factors and sources of error would contribute for such difference (Arendt-Nielsen and Zwarts 1989; Zwarts 1989). Moreover, Broman et al. (1985) reported the presence of non–propagating waveforms in the surface EMG signals which biased the conduction velocity estimates toward higher values. Since then, a number of simulation and experimental studies have investigated the effects of many generation and detection EMG system parameters on conduction velocity estimation (Broman et al. 1985; Farina et al. 2002a; Farina et al. 2002b; Farina and Mesin 2005; Schneider et al. 1991). The major factors were listed in Farina and Merletti 2004b and are presented in the table 2.

Table 3-2 – Factors that influence the conduction velocity. The primary factors are those that directly affect conduction velocity estimation. The effects of the primary factors are dependent of other parameters of the surface generation and detection [from Farina and Merletti (2004b)]

Primary factors that affect CV estimates	Factors that influence sensitivity of the conduction velocity estimates to primary factors
Inclination of the fiber with respect to detection systems	1. Electrode shape and size 2. Spatial filter 3. Distance between fiber and detecting electrodes 4. Inter-electrode distance 5. Subcutaneous tissue layer thickness 6. Electrode location
End-plate and end-of-fiber components	Factors 1-6 7. Muscle fiber length 8. Spread of end-plates and tendon junctions within motor units 9. Spread of innervation zones and tendon regions among motor units
Tissue inhomogeneities Additive noise Non-propagating potentials from sources other than end-plate and end-of- fiber effects	10. Electrode-skin interface and electronics 11. Duration of signal epoch (for interference EMG signals) 12. Cross-talk from nearby muscles 13. Presence of more than one pennation angle

Although Broman et al. (1985) suggested that the non-propagating components might arise from the inhomogeneity and anisotropic properties of the volume conductor, recent work indicated that non- propagating waveforms are mainly due to the end-plate and end-of-fiber effects arising from the generation and extinction of the intracellular action potentials (Arabadzhiev et al. 2003; Dimitrov and Dimitrova 1998). The relative weight of the non-travelling signals depends on the thickness of subcutaneous layers, distance between the detection system and the innervation and tendon region and cross-talk from the nearby muscles (Dimitrova et al. 1991; Farina et al. 2002b; Schulte et al. 2003). The application of different spatial filters may suppress to a different extent the non-travelling components (Arabadzhiev et al. 2003; Farina et al. 2002b; Schulte et al. 2003). Figure 3-10 shows some of the spatial filters investigated.

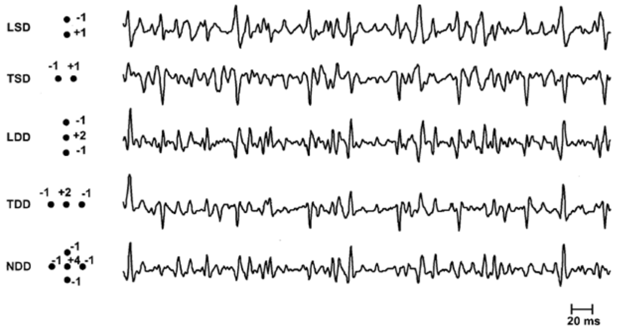

Figure 3-10 – Scheme and raw surface EMG signals detected with longitudinal and transversal single and double differential (LSD, TSD, LDD, TDD, respectively), Laplacian filter (NDD) in the tibialis anterior [adapted from Farina et al. (2003a)].

The application of spatial filters such as longitudinal double difference filters (Broman et al. 1985; Farina et al. 2002b) or bipolar transversal double difference filters (Arabadzhiev et al. 2003) have been shown to be effective in reducing the effects of non-travelling components on the conduction velocity estimates. However, the application of such filters still presents limitations on estimation of the conduction velocity from signals detected near the end-place and end-of-fiber regions (Arabadzhiev et al. 2003). Moreover, the application of multichannel techniques does not reduce the sensitivity to this phenomenon or to the inclination of detection systems with respect to muscle fiber direction, showing similar performance to two-channel techniques (Farina et al. 2002b; Farina et al. 2000). For this reason, the electrodes should be well aligned with the muscle fiber direction and placed in the most centered area between the innervation zone and tendon region, where the smallest variations of the estimation can be observed (Farina et al. 2002b). The clear identification of the referred anatomical landmarks is of relevance for reliably estimating the conduction velocity. This can be made by using a multichannel detection system (Fig. 3-11).

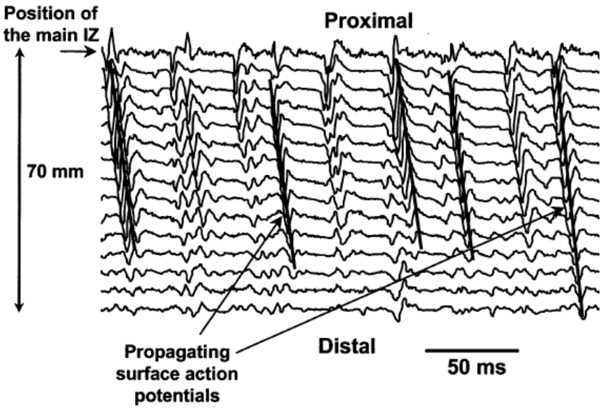

Figure 3-11 – Representative EMG signal acquired with a linear surface array (16 channels and 10 mm inter-electrode distance) during an isometric contraction of the tibialis anterior at 25% of the maximal voluntary force. Clear propagation of MUAPs along the array can be recognized by visual inspection of the acquired signals [adapted from Farina et al. (2002a)].

The correct alignment of the detection system with respect to the muscle fiber direction is a critical issue and not easy to detect in in-vivo tests (Pozzo et al. 2004). It requires expert operators that are able to visually detect a clear propagation of the detected signals from the innervation zone to the tendon region (Farina et al. 2002a; Pozzo et al. 2004). The channels that proportionate the most similar propagating waveform signals should be selected (Fig. 3-11). This constrains the number of muscles in which the conduction velocity can be study. Thus, for muscles with more than one fiber orientation or with spread innervation zones (e.g. rectus femoris or deltoid muscle), the conduction velocity estimation is very poor (Mesin et al. 2007).

Another issue in the conduction velocity estimation is the repeatability and reproducibility of the measures. The initial studies in surface conduction velocity, reported a “remarkable” variability of the conduction velocity values depending on the recording site along the muscle fibers which was attributed to tissue inhomogeneities (Schneider et al. 1991). Although the proper placement of the electrodes can reduce the variability, a substantial reduction can be observed when the number of spatially filtered signals used to estimate either global or motor unit conduction velocity is increased (Farina et al. 2002a; Farina et al. 2004f) (Fig. 3-12).

The inter-electrode distance is also a critical parameter that may have opposite effects (Farina et al. 2004f). Simulation and experimental studies showed that, an increase of the distance between channels leads to an improvement of the stability of the conduction velocity estimates and increases reproducibility (Farina and Mesin 2005; Farina et al. 2004f). However, this reduces the number of available signals which is also determinant to reduce variance and increased reproducibility (Farina et al. 2004f). The effect of this combination of factors is difficult to predict and depends on the fiber length and muscle anatomy (Farina et al. 2004f). For instance, shorter muscles will require smaller inter-electrode distances to detect the necessary number of signals, whereas for longer muscles with the innervation zones concentrated in a small region, larger distances may be used. Depending on the experimental conditions and muscle to be analyzed the researcher or clinician needs to find the best compromise between number of detected signals and inter-electrode distance.

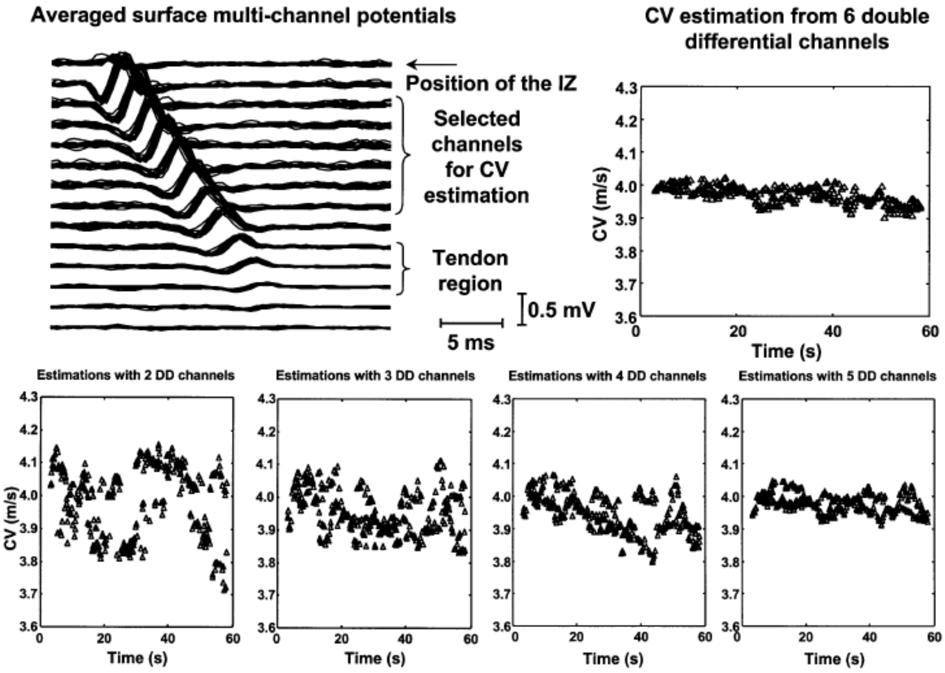

Figure 3-12 – Example of estimation of single motor unit CV from twenty averaged MUAPs, using different number of surface EMG channels. From the figure, it is evident the difference between the optimal number of channels (six channels) and the classic two-channel regarding the variance of the CV estimation [from Farina et al. (2002a)].

The variance of the conduction velocity estimation can also be reduced if appropriate computational methods are used. The few studies comparing different methods reported lower variances for the maximum likelihood estimate than for other methods (Farina et al. 2002a; Schulte et al. 2003). This approach can be applied either to estimate the global or the motor unit conduction velocity.

3.2.3.2 Motor unit vs. global conduction velocity estimation

The motor unit conduction velocity is estimated from individual motor unit action potentials extracted from the interference surface EMG signals, providing information on the muscle fiber membrane properties. On the other hand, the global conduction velocity corresponds to a weighted average of the delays of propagation of the action potentials of all the active motor units firing during the selected epoch (Farina and Merletti 2004a). Thus, variations of the global measures might result from changes in the muscle fiber membrane properties and/or alterations in the motor unit recruitment (Gazzoni et al. 2001). For instance, during submaximal contractions the global conduction velocity may not change or even increase over time due to recruitment of fast motor units with higher conduction velocities (Gazzoni et al. 2001). This was also observed in our investigations on conduction velocity during submaximal sustained contractions (Fig. 3-13).

In order to distinguish the central and peripheral effects on the conduction velocity estimation, the analysis should be at motor unit level (Cescon and Gazzoni 2010; Gazzoni et al. 2001). Such measures imply the extraction of single motor unit action potentials from the surface interference signals.

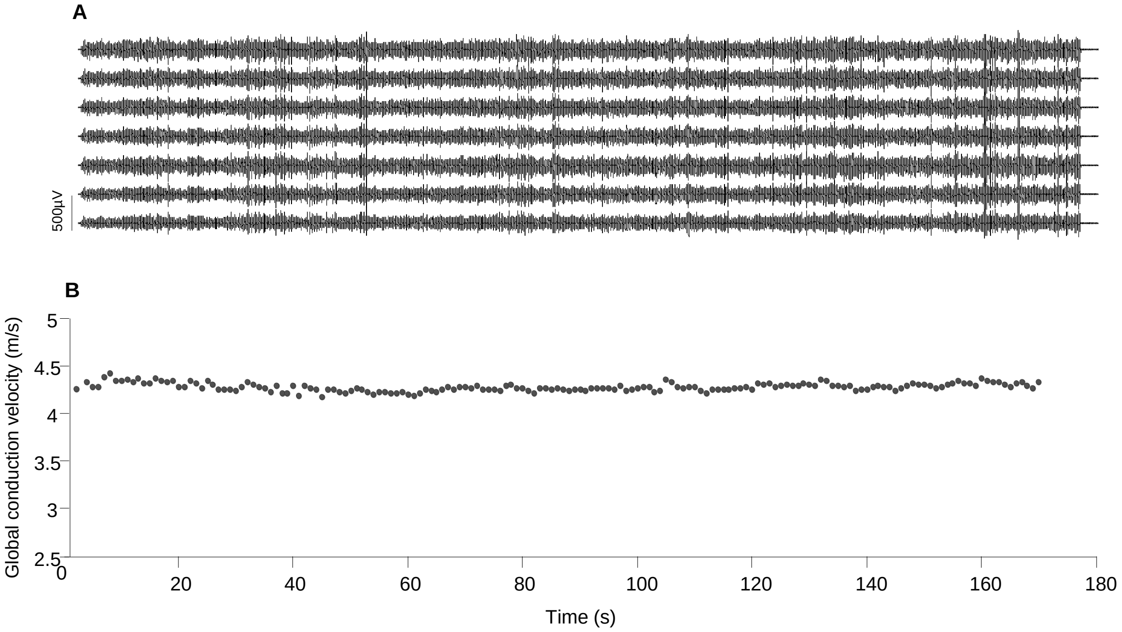

Figure 3-13 – Representative EMG signal acquired with a linear surface array (8 channels and 5mm inter-electrode distance) during an isometric contraction of the vastus medialis obliquus at 30% of the maximal voluntary force (A) and estimation of global conduction velocity in consecutive non-overlapping epochs of 1s (B). Figure plotted with data from the study II.

Recently, Farina et al. (2002a) proposed a combination of intramuscular and multichannel surface techniques to estimate single motor unit conduction velocity. The intramuscular recordings provide information on the MUAPs occurrences that can be used to trigger the averaging of the surface EMG signals and thus to estimate the surface representation of the single motor unit action potentials (Farina et al. 2002a; Roeleveld et al. 1997) (Fig. 3-14).

The signal-noise ratio can be enhanced by increasing the number of averages. With this technique a reliable estimation of single motor unit conduction velocity can be achieved with a low number of averages (20 firings), allowing the detection of changes of the motor unit conduction velocity over time with a good temporal resolution (Farina et al. 2002a). This was not possible with the classical approaches applied in the past, due to larger variability of the motor unit conduction velocity estimates.

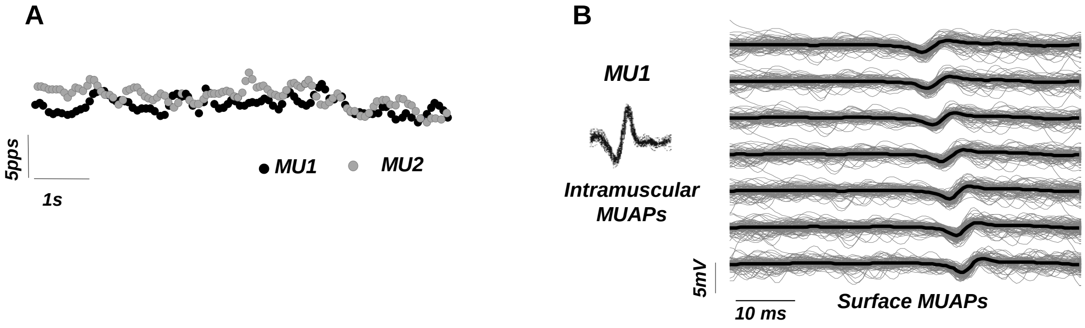

Figure 3-14 – Example of the instantaneous discharge rate of two motor units identified for the vastus medialis obliquus at 30% maximal force (A). The time of occurrence of the individual motor unit action potentials in the intramuscular signals were used as trigger for averaging the multichannel surface EMG signals. (B) Example of surface averaged action potentials belonging to a motor unit (MU1). The gray lines represent the superimposed action potentials belonging to the target motor unit and the black line indicate the average potential over the seven bipolar channels. Figure plotted with data from study II.

The variance is substantially reduced by increasing the number of detected surface signals along the muscle fiber (Fig 3-12) and by computing the motor unit conduction velocity through the maximum likelihood estimate method, as previously referred. Moreover, the electrodes should be correctly aligned and at middle distance between the innervation and tendon zone. Due to the spatial selectivity multichannel detection systems, the intramuscular wires should be inserted in the muscle close to the surface electrodes and the depth should be just a few millimeters below the muscle fascia. This maximizes the number of common motor units detected by both intramuscular and surface multichannel detection systems and thus the conduction velocity can be estimated for a large number of motor units.

Despite the complexity and limitations, the combination of intramuscular and surface multichannel techniques is of particular interest, since that can provide reliable information on both central and peripheral properties of the single motor units (Farina et al. 2002a; Farina et al. 2004a). This allows a simultaneous investigation of the physiological and motor control adjustments of the motor units in response to particular environments or motor training. Such information is relevant in applied fields such as ergonomics, exercise physiology or rehabilitation.

3.3 Electromyographic responses to peripheral nerve stimulation

Since the pioneer work of Paul Hoffman in the early 20th century, the electrical peripheral nerve stimulation has been used to assess changes in the input to spinal motoneurons (Magladery and Mc 1950; Misiaszek 2003; Pierrot-Deseilligny and Burke 2005). It is known that changes in any of the spinal pathways would result in changes of the excitability of the motoneurons, "the final common pathway", conditioning the motor unit behavior (Pierrot-

Deseilligny and Burke 2005). However, the ongoing EMG traces are not sensitive enough to reveal subtle changes in motoneuron excitability (Misiaszek 2003; Pierrot-Deseilligny and Mazevet 2000). Such might be possible by applying an electrical stimulation to a peripheral nerve and recording the evoked compound muscle action potentials in the homonymous muscle as an EMG response (Magladery and Mc 1950; Zehr 2002). A number of methods have been developed to investigate different spinal pathways. In the following subsections the methods applied in the PhD project are described.

3.3.1 Electrically evoked potentials

The electrical stimulation of a mixed peripheral nerve, above the motor threshold, elicits two synchronous EMG responses: (i) a direct M-wave and; (ii) a reflexive response (H-reflex). The underlying principle relies on the monosynaptic reflex arc, which is responsible for the tendon Jerk (Knikou 2008; Pierrot-Deseilligny and Mazevet 2000). The Ia afferent fibers that originates from the muscle spindles have monosynaptic excitatory projections to the motoneurons innervating the homonymous muscle (Pierrot-Deseilligny and Burke 2005). The major difference between the electrically elicited reflex (H-reflex) and the spinal stretch reflex is that the H-reflex bypasses effects of γ–motoneurons and of the muscle spindle discharge (Pierrot-Deseilligny and Burke 2005). A simplified sketch of the monosynaptic reflex is shown in the figure 3-15 A.

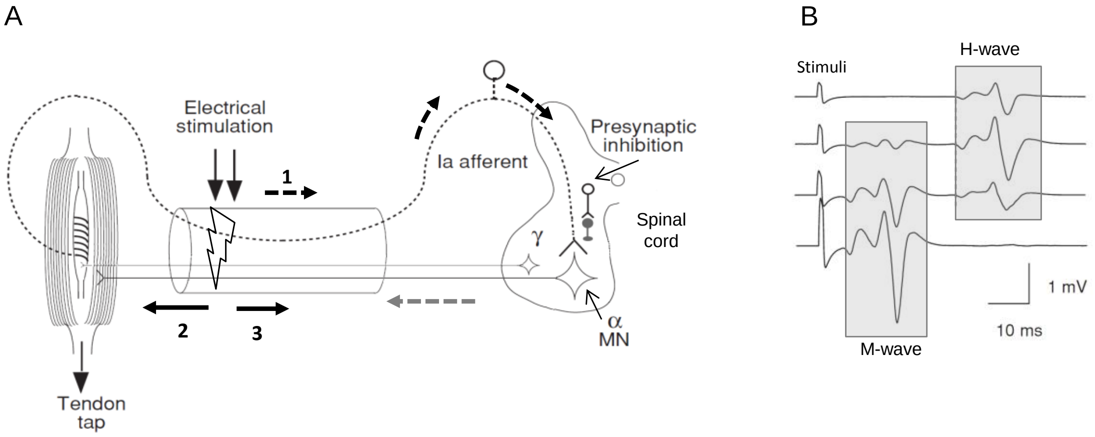

Figure 3-15 – (A) Illustration of the pathway of the monosynaptic reflex. Ia afferents from muscle spindle primary endings (dotted line) have monosynaptic projections to α-motoneurons innervating the corresponding muscle (homonymous MNs). The H reflex is produced by electrical stimulation of Ia afferents (1) that reach the spinal cord, which travel down the α-motoneurons axons to the muscle. Gradually increasing the stimulus intensity elicits a direct motor response (2) and simultaneously action potentials propagate antidromically (2) in the α-motoneurons axons toward the spinal cord (3) to collide with action potentials of the evoked reflex response (1), resulting in partial cancellation of the reflex response. (B) Representation of the volleys in the Ia afferents and motor axons when the stimulus intensity is progressively increased [adapted from Pierrot-Deseilligny and Burke (2005)].

When an electrical stimulus is applied to the nerve the Ia afferent fiber are the first to be activated due to their larger diameter. Subsequently the afferent volley propagates towards the spinal cord leading to a monosynaptic excitation of the target motoneurons. The action potentials generated by the excited motoneurons travel down into the muscle and a compound response with a latency of 30-40ms (in the soleus muscle) can be observed in the EMG trace (Fig. 3-15 B). With increasing stimulus intensity the thinner motor axons are progressively activated generating action potentials that: (i) travel down to the muscle as a direct response (M-wave; latency of 5-8 ms in the soleus) and; (ii) antidromically in direction to spinal cord, colliding with the evoked reflex response and resulting in a partial cancellation of the H-reflex (Aagaard et al. 2002b; Tucker et al. 2005). Thus, with increasing stimulus intensity the M-wave increases, while the H-reflex amplitude gradually decreases due to the antidromic collision (Fig. 3-15 B). When applied an incremental intensity from zero an H and M recruitment curve can be obtained (Fig. 3-16).

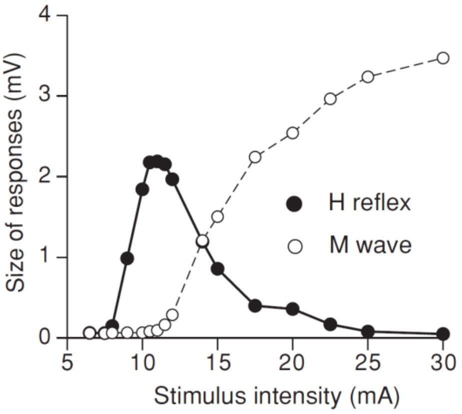

Figure 3-16 – H and M-wave recruitment curve. The amplitudes are plotted against stimulus intensity [adapted from Pierrot-Deseilligny and Burke (2005)].

3.3.2 Changes in spinal input investigated by electrically evoked potentials

The responses of both M and H waveforms to changes in intensity of stimulation describe the input/output relation of each phenomenon which can be quantified by specific parameters (Zehr 2002). The ascending limb of the H-reflex is of particular interest and parameters such as H-reflex threshold, the maximum H-reflex response and the slope of the ascending limb are normally used to represent the alterations in the H-reflex input/output function, providing information on the gain and recruitment of motor units of different thresholds (Klimstra and Zehr 2008; Misiaszek 2003). Because, these parameters might be differentially affected by different experimental protocols, it is frequently convenient to acquire many measures of the H-reflex excitability (Carroll et al. 2011; Misiaszek 2003; Zehr 2002). The maximal M-wave occurs when all motor axons are recruited and thus provides an estimate of the response given by the whole motoneuron pool (Pierrot-Deseilligny and Mazevet 2000). This parameter is normally used as a normalization factor, in which the H-reflex is expressed as a percentage of the M_{max} (H/ M_{max}). By this process, it is possible to remove influence of the changes in muscle geometry related to muscle length and contraction, as well changes in the muscle fibre membrane properties (Nielsen and Clausen 2000; Pierrot-Deseilligny and Mazevet 2000; Tucker et al. 2005). The magnitude of the H-reflex amplitude is influenced by other methodological factors, including the size of the afferent volley elicited by the stimulation pulse, background level of muscle activation, muscle contraction intensity, posture and attention level of the subjects (Carroll et al. 2011; Klimstra and Zehr 2008; Misiaszek 2003; Zehr 2002). Therefore, appropriate interpretation of the H-reflex response requires that particular recording conditions are achieved (Knikou 2008; Zehr 2002). These methodological issues and recommendations are described in the paper *"Changes in H-reflex and V-wave following short-term endurance and strength training"* (part II).

Due to the direct synaptic connection of Ia afferents and α-motoneurons, it has been tempting for the researchers to use the elicited H-reflex as a direct measure of the excitability of the α-motoneurons (Misiaszek 2003; Pierrot-Deseilligny and Mazevet 2000). However, when the muscle is voluntary activated, the H-reflex amplitude is dependent of several physiological factors such as presynaptic inhibition, level of efferent and descending neural drive and recurrent inhibition (Crone et al. 1990; Hultborn et al. 1987; Pierrot-Deseilligny and Mazevet 2000). Moreover, the H-reflex shape and specially the latter part of the reflex, is known to have oligosynaptic contributions from the spindles afferents as well as the involvement of inhibitory Ib effects from Golgi tendon organs (Hultborn et al. 1987; Pierrot-Deseilligny and Mazevet 2000).

As main caveat of the H-reflex measures is that might not be possible to discriminate the contributions of different synaptic inputs to the H-reflex amplitude (Misiaszek 2003; Pierrot-Deseilligny and Burke 2005). Nevertheless when combined with other methods the participation of particular mechanisms can be distinguished (Misiaszek 2003). For example, the V-wave which is an electrophysiological variant of the H reflex recording during maximal voluntary effort, has been shown to be more sensitive to changes in supraspinal input to the motor neuron pool (Aagaard et al. 2002b; Duclay and Martin 2005; Upton et al. 1971), while the H-reflex is more sensitive to altered presynaptic inhibition and motoneuron excitability (Ekblom 2010; Hultborn et al. 1987; Misiaszek 2003; Nordlund et al. 2002; Pierrot-Deseilligny and Mazevet 2000). Thus, combined measures of the H-reflex and V-wave may provide a discrimination between changes in the motoneuron excitability elicited supraspinal or spinal inputs, helping to understand the sites and mechanisms of the neural adaptations (Aagaard et al. 2002b; Upton et al. 1971). This is discussed in the paper *"Changes in H-reflex and V-wave following short-term endurance and strength training"* (part II).

PART II

Experimental Studies,
General Discussion and Conclusion

CHAPTER

4

Study I

Motor unit behavior during submaximal contractions following six weeks of either endurance or strength training program.

Carolina Vila-Chã, Deborah Falla, Dario Farina

J Appl Physiol. 2010 Nov;109 (5):1455-66.

Abstract

The study investigated changes in motor output and motor unit behavior following 6 weeks of either strength or endurance training programs commonly used in conditioning and rehabilitation. Twenty-eight sedentary healthy men (age, mean ± SD, 26.1 ± 3.9 yrs) were randomly assigned to strength training (ST) ($n = 9$), endurance training (ET) ($n = 10$) or a control group (CT) ($n = 8$). Maximum voluntary contraction (MVC), time to task failure (isometric contraction at 30% MVC), and rate of force development (RFD) of the quadriceps, were measured before (week 0), during (week 3), and after a training program of 6 weeks. In each experimental session, surface and intramuscular EMG signals were recorded from the vastus medialis obliquus (VMO) and vastus lateralis (VL) muscles during isometric knee extension at 10% and 30% MVC. After 6 weeks of training, MVC and RFD increased in the ST group (17.5±7.5 % and 33.3±15.9 %, respectively; $P < 0.05$) whereas time to task failure was prolonged in the ET group (29.7±13.4 %; $P < 0.05$). The surface EMG amplitude at 30% MVC force increased with training in both groups but the training-induced changes in motor unit discharge rates differed between groups. After endurance training, the motor unit discharge rate at 30% MVC decreased from 11.3 ±1.3 pulses per second (pps) to 10.1 ± 1.1 pps ($P < 0.05$) in the vasti muscles, whereas after strength training it increased from 11.4 ± 1.2 pps to 12.7 ± 1.3 pps ($P < 0.05$). Finally, motor unit conduction velocity during the contractions at 30% MVC increased for both the ST and ET groups, but only after 6 weeks of training ($P < 0.05$). In conclusion, these strength and endurance training programs elicit opposite adjustments in motor unit discharge rates but similar changes in muscle fiber conduction velocity.

4.1 Introduction

Motor performance is enhanced by repeated exposure to exercise training. Depending on the desired goal, exercise paradigms may include strength, sprint, endurance or skill training. The muscular and neural adaptations induced by each type of exercise approach are highly specific and may vary for different training paradigms. Typically, endurance training involves generalized muscle activation performed over many repetitions (34, 62). Exercises such as running or cycling are classic examples of endurance training and are known to improve the ability to sustain rhythmic movements for longer periods, mainly due to increased maximal oxygen uptake and increased ability of skeletal muscles to generate energy via oxidative metabolism (28). At the other extreme, strength training typically involves exercises for specific muscle groups which are performed over a short-duration, e.g. performing few repetitions at high force levels (34, 58, 62).

Distinct anatomical and physiological adaptations in response to conventional strength and endurance training have been documented (for review see Refs. 21-23, 28). Because these two types of exercise programs represent extremes of physical activity (58), they may also elicit different neural adaptations. Accordingly, the increases in maximal strength and rate of force development achieved with strength training appear to be impaired when endurance and strength training are applied concurrently (27, 49). This effect has been mainly attributed to an opposite influence of the two training regimes on the neural control of muscles (16, 27). For example, Hakkinen et al (27) showed that muscle activity at the onset of a rapid isometric explosive contraction was impaired by concurrent endurance and strength training even though similar morphological adaptations occurred with respect to strength training only. In addition to muscular adaptations, the effects of strength and endurance training on motor performance reflect supraspinal and spinal adjustments (6, 15), which ultimately influence the neural drive to the muscles, i.e. the behavior of motor units. However, as recently discussed (15), *in vivo* data on motor unit properties following training are scarce. Only a few studies have investigated motor unit behavior following strength training (31, 51, 56, 61) and the results remain controversial. For example, increased motor unit discharge rates have been observed after explosive (61) and dynamic strength training (31), however no changes were observed after isometric training (54, 56). Furthermore, the effects of training on the discharge rates assessed during maximal and submaximal contractions show mixed results (31, 54, 56).

Currently there are no available data on changes in motor unit discharge behavior following endurance training. Although there are speculations on changes in motor unit recruitment and discharge rates with endurance training, these conclusions are largely based on reflex studies (37, 42, 52) and animal experiments (8, 9). For example, Pérot et al. (52) reported an increase of the H-reflex after 8 weeks of endurance training, indicating a potential increase in motor neuron pool excitability. Accordingly, for the same relative force level, endurance training was shown to increase the proportion of recruited low-threshold motor units (52). However, it is not possible to draw firm conclusions on changes in motor unit behavior based on the H-reflex response (24, 63).

The specificity of adjustments in motor unit behavior with different types of training is poorly understood, mainly due to a limited number of studies in this field in addition to a lack of studies which have employed the same methodology to compare changes in motor unit behavior following different training paradigms. Therefore, the purpose of this study was to investigate and compare changes in muscle activity and motor unit behavior of synergistic knee extensor muscles following a strength and an endurance training program. It was hypothesized that differences in motor output (maximal strength, rate of force development, and resistance to fatigue) induced by the two training programs would be associated with different adjustments in motor unit discharge rates. In addition, muscle fiber conduction velocity was measured for individual motor units as an indicator of changes in muscle properties following the training programs.

4.2 Methods

Subjects

Thirty healthy men (age, mean ± SD, 26.0 ± 3.8 yrs) with no history of lower limb disorders participated in the study. None of the subjects were involved in regular strength or endurance training. All subjects gave their informed consent to the procedures of the study. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (N-20090032). After the first experimental session (pre-training session), the subjects were randomly assigned to one of three groups: strength training (ST) (n = 10); endurance training (ET) (n = 10); and control group (CT; no exercise intervention) (n = 10).

Training programs

Progressive endurance or strength training was performed over 6 weeks. Subjects attended three training sessions per week. All training sessions were supervised by an investigator of the study (CV-C).

Endurance training. Endurance training was performed on a bicycle ergometer and the exercise intensity was prescribed based on the percent of the heart rate reserve (HRR). The lower and upper limits of HRR [target heart rates (THR)] were calculated with the Karvonen method [(maximal heart rate - resting heart rate) × % Intensity + resting heart rate] (32). The use of %HRR has been recommended for prescribing exercise intensity in cycling activities (39), since it provides accurate target workloads, especially for individuals with a low fitness level (60).

To maintain the exercise intensity within the required THR, each subject used a pulse meter during the training sessions. From week 1 to week 2, the THR was set between 50% and 60% of the HRR and each training session lasted 20-30 min. On weeks 3-4, the duration of the sessions was 30-40 min and THR was 60%-70% of the HRR. For the last two weeks, the time per session increased to 40-50 min and the THR was maintained at 65-75% of the HRR.

Strength training. Each training session involved three bilateral leg exercises (leg press, leg extension, and leg curl) and four additional exercises for the other main muscle groups of the body (lateral pull down, bench press, exercise for the trunk flexors and for trunk extensors). For the leg exercises, the subjects trained with loads of 60-70% of the one-repetition maximum

(1RM) and performed 3 sets of 13-15 repetitions during weeks 1 and 2. During weeks 3 and 4, the load intensity was increased to 70-75% of the 1RM, and subjects performed 3-4 sets of 10-12 repetitions. Finally, during weeks 5 and 6, the load intensity varied in the range 70-85% of the 1RM and the participants performed 3-4 sets of 8-12 repetitions. The additional exercises for the upper limbs and trunk were performed over 2-3 sets of 13-18 repetitions at an intensity corresponding to 60-70% of the 1RM. The training load was continuously monitored allowing the necessary adjustments to keep the intensity at the required level.

Procedure

The subjects attended three laboratory sessions, immediately before (session 1 – week 0), 3 weeks after the beginning of training (session 2 – week 3), and after completion of the 6-week training period (session 3 – week 6). In order to avoid an effect of fatigue on the results, sessions 2 and 3 were performed at least 48 hours after a training session.

In each experimental session, the subject was comfortably seated on an Isokinetic dynamometer (KinCom Dynamometer – Chattanooga, TN, USA) with their trunk reclined to 15° in an adjustable chair and their hip and distal thigh firmly strapped to the chair. The rotational axis of the dynamometer was aligned with the right lateral femoral epicondyle and the lower leg was secured to the dynamometer lever arm, above the lateral malleolus. Maximal and submaximal isometric knee extensions were exerted with the knee flexed to 90º.

Following placement of the surface EMG electrodes (as described below), the subject performed two maximal voluntary contractions (MVC) of knee extension over a period of 5 s. These trials were separated by 2 min of rest. The highest MVC value was used as a reference for the definition of the submaximal force levels. In each of the three experimental sessions, the submaximal forces were relative to the MVC measured during the same session. After the maximal contractions, the subjects performed two explosive isometric contractions and they were encouraged to exert their maximal force as fast as possible.

Intramuscular EMG electrodes were then inserted into the vastus lateralis (VL) and vastus medialis obliquus (VMO), as described below. The subjects performed two isometric knee extensions at 10% and 30% MVC (random order) for 10 s, with 2 min of rest in between. Subjects were provided with online visual feedback of the force exerted which was displayed on an oscilloscope. Subjects then performed a further isometric knee extension contraction at 30% MVC maintaining the force for as long as possible. Time to task failure was defined as a drop in force greater than 5% of the target force level for more than 5 s, after strong verbal encouragement to the subject to maintain the target force. During the submaximal contractions, knee extension force and intramuscular and surface EMG of the VMO and VL were recorded concurrently (Fig.4-1).

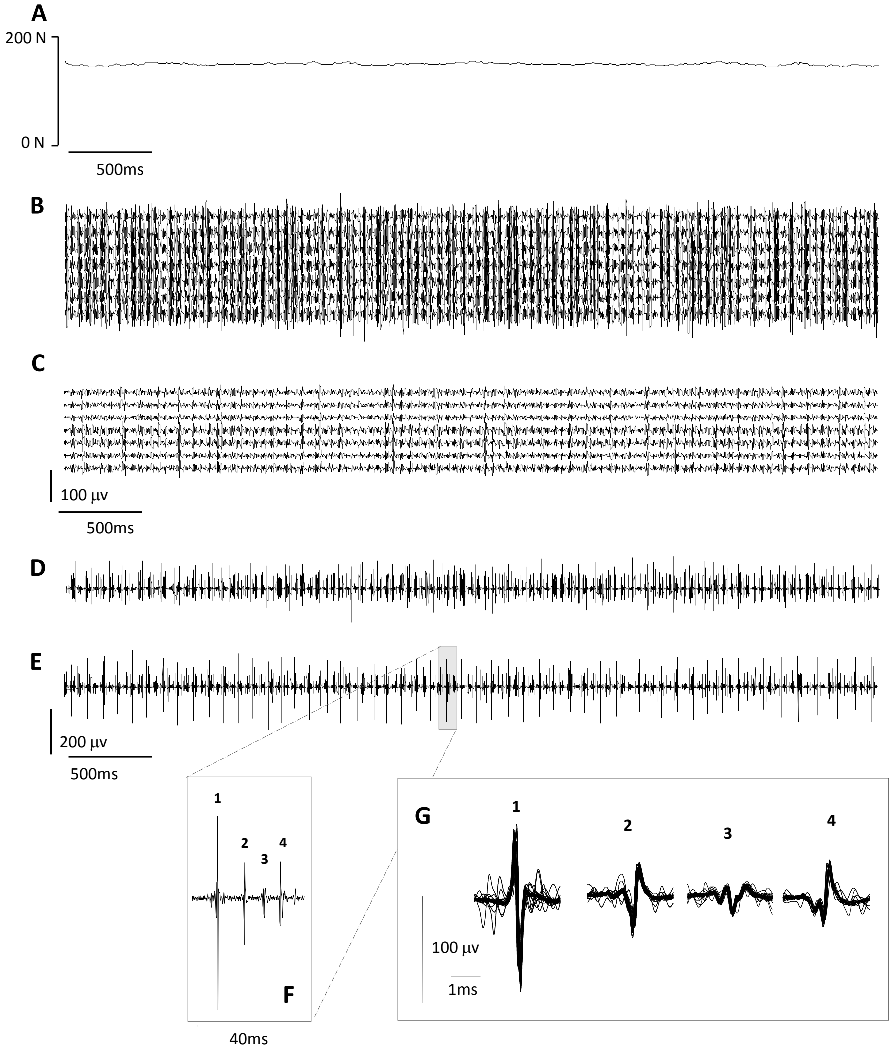

Figure 4-1 – Representative recordings obtained during an isometric knee extension contraction performed at 30% of the maximum voluntary force. ***A***: force exerted by the knee extensors. ***B***: surface EMG signals from the vastus medialis obliquus. ***C:*** surface EMG signals from the vastus lateralis. ***D:*** intramuscular EMG signal from the vastus lateralis ***E:*** intramuscular EMG signal from the vastus medialis obliquus. ***F:*** interval of 40 ms of intramuscular EMG signal from the vastus medialis obliquus. ***G***: superimposed action potentials generated by the target motor units observed in the time interval illustrated in ***F***.

EMG recordings

Surface EMG signals were acquired from the VL, VMO and biceps femoris (BF) muscles during the maximal and submaximal isometric contractions. Signals from the VL and VMO were detected with adhesive linear arrays of 8 electrodes (5×1 mm size, 5-mm interelectrode; SPES Medica, Salerno, Italy). The arrays were located between the innervation zone and the distal tendon, along the direction of the muscle fibers. The tendon regions and innervation zones were identified during preliminary brief knee extensions (45). Signals from the BF were recorded with Ag–AgCl electrodes (Ambu Neuroline, conductive area 28 mm2), located as recommended by Hermens et al (29). Prior to placement of the electrodes, the skin was shaved, lightly abraded and cleansed with water. A ground electrode was placed around the right ankle. Surface EMG signals were amplified as bipolar derivations (EMG amplifier, LISiN-OT Bioelettronica, Torino, Italy), band-pass filtered (-3dB bandwidth, 10-500Hz), sampled at 2048 samples/s, and converted to digital data by a 12-bit A/D converter board.

Intramuscular EMG signals were recorded from the VMO and VL with two pairs of wire electrodes (50-μm-diameter) made of Teflon coated stainless steel (A-M Systems, Carls, WA, USA). Electrodes were inserted with a 23 G needle, 10-20 mm proximal to the surface electrodes. The wires were uninsulated for ~1mm at the tip to detect intramuscular EMG signals. The angle of insertion of the needle was ~ 45°, and the depth was a few millimeters below the muscle fascia. The needles were removed after insertion and the wire electrodes were left inside the muscle. Intramuscular EMG signals were amplified as bipolar derivations (Counterpoint EMG, DANTEC Medical, Skovlunde, Denmark), band-pass filtered (500 Hz – 4 kHz), sampled at 10,000 Hz, and stored after 12-bit A/D conversion.

Signal analysis

During the submaximal contractions of 10-s duration, the average rectified value (ARV) was computed from the surface EMG in intervals of 1 s. These values were averaged over the entire duration of the contraction and over all channels of the electrode array. During the explosive contractions, ARV was calculated in two intervals of 50-ms duration and averaged over all channels of the electrode array. The first interval started 70-ms prior to the onset of force (2, 13). The onset for force was defined as the time instant when force exceeded 8.5 N. The second interval was centered at the time instant of the maximal slope in force (Fig. 4-2). Rate of force development (RFD) was calculated from the explosive contractions as the maximum slope of the force-time curve (Δforce/Δtime).

The intramuscular EMG signals recorded during the 10-s submaximal contractions were decomposed with a decomposition algorithm (47). The software displays a segment of the EMG signal, the templates of the action potentials of the identified motor units, the discharge patterns, and a close-up of the signal for resolving missed discharges and superimpositions. Accuracy of the automatic decomposition was achieved by inspection of the identified discharge patterns.

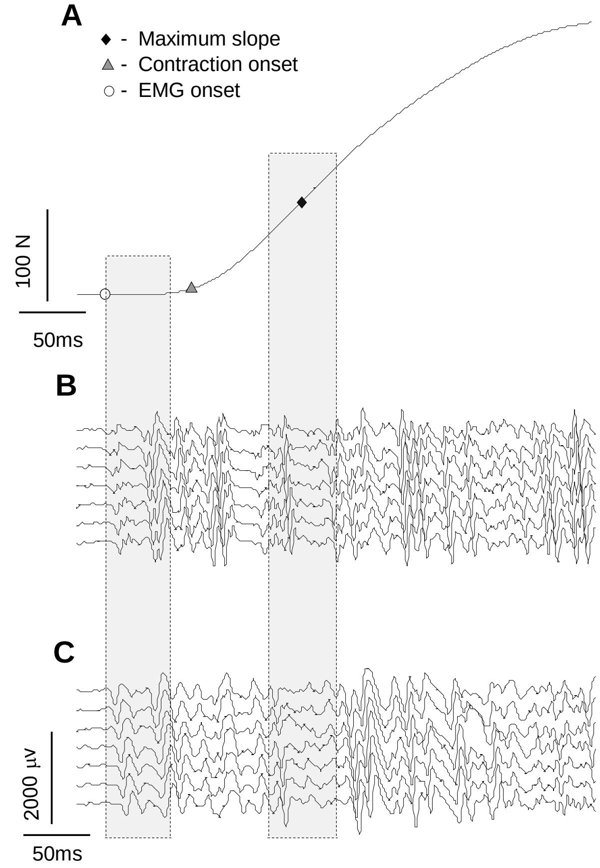

Figure 4-2 - Representative recordings obtained during the explosive isometric knee extension contraction. ***A***: force exerted by the knee extensors. ***B***: surface EMG signals recorded from the vastus medialis obliquus. ***C:*** surface EMG signals recorded from the vastus lateralis. Surface EMG signals were analyzed in two intervals of 50-ms *(gray boxes).* The first interval started 70 ms prior to the onset of force (*open circle*) and the second interval was centered at the time instant of the maximal slope in force (*black diamond)*. The onset of force was defined as the time instant when force exceeded 8.5 N *(gray triangle).*

Full, regular patterns provided confidence that the decomposition was correct, whereas gaps, extra discharges or uneven intervals provided an indication of possible decomposition errors. To assist in identifying missed discharges the program displays bars in the signal panel that indicate the expected discharge times of each motor unit. The signal portion can then be viewed in the close-up panel which displays the signal at an expanded scale and allows matching motor unit templates to be selected. The close-up panel also displays superimpositions at an expanded scale which allows verification of the result or allows different sets of templates to be selected and adjusted to find the correct fit. Commands are also available for undoing identifications and deleting or merging templates. Unusually short (<20 ms) or long (>200 ms) interspike intervals (ISIs) were manually reanalyzed and corrected when necessary.

The mean discharge rate [pulses per second (pps)] and ISI variability (SD of ISI divided by mean ISI, %) were computed from the motor unit spike trains. For the motor units that could be identified during both the contraction at 10% and 30% MVC in the same session (by visual inspection of superimposed action potential templates), the increment in mean discharge rate between 10% and 30% MVC (Δ MDR) was also computed.

Electrophysiological membrane properties of the muscle fibers of individual motor units were investigated by estimating motor unit conduction velocity (MUCV). This was achieved by extracting the averaged multi-channel surface EMG signals with the intramuscular action potentials as triggers (18). The MUCV was estimated from the averaged surface EMG with a multi-channel technique previously described (18). MUCV values were obtained from the 10-s isometric knee extensions at 10% and 30% MVC.

Statistical analysis

The effects of the two training programs on strength (MVC), time to task failure, rate of force development (RFD), and ARV of the BF were assessed with two-way repeated measures ANOVA with factors group (control, endurance, and strength) and time (week 0, 3, and 6). Additionally, changes in motor unit discharge rates, ISI variability, ARV of the VMO and VL and MUCV were evaluated with three-way repeated measures ANOVA with factors group, time, and muscle. Pair-wise comparisons were performed with the Student-Newman-Keuls post-hoc test when ANOVA was significant. The significance level was set to $P < 0.05$. Results are reported as means and SD in the text and mean and SE in the figures.

4.3 Results

The three groups initially consisted of 10 subjects each; however 1 subject from the ST group and 2 subjects from the CT group did not complete the final experimental session and were excluded from the analysis. Thus, the results are presented for 9 subjects in the ST (age, 25.4 ± 4.2 yrs; height, 183.4 ± 6.9 cm; weight, 80.3 ± 16.3 kg), 10 subjects in the ET (age, 26.1 ± 2.8 yrs; height, 180.6 ± 6.2 cm; weight, 78.3 ± 14.1 kg) and 8 subjects in the CT group (age, 27.0 ± 5.0 yrs; height, 175.3 ± 3.4 cm; weight, 78.0 ± 13.0 kg). No differences were observed between groups for age, height and weight. Furthermore, no differences were observed between the groups for any of the motor output or electrophysiological parameters assessed in the first experimental session (prior to training). Although not statistically significant, the ST group showed a trend towards a relatively longer time-to-task-failure compared to both the ET and CT group (Fig. 4-3). This was mainly attributed to the values for time-task-failure from two subjects, which was substantially above the average of the group at baseline (243.2s and 316.7s).

Motor output

As expected, endurance and strength training induced specific changes in motor performance over the training period (Fig. 4-3). Six weeks of endurance training increased the time to task failure by 29.7% ± 13.4 (Fig. 4-3*A*), but did not change MVC or maximal RFD (Fig.4-3 *B,C*). In contrast, 6 weeks of strength training induced an increase in the MVC force by 17.5 ± 7.5% (Fig. 4-3 *B*) and maximal RFD by 33.3% ± 15.9 (Fig. 4-3*C*) but did not influence time to task failure (Fig. 4-3*A*). None of motor output parameters changed in the CT group (Fig. 4-3). Although not statistically significant, both training groups showed changes in MVC after only 3 weeks of training. The trend for a decrease in MVC for the endurance group was mainly due to two subjects. These subjects showed a decrease of the MVC which was much greater (30.0 ± 0.1%) than the rest of the group (1.6 ± 10.6%).

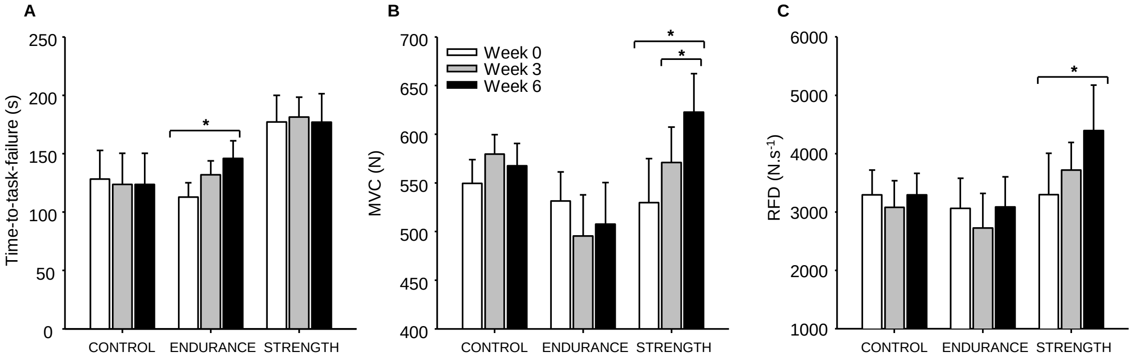

Figure 4-3 - Changes in motor performance across the 6 week training intervention. Values are mean ± SE. ***A***: Time to task failure assessed during isometric contractions at 30% MVC. **B:** Maximal voluntary contraction (MVC) of the knee extensors. ***C:*** Contractible rate of force development (RFD). *: $P < 0.05$.

Conduction velocity

Figure 4 shows the MUCV for the VM and VMO (average over both vasti) during the submaximal contractions at 10 and 30% MVC for each experimental session. In the first 3 weeks no statistically alterations of the MUCV were observed (p = 0.25). For the contractions at 30% MVC, MUCV increased from baseline to 6 weeks post training for both training groups (P < 0.05; Fig. 4-4) without differences between the two training groups ($P > 0.11$). Despite the trend for increased MUCV over time for the contractions at 10% MVC, no statistical differences were observed at that contraction level (main effect for time; P = 0.08).

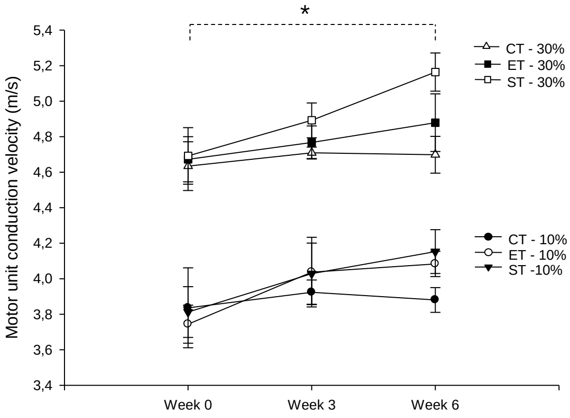

Figure 4-4 - Mean ± SE of motor unit conduction velocity for the vasti muscles (average of the vastus medialis obliquus and vastus lateralis) during isometric knee extensions at 10% and 30% of MVC for the control (CT), endurance (ET) and strength (ST) group. * $P < 0.05$

Surface EMG

Fig. 4-5 shows the EMG amplitude for the VMO and VL during maximal (MVC) and submaximal isometric knee extension contractions, for each experimental session. In the two training groups, the ARV of VMO and VL showed similar changes over the training period (interaction: time × muscle; $P > 0.42$ for all isometric contractions). Further, the activity of the BF muscle did not differ across sessions in any of the groups (interaction: time × group; $P > 0.55$ for all isometric contractions).

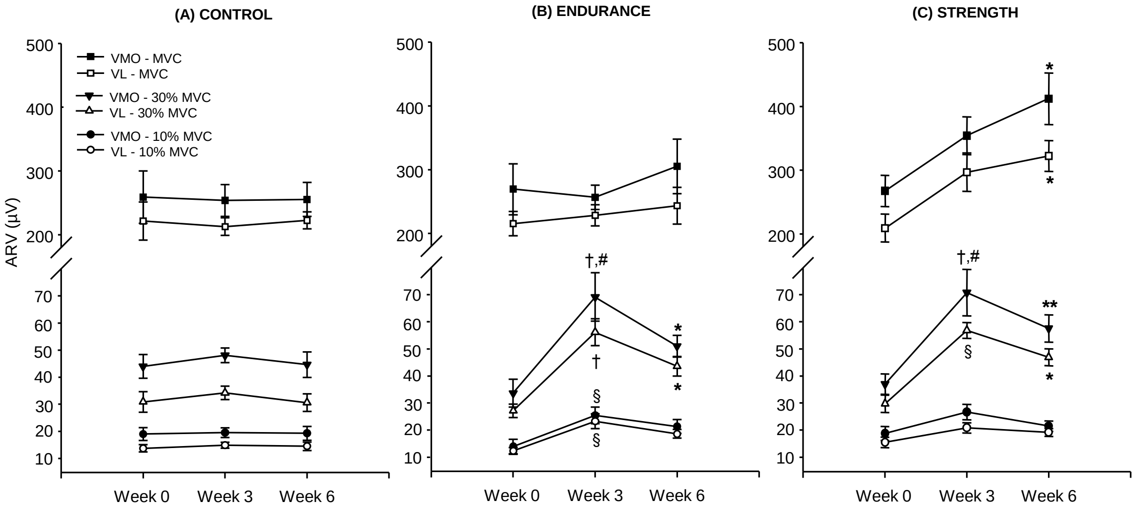

Figure 4-5 – Mean ± SE for the average rectified value (ARV) of the vastus medialis obliquus (VMO) and vastus lateralis (VL) obtained during maximal (MVC) and submaximal (10% and 30% MVC) isometric knee extension contractions, across each experimental session for: - **(A)** ***Control group***; - **(*B*)** ***Endurance Group***. §: $P < 0.01$ from week 0 to week 3. †: $P < 0.0001$, from week 0 to week 3. #: $P < 0.01$ from week 3 to week 6. *: $P < 0.05$ from week 0 to week 6; **- (*C*)** ***Strength group.*** †: $P < 0.0001$ from week 0 to week 3. #: $P < 0.05$ from week 3 to week 6. **: $P < 0.001$ from week 0 to week 6. *: $P < 0.05$ from week 0 to week 6.

For the contraction at 30% MVC, both training groups displayed increased values of VMO and VL ARV after 3 weeks of training (Fig. 4-5*B,C*). The ARV increased by 101.8 ± 52.4% (average of both vasti) for the ET group (Fig. 4-5*B*) and 92.6 ± 54.6% for the ST group (Fig. 4-5*C*). In the second half of the training program (from week 3 to week 6), the ARV declined in both training groups (average over group and muscles, 45.9 ± 35.0%), however the changes were only significant for the VMO muscle (Fig. 4-5 *B*,*C*).

Despite this reduction, ARV remained 47% to 62% higher at week 6 compared to baseline (significantly different between week 0 and 6 for all muscles in both groups; Fig. 4-5). A similar pattern was observed for the isometric contractions at 10% MVC, especially for the ET group (Fig. 4-5*B*). No significant changes in EMG ARV were observed for the CT group for any of the submaximal contractions (Fig. 4-5*A*). In contrast to the submaximal contractions, only the ST group showed a significant change in ARV during the maximal contractions (Figs. 4-5*C*). After 6 weeks of strength training, the ARV during MVC increased by 60.7 ± 36.7 % and 55.4 ± 30.9% for the VMO and VL, respectively (Fig. 4-5*C*).

The ARV for the VMO and VL in the early phase of the explosive contractions (0-50ms) increased progressively over the 6 week training period for the ST group (Fig. 4-6*A*). By the end of the strength training program, the ARV had increased by 167.1 ± 99.8% for the VMO and by 107.2 ± 75.3 % for the VL (Fig. 4-6*A*). The ARV also increased in the second time interval analyzed (50 ms centered on maximum slope) (Fig. 4-6*B*). Contrary to the ST group, the ET and CT groups did not show any significant change in EMG ARV during the explosive contractions (Fig. 4-6*A*,*B*).

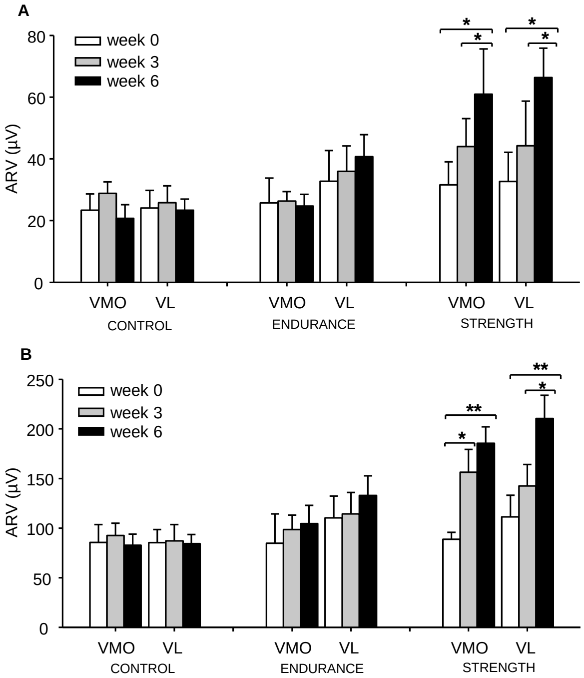

Figure 4-6 - Mean ± SE of the EMG average rectified value (ARV) for the vastus obliquus (VMO) and vastus lateralis (VL) obtained during the isometric explosive contractions of the knee extensors at week 0, 3 and 6 for all groups. **A:** ARV calculated in the time interval 0-50ms, relative to the onset of the EMG. **B:** ARV assessed during a time interval of 50 ms centered at the time instant of the maximum slope. *: $P < 0.05$; **: $P < 0.001$.

Motor unit behavior

Discharge rate and ISI variability were obtained from a total of 1398 motor units during the 10-s isometric contractions at 10% and 30% MVC (Table 4-1). The number of motor units recorded per subject in each experimental session ranged between 1 and 10 at each force level and for each muscle (average: 4 motor units in each condition).

Fig. 4-7 illustrates the mean motor unit discharge rate for the VMO and VL during the submaximal contractions at 10% and 30% MVC. As observed for the surface EMG ARV, in each group, no differences in the mean motor unit discharge rate were observed between the VMO and VL in each experimental session (interaction: time × muscle; $P > 0.72$ for both contractions).

For the contractions at 10% MVC, the average motor unit discharge rate was not influenced by training, except for the VMO in the ST group (Fig. 4-7*C*). However, at 30% MVC force, significant changes in motor unit discharge rate were observed at the end of the first half of the training program, in both groups (Fig. 4-7*B,C*). From week 0 to week 3, mean motor unit discharge rate of the vasti declined by 8.3 ± 4.9 % in the ET group (average over VMO and VL; both $P < 0.05$) whilst an increase of 8.4 ± 5.2% was observed in the ST group (for VMO $P < 0.05$; changes did not reach significance for VL, $P = 0.12$).

Table 4-1 – Interspike interval (ISI) variability for the motor units identified for each group (control, endurance and strength), muscle [vastus medialis obliquus (VMO) and vastus lateralis (VL)], load (10% and 30% MVC) and session (week 0, 3 and 6).

Group	Load/ muscle	week 0		week 3		week 6	
		VMO	VL	VMO	VL	VMO	VL
Control	10%	13.5 ± 4.6 (n = 27)	12.8 ± 4.4 (n = 27)	10.4 ± 1.4 (n = 26)	11.5 ± 3.5 (n = 26)	11.9 ± 0.9 (n = 32)	12.6 ± 4.3 (n = 25)
	30%	12.9 ± 3.8 (n = 38)	12.4 ± 3.2 (n = 35)	11.8 ± 1.2 (n = 39)	10.5 ± 1.2 (n = 38)	12.9 ± 2.2 (n = 36)	11.8 ± 0.8 (n = 32)
Endurance	10%	13.4 ± 2.6 (n = 39)	12.0 ± 3.8 (n = 38)	12.1 ± 3.6 (n = 49)	11.2 ± 3.4 (n = 40)	12.0 ± 4.6 (n = 40)	10.7 ± 4.6 (n = 32)
	30%	11.6 ± 2.8 (n = 41)	11.0 ± 2.8 (n = 40)	11.5 ± 1.3 (n = 52)	10.1 ± 1.2 (n = 48)	12.3 ± 3.6 (n = 50)	11.3 ±2.6 (n = 46)
Strength	10%	16.5 ± 4.4 (n = 32)	13.4 ± 3.4 (n = 33)	12.5 ± 2.1 * (n = 33)	11.4 ± 3.8 (n = 41)	10.8 ± 1.7 ** (n = 45)	10.5 ± 2.7 (n = 41)
	30%	15.7 ± 2.9 (n = 40)	12.9 ± 3.3 (n = 49)	14.5 ± 3.6 (n = 39)	12.6 ± 2.6 (n = 50)	12.2 ± 3.1 # (n = 43)	12.5 ± 2.8 (n = 45)

*: $P < 0.05$ comparing week 0 to week 3; **: $P < 0.01$ comparing week 0 to week 6; #: $P < 0.05$ comparing week 0 to week 6.

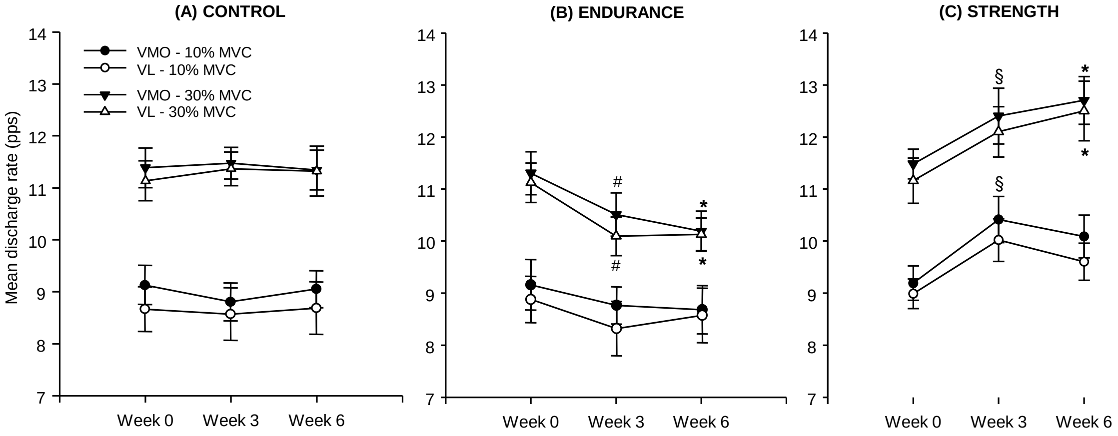

Figure 4-7 - Mean ± SE of motor unit discharge rate for the vastus medialis obliquus (VMO) and vastus lateralis (VL) obtained during submaximal isometric contractions of the knee extensors across experimental sessions for: - ***(A) Control group***; - ***(B) Endurance group***. Significant changes were only observed at 30% MVC. #: $P < 0.05$ from week 0 to week 3. *: $P < 0.05$ from week 0 to week 6. - ***(C) Strength group***. §: $P < 0.01$ from week 0 to week 3. *: $P < 0.01$ from week 0 to week 6.

During this period the small changes in absolute loads applied during the contractions at 30%MVC did not correlate with the changes in discharge rates observed in both training groups ($0.11 < P < 0.79$, for both training programs and muscles). In the following 3 weeks (between week 3 and 6), smaller and non-significant changes of the average motor unit discharge rate were observed for both training groups (Fig. 4-7*B,C*). Over the total training time of 6 weeks, the average motor unit discharge rate decreased by 10.9 ± 6.2 % (VMO) and 10.6 ± 5.6 % (VL) in the ET group and increased by 10.7 ± 4.9 % (VMO) and 12.8 ± 4.7% (VL) in the ST group (Fig. 4-7*B,C*).

The ISI variability did not change significantly with training, with the exception of a moderate decrease in the VMO muscle of the ST group (Tab. 4-1). A subset of 242 motor units were identified during contractions at both 10% and 30% MVC within the same experimental session. For each experimental session and muscle the number of motor units identified at both force levels ranged between 9 and 22. At least one motor unit was identified at both contraction forces for each subject and experimental session. Fig. 4-8 shows motor unit data recorded from the VMO during the contractions at 10% and 30% MVC, both pre- and post-training from a representative subject of each group. In this example, the difference in rate between the lower and the higher force (ΔMDR) decreased following endurance training (Fig. 4-8*B*) and increased following strength training (Fig. 4-8*C*).

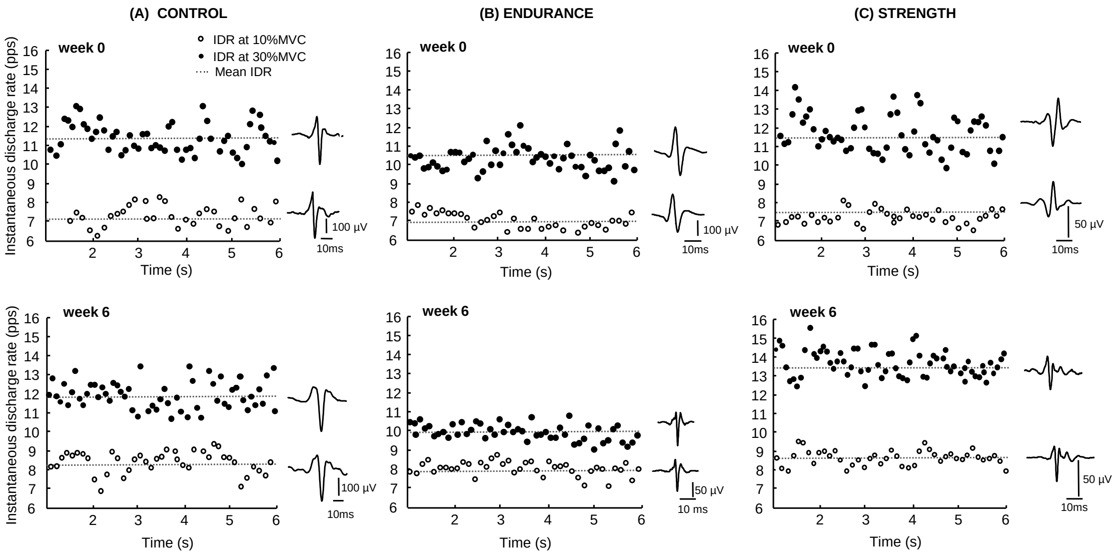

Figure 4-8 - Instantaneous discharge rate of motor units identified for the VMO muscle during isometric contractions at both 10% and 30% MVC in the experimental session prior to training (week 0) and after training (week 6) for representative subjects in the: **(*A*) *Control group*, (*B*) *Endurance group*** and **(*C*) *Strength group***. Motor unit action potential templates are provided to the right of each graph which confirms that within the same experimental session (week 0 or week 6), the action potentials are presumably generated by the same motor unit monitored for both the 10% and 30% MVC contractions. In the pre-training session, the ISI variability, quantified as coefficient of variation of the ISI, for the motor units shown in this figure for the control, endurance and strength group was 21.9%, 9.6%, and 16.5% at 10% MCV and 17.1%, 9.3%, and 12.4% at 30% MVC. In the post-training session and for the same groups the coefficient of variation of the ISI was 10.1%, 6.1% and 8.9% at 10% MVC and 9.8%, 5.2% and 8.3% at 30% MVC, respectively.

Accordingly, the group data showed that 6 weeks of endurance training decreased the ΔMDR of the VMO by 1.12 ± 0.8 pps (Fig. 4-9*B*) whereas strength training evoked an increment of 1.28 ± 0.7 pps in the same muscle (Fig. 4-9*C*). For the VL muscle, ΔMDR increased by 1.60 ± 0.8 pps in the ST group and decreased (although non significantly) by 0.7 ± 0.6 pps in the ET group (Fig. 4-9*B,C*). No changes were observed over time for the CT group (Fig. 4-8*A* and 4-9*A*).

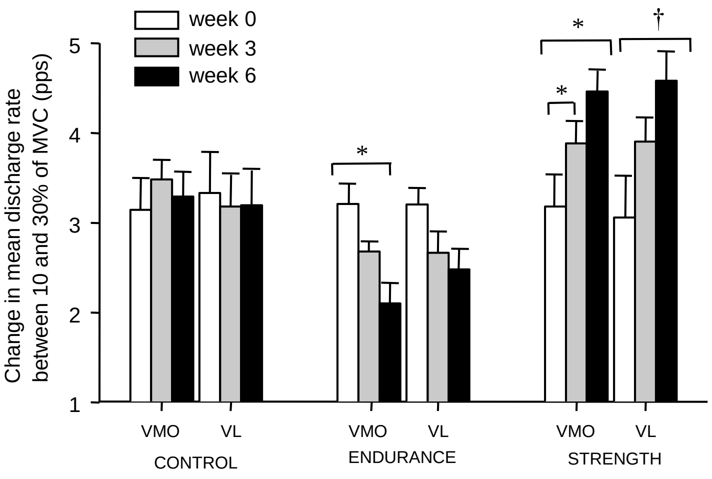

Figure 4-9 - Mean ± SE of the change in mean discharge rate for motor units detected at both 10% and 30% MVC (ΔMDR) for the vastus medialis obliquus (VMO) and vastus lateralis (VL). Data are presented only for motor units that could be identified during both the contraction at 10% and 30% MVC in the same session (by visual inspection of superimposed action potential templates). (A) Control group, (B) Endurance group, (C) Strength group. *: $P < 0.05$; †: $P < 0.001$.

4.4 Discussion

This study shows distinct adjustments in motor unit discharge rates and similar changes in motor unit conduction velocity for the knee extensors following strength and endurance training.

Training programs

Conventional endurance and strength training programs were used in this study since they represent common training programs applied in both conditioning and rehabilitation fields. Typically, endurance training implies alternated rhythmic extension and flexion of the limbs, such as running or cycling, which are performed for long periods at low-force levels (27, 58). On the other hand, common strength training programs involve rhythmic extension and flexion of either one or both limbs, performed at high-force levels for short periods of time (27, 58). Given these differences between strength and endurance training, the changes in motor performance are also expected to be divergent (27). Manipulation of specific training stimulus variables, such as intensity and time of exposure, evokes multiple physiological and neurological adaptations, contributing to motor performance enhancement. Furthermore, the magnitude of these alterations is strongly dependent on the progressive increment of training variables. In the present study, both training programs were designed to introduce progressive increments in volume and intensity to allow progressive adaptation, avoiding the risk of overtraining. Moreover, a systematic increase of demands placed upon the body is necessary for progressive improvement (36). This model of training may have contributed to differences between our results and those of previous studies on motor unit adaptations to strength training (31, 54, 56). Another important consideration is the type of movement pattern used in the different studies. Previous studies on motor unit adaptations to training involved only unilateral and single-joint exercises (31, 54, 56) whereas in the present study bilateral and multi-joint exercises were selected. Although both training programs in the present study involved rhythmic flexion and extension of both lower limbs, in the strength training program the movement was performed simultaneously with both legs, while in the endurance training the task involved alternated leg movements. It cannot be excluded that this difference in the tasks had an effect on the different adaptations (see also “Limitations”).

Motor output

Six weeks of either strength or endurance training induced significant changes in motor performance. In the ET group, resistance to fatigue was enhanced but maximal force and RFD remained unchanged. In contrast, the ST group experienced increases in MVC and RFD and no change in the resistance to fatigue. These distinct effects on motor output following strength and endurance training are in agreement with previous results (27, 46) and demonstrate the efficacy of the applied training programs.

Maximal contractions and surface EMG amplitude

The EMG amplitude of the VMO and VL muscles measured during maximal and explosive knee extension was affected by strength but not by endurance training (Fig. 4-5 and 4-6*A*). Six weeks of strength training induced marked increments in the EMG amplitude of the VMO and VL muscles which was accompanied by increments in MVC and maximal RFD. Furthermore, the ARV of the VMO and VL increased at the onset of the explosive contractions for the strength group (Fig. 4-6*A*). Since gains in force have been observed in the early phase of strength training in the absence of morphological changes of the muscle (23, 57), it has been suggested that neural factors, such as increased agonist muscle activation (3, 48, 57) and decreased antagonist muscle activation (11), are the main determinants of the increase in force. However, these effects have not been demonstrated consistently, either for the agonist (30, 56) or antagonist (4, 27) muscles.

In the present study, the gains in force output were accompanied by marked increments in the EMG amplitude of the agonist muscles (VMO and VL) without changes of the antagonist muscle (BF). The changes observed in the surface EMG amplitude may reflect both neural (e.g., motor unit behavior) and muscular (e.g., increased fiber CSA, sarcolemmal excitability) adaptations. Some studies have reported early changes (i.e., after 5 to 14 weeks) in the muscle architecture, such as increased fiber CSA and pennation angle (10, 35, 59) in response to high-intensity strength training. However, in the current study, high-intensity loads were only used in the last 2 weeks of training, therefore significant alterations of the muscle architecture were not expected. Nonetheless, changes in the conduction velocity of motor unit action potentials (MUCV) were observed during submaximal contractions at 30% MVC after 6 weeks of training. Therefore, both training programs had an influence on the muscle fiber electrophysiological properties, which indicates that some peripheral adaptations occurred in response to training. The changes in MUCV were in the same direction for the two training programs, which may be explained by similar changes at the membrane level. For example, MUCV depends on the Na^+-K^+ pump capacity (19) and both strength and endurance training up-regulate the Na^+-K^+ pump capacity in the skeletal muscle (12, 41).

Although muscle fiber conduction velocity has been shown to be associated with fiber diameter and correlated to the peak of the fiber twitch force (7), changes in MUCV do not necessary imply changes in the contractile properties (see, e.g., Ref. 17), therefore it is not possible to infer changes in contractility from the current data (see also Limitations).

Submaximal contractions and motor unit behavior

Following training, the ET and ST groups showed similar changes in surface EMG amplitude (Figs. 5 *B,C*), but opposite adjustments in motor unit behavior (Figs 4-7 *B,C*). After 6 weeks of training, the average motor unit discharge rate decreased in the ET group (Fig. 4-7*B*) and increased in the ST group (Fig. 4-7*C*). The same trends were also observed for the increment in discharge rate from 10% to 30% MVC following training (Fig 4-9). These observations suggest that the motor training programs used in this study evoked specific adjustments in the behavior of motor units. Furthermore, these changes were observed after only 3 weeks of training for both training approaches. The potential mechanisms involved in these early and opposite adaptations will be discussed in the following sub-sections for each training program.

Endurance training

This is the first study that investigates motor unit adaptations to endurance training in humans *in vivo*. The main observation is that for the same relative submaximal force level, the motor unit discharge rate decreased following training. Despite the decrease in discharge rate, the surface EMG amplitude increased. An increase in EMG amplitude reflects an increase in the motoneuron pool output (20). Thus, because the discharge rate of individual motor units decreased, it is likely that more motor units were active. However, alterations of the surface EMG signals should be interpreted with caution and may also be associated, e.g., with changes in the intracellular action potential shape or volume conductor (for review see Ref. 20).

Interestingly, Adams et al. (5) observed a similar difference in motor unit discharge rate during submaximal contractions of the dominant versus the non-dominant hand of healthy volunteers. In addition to a reduction in discharge rate, the motor units of the dominant hand showed lower and more clustered recruitment thresholds (5). Their results were interpreted as the effect of long-term preferential use of the dominant hand, which can be viewed as a moderate form of exercise (5). Those observations are in agreement with the results of the current study, although important differences, such as the range of motor unit recruitment in hand and leg muscles, exist between the two studies.

Increased net motoneuron excitability, assessed by classic H-reflex techniques, has been shown in endurance-trained athletes compared to power-trained athletes (37, 42) and has also been observed after a short period of endurance training (52). Based on these results, it was suggested that a larger proportion of low-threshold motor units would be recruited for the same relative force level after endurance training (37, 52). However, an increased H-reflex has also been observed after strength training (3) and immobilization (40). Since the H-reflex is modulated by several neural mechanisms which can alter motor unit behavior, its interpretation is limited. In addition to studies on the H-reflex, animal studies have also directly analyzed motor neuron properties following endurance training and have shown that moderate training results in a more hyperpolarized resting membrane potential and voltage threshold, greater after-hyperpolarization amplitude, and decreased spike rise time in low-threshold motor neurons (8, 9). Although their functional consequences are not fully known (24), a greater after-hyperpolarization amplitude and decreased spike rise time in low-threshold motor neurons would alter the motor neuron frequency-current relation and decrease the discharge rates (25), in agreement with the present results.

In addition to this interpretation, the changes in motor unit behavior may also be a consequence of changes in contractile properties of the muscle fibers following training. The time course of the twitch force partly reflects the time course of rise and fall of calcium released from the sarcoplasmic reticulum (33). It has been shown that 5 weeks endurance training induces a down-regulation of the calcium cycling due to a reduction of the Ca^{2+} -ATPase activity (43). These adaptations might contribute to a lengthening of the twitch duration, which would result in a similar twitch fusion at lower discharge rates. In this study the contractile properties of the muscle were not directly assessed, which represents a limitation of the study. However, Grosset et al. (26) did not observe changes in the muscle twitch contraction time or half relaxation time after 10 weeks of endurance training. Furthermore, the unloaded shortening velocity (V_0) of type I fibers increases after 13 weeks of endurance training (21, 44). These previous results indicate that it is unlikely that muscle contractility changes caused the observed changes in motor unit discharge rate after only 3 weeks of training. In addition, in this study we measured the conduction velocity in individual motor units. This measure provides information on the muscle fiber electrophysiological membrane properties at the single motor unit level. Although the measured MUCV did not change during the first 3 weeks of training., a significant increment in MUCV was observed at the end of the training program, although only for the contractions at 30% MVC. Therefore, some peripheral adaptations did eventually occur after 6 weeks of training. However, the time course of these adaptations was different from the observed changes in discharge rates.

Finally, it is worth noting that, despite the MVC decreased slightly for the ET group after 3 weeks of training, albeit not significantly. Consequently, the absolute load applied during the relative submaximal force contractions was smaller. This difference might have contributed to the observed changes in motor unit discharge rates. However, the small change in absolute loads applied during the contractions at 30%MVC did not correlate with the change in discharge rates observed in both training groups.

Strength training

Motor unit discharge rates of the vasti increased following strength training during contractions performed at 30% MVC and were accompanied by an increase of the surface EMG signal amplitude. The increase in motor unit discharge rate during submaximal contractions following strength training was also in agreement with the increase in EMG signal amplitude during the maximal contractions. These results suggest that the changes in motor unit discharge rates contributed to changes in force output at least in the initial phase of training (1, 51, 61).

Maximal discharge rates have been shown to increase during both ballistic contractions (61) and maximal isometric contractions (31, 51) following strength training. Nevertheless, for submaximal contractions, these observations are not confirmed by all studies (31, 56). The different results observed in the present study compared to some previous studies (31, 56) are likely due to differences in the exercise paradigms. For example, in the studies by Kamen and Knight (31) and Rich and Cafarelli (56) the training paradigm involved one unilateral exercise (leg extension) and the training variables (load intensity, number of repetitions and series) were maintained constant across the entire training program. On the contrary, in the present study we applied a progressive strength training program across the 6 week period. The total volume of training reached at the end of the 6 week training period was higher in the present study compared to the aforementioned studies. Differences in exercise paradigms are relevant since the intensity and time of exposure to exercise influences motor neuron properties and consequently the motor unit behavior (25). Other training variables, such as the type of exercise (single vs. multi-joint exercise) or type of contraction (isometric, vs. dynamic), may also contribute to different training outcomes. The strength training program employed in this study involved both single (leg extension) and multi-joint (leg press) exercises of the quadriceps, performed during dynamic contractions (both concentric and eccentric actions). Additionally, the subjects performed a single-joint exercise for the hamstrings muscles.

Changes in motor unit behavior following strength training may include changes in the recruitment threshold of motor units. Van Cutsem et al (61) reported lower recruitment thresholds and higher discharge rates after 12 weeks of explosive training in the absence of changes in the contractile properties of the motor units. In another study, three weeks of isometric training was shown to increase the maximal surface EMG of the VL, M-wave and maximal activation (measured with twitch interpolation techniques) whereas the discharge rate of motor units remained unchanged (54).

Despite limitations associated with surface EMG, the increased EMG amplitude observed in this study with strength training is in accordance with the observed increased motor unit discharge rates. Changes in MUCV may also contribute to changes in the surface EMG signal amplitude (20). However, MUCV did not change after 3 weeks of training, contrary to surface EMG amplitude and motor unit discharge rates.

Early adaptations to training

Both training programs showed significant changes in motor unit behavior after only 3 weeks of training. In this period, MUCV did not change significantly, thus the electrophysiological membrane properties were not influenced by training. Only a few other studies have assessed changes in motor unit behavior across a training period (31, 51). In these studies, maximal motor unit discharge rates of the abductor digiti minimi and vastus lateralis increased after 2 and 7 days of strength training, respectively (31, 51). Interestingly, after this peak, maximal discharge rates decreased returning to baseline levels after 6 weeks of training (51). In the current study, the greatest rate of change in motor unit discharge rate occurred in the first 3 weeks of training. In the subsequent 3 weeks, smaller changes were observed, however neither the strength nor the endurance training group showed a reversal of the adaptations which had occurred within the first half of the training program. The surface EMG amplitude of the vasti also showed a rapid increment in the first half of the training program but was followed by a decrease in the second half of training; nevertheless, by the end of week 6 the EMG amplitude still remained greater compared to baseline values. These results are in agreement with previous observations on more rapid changes in the early phase of strength training with respect to later phases (22). These early adaptations likely involve changes in supraspinal excitability, spinal pathways or changes in the membrane properties of the motoneurons (15). However, the nature and exact sites of the neural adaptations to strength training in this early phase remains unknown (6, 15).

Synergistic muscles

Both the VMO and VL showed similar neural adaptations to strength training. Some studies have reported differences in the magnitude and time course of muscle architecture adaptations of the VL and VMO to high-intensity strength training (10, 59). A significant increase of the cross-sectional area of the VL was observed following 20 days of training (equivalent of 6.5 weeks of training) whilst it was only observed after 35 days (equivalent of 11 weeks) for the VMO muscle (59). It has been suggested that this dissimilarity is related to differences in activation of the muscles within the quadriceps group depending on the speed and angle of the contraction (10). Conversely, in the present study, no differences in the surface EMG, motor unit behavior, or MUCV were observed between the VMO and VL in any subject group. However, in this study the vasti were investigated during knee extension contractions performed with the knee in 90° of flexion, a condition that corresponds to a similar level of activity of the vasti (50, 53).

Limitations

Training-induced improvements in motor output are dependent on a number of physiological, morphological and neural factors (62). In the present study we mainly investigated changes in motor unit discharge rate and MUCV, which constitute only a part of a number of possible adaptations. Adjustments in motor unit discharge rate are also dependent of several factors including muscle contractile properties and motor unit recruitment thresholds. Although MUCV partly correlates to the force expressed by the motor units (7), changes in the MUCV do not necessarily reflect alterations of the contractile properties and direct measures of contractility would provide further clarification on motor unit adaptations to training programs. Alterations of the discharge rates may indeed represent an adjustment to the modified contractile properties of the muscle fibers. For example, in endurance training there may be a lengthening of the twitch force so that twitch fusion force can be produced at lower discharge rates. This hypothesis cannot be tested from the current data.

The different types of exercise used in each training program (i.e., alternating vs. simultaneous flexion and extension of the lower limbs) might have accounted for the opposite adaptations observed at the motor unit level since they involve different motor control strategies. Cycling is generated by the integrated activity of several control systems at various levels of the central nervous system and differences exist when comparing the soleus H-reflex and motor-evoked potentials during cycling and tonic plantar flexion (55).

Finally, changes in voluntary activation may have an influence on motor unit discharge rates. Voluntary activation has been reported to change with training in some studies (14, 54), although in other studies this was not observed (38, 61). The current study did not include the measure of voluntary activation, whose potential changes with training may explain some of the results presented.

4.5 Conclusion

The findings in this study suggest that two specific endurance and strength training programs elicit early and opposite adjustments in motor unit discharge rates. Endurance training increased resistance to fatigue and was accompanied by a decrease in motor unit discharge rates. In contrast, strength training enhanced maximum force output and was accompanied by an increase in motor unit discharge rates. By the end of 6 weeks of training, both programs elicited increases in MUCV, revealing electrophysiological adaptations of the muscle fiber membrane properties in similar directions. However in the first 3 weeks of training, when changes in motor unit discharge rates were most marked, changed in MUCV were not observed. These findings reveal different time courses of some of the neural and peripheral adaptations in response to different motor training programs.

Acknowledgments

C. Vila-Chã was supported by Fundação para a Ciência e a Tecnologia (FCT) of Portugal (Grant ID - SFRH / BD / 31796 / 2006). The study was partly supported by the Danish Technical Research Council.

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CHAPTER

5

Study II

Adjustments in Motor Unit Properties during Fatiguing Contractions Following Training

Carolina Vila-Chã, Deborah Falla, Miguel Velhote Correia, Dario Farina

Med Sci Sports Exerc. Sep 7, 2011. (Epub ahead of print)

Abstract

Introduction: To investigate the effect of strength and endurance training on muscle fiber membrane properties and discharge rates of low- threshold motor units of the vasti muscles during fatiguing contractions. **Methods**: Twenty-five sedentary healthy men (age, mean ± SD, 26.3 ± 3.9 yr) were randomly assigned to one of 3 groups: strength training, endurance training, or a control group. Conventional endurance and strength training was performed three days per week, over a period of 6 weeks. Motor unit conduction velocity and EMG amplitude of the vastus medialis obliquus and lateralis muscles and biceps femoris were measured during sustained isometric knee extensions at 10% and 30% of the maximum voluntary force (MVC) before and immediately following training. **Results**: After 6 weeks of training, the reduction in motor unit conduction velocity during the sustained contractions at 30%MVC occurred at slower rates compared to baseline ($P < 0.05$). However, the rate of decrease was lower following endurance training compared to strength training ($P < 0.01$). For all groups, motor unit discharge rates declined over the duration of the sustained contraction ($P < 0.001$) and their trend was not altered by training. In addition, the biceps femoris/ vasti co-activation ratio declined after the endurance training. **Conclusion:** Short-term strength and endurance training induce alterations of the electrophysiological membrane properties of the muscle fiber. In particular, endurance training lowers the rate of decline of motor unit conduction velocity during sustained contractions more than strength training

5.1 Introduction

Impairment of neuromuscular function develops gradually during submaximal fatiguing contractions (8, 15). Although voluntary effort is progressively increased, task failure will eventually occur even if muscles have not been fully activated (8, 9, 15). The etiology of such impairment is complex and can occur at any site ranging from the cortex to the actin-myosin cross bridges (15).The site of the impairment depends on the task being performed, resulting in different times to failure (9, 15). Furthermore, the subject's training background will affect the endurance time limit (16, 36).

Several cross-sectional studies have shown that during submaximal sustained contractions power-trained athletes have lower times to task failure, reaching exhaustion earlier than endurance-trained athletes (6, 36). Likewise, following submaximal fatiguing exercise, power-trained athletes show a greater decline in maximal isometric force of the knee extensors than endurance-trained athletes (16, 21, 24), which is accompanied by a greater decline in peak twitch torque (16) and in conduction velocity (37), but not in changes in the EMG activity pattern of the vasti muscles (16, 37). These studies suggests that the distinct neuromuscular fatigue profiles between power- and endurance-trained athletes are mostly due to differences at the peripheral level, in particular the muscle fiber composition (6, 24, 36, 37). The percentage of slow twitch fibers in the skeletal muscle are positively correlated with endurance time (21), which explains longer times to task failure for the endurance-trained athletes. However, longitudinal studies, show that as little as three sessions of practice of a sustained submaximal isometric contraction of the elbow flexors enable sedentary subjects to prolong their time to exhaustion (22, 38). This was accompanied by a lower rate of increase in agonist activity (22) and reduced antagonist inhibition during the prolonged sustained task (38). Therefore, other mechanisms than those occurring at the periphery contribute to an increase in time to failure.

A recent study of our group (40) showed that 6 weeks of endurance training results in prolonged time to task failure which is accompanied by a decrease of motor unit discharge rates during brief submaximal contractions. On the contrary the strength training group showed increased discharge rates during the brief contractions and both training programs induced similar increments in surface EMG amplitude and muscle fiber conduction velocity in un-fatigued muscles. Although neuromuscular adaptations observed in non-fatigued muscles may contribute to differences in time to failure, it is the time course of changes in peripheral and/or central mechanisms that will dictate task failure. Nonetheless, the training-induced adjustments in the neuromuscular system when fatigue progressively develops during submaximal contractions are not well understood (16). Therefore, the purpose of this study was to investigate the effects of a short-term endurance and a strength training program on the time course of the muscle fiber membrane properties and firing rates of low- threshold motor units of the vastus medialis obliquus and lateralis muscles during prolonged isometric contractions.

5.2 Methods

Subjects

Thirty healthy men (age, mean ± SD, 26.0 ± 3.8 yrs) with no history of lower limb disorders participated in the study. None of the subjects were involved in regular strength or endurance training. All subjects gave their informed consent to the procedures of the study. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (N-20090032). After the first experimental session (pre-training session), the subjects were randomly assigned to one of three groups: strength training (n = 10); endurance training (n = 10); or a control group (no exercise intervention) (n = 10). Not all subjects completed the training program. One subject from the strength group and 2 subjects from the control group did not complete the final experimental session and were excluded from the analysis. Additionally, due to technical problems during session 1, the data collected from two subjects (1 from each training group) could not be used. Thus, the results are presented for 8 subjects in the strength group (age, 25.8 ± 4.2 yrs; height, 184.0 ± 6.9 cm; weight, 82.5 ± 14.3 kg), 9 subjects in the endurance group (age, 25.8 ± 2.8 yrs; height, 180.9 ± 6.5 cm; weight, 78.9 ± 15.0 kg) and 8 subjects in the control group (age, 27.0 ± 5.0 yrs; height, 175.3 ± 3.4 cm; weight, 78.0 ± 13.0 kg).

Training programs

The training regime has been described in detail previously (40). Briefly, endurance or strength training was performed over 6 weeks for a total of 18 sessions. All training sessions were supervised by an investigator of the study. Endurance training was performed on a bicycle ergometer and the exercise intensity was prescribed based on the percent of the heart rate reserve (HRR) according to the Karvonen method (23). During the training period the load intensity ranged between 50 to 70% of the HRR and the duration of the sessions between 20 and 50 min. The strength training involved three bilateral leg exercises (leg press, leg extension, and leg curl). The subjects trained with loads of 60-85% of the one-repetition maximum (1RM) and performed 3-4 sets of 8-15 repetitions. For both training programs the load intensity were applied progressively over the training period.

Procedure

The subjects attended two laboratory sessions, immediately before (session 1 – week 0) and after completion of the 6-week training period (session 2 – week 6). For each laboratory session, the subject was comfortably seated on an Isokinetic dynamometer (KinCom Dynamometer – Chattanooga, TN, USA) with his trunk reclined to 15º in an adjustable chair and hip and distal thigh firmly strapped to the chair. The rotational axis of the dynamometer was visually aligned with the lateral femoral epicondyle. The right leg was secured to the dynamometer's attachment above the lateral malleolus with the knee at 90º of flexion. Progressive maximal voluntary contractions (MVCs) of the knee extensors were measured twice, with 2-min rest in between. The maximum of the two force measures was used as a reference for the definition of the submaximal force levels. In both experimental sessions, the submaximal forces were relative to the MVC measured during the same session. Subjects were then asked to maintain a constant isometric contraction at 10%MVC for 70s. This duration was proven in previous studies to be sufficient for eliciting changes in muscle fiber conduction velocity in the vasti muscles (19). Subjects were provided with online visual feedback of the force exerted which was displayed on an oscilloscope. The knee extensor force was represented by a bold horizontal line parallel to the target line. Across target forces, the vertical gain on the oscilloscope screen was adjusted in order to keep the target line approximately at the same position. After 15-min of rest, the subjects performed an isometric knee extension at 30% MVC maintaining the force for as long as possible (endurance task). Time to task failure was defined as a drop in force greater than 5% of the target force level for more than 5 s, after strong verbal encouragement to the subject to maintain the target force. During all submaximal contractions, knee extension force, intramuscular and surface EMG signals were recorded concurrently.

EMG recordings

Surface EMG. After skin preparation (shaving, abrasion and cleaning with water), two adhesive linear arrays of 8 electrodes (5×1 mm size, 5-mm interelectrode; SPES Medica, Salerno, Italy) were placed on the vastus lateralis and vastus medialis obliquus and a pair of surface Ag–AgCl electrodes (Ambu Neuroline, conductive area 28 mm^2) on the biceps femoris (BF). The arrays of electrodes were located between the innervation zone and the distal tendon, along the direction of the muscle fibers (19). The tendon regions, innervation zones and propagation of the detected motor unit action potentials were identified during preliminary brief knee extensions. For this purpose, a linear array of 8 equi-spaced silver electrodes (LISiN-OT Bioelettronica, Torino, Italy) was used and oriented in order to observe a clear propagation of the detected MUAPs from the innervation zone to the tendon region, as described previously (10, 28). For the biceps femoris muscle, the electrodes were placed according to Hermens et al. (20). A ground electrode was placed around the right ankle. Surface EMG signals were amplified as bipolar derivations (EMG amplifier, LISiN-OT Bioelettronica, Torino, Italy), band-pass filtered (-3dB bandwidth, 10-500Hz), sampled at 2048 samples/s, and converted to digital data by a 12-bit A/D converter board.

Intramuscular EMG. Two pairs of wire electrodes (50-µm-diameter) made of Teflon coated stainless steel (A-M Systems, Carls, WA, USA) were inserted with a 23 G needle, 10-20 mm proximal to the surface electrode arrays (10). The wires were uninsulated for ~1mm at the tip to detect intramuscular EMG signals. The angle of insertion of the needle was ~ 45°, and the depth was a few millimeters below the muscle fascia. The needles were removed after insertion and the wire electrodes were left inside the muscle. Intramuscular EMG signals were amplified as bipolar derivations (Counterpoint EMG, DANTEC Medical, Skovlunde, Denmark), band-pass filtered (500 Hz – 4 kHz), sampled at 10,000 Hz, and stored after 12-bit A/D conversion.

Subcutaneous fat layer thickness

Estimation of motor unit conduction velocity is affected by the thickness of the subcutaneous fat layer (11). With training this factor can be altered resulting in changes of the motor unit conduction velocity. Thus the subcutaneous tissue thickness of the subjects participating in the training programs was assessed pre and post training by ultrasound B-mode imaging (Acuson xp 180). The probe (10 Mhz, linear 38mm) was placed both proximal and distal to the location of the array electrodes and the thickness was measured between the dermis of the skin and fascia.

Signal analysis

Motor unit conduction velocity and average rectified value were calculated in two intervals of 10 s duration. For both isometric contractions (at 10 and 30% MVC) the first interval (T_1) was defined at the beginning (from 0 to 10 s) of the contraction and the second interval (T_2) corresponds to the time after one minute of the sustained contraction (from 60 to 70 s).

For the contraction at 30% MVC sustained until task failure, two additional intervals of 10 s were used to analyze the average rectified value. These intervals were set 10 s prior to 50% and prior to 100% of the time to task failure (designated as T_3 and T_4, respectively). Motor unit conduction velocity were not estimated for these additional intervals since the intramuscular signal became progressively more interference, making it difficult to accurately track the same motor units over time.

The time course of the average rectified value of the vastus lateralis and vastus medialis obliquus, over different time intervals (T_2 , T_3 and T_4) , were calculated by subtracting the values of the respective interval (T_2 , T_3 and T_4) from the initial values (first interval -T_1). The time course of motor unit conduction velocity and motor unit discharge rates were estimated only for the time interval T_2.

Decomposition of intramuscular signals and motor unit conduction velocity estimation. The recorded intramuscular signals were decomposed into the constituent motor unit action potentials by a decomposition algorithm previously validated (29). Unusually short (<20 ms) or long (>200 ms) time intervals between subsequent detected discharges of the individual motor units were manually reanalyzed and corrected when necessary. For both sustained contractions, only the motor units that were active during the first (T_1) and second (T_2) time intervals were used to compute the motor unit conduction velocity. The mean discharge rate [pulses per second (pps)] and interspike interval variability were computed from the motor unit spike trains. Conduction velocity of the individual motor units was estimated from the averaged multichannel surface potentials by a spike-triggered average technique previously described (10). Briefly, the detected times of occurrence of individual motor unit action potentials in the intramuscular signals were used as trigger for averaging the multichannel surface EMG signals from which a multi-channel maximum likelihood methods was used to estimate motor unit conduction velocity (13). A minimum of 70 triggers were used for each motor unit in each of the time intervals analyzed. A window of 50 ms centered on the time instant of detected motor unit action potentials was used for the average of the surface signals. In order to reduce the effects of the non-propagating components, double differential signals (obtained by subtracting two adjacent single differential signals) were used for the estimation. The channels used for the conduction velocity estimation were manually selected. Only the channels showing similar propagating motor unit action potentials were accepted for analysis. For each experimental session, the same channels were selected for all motor units recorded from the same subject.

Surface EMG amplitude. Average rectified value was estimated from the same channels used for motor unit conduction velocity estimation. For each time interval, the average rectified value was estimated in consecutive non-overlapping epochs of 1s and then averaged. The level of coactivation was quantified by computing the ratio between the antagonist and agonist (average across both vasti muscles) EMG signals.

Statistical analysis

The effects of the training programs on the average rectified value of the vastus lateralis, vastus medialis obliquus and biceps femoris, the motor unit conduction velocity and motor unit discharge rates of the the vastus lateralis, vastus medialis obliquus were evaluated with four-way repeated measures ANOVA with factors group (control, endurance, and strength), training period (week 0 and 6), time intervals [(T_1, $T_{2,}$ $T_{3,}$ and T_4) or (T_1 and $T_{2,}$)] and muscle (vastus lateralis and vastus medialis obliquus). The effects of training on the time course of motor unit conduction velocity and discharge rates of the vastus lateralis, vastus medialis obliquus over time were assessed with three-way repeated measure ANOVA with factors group, training period and muscle. In addition, changes in the time course of the vasti ARV was assessed with a four-way repeated measures ANOVA with factors group, training period, time intervals and muscle. A two-way repeated measure ANOVA with factors group and training period was applied to assess changes in the MVC, time to task failure and subcutaneous tissue thickness. Before conducting each repeated measure ANOVA, normality and equality of covariance matrices was tested. Due to the sample size and because few cells of some dependent variables violated the normality assumption, Pillai's Trace omnibus statistic was used in preference to Wilks' lambda (39). In addition, Mauchly's sphericity test was computed and when the sphericity assumption was violated Greenhouse-Geisser adjustment was made.

When ANOVA was significant, the post-hoc Student-Newman-Keuls test for pair-wise comparisons was applied. Statistical significance was designated at $P < 0.05$ for all comparisons. Results are reported as means and SD in the text and mean and SE in the figures.

5.3 Results

No differences were observed between groups for age, height and weight. Moreover, no differences were observed between the groups for any of the motor output or electrophysiological parameters assessed in the first experimental session (prior to training).

Prior to training the thickness of the subcutaneous tissue was 5.1 ± 1.7mm and 5.2 ± 1.6mm for the endurance and strength groups respectively (average of the distal and proximal measures obtained for the lateral and medial side of the leg). Following 6 weeks of training the subcutaneous tissue thickness remained unchanged (endurance group: 5.2 ± 1.6mm; strength group: 5.3 ± 1.7mm; $P > 0.56$, for each group).

Motor output

Following 6 weeks of endurance training, time to task failure increased by 29.2 ± 13.7% (from 119.7 ± 33.8s to 154.3 ± 42.2s, $P < 0.05$) but no significant changes were observed in MVC (from 525.1 ± 98.9N to 500.4 ± 92.3N). In contrast, strength training induced an increase in the MVC force by 16.8 ± 8% (from 520.1 ±112.1N to 603.7 ± 111.2N, $P < 0.05$) but did not influence time to task failure (from 159.8 ± 46.9s to 170.9 ± 74.4s). None of the motor output parameters changed in the control group (MVC: from 549.5 ± 68.5N to 554.4 ± 47.2N; time task failure: from 128.8 ± 69.1s to 123.7 ± 73.3s).

Motor unit behavior

The alterations induced by the training programs showed similar trends for the vastus lateralis and vastus medialis obliquus muscles. Since no interactions between muscle and time were observed ($P > 0.51$, for all variables), the results presented in the figures correspond to the average of both vasti muscles.

From all sessions, a total of 820 motor units were identified in the first time interval and only 536 motor units could be tracked in the second time interval. The motor unit conduction velocity was computed only for the motor units that could be identified in both time intervals. The number of motor units identified for each subject in each experimental session ranged between 1 and 7 at each force level and for each muscle (average: 2.7 motor units in each condition).

The discharge rate of the detected motor units decreased over time, in particular at 30%MVC (*P*< 0.001). After 60s of sustained contraction at 30%MVC, the discharge rate of vasti motor units decreased between 0.6 ± 0.2 pps and 1.1 ± 0.3 pps (Fig. 1). The decline of the discharge rate over time was not influenced by any of the training programs (interaction between group and time: *P* > 0.60; Fig. 5-1).

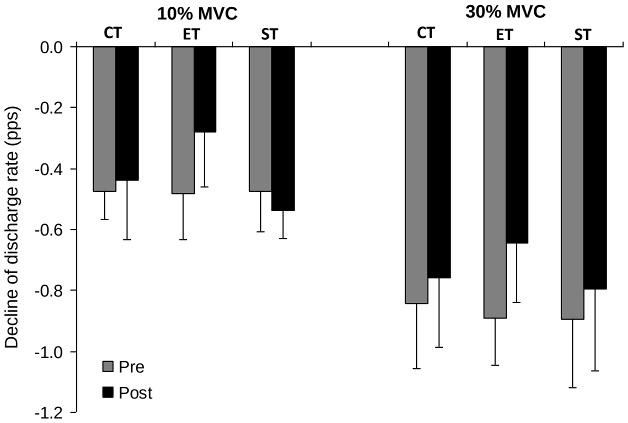

Figure 5-1 - Mean ± SE for the decline of the discharge rates of motor units of the vasti muscles (average of vastus medialis obliquus and vastus lateralis) recruited at the onset of the sustained knee extension contractions at 10% and 30% MVC for all groups, before and after training.

Motor unit conduction velocity.

Figure 2 shows the motor unit conduction velocity for the vasti muscles during the submaximal isometric contractions. The motor unit conduction velocity values at the beginning of the contractions were dependent on the force level and training period. At 30% MVC, the initial motor unit conduction velocity was 74.0 ± 10.2% higher than at 10% MVC (*P* < 0.001). The effect of the training programs was only observed at the higher force level (Fig. 5-2). Following six weeks of endurance and strength training the initial motor unit conduction velocity of the vasti muscles increased by 10.3 ± 8.8% and 11.1 ± 10.0%, respectively, while no changes were observed for the control group (Fig. 5-2).

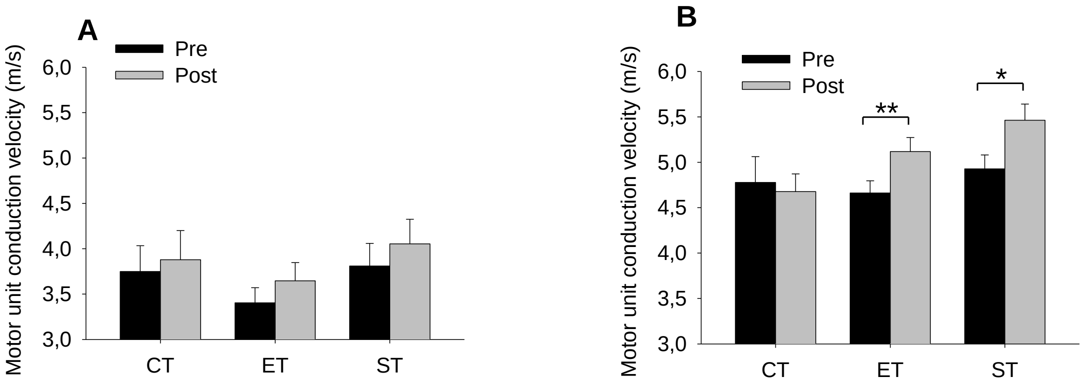

Figure 5-2 - Mean ± SE for the initial values of motor unit conduction velocity of the vasti muscles at (A) 10% MVC and (B) 30% MVC, for all groups, before and after training. *- $P < 0.05$ from week 0 to week 6. ** - $P < 0.001$ from week 0 to week 6.

Motor unit conduction velocity decreased during the sustained contractions. The decline in motor unit conduction velocity over time was load- and training-dependent ($P < 0.05$, Fig. 5-3). At 30% MVC the decline was greater (-5.3 ± 2.3%) than at 10% MVC (-3.4 ± 1.9%).

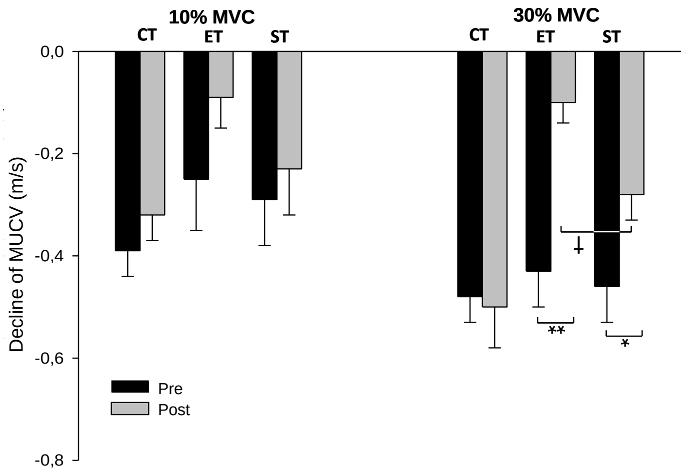

Figure 5-3- Mean ± SE for the decline in motor unit conduction velocity in the vasti muscles (average of vastus medialis obliquus and vastus lateralis) during sustained knee extension contractions at 10% and 30% of MVC for all groups, before and after training. *- $P < 0.05$ from week 0 to week 6. ** - $P < 0.001$ from week 0 to week 6. †- Decline in motor unit conduction velocity after training was lower for the endurance than for the strength group ($P < 0.01$).

At 30%MVC, the reduction in motor unit conduction velocity over time was lower after both training programs (Fig. 5-3). Nonetheless, for the sustained contraction at 30%MVC, the endurance group showed a lower decline in motor unit conduction velocity than the strength group ($P < 0.01$; Fig. 5-3). No alterations were observed in the control group.

Surface EMG amplitude

Figure 5-4 shows the EMG amplitude of the vasti muscles during the submaximal contractions for all groups, over each experimental session. After both training programs, the average rectified value of the vasti muscles increased in all time intervals analyzed for the contraction at 30% MVC (interaction between time, interval and group; $P < 0.05$; Fig. 5-4 *B,C*) whereas no changes were observed in the control group (Fig. 5-4 *A*).

The average rectified value of the vasti muscles increased progressively throughout the endurance task (main effect interval; $P < 0.0001$). For all groups, at the end of the endurance task, the average rectified value of the vasti was 49.2 ± 22.9% to 66.1 ± 17.2% higher than at the beginning of the contraction (Fig. 5). No interaction between group and time was observed for the rate of increase of the vasti average rectified value ($P = 0.09$; $\eta_p^2 = 0.14$ and observed power = 0.58) (Fig. 5-5).

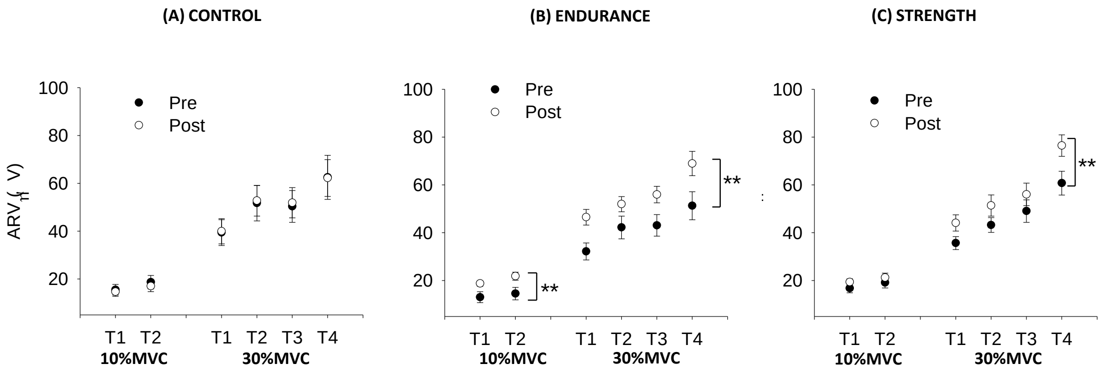

Figure 5-4 - Mean ± SE for the average rectified value of the vasti muscles obtained during submaximal isometric knee extension contractions at 10% and 30% MVC, across experimental sessions for: - **(A)** ***Control group***, **(*B) Endurance Group*** and **(*C) Strength group.*** The data represent the average of the average rectified value of the vasti muscles obtained for the time intervals T_1, T_2, T_3 and T_4.. * - $P < 0.05$ from week 0 to week 6. **-$P < 0.001$ from week 0 to week 6.

The EMG amplitude of biceps femoris also increased progressively throughout the sustained contractions (interval effect $P < 0.001$, for both isometric contractions) and was influenced by both training programs (interaction group × time × interval: $P < 0.001$; Fig. 5-6). After endurance training the average rectified value of the biceps femoris was lower than prior to training, in particular during the time intervals T_2 and T_3 ($P < 0.01$ for both time intervals; Fig. 5-6 *B*). In contrast, after strength training, the average rectified value of the biceps femoris increased, particularly during the last two time intervals ($P < 0.01$ and $P < 0.001$ for T_3 and T_4, respectively; Fig. 5-6 *C*).

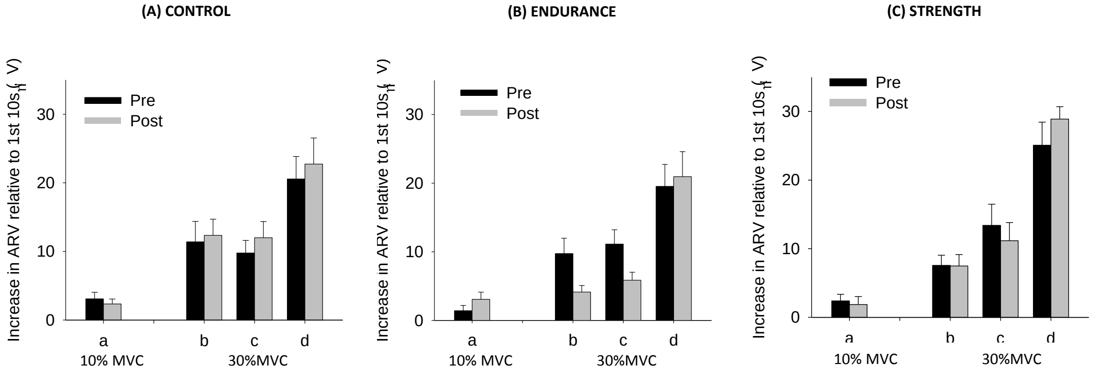

Figure 5-5 -Mean ± SE for the changes in average rectified value of the vasti muscles at two submaximal isometric knee extension contractions (10 and 30% MVC) over experimental sessions for: - **(A)** ***Control group***, **(*B) Endurance Group*** and **(*C) Strength group.*** The changes over different time intervals (T_2, T_3 and T_4) were computed relative to the initial time interval (T_1). * - $P < 0.05$ from week 0 to week 6.

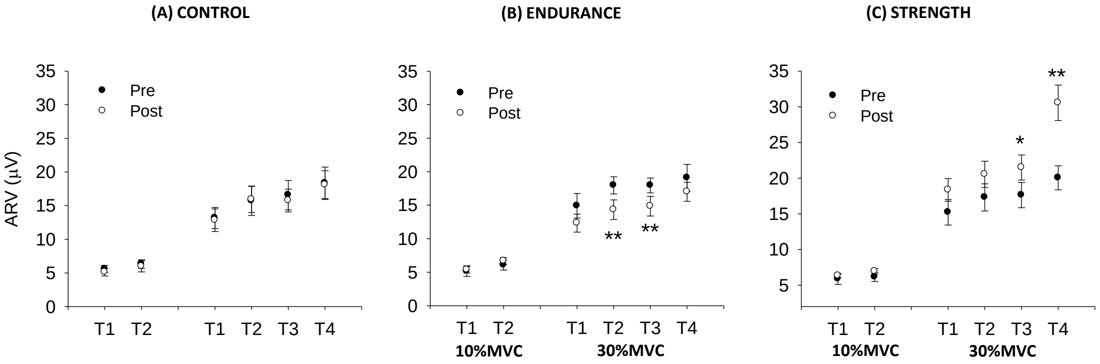

Figure 5-6- Mean ± SE for the average rectified value of the biceps femoris obtained during submaximal isometric knee extension contractions at 10% and 30% MVC, across experimental sessions for: - **(A)** ***Control group***, **(*B) Endurance Group*** and **(*C) Strength group.*** The data represent the average of the average rectified value of the vasti obtained for the time intervals T_1, T_2, T_3 and T_4.. * - $P < 0.05$ from week 0 to week 6. **-$P < 0.001$ from week 0 to week 6.

The alterations in both the agonist and antagonist muscle activity observed after endurance training influenced the co-activation ratio. After 6 weeks of endurance training, the level of co-activation was reduced by 36.8 ± 22.3 % ($P < 0.05$). No changes in the co-activation were found for the strength or control group ($P > 0.80$, for both groups).

5.4 Discussion

The purpose of the study was to investigate the effects of distinct training programs on the time course of the muscle fiber membrane properties of low-threshold motor units and EMG activity of the vasti muscles during submaximal fatiguing contractions. The main finding is that, following six weeks of either endurance or strength training, the rate of decline over time of the motor unit conduction velocity was reduced, mostly for the endurance group. Additionally, the biceps femoris/vasti co-activation ratio decreased, following endurance training.

Motor performance

Six weeks of endurance and strength training induced specific changes in motor performance. Endurance training improved time to task failure with no changes in MVC whereas strength training enhanced MVC and did not affect time to task failure.

Electrophysiological variables during sustained submaximal contractions

Time course of motor unit conduction velocity

Changes of the peripheral neuromuscular system induced by fatiguing contractions can be detected by investigating changes in motor unit conduction velocity (12). During the sustained submaximal contractions, the motor unit conduction velocity decreased likely due to alterations of the sarcolemma excitability (25). The generation of action potentials induces cellular K^+ efflux and Na^+ and Cl^- influx, causing perturbations in the intracellular and extracellular K^+ and Na^+ gradient concentrations (31). These alterations depolarize the sarcolemmal and t-tubular membranes which reduce membrane excitability (7, 25). The loss of membrane excitability is partly counteracted by the Na^+-K^+ pump activity, which however is not sufficient to fully balance the K^+ efflux (7, 33). Thus during sustained contractions, the velocity of propagation of the action potential decreases, even at very low force levels (33). Additionally, with continued muscle contraction an increase in the concentration of H+ ions can occur, contributing to the change in membrane excitability (15).

This study showed that the rate of decline of motor unit conduction velocity during sustained contractions was slower following six weeks of either endurance or strength training with respect to baseline. Although both groups showed similar adaptations, the decline in motor unit conduction velocity was reduced to a greater extent following endurance training. The observed alterations may reflect changes in the Na^+-K^+ pump concentration (content and/or activity). The Na^+-K^+ pump is a determinant for the regulation of the Na^+-K^+ distribution and maintenance of membrane excitability during muscle contractions (30, 33). Thus, an up-regulation of pump concentration would prolong the balance of Na^+-K^+ concentration gradients, protecting the muscle contractibility against fatigue (7, 31).

It has been shown that regular physical activity of moderate to high intensities increases the Na^+-K^+ pump concentration, independently of the type of training (7). Increased Na^+-K^+ pump concentration has been observed after endurance-training (17), strength-training (17), and sprint-training (32). Nevertheless, the time course of this adaptation may differ between different types of training. Green et al. (17), demonstrated that after 3 weeks of high volume cycling (2 h, 6 times per week), the Na^+-K^+ pump concentration increased significantly in the vastus lateralis muscle, whereas 7 weeks of strength training (3 leg exercises, 3 times per week) were required to induce similar adaptations. The adaptation of the Na+-K+ pump concentration is associated with contractile activity and with a concomitant up-regulation of oxidative metabolism (17, 18), which may explain the faster adaptations following endurance training compared to strength training. Different time courses of the Na^+-K^+ pump adaptation may explain the differences in the rate of decline of motor unit conduction velocity between the endurance and strength group in the present study. Furthermore, such adaptation of the

Na^+-K^+ pump enables fast conduction of the action potentials (7), which may have contributed towards the higher initial motor unit conduction velocity values following both training programs.

Alterations of the conduction velocity may also be due to other training effects, such as an increase in the diameter of the muscle fibers (2) or reduction in the thickness of the fat layer beneath the surface electrodes (11). However, an increase in muscle fiber diameter is typically induced by strength training, not endurance training, which may even reduce fiber diameter (26). Alterations in subcutaneous tissue thickness can also be ruled out since the thickness of tissue beneath the electrode arrays was not affected by either training program.

Conduction velocity is also influenced by changes in motor unit discharge rates (12, 34). However, in the present study, the initial motor unit conduction velocity values showed a similar increment following both training programs, despite opposite adjustments in the initial mean discharge rates. Thus, it is unlikely that the changes observed in motor unit conduction velocity following training are due to changes in motor unit discharge rates.

Time course of the EMG amplitude

During the endurance task at 30%MVC, the activity of the vasti muscles increased progressively reaching approximately two-fold the initial values by the end of the task, independently of the group or training period. The progressive increment of the EMG amplitude reflects an increase of the excitation of the motoneuron pool (3, 14). As fatigue develops, recruitment of additional motor units occurs to compensate for the reduction of the force-generating capacity of the initially recruited motor units (15). Concomitantly the motor unit discharge rates may remain constant, increase or decrease (1, 5), however typically motor units recruited at the beginning of the task will reduce their discharge rate over a sustained contraction (1, 3). In the present study, the surface EMG amplitude of the vasti muscles increased while the discharge rates of the initially recruited motor units decreased significantly after 60 s of the sustained contraction. This data suggests that the submaximal force level was maintained due to recruitment of additional motor units, which is in accordance with previous studies (3, 5). Both the rate of decline in discharge rate of the initial active motor units and the rate of increase of the EMG amplitude was not significantly affected by either training program. Nevertheless, it has been shown that a short interval of practice of a fatiguing sustained contraction improves the endurance time and reduces the rate of increase of the EMG activity of the agonist muscles (22, 38). Despite the nonlinear summation of the motor unit action potentials in the EMG signal (13), this might indicate that following endurance training the recruitment of the motoneuron pool occurred at slower rates than before training, contributing to a longer endurance time (22).

Endurance training induces various physiological adaptations, such as increased Na^+-K^+ pump activity and increased energy metabolic potential (7, 17) which leads to a slower rate of increase of metabolic by-products during sustained contractions. Afferent feedback on muscle milieu is provided by intramuscular receptors, in particularly by group III-IV muscle afferents. These afferents are sensitive to metabolic by-products of the muscle contraction, such as increase in the extracellular potassium (4). The discharge of these afferents in response to such stimuli acts in a complex manner over several points of the pathways responsible for force production (15). Their activity contributes to a compression of the recruitment thresholds of the high threshold motoneurons (27) which would lead to recruitment of additional motor units but with lower firing rates in generating the target force level (4). Thereby, a slower rate of increase of metabolic by-products of the contraction, would presumably account for a slower rate of increase of the discharge rates of the group III-IV afferents and consequently a slower recruitment of the motoneuron pool over the sustained contraction. Such alterations would contribute to increased endurance time following training.

Other mechanisms may also account for improvements in the endurance capacity during sustained contractions (9, 22). In the present study, the co-activation of the biceps femoris was lower after endurance training while it did not change after strength training. Some cross-sectional studies report a lower co-activation ratio in endurance-trained athletes compared to power-athletes (35) however others report no difference (16) during un-fatigued contractions. During dynamic fatiguing exercise the co-activation of the hamstrings muscles increases in power-trained athletes but not in endurance-trained athletes, while during isometric contractions no change was reported for either group (16). While the results from cross-sectional studies are inconsistent, a longitudinal study showed that reduced antagonist inhibition was associated to increased time to task failure (38). Thus, reduced co-activation may have also contributed to the increased endurance time observed for the endurance group in this study.

5.5 Conclusion

The rate of decline of motor unit conduction velocity during sustained contractions was reduced following six weeks of both endurance and strength training, however a greater reduction is observed following endurance training. In addition, endurance training reduces the level of co-activation between the vasti and biceps femoris during sustained contractions of knee extension. These alterations likely contribute to longer times to task failure following endurance training.

Acknowledgements

Carolina Vila-Chã was supported by Fundação para a Ciência e Tecnologia (FCT) of Portugal (PhD Grant ID – SFRH/BD/31796/2006). The results of the present study do not constitute endorsement by ACSM. The authors declare no conflict of interest.

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CHAPTER

6

Study III

Changes in H-reflex and V-wave following short-term endurance and strength training

Carolina Vila-Chã, Deborah Falla, Miguel Velhote Correia, Dario Farina

J Appl Physiol. Oct 13, 2011 (Epub ahead of print)

Abstract

This study examined the effects of 3 weeks of either endurance or strength training on plasticity of the neural mechanisms involved in the soleus H-reflex and V-wave. Twenty-five sedentary healthy subjects were randomized into an endurance group (n = 13) or strength group (n = 12). Evoked V-wave, H-reflex and M-wave recruitment curves, maximal voluntary contraction (MVC) and time-to-task-failure (isometric contraction at 40% MVC) of the plantar flexors were recording before and after training. Following strength training, MVC of the plantar flexors increased by 14.4 ± 5.2% in the strength group ($P < 0.001$), whereas time-to-task-failure was prolonged in the endurance group (22.7 ± 17.1%; $P < 0.05$). The V/M_{max} ratio increased significantly (55.1 ± 28.3 %; $P < 0.001$) following strength training but the H_{max}/M_{max} ratio remained unchanged. Conversely, in the endurance group the V/M_{max} ratio was not altered whereas H_{max}/M_{max} ratio increased by 30.8 ± 21.7% ($P < 0.05$). The endurance training group also displayed a reduction in the H-reflex excitability threshold while the H-reflex amplitude on the ascending limb of the recruitment curve increased. Strength training only elicited a significant decrease in H-reflex excitability threshold while H-reflex amplitudes over the ascending limb remained unchanged. These observations indicate that the H-reflex pathway is strongly involved in the enhanced endurance resistance which occurs following endurance training. On the contrary, the improvements in MVC following strength training are likely attributed to increased descending drive and/or modulation in afferents other than Ia afferents.

6.1 Introduction

The human nervous system is highly adaptive in response to training (2). Neural adaptations occur in response to both endurance (34, 39) and strength training (1, 7, 8, 10, 11, 27, 39) and are thought to contribute to enhanced motor performance (11, 34). Although efforts have been made to elucidate the mechanisms underlying these adaptations, the results are not clear (5, 11). The sites and mechanisms underlying neural adaptations to motor training can be investigated by measuring reflex responses, in particular the H-reflex and V-wave (1, 7, 11, 15, 18, 34). Although these evoked responses are affected by common neural mechanisms, during voluntary contractions, the H-reflex is more sensitive to altered presynaptic inhibition and motoneuron excitability (11, 19, 29, 31, 36), whereas the V-wave is more sensitive to changes in supraspinal input to the motor neuron pool (1, 9, 38). Thus, combined measures of the H-reflex and V-wave may provide a better understanding of the neural adaptations elicited by specific motor training programs.

A number of cross-sectional studies have shown that the H-reflex excitability measured at rest is higher in endurance-trained athletes than in power-trained athletes (6, 25, 28, 32, 37), and lower in power-trained athletes than in sedentary people (6). Another study reported that H-reflex excitability was higher in moderately and well-trained individuals compared to sedentary people but was lowest in a group of ballet dancers (30). Taken together, these observations suggest that spinal reflex circuits adapt specifically to the training demands. However, results from cross-sectional studies should be interpreted with caution since it is not possible to fully dissociate intrinsic genetic endowment from actual training adaptations (1, 40).

Reflex investigations have been performed in few longitudinal strength training studies. Some studies have reported increases in the V-wave, without changes of the H-reflex (7, 11-13), while others observed increases in both V-wave and H-reflex responses (1). The different results may be attributed to diverse training protocols and/or methodological approaches (5, 7). The only longitudinal study on the effects of endurance training on the size of the H- reflex revealed that 75% of the subjects increased both spinal excitability and maximal aerobic capacity while for the remaining participants these variables were barely modified or even decreased (34). The heterogeneity of the results were likely due to differences in the subject's fitness level and/or their involvement in other sport activities (34).

Understanding the extent to which the nervous system can adapt to specific motor training programs is of extreme importance in both rehabilitation and exercise training (41). However based on the current literature it is difficult to compare and interpret training-induced neural adjustments since studies have employed subjects with different physical fitness levels and applied different experimental methodologies, which contributes to the observed discrepancies between studies. To overcome this limitation, we selected to investigate, longitudinally, spinal reflex plasticity in two training groups with similar baseline characteristics, using the same experimental methodology so that the adaptations to motor training could be directly compared. Thus the present study intended to investigate if endurance and strength training induce parallel changes in motor performance, H-reflex and V-wave responses during voluntary contractions of the soleus muscle and, if so, whether there are associations between changes in motor performance (maximal strength and resistance to fatigue) and changes in reflex responses.

6.2 Methods

Subjects

Twenty six healthy volunteers (4 women and 22 men, age: mean ± S.D. 24.0 ± 2.6 yrs) with no history of lower limb disorders participated in the study. None of the subjects were involved in regular strength or endurance training. All participants gave their informed written consent before inclusion in the study. The study was approved by the local ethics committee (N-20090032) and conducted in accordance with the Declaration of Helsinki.

Once the baseline measures were completed, the subjects were matched in pairs based on their age, sex and fitness level and from each pair one subject was randomly allocated to the endurance and one to the strength training group.

Study design

The subjects participated in 3 laboratory sessions, on 3 separate days, and in 9 training sessions over a 3-wk training period (3/wk). A short period of training was chosen since neural adaptations to training can be observed within a few sessions of training (7, 39) and a short duration of training would limit the bias of potential peripheral adaptations on neural responses (7). In order to assess day-to-day variability, two laboratory sessions were completed 1-2 weeks before training (*PRE-S1*and *PRE-S2*), with at least 3 days between sessions. The subjects performed the last laboratory session (*POST*), 24 to 48 hours after the last training session.

Training programs

For both endurance and strength training programs the load intensity increased progressively over the 3-week period. The load was continuously monitored to keep the intensity at the required level. All training sessions were supervised by an investigator of the study.

Endurance training. Endurance training was performed on a cycle ergometer and the exercise intensity was prescribed based on the percent of the heart rate reserve (HRR) according to the Karvonen method (20). Each subject maintained the exercise intensity within the required target heart rate range (THRR) by using a pulse meter during the training sessions. During the first week of training, THRR was set between 55-65% of the HRR and each training session lasted 30-40 min. On weeks 2 and 3 the duration of the training sessions was 40-50 min and the THRR was 60-75% and 65-75% of the HRR, respectively.

Strength training. The strength training program included three bilateral leg exercises (leg press, seated calf raise and calf raise on the leg press) and four extra exercises for the main muscle groups of the trunk and upper body (lateral pull down, bench press, exercise for the trunk flexors and for extensors [abdominal crunch and back extensions]). In order to avoid muscle soreness and to get the subjects familiarized with the lateral pull down, bench press and lower limb exercises, in the first week the load intensity used varied between 60-65% of the one-repetition maximum (1RM) and the number of repetitions between 15 and 18 repetitions, performed over 3 sets. On the week 2 the load intensity for the lower limb exercises ranged between 65-70% of the 1RM and the participants performed 3 sets of 13-15 repetitions and on the last week the load intensity varied between 70-80% of the 1RM, performed over 3 sets of 8-12 repetitions. For the lateral pull down and bench press exercises, the load intensity used in the last two weeks was 65-70 % of the 1RM, performed over 2-3 sets of 15-18 repetitions. The subjects finalized each training session with abdominal crunch and back extensions exercises performed over 2-3 sets of 15-20 repetitions, with no additional load.

Instrumentation

All tests were performed on the right leg while the subjects were comfortably seated in an Isokinetic dynamometer (KinCom Dynamometer – Chattanooga, TN, USA) with their trunk, hips and right thigh firmly strapped to an adjustable chair. The subjects maintained their hips and knee flexed at 120º and ankle at 110º of plantar flexion. The right foot was firmly attached to a force plate mounted in the dynamometer. The foot plate was adjusted so that the lateral malleolus was aligned with the rotational axis of the dynamometer. Particular care was taken to monitor the posture of the subjects. During reflex testing, all subjects were asked to focus on the task and to not alter their posture.

Surface EMG. Surface EMG signals were recorded from the tibialis anterior (TA) and soleus (SOL) muscles with Ag–AgCl electrodes (Ambu Neuroline 720, Ambu A/S, Ballerup, Denmark; conductive area 28 mm^2, inter-electrode distance 20mm), located as recommended by Hermens et al. (17). Prior to electrode placement, the skin was shaved, lightly abraded and cleansed with water. A ground electrode was placed around the right ankle. Surface EMG signals were amplified as bipolar derivations (custom-built EMG amplifier), band-pass filtered (-3dB bandwidth, 10-2000 Hz), sampled at 10000 samples/s, and converted to digital data by a 12-bit A/D converter board.

Stimulation. The H-, M- and V- wave of the SOL muscle were elicited by stimulation of the common posterior tibial nerve. The electrical stimulus was provided by an isolated stimulator (Noxitest IES 230). A monopolar stimulation of the common posterior tibial nerve of the right leg was elicited by a cathode (custom built silver ball with 10 mm diameter) located in the popliteal fossa and the anode (PALs platinum rectangular electrode, 75 × 100 mm, Axelgaard Man) proximal to the patella. Before the cathode electrode placement, the optimal position was identified using a handheld cathode ball electrode (10 mm diameter). The position eliciting the greatest response with the minimum stimulus intensity was chosen.

Experimental procedures

During laboratory sessions, the subject was comfortably seated in the Isokinetic dynamometer as described above. The subject's position was saved in the dynamometer device in order to maintain similar postures over experimental sessions. After placement of the surface electrodes, the subjects performed a warm-up which consisted of multiple submaximal isometric contractions of the plantar flexors (3-4 repetitions at ~50% MVC) and then the following measures we collected:

1. Strength. The subjects performed 3 progressive maximal voluntary contractions (MVCs) of the plantar flexors of 5 s of duration, separated by 2-min rest. Subjects were verbally encouraged to produce maximal force and visual feedback was displayed by a moving bar on a computer monitor. The highest plantar flexor MVC in each experimental session was used to compute the submaximal target force levels.

2. H and M recruitment curve recordings. The motor response (M-wave) and H-reflex of the SOL muscle were elicited while the subject was performing a low-level tonic contraction of the plantar flexors (10% MVC). Subjects were provided with online feedback of the force exerted which was displayed on a computer monitor. The testing procedure and recordings started by progressively increasing the current intensity in 5 mA increments until both peak-to-peak amplitude of the M-wave and peak of the twitch force reached their maximum size during rest condition. A total of three trials at each current intensity were recorded. Then at each current intensity, the preceding M-wave peak-to-peak amplitude was compared with the new M-wave peak-to-peak amplitude. Once the preceding M-wave peak-to-peak amplitude and new M-wave peak-to-peak amplitude had reached a plateau over the three trials, the current intensity of the previous stimulation was considered the maximum current intensity. Then, the upper current intensity used was 5 mA higher than necessary to elicit the maximal M-wave to ensure that the last 2 points of the H and M recruitment curve represented the plateau of the M-wave amplitude and no further increments of the M-wave could be observed. To construct the M and H recruitment curves, the upper current intensity was divided into 22 segments which were equally separated on a logarithmic scale (3). For each current intensity, a total of 16 stimuli were delivered at random time intervals between 2 s and 3 s. To avoid fatigue and mental distraction of the participants, rest periods of 2 min were given every 88 stimuli. Moreover, the subjects were given the possibility to pause the experiment at any time if they reported fatigue.

3. Contractile properties. Maximal twitch amplitude, time to peak and half-relaxation time were measured from the twitch evoked by supramaximal electrical stimulation. After H-M measurements, 4 single pulses (1ms square pulse) at supramaximal intensity (150% of the current needed to evoke peak twitch amplitude) were delivered to the tibial nerve every 5–7 s.

4. V-wave recordings. The subjects were asked to perform 7-10 progressive MVCs of ~5 s duration, with 2 min of rest in between. During the progressive contraction, a supramaximal stimulus (150% of the current needed to evoke maximal M-wave; 1-ms square pulse) was applied to the tibial nerve at the instant that the force exceeded 90% of the MVC (1).

5. Time-to-task-failure. Resistance to fatigue was assessed by performing a sustained contraction at 40% MVC. The subjects were asked to maintain the isometric contraction of the plantar flexors for as long as possible. Task failure was defined as a drop in force greater than 10% of the target force level for more than 5 s, after strong verbal encouragement to the subject to maintain the target force.

Data analysis

Evoked Potentials. Peak-to-peak amplitude of the H-reflex, M-wave, and V-wave was computed offline from the unrectified EMG signals. To reduce inter-subject variability, H-, M-, and V-waves were normalized to the corresponding maximal M-wave (M_{max}) and the H_{max}/M_{max} and V/ M_{max} ratios were computed. For each recruitment curve, the current intensity at H_{max} and M_{max} was identified. Since the size of the M-wave is affected by contraction intensity (33), the M_{max} wave elicited concomitantly either with H-reflex or V-wave was used for the respective normalization.

The ascending part of recruitment curve was fit by a general least squares model, as described by Klimstra and Zehr (23). From the curve fit analysis, the following parameters were analyzed: current intensity at H-reflex threshold (H_{thresh}); current intensity at 50% of the H_{max} (50%H_{max}) and; the slope of the ascending limb of the recruitment curve at 50% of the H_{max} (H_{slope}) (Fig. 6-1).

Additionally, the relative current intensities coinciding with the H-reflex variables computed from the *PRE-S1*recruitment curves were utilized as inputs to the equations describing the *PRE-S2* and *POST* training recruitment curves. According to Darget and Zehr (8), this procedure is more sensitive for detecting training-induced changes since it allows comparison of reflex amplitudes at the same relative current intensities. In order to differentiate the reflex parameters obtained from the fitted curves and from the standard recruitment curve, the predicted parameters are identified with "@", as earlier defined by Klimstra and Zehr (23). Only the recruitment curves with the *r*-square > 0.90 were used for analysis.

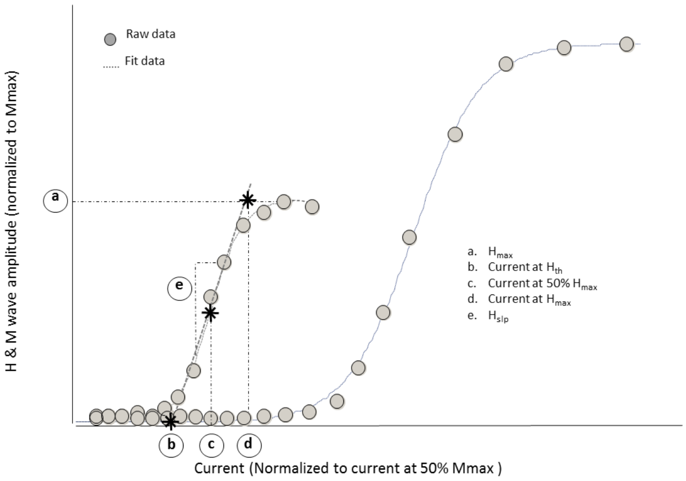

Figure 6-1 - Representation of the H-reflex parameters investigated in this study. The parameters are taken from the ascending limb of the H-reflex recruitment curve. The following parameters were analyzed: (a) Maximum amplitude of the H-reflex (H_{max}); (b) current intensity at H-reflex threshold (H_{thresh}); (c) current intensity at 50% of the H_{max} (50%H_{max}); (d) current intensity at H_{max} and; (e) the slope of the ascending limb of the recruitment curve at 50% of the H_{max} (H_{slope}).

Surface EMG background level. Average rectified value (ARV) of the SOL EMG was estimated from each sweep for an epoch of 500 ms prior to the stimulation and then averaged. The ARV values were normalized with respect to the ARV computed from the highest MVC and expressed as a percentage. During the MVCs performed with supramaximal stimulation the EMG were analyzed over a 500 ms period before stimulation and then normalized to the corresponding amplitude of the M_{max}. The level of co-activation was quantified by computing the ratio between the agonist (SOL) and antagonist (TA) EMG ARV and multiplying by 100.

Statistical analysis

Prior to statistical comparison, all data were tested for normal distribution by a Kolmogorov-Smirnov test. All pre- to post training changes were evaluated using a two-way repeated measures ANOVA with factors group (endurance and strength) and session (*PRE-S1, PRE-S2* and *POST*). For multiple comparison analysis, post-hoc Student-Newman-Keuls test was used when ANOVA was significant. Statistical significance was set at $P < 0.05$ for all comparisons.

Results are reported as means and SD in the text and mean and SE in the figures. To investigate associations between the variables affected by training a multiple regression was performed with changes in the evoked potentials parameters as the independent variables and maximal strength or time-task-failure as dependent variables. The regressions were performed separately for each training group.

6.3 Results

One subject from the strength group did not complete the final laboratory session and was excluded from the analysis. Therefore, the results are presented for 12 subjects in the strength group (age, 23.6 ± 2.2 yrs; height, 177.5 ± 8.7 cm; weight, 73.2 ± 13.5 kg) and 13 subjects in the endurance group (age, 24.4 ± 3.8 yrs; height, 176.5 ± 10.5 cm; weight, 76.5 ± 13.6 kg). No differences were observed between groups for any of the anthropometrical characteristics, motor output or electrophysiological parameters assessed in the *PRE-S1* and *PRE-S2* sessions.

Motor performance

During the first two sessions, neither MVC nor time-to-task-failure was altered in either training group (*P* > 0.87; Fig. 2). Following 3 weeks of training the MVC increased by 14.4 ± 5.2% in the strength training group (*P* < 0.0001), whereas no significant change in MVC was observed for the endurance group (*P* > 0.68; Fig. 2). Conversely, the time-to-task-failure increased by 22.7 ± 17.1% following endurance training (*P* < 0.04) and was not affected by strength training (*P* > 0.68; Fig. 6-2).

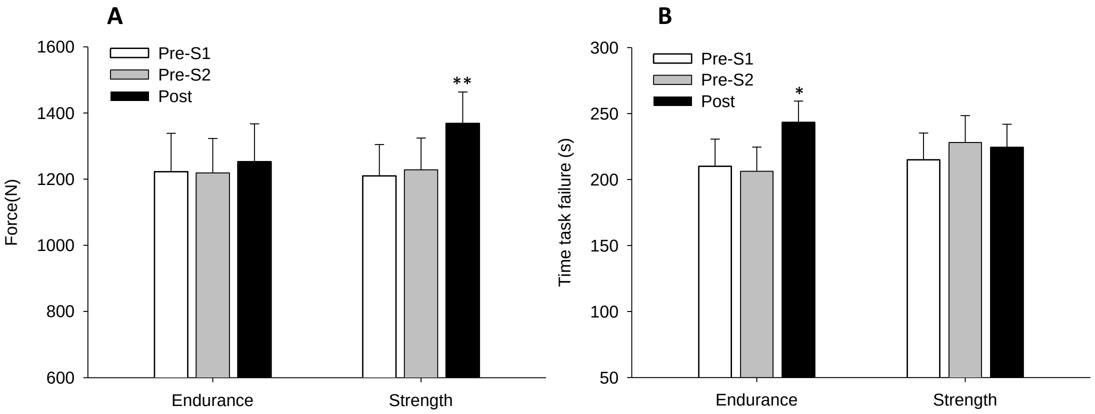

Figure 6-2 - Changes in motor performance following 3 weeks of strength or endurance training. Values are mean ± SE. ***A***: Maximal voluntary contraction (MVC) of the plantar flexors. ***B***: Time to task failure assessed during isometric contractions at 40% MVC. *: *P* < 0.05 and **: *P* < 0.01 when comparing *POST* to *PRE-S1* and *PRE-S2*.

Contractile properties

There were no main effects or interaction between group and session for the contractile parameters analyzed in this study ($P > 0.13$ for all conditions and parameters). Across the 3 laboratory sessions the averaged value of each group for the maximal twitch amplitude ranged between 117.3 ± 32.5 N and 121.3 ± 46.4 N ($P > 0.80$ for all conditions), for the time to peak force ranged between 89.3 ± 6.3 ms and 92.6 ± 9.1 ms ($P > 0.43$ for all conditions) and for the half-relaxation time varied between 254.8 ± 18.2 ms and 263.7 ± 21.1 ms ($P > 0.28$ for all conditions).

Evoked potentials

The peak-to-peak amplitude of the SOL maximal compound action potential (M_{max}) did not change significantly following training ($P > 0.52$ for all conditions; see Table 1) and was not different between the two baseline sessions (*PRE-S1*and *PRE-S2;* $P > 0.22$ for all parameters).

V-wave amplitudes during MVC. Fig. 6-3 shows representative V-waves for one subject of the endurance group and one of the strength group pre- and post-training. In these examples, the V-wave increased following strength training while it did not change substantially following endurance training. This result was confirmed by the group analysis. There was a significant interaction between session and group for the normalized V-wave amplitude (V/M_{max}) assessed during MVC ($P < 0.01$).

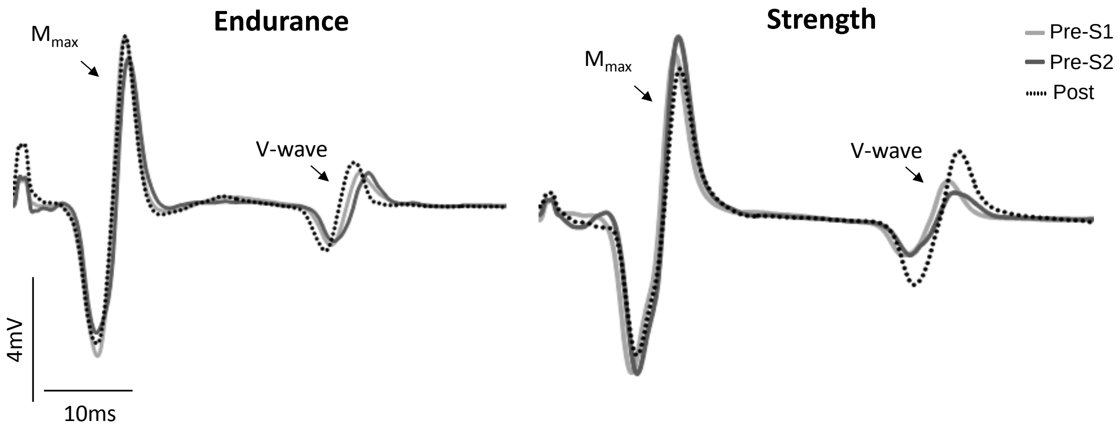

Figure 6-3 - Evoked V-wave before (*PRE-S1* and *PRE-S2*) and after (*POST*) training for a representative subject of the endurance and strength training group.

Following strength training the V/M_{max} ratio increased in average by 55.1 ± 28.3 % compared to pre-training sessions ($P < 0.001$ when compared *POST* to *PRE-S1* and *PRE-S2*). For the endurance group, the V/M_{max} ratio did not change significantly over time ($P > 0.34$; Fig. 6-4).

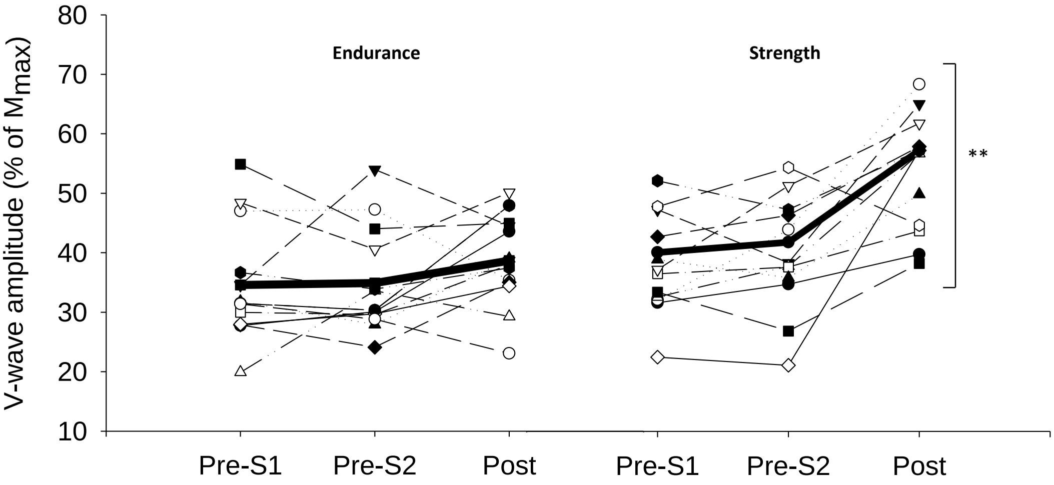

Figure 6-4 - V-wave peak-to-peak amplitude normalized to M_{max} measured during maximal isometric contraction of the plantar flexors before (*PRE-S1* and *PRE-S2*) and after (*POST*) training. Each line represents one subject. The thick black line represents the mean of the group. $P < 0.001$ when comparing *POST* to *PRE-S1* and *PRE-S2* in the strength group.

Low-level tonic contractions and H-reflex parameters. Fig. 6-5 illustrates the M-wave and H-reflex recruitment curve for one subject of the endurance group and one of the strength group, pre- and post-training. In these examples, the endurance training elicited an increase H-reflex excitability, while in the strength training only slight changes can be observed at H_{thresh}. These observations were confirmed by the group analysis. A significant increment of the SOL H-reflex amplitude was observed following endurance training but not strength training (interaction between group and session: $P < 0.05$; Fig. 6-5; Table 6-1).

Since the M_{max} did not change for either training group (Table 6-1), the endurance group also revealed changes in the H_{max}/M_{max} ratio, which was not observed in the strength training group (interaction between group and session: $P < 0.001$; Fig. 6-5). Following endurance training, the H_{max}/M_{max} ratio increased by 30.8 ± 21.7%, when compared to pre-training sessions ($0.001 < P < 0.05$; Fig. 6-6).

Similarly, the slope of the ascending limb of the recruitment curve at 50% of the H_{max} (H_{slope}) was only affected by the endurance training intervention (interaction between group and session: $P < 0.01$; see Table 6-1). After 3 weeks of endurance training a significant increment of the H_{slope} was observed when compared to both pre-training sessions ($P < 0.05$ compared to both pre-training sessions; see Table 6-1).

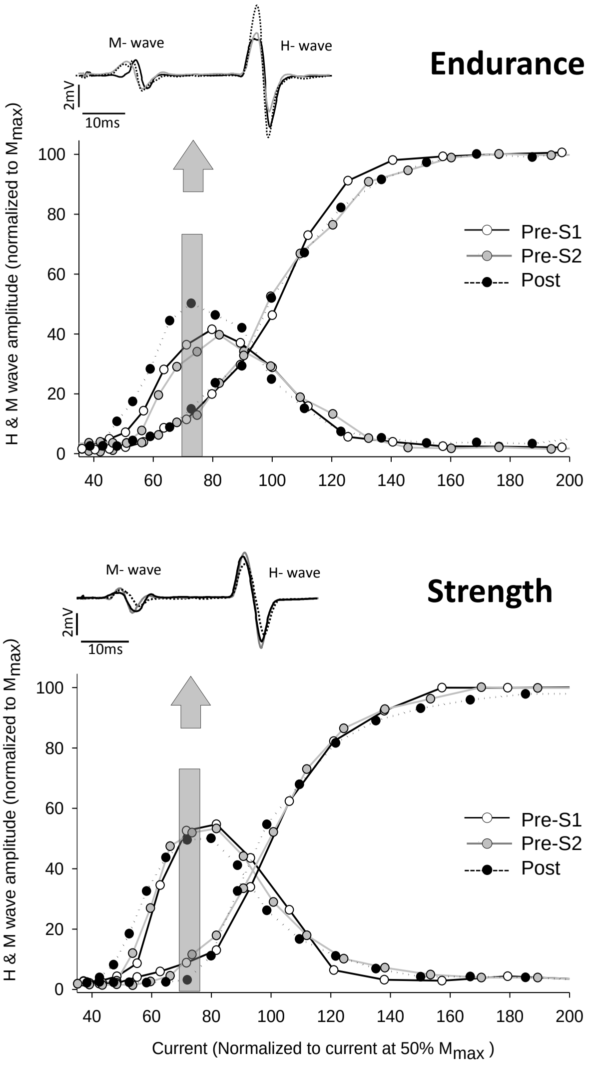

Figure 6-5 – H and M-wave recruitment curves before (*PRE-S1* and *PRE-S2*) and after (*POST*) training for a representative subject of the endurance and strength training group. The abscissa represents the stimulation intensity normalized to the current at 50% M_{max} and ordinate illustrates response amplitude normalized to the corresponding M_{max}. In the left superior corner is represented the average of 8 H and M waves evoked by a current of 70-75% of 50% M_{max}, before [*PRE-S1 (black solid line)* and *PRE-S2 (gray solid line)]* and after [*POST (black dotted line)*].

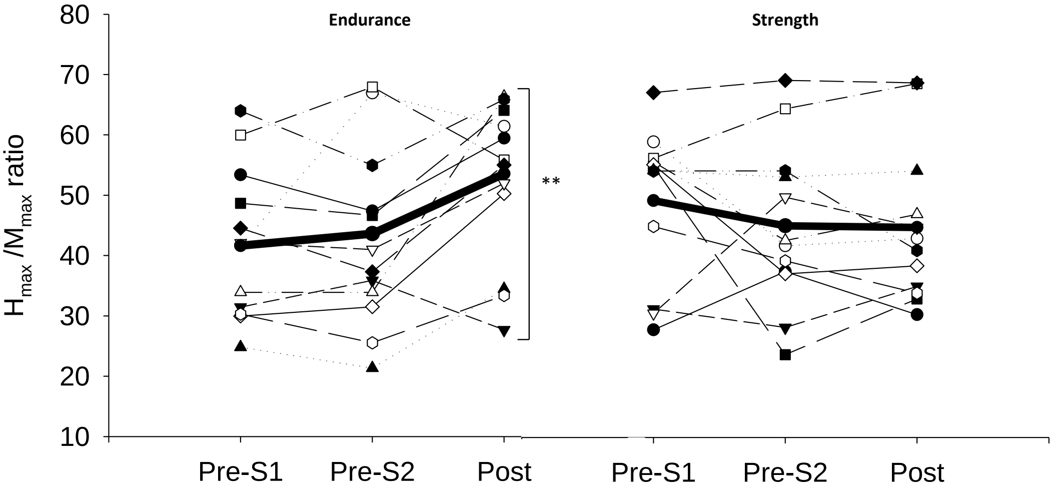

Figure 6-6.– H_{max}/M_{max} ratios measured during isometric contractions of the plantar flexors at 10% of MVC before (*PRE-S1* and *PRE-S2*) and after (*POST*) training. Each line represents one subject. The thick black line represents the mean of the group. *P* < 0.01 when comparing *POST* to *PRE-S1* and *PRE-S2.*

Table 6-1- Mean and standard deviation of the maximal M-wave (M_{max}), H-reflex (H_{max}), slope of the ascending limb of the recruitment curve at 50% of the H_{max} (H_{slope}), normalized current (normalized to current at 50%M_{max}) at H-reflex threshold (H_{thresh}), at 50% of H_{max} and at H_{max}, soleus EMG activity during MVC and soleus EMG background during the low-level tonic contractions

Parameters	**Endurance Group**			**Strength Group**		
	Pre-S1	**Pre-S2**	**Post**	**Pre-S1**	**Pre-S2**	**Post**
M_{max} (mV)	7.0 ± 2.7	6.5 ±1.5	7.2 ± 1.9	7.1 ± 4.0	7.2 ± 4.1	6.9 ± 3.6
H_{max} (%M_{max})	43.7 ± 16.2	43.6 ± 14.5	54.3 ± 14.2*	53.7 ± 10.9	50.5 ±11.7	52.2 ± 12.0
H_{slope} (mV.s^{-1})	2.3 ± 0.7	2.2 ± 0.9	3.1 ± 1.0*	2.9 ± 1.3	2.8 ± 1.4	2.5 ± 1.1
Current at H_{thresh} (% current at 50% M_{max})	50.5 ± 8.2	49.1 ± 6.9	46.0 ± 7.0†	47.4 ± 5.6	47.6 ± 6.6	45.4 ± 6.9†
Current at 50%H_{max} (% current at 50% M_{max})	61.1 ± 7.9	59.6 ± 7.4	55.4 ± 6.5‡	57.8 ± 6.0	56.4 ± 7.2	55.3 ± 7.7
Current at H_{max} (% current at 50% M_{max})	71.9 ± 8.7	70.3 ± 7.5	65.5 ± 7.1*	68.6 ± 8.6	66.9 ± 8.4	66.1 ± 9.2
Soleus EMG						
MVC (% M_{max})	2.3 ± 0.5%	2.4 ± 0.5%	2.5 ± 0.6%	2.5 ± 0.6%	2.5 ± 0.8%	3.2 ± 1.5%#
Background (% MVC)	24.8 ± 8.4%	26.6 ± 7.3%	28.0 ± 7.8%	24.3 ± 5.7%	25.2 ± 6.2%	27.2 ± 11.3%

* - $P< 0.05$ when comparing *POST* to *PRE-S1* and *PRE-S2*. †- Main effect for session; $P< 0.05$ when comparing *POST* to *PRE-S1.* ‡- $P< 0.01$ when comparing *POST* to *PRE-S1* and $P< 0.05$ when comparing *POST* to *PRE-S2*. # - $P< 0.01$ when comparing *POST* to *PRE-S1* and *PRE-S2*

A session effect was obtained for the normalized current intensity at H-reflex threshold (H_{thresh}) ($P < 0.01$, Table 6-1). Following both endurance and strength training, the H_{thresh} was significantly lower than at the pre-training sessions (-7.7± 7.1% and -4.7 ± 6.7%, respectively; $0.01 \leq P < 0.05$ for all conditions; Table 6-1). An interaction between group and session was observed for the current intensity at 50% of the H_{max} (50%H_{max}) and at H_{max} ($P < 0.05$ for both parameters; Table 6-1).

The normalized current intensity required to evoke 50%H_{max} and the H_{max} was significantly reduced following endurance training (on average -8.6 ± 6.2%) when compared to the pre-training sessions (for both parameters: $P < 0.01$ when compared to pre-training sessions; see Table 6-1). Both 50%H_{max} and H_{max} parameters were not affected by strength training ($P > 0.59$ for all conditions).

Figure 6-7 illustrates the predicted H-reflex amplitudes evoked by the current intensities associated to the H_{thresh}, intensity at 50%H_{max} and at H_{max} observed before training. The predicted values are distinguished by "@". The $H_{@thresh}$ was significantly affected by both training programs (main effect session: $P < 0.001$; Fig. 6-7 *A*), but no interaction between group and session was observed ($P = 0.26$). Compared to the pre-training session, the $H_{@thresh}$

increased by 182.6 ± 121.4% and by 105.6 ± 88.9% following endurance and strength training respectively (0.0001 < P <0.001 for all conditions*;* Fig. 6-7 *A*).

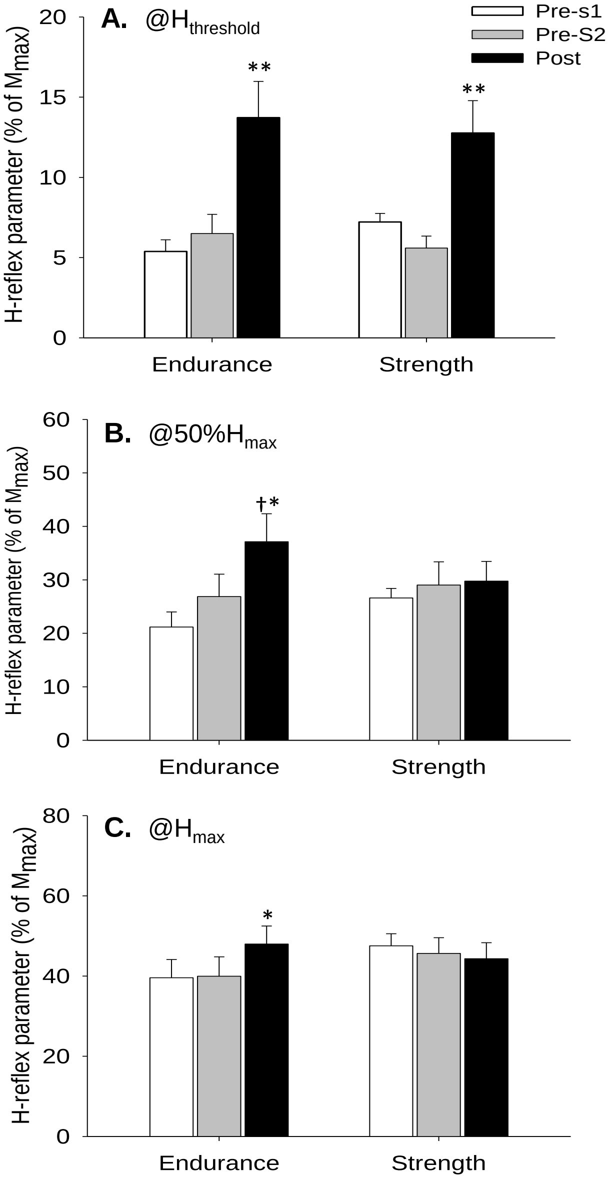

Figure 6-7 - Predicted H-reflex amplitudes evoked by the current intensity associated to the H_{thresh}, intensity at 50%H_{max} and at H_{max} observed before training. Values are mean ± SE. ***A***: Predicted H-reflex amplitude at H_{thresh}. ***B***: Predicted H-reflex amplitude at 50%H_{max}. ***C***: Predicted H-reflex amplitude at Hmax *: P < 0.05 and **: P < 0.001 when comparing *POST* to *PRE-S1* and *PRE-S2*. †: P< 0.001 when comparing Post to *PRE-S1*.

For the 50%$H_{@max}$ and $H_{@max}$ parameters, an interaction between group and session was observed (P < 0.05 for both parameters; Fig. 6-7 *B* and *C*). After 3 weeks of endurance training the 50%$H_{@max}$ was on average 80.7 ± 42.6% greater than in the pre-training sessions (0.001 < P <0.05; Fig. 6-7 *B*). Further, the $H_{@max}$ observed following endurance training increased by 60.2 ± 34.8% when compared to pre-training values (P < 0.05*;* Fig. 6-7 *C*). No changes of the 50%$H_{@max}$ and $H_{@max}$ parameters were observed with strength training.

EMG activity and background level. During the MVC, the SOL normalized EMG was only affected by the strength training program (interaction between group and session: $P < 0.05$, Table 6-1). Following strength training, the SOL normalized EMG was significantly higher compared to baseline ($P < 0.01$; Table 6-1). The background EMG level of the SOL muscle during the low-level tonic contractions was not affected by either training program ($P > 0.12$ for all conditions, Table 6-1). Moreover, the level of co-activation was not affected by training ($P > 0.45$). For the endurance group the co-activation ratio in the *PRE-S1*, *PRE-S2* and *POST* sessions was 38.1 ± 10.9%, 39.2 ± 13.4% and 35.5± 11.6% and for strength training it was 42.0 ± 11.6%, 39.3 ± 9.9% and 38.9± 14.6% respectively.

Associations between changes in strength, resistance to fatigue and evoked potential parameters

For the strength training data we examined the association between changes in maximal force and V/M_{max} ratio, while for endurance training the association between changes in time-to-task-failure and changes in parameters obtained from the fitted curves ("@"parameters) and from the standard recruitment curve (H_{max}/M_{max}; H_{slop} and H_{thresh}; $50\%H_{max}$; H_{max}) were examined.

A significant association ($P<0.01$) was observed between the increase in MVC and increase in V/M_{max} ratio (β= 0.23; $F_{1,3}=4.25$; adjusted $R^2=0.20$) for the strength training group. In the endurance training group, the multiple linear regression showed a significant association between the increase in time-to-task-failure and increase in $@H_{thresh}$, in $@50\%H_{max}$ and in $@H_{max}$ (β= 0.32; β= 0.05; and β= 0.26, respectively; $P<0.01$; $F_{3,3}=4.7$; adjusted $R^2=0.24$]. However, no associations were found between the increase in time-to-task-failure for the endurance group and changes in H_{max}/M_{max}; H_{slop} and H_{thresh}; $50\%H_{max}$; H_{max}.

6.4 Discussion

This study investigated spinal reflex plasticity following 3 weeks of either endurance or strength training. For the first time these changes were concurrently investigated in two training groups with similar characteristics, so that a direct comparison of the neural adaptations was possible. The results show that improvement in time to task failure following endurance training is accompanied by significant changes of the H-reflex recruitment curve whereas no changes in the V/M_{max} ratio are observed. Conversely, following strength training, an increase of the MVC occurs and is accompanied by a significant increase of the V/M_{max} ratio whereas changes in the H-reflex recruitment curve were only observed at H_{thresh}.

Motor performance

Strength and endurance training result in specific adaptations of motor performance. Following endurance training, the resistance to fatigue increased while the maximal strength of the plantar flexors did not change and strength training improved MVC of the plantar flexors resistance to fatigue remained unchanged.

The results showed that 24% of the improvement in resistance to fatigue could be explained by increased H-reflex excitability, while the increase in V/M_{max} ratio explained 20% of the improvement in MVC. Few other studies have shown positive associations between measures of motor performance and the degree of spinal excitability (11, 34). Ekblom (11) found that 66% of the improvement in maximal isometric force of the plantar flexors following 5 weeks of dynamic strength training, was explained by increased voluntary activation and V/M_{max} ratios. The only longitudinal study on endurance training and reflex excitability, has shown that following 8 weeks of training, 75% of the subjects presented positive associations between the H_{max}/M_{max} ratio and maximal aerobic power (34). In the current study, the low level of association may be due to the different condition of the measures since both endurance and strength training involved dynamic work around several joints while the tasks for motor performance and the neural plasticity tests were performed under isometric conditions. Despite the weak association, the present study indicates that neural plasticity occurred in response to specific motor training and such alterations contributed to the specific motor performance improvements.

Methodological considerations

H-reflex responses are influenced by several methodological factors, including the size of the afferent volley elicited by the stimulation pulse, background level of muscle activation, muscle contraction intensity, H-reflex normalization, posture and attention level of the subjects (for review see (29, 40)). Therefore, appropriate interpretation of the H-reflex response requires that particular recording conditions are achieved (24, 40). In the present study, the posture of the subject and their attention level were carefully controlled.

The H-reflex measures in this study were provided by M and H-reflex recruitment curves obtained during low-level tonic contraction of the plantar flexors. This procedure has been recommended (5, 29, 40), since it allows the best control over the effect of afferent volley size and attenuates the effect of activity-dependent changes in axonal excitability (4, 5, 29, 40). Moreover, measurement of the H-reflex recruitment curve allows the H-reflex to be assessed at different stimulus intensities thereby providing information on the recruitment of motor units of different thresholds (23). Alterations of the contraction intensity and EMG background level of the target muscle can also induce changes in the H-reflex amplitude. Nonetheless, similar contraction intensity across each experimental session was ensured and the background EMG activity during low-level tonic contractions did not differ between experimental sessions in this study.

Effects of training on V/M_{max} ratio and EMG activity

The present study showed that SOL V/M_{max} ratio increased by 55% after 3 weeks of strength training but remained unchanged following an equal period of endurance training. Changes in the neural mechanisms underlying the V-wave responses are thus specific to strength training. Enhanced SOL V-wave amplitude following strength training has been reported by other longitudinal studies, showing increments of 55% to 81% compared to the pre-training condition (1, 7, 11, 12, 15). The V-wave results from a supramaximal electrical stimulus applied to the nerve during ongoing muscle contraction which elicits action potentials in all Ia afferent fibers and motor axons (9, 35, 38). In the motor axons, the action potentials will travel to the muscle, generating the M-wave, and antidromically toward the spinal cord. The antidromic potentials collide with orthodromic motor action potentials elicited by the descending voluntary input to the motoneuron pool. This results in cancellation of the two potentials so that the H-reflex can pass to the muscle where it is recorded as V-wave (1, 38). An increase of the efferent motor output would increase the probability of antidromic collision and thus allow a larger part of the evoked afferent volley to reach the muscle. Consequently, it has been assumed that the increase of V-wave amplitude reflects an increase of supraspinal activation, which enhances descending volitional drive (i.e., recruitment and/or discharge rate of the motor neurons) to the muscle (1, 7, 10). Increased descending drive may also explain the increased SOL normalized EMG during the MVC, which was observed following strength training in this study but not following endurance training. The V-wave amplitude can also be altered by other mechanisms, including changes in motor neuron responsiveness (e.g., changes of intrinsic membrane properties and discharge rate), synaptic transmission efficacy at Ia afferent terminals (e.g. pre-synaptic inhibition), and/or post-synaptic inhibition (5, 35). Nonetheless, these mechanisms would also contribute in a very similar way to the H-reflex (35, 41). In the present study, the strength training program induced a significant increase in V-wave amplitude while alterations of the H-reflex were only observed at H_{thresh}. These observations indicate that increased V-wave amplitude mostly reflects changes in the descending neural drive to the muscle.

Effects of training on H-reflex responses

Following endurance training, the normalized current intensity at H_{thresh}, 50%H_{max} and at H_{max} was significantly lower, confirming increased H-reflex excitability. These results were supported by the significant change of the predicted amplitudes (@H_{thresh}, @50%H_{max} and @H_{max}), which are more sensitive to training-induced changes (8, 23). An increase of the predicted amplitudes indicates that the current intensities at H_{thresh}, 50%H_{max} and at H_{max} observed before training enhanced the H-reflex after endurance training. The H_{max}/M_{max} ratio and the slope of the ascending limb also increased following endurance training only. Taken together, the above findings reveal that endurance training elicited a leftward shift of the ascending limb of the H-reflex recruitment curve (Fig. 5), increased H-reflex amplitudes and H-reflex gain (measured as H_{slp}), confirming plastic adaptations in the Ia spinal reflex pathway. Furthermore, this adaptation appeared to contribute for improved resistance to fatigue following training.

These alterations suggest that endurance training lowers the recruitment threshold of motor neurons to Ia afferent input (28, 34) and increases the recruitment gain (i.e., the magnitude of the threshold differences between motor neurons decreases) (21, 22). Several cross-sectional studies have also shown increased H-reflex excitability in endurance-trained athletes compared to strength and power-trained athletes (6, 26, 28, 37). These results were partly explained by differences in the muscle fiber type distribution between endurance and power-trained athletes. Endurance-trained athletes have a higher percent of slow-twitch fibers (14, 16) and the excitatory postsynaptic potentials are largest in small motor neurons innervating slow-twitch motor units (35), which would explain greater H-reflex responses in endurance - trained athletes (26, 28). However, in the present study the contractile properties were not influenced by training, which indicates that 3 weeks of training was probably not sufficient to induce peripheral changes. Thus, it is likely that the enhanced H-reflex excitability resulted from a change in the distribution of motor neuron excitability and synaptic inputs across the motor neuron pool (21, 22). In a previous study (39), it was reported that for the same relative load (30% MVC), the motor unit discharge rate of the vasti muscles decreased following endurance training. Based on these results it seems that to maintain the same relative load, the decreased discharge would be compensated by increased number of recruited motor units. It can be speculated that these changes represent a mechanism to reduce fatigue, which would optimize motor performance during prolonged exercise. Lower discharge rates and increased motor unit recruitment would increase the energy efficiency (ATP supply through the aerobic metabolism) and counteract muscle fatigue without affecting force and rate of force development (21). The neural mechanisms that may contribute to these observed changes include decreased presynaptic Ia and reciprocal inhibition, increased motor neuron excitability and supraspinal activation. Based on the V-amplitude measures, it is unlikely that changes in the descending drive occurred following endurance training. Furthermore, the co-activation ratio was not altered by endurance training, suggesting that changes in the H-reflex response were not due to a change in reciprocal inhibition. Thus, the training induced alterations of the H-reflex were most likely due to adjustments in motor neuron responsiveness and/or in presynaptic inhibition of Ia terminals.

Contrary to endurance training, strength training only elicited a decrease in the normalized current intensity at H_{thresh}, while no changes were observed in other measures of the H-reflex. It is well known that the motor neurons are recruited in an orderly fashion by the Ia input from the smallest to the largest, according to the size principle (35). The present results suggest that the net excitability was only altered for the low-threshold motor units while no changes were observed for the higher-threshold motor units involved in the maximal H-reflex response. Other studies involving strength training, also reported increased H-reflex excitability at very low stimulus intensities (at H_{thresh}, and 5%M_{max}) and no change for the H_{max}/M_{max} ratios (8, 27). While unchanged H_{max}/M_{max} ratios have been reported in several studies (10, 11, 15), some report increased H-reflex response at stimulus intensities of 20%M_{max}(1, 18) or no change at 10% M_{max} (12). Such conflicting results may partly be due to differences in the methodologies applied to elicit and measure the H-reflex (7, 27). In addition, the contraction level used varies across studies, thus the activation of different motor unit populations may contribute to the inconsistency of the results.

6.5 Conclusion

The current work showed that following 3 weeks of endurance training the excitability in the H-reflex pathway increased but the V-wave amplitude remained unchanged. In contrast, following strength training the V-wave amplitude increased whereas subtle changes were observed in the H-reflex pathway. Moreover, although weakly, the improvement in time-to-task-failure of the plantar flexors was associated with increased H-reflex excitability while the increase in MVC was associated with increased V-wave amplitude. These results suggest that the elements of the H-reflex pathway are strongly involved in chronic adjustments in response to endurance training, contributing to enhance resistance to fatigue. Conversely, following strength training, it is more likely that increased descending neural drive during MVC and/or modulation in afferents other than Ia afferents contributed to increased motoneuron excitability and MVC of the plantar flexors.

Acknowledgements

We thank Dr. Natalie Mrachacz-Kersting, Center for Sensory-Motor Interaction (SMI), University of Aalborg, Denmark and Professor Kemal Türker, University School of Medicine, Izmir, Turkey for their helpful comments on the experimental design. We are also grateful to Mostafa Kamel and Saba Gervasio, SMI, University of Aalborg, Denmark, for their assistance in Matlab programming. Carolina Vila-Chã was supported by Fundação para a Ciência e Tecnologia (FCT) of Portugal (PhD Grant ID – SFRH/BD/31796/2006 and PROLIMB project: PTDC/EEA-ELC/103683/2008). Part of the study was supported by the ERC Advanced Research Grant DEMOVE ("Decoding the Neural Code of Human Movements for a New Generation of Man-machine Interfaces"; no.: 267888) (DF).

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CHAPTER

7

General Discussion and Conclusions

The main objective of this dissertation was to systematically investigate the changes in neural mechanisms, motor unit behavior and in the electrophysiological properties of the muscle fibers following endurance and strength training by applying advanced electrophysiological techniques. In this section it will be present an integrated discussion outlining the major findings of each study and their potential contribution for improvement in motor performance. The discussion will be concluded with limitations of the present work and suggestions of future work.

7.1 General discussion

The study I presented in Part II of the thesis showed that specific endurance and strength training programs elicit opposite adjustments in motor unit discharge rates, but similar adaptations of the electrophysiological muscle fiber properties, as it was revealed by the motor unit conduction velocity estimates. Endurance training increased resistance to fatigue and was accompanied by a decrease in motor unit discharge rates. In contrast, strength training enhanced maximum force output and was accompanied by an increase in motor unit discharge rates. By the end of 6 weeks of training, both programs elicited increases in motor unit conduction velocity, revealing electrophysiological adaptations of the muscle fiber membrane properties in similar directions. However, in the first 3 weeks of training, when changes in motor unit discharge rates were more pronounced, changes in motor unit conduction velocity were not observed. These findings showed different time courses of some of the neural and peripheral adaptations in response to motor training.

Despite the opposite adaptations of the mean discharge rates (study I), during prolonged contractions, motor unit discharge rates decrease as fatigue develops and neither endurance nor strength training altered this trend (study II). The motor unit conduction velocity was also shown to decline as fatigue develops, which reveals gradual change of the muscle fiber properties due to alterations in the extracellular and intracellular K^+ and Na^+ concentrations and accumulation of intracellular lactate and hydrogen ions. This reduces membrane excitability and impairs the velocity of propagation of the action potentials along the muscle fibers. With both types of training the rate of decline of motor unit conduction velocity during sustained contractions was reduced following six weeks of both endurance and strength training, however a greater reduction is observed following endurance training. Moreover, endurance training reduces the level of co-activation between the vasti and biceps femoris during sustained contractions of knee extension. These alterations likely contribute to longer times to task failure following endurance training.

Following up the evidences encountered in the previous research, study III was designed to investigate the adjustments in the spinal cord input that may contribute for changes in the motor unit behavior following endurance and strength training. For this purpose we analyzed the evoked V-wave, H-reflex and M-wave recruitment curves before and after 3 weeks of training. The main findings of study III suggest that following endurance training the excitability in the H-reflex pathway increased but the V-wave amplitude remained unchanged, conversely, following strength training the V-wave amplitude increased whereas subtle changes were observed in the H-reflex pathway. Moreover, the improvement in time-to-task-failure of the plantar flexors was associated with increased H-reflex excitability while the increase in MVC was associated with increased V-wave amplitude. Such adaptations occurred in the absence of changes in the contractile properties of the trained muscles. The results indicate that the elements of the H-reflex pathway are strongly involved in chronic adjustments in response to endurance training, contributing to enhance resistance to fatigue. In contrast, following strength training, it is more likely that increased descending neural drive during MVC and/or modulation in afferents other than Ia afferents contributed to increased motoneuron excitability and MVC of the plantar flexors.

The collective results of this thesis indicate the central nervous system adapts differently to match the endurance and strength training demands. Moreover, the distinct adjustments in the motor unit behavior, seems to result from changes in different neural mechanisms located at supraspinal and spinal level. These adaptations were observed after only 3 weeks of training, while no significant changes in the contractile and electrophysiological properties of the muscle fibers were detected. This indicates that the initial gains in endurance and strength capacity partially result from changes in the central nervous system. But the question on how these changes may contribute to increase motor performance remains.

Regarding to endurance training, we observed that to maintain the same relative force level the motor unit discharge rates were lower and surface EMG amplitude higher following training (study I). Study III revealed that this type of training increases H-reflex excitability, suggesting that endurance training lowers the recruitment threshold of motor neurons to Ia afferent input (Maffiuletti et al. 2001; Perot et al. 1991) and increases the recruitment gain (i.e., the magnitude of the threshold differences between motor neurons decreases) (Kernell 2006; Kernell and Hultborn 1990). Based in these results, we may speculate that endurance training alters both recruitment and discharge rate behavior of the motor units. Accordingly, to maintain the same relative force level, the decrement in discharge rates would be compensated by an increment of the number of motor units recruited (Adam et al. 1998; Gardiner 2011). Lower discharge rates and increased motor unit recruitment would increase energy efficiency without affecting force and speed output (Kernell 2006). These changes may represent a mechanism to reduce fatigue, which would optimize motor performance during prolonged exercise (Gardiner 2011; Kernell 2006). It is well known that endurance training increases the oxidative capacity of the muscle promoting the ATP supply through the aerobic metabolism (Costill 1970; Hawley 2002). Although energetically efficient, the rate of ATP production is slower than the anaerobic metabolism. Thus, decreasing the rate of cross-bridge cycles (i.e. lowering motor unit discharge rates) would balance the ATP production/consumption process and protect the cell energy homeostasis during prolonged exercise. The neural mechanisms that may contribute to these observed changes include decreased presynaptic Ia and reciprocal inhibition, increased motor neuron excitability and supraspinal activation. Based on the V-amplitude measures, it is unlikely that changes in the descending drive occurred following endurance training. Thus, the training induced alterations of the H-reflex were most likely due to adjustments in intrinsic properties of the motoneurons (e.g. AHP duration) and/or in presynaptic inhibition of Ia terminals.

Later adjustments in the peripheral properties, as evidenced in study II, may also contribute for increased endurance resistance. Following six weeks of endurance, the slower decline of motor unit conduction velocity during sustained contractions, reflects a slower rate of increase of metabolic by-products of the contraction. The observed alterations may be due to changes in the Na^+-K^+ pump concentration (content and/or activity), which is considered an important mechanism for the regulation of the Na^+-K^+ distribution and maintenance of membrane excitability during muscle contractions (McKenna 1995; Nielsen and Clausen 2000). Thus, an up-regulation of pump concentration would prolong the balance of Na^+-K^+ concentration gradients, protecting the muscle contractibility against fatigue (Clausen 2003; McKenna et al. 2008). Other mechanisms, such as decreased co-activation during the sustained contractions (study II) may also account for improvements in the endurance capacity during sustained contractions (Enoka and Duchateau 2008; Hunter and Enoka 2003).

Contrary to endurance training, strength training increased the motor unit discharge rates (study I). Such alterations likely have an important role in the enhancement of maximal strength and maximal rate of force development (Aagaard 2003; Duchateau et al. 2006; Van Cutsem et al. 1998). Study III showed that strength training increased V-wave amplitude while alterations of the H-reflex were only observed at H-reflex threshold. Altogether, these observations suggest that increased spinal cord output following strength training likely reflect changes in the descending neural drive to the muscle. Increased V-wave amplitude has been consistently observed following strength training (Aagaard et al. 2002b; Del Balso and Cafarelli 2007; Duclay and Martin 2005; Ekblom 2010; Fimland et al. 2009), nevertheless, the TMS studies are not revealing a clear pattern of changes of the corticospinal excitability (Beck et al. 2007; Carroll et al. 2009; Carroll et al. 2002; Griffin and Cafarelli 2007). Following strength training, the size of MEPs has been shown to increase in the plantar flexors (Beck et al. 2007; Griffin and Cafarelli 2007), be unchanged in finger muscles (Carroll et al. 2009; Carroll et al. 2002) or trend lower in arm muscles (Jensen et al. 2005). Caroll et al. (2011), suggests that the inconsistent results might be due to a lack of sensitivity of the TMS to detecte subtle changes with MEP approaches over different experimental contexts. A recent study used a different approach to address the possible role of the primary motor cortex in response to strength training (Hortobagyi et al. 2009). These authors applied low-frequency repetitive magnetic brain stimulation (rTMS) after every session in a 4 week of strength training first dorsal interosseus, in order to induce corticospinal inhibition to primary motor cortex. The results revealed that although no significant changes in corticospinal excitability measured with TMS were observed, the subjects that received rTMS showed lower strength gains in comparison to the control group. Thus, the rTMS of the primary motor cortex interfered with strength gains and, moreover, the impairment in strength gain was associated with the reductions in primary motor cortex excitability. This indicates that primary motor cortex may play a role in mediating neural adaptations to strength training, although contributions from spinal sources cannot be excluded (Hortobagyi et al. 2009).

The influence of potential changes in the spinal circuitry on the V-wave amplitude measures cannot also be discarded. Although combined measures of V-wave and H-reflex contributes for identifying the potential mechanisms and sites involved in the adaptations, both responses involve different stimulus intensities that activate different populations of afferent and motoneurons (antidromically), which might also result in different oligosynaptic influences on the reflex amplitude (Carroll et al. 2011). At the moment, there are no methods that unequivocally would allow distinguishing the adaptations occurring at supraspinal and spinal level in humans. Although also based in some neurological assumptions, the combined methods such as TMS and cervicomedullar stimulation of the corticospinal tract are referred as the best methodology to dissociate cortical from subcortical effects (for review see Taylor and Gandevia 2004). However, cervicomedullar stimulation is very painful which difficult the recruitment of subjects for the studies (Carroll et al. 2011). Despite this, the studies based on different electrophysiological techniques provide some evidence that strength training increases the capacity of the motor cortex to drive the motoneurons during maximal effort (Carroll et al. 2011).

7.2 Conclusions

The aim of this thesis was to systematically investigate the neuromuscular adaptations to distinct motor training programs, such as endurance and strength training, with particular emphasis on the neural mechanisms. To achieve this purpose different electrophysiological techniques were combined and applied, which allowed to concurrently assess both central (i.e. motor unit behavior) and peripheral (muscle fiber membrane properties) adaptations to specific motor training programs. Due to the high spatial selectivity, intramuscular EMG signals were used to investigate changes in spinal cord output by assessing motor unit behavior. Information on the discharge rate patterns was extracted from the intramuscular signals by employing digital signal processing and pattern recognition techniques recently developed. Based on this data, the present work showed that endurance and strength training elicits opposite adjustments in the spinal cord output. These distinct changes seem to match the divergent motor output expected for the two training programs. Endurance training increases resistance to fatigue and is accompanied by decreased motor unit discharge rates. In contrast, strength training enhances maximum force output and is accompanied by increased motor unit discharge rates. This distinct adjustments in the spinal cord output, seems to result from changes in different neural mechanisms located at supraspinal or spinal level. Based on the V-wave and H-reflex measures obtained with electrical stimulation protocols, the neural adjustments following endurance training seems to result from changes at spinal level whereas the adjustments following strength training are likely due to changes at supraspinal level. These adaptations occurred in the absence of changes in the contractile and electrophysiological properties of the muscle fibers. Changes at peripheral level were only following a longer period of training. The combination of intramuscular and multichannel surface techniques, by means a spike triggered averaging technique, allowed to estimate the motor unit conduction velocity which reflects the electrophysiological properties of the muscle fibers. This data revealed similar increments of the motor unit conduction velocity for both training programs, but only after a period of six weeks. In addition, the rate of decline of the motor unit conduction velocity during sustained contraction was reduced following six weeks of both endurance and strength training, however a greater reduction was observed after endurance training. All together, these findings indicate different time courses of the neural and peripheral adaptations in response to motor training.

7.3 Limitations and recommendations for future work

Although the results of this thesis indicate that endurance and strength training evokes distinct adjustments in the motor unit behavior and that different neural mechanisms would contribute for such changes, the exact nature and loci of the neural changes could not be identified. The present work only evidenced that strength training mainly affects the V-wave amplitude, suggesting changes at supraspinal level, while endurance training changes the H-reflex excitability, indicating changes at spinal level. Moreover, the results are confined to the specific training programs used in this project (please see below).

An understanding of the exact neural mechanisms involved in the adaptation to motor training would be relevant not only in sports but most importantly in the rehabilitation field, since it would allow to design better motor training interventions. The comprehension of the specific mechanisms involved in the neural adaptation can be advanced by the application of conditioning-test approaches and by the study of discharge rate probability of single motor units. These techniques can provide information on neural circuits such as dysinaptic inhibition arising at the antagonist muscles and presynaptic inhibition exerted on Ia afferent terminals of the motoneurons of agonist muscle. In addition, techniques based on the motor unit inter-spike intervals can provide information on the AHP duration, which is an important motoneuron property related with discharge rate (Gossen et al. 2003; Matthews 1996). Although these techniques are powerful, they are technically challenging. Such methods imply a strict control of the: (i) subject's posture and concentration, (ii) time intervals between electrical stimulus on different peripheral nerves (can be lower than 0.2ms) and, (iii) size amplitude of the post-potential generated, which have high variability. In addition, the study of discharge rate probability of single motor units requires: (i) that the subjects are able to isolate and maintain a single motor unit active for longer periods (~20-30 min) and (ii) a real-time signal processing method able to reliably decompose the intramuscular signal in order to provide feedback in real time to the subjects. Recent technological developments might be applied to overcome some of these limitations, by automatically controlling some factors or by improving the quality of the real time feedback .

A part from the neural mechanism involved in the regulation of the motor unit behavior of agonist muscle, it would be relevant to provide new insights on the role of the coordination of the multiple synergist muscles in the motor performance gains (either endurance or strength capacity). The new developments of the EMG systems and signal processing provides promising avenues for advancing knowledge in this field and that should be explored in future studies. Moreover, although debate remains regarding the decomposition of the surface EMG signal into its constituent action potentials, the continued advances have shown that is possible to assess with accuracy to motor unit behavior by decomposing the surface EMG signals (Farina et al. 2010; Holobar et al. 2009). This opens the possibility to study a greater number of motor units on several muscles simultaneously, which would offer a better picture of the neuromuscular changes occurring in response to training.

Another limitation of the present work is the specific characteristics of the motor training used. As discussed in the study I (cf, chapter 4, section 4.5), previous studies used mostly unilateral and single joint exercises, while we used bilateral and multipoint exercises. Both types of exercises imply different movement patterns which may contribute for changes in different neural mechanisms and thus dissimilar results. This factor also represents a limitation within our studies. We used endurance and strength training programs that are commonly applied in both conditioning and rehabilitation fields. Thus, endurance training was performed in a cycle ergometer which implied alternating flexion and extension of the lower limbs, while strength training was performed with bilateral exercises that involved simultaneous flexion and extension of the lower limbs. The different motor patterns involved in both training programs may have *per se* contributed for the opposite neural adaptations observed in our studies. Another confounding factor may be associated to the physiological demands imposed by the selected training programs. The conventional endurance and strength training are not typically restricted to a specific muscle or muscle group. These training programs are designed to increase demand over all body and typically they also lead to systemic adaptations. These systemic adaptations involve changes in several metabolic and neurochemical pathways among skeletal muscle, spinal cord and brain which affects the motoneuron excitability. These adaptations all together might have contributed for the motor unit behavior adaptations, which might not happen under very restrict training programs, such as those reported in the literature. Therefore, from the present work it is difficult to discriminate the neural from the systemic adaptations induced by endurance and strength training. Nonetheless, despite of the research limitations associated to these types of training programs they are the ones commonly applied in the conditioning and rehabilitation programs. For this reason we decided to assume the specificity of the common training programs [i.e., motor capacity trained (endurance *vs.* strength) and movements patterns (alternating *vs.* simultaneous extension/flexion of the lower limbs)], even if they might limit the interpretation of the results. Future work designed to explore the effects of the systemic adaption to training on the neural mechanism would certainly contribute for an understanding of the specificity of the training programs at their impact on the diverse biological systems and how they interact to improve motor performance.

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