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Mechanism of Muscle Vibrations During Stimulated and Voluntary Isometric Contractions of Mammalian Skeletal Muscle

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a dissertation entitled "Mechanism of Muscle Vibrations During Stimulated and Voluntary Isometric Contractions of Mammalian Skeletal Muscle" submitted by Marco Aurélio Vaz in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Abstract

When a muscle contracts it produces vibrations. The origin of these vibrations is not known in detail. The purpose of this study was to determine the mechanism associated with muscle vibrations. Mechanisms which have been proposed in the literature were described as theories (*cross-bridge cycling, vibrating string* and *unfused motor unit theories*). Specific predictions were derived from each theory, and tested in three conceptually different studies. In the first study, the influence of recruitment strategies of motor units (MUs) on the vibromyographic (VMG) signal was studied in the in-situ cat soleus using electrical stimulation of the soleus nerve. VMG signals increased with increasing recruitment and decreased with increasing firing rates of MUs. Similar results were obtained for the human rectus femoris (RF) muscle using percutaneous electrical stimulation of the femoral nerve. The influence of MU activation on muscle vibrations was studied in RF by analyzing VMG signals at different percentages (0-100%) of the maximal voluntary contraction (MVC). In our second study, we tested the effects of changing the material properties of the in-situ cat soleus (through muscle length changes) on the VMG signal. The magnitude of the VMG signal was higher for intermediate muscle lengths compared to the longest and the shortest muscle lengths. The decreased magnitude of the VMG signal at the longest and at the shortest muscle lengths was associated with increased passive stiffness and with decreased force transients during unfused contractions, respectively. In the third study, the effect of fatigue on muscle vibrations was studied in human RF and vastus lateralis (VL) muscles during isometric voluntary contractions at a level of 70% MVC. A decrease in the VMG signal magnitude was observed in RF

(presumably due to derecruitment of MUs) and an increase in VL (probably related to the enhancement of physiological tremor, which may have occurred predominantly in a medio-lateral direction) with fatigue. The *unfused MU theory,* which is based on the idea that force transients produced by MUs during unfused tetanic contraction is the mechanism for muscle vibrations, was supported by the results obtained in the above three studies.

Preface

Chapters 3, 4 and 5 of this dissertation are based on the following four manuscripts:

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AMG:	acoustic myography, acoustic myographic
CMAP:	compound muscle action potential
(C)MUAP(s):	(compound) motor unit action potential(s)
EMG:	electromyography, electromyographic
FFT:	Fast Fourier Transform
MDF:	median frequency
MMG:	mechanomyography
MU(s):	motor unit(s)
MVCs:	maximal voluntary contraction(s)
RF:	rectus femoris
RMS:	root mean square
SOL:	soleus
VL:	vastus lateralis
VMG:	vibromyography, vibromyographic

Chapter 1

Introduction

Upon contraction, a skeletal muscle produces sounds and vibrations. This observation was made over three hundred years ago (Grimaldi, 1665; Wollaston, 1810). However, the study of muscle vibrations (vibromyography, VMG) did not receive much attention by scientists up to three decades ago. The reason for this gap in time was that, until recent years, the technology to detect and process VMG signals effectively was not available. The main tool that could have been used to possibly detect muscle sounds, the stethoscope, had the limitation of eliminating most of the physiological signal due to its frequency range (Oster and Jaffe, 1980). Only recently, with the availability of electronic sensors (such as piezoelectric transducers, condenser microphones, piezoceramic membranes and accelerometers) and computers that could collect, store and numerically process large amounts of signals, have VMG signals been used to study the workings of muscle (Orizio, 1993).

In this century, many studies described the behavior of muscle sounds (vibrations) in both voluntary and stimulated contractions (Herreoun and Yeo, 1885; Gordon and Holbourn, 1948; Oster and Jaffe, 1980; Lammert et al. 1976; Barry, 1991). VMG measurements have been used for many different purposes in the past; however, the precise mechanism underlying muscle vibrations has not been determined conclusively. Most of the interpretations of results in the area of VMG research were based on observed phenomena or intuition. Therefore, the full benefit of using VMG measurements as a noninvasive tool to study muscle contraction may only be achieved after the mechanism(s) underlying these vibrations is (are) identified.

The sounds and the vibrations produced by a contracting muscle can be measured on the surface of the skin by means of microphones (Barry, 1987, 1991; Barry and Cole, 1990; Barry and Gooch, 1986; Barry et al. 1985; Bolton et al. 1989; Orizio, 1993; Oster and Jaffe, 1980) and accelerometers (Barry, 1992; Barry et al. 1992; Jorgensen and Lammert, 1976; Lammert et al. 1976; Zhang et al. 1992; Zwarts and Keidel, 1991), respectively. According to the type of transducer used to detect muscle vibrations, different names have been proposed to describe the VMG signals (e.g. accelerometermyography, Jorgensen and Lammert, 1976, and Lammert et al. 1976; acoustic myography, Barry et al. 1985; phonomyography, Marchetti et al. 1974, and Maton et al. 1990; soundmyography, Orizio et al. 1989a, 1989b). Recently, two terms have been suggested as being more appropriate than the other names to describe the measured signal: vibromyography (VMG; Keidel and Keidel, 1989; Barry et al. 1992) and mechanomyography (MMG; Orizio, 1993). The term vibromyography will be used throughout the text as being representative of the previous names used. The use of different types of transducers (electronic stethoscope, hydrophone, contact sensor microphone, accelerometer), however, is one of the limitations in this field of research, as comparisons of the results from different studies become difficult because of the different measuring units used to report the results, and because of the technical differences which exist between different types of transducers. The fact that most studies were descriptive seems to be related to the lack of a theory explaining the mechanism of muscle vibrations; this lack of a theory also seems to be the reason for the different names proposed and the different types of transducers used in different studies.

Several hypotheses have been proposed to explain the mechanisms responsible for the production of muscle sounds/vibrations. Oster and Jaffe (1980) have described the dominant frequency of muscle sounds during voluntary isometric contractions or isometric contractions elicited by electrical stimulation to be approximately 25±2.5 Hz. They associated the sound frequencies recorded with the rate of cross-bridge cycling. However, no evidence was provided by the authors or in the literature to support this hypothesis.

The model of a vibrating string has been proposed to explain the sound pressure generated by an isolated muscle. Frangioni et al. (1987) showed that plucking of the isolated muscle produced a decaying pressure oscillation in which the first half cycle was the largest, just as would be expected from a tense string.

Jorgensen and Lammert (1976) showed that the vibratory signal of a single motor unit (MU) can be recorded at very weak contractions using an accelerometer, and suggested that the recorded signal was closely related to the twitch of MUs. Lammert et al. (1976) proposed the existence of a relationship between recruitment strategies of MUs and the VMG signal obtained during different levels of voluntary effort.

Possibly because the field of research about VMG is still in its early stages, most of the studies reported in the literature are descriptive in nature. Although some characteristics of the signal have been described, a detailed and systematic study aimed at determining the origin of muscle vibrations has not been done. This lack of research manifests itself in a lack of hypotheses and corresponding predictions in most VMG studies.

The general purpose of this project was to identify the possible mechanism(s) behind muscle vibrations of mammalian skeletal muscles. If muscle vibrations were related to the force transients of unfused MU contractions, then, by stimulating the muscle at different rates and at different voltage levels, it might be possible to assess the contractile properties of active MUs and detect the influence of these properties on the VMG signals. Therefore, our first specific objective was to assess the effect of MU recruitment and MU firing rates on the VMG signal using electrical stimulation in two different models (i.e. animal and human; Chapter 3). By knowing the effects of MU recruitment strategies on the VMG signal during electrically elicited contractions, interpretation of the results obtained during voluntary effort might become possible. The second specific purpose was to assess the effects of changing MU firing rates and number of active MUs on the VMG signal during voluntary contractions (Chapter 3). Also, derecruitment or loss of force of MUs during fatiguing contractions might affect the VMG signal. Therefore, the specific purpose of Chapter 5 was to determine the effects of fatigue on the VMG signals.

If muscle vibrations were produced by cross-bridge cycling or were simply determined by the elastic properties of the muscle, we expected that the specific characteristics of the VMG signal could be associated with different stimulation strategies and with changes in the material properties (as determined by length changes) of the muscle. These ideas gave impetus for the specific objective of Chapter 4.

In order to identify the mechanisms underlying VMG, specific theories about the origin of muscle vibrations need to be formulated, and testable predictions must be derived based on three theories.

Theories

Three primary theories have been proposed in the literature to explain the origins of muscle vibratory signals. These three theories may be referred to as (a) the *cross-bridge cycling theory* (Oster and Jaffe, 1980), (b) the *vibrating string theory* (Frangioni et al. 1987), and (c) the *unfused MU theory* (Jorgensen and Lammert, 1976). None of these theories has been tested systematically; rather, they were proposed based on observed phenomena or intuition. Furthermore, no specific predictions based on these theories were ever proposed. In the following, it is attempted to make specific predictions about muscular vibrations based on each of the three proposed theories. Only predictions which can be tested experimentally are proposed, so that a systematic testing of the predictions may help support one (or more, if any) of the proposed theories.

Cross-bridge Cycling Theory

The *cross-bridge cycling theory* (Oster and Jaffe, 1980) is based on the notion that muscle vibrations are caused by (or at least associated with) cross-bridge cycling during contractions. The theory was born based on the observation that the dominant frequency of muscle sounds was about 25 Hz, a frequency that Oster and Jaffe (1980) associated with cross-bridge cycling frequencies. Using the cross-bridge cycling theory, the following testable predictions may be made:

a) a muscle at rest should not produce any sounds (vibrations);
b) an active muscle should always produce sounds (vibrations);
c) during an isometric contraction where the force is gradually increased from zero to maximal, the sound (vibratory) signal should increase in amplitude. This prediction assumes that the average cross-bridge cycling rate during an isometric contraction is constant (on average), and that the sound (vibratory) signal should reflect the increasing number of cross-bridges participating in force production as the force is increased. In fact, if it is assumed that each cross-bridge acts independent and exerts (on average) the same force as all the other cross-bridges (which is assumed in the cross-bridge theory, Huxley, 1957; Huxley and Simmons, 1971), and if the sound (vibratory) signals from each cross-bridge add algebraically, then the magnitude of the sound (vibratory)

signal should be related linearly to muscular force.

d) shortening contractions should produce a sound (vibratory) signal of higher frequency content than isometric contractions, because based on results on the energetics of muscle contraction, it may be presumed that the cross-bridge cycling rate is increased for shortening compared to isometrically contracting muscle (Woledge et al. 1985).

Predictions a), b) and c) may be tested readily; prediction d) requires that a frequency analysis of the sound (vibratory) signal is made using an algorithm that can handle non-stationary signals, because the sound (vibratory) signals for the shortening contractions, most likely, are non-stationary. Moreover, one may argue that analysis of VMG signals during dynamic contractions may not provide a good indication of the physiological processes which occur in the muscle because changes in the position of muscle fibers underneath the recording sites prevent recordings from the same location. In addition, movement artifacts may interfere with the sound (vibratory) signals. Therefore, prediction d) cannot be tested readily.

Vibrating String Theory

The *vibrating string theory* (Barry, 1987; Frangioni et al. 1987) implies that the sound (vibratory) signals are associated with vibrations of the entire muscle, like vibrations of a guitar string. The theory was born based on the observation that the sound (vibratory) signals of an isolated muscle have the appearance of a damped sinusoid; that is, the signal amplitude is large initially and then decays. Using the vibratory string theory, the following testable predictions may be made:

a) a muscle at rest should not produce any sound (vibration), because there is no forcing function that makes the muscle vibrate;

b) for increasing muscular forces, and thus presumably increasing stiffness of the muscle, the amplitude of the sound (vibratory) signal should decrease and the frequency content of the signal should increase.

Unfused MU Theory

The *unfused MU theory* (Lammert et al. 1976; Jorgensen and Lammert, 1976; Orizio, 1993) is based on the idea that MUs produce unfused tetani when activated at frequencies below about 20-30 Hz for slow twitch MUs and 40-60 Hz for fast twitch MUs (Burke et al. 1973; McPhedran et al. 1965; Wuerker et al. 1965). The changes in force of unfused MU contractions have been proposed as the origin of muscle sounds (vibrations). Using the unfused MU theory, a series of predictions may be made for electrically stimulated and voluntary muscular contractions. The first series of predictions apply to experiments using electrical stimulation.

a) there should be no sound (vibratory) signal when the muscle is at rest;
b) if the nerve of a muscle is stimulated, a sound (vibratory) signal should be seen at all unfused frequencies of stimulation, and the sound (vibratory) signal should disappear at high frequencies of stimulation when all MUs are contracting tetanically;
c) if the nerve of a muscle is stimulated at a constant low frequency (i.e. a frequency not producing a fused tetanic contraction) and the stimulating voltage is gradually reduced, the VMG signal should decrease;
d) at subtetanic frequencies of stimulation, one distinct sound (vibratory) signal should be seen per stimulation pulse;
e) for a non-fatiguing series of stimulations, the sound (vibratory) signal of stimulated muscle should be the same for repeat stimulation protocols.

The second series of predictions applies to tests using voluntary contractions:

f) there should be no sound (vibratory) signal when the muscle is at rest;
g) for isometric contractions at increasing levels of force (from 0% - 100% of the maximal force), it is predicted that the amplitude of the sound signal is small initially (at low levels of force), that it increases and reaches a maximum at medium levels of force, and then decreases again towards maximal force. This prediction is based on the known recruitment process of MUs. For low levels of force, only a few MUs are activated; at intermediate levels of force, most MUs are activated and many of them at sub-tetanic levels; and towards maximal force, all MUs are activated, but most of them at tetanic levels where they do not contribute towards the sound (vibratory) signal;

h) during fatiguing isometric contractions, a decrease in the magnitude of the VMG signal should be observed because of (1) the increased relaxation time of MU with fatigue, which produces tetanic contractions at decreasing activation frequencies (Bigland-Ritchie et al. 1983, 1986), (2) a decrease in twitch amplitude, which produce smaller force transients, and (3) a decrease in muscle activation, which is probably responsible for a decrease in motoneurones firing rate with fatigue. Also, increasing fatigue will result in a loss of force production in specific MUs; therefore, the number of MUs contributing to the vibratory signal decreases.

In this project an attempt was made to explore systematically the proposed mechanisms of muscle vibrations using two different approaches. In the first approach, an animal model was chosen in order to conduct invasive studies in an in-situ muscle preparation. The in-situ preparation had the advantage over in-vitro preparations used previously (e.g. Barry, 1987; Frangioni et al. 1987) in that it was more physiological and probably more meaningful when comparing its results to the results obtained in-vivo.

The cat soleus (SOL) muscle was selected for the in-situ study, because it is a homogeneous muscle comprised of slow twitch fibers (Ariano et al. 1973) which are fatigue resistant, and therefore could be used for lengthy protocols involving neuromuscular stimulation with minor fatigue effects.

The in-vivo research was performed using the rectus femoris (RF) and vastus lateralis (VL) muscles because of the previous research performed with these muscles (Dalton et al. 1992; Dalton and Stokes, 1993; Lammert et al. 1976; Stokes and Dalton, 1991b; Zhang et al. 1992; Herzog et al. 1994).

Project

In order to accomplish the general and specific purposes stated previously, four studies were conducted using three different approaches. Initially, the effect of increasing the number of activated MUs at one specific stimulation rate, and the effect of stimulation rates on the VMG signals were studied using an acute animal preparation. Using a nerve cuff electrode around the soleus nerve, changes in the recruitment of MUs were achieved by changing the voltage output to the nerve electrode. The effect of frequency of activation on the VMG signal was studied by changing the stimulation rates. This protocol allowed for testing of predictions made for all three theories during electrically elicited contractions.

In the second study, femoral nerve stimulation in humans was used to study the behavior of in-vivo muscles with controlled activation. Also, VMG was measured during voluntary efforts to see if changing force was associated with changing VMG amplitude. Predictions made for both artificially stimulated and voluntary contractions in a non-fatigued muscle could be tested in the second study. The above two studies are presented in Chapter 3.

In the third study (Chapter 4), electrical stimulation was used to assess the effect of muscle length changes on the VMG signal during isometric contractions. Contractions were obtained at two to four different SOL lengths in three animals. Although it was hard to predict a priori the effects of muscle length changes on the VMG signal, it was expected that the muscle material and mechanical properties would be the principal factors to produce possible changes in the VMG as the stimulation to the muscle was similar for the different muscle lengths studied.

In the fourth study (Chapter 5), the effect of fatigue and recovery on the VMG signal was assessed during isometric voluntary efforts performed at a level of 70% MVC in eleven human subjects. The last prediction made for voluntary contractions in the *unfused MU theory* was tested in this study.

Chapter 2

Review of the Literature

Selected studies aimed at explaining the origin of muscle sounds/vibrations are reviewed in chronological order in this chapter. A summary of the literature reviewed is presented at the end of the chapter.

Origin of Muscle Sounds/Vibrations

The fact that contracting muscles produce vibrations or sounds has been known for more than three centuries. The first account of listening to muscle sounds was presented by Grimaldi (1665). By inserting one's thumbs in the ears so as to cover the ear canal, muscle sounds were perceived as rumbling sounds when making a fist with the elbows raised, and were interpreted by Grimaldi as representing the continuous tremors and movements occurring in finger, arm and in the whole body due to the continuous hurried motion of the spirits (Orizio, 1993; Wollaston, 1810).

Wollaston (1810) compared the sounds he heard during muscle contraction to those produced by rubbing a round piece of wood along notches of equal size cut on a board at different speeds. He was able to determine the frequency range of these rumbling sounds, and described the upper and lower limits of muscle sound frequencies to be 36 and 14 Hz, based on increased or decreased voluntary effort with which the contraction was performed, respectively. Using a stethoscope, Wollaston also observed that leg muscles produced the same rumbling sounds during contraction as the sounds observed for the muscles of the thumb. In addition, he compared the sound frequencies produced by muscle during contraction (as obtained in Grimaldi's experiment) with that of carriages

moving over cobblestones at different speeds. By knowing the dimensions of the cobblestones (i.e the breadth was about 6 inches) and by assuming the number of vibrations to be twenty four in a second (i.e. an average number within the frequency range of 14-36 Hz), he estimated the speed of the carriages (whose sounds resembled the sounds produced by muscle contraction) to be 8 miles/hour, which agreed with the true speed. It is extraordinary how close Wollaston's estimates of the frequency range were compared with the frequency range reported nowadays, despite the fact that computers and sound transducers were unavailable at the time he conducted his experiments.

Herreoun and Yeo (1885) were the first to compare muscle sounds obtained during electrically elicited and voluntary contractions. They found a similarity between the pitch and quality of muscle sounds/vibrations produced during voluntary and electrically elicited contractions at stimulation rates up to about 30 Hz. However, Herreoun and Yeo (1885) rejected the idea that muscles produced sounds during contraction, and related the rumbling sounds to pressure changes in the ear caused by the fingers which were used to close the aperture of the ear canal. They associated the observed muscle motions or vibrations to the resonant tone of the membrana tympani.

Gordon and Houlbourn (1948) studied the sounds produced by single MUs of the muscle orbicularis oculi during voluntary contractions, and suggested that the sounds observed were the mechanical counterparts of the muscle action potentials, and were probably produced by the thickening of muscle fibers during contraction. These authors were able to show that there was a sound signal, as well as a mechanical movement, for each action potential recorded from this muscle. They associated the sound signals with the individual motor unit action potentials (MUAPs). Thickening of the muscle during contraction was later disproved as the mechanism responsible for the production of muscle sounds (see Barry, 1987 below). Although Gordon and Houlbourn (1948) did not make any measurements for any other muscles, they suggested that the complex rumbling sound produced by large limb muscles must be caused by the asynchronous activation of many MUs. This was probably the first time that a relationship was proposed between MU activation and muscle sounds/vibrations during voluntary effort.

The above relationship between MU activation and muscle sounds/vibrations was followed by Lammert et al. (1976) and by Jorgensen and Lammert (1976) who used an accelerometer to measure muscle vibrations. Lammert et al. (1976) were able to show that, at the same voluntary efforts, the root mean square (RMS) of the VMG signal as a function of voluntary effort was different for different muscles (i.e. for RF and biceps brachii

muscles) and for the same muscle of subjects with different muscle fiber type composition. Subjects with a high percentage of fast twitch fibers in vastus lateralis (VL) showed a marked increase in the RMS of the VMG signal from 10-60 % MVC, while the RMS values remained about constant from 60-100% MVC, whereas the slow twitch fibered subjects showed an almost linear increase for the RMS-voluntary effort relationship from 50-100% MVC, while the RMS remained about constant from 10-50% MVC. However, the fiber type distribution was calculated from samples of the VL rather than RF which was used for the VMG measurements. Also, the small sample size (n=2 for subjects with slow and fast twitch fiber composition, respectively) precluded a generalization of the results. Lammert et al. (1976) also showed that there was an increase in the frequency content of the VMG signal with increasing voluntary effort, while the electrically elicited contractions showed a very narrow concentration of the VMG frequencies around the stimulation frequency. Mathematical algorithms (Zhang et al. 1994) showed that signals obtained using periodic stimulation will have their frequency content highly influenced by the stimulation frequency due to the regular interval between stimulation pulses characteristic of periodic stimulation.

Jorgensen and Lammert (1976) showed that mechanical vibrations were associated with action potentials from single MUs obtained at very weak voluntary efforts. They were also able to show that the VMG amplitude was reduced as the mean spike interval of MUs was reduced. They assumed that a decrease in the mean spike interval was associated with increased firing rates of MUs, suggesting, therefore that this increase in the firing rates of MUs was responsible for the decrease in the VMG signal amplitude. When MUs with similar firing rates and with different action potential amplitudes were compared, they found an increase in the VMG signal amplitude with increasing EMG spike amplitude.

Lammert et al. (1976), based on their results and on the results of Jorgensen and Lammert (1976), suggested that an increase in VMG, as a function of voluntary effort, could be interpreted as an increased contribution from newly recruited MUs, while a levelling off in the VMG-voluntary effort relationship could be interpreted as an increase in frequency of the already active MUs with minor new recruitment. The above interpretation gave origin to the *unfused MU theory*.

Oster and Jaffe (1980), using an electronic stethoscope (which has both an air-coupled and a contact microphone), were able to detect sounds from several human skeletal muscles during different tasks. Their findings can be summarized as follow: (a) muscle sounds are periodic with a frequency of 25 $\pm$ 2.5 Hz; (b) the amplitude of the sounds

increases linearly with tension; (c) the quality of the sound is the same for different muscles tested; (d) the quality of muscle sounds is unaffected by changes in tension, temperature and blood flow; (e) sounds produced during electrically elicited and voluntary contractions are indistinguishable; (f) there is no synchrony between the amplitude of the sound and the EMG activity obtained simultaneously during voluntary contractions. These authors suggested that muscle sounds are related to the intrinsic properties of muscle contraction, and concluded by saying that the sound frequencies found in their study were close to the frequency of cross-bridge cycling.

This study is probably the only one reported in the literature to associate muscle sound characteristics with cross-bridge cycling rate, giving origin to the *cross-bridge cycling theory*. Oster and Jaffe (1980) suggested that the quality of the sound was similar for different muscles, and proposed a linear relationship between sound amplitude and tension. Although the significance of the expression *quality of the sound* was not defined, their results contradict the results from Lammert et al. (1976), who found different relationships between the VMG signal RMS and voluntary effort in different muscles, suggesting that muscle sounds/vibrations are muscle dependent. Also, Oster and Jaffe (1980) based their relationship between sound signal amplitude and voluntary effort on results presented solely for the biceps brachii muscle. Finally, Oster and Jaffe (1980) disputed the results by Gordon and Holbourn (1948) of a relationship between recruitment strategies and sound/vibration amplitude, as Oster and Jaffe (1980) did not find any synchrony between EMG and sound signals.

The principal characteristic of Oster and Jaffe's (1980) manuscript is descriptive and exploratory. They tried to describe as many factors as possible which might influence muscle sounds (e.g. signal characteristics; temperature, blood flow, level of effort; type of contraction; type of muscle; EMG). Much of the methodology used, however, is not clear and is described in a convoluted way in the results section, while the discussion of their results does not lead the reader to any concrete conclusion about their findings. The main contribution of their work is probably that, by denying results from previous studies, they called attention to the area of VMG. Also, by suggesting a close relation between the intrinsic properties of muscle and muscle sound characteristics, this study was probably the motivator of many of the in-vitro and in-vivo muscle sound studies since it was cited in most of the studies to come. Finally, they were able to exclude artifacts and nonmyogenic phenomena such as vascular sounds, microphone rubbing on the skin and nerve conduction as possible factors giving origin to muscle sounds/vibrations.

Brozovich and Pollack (1983), using a broad-band piezoelectric sensor on isolated frog sartorius muscles, found that muscle contraction produces discrete sound bursts of short duration (0.4 ms) during shortening. They were also able to show that temperature changes influenced the interval of successive bursts in such a way that, for each increase in muscle temperature of 10°C, there was a decrease of 50% in the inter-spike intervals, and concluded that muscle contraction may occur in a discrete, synchronous manner. Their results, however, did not agreed with the results of Gordon and Holbourn (1948), who found similar discrete sound bursts of longer duration (5-15 ms) during voluntary contractions of the muscle orbicularis oculi. Also, the band-pass filter used in Brozovich and Pollack's (1983) study for the sound signals (i.e. from 500 Hz to 250 kHz) eliminated any sound signal with frequencies between 14-40 Hz which were typically reported in earlier studies. The short duration of the sounds recorded in Brozovich and Pollack's (1983) experiment is not related to the low-frequency or rumbling sounds reported in any of the previous studies. Nevertheless, Brozovich and Pollack's (1983) study was probably the first to use an in-vitro muscle preparation to investigate muscle sounds.

Barry et al. (1985) studied the effects of load increases and fatigue on the VMG signal of the human biceps brachii. They found that the VMG signal amplitude increased with increasing force of contraction, whereas the VMG signal amplitude paralleled the decline in force with fatigue. They also studied the VMG signals obtained from two subjects with motor nerve injuries (i.e. patients with decreased numbers of MUs and increased MUAP size), and were able to show simultaneous recordings of MUs in both VMG and needle EMG recordings from the VL. However, they were not able to find similar recordings from healthy subjects. This was probably due to the levels of voluntary effort that they used in the study, which were too high to resolve acoustically individual MUs in healthy subjects.

Barry (1987) used a hydrophone to record sound signals elicited by electrical stimulation (maximal isometric twitches) of the frog gastrocnemius muscle in an in-vitro muscle preparation. He was able to show that the initial deflection of the acoustic signals appeared before the initial deflection in isometric force, but always followed the compound muscle action potential (CMAP) by approximately 2.0 ms. Increases in muscle length up to 90% of the optimal length (i.e. the length at which the largest force was obtained) produced an increase in the sound signal amplitude, whereas further length increases produced a decrease in the signal amplitude. Distinct sound signals were associated with individual stimulation pulses when the contraction was produced using a stimulation

frequency of 5 Hz, whereas a single sound signal was observed for single twitches or when the contraction was a fused tetanus produced by a frequency of stimulation of 100 Hz. Barry (1987) proposed that sound signals were produced by lateral movements of the muscle which should occur at the resonant frequency of the muscle, but could not explain the reduction in the signal amplitude at lengths shorter than 90% of the optimal length. A possible explanation for this decrease is that, if muscle sounds/vibrations are related to the force transients caused by the unfused contraction of MUs, decreases in length produced a decrease in these force transients, which was probably responsible for the signal amplitude decrease at muscle lengths shorter than 90% of the optimal length (see Chapter 4).

Frangioni et al. (1987), using a similar experimental set-up and the same muscle as Barry (1987), found results similar to those obtained by Barry (1987). In brief, they found that (a) there was a sound signal associated with a single twitch, which always began after the stimulus and before the tension began to rise; (b) the first force development elicited the typical sound signal of a twitch during both unfused (10 Hz stimulation rate) and fused (30-50 Hz stimulation rate) tetani; (c) there was no sound signal produced during the plateau of the fused tetani; (d) the sounds accompanying subsequent force developments in the unfused tetanus had a smaller initial amplitude than the initial sound produced, and these sound signals decayed in fewer half-cycles of vibration than the initial sound; (e) there was a decline in the sound signal amplitude which paralleled the decrease in the force increment after each twitch stimulation during a fatigue protocol (a twitch every 10 s over a period of 15 min); (f) the sound signal amplitude increased with increasing muscle length up to 95% of the optimal length, decreasing at longer lengths; (g) there was an increase in the frequency content of the sound signal with increasing muscle length; (h) plucking the muscle at rest showed a sound signal of smaller frequency content than the one produced during a twitch; (i) plucking the muscle during a tetanus produced a signal with a higher frequency content than that produced by plucking the muscle at rest; (j) in addition to the ringing sounds that were recorded after twitch stimulations given to the muscles, a second sound signal was often present immediately after the peak of the force record. It always had a lower peak-to-peak amplitude than the first sound signal; (k) larger sound signals were always obtained close to the centre compared to the ends of the muscle belly.

Frangioni et al. (1987) and Barry (1987) rejected the idea proposed by Gordon and Holbourn (1948) that sounds were produced by thickening of muscle fibers during contraction. Although thickening may occur during contraction, the main mechanism of muscle sound production could not be attributed to thickening of the fibers, as two

hydrophones placed on opposite sides of the muscle produced signals that were 180° out of phase. If thickening was the principal mechanism, then all sound signals should reveal a similar phase pattern at any position around the muscle.

The results of the in-vitro experiments discussed above cannot be used directly to interpret the results obtained during in-vivo experiments. Frangioni and co-workers (1987) stated that the fascia, skin and bones of the leg did not play a critical role in sound production, since similar sounds of the ones obtained in the in-vitro preparation were observed in an intact preparation. This similarity between sound signals of in-vitro and in-vivo preparations may be true for superficial muscles. However, a muscle in its proper position such as the human SOL cannot vibrate freely because of the tissues surrounding it and because of its attachment to bones.

The studies by Barry (1987) and Frangioni et al. (1987) were the first to show that an isolated muscle was able to vibrate freely when stimulated or plucked, and that in-vitro muscle preparations (as well as muscles from intact legs) produced low-frequency sounds. According to Frangioni et al. (1987), the amplitude of the lateral motion of the muscle was greatest in the middle, as would be the case for a shoelace excited into vibration by starting from a slack position and pulling it taut. The ideas by Barry (1987) and Frangioni et al. (1987) gave origin to the theory referred to in Chapter 1 as the *vibrating string theory*.

Barry and Cole (1988) followed the idea that muscle sounds are related to the lateral movement of muscles, as presented in the studies by Barry (1987) and by Frangioni et al. (1987). By using a similar preparation as used in the above two in-vitro studies, they showed (a) that pressure waves were generated by lateral vibrations or movements produced by the isolated frog muscle during isometric contraction, and (b) that the pressure wave form was directly related to the lateral acceleration of the muscle when contracting. Barry and Cole (1988) did not agree with the idea proposed in previous in-vitro studies (where the muscle was immersed in a bath solution) that the recorded signal was a sound signal, but a pressure wave which, when encountering a boundary such as a skin-air interface, could produce the described muscle sounds. They also disagreed from Frangioni et al. (1987) that intact frog legs produced signals that were qualitatively the same as the ones produced in-vitro. They suggested that in-vivo muscle preparations produce sounds with lower frequencies than in-vitro because of the presence of adjacent muscles which dampen the signal, which was not the case of the in-vitro studies. Finally, Barry and Cole (1988) related the VMG signals to two different types of movement: a large lateral movement which was accompanied by smaller, superimposed oscillations. They suggested

that the large, slow muscle movement was associated with the asymmetrical distribution of muscle fibers which were contracting asynchronously, whereas the high frequency and small amplitude oscillation was related to, and in phase with the lateral acceleration of the muscle. Also, Barry and Cole (1988) suggested that these superimposed high frequency oscillations, which dominated the recorded signal, represent the natural mechanical response of the muscle to a step function input.

Keidel and Keidel (1989) were probably the first to propose the term vibromyography to describe the measurements of muscle vibrations. These authors studied muscle vibrations in ten healthy subjects by placing an accelerometer over the skin of the masseter, biceps brachii, wrist extensor group, and tibialis anterior muscles. By asking the subjects to perform specific tasks, they recorded signals from these muscles for a variety of conditions (e.g. rest, fatigue, MVCs performed isometrically, isometric contractions with increasing loads). Their main findings were that (a) different muscles produce different VMG signals, (b) there is an increase in the power-spectra of all muscles during contraction compared with the rest state, and (c) different peaks could be observed in the power spectrum during contraction and at rest. They discussed many of the possible physiological factors that might be involved in the production of muscle vibrations, and did not exclude the possibility that these vibrations might be related to some aspect of cross-bridge cycling as suggested by Oster and Jaffe (1980). However, they considered neuronal activity (or the net activity of MUs) as being the basic mechanism underlying muscle vibrations. By looking at the changes that occurred in the power-spectra of the VMG during contraction, they suggested that their study demonstrated that VMG gave information beyond that obtained using EMG regarding the amount of recruitment of MUs, MU rotation, recruitment and derecruitment order, the firing rate, modulation of the firing rate, tetanic fusion frequency, and especially, synchronization of MUs. The main limitation of their study was that they drew unsupported conclusions about the physiological mechanisms that may cause muscle vibrations. Although VMG signals are probably related to most of the above cited neuronal mechanisms, it seems difficult to accept that, by looking only at the power-spectra of the VMG signal, all these mechanisms could be extracted from a frequency analysis of the signal.

Barry (1991,1992) studied sounds and vibrations produced by electrical stimulation during a supramaximal twitch in the human abductor pollicis brevis and abductor digiti minimi. By measuring EMG, force and VMG signals simultaneously, it was possible to describe and compare the latencies and amplitudes of the EMG and VMG signals. Barry

(1992) stated that the advantage of using electrical stimulation over voluntarily elicited contractions was that physiological tremor artifact could be eliminated. He also emphasized some of the advantages of using accelerometers over microphones, and proposed, as the goal of his study, to establish procedures for the use of VMG that could be implemented by all laboratories in an easy way. This was probably the first time that an attempt was made to establish a reference frame for the use of accelerometers to study muscle vibrations.

Stokes and Cooper (1992) studied the relationship between force, EMG and VMG for voluntary and stimulated isometric contractions of the adductor pollicis muscle in six human subjects. By using frequencies of stimulation in the physiological range, they were able to show that (a) increasing frequencies of stimulation were responsible for a decrease in the amplitude of the VMG signal, (b) the behavior of the integrated VMG values paralleled the behavior of the force oscillations produced by electrical stimulation, and (c) the frequency of the VMG signal matched the stimulation frequency. Also, they showed a curvilinear relationship for the integrated EMG and integrated VMG-voluntary effort relationships, and suggested that the sounds/vibrations produced during muscle contraction were determined by motor control mechanisms rather than intrinsic contractile processes.

They rejected the *cross-bridge cycling theory* proposed by Oster and Jaffe (1980), because the relaxation rate and the energy turnover rate were not influenced by the stimulation frequency (Cooper et al. 1988), whereas the VMG signal was, as shown by Stokes and Copper's (1992) results. If the rate of cross-bridge cycling (which is assumed to partly reflect the energy consumption during contraction) was responsible for the production of muscle sounds, then at high frequencies of stimulation where the VMG signal amplitude is smaller than at low frequencies, the cross-bridge cycling rate should be smaller as well than the one at low frequencies of stimulation, which does not appear to be the case.

Based on the results of the electrically elicited contractions, Stokes and Cooper (1992) suggested that the similarities between the VMG signal and force fluctuations indicated that the characteristics (i.e. amplitude, frequency and wave form) of the VMG signal were directly determined by the manner the muscle was activated. Muscles of the hands (such as the one studied by Stokes and Cooper, 1992), however, depend primarily on recruitment of MUs up to a level of about 50% MVC, whereas rate modulation is the principal mechanism to produce force at higher levels of voluntary effort (Basmajian and De Luca, 1985). Based on the results of the electrically elicited vibrations, it was shown that the force transients and the amplitude of the VMG signal decreased with increasing

stimulation frequencies. Therefore, if we assume that the VMG signal reflects the force transients caused by the unfused contraction of MUs, we should expect to see a decrease in the VMG signal amplitude from about 50% to 100% MVC in the above muscle, as opposed to the curvilinear increase shown with increasing voluntary effort, as no recruitment should occur above 50% MVC. It may be, however, that the above curvilinear relationship was produced by synergistic muscles of the hands (as mentioned by the authors), which might have influenced the results during high-force contractions. Stokes and Cooper's (1992) results gave support to the *unfused MU theory,* because of the close relationship demonstrated between force fluctuations (obtained at unfused tetanic contractions) and muscle vibrations during electrically elicited contractions.

Orizio (1993) suggested that the time and frequency domain properties of the VMG signal are clearly related to the number, the type, and the firing rates of the recruited MUs. Although a detailed description of the experimental set-up was not provided, he showed (a) simultaneous recordings from electrical (obtained via needle electrode) and vibratory (obtained with an accelerometer) signals recorded from individual MUs of the human extensor digitorum brevis, and (b) that there was a summation of the mechanical activity when a new MU was recruited. He concluded that, during steady voluntary contractions, the main generation mechanism of muscle sounds/vibrations was related to the summation of the twitching of each individual MU.

The above relationship between the VMG signals and the twitching of MUs was later followed by Petijean and Maton (1995) and by Orizio et al. (1996). Petijean and Maton (1995) showed that MUs were responsible for the generation of the VMG signals obtained at very weak voluntary effort. They used a loud speaker and an oscilloscope to give feedback to their subjects, and recorded VMG signals whenever the subjects performed voluntary contractions which were weak enough to obtain single, low frequency clicks from the loudspeaker, and which corresponded to the spike trains of a single MU on the oscilloscope. EMG and VMG signals of a single MU were then obtained while the firing rate of that MU was maintained for as long as possible. Increased recruitment of MUs was performed by asking the subjects to increase voluntary effort, while a second spike train was added to the previous individual MUAP signal. The average of the elementary VMG signals was then determined from 87 MUs in the anconeus muscle of 14 subjects during elbow extension. Spike-triggered averaging technique was used to isolate the specific acoustic contribution from the active MUs. The results agreed with previous results provided in the literature. In brief, (a) the electro-acoustic delay (i.e. a mean value

of 3.5 ms between the onset of the MUAP and the VMG signal) agreed with the results by Barry (1991), (b) the bipolar shape of the VMG signals agreed with results by Gordon and Holbourn (1948), and (c) the strict one-by-one mapping between MUAP and VMG signals was similar to the results by Gordon and Holbourn (1948) and by Barry et al. (1985). Petijean and Maton (1995) discussed some of the possible factors that may affect the signal characteristics (e.g. relation between MU size and signal amplitude, the relation between thickening of MUs and the polarity of the signal; attenuation of the signal by surrounding tissues to the active MUs; decrease in VMG signal magnitude caused by increasing firing rates of MUs), and concluded by saying that the VMG signal represents the activity of individual MUs during voluntary isometric contractions. However, they could not solve the problem of summation of individual VMG signals produced during high levels of voluntary effort, and the relation between recruitment and firing rates of MUs and this summation process.

Orizio et al. (1996) studied how the summation of the mechanical activity (i.e. the pressure waves generated by the dimensional changes of the fibers of the active MUs) of different MUs was reflected on the VMG signal of the extensor digitorum communis in three human subjects. By stimulating two different motor points in the above muscle they were able to activate two different groups of muscle fibers, which they called artificial MUs. These authors then administered separate stimulation protocols to each of these MUs. In their first protocol they administered stimulation frequencies of 3 Hz for one MU and 8 Hz for the second MU. They then generated an artificial VMG signal by linear summation of the two obtained signals from these artificial MUs and compared to the signal obtained by simultaneous stimulation of MU1 at 3 Hz and MU2 at 8 Hz. Orizio et al. (1996) were able to show that there was a close agreement between the two generated VMG signals, and concluded that, at the above frequencies of stimulation, the mechanical activities (as represented by the VMG signals) could be summed linearly. However, by using a similar protocol with higher stimulation frequencies (i.e. 9 Hz for MU1 and 20 Hz for MU2) they were not able to reproduce the VMG signal generated by simultaneous stimulation of the artificial MUs with the linearly summation procedure. The VMG signal generated by the linear summation had a larger amplitude than the one generated by the simultaneous stimulation of the MUs. Orizio et al. (1996) suggested that, at higher frequencies of stimulation, the VMG signal had a non-linear behavior in the extensor digitorum communis, which was probably related with the higher stiffness obtained by the higher stimulation frequencies, therefore producing the signal with decreased amplitude.

They concluded by saying that the VMG signal could be considered as a compound signal in which the mechanical activities of the muscle fibers was summed, but the linear summation process could not be applied over the full physiological range of MU firing rates.

Three main limitations follow from the study of Orizio et al. (1996): (a) we have not found any study in the literature in which MU recruitment during voluntary contractions has been shown to occur at frequencies in the range of 0-3 Hz; therefore, the stimulation frequency of 3 Hz used in their study is probably not within the physiological range in which recruitment and firing rates of MUs occur, (b) periodic stimulation was used to activate both MUs, which is not the case in voluntary contractions, and (c) the mechanical response from these artificial MUs, where muscle fibers of different types and MUs of different sizes were recruited simultaneously by the artificial stimulation, will not reflect the VMG signal obtained during voluntarily elicited muscle vibrations. However, this was the first study aimed at developing a model to study the frequency domain of the VMG signals based on the power-spectra obtained by the above stimulation protocols.

Summary

The fact that contracting muscles produce vibrations or sounds has been known for more than three centuries. The characteristics of these sounds/vibrations were first described as low frequency rumbling sounds that were produced by muscle contraction, and which resembled the sounds of carriages running over cobblestone. The improvement of transducers and the availability of computers allowed for a better description and identification of the sound/vibratory signal. In-vitro studies related these vibrations with pressure waves or lateral movements which corresponded to accelerations that were produced during muscle contraction. In-vivo studies related the VMG signal to cross-bridge cycling rates and to the force fluctuations produced during voluntarily elicited muscle contractions. Evidence for a relation between MU activation and sounds/vibrations was provided by studies where electrical (artificial) stimulation was used, as the VMG signal was highly dependent on the frequency of stimulation, and by voluntary contractions at weak voluntary effort, as VMG signals were found to be associated with the individual MUAPs. Also, the VMG signal was shown to be associated with the mechanical properties of a contracting muscle as shown by the correlation between the force behavior

(i.e. force oscillations) and the recorded signal.

Purpose

Up to 1992, three ideas were proposed to describe the possible mechanisms involved in the production of muscle sounds. However, none of the studies that proposed these ideas was based on specific theories. Also, no testable predictions were proposed based on the ideas that were put forward in these studies. Therefore, the purpose of this project was to determine the mechanism of muscle vibrations during electrically elicited and voluntary isometric contractions in mammalian skeletal muscle. We described theories, suggested testable predictions, and tested these predictions in a series of experiments. This approach allowed us to accept or reject any of the theories based on the experimental results of the proposed studies (which will be described in the following three chapters), as well as establish a framework from which future work may evolve.

Chapter 3

Mechanism of Muscle Vibrations

In Chapter 1, specific predictions were made based on the theories proposed in the literature and our own ideas about the mechanisms of muscle vibrations. In the experiments introduced in this chapter, specific predictions related to three theories were tested. The results presented in this chapter will be used (Chapter 6) to evaluate all proposed models and their predictions.

The experiments described below were made on in-situ cat SOL and in-vivo human RF muscles.

Animal Experiment

(Vaz et al. Mechanism of electrically elicited muscle vibrations in the in-situ cat soleus muscle. *Muscle & Nerve*, 19:774-776, 1996.)

Introduction

The fact that active muscles produce sounds has been known for almost two centuries (Wollaston, 1810). These sounds are produced by vibrations which probably originate from inside the muscle and can be measured on the surface of the muscle using accelerometers (Lammert et al. 1976; Keidel and Keidel, 1989; Zwarts and Keidel, 1991; Barry, 1992). Muscle sounds (acoustic myography, AMG) or muscle vibrations (vibromyography, VMG) have been thought to reflect some intrinsic mechanical activity of muscle (Barry et al. 1985; Barry and Gooch, 1986; Barry, 1991; Zhang et al. 1992; Orizio, 1993; Herzog et al. 1994), therefore knowledge of the relation between AMG or VMG

signals and activation or force production may provide useful insight into the workings of muscles, particularly since AMG and VMG measurements are non-invasive and simple to perform.

The likely mechanism of muscle vibrations resulting from electrical stimulation of isolated muscle (i.e. in-vitro) preparations has been suggested to be lateral vibrations at the natural frequency of the muscle (Barry and Cole, 1990; Dobrunz et al. 1990). These lateral vibrations have been described as damped oscillations and were represented in a way similar to that when a guitar string is plucked (Frangioni et al. 1987). Since the muscle can change its stiffness depending on its activation and length, the magnitude and frequency response of the vibrating muscle should relate in a predictable way to the activation and length of the muscle, as described elsewhere (Barry, 1987; Frangioni et al. 1987).

In physiological models of muscle vibrations, it has been suggested that the vibrations may be produced by the force changes associated with the contraction of unfused MUs (Jorgensen and Lammert, 1976; Orizio, 1993). The unfused MU hypothesis of muscle vibrations allows for testable predictions. For example, it is known that muscle force is increased by increasing the number of activated MUs, and/or by increasing the firing rates of the active MUs (De Luca and Erim, 1994). According to the unfused MU hypothesis, one would expect only a few MUs to be active at low force levels, and so, the magnitude of the muscle vibrations should be small. At intermediate levels of force, the magnitude of the vibratory signal should reach a peak, because one would expect that a large number of MUs are recruited at unfused frequencies. Towards maximal force production of the muscle, one would expect a small (or no) VMG signal because most MUs should fire at (or near) tetanic frequencies where the MUs produce a fused contraction.

The purpose of this study was to test the hypothesis that muscle vibrations are caused by the movements produced by unfused MUs. Experiments were performed on the in-situ cat soleus (SOL) muscle. The preparation allowed for the precise control of activation and length changes of the SOL, as well as for direct force measurements. An in-situ preparation was chosen because it probably represents the actual physiological vibrations better than in-vitro preparations. The cat SOL muscle was chosen, because it is a primarily slow-twitch fibered mammalian muscle (Ariano et al. 1973), and its results may provide for interesting comparison with results obtained from cat gastrocnemius experiments (Orizio et al. 1993), the only published VMG results of non-human, mammalian skeletal muscle.

Material and Methods

Preparation and Measurements

All experiments were approved by the Ethics Committee of the University of Calgary. Electromyographic (EMG), VMG and force measurements were obtained from in-situ cat SOL muscle using electrical stimulation of the SOL nerve. Four adult male cats were anesthetized and the SOL muscle was exposed in its entirety. The target hindlimb was fixed in a stereotaxic frame. A warm saline solution was applied to the SOL muscle and nerve throughout the experiment to prevent tissues from drying. Body temperature was monitored and maintained near 37°C. Force measurements were obtained from E-shaped tendon force transducers which were calibrated using static weights in a terminal experiment (Herzog et al. 1993a), EMG signals were recorded with bipolar, fine wire, indwelling electrodes (Herzog et al. 1993b), and VMG signals were measured from the mid-belly of the SOL muscle using a miniature, unidirectional accelerometer (Dytran 3115A). The accelerometer had a sensitivity of 1 mV/m/s^2 and a frequency response of 0 to 20 kHz, and was fixed to the epimysium of the SOL with cyanoacylate in such a way that its sensing axis was perpendicular to the surface of the soleus muscle (Zhang et al. 1992). All signals were digitized at a frequency of 2500 Hz per channel using an analogue-to-digital-board and stored on a 386 personal computer for further signal analysis.

Nerve Stimulation

Electrical stimulations were performed for six seconds each using a Grass stimulator (S88, Quincy, Mass., USA), and a bipolar nerve cuff electrode that was placed around the SOL nerve (Herzog et al. 1992). In a first set of experiments, the nerve was stimulated with a voltage of 1.5-2 times the motor threshold (i.e. supramaximally, 1.8 - 2.0 Volts), and average stimulation rates of 4,8,12,16,20,25,30,35 and 60 Hz. The coefficient of variation of the interpulse intervals was 0.15 (Zhang et al. 1990). In a second set of experiments, the stimulation frequency was kept constant at a specific rate, and the stimulation voltage was decreased systematically to achieve one to three submaximal levels of activation. Therefore, in the first set of experiments the number of activated MUs was kept constant (maximal) while the rate of stimulation was changed; whereas in the second

set of experiments the rate of stimulation was kept constant while the number of MUs was changed.

Protocol

Throughout testing, the hip and knee joint angles were kept constant at approximately 90 degrees. All tests were performed at two to four different ankle angles within the normal physiological range (80 to 140 degrees, included angles between the foot and the shank). The first and the second set of the experiments were always completed at one ankle angle before proceeding to the next. One minute intervals were given between each new trial to prevent muscle fatigue.

Data Analysis

EMG, VMG, and force signals were extracted for segments of five seconds from the middle of the trials in order to ensure that transient signals at the beginning and end of the contraction were excluded. EMG signals were band-pass filtered at 10 Hz to 1 kHz, and notch filtered from 59 to 61 Hz in order to eliminate 60 Hz noise. VMG signals were filtered using a second order Butterworth high-pass (5 Hz) filter. Root mean square (RMS) values were calculated from the EMG and VMG signals (Basmajian and De Luca, 1985). Median frequencies (MDF) of the VMG signal were calculated using a Fast Fourier Transform (FFT) algorithm.

Results

Complete data were obtained from animals 3 and 4. From animal 1, force and VMG signals were obtained for tests involving supramaximal nerve stimulation at two muscle lengths; from animal 2, force and EMG measurements were obtained for supramaximal and one submaximal test at four muscle lengths.

The findings presented here were obtained from experiments on animals 3 and 4 at one specific muscle length (i.e. at an ankle angle of 100 degrees). The results for the remaining muscle lengths were consistent with those obtained at the ankle angle of 100 degrees. As expected, the average force increased substantially when the rates of

stimulation were increased from 4 Hz to about 16-20 Hz. At rates of stimulation beyond 20 Hz, the increases in force became small at all levels of activation (Figure 3.1a). In animal 3, fused tetanic contractions were achieved at stimulation frequencies of 16-20 Hz, while in experiment 4 fusion occurred at 12-16 Hz.

The RMS values of the EMG signal increased steadily with increasing rates of stimulation for the experiments performed at supramaximal levels of activation. For the trials performed at submaximal levels of activation, the RMS values became constant for frequencies of stimulation exceeding 12 or 16 Hz (Figure 3.1b). This was true for approximately 70% of the cases studied, while in the other 30%, the RMS values of the EMG signal increased continuously with increasing rates of stimulation, but to a lesser extent than in the supramaximal levels of activation. The EMG signals showed that the compound motor action potentials (CMAPs) were of approximately constant magnitude at the supramaximal and intermediate submaximal levels of activation, whereas the magnitude of these potentials varied somewhat more at the lowest submaximal level compared to the supramaximal level of activation (Figure 3.2). For animal 3, the magnitudes of the CMAPs varied for all submaximal levels while they remained nearly constant for the supramaximal levels of activation (Figure 3.3).

The RMS of the VMG signals decreased with increasing frequencies of stimulation in the range from 4 to 12 Hz and reached values close to the baseline value (no stimulation) at 12 Hz and beyond (Figure 3.4a-c). Similar results were seen for animal 3, in which the RMS values reached baseline values at 16 Hz. As might have been expected based on the results shown in Figure 3.4, the amplitude of the VMG signal decreased with increasing stimulation frequencies (Figure 3.5). In addition, the shape of the VMG signal changed with increasing frequencies of stimulation and became indistinguishable from the baseline in the range of 16-60 Hz for animal 3, and 12-60 Hz for animal 4. RMS values recorded at a given frequency of stimulation typically decreased with decreasing levels of activation (Figures 3.4a-c, 4 Hz and 8 Hz).

The MDF of the VMG signal tended to increase with increasing stimulation rates at given levels of activation, and tended to decrease from the maximal to the lowest submaximal level of activation at given frequencies of stimulation (Figures 3.6a and 3.6c).

Little VMG signal is seen above 8 Hz for animal 4 (Figure 3.7) at the lowest submaximal level of activation, while although small, the VMG signal can be seen for frequencies as high as 30 and 60 Hz for animal 3 (Figure 3.8).

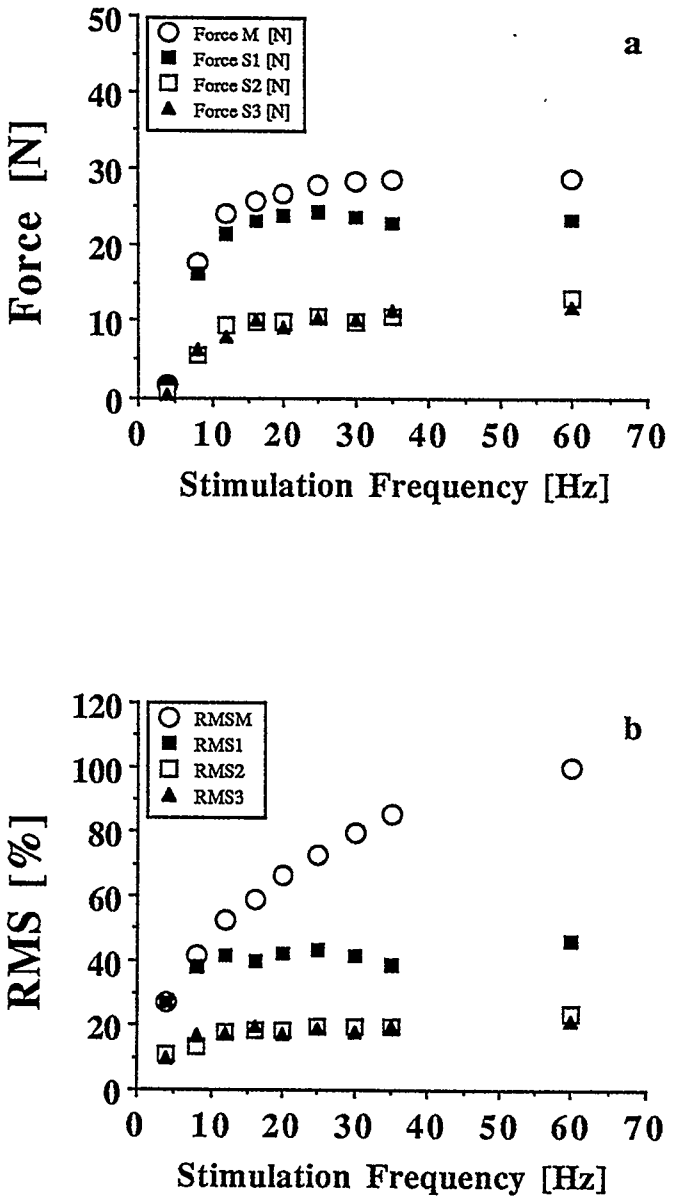

Figure 3.1. Force and RMS values of the EMG signal for the entire protocol of stimulation at one specific ankle angle (100 degrees) for animal 3. The frequencies of stimulation performed were 4,8,12,16,20,25,30,35, and 60 Hz. In (a), M = maximal level; S1 = first submaximal level; S2 = second submaximal level; S3 = third submaximal level. In (b), RMSM = RMS at maximal contraction; RMS1 = RMS at first submaximal level; RMS2 = RMS at second submaximal level; and RMS3 = RMS at third submaximal level.

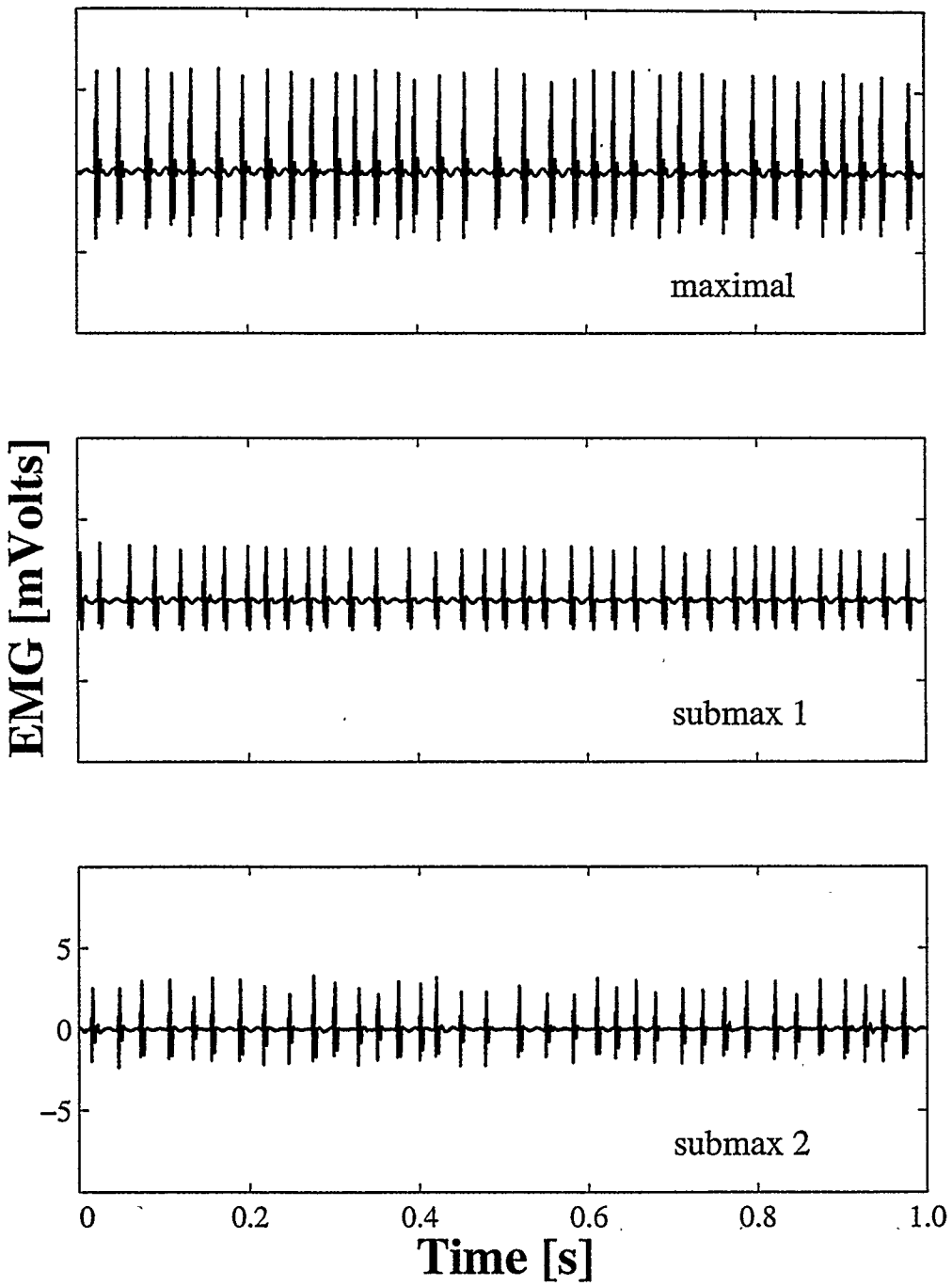

Figure 3.2. Representative data showing the EMG recordings from soleus muscle at the three different levels of stimulation (from bottom to top, maximal level, and submaximal levels 1-2) for animal 4. Note, the decrease in the amplitude of the signal with decreasing levels of activation.

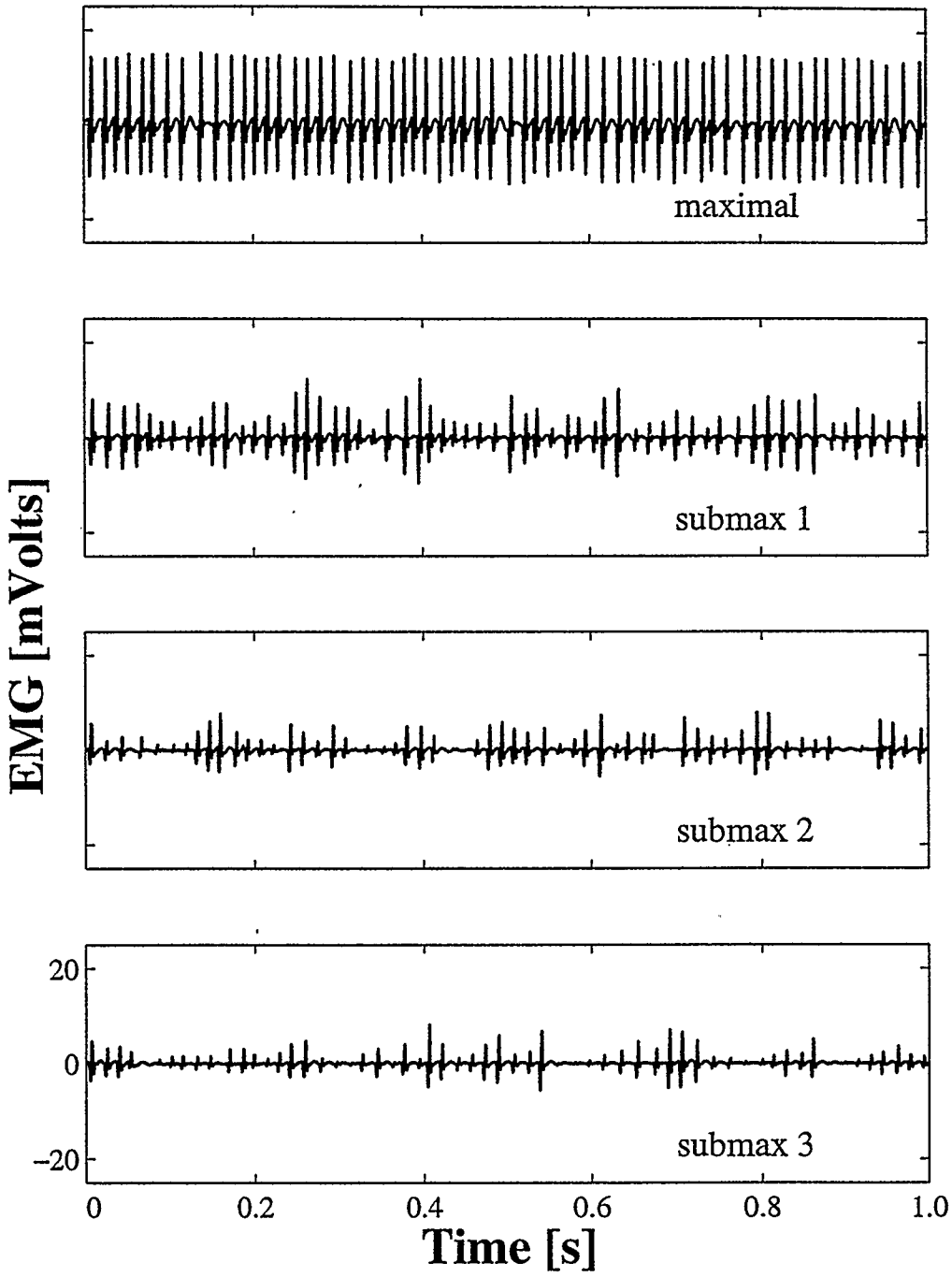

Figure 3.3. EMG recordings from the soleus muscle of animal 3 at four different levels of activation (from bottom to top, maximal level, and submaximal levels 1-3). Note, the variability of the compound motor action potential (CMAP) amplitude at all submaximal levels.

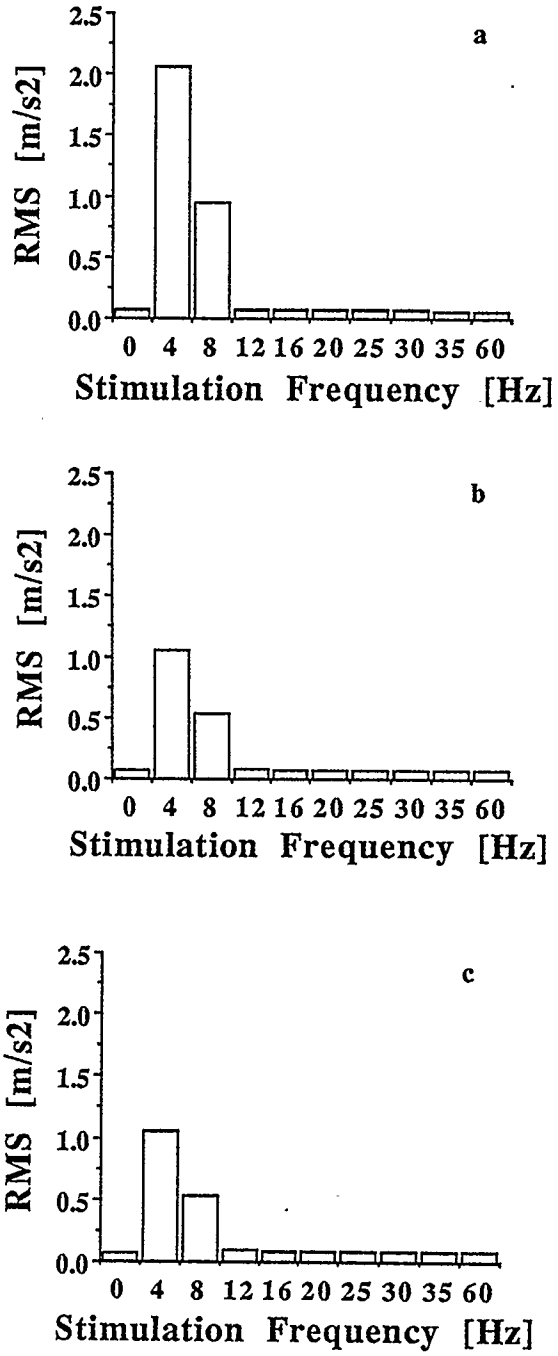

Figure 3.4. RMS of the VMG signal for the entire protocol of stimulation at an ankle angle of 100 degrees (animal 4). Note, the similarity of the RMS of the VMG signal at rest (0 Hz) and at frequencies in the range of 12-60 Hz. (a) = maximal level; (b) = first submaximal level; and (c) = second submaximal level.

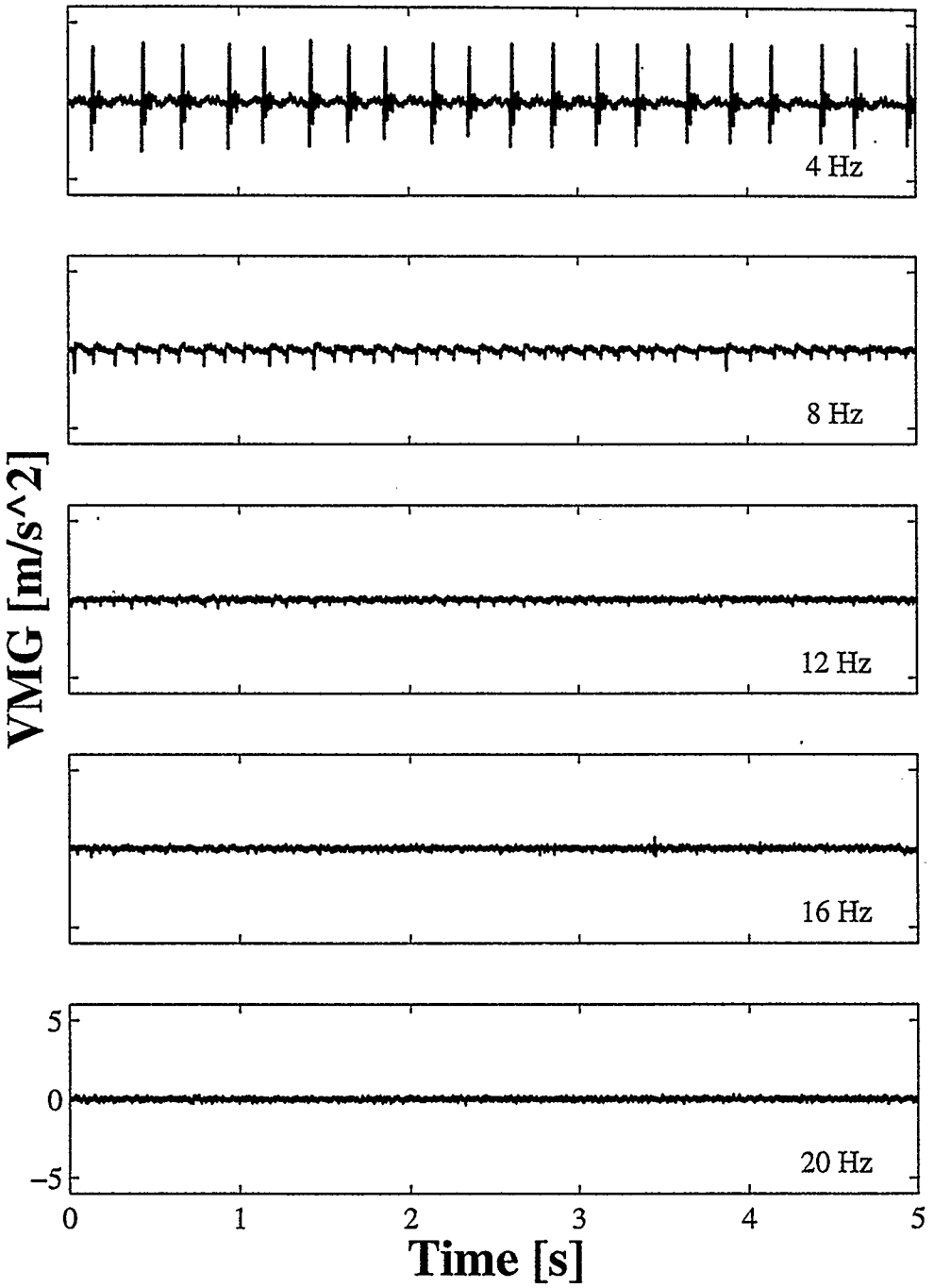

Figure 3.5. Representative data showing VMG recordings at five different stimulation rates (from bottom to top, 4, 8, 12, 16, and 20 Hz) and maximal level of contraction for animal 3.

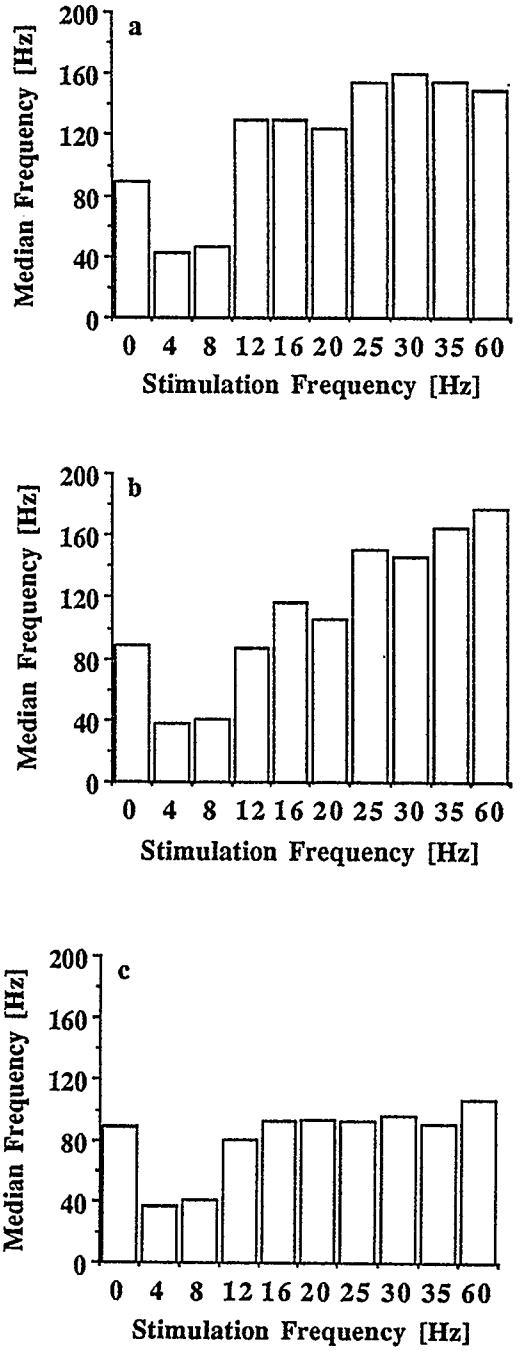

Figure 3.6. MDF of the VMG signal for the entire protocol of stimulation at an ankle angle of 100 degrees of (a) maximal contraction, (b) first submaximal level, and (c) second submaximal level in animal 4.

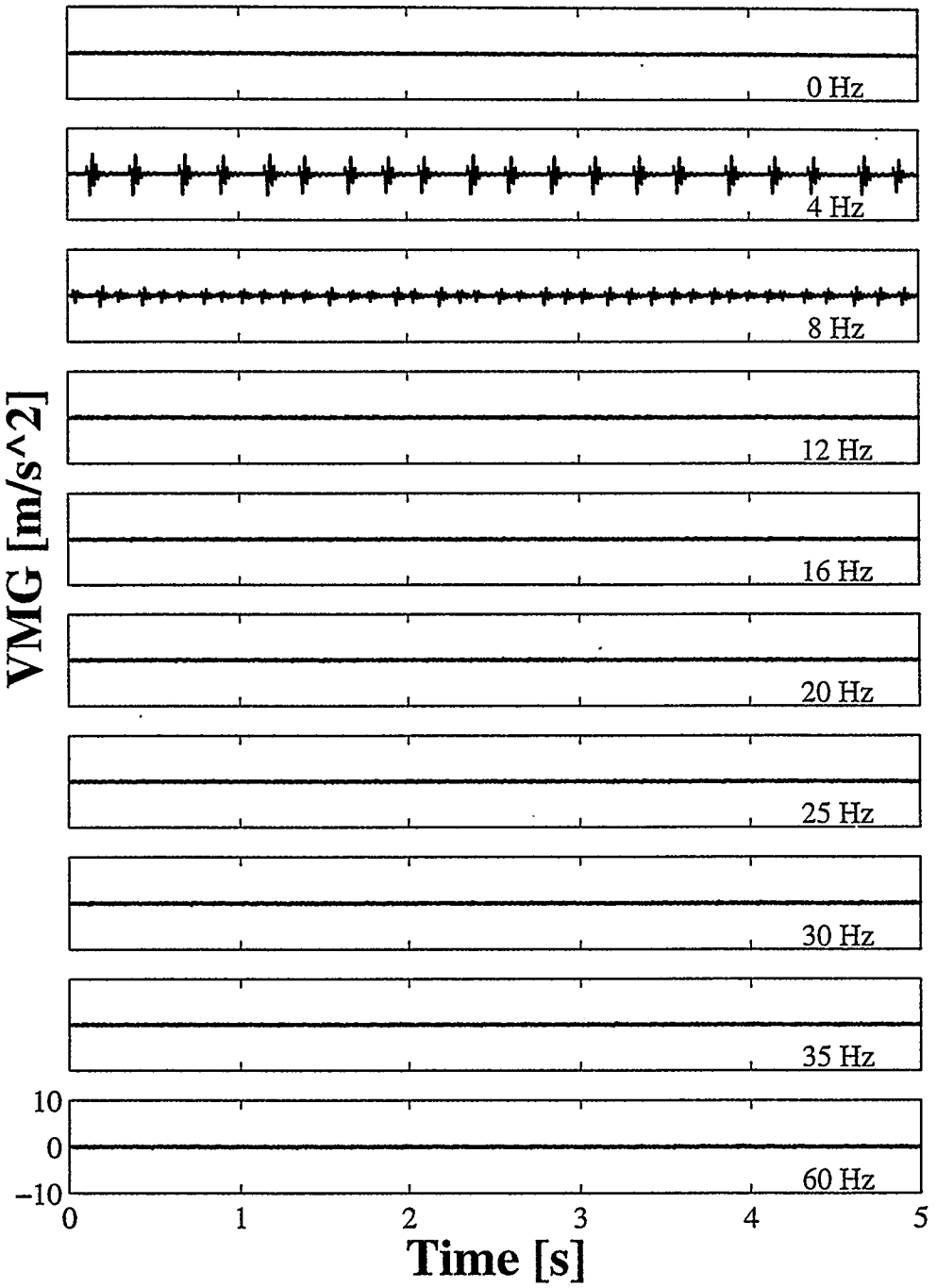

Figure 3.7. VMG signal of the lowest submaximal level of activation for all the frequencies used in the protocol (animal 4). Note, that the VMG signals are only present at low frequencies of stimulation

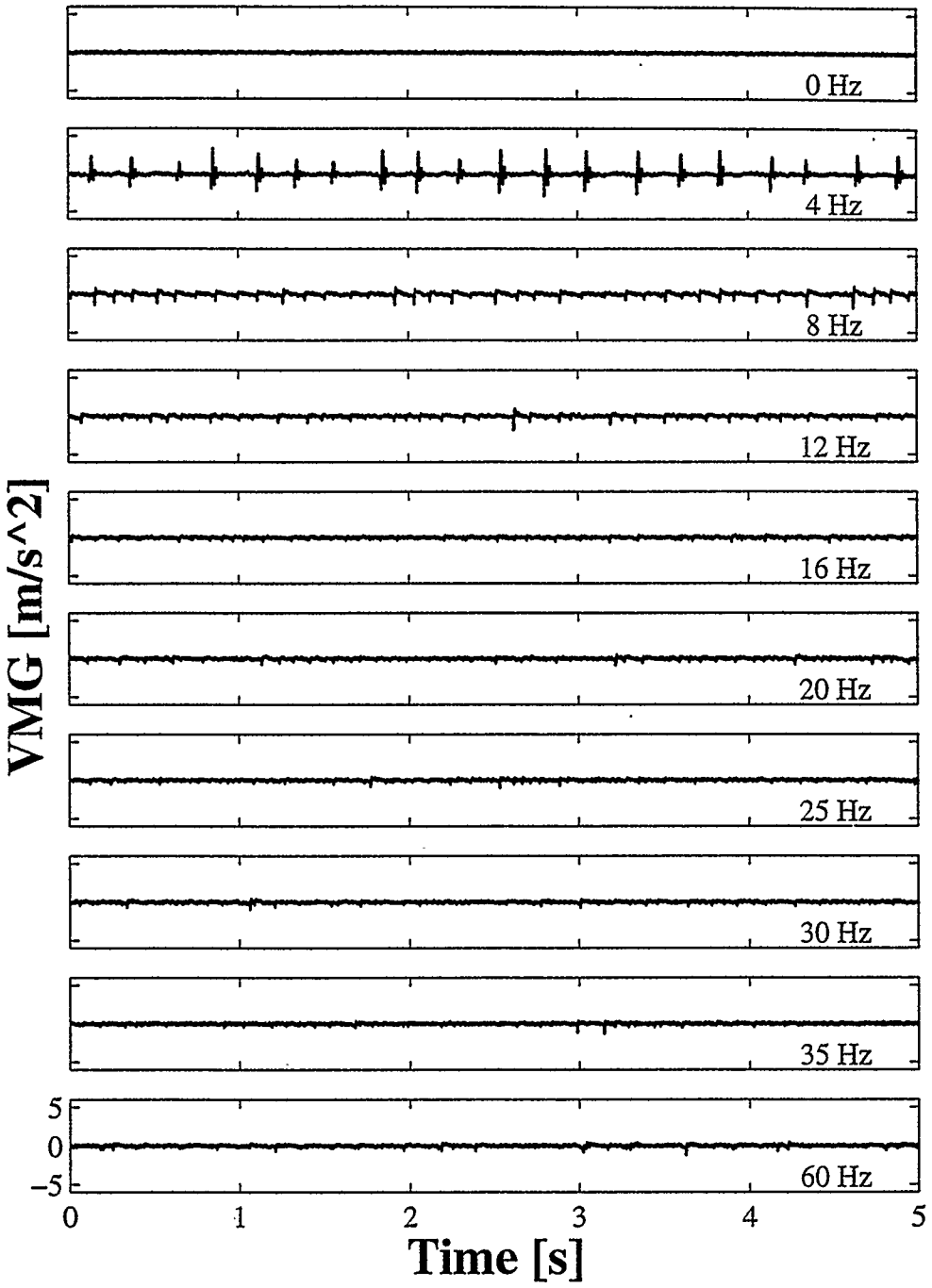

Figure 3.8. VMG signal of the lowest submaximal level of activation for all the frequencies used in the protocol (animal 3). Note, that the VMG signals appear at all frequencies of stimulation.

Discussion

The purpose of this study was to test the hypothesis that muscle vibrations are caused by the movements produced by unfused MUs. Two basic experimental protocols were performed. In the first protocol, the activation to the muscle was (supramaximal) constant and the frequency of stimulation was changed from 4 Hz to 60 Hz. In the second protocol, the stimulation frequencies were held constant while the voltage to the nerve cuff electrode was decreased from a supramaximal level (i.e. all MUs were recruited) to a series of submaximal levels (i.e. only part of the MU pool was recruited).

Constant Activation and Change in Stimulation Rates

The results presented in Figures 3.4 and 3.5 support the idea that each electrical stimulus produces a distinct vibratory signal, and that increases in the rates of stimulation decrease the amplitude of the VMG signal. In addition, no vibratory signals beyond background noise were detected when the muscle was at rest, and at or above stimulation frequencies of 12 to 20 Hz during supramaximal stimulations. It has been shown that 89% of the MUs of the slow twitch SOL muscle of the cat produce more than 70% of their maximum force at a stimulation rate of 20 Hz (McPhedran et al. 1965); i.e. most of the MUs are contracting tetanically, and therefore no vibratory signals were produced at or above 20 Hz. The results of Figure 3.4a (animal 4) further support this idea as the RMS of the VMG signal is similar for the resting state and at frequencies of about 12 Hz and higher. Similar results were found in animal 3, although VMG signals could be seen at frequencies of 16 Hz. This may be explained with the different frequencies at which the SOL muscle achieved fused tetanic contractions in each experiment (i.e. 12 Hz in animal 4, and 16-20 Hz in animal 3, respectively).

Constant Stimulation Rate and Change in Activation Level

As the number of activated MUs decreased when going from maximal to submaximal levels of activation (Figures 3.2 and 3.3), it was expected that (a) the magnitude and (b) the frequency content of the vibratory signals should decrease with decreasing number of activated MUs to reflect that non-active units will have to be

passively moved along by the active MUs, and therefore damp the signal. The RMS and the MDF of the VMG signal typically decreased from maximal to submaximal levels of activation (Figures 3.4 and 3.6, respectively) for a given sub-tetanic frequency, therefore supporting expectations (a) and (b) above.

The presence of muscle vibrations at frequencies as high as 30-60 Hz for the lowest submaximal level of activation in animal 3 (Figure 3.8) appears to be associated with fluctuations in the activation level in this particular experiment. Figure 3.3 supports the idea that activation at the submaximal levels was not constant in animal 3, as the magnitude of the CMAPs changed continuously. Since the activation parameters were kept constant, fluctuations in the activation may have been produced by a change in contact of the stimulating electrode with the nerve possibly caused by SOL vibrations. The non-constant activation in this experiment may also explain the small increase in the RMS of the VMG signal at most frequencies (i.e. 8-60 Hz) when going from the maximal to submaximal levels of activation (results not shown). Such variations in CMAPs and force were not seen in animals 2 and 4, thus explaining why no vibratory signals were seen at frequencies higher than 12 or 16 Hz at all levels of activation in animal 4.

The results of this study are in accordance with the idea that muscle vibrations are caused by the force fluctuations of unfused contraction of MUs. Also, they support the evidence provided from isolated amphibian muscle preparations (Barry, 1987; Frangioni et al. 1987) about the mechanism of muscle vibrations. The decrease in the VMG signal amplitude observed when the stimulation rates were increased agree with the results shown for electrically elicited mammalian muscle contractions in both human (Bolton et al. 1989; Stokes and Cooper, 1992) and non-human preparations (Orizio et al. 1993). However, in the non-human mammalian case, sound signals were recorded up to a frequency of 50 Hz (the maximal stimulation frequency used) in the cat gastrocnemius, a muscle composed primarily of fast MUs (Ariano et al. 1973; Burke and Tsairis, 1973). It has been shown that 70% of the MUs of gastrocnemius did not reach 90% of their maximum force at a stimulation frequency of 50 Hz (Wuerker et al. 1965). Therefore, one might expect fusion frequencies of some MUs to be above 50 Hz in this muscle, which may explain the existence of sound signals at higher frequencies than observed here for the cat SOL.

Analysis of the VMG signals in the frequency domain during electrically elicited muscle contractions in in-vivo muscle preparations have described the signal frequency as matching the stimulation frequency (Stokes and Cooper, 1992), or having main frequencies of 2 and 13 Hz (Bolton et al. 1989). This was not the case in our study (Figure 3.6) where

the MDFs were between 30 and 50 Hz during unfused contractions. This higher frequency range may be due to (a) the non-periodical stimulation that was used, which does not influence the MDF of the VMG signal as it does when periodical stimulation is used (Zhang et al. 1994), and (b) the skin and fat tissues, which could cause damping of the signal, were not present in our preparation. Analysis of the VMG signal revealed a very good signal-to-noise ratio at unfused frequencies of stimulation, whereas the signal-to-noise ratio decreased at stimulation frequencies where the contraction was a fused tetanus; at these stimulation frequencies, where a fused tetanic contraction was produced, the VMG signals could not be distinguished from noise anymore (e.g. Figures 3.5, 3.6 and 3.7). Therefore, the MDFs shown in Figure 3.6 do not directly reflect the vibratory signals.

If the hypothesis about the origin of muscle vibrations should hold, then VMG may become a powerful tool for studying MU recruitment patterns with increasing force production. For example, it should be possible to use VMG to estimate the force level at which all (or most) MUs are producing fused contractions, and thus, to estimate when all MUs of a muscle are activated.

Human Experiment

(Vaz et al. Mechanism of human skeletal muscle vibrations during stimulated and voluntary isometric contractions. Submitted. *J. Appl. Physiol.*, 1996.)

Introduction

Early vibromyographic (VMG) studies showed that muscles produced sounds during contraction (Grimaldi, 1665; Wollaston, 1810). The sounds, and the corresponding vibrations produced by a contracting muscle, can be measured on the surface of the skin by means of microphones (Bolton et al. 1989; Orizio, 1993; Stokes, 1993) and accelerometers (Zwarts and Keidel, 1991; Zhang et al. 1992), respectively. In the last century, muscle vibrations have been described extensively (Herreoun and Yeo, 1885; Gordon and Holbourn, 1948; Oster and Jaffe, 1980; Barry, 1991). However, the physiological mechanism associated with these vibrations has not been determined conclusively in voluntary and stimulated contractions.

Different models have been used to describe the mechanisms of muscle vibrations (Oster and Jaffe, 1980; Frangioni et al. 1987). However, the most likely mechanism is the

force changes associated with the unfused contraction of MUs (the unfused MU hypothesis; Jorgensen and Lammert, 1976; Orizio, 1993; Vaz et al. 1996a). Evidence in support of the unfused MU hypothesis has been provided for in-situ muscle preparations (Orizio et al. 1993; Vaz et al. 1996a). In these studies (Orizio et al. 1993; Vaz et al. 1996a), the influence of MU recruitment and changing firing rates on the VMG signals were studied for electrically induced contractions in the cat gastrocnemius and SOL muscles, respectively, and involved the placement of the transducer directly on the muscle. Because of the differences between electrically elicited and voluntary contractions, the results of the in-situ studies cannot be directly applied to in-vivo situations.

It is well known that muscle force is increased by increasing the number of activated MUs, and/or by increasing the firing rates of the active MUs (De Luca and Erim, 1994) during electrically elicited and voluntary contractions. Assuming that the unfused MU hypothesis is true, one would expect that during electrically elicited muscle contractions an increase in the voltage of stimulation (which is associated with an increase in the number of recruited MUs) should produce an increase in the amplitude of the VMG signal, whereas an increase in the stimulation rate (which is associated with an increase in fusion of the MUs) should produce a decrease in the amplitude of the VMG signal. Stokes and Cooper (1992) have shown that the integrated values of the acoustic myographic (AMG) signal increase in a curvilinear fashion over the entire range of voluntary contractile force for a hand muscle, the adductor pollicis. These authors also demonstrated that, during electrically elicited contractions, the AMG decreased in amplitude with increasing frequency of stimulation, and concluded that the increase in magnitude of the AMG signal during increasing force of voluntary contractions was evidence of a MU recruitment strategy. Since it is known that the MU recruitment strategy is different for large leg muscles compared to small hand muscles (Basmajian and De Luca, 1985), it is of interest to evaluate the VMG response of large leg muscles to see if this known difference in recruitment strategy is reflected in the VMG signal.

The purpose of this study was to test the hypothesis that an increase in the number of active MUs will increase, and an increase in MU firing frequency will decrease the magnitude of muscle vibrations. Changes in the magnitude of the VMG signal were also recorded for different levels of effort during voluntary contractions in anticipation that this procedure would provide qualitative information on the MU recruitment strategies of large skeletal muscles.

Material and Methods

Preparation and Measurements

Informed consent was obtained from all subjects prior to testing. VMG signals from the rectus femoris (RF) and knee extensor moments from the entire quadriceps group were obtained from twenty healthy subjects (age 20 to 45 years) during electrically elicited and voluntary isometric contractions. RF was selected for this study because of the previous vibromyographic research performed using this muscle (Jorgensen and Lammert, 1976; Lammert et al. 1976; Stokes and Dalton, 1991b; Zhang et al. 1992; Dalton and Stokes, 1993). Isometric knee extensor moments were measured using a Cybex II isokinetic dynamometer. The hip and knee joint angles were kept constant at approximately 90 degrees.

A cylindrical (6 mm diameter, 7 mm height) unidirectional accelerometer (Dytran 3115A) with a sensitivity of 1 mV/(m/s^2) and a frequency response of 0 to 20 kHz was fixed to the skin with thin, double-sided adhesive tape on the distal third of RF.

Percutaneous electrical stimulation (square wave pulses of 0.8 ms duration) of the femoral nerve was performed for one second using a Grass stimulator (S88, Quincy, Mass., USA) and an isolation unit (Model SIU8T) approved for human use. The stimulating surface electrodes (4.5 x 10 cm) were placed (a) proximally, on the antero-medial surface of the thigh, over the approximate anatomical site of the femoral nerve, and (b) distally, over the distal portion of the quadriceps, respectively. VMG signals and knee extensor moments were digitized at a frequency of 2500 Hz using an analogue-to-digital board, and were stored on a 486 personal computer for further signal analysis.

Protocol

In a first set of measurements, percutaneous stimulations of the femoral nerve were performed for one second each at a frequency of 20 (n=5), 25 (n=5) or 30 Hz (n=10), while the stimulation voltage was increased systematically in steps of 10 Volts to achieve five to seven submaximal levels of activation. The number of submaximal levels (or the voltage range) and the stimulation frequency were limited by the subject's comfort level. In a second set of experiments, percutaneous nerve stimulations were performed (one

second) at a constant submaximal voltage (40,50,60,70,80 or 90 Volts), while the stimulation rates were increased from 10 to 40 Hz in steps of 5 Hz. In the first set of measurements, the rate of stimulation was kept constant while the number of MUs was changed. In the second set of measurements, the number of activated MUs was kept approximately constant while the rate of stimulation was changed. In a third set of tests, voluntary contractions were performed for six seconds each at levels ranging from 0 to 100% MVC at intervals of 10%.

Data Analysis

Electrically Elicited Contractions: VMG and force signals were extracted for segments of seven hundred milliseconds from the one second trials starting 300 ms after the recorded stimulation pulse in order to avoid the initial and final transient effects coinciding with the increase (Cole and Barry, 1994) and the decrease in force, respectively. VMG signals were filtered (second order Butterworth, band-pass 5-100 Hz) and RMS values (Basmajian and De Luca, 1985) were calculated.

Voluntary Contractions: VMG and force signals were extracted for segments of five seconds from the middle of the voluntary trials, in order to ensure that transient signals at the beginning and end of the contraction were excluded. VMG signals were filtered (second order Butterworth, band-pass 5-100 Hz) and RMS values were calculated from the VMG signals (Basmajian and De Luca, 1985). Two second segments of VMG signals were extracted while RF was at rest, and the RMS values were calculated.

Results

All results were consistent across subjects, except where specifically pointed out. The knee extensor moment increased with increasing stimulation voltages, increasing stimulation rates, and increasing voluntary effort, as expected. There was a decrease in the amplitude of the force fluctuations with increasing stimulation frequencies (Figure 3.9), while the magnitude of the force fluctuations increased with increasing stimulation voltages (Figure 3.10).

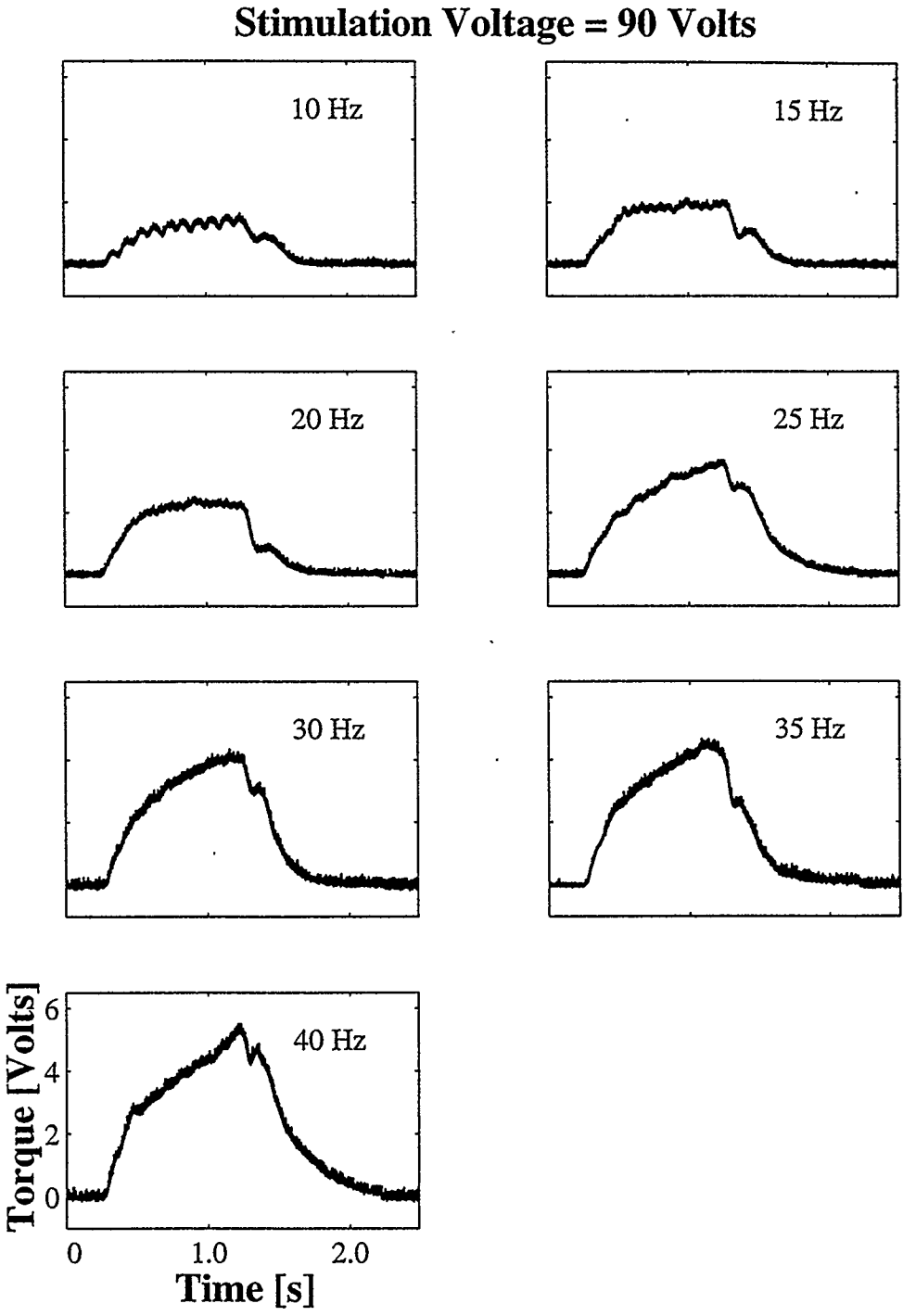

Figure 3.9. Representative raw data illustrating the torque behavior as a function of stimulation frequency. Observe the decrease in the force transients with increasing frequencies of stimulation.

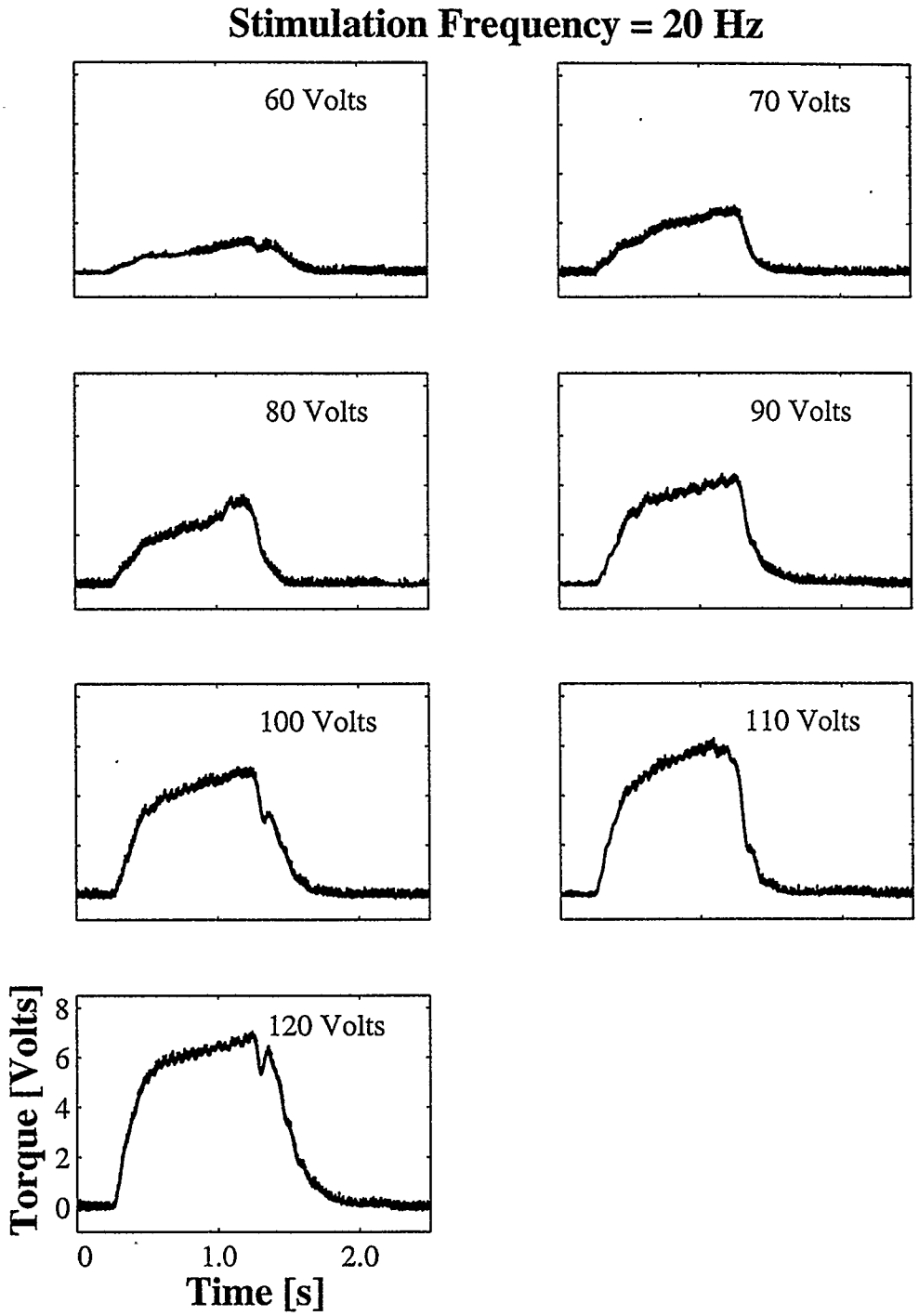

Figure 3.10. Representative raw data illustrating the torque behavior as a function of stimulation voltage. Observe the increase in the force transients with increased voltage of stimulation.

For a given frequency of stimulation, the RMS of the VMG signal increased with increasing stimulation voltages (Figure 3.11a), whereas for a given voltage of stimulation the RMS decreased with increasing frequencies of stimulation (Figure 3.11b). During voluntary contractions, the RMS of the VMG signal increased in a step-wise fashion with increasing voluntary effort (Figure 3.11c). From 0 to 20% and from 40 to 80% MVC, there was a substantial increase in the RMS of the VMG signal, whereas from 20 to 40% and from 80 to 100% MVC, the RMS values remained about constant.

In order to visualize the changes of the VMG signal contributing to the RMS, selected raw data of the VMG signals are presented in Figures 3.12 - 3.14. No vibratory signals beyond background noise were detected when the muscle was at rest, while each electrical stimulus produced a distinct vibratory signal (Figure 3.12). The magnitude of the VMG signals increased with increasing stimulation voltage (Figure 3.12), and decreased with increasing stimulation frequencies (Figure 3.13). During voluntary contractions (Figure 3.14), changes in the magnitude of the signal were difficult to assess from 10-40% MVC, an increase was observed from 40-80% MVC, and a decrease in the magnitude of the signal was apparent from 80-100% MVC.

Discussion

The purpose of this study was to test the hypothesis that muscle vibrations in human subjects are increased by recruitment of additional MUs, and decreased by increasing frequency of activation of MUs.

Constant Stimulation Rate and Change in Activation Level

As the number of activated MUs was increased during the electrically induced RF contractions, it was expected that the magnitude of the VMG signals should increase, thereby reflecting the contributions from an increasing number of simultaneously activated MUs. Damping of the signal by the inactive MUs would decrease as more units were activated. The RMS of the VMG signal typically increased with increasing levels of activation (Figure 3.11a) for a given stimulation frequency, therefore supporting the above prediction. Similar results were also found for the cat SOL (Vaz et al. 1996a) and for the human thenar muscle group (Bolton et al. 1989).

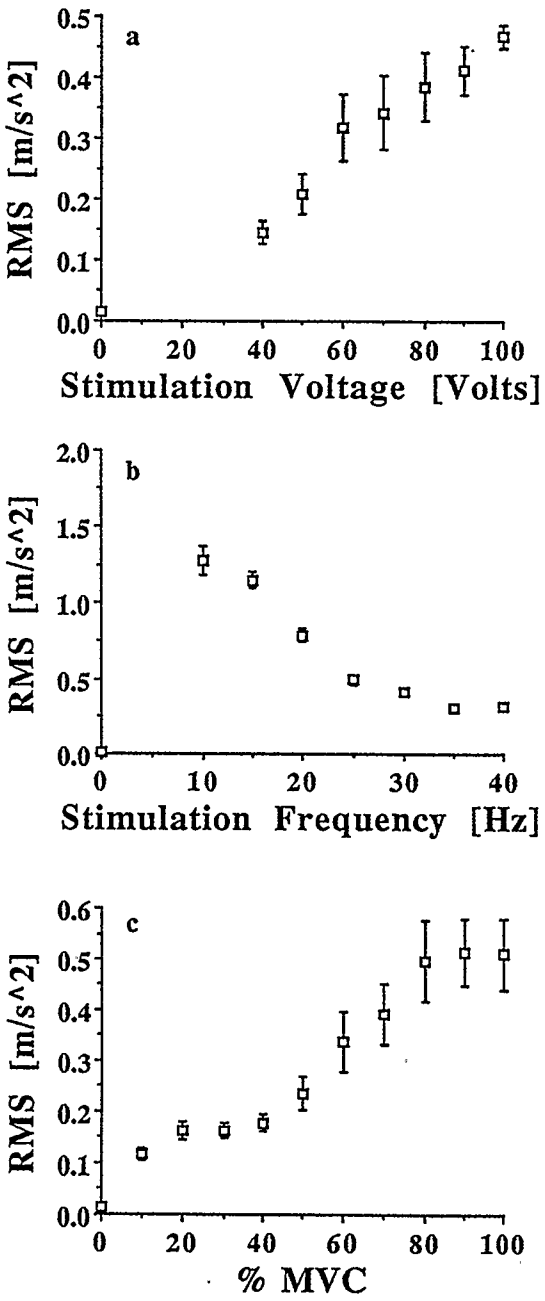

Figure 3.11. RMS of the VMG signal (mean ± standard error) as a function of (a) stimulation voltage (n=10 for stimulation voltages of 60-80 Volts, and 5,6,7,and 8 for stimulation voltages of 90,40,50 and 80 Volts, respectively; stimulation frequency = 30 Hz), (b) stimulation rate (n=20 for stimulation frequencies of 10-30 Hz, and 19 and 14 for frequencies of 35 and 40 Hz, respectively; stimulation voltage range = 40-90 Volts), and (c) percentage of the maximal voluntary contraction (MVC; n=8).

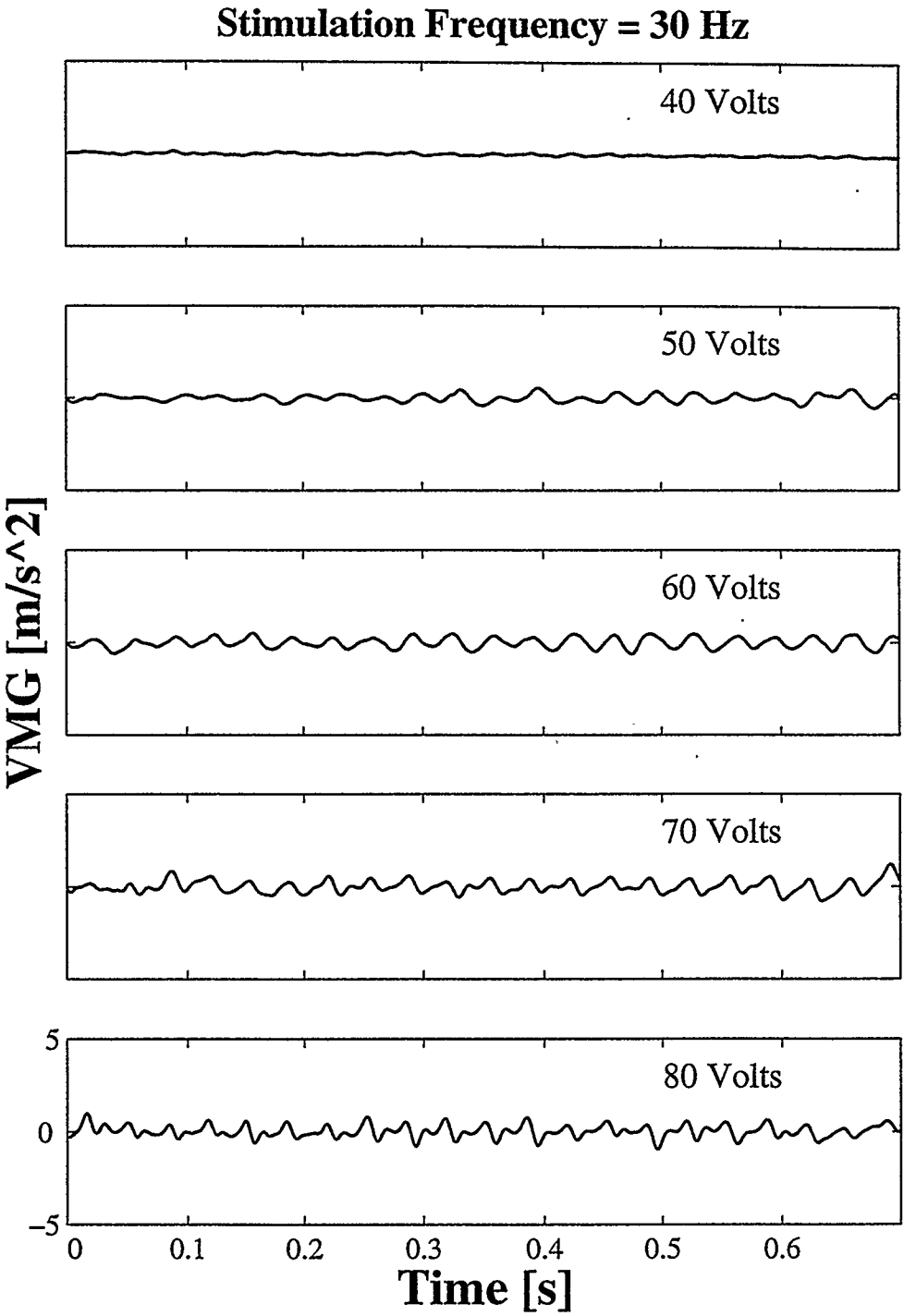

Figure 3.12. VMG signals as a function of stimulation voltage.

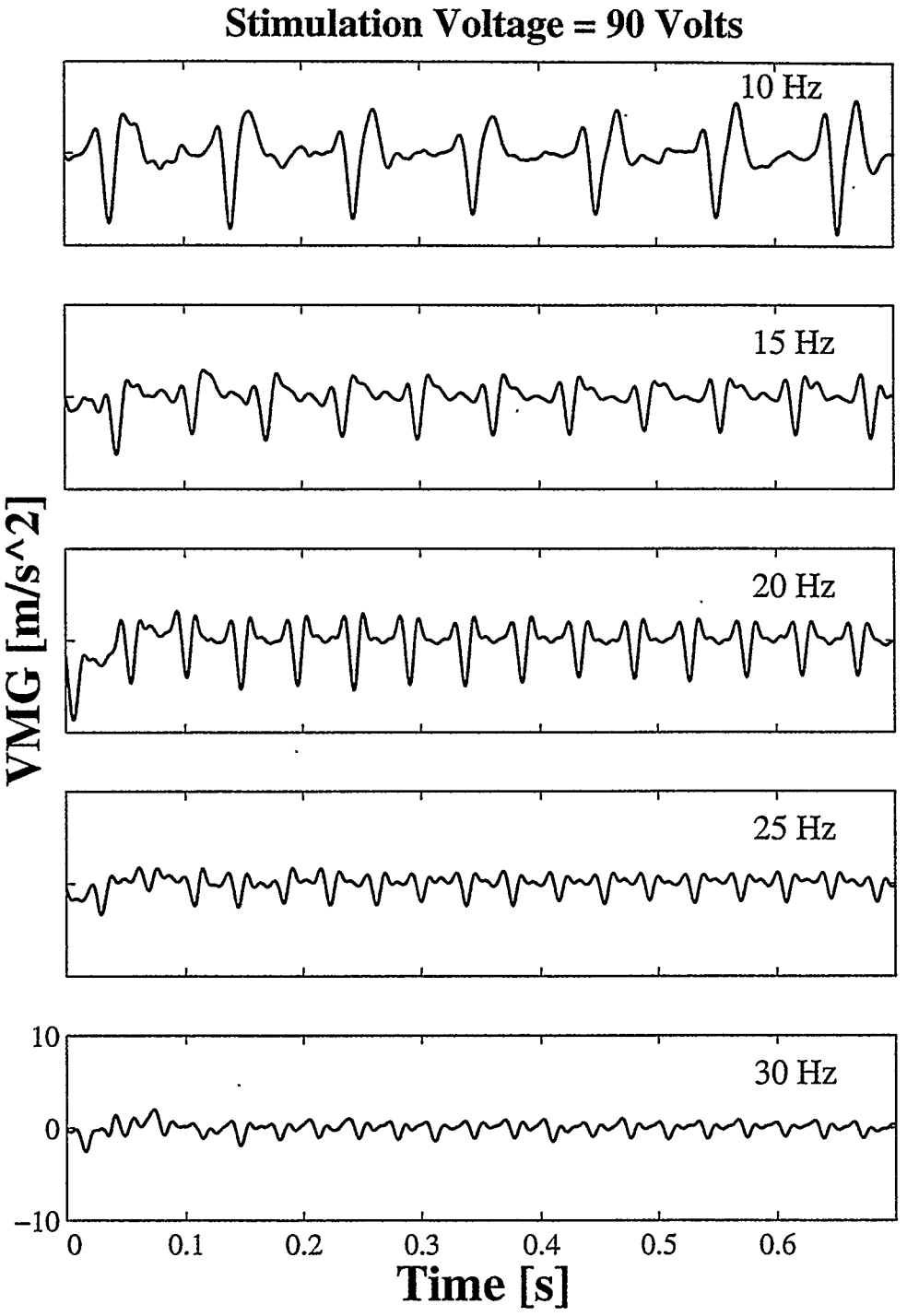

Figure 3.13. VMG signals as a function of stimulation frequency.

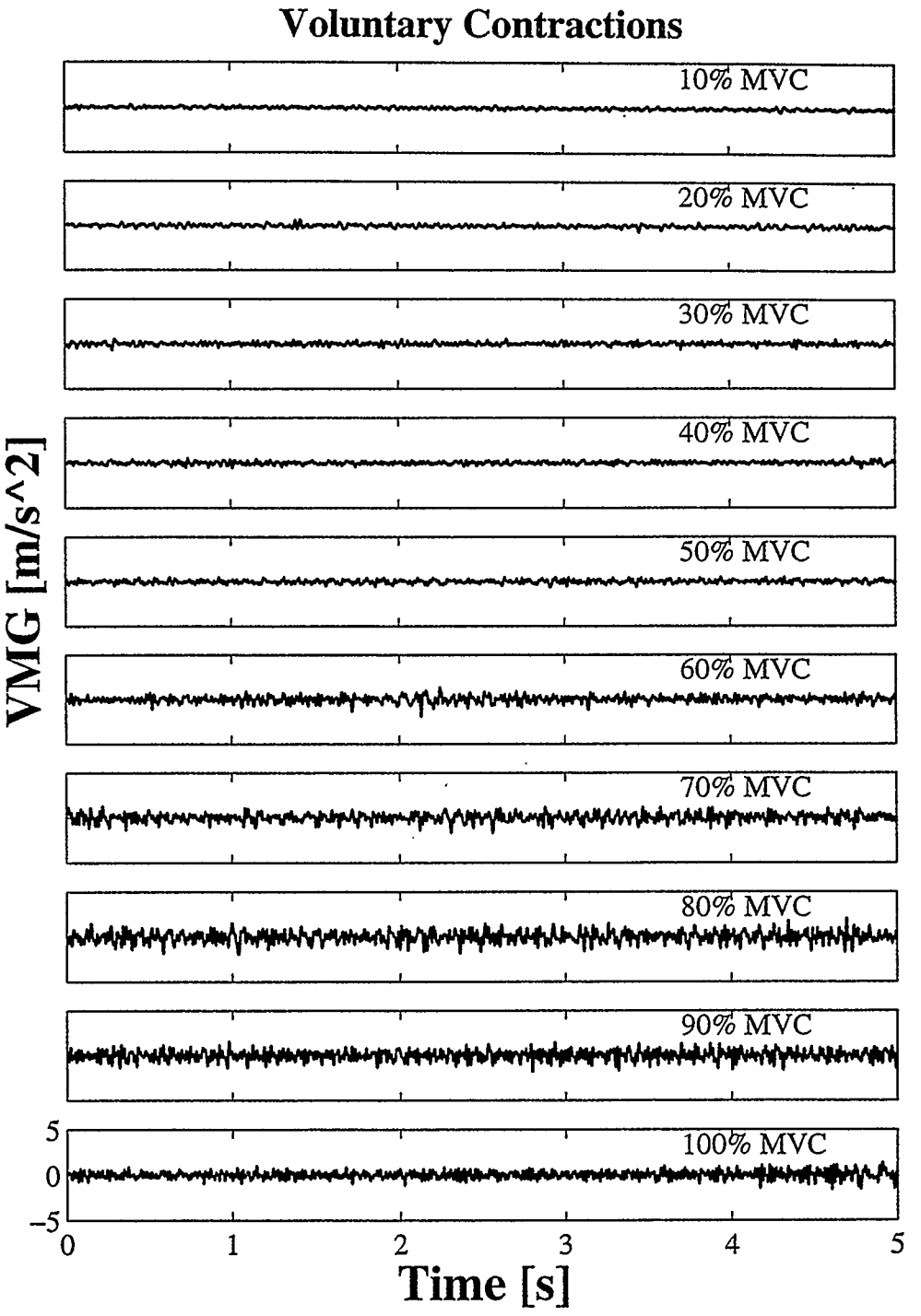

Figure 3.14. VMG signals as a function of percentage of MVC.

Constant Activation and Change in Stimulation Rates

The distinct vibratory signals observed at subtetanic frequencies of stimulation were assumed to reflect a vibration produced by the transient force response of the muscle to each stimulus (Vaz et al. 1996a). Increases in the stimulation rate (which is associated with an increase in the fusion of sequential activation responses) were expected to produce a decrease in the magnitude of the VMG signal. In accordance with this expectation, the RMS of the VMG signal decreased with increasing stimulation rates (Figure 3.11b), supporting the hypothesis that increases in the rates of stimulation decrease the magnitude of the VMG signal. Also, the decreased force fluctuations with increasing stimulation frequencies (Figure 3.9) parallelled the decreased magnitude of the vibratory signals (Figure 3.13), further emphasizing the assumption made above that muscle vibrations are closely related to the transient force responses of a muscle. Similar results were found for in-vitro (Barry, 1987; Frangioni et al. 1987), in-situ (Orizio et al. 1993; Vaz et al. 1996a, 1996b), and in-vivo muscle preparations (Bolton et al. 1989; Stokes and Cooper, 1992).

Voluntary Contractions

During voluntary contractions, the RMS-values of the VMG signal increased with increasing torque values from 0-20% and from 40-80% MVC, whereas they remained about constant with increasing torque values from 20-40% and from 80-100% MVC. Force increases during voluntary contractions occur through an increase in the number of recruited MUs and a simultaneous increase in the firing rates of the active MUs (De Luca and Erim, 1994). The results of the electrically elicited muscle contractions suggest that an increase in the number of MUs is associated with an increase in the VMG signal, whereas an increase in the firing rates of the MUs is associated with a decrease in the VMG signal. Therefore, it appears that during voluntary contractions, the effect of increasing numbers of MUs on the VMG signal is stronger than the effect of increasing firing rates for voluntary contractions ranging from 0-20 and from 40-80% MVC. The two effects appear to cancel each other for voluntary contractions between 20-40 and between 80-100% MVC.

It has been shown that large muscles of the limbs recruit MUs in the full range of voluntary effort, as opposed to small muscles which depend primarily on MU recruitment from 0-50% MVC, and depend primarily on increases in firing rate from 50-100% MVC

(Basmajian and De Luca, 1985). At low levels of force production (0-20% MVC), recruitment of small slow-twitch MUs would be expected to be the main strategy in large muscles, therefore producing the observed increase in the RMS of the VMG signal. As additional force is required to go from 20-40% MVC, the influence of increasing firing rates appears to offset the influence of additionally recruited MUs; as a consequence, the VMG signal amplitude does not increase further. The RMS plateau between 20-40% MVC may be interpreted as a possible strategy of the central nervous system aimed at satisfying the increasing force demands in that region primarily by increasing the firing rates of the (presumably slow) active MUs, rather than recruiting new (presumably fast) MUs. Such a strategy may increase the endurance capacity of a muscle by avoiding an early recruitment of fast fatiguable MUs.

At intermediate voluntary efforts (i.e. 40-80% MVC), additional recruitment of MUs (presumably of large fast-twitch MUs) is necessary to satisfy the increasing force demands, explaining the large increase in the RMS of the VMG signal. Towards maximal effort (80-100% MVC), increases in the firing rate seem to offset the effects of MU recruitment on the VMG signal. This result could indicate that most MUs were recruited at levels below 80% MVC, and frequency modulation was the principal mechanism to increase force from 80-100% MVC.

The results of this study give support to the hypothesis that muscle vibrations are increased by recruitment of additional MUs and decreased by increasing frequency of activation during electrically elicited contractions of human skeletal muscle. Also, these vibrations appear to reflect the control strategies used by the nervous system to produce force during voluntary isometric contractions. If the hypothesis about the origin of muscle vibrations should hold, then VMG may become a powerful tool for studying MU recruitment patterns during voluntary contractions.

Chapter 4

Effect of Muscle Length on Muscle Vibrations

(Vaz et al. The effect of muscle length on electrically elicited muscle vibrations in the in-situ cat soleus muscle. In press. *J. Electromyogr. Kinesiol.*, 1996.)

In the preceding chapter, the mechanism of muscle vibrations was studied in in-situ and in-vivo muscle preparations at a specific muscle length. When performing isometric contractions at different muscle lengths, the material properties of a muscle change. In the present study, the effects of changing muscle lengths (and therefore, presumably muscle mechanical properties) on muscle vibrations are studied in-situ.

Introduction

Muscle sounds (acoustic myography, AMG) are produced by vibrations which originate from inside the muscle (Barry, 1987; Frangioni et al. 1987). Vibromyography (VMG) is a new technique that has been used to record these vibrations on the surface of muscles during contraction. It has been suggested that muscle sounds/vibrations originate from the movements produced by the unfused contraction of MUs (Orizio, 1993; Orizio et al. 1993; Vaz et al. 1996a). If so, VMG may possibly be used to assess qualitatively the changing behavior of MUs with increasing muscle forces.

A mechanical vibration is the motion of a body which oscillates about a position of equilibrium (Beer and Johnson, 1987). This motion, or oscillation, is usually caused by a forcing function, and depends on the material properties of the system. When this oscillatory motion reaches the surface of the system, areas of high and low pressure occur because of displacements of molecules of the medium (e.g. air, water) in which the system

is immersed (Bolton et al. 1989). This oscillatory behavior of the molecules in the medium surrounding the system causes sound waves (Serway and Faughn, 1985).

The material properties of a muscle are determined by the mechanical properties of its constituent tissues. According to the cross-bridge model (Huxley, 1957; Huxley and Simmons, 1971), the stiffness of a muscle is determined primarily by the active contractile components of a muscle. In addition, passive stiffness, which is primarily produced by the stretch of connective tissues, and therefore, is muscle length dependent, influences the total muscle stiffness (Wilkie, 1968). Muscle sounds have been related to the stiffness of the active contractile (Barry, 1987; Barry and Cole, 1988, 1990; Dobrunz et al. 1990; Frangioni et al. 1987) and the parallel, passive elastic components of muscle (Bolton et al. 1989) during electrically elicited twitches. If so, the amplitude and frequency content of the VMG signal might reflect active and passive properties of muscle (Barry, 1991; Barry and Gooch, 1986; Barry et al. 1985; Herzog et al. 1994; Orizio, 1993; Zhang et al. 1992). Assuming that increases in the stimulation frequency of a muscle produce an increase in force and active stiffness, it may be possible to monitor changes in muscle stiffness as an increase in the frequency content of the VMG signal.

The active and passive contractile properties of muscle are affected by changes in muscle length (Gordon et al. 1966). Evidence for a length effect on muscle sounds has been shown using in-vitro muscle preparations (Barry, 1987; Frangioni et al. 1987). However, no systematic research has been performed aimed at identifying the precise effects of changes in muscle lengths on muscle vibrations using in-situ preparations. Knowledge of the effects of length changes on muscle sounds or muscle vibrations may allow for the evaluation of muscle performance/properties (e.g. the magnitude of the force transients, muscle stiffness, MU recruitment) in an easy, noninvasive way. The purpose of this study was to evaluate the influence of changes in the active and passive properties of a contracting muscle on the VMG signal which occur when the length of a muscle is altered. The in-situ cat SOL muscle was used as the experimental preparation. Stimulation of the SOL could be controlled accurately, forces could be measured easily, and length changes could be performed throughout the range of motion of the ankle joint.

Material and Methods

Preparation and Measurements

All procedures were approved by the Committee for Animal Ethics of The University of Calgary. VMG and force measurements were obtained from in-situ cat SOL muscle using electrical stimulation of the SOL nerve. Three adult male cats were given acepromazine maleate (0.5 mg/Kg, I.M.) and atropine sulfate (0.02 mg/Kg, S.C.) 30 minutes prior to anesthesia. Anesthesia was via halothane in a 1:1 mixture of medical oxygen and nitrous oxide. Induction level of halothane was 5%, reduced to the minimum required to maintain surgical depth (approximately 1-1.5%). The body of the animal was suspended in a sling from a metal frame and the hips and target hindlimb were fixed in a stereotaxic frame. The frame was built in-house and was a design incorporating the general principles found in the models 1450 and 1780 stereotaxic instruments made by Kopf Instruments (Tujunga, CA). The midpoint of the femur, the head of the tibia and the calcaneous were all fixed with respect to the external frame by diametrically opposed, sharpened metal rods. The SOL muscle was exposed in its entirety and a warm saline solution (38-39°C) was applied to the SOL muscle and nerve throughout the experiment to prevent tissues from drying. The sling that the animal was suspended in contained a circulating warm water heating pad (39°C) to help maintain body temperature. Muscle temperature was monitored by a digitally displayed thermistor and a radiant heat applied to help maintain a constant temperature at 37°C. Force measurements were obtained from E-shaped (stainless steel) tendon force transducers (Walmsley et al. 1978) which were calibrated using static weights in a terminal experiment (Herzog et al. 1993a). These force transducers had a high linearity (r^2 = 0.99-1.00) and a sensitivity of approximately 0.01 N/mV. Compliance of the transducer was assumed to be negligible based on the dynamic calibration results presented elsewhere (Walmsley et al. 1978; e.g. Figure 4.1).

VMG signals were measured from the mid-belly of the SOL muscle using a miniature, unidirectional accelerometer (Dytran 3115A). The accelerometer had a sensitivity of 1 mV/(m/s^2) and a frequency response of 0 to 20 kHz, and was fixed to the epimysium of the SOL with cyanocylate (Crazy Glue) in such a way that its sensing axis was perpendicular to the surface of the SOL (Zhang et al. 1992). All signals were digitized at a frequency of 2500 Hz per channel using an analogue-to-digital-board and stored on a

386 personal computer for further signal analysis.

Electrical Stimulation

Electrical stimulations were performed each using a Grass stimulator (S88, Quincy, Mass., USA), and a bipolar nerve cuff electrode that was placed around the SOL nerve (Herzog et al. 1992). The nerve was stimulated for six seconds with a voltage of 2-3 times the motor threshold (i.e. supramaximally, 1.8-2.0 Volts), and with a stimulus pulse duration of 0.1 ms. Average stimulation rates of 4,8,12,16,20,25,30, and 35 Hz, with a coefficient of variation of the interpulse intervals of 0.15 were used (Zhang et al. 1990). The coefficient of variation of the interpulse intervals was controlled via a pseudorandom pulse generator which was built in-house. The pulse generation was software derived, based on a mathematical algorithm with two constant inputs: the mean frequency of stimulation and the coefficient of variation of the interpulse intervals.

Protocol

Throughout testing, the hip and knee joint angles were kept constant at approximately 90 degrees. All tests were performed at two to four different ankle angles within the normal physiological range (80 to 140 degrees, included angles between the foot and the shank; therefore 80 degrees corresponds to the longest and 140 degrees to the shortest muscle length, Table 1). The range of stimulation frequencies (4-35 Hz) and total contraction time per trial (i.e. 6s for each frequency of stimulation used) were not expected to produce fatigue in the slow twitch fibered cat soleus. The experiments for one ankle angle were always completed before proceeding to the next. One minute intervals were given between contractions to prevent muscle fatigue. Also, 5-10 minutes of rest intervals were given when going from one muscle length to the next. These intervals were chosen based in previous work performed in our laboratory, where similar rest intervals had been used with no apparent sign of fatigue (Guimaraes et al. 1994a, 1994b).

Table 1. Ankle angles studied and measurements obtained for each animal.

Animal	Ankle Angle	Stimul. Rates	Force	VMG
1	80	all	yes	yes
1	120	all	yes	yes
2	100	all	yes	yes
2	120	all	yes	yes
2	140	all	yes	yes
3	80	all	yes	yes
3	100	all	yes	yes
3	120	all	yes	yes
3	140	all	yes	yes

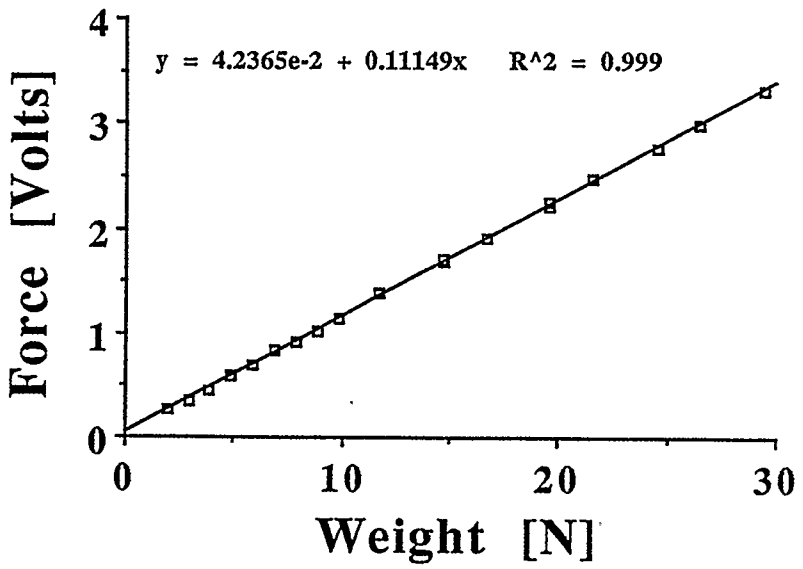

Figure 4.1. Calibration curve for the E-shaped force transducer used for animal 3.

Data Analysis

In order to ensure that transient signals at the beginning and end of the contraction were excluded, VMG and force signals were extracted for segments of five seconds from the middle of the trials. VMG signals were filtered using a second order Butterworth high-pass (5 Hz) filter before any data analysis was performed in order to eliminate any low frequency (1-4 Hz) movement artifact that is usually recorded when using accelerometers. Root mean square (RMS) values were calculated from the VMG signals. Median frequencies (MDFs) of the VMG signal were calculated using a Fast Fourier Transform (FFT) algorithm (2048 points). For the spectral estimation, the five second segments were divided into seven segments of 714 ms each from which MDFs were calculated. Zero padding was used (263 zeros were added), and the resolution obtained was 1.2 Hz. As the stimulation was non-periodic (i.e. the coefficient of variation of the interpulse interval was equal to 0.15) it was not necessary to average individual vibratory responses because the power-spectrum of the chosen non-periodic stimulation is not influenced appreciably by the stimulation rates (Zhang et al. 1994).

Results

All results were consistent across animals, except where specifically pointed out; therefore, the results presented here are subsamples of all results (cats 2 and 3). The VMG signals became zero at fused tetanic frequencies, therefore only results from unfused contractions are shown and discussed.

The average force of the SOL increased with increasing muscle length and with increasing rates of stimulation (Figure 4.2). Fused tetanic contractions were achieved at stimulation frequencies of 20-25 Hz in animal 1, 16-20 Hz in animal 2, and 12-16 Hz in animal 3.

For sub-tetanic contractions (4 and 8 Hz; Figures 4.3a and 4.3b, respectively), the RMS of the VMG signal was small at the shortest and the longest muscle lengths tested, and was large at intermediate muscle lengths. Towards fused tetani (12 Hz, Figure 4.3c), there was an increase in the RMS of the VMG signal with decreasing muscle length. In order to visualize the changes occurring in the RMS of the VMG signal, selected raw data of the VMG signals are presented in Figures 4.4, 4.5 and 4.6 for frequencies of stimulation

of 4, 8 and 12 Hz and ankle angles of 100, 120 and 140 degrees for animal 2, and in Figures 4.7, 4.8 and 4.9 for the same stimulation frequencies and ankle angles of 80, 100, 120 and 140 degrees for animal 3, respectively. As might have been expected from Figure 4.3, the amplitude of the VMG signal was greater at intermediate muscle lengths and sub-tetanic frequencies of stimulation (Figures 4.4, 4.7 and 4.8), while it increased with decreasing muscle length when the contraction approached a fused tetanus (Figures 4.5, 4.6 and 4.9).

In order to assess the degree of fusion of the force signals, the forces corresponding to the VMG signals shown in Figures 4.7 - 4.9 are presented in Figures 4.10 - 4.12, respectively. As expected from Figure 4.2, there was a decrease in the SOL force with decreasing muscle length. For frequencies of stimulation of 4 and 8 Hz, forces in the SOL muscle remained close to zero for the two shortest muscle lengths tested. For the two longest muscle lengths, the 4 Hz stimulation caused individual twitches, and the 8 Hz stimulation caused an unfused contraction in which the force did not return to baseline after each stimulus. At a stimulation frequency of 12 Hz, force at the shortest muscle lengths remained nearly zero, whereas the forces became substantial for the remaining lengths and fluctuated less than for the 4 and 8 Hz stimulation protocols.

VMG signals preceded force signals by a few milliseconds, and ended always before the termination of the relaxation phase of the force (Figure 4.13).

MDFs of the VMG signal tended to increase with increasing muscle lengths (Figures 4.14a,b,c). The spectrum for the non-periodic stimulation was not discrete, did not exhibit peaks at multiples of the mean frequency, and did not increase because of increases in the stimulation frequencies (Zhang et al. 1994; Figure 4.15).

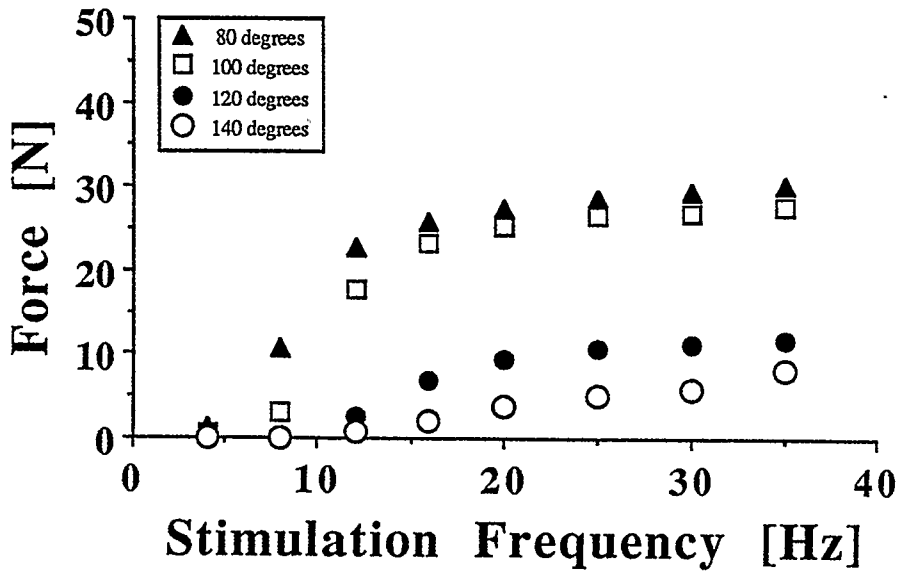

Figure 4.2. Force values for stimulation rates of 4 to 35 Hz at four different ankle angles (80, 100, 120, and 140 degrees) for animal 3.

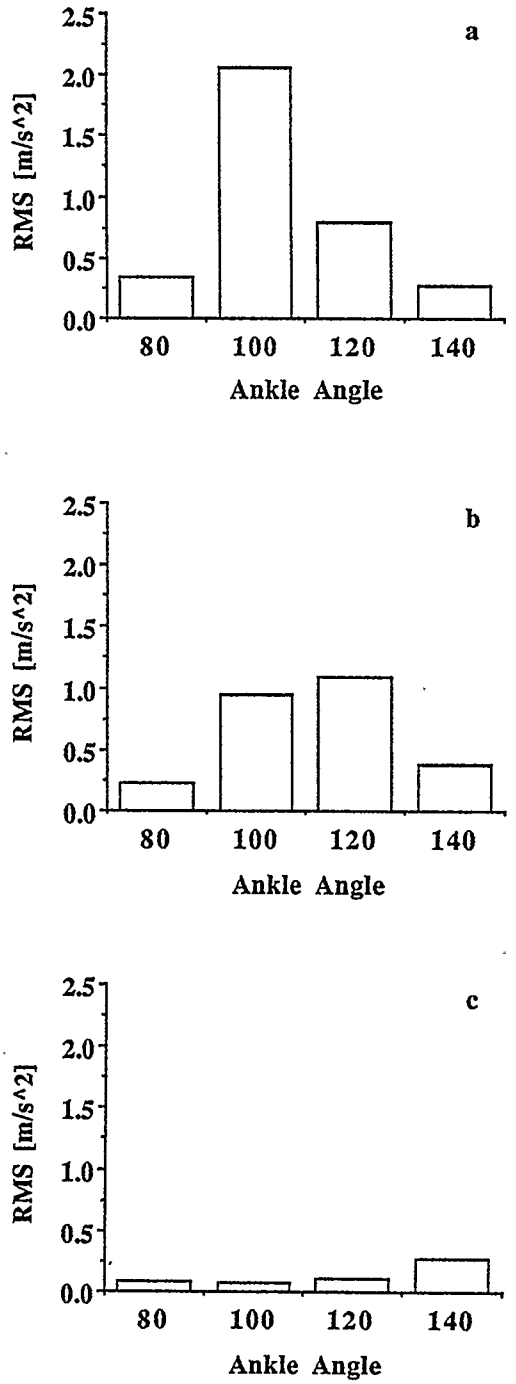

Figure 4.3. RMS of the VMG signal for three stimulation rates at four different ankle angles (animal 3); (a) stimulation frequency = 4 Hz, (b) stimulation frequency = 8 Hz, and (c) stimulation frequency = 12 Hz.

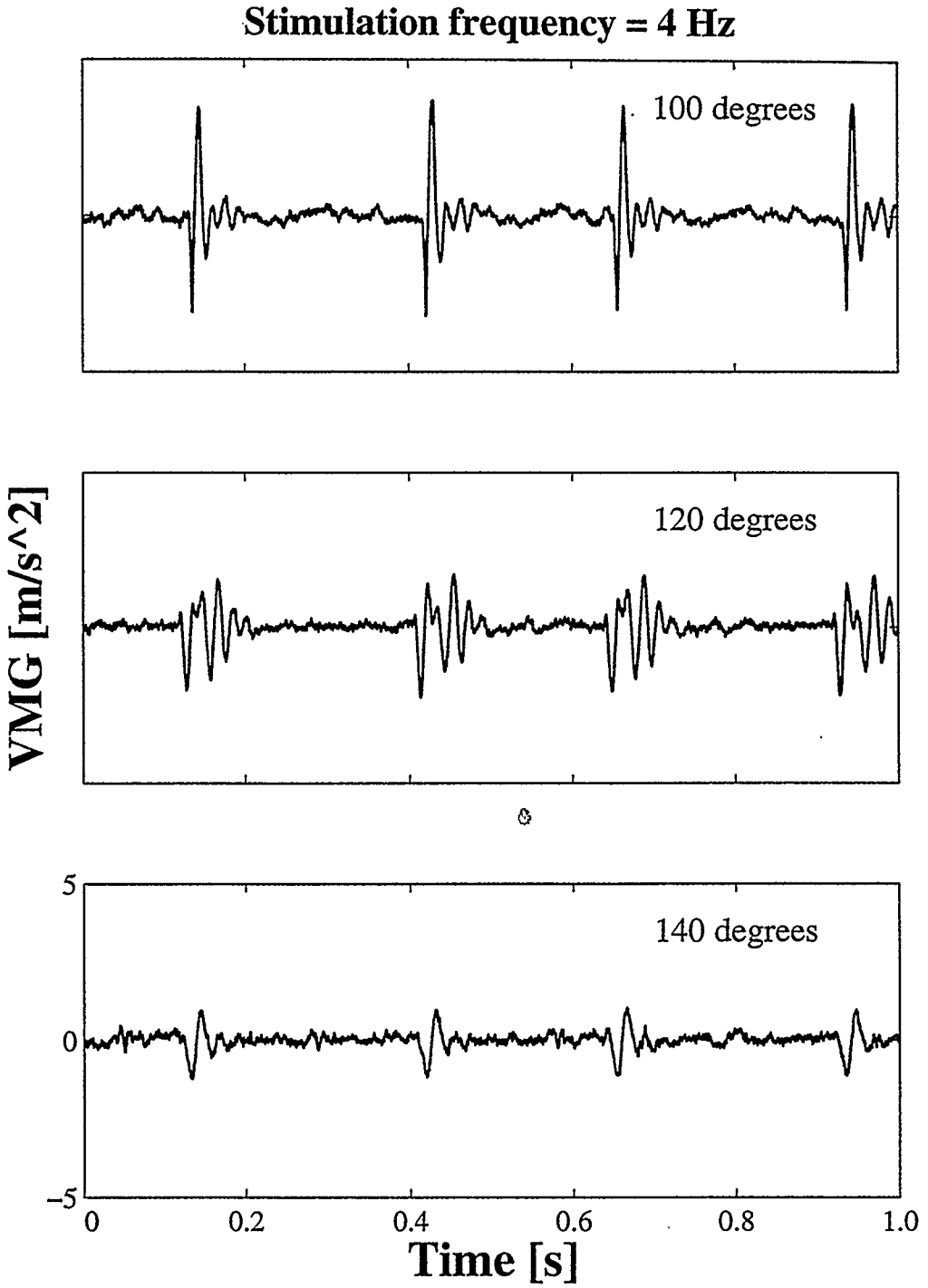

Figure 4.4. Representative data showing VMG recordings at three different muscle lengths and stimulation frequency of 4 Hz (animal 2).

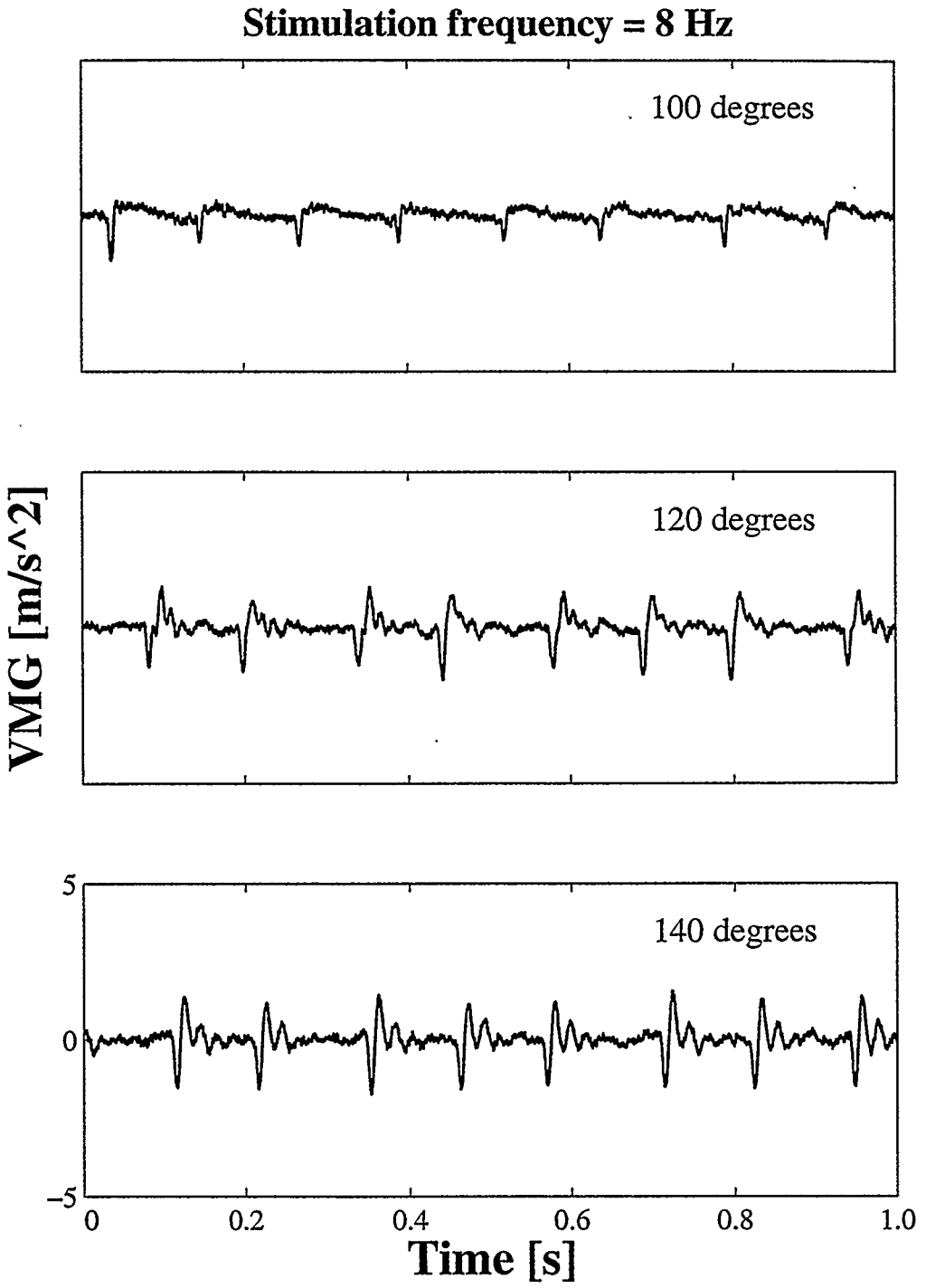

Figure 4.5. Representative data showing VMG recordings at three different muscle lengths and stimulation frequency of 8 Hz (animal 2).

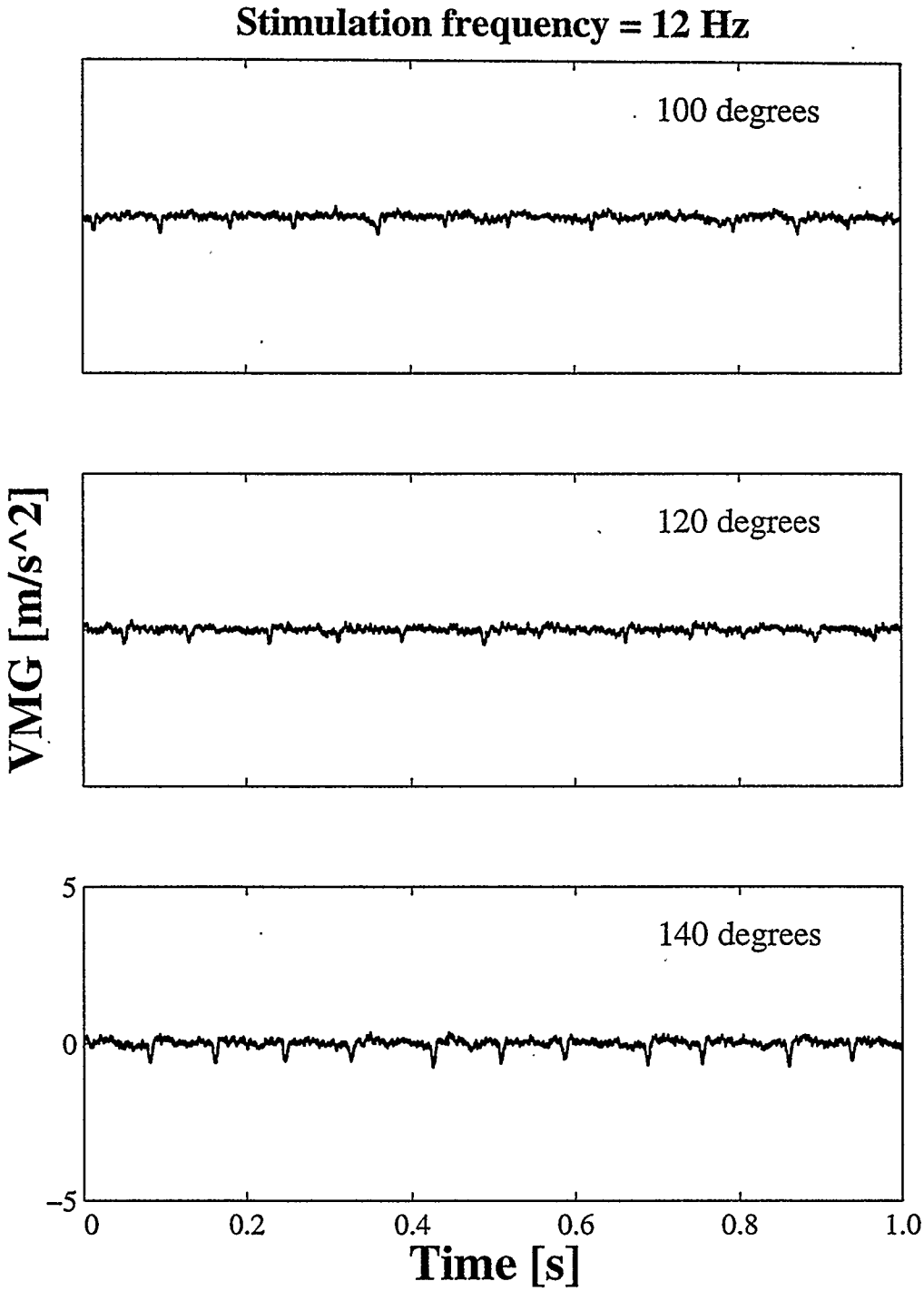

Figure 4.6. Representative data showing VMG recordings at three different muscle lengths and stimulation frequency of 12 Hz (animal 2).

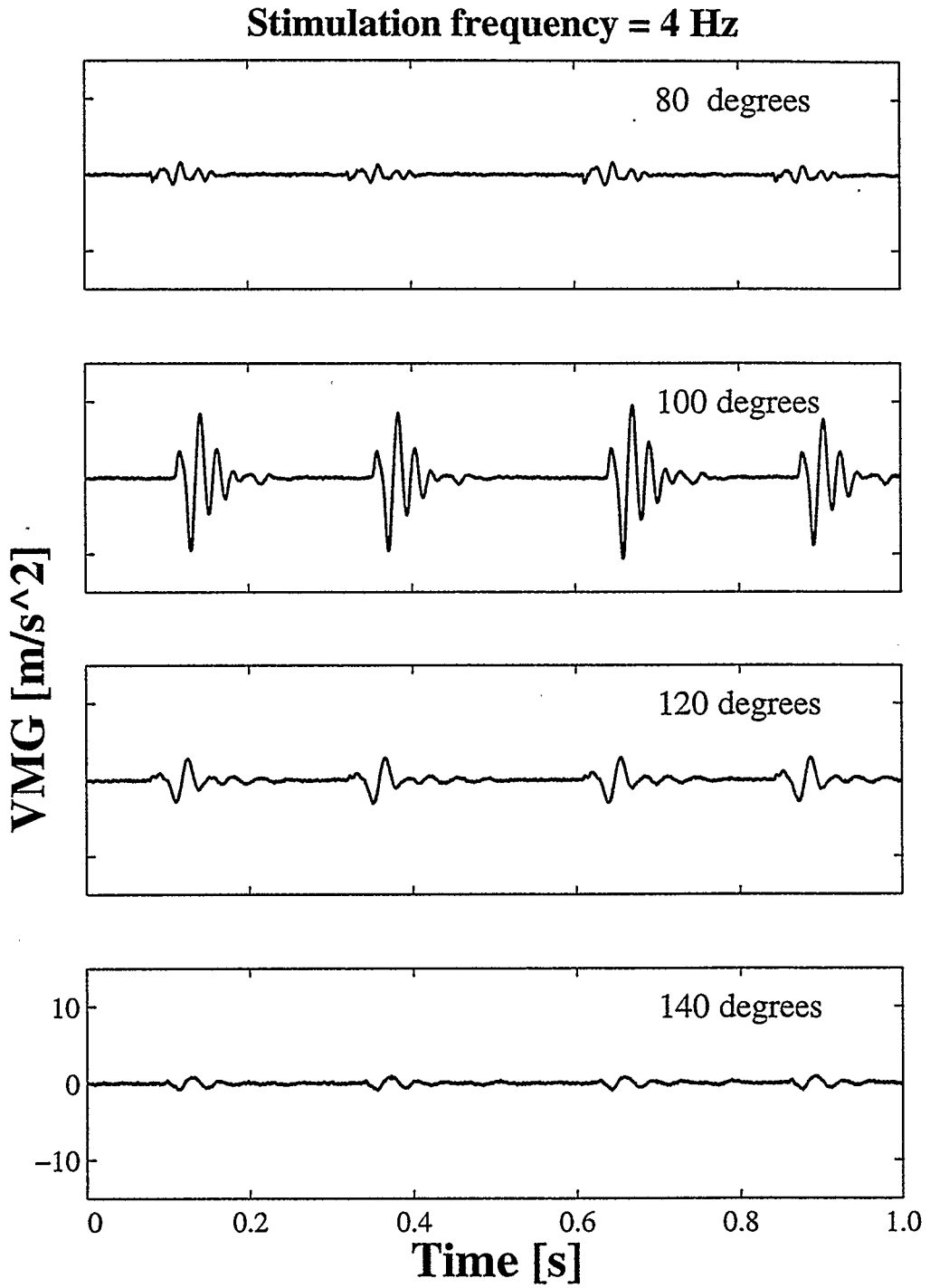

Figure 4.7. Representative data showing VMG recordings at four different muscle lengths and stimulation frequency of 4 Hz (animal 3). Observe the large VMG signal amplitudes at intermediate muscle lengths during unfused tetanic contractions.

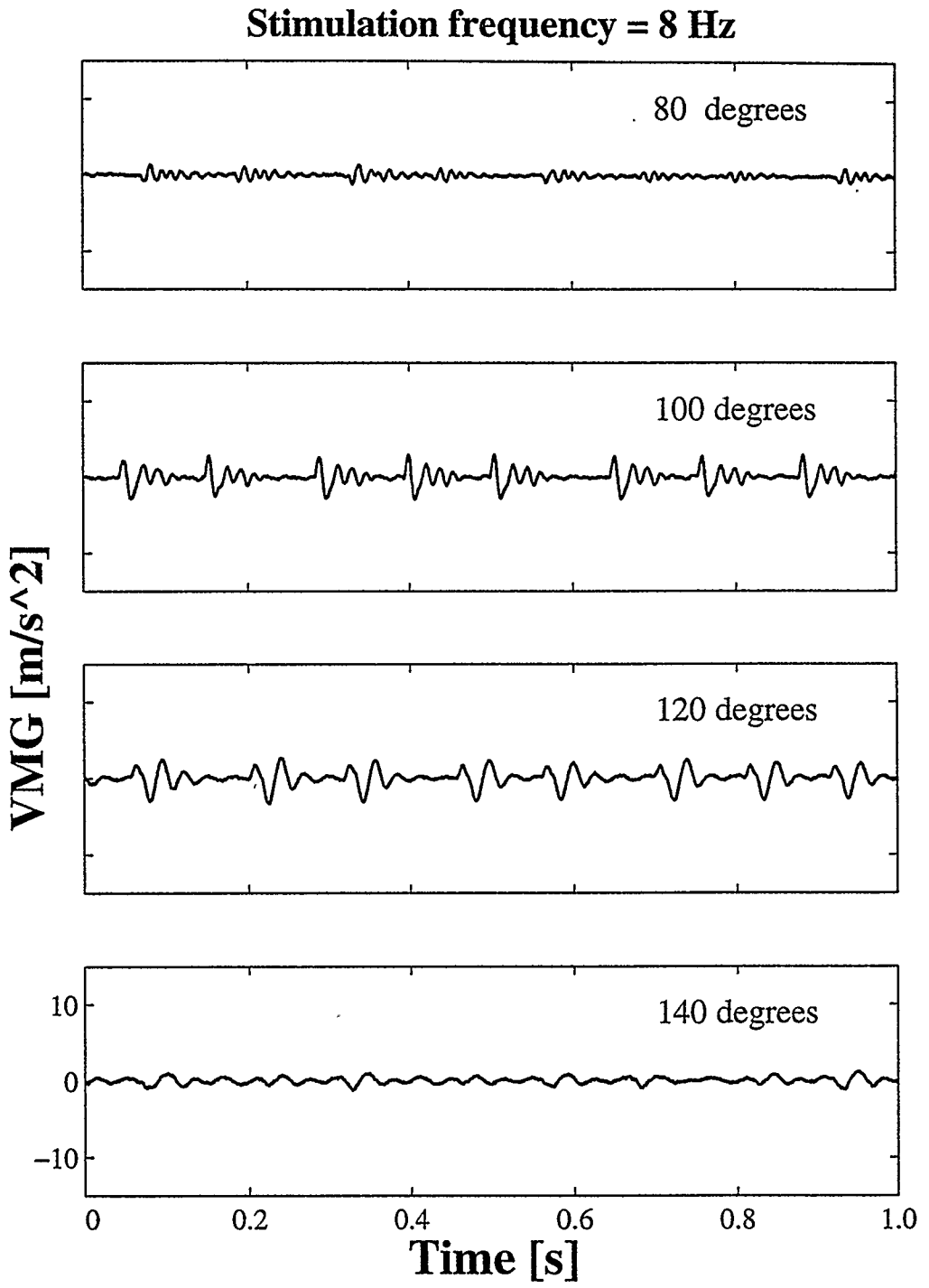

Figure 4.8. Representative data showing VMG recordings at four different muscle lengths and stimulation frequency of 8 Hz (animal 3). Large VMG signal amplitudes can be observed at intermediate muscle lengths during unfused tetanic contractions (as in Figure 4.7).

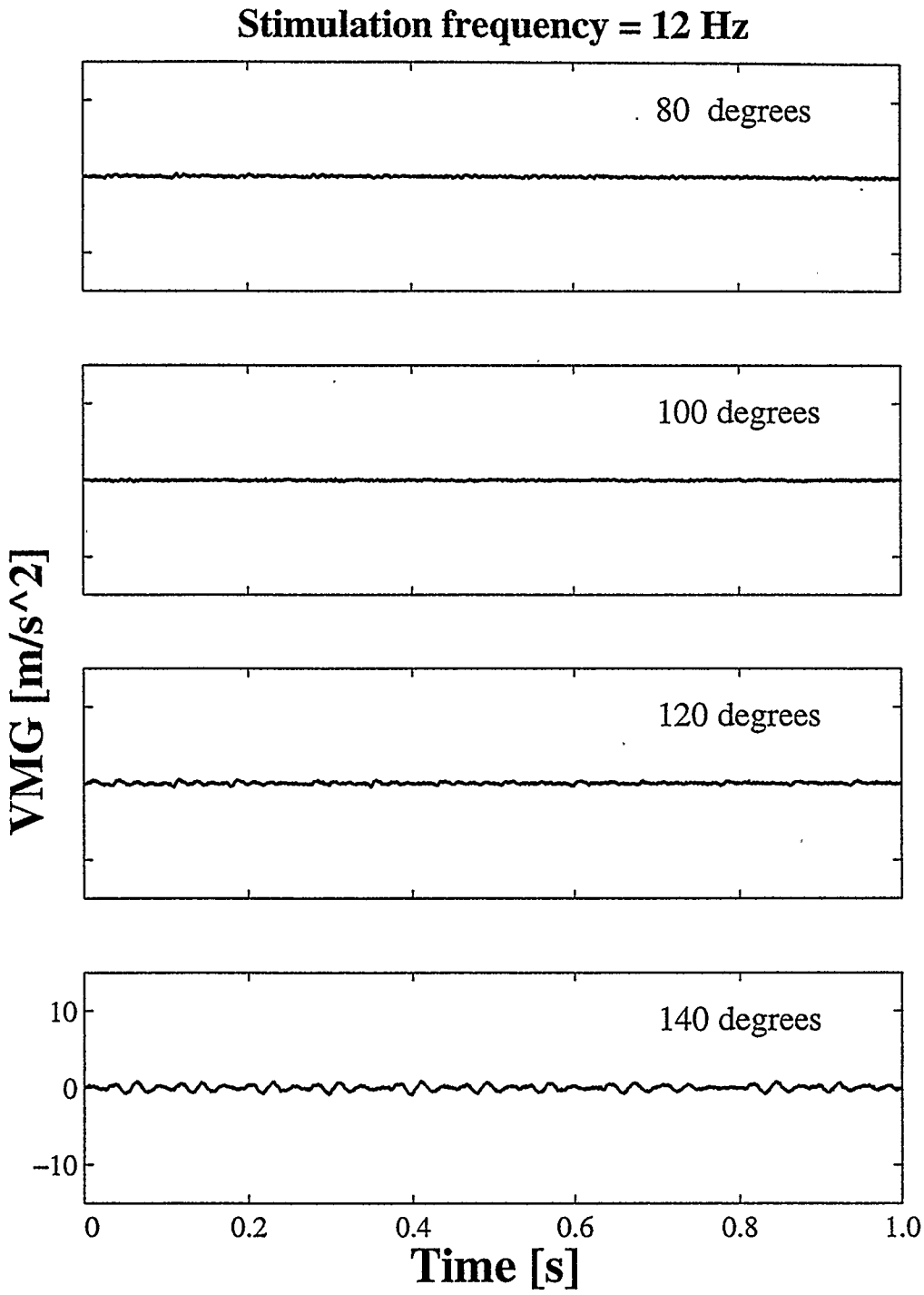

Figure 4.9. Representative data showing VMG recordings at four different muscle lengths and stimulation frequency of 12 Hz (animal 3). Observe the increase in VMG signals with decreasing muscle length when the contraction was almost fused.

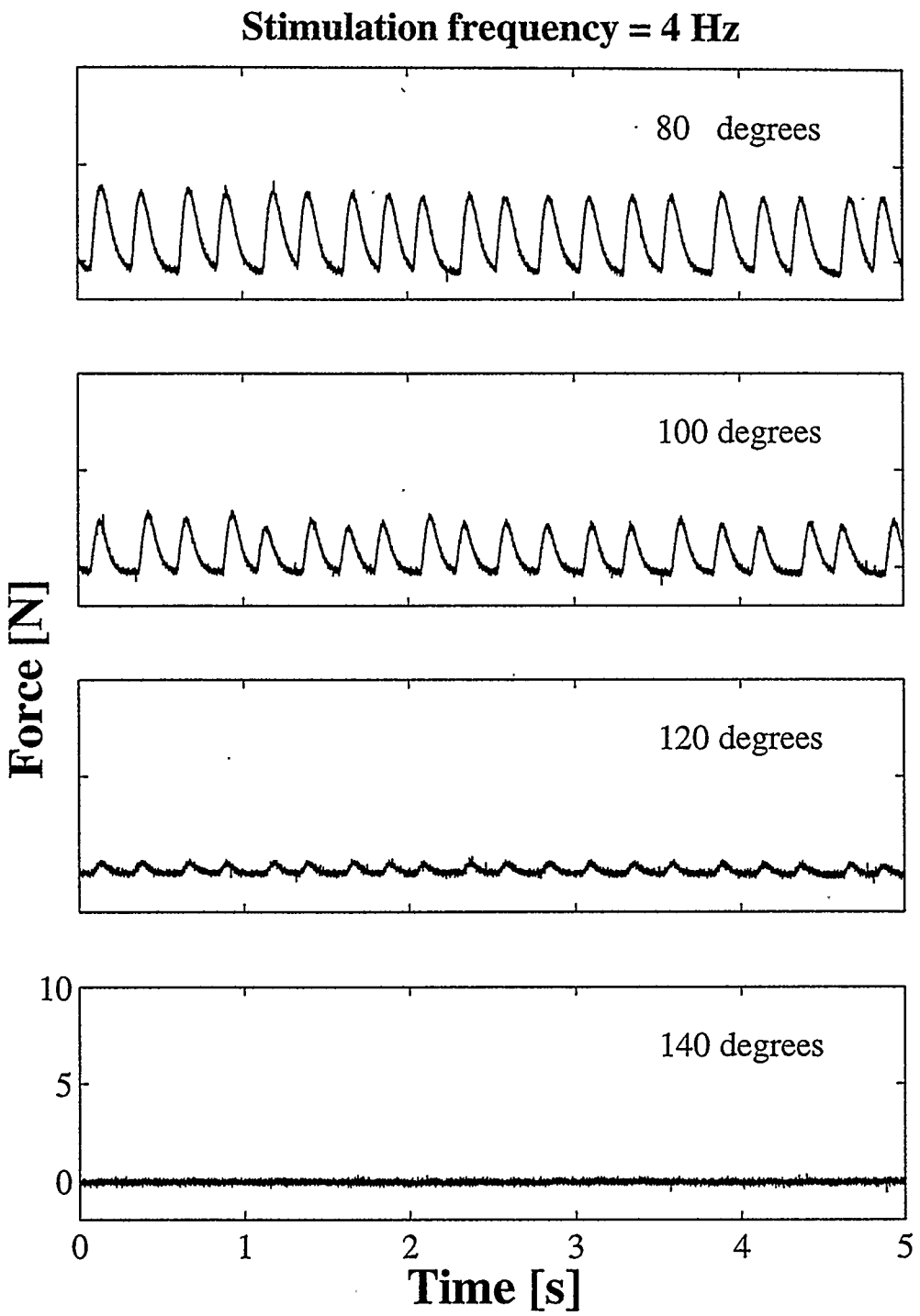

Figure 4.10. Representative data showing force recordings at four different muscle lengths and stimulation frequencies of 4 Hz (animal 3). Observe the increase in the force fluctuations and in the total force produced during the unfused tetanus with increasing muscle length.

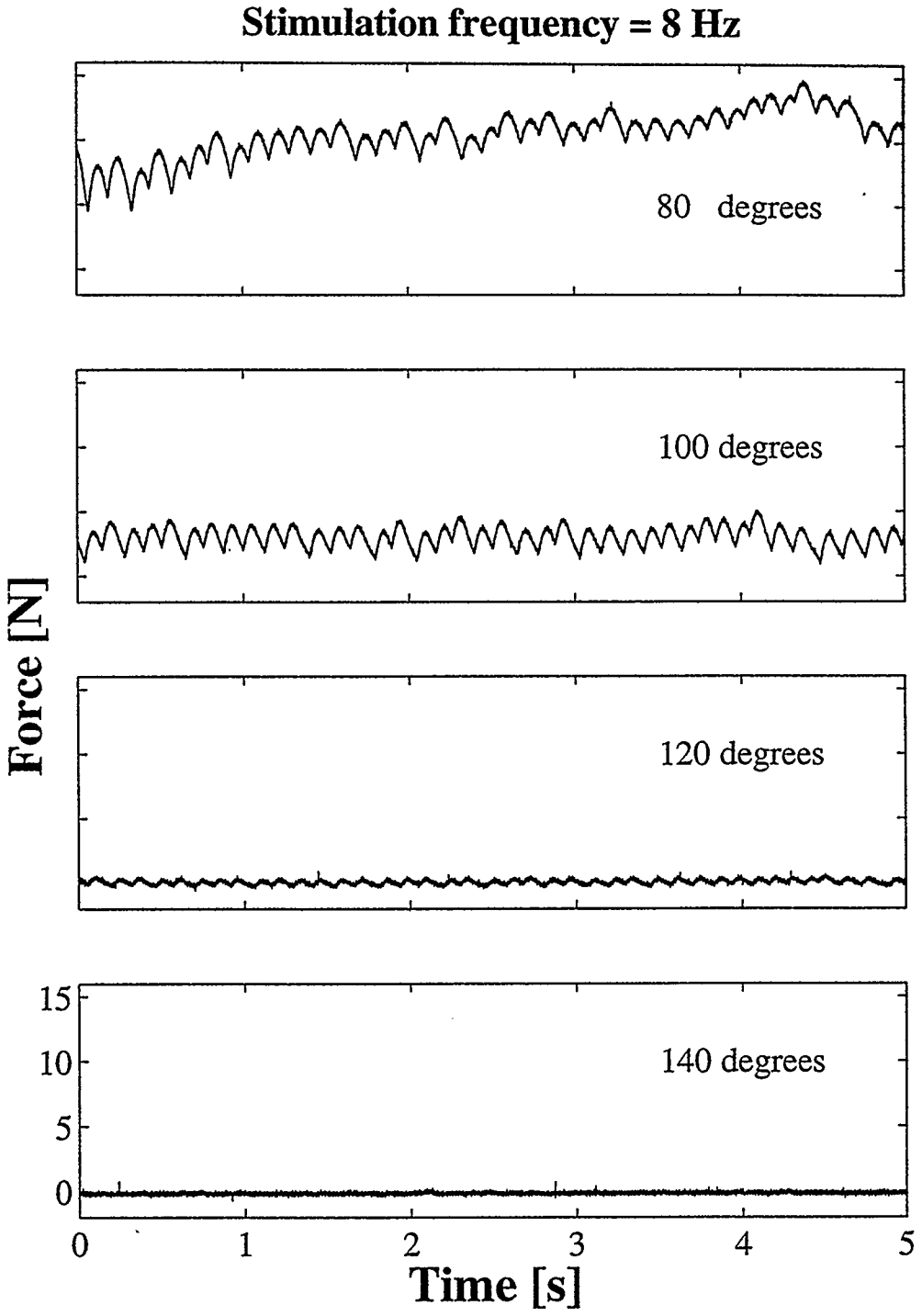

Figure 4.11. Representative data showing force recordings at four different muscle lengths and stimulation frequency of 8 Hz (animal 3). Observe the increase in the force fluctuations and in the total force produced during the unfused tetanus with increasing muscle length.

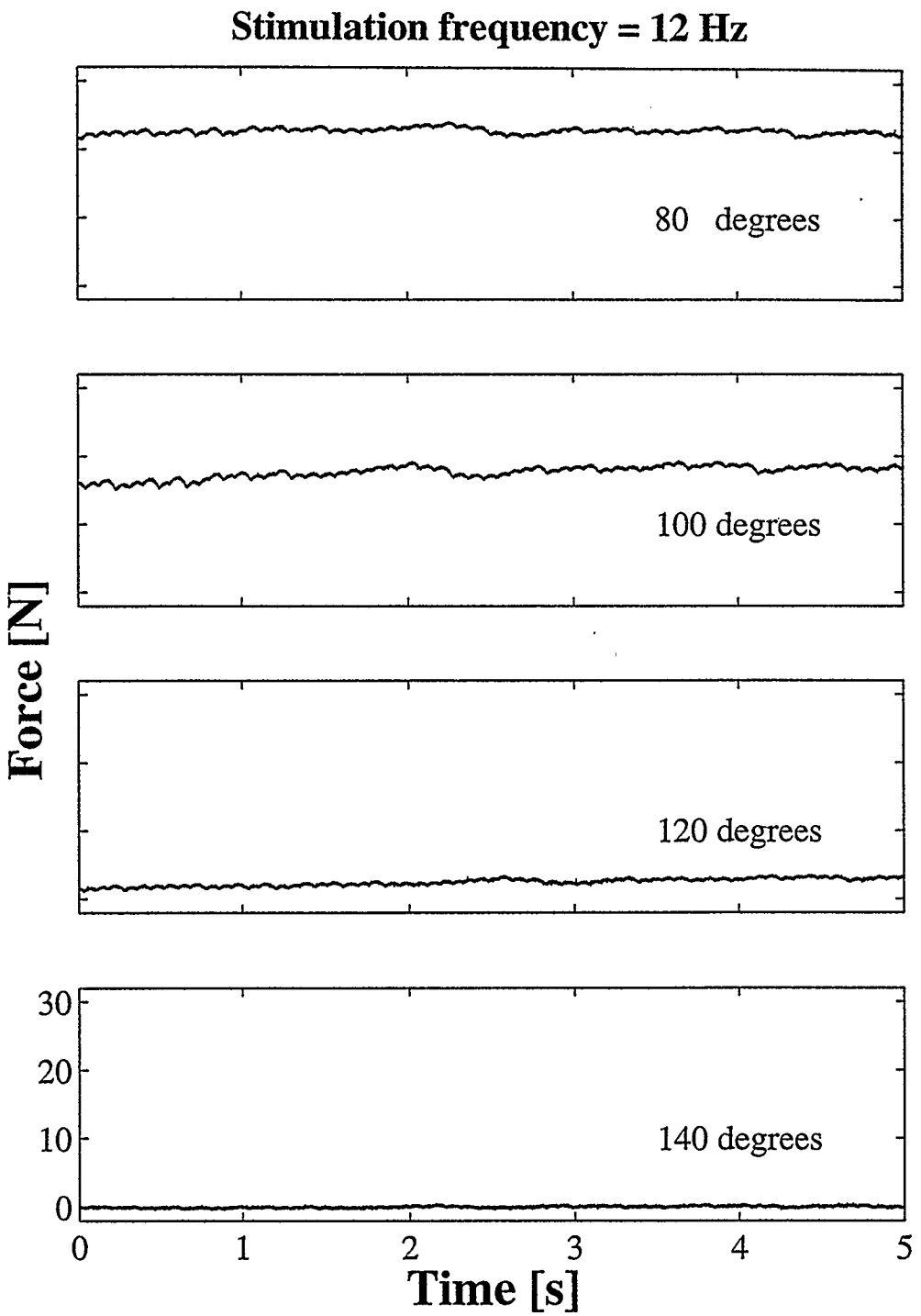

Figure 4.12. Representative data showing force recordings at four different muscle lengths and stimulation frequency of 12 Hz (animal 3). Observe the little change in the magnitude of the force fluctuations as the contraction is almost completely fused.

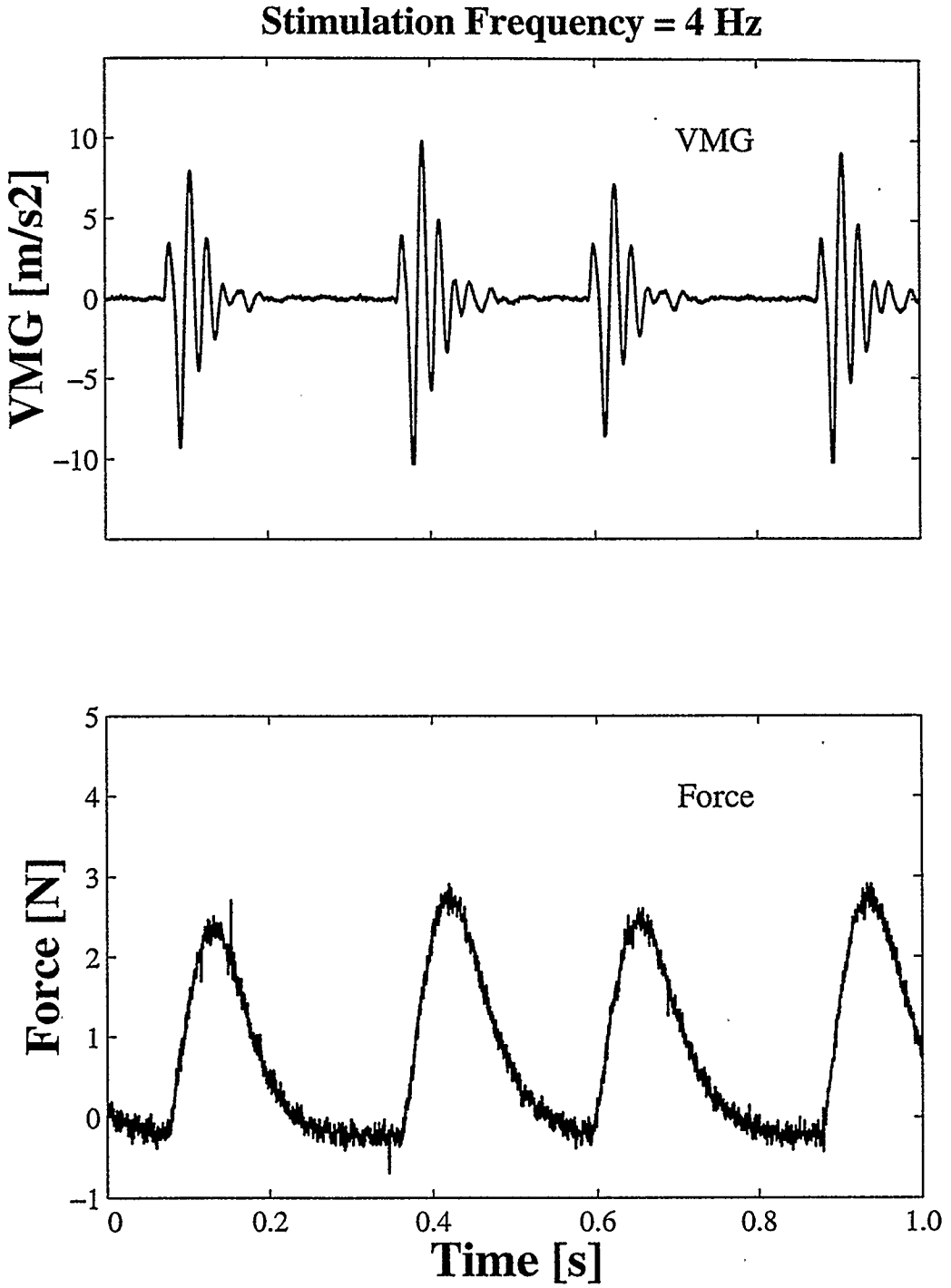

Figure 4.13. Simultaneous VMG and force recordings at one specific muscle length (i.e. at an ankle angle of 100 degrees) and a stimulation frequency of 4 Hz (animal 3).

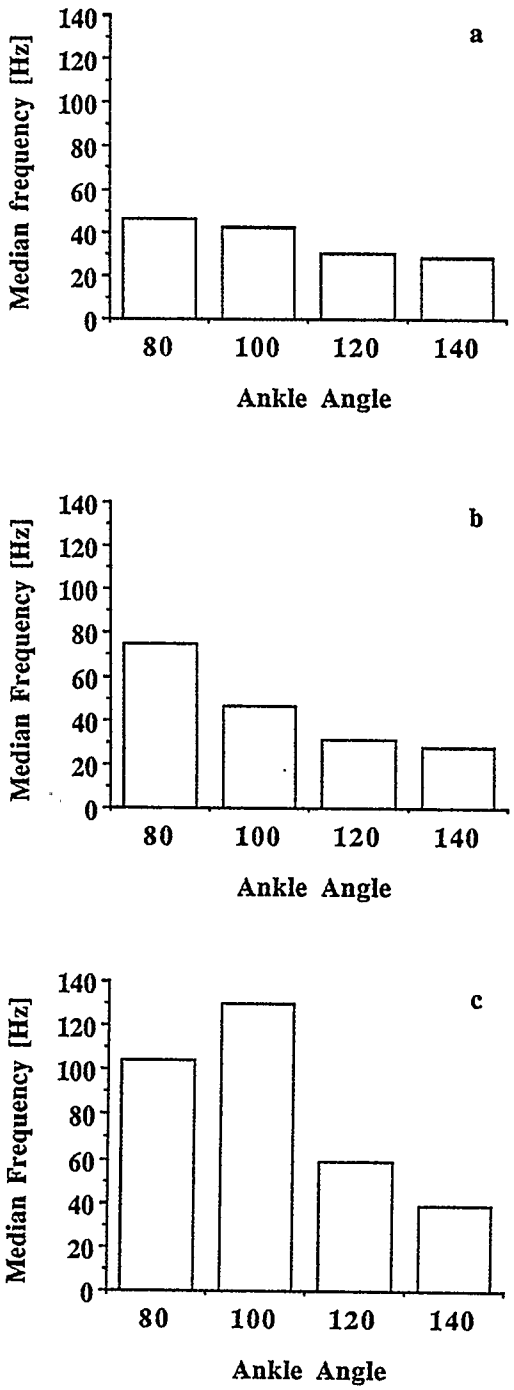

Figure 4.14. MDF of the VMG signal for three stimulation rates at four different ankle angles (animal 3); (a) stimulation frequency = 4 Hz, (b) stimulation frequency = 8 Hz, and (c) stimulation frequency = 12 Hz. Observe the increase in the MDF with increasing muscle length.

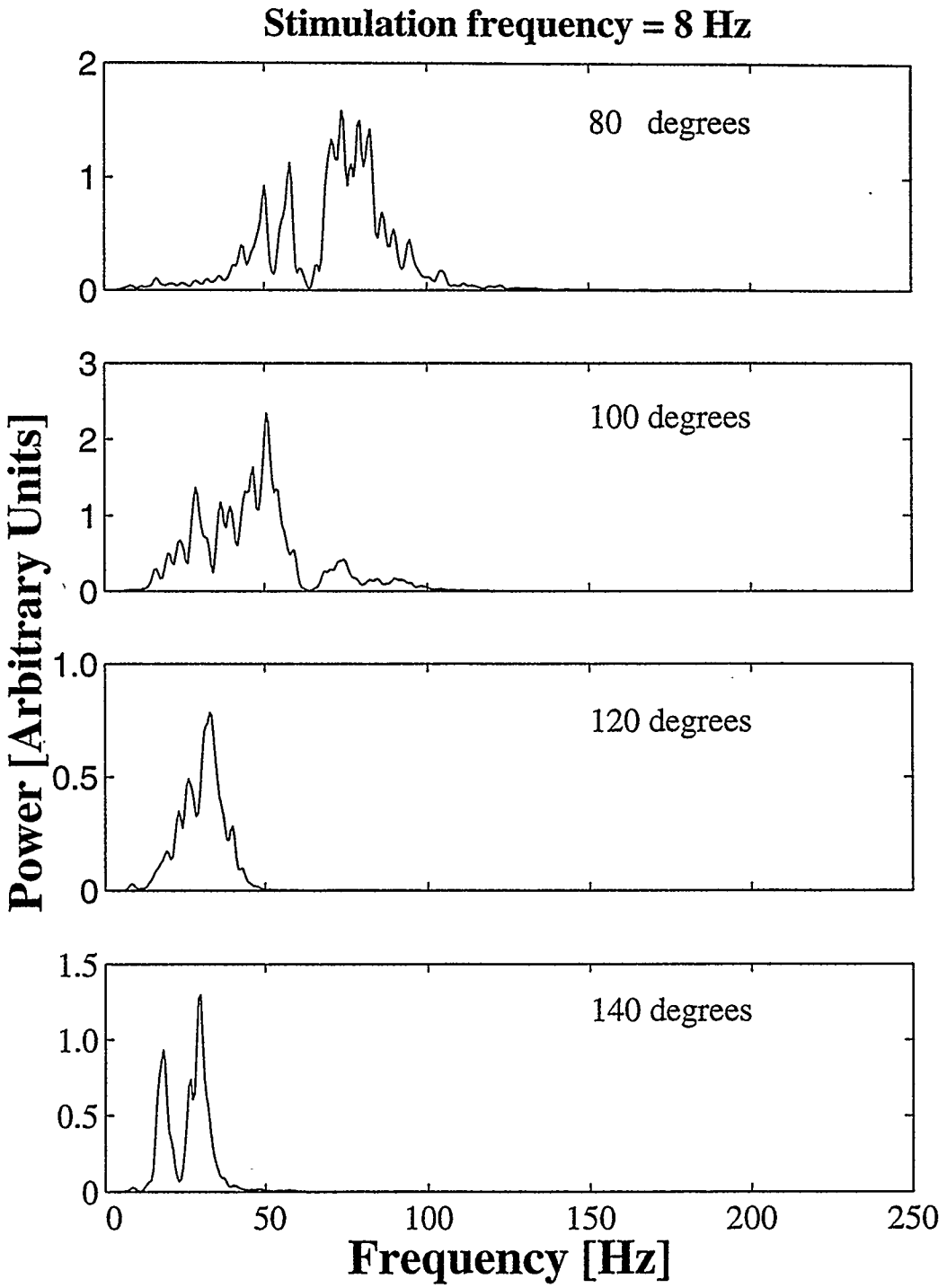

Figure 4.15. Power-spectra from the data shown in Figure 4.14b. As expected from Figure 4.14b, there was a shift towards lower frequencies with decreasing muscle length. The scales for the power in each figure are different (x10^2 for 80 degrees, x10^4 for 100 and 120 degrees, and x10^3 for 140 degrees).

Discussion

The purpose of this study was to test the effects of changes in passive and active properties, which occur due to muscle length changes, on electrically elicited muscle vibrations. It was hypothesized that muscle vibrations are produced by the unfused contraction of MUs (Orizio, 1993; Orizio et al. 1993; Vaz et al. 1996a), and reflect some intrinsic mechanical activity of muscle (Barry, 1991; Barry and Gooch, 1986; Herzog et al. 1994; Orizio, 1993; Zhang et al. 1992). Also, it was assumed that variations in muscle length influence the stiffness of the contractile elements in accordance with the cross-bridge model (Huxley, 1957; Huxley and Simmons, 1971), and change the stiffness associated with the parallel elastic elements. Therefore, length changes were anticipated to influence the VMG signal. Increases in length were associated with increased force because the cat SOL works on the ascending limb of the force-length relation (Herzog et al. 1992; Rack and Westbury, 1969). Increasing force was hypothesized to cause an increase in the RMS values of the VMG. However, this increase in VMG amplitudes may be offset, partly or completely, by the increased active and passive stiffness of the SOL with increasing length. Therefore, it was hard to predict a priori how the RMS values of the VMG would change with increasing SOL length. The MDFs of the VMG power-spectrum were expected to increase with increasing SOL length because of the corresponding increase in muscle stiffness.

In accordance with these expectations, the RMS values and MDFs of the VMG signals changed substantially across lengths, even when the stimulation delivered to the SOL nerve was kept constant (Figures 4.3a,b,c and 4.14a,b,c). During unfused contractions, each stimulus produced a distinct vibratory signal at each muscle length. Similar results have been shown for in-vitro muscle preparations (Barry, 1987; Cole and Barry, 1994; Frangioni et al. 1987). It was assumed that these distinct signals reflect a vibration of the muscle produced by the transient force responses of the muscle to each stimulus (Vaz et al. 1996a). At stimulation frequencies of 4 and 8 Hz, the VMG signals had the smallest amplitude at the shortest (140 degrees) and longest (80 degrees) lengths, and the highest amplitude at the intermediate lengths (at 100 degrees for 4 Hz in Figures 4.4 and 4.7, and at 120 degrees for 8 Hz in Figure 4.8, respectively). These results agree with findings reported in the literature which suggest that the acoustic signal amplitude increases with increasing muscle length up to 90-95% of the optimal length and then decreases for in-vitro muscle preparations (Barry, 1987; Frangioni et al. 1987).

Two factors must be considered when attempting to explain the results of this study: the magnitude of the force transients, and the active and passive stiffness of the muscle. Intuitively, it appears appealing to associate large amplitude VMG signals with large amplitude force transients and small muscle stiffness, and to associate small VMG amplitudes with small amplitude force transients and high muscle stiffness. Going from short (140 degrees) to long (80 degrees) muscle lengths, the force potential of the SOL increases (Figure 4.2), and so does the amplitude of the force transients at frequencies of stimulation of 4 and 8 Hz (Figures 4.10 and 4.11). Based on this observation, one would expect the VMG signals to increase in amplitude with increasing muscle length. However, increasing muscle length is associated with an increasing active and passive muscle stiffness because of the increased contractile and passive forces (Herzog et al. 1992; Rack and Westbury, 1969). Based on the stiffness properties, one would expect the VMG amplitudes to decrease with increasing muscle lengths; the combination of increased force and increased stiffness with increasing SOL length, therefore, appears to cause the observed behavior: peak VMG amplitudes at intermediate muscle lengths. The single exception to this behavior was observed at a stimulation frequency of 8 Hz (Figure 4.5) where peak amplitudes were observed at the shortest muscle length (similarly to what occurred at a stimulation frequency of 12 Hz, see below).

At a frequency of stimulation of 12 Hz (Figure 4.3c), the RMS values of the VMG signals are smaller than the corresponding values at 4 and 8 Hz (Figures 4.3a and 4.3b). This result may be caused by two factors: first, the force transients at 12 Hz (Figure 4.12) are smaller than those at 4 and 8 Hz (Figures 4.10 and 4.11, respectively) because the muscle is almost contracting in a fused fashion; second, the active forces (and therefore, the active stiffness) are much higher at 12 Hz compared to 4 and 8 Hz. At 12 Hz, the stiffness properties of the muscle appear to dominate the vibratory signal; stiffness is smallest at the shortest (140 degrees) muscle length, the corresponding VMG signal is largest. This result also seems to explain the results presented in Figure 4.5 (8 Hz), where the amplitude of the signal increases with decreasing muscle length.

The MDFs of the VMG signals tended to increase with increasing frequencies of stimulation (Figures 4.14a,b,c). This behavior is likely associated with the increased forces and stiffness at increased stimulation frequencies, and not with factors related to the stimulation frequency itself as reported in studies in which the stimulation was periodic (Stokes and Cooper, 1992). For a given frequency of stimulation, the MDFs tended to decrease with decreasing muscle length, and thus decreasing force and stiffness. Again,

this behavior appears intuitively correct, except for the single case where the MDF did not decrease with decreasing muscle length (12 Hz, from 80 to 100 degrees, Figure 4.14c).

On many occasions it has been suggested that muscle sounds/vibrations are linearly related with force production during voluntary contractions (Barry et al. 1985, 1992; Oster and Jaffe, 1980; Stokes and Dalton, 1991b; Zwarts and Keidel, 1991). This statement cannot be supported by the findings presented here for electrically elicited vibratory signals. If anything, increases in the stimulation frequency (and thus the force) at a given muscle length were associated with decreasing VMG signal amplitudes. Similarly, peak VMG amplitudes at a given frequency of stimulation were not associated with the muscle length at which peak forces were produced (i.e. the longest muscle length, 80 degrees). It may be, however, that the suggested linear relationship between muscle sounds and force exists during voluntary contractions when only the initial transient VMG signal that coincides with the increase in force is considered (as suggested for in-vitro studies; Cole and Barry, 1994), or when other physiological processes such as fatigue and/or muscle tremor are involved in the observed signals (Barry et al. 1985, 1992; Oster and Jaffe, 1980; Zwarts and Keidel, 1991).

The results of this study support the idea that muscle vibrations are dependent on the way a muscle is activated, and on its mechanical properties as determined by muscle length. Information about MU activation, muscle stiffness, and force transients can be retrieved from the VMG signal, allowing this technique to be used for muscle performance/properties evaluation. Changes in muscle length directly affect the active and passive properties of muscle contraction, and therefore change the characteristics of the VMG signal. Muscle active contractile and passive stiffness seem to influence the amplitude and the frequency content of the vibratory signals; thus, muscle length is an important variable that should be considered when describing muscle vibrations.

Chapter 5

Effect of Fatigue on Muscle Vibrations

(Vaz et al. The behavior of human rectus femoris and vastus lateralis during fatigue and recovery: an electromyographic and vibromyographic study. *Electromyogr. Clin. Neurophysiol.*, 36:221-230, 1996.

Vibromyography has been used recently as an additional non-invasive tool to EMG that might be useful in detecting muscle fatigue. In a previous study (Herzog et al. 1994), we have shown that the VMG signal could be used to observe on line when muscle fatigue occurred. In this study, the effects of fatigue and recovery on the VMG signal are assessed during a fatigue/recovery protocol.

Introduction

Surface electromyography (EMG) is a well established technique which has been used widely as a non-invasive tool to detect muscle fatigue (Lindstrom et al. 1977; Luciani et al. 1983; Arendt-Nielsen and Mills, 1988; Dolmage and Cafarelli, 1991; Grabiner et al. 1991). Several EMG parameters have been used to evaluate fatigue; the most frequently used parameters are the median frequency (MDF) of the power spectrum and the root mean square (RMS) of the raw signal (Basmajian and De Luca, 1985).

During fatiguing contractions, the frequency content of the EMG shows a clear shift towards lower frequencies (Cobb and Forbes, 1923). This can be observed as a decrease in the MDF (Nagata et al. 1990). Although attempts have been made to relate the decrease in the MDF to changes in frequency of activation, theoretical and experimental evidence

indicates a strong correlation between the median frequency of the power spectrum and muscle fiber conduction velocity (Lindstrom et al. 1970; Lindstrom et al. 1977; Stulen and De Luca, 1981; De Luca, 1984; Merletti et al. 1984; Arendt-Nielsen and Mills, 1985 and 1988; Merletti et al. 1990). During repeated muscle contractions leading to fatigue, the speed of propagation of the action potential decreases, and this decrease is paralleled by a decrease in MDF.

The RMS of the EMG signal also changes with fatigue (Stulen and De Luca, 1978). Two mechanisms have been associated with the changes in the RMS: (1) increased recruitment and firing rates of motor units (Edwards and Lippold,1956; Viitasalo and Komi, 1977), and (2) the leftward shift in the power frequency spectrum which contributes to an increase in the RMS of the EMG signal, because of the low-pass filter effect of the skin and the subcutaneous tissues (De Luca, 1984).

Vibromyography (VMG) is a new technique that records the vibrations of the muscle that occur during contraction. The precise origin of these vibrations remains to be explained. Nevertheless, there are preliminary indications that VMG might be a useful tool to detect muscular fatigue (Barry et al. 1992; Zhang et al. 1993; Orizio, 1993). In particular, it has been suggested that the frequency domain of the VMG signal is related to the frequency of activation of motor units (Orizio, 1993), and to the levels of voluntary effort during muscle contraction (Zhang et al. 1992). If so, the VMG, like the EMG, may be a useful tool to evaluate fatigue. However, VMG and EMG recordings have been used rarely in combination during fatigue and recovery experiments (Zwarts and Keidel, 1991).

The purpose of this study was to describe and compare the behavior of EMG and VMG signals of rectus femoris (RF) and vastus lateralis (VL) muscles during a fatigue-recovery protocol by analyzing the MDF and the RMS values obtained from these signals. We hypothesized that during fatiguing isometric contractions, a decrease in the magnitude of the VMG signal should be observed because of (a) a decrease in the twitch amplitude due to fatigue, which should decrease the magnitude of the force transients contributing to the VMG signal, (b) the increased relaxation time of MU with fatigue, which produces tetanic contractions at decreasing activation frequencies, and (c) a decrease in muscle activation due to fatigue, which may be responsible for a decrease in MU firing rates (Bigland-Ritchie et al. 1983, 1986). Also, increasing fatigue will result in a loss of force production in specific MUs; therefore, the number of MUs contributing to the vibratory signal decreases.

Material and Methods

Eleven healthy male subjects without history of neuromuscular disease or knee joint injury (age = 22-35 years; mass = 65-90 kg; height = 1.69-1.83 m) attended one testing session, and four of the eleven subjects repeated all tests one week after the first session. Subjects gave written informed consent to participate in this study.

Bipolar surface electrodes (3 cm apart) were placed on the distal third of RF and VL muscles. VL electrodes were positioned in the approximate direction of the muscle fibers; RF electrodes were positioned along the assumed longitudinal direction of the muscle. Ground electrodes were placed on the tibia. The skin underneath the recording electrodes was prepared for EMG recording using standard procedures (e.g. Basmajian and De Luca, 1985), and the interelectrode impedance was kept below 2.0 kOhms. EMG signals passed through a pre-amplifier, located no farther than 10 cm from the electrodes, and then passed through a main amplifier. EMG signals were band-pass filtered using cut-off frequencies of 10 Hz and 1 kHz.

Two miniature accelerometers (Dytran 3115A) with a frequency response of 1 Hz to 20 kHz were used to obtain the VMG signals. The accelerometers were fixed with thin, double-sided adhesive tape between the EMG electrodes of the RF and VL muscles. VMG signals were band-pass filtered using cut-off frequencies of 3 Hz and 100 Hz.

Knee extensor moments were measured using a Cybex II isokinetic dynamometer. EMG, VMG and knee extensor moments were digitized at a frequency of 3360 Hz using an analogue-to-digital-board and stored on a 386 personal computer for further signal analysis.

Protocol

Subjects were seated on a Cybex II isokinetic dynamometer chair. The hip and knee joint angles were kept at 83 and 90 degrees from full extension, respectively. Three maximal isometric voluntary knee extensor contractions (MVC) were performed for a period of 2 seconds each. The largest knee extensor moment obtained during these three trials was used to calculate the moment representing 70% of MVC. Following the maximal trials, three submaximal knee extensor contractions were performed at an effort of 70% of MVC, each lasting 10 seconds. Two minute intervals were given between each trial to

prevent fatigue. The target moment of 70% of MVC was displayed on an oscilloscope to provide the subjects with visual feedback. Following the three 70% of MVC trials, the subjects performed the fatigue protocol, which consisted of an isometric knee extensor contraction at 70% of MVC maintained for as long as possible. Once the subjects could not maintain a moment of 70% of MVC, they continued the test until the knee extensor moments dropped to a level of approximately 50% of MVC, when the test was stopped. The duration of these tests ranged from 52 to 107 seconds. During the recovery period, measurements of 10 second contractions (70% of MVC) were obtained at 20 seconds, 50 seconds, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 15 minutes after termination of the fatigue test.

Data Analysis

EMG and VMG data were extracted for a segment of 1 second for the MVC trial, and for segments of 5 seconds from each trial before and after the fatigue protocol. These segments were taken from the middle of the original segments in order to ensure that the signals were stationary (i.e. from the middle of the 2 seconds MVC trials, and from the middle of the 10 seconds, 70% of MVC trials, before and after fatigue). For the fatigue trial, consecutive segments of 5 seconds were extracted throughout the fatigue protocol, except for the segment preceding the drop of the knee extensor moments and the last segment of the fatigue test. These segments were typically shorter than 5 seconds, but always equal to or longer than 1 second.

The instant when the subject could not maintain the target level of 70% of MVC during the fatigue test was determined by inspection of the knee extensor moments. RMS-values were obtained from the raw EMG and VMG signals. Frequency analysis of the EMG and VMG signals were performed using a Fast Fourier Transform (FFT; 2048 points for the EMG and 4096 points for the VMG). MDF was calculated from the power frequency spectrum. A butterworth high-pass filter (cut-off frequency = 5 Hz; second order) was used on the raw VMG signal before the FFT analysis to eliminate low frequency noise (Wee and Ashley, 1989).

Statistical Analysis

In order to determine possible changes in RMS and MDF of the EMG and VMG signals during the fatigue-recovery protocol, the RMS and MDF values were evaluated for statistically significant changes for six distinct intervals of the experiment (trials 1-6). Values for trial 1 were obtained from the MVC; values for trial 2 were obtained from the first 5 seconds of the fatigue protocol (i.e., at a level of 70% of MVC when the muscle was not fatigued yet); values for trial 3 were obtained from the last 5 seconds at which 70% of MVC was maintained (i.e. just before a substantial decrease in torque was observed); values for trial 4 were obtained from the last 5 seconds of the fatigue trial at a level below 70% of MVC; values for trial 5 were obtained from the first trial after the fatigue protocol (i.e. 20 seconds after the termination of the fatigue protocol); and values for trial 6 were obtained from the last trial of the recovery period (i.e. 15 minutes after the fatigue trial).

A two way analysis of variance for repeated measures (EMG and VMG; with trials) was used to determine statistical difference for each measured parameter and to test for an interaction effect between the methods and the trials. Contrasts were used to evaluate differences between any two trials. Trial 2 was taken as the non-fatigued 70% of MVC control. One way analysis of variance for repeated measures was used to determine statistical difference within each method when interaction was significant and for the ratio RMS/torque for the same intervals as described above. The level of significance was chosen as 0.05 for all statistical tests. Mean values are always presented with the corresponding standard deviations.

Results

Figures 5.1 (a,b) and 5.2 (a,b) show the behavior of the MDF of the EMG - and VMG - time histories of RF and VL during the entire fatigue-recovery protocol for two sessions of one representative subject. The MDF of the EMG and VMG signals for both muscles tended to decrease during the fatigue protocol, reached minimal values towards the very end of the fatigue protocol, and returned to about normal values during the recovery period. Test and retest were similar in all cases.

Figures 5.3 (a,b) and 5.4 (a,b) show mean values and standard deviations of the MDF of EMG and VMG signals for RF and VL at selected intervals during the experimental protocol (see statistical analysis in the methods section for a description of these intervals). The MDF values for trials 1,3,4 and 5 were different from trial 2 in all cases, while the MDF values for trial 6 were not (i.e. the MDF returned to the pre-fatigue values at the end of the recovery period).

Figures 5.5 (a,b) and 5.6 (a,b) show the mean values and standard deviations of the RMS values of EMG and VMG signals for RF and VL for the trials 1-6, respectively. As the two way analysis of variance revealed an interaction between the RMS of the EMG and of the VMG, a one way analysis of variance was used in this case to evaluate the differences within each technique. The RMS of the EMG signal showed a similar behavior in RF and VL. The RMS values of both muscles were significantly different between the MVC (trial 1) and the 70% MVC (trial 2) contractions. The RMS values for trials 3, 5 and 6 were higher, and the RMS values for trial 4 were similar to the control values. The RMS values of the VMG signal followed a different pattern for the two muscles late in the fatigue protocol. For the RF, there was a decrease in the RMS values from trial 2 to trial 3, and a significant increase from trial 3 to trial 4, while in VL there was an increase in the RMS values with decreasing force production. Both muscles returned to the pre-fatigue values by trial 6.

The torque as a percentage of MVC is shown in Figure 5.7 for the six trials used in the statistical analysis. Subjects were not able to maintain 70% of MVC in trial 4, whereas (by definition) the 70% of MVC level was maintained in all other submaximal trials. The mean values for the RMS/torque of the EMG signals for trials 1 to 6 are shown in Figures 5.8a for RF and 5.8b for VL. Trial 2 is the only one to show a decrease in this ratio in both muscles, while in all the other trials there was a similar normalized RMS (with the exception of trials 1 and 3 that were different from trial 6 in the case of RF, and trial 3 that was different from trial 4 in VL).

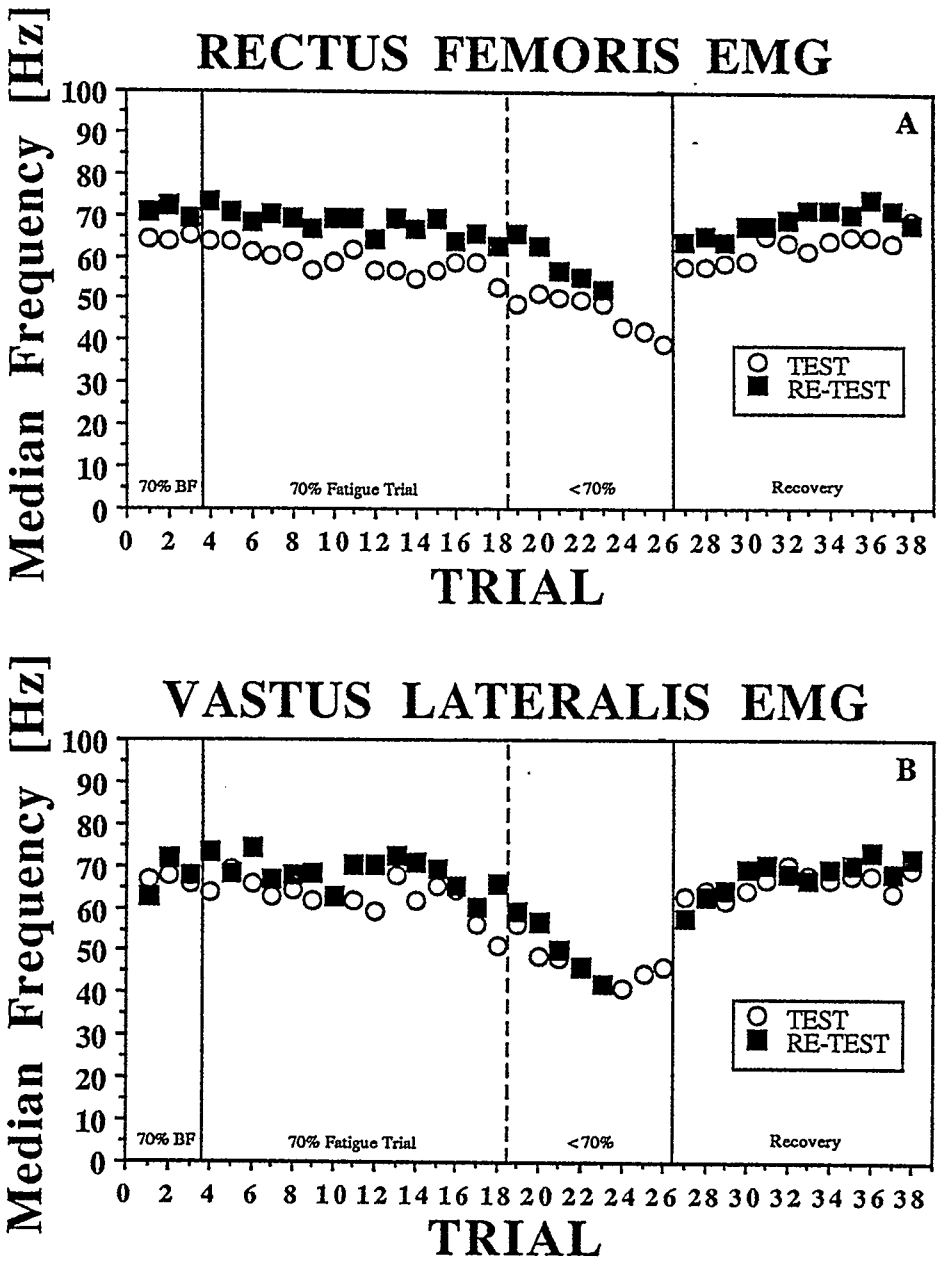

Figure 5.1. MDF for the entire protocol of RF and VL of one representative subject (open circles = test; filled squares = re-test). Solid lines separate the periods before, during and after fatigue, respectively; dashed line represents the limit where the contraction could not be maintained at the 70% of MVC level during the fatigue phase (based on a qualitative analysis of the drop in the knee extensor moments); (a) results for EMG of RF, and (b) results for EMG of VL.

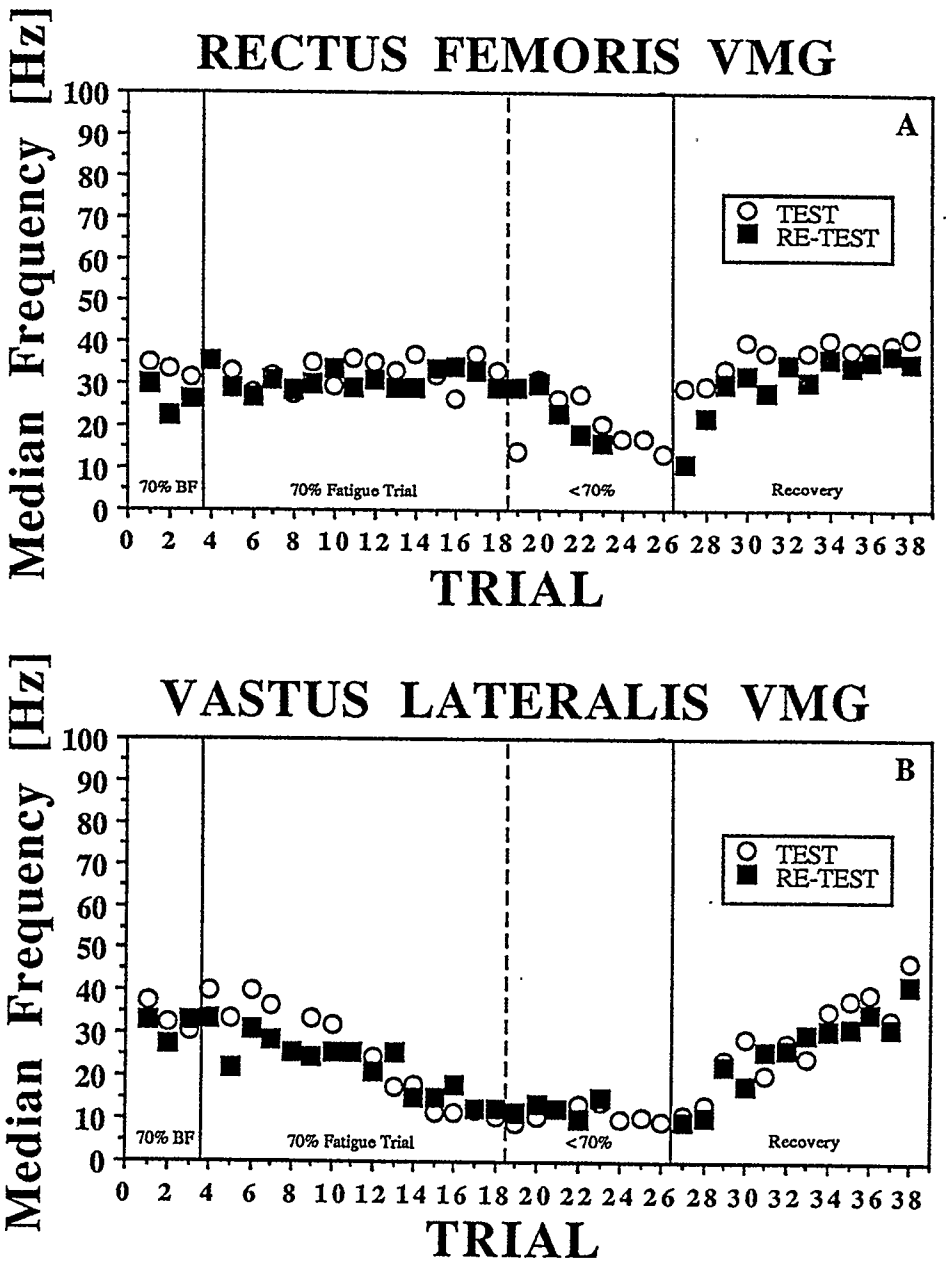

Figure 5.2. MDF for the entire protocol of RF and VL of one representative subject (open circles = test; filled squares = re-test). Solid lines separate the periods before, during and after fatigue, respectively; dashed line represents the limit where the contraction could not be maintained at the 70% of MVC level during the fatigue phase (based on a qualitative analysis of the drop in the knee extensor moments); (a) results for VMG of RF, and (b) results for VMG of VL.

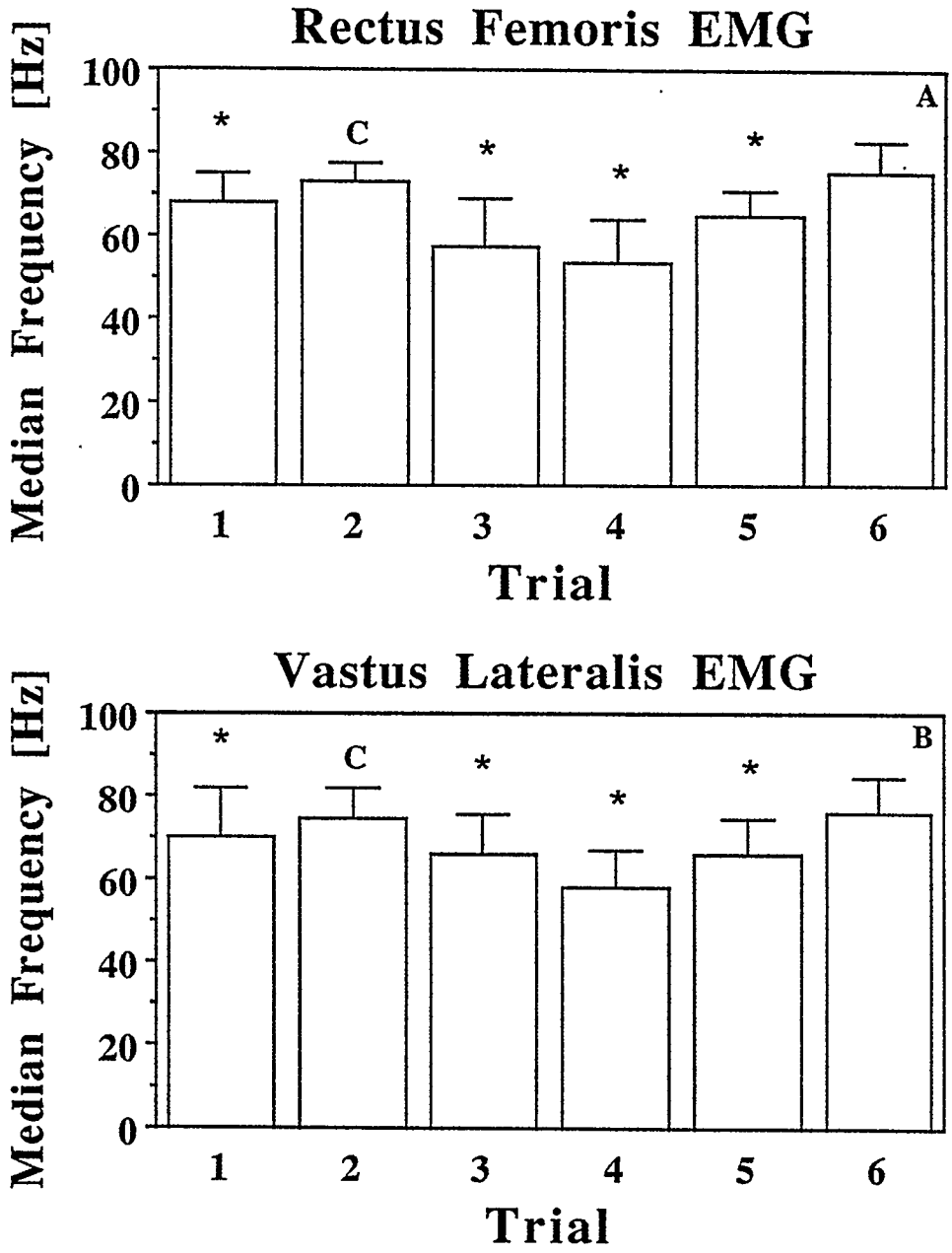

Figure 5.3. Mean values and standard deviations of the MDF of (a) RF EMG, and (b) VL EMG, where trial 1 corresponds to MVC, trial 2 = first 5 seconds of the fatigue protocol, trial 3 = last 5 seconds of the fatigue protocol at a level of approximately 70% of MVC, trial 4 = last 5 seconds of the fatigue protocol at an approximate level of 54% of MVC, trial 5 = first 5 seconds obtained from the first trial of the recovery period, and trial 6 = last 5 seconds of the recovery period. Letter "C" above the standard deviation bars indicates the control trial, while "*" indicates the trials that had MDF values significantly different from the control trial ($p<0.05$).

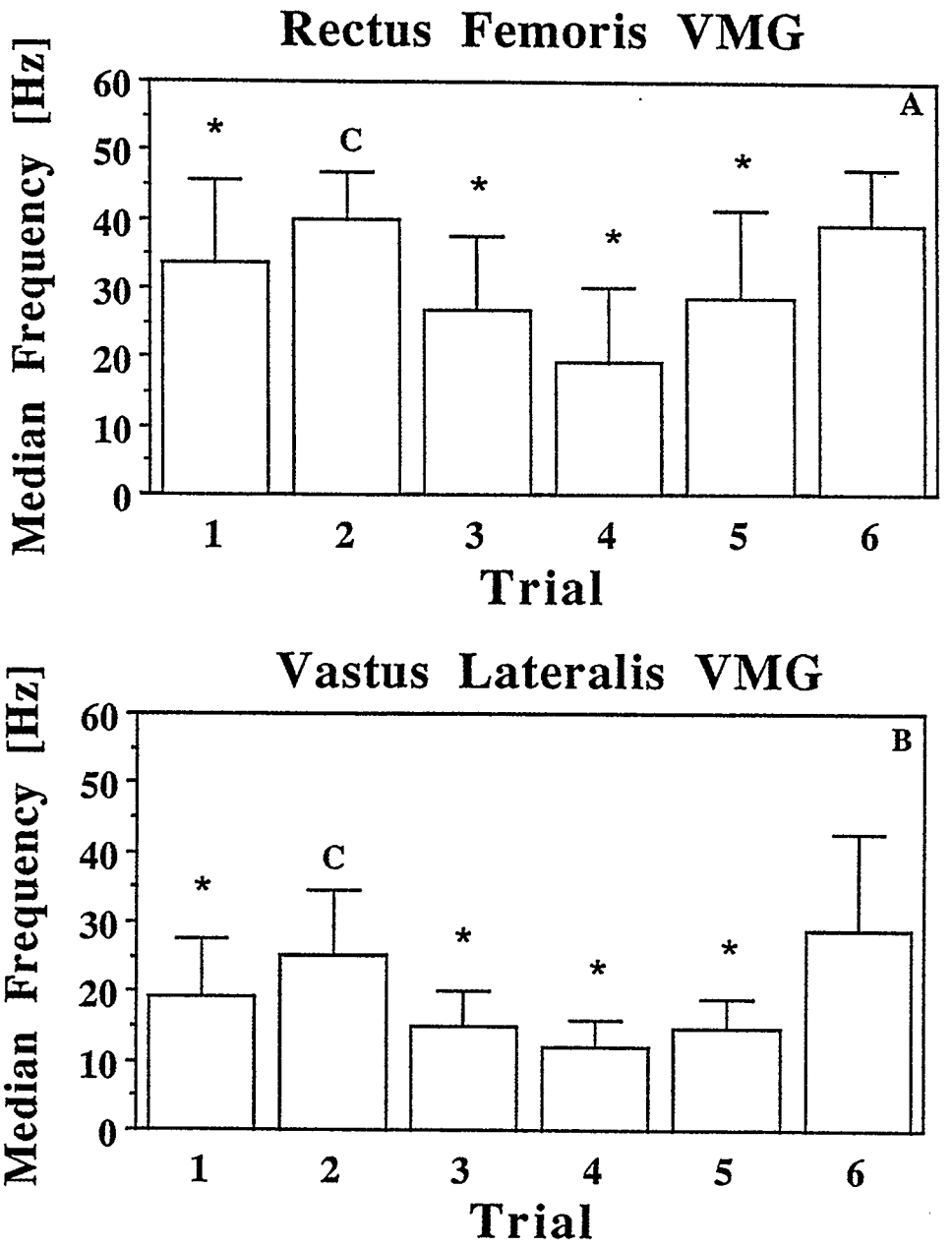

Figure 5.4. Mean values and standard deviations of the MDF of (a) RF VMG, (b) VL VMG, where trial 1 corresponds to MVC, trial 2 = first 5 seconds of the fatigue protocol, trial 3 = last 5 seconds of the fatigue protocol at a level of approximately 70% of MVC, trial 4 = last 5 seconds of the fatigue protocol at an approximate level of 54% of MVC, trial 5 = first 5 seconds obtained from the first trial of the recovery period, and trial 6 = last 5 seconds of the recovery period. Letter "C" above the standard deviation bars indicates the control trial, while "*" indicates the trials that had MDF values significantly different from the control trial ($p<0.05$).

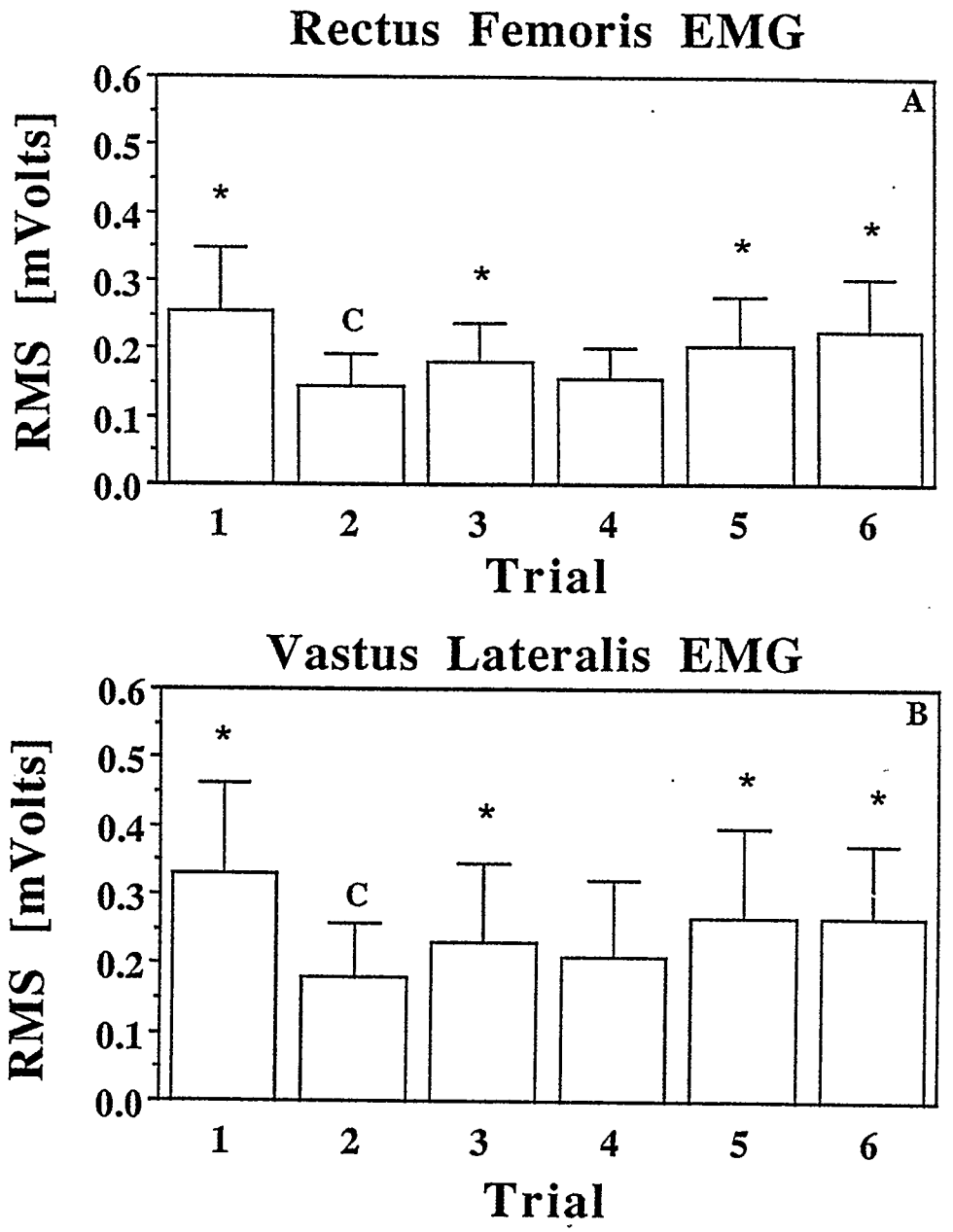

Figure 5.5. Mean values and standard deviations of the RMS of (a) RF EMG, (b) VL EMG, where trial 1 corresponds to MVC, trial 2 = first 5 seconds of the fatigue protocol, trial 3 = last 5 seconds of the fatigue protocol at a level of approximately 70% of MVC, trial 4 = last 5 seconds of the fatigue protocol at an approximate level of 54% of MVC, trial 5 = first 5 seconds obtained from the first trial of the recovery period, and trial 6 = last 5 seconds of the recovery period. Letter "C" above the standard deviation bars indicates the control trial, while "*" indicates the trials that had RMS values significantly different from the control trial ($p<0.05$).

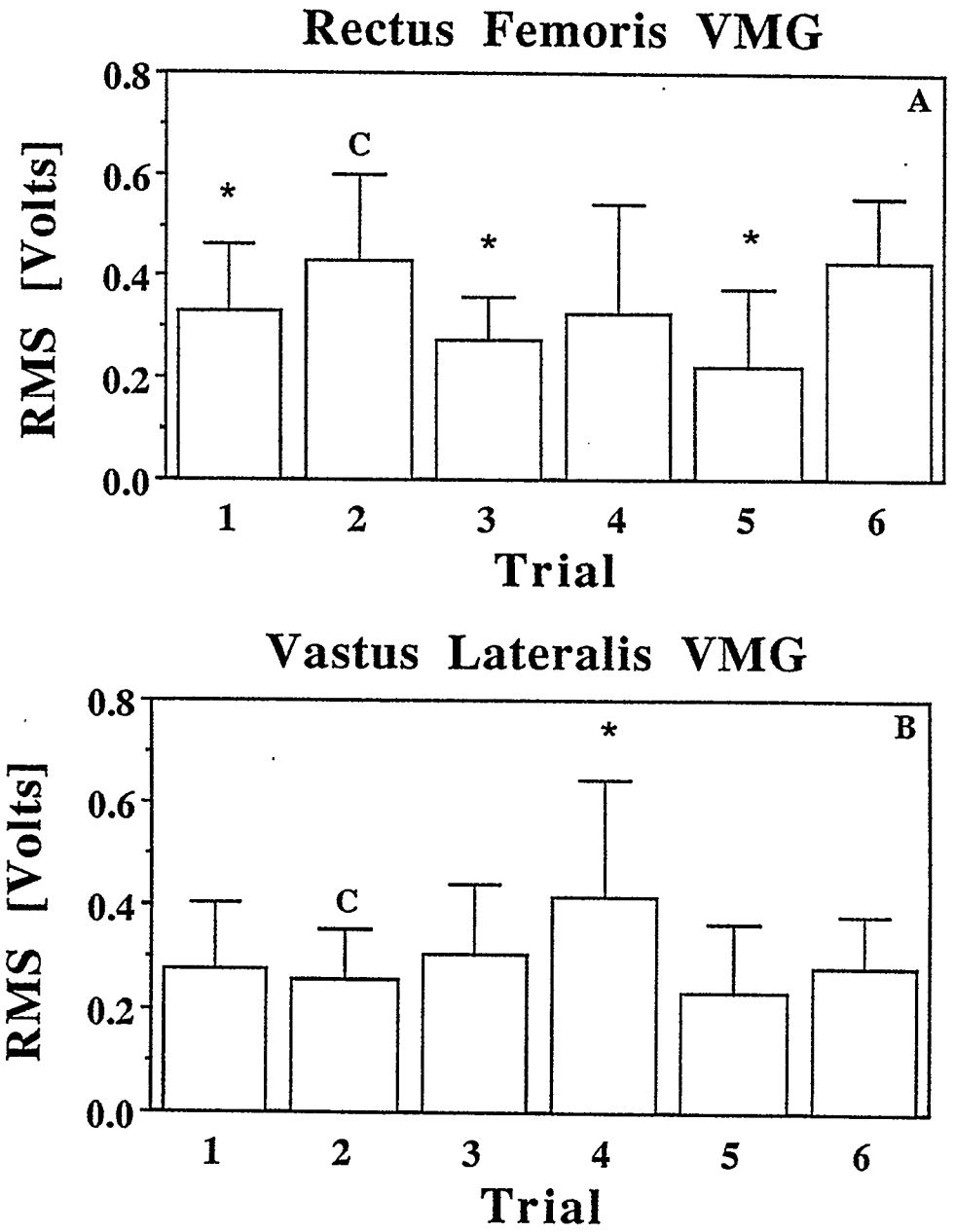

Figure 5.6. Mean values and standard deviations of the RMS of (a) RF VMG, (b) VL VMG, where trial 1 corresponds to MVC, trial 2 = first 5 seconds of the fatigue protocol, trial 3 = last 5 seconds of the fatigue protocol at a level of approximately 70% of MVC, trial 4 = last 5 seconds of the fatigue protocol at an approximate level of 54% of MVC, trial 5 = first 5 seconds obtained from the first trial of the recovery period, and trial 6 = last 5 seconds of the recovery period. Letter "C" above the standard deviation bars indicates the control trial, while "*" indicates the trials that had RMS values significantly different from the control trial ($p<0.05$).

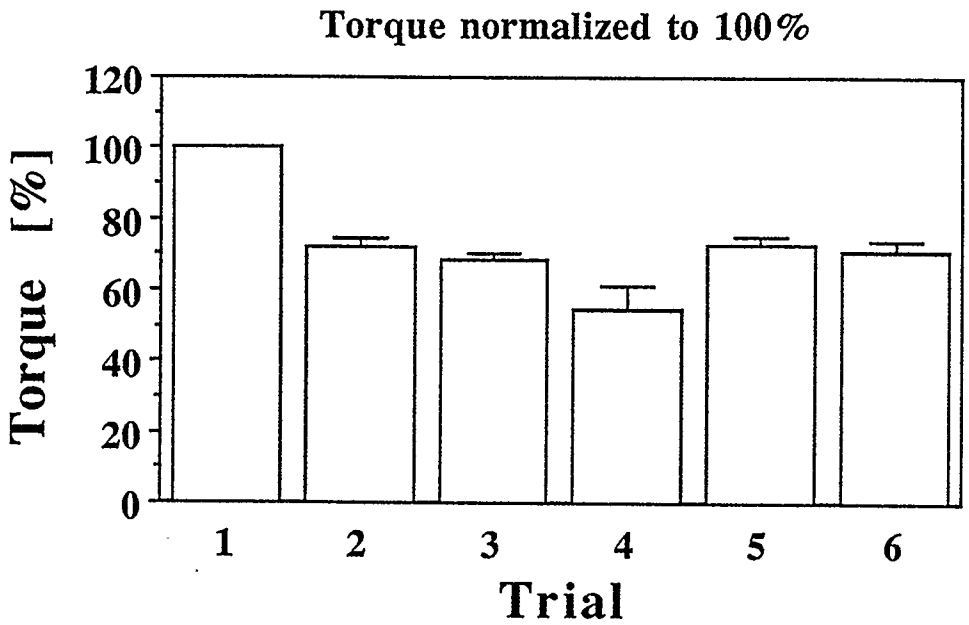

Figure 5.7. Mean values and standard deviations of the percentage torque produced during trials 1-6 (see Figure 5.3 for description) normalized to the MVCs.

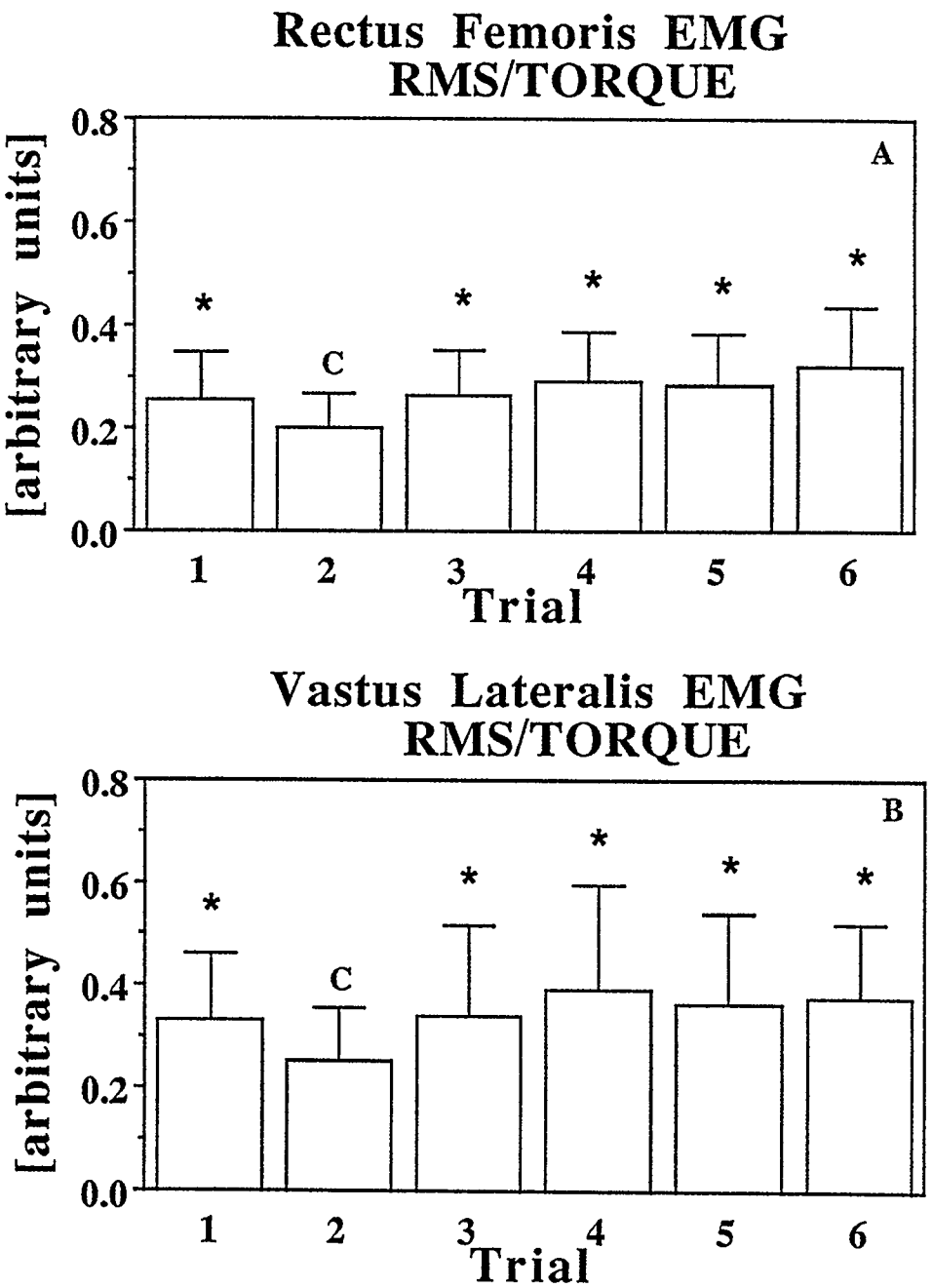

Figure 5.8. Ratio between RMS and percentage change in torque. The ratio was calculated for each subject. Intervals are the same as in Figure 5.3 (results for RF in "a" and for VL in "b"). Letter "C" above the standard deviation bars indicates the control trial, while "*" indicates the trials that had values significantly different from the control trial ($p<0.05$).

Discussion

MDF of the EMG signal

Spectral alterations in the surface EMG signal have been studied by reducing the spectral data to a single index, such as the MDF or the mean power frequency (Hagg, 1992). The EMG results obtained in this study agree with those reported in the literature, i.e. a decrease in the MDF with fatigue (Sadoyama and Miyano, 1981; Moritani et al. 1982; Gamet and Maton, 1989) in both muscles. Many mechanisms have been used to explain the decrease in the MDF of the EMG signal with fatigue: derecruitment of MUs, synchronization of the firing patterns of MUs, changes in the coefficient of variation of MU discharge, and changes in the conduction velocity of the muscle fibers (Basmajian and De Luca, 1985). However, it seems that a decrease in the conduction velocity of MU action potentials may account for most of the decrease in MDF during fatigue (Hagg, 1992).

It has been suggested that the MDF provides a reliable, consistent and relative unbiased estimate of a parameter that is related to the muscle fiber conduction velocity (Stulen and De Luca, 1981). Since the MDF of the EMG returns to the pre-fatigue values from trial 4 to trial 6 (Figures 5.3a and 5.3b), it seems reasonable to assume that the conduction velocity of muscle fibers has returned to pre-fatigue levels within the fifteen minutes of the recovery period. The power spectrum has been observed to recover within two to five minutes after the cessation of the exercise that induces localized muscle fatigue (De Luca, 1984; Kuorinka, 1988). The results of this study concur with this observation, since the MDF had returned to the control levels at about five minutes into the recovery protocol for all eleven subjects (e.g. Figures 5.1a,b).

MDF of the VMG signal

The MDF of the VMG signals showed a decrease with fatigue and a fast return to control values during the recovery period in both RF and VL. Orizio et al. (1992), using soundmyogram to record muscular vibrations in the biceps brachii, reported an initial increase, followed by a decrease in the MDF during fatiguing contractions at 60 and 80% of MVC. At 20% of MVC the frequency content remained constant for long lasting contractions. The decrease in the MDF of the vibratory signals has been related to changes

in MU activation strategy (Orizio, 1993). Also, it has been suggested that muscle tremor that occurs with fatigue may affect muscle vibrations (Barry et al. 1985; Stokes and Cooper, 1992). As muscle tremor was observed during the experiments, and since muscle tremor has a low-frequency content (6-12 Hz; Allum et al. 1978; Elble, 1986), the decrease in the MDF of the VMG (Figures 5.4a,b) was probably associated with muscle tremor. However, as the mechanisms behind muscle vibration are not completely understood, further investigations need to be done.

RMS of the EMG signal

According to Basmajian and De Luca (1985), the RMS of the EMG signal is related to the number of active MUs (recruitment), the firing rate of MUs, the shape of the MU action potential, and the cross-correlation of MU discharge during contraction.

The RMS of the EMG signal increases from the pre-fatigue control condition to the fatigued condition at the 70% of MVC level (trial 2 vs trial 3 in Figures 5.5a and 5.5b). Similar results have been reported in the literature (Stulen and De Luca, 1978). An increase in the RMS during a fatigue protocol where a submaximal force is maintained has been associated with changes in the shape of the MU action potentials (Stulen and De Luca, 1978), and with an increased recruitment and firing rate of the MUs (Viitasalo and Komi,1977; Moritani et al. 1986). When the 70% of MVC cannot be sustained, the RMS starts to decrease (i.e. a significant decrease in the RMS from trial 3 to trial 4) in parallel with the decrease in force production (see the drop in torque from trial 3 to trial 4 in Figure 5.7). Similar observations have been made previously (Bigland-Ritchie et al. 1983; Bigland-Ritchie and Woods, 1984; Moritani et al. 1986). The decrease in the RMS has been associated with a loss in the ability to activate MUs, and a possible decrease in the average MU firing rate with increasing fatigue when a force cannot be maintained.

An alternative explanation for the increase in the RMS associated with fatigue has been offered by De Luca (1984). He suggested that an increase in the low-frequency component of the EMG signal contributed to an increase in the RMS because body tissues allow for easier transmission of low-frequency compared to high-frequency signals. If body tissues act as low-pass filters, one might expect a decrease in the RMS values during the recovery period when the MDF increases. From the very end of the fatigue protocol (trial 4) to the first measurement in the recovery period (trial 5), the RMS of the EMG signals increased in both muscles (Figures 5.5a,b) which may be associated with the higher

torque that was produced in trial 5 (about 70% MVC) compared to trial 4 (on average about 54%). After fifteen minutes of recovery (trial 6), the RMS values of RF and VL were still elevated above the control levels. Since the MDF had recovered by this time, the increased RMS values are not likely due to a decrease in the frequency content of the EMG signal. Therefore, the elevated RMS values probably indicate that more MUs are activated and/or higher average firing rates are used to achieve the target force level compared to the control trial. This is confirmed by the results of Figures 5.8a and 5.8b, where the activation level seems to be similar in all trials, except for the control trial (trial 2). This shows that the decrease in RMS of the EMG in trial 4 of Figures 5.5a and 5.5b was due to a decrease in torque at that instant. There is still a relative increase in RMS compared to the control value at this instant during fatigue.

RMS of the VMG signal

The RMS of the VMG signals showed a complex behavior. There was a decrease in the RMS of RF with fatigue (from trial 2 to trial 3 of Figure 5.6a), and a return to values similar to those of the control trial with decreasing force (trial 4). In VL, a significant increase occurred when the target force could not be maintained (trial 4 of Figure 5.6b). The recovery protocol revealed an increase in the RMS of VMG of RF towards the end of this period (i.e. between trials 5 and 6 of Figure 5.6a), while VL showed no change in the RMS. In both muscles, the RMS decreased from trial 4 to trial 5.

The process responsible for muscle vibration is not fully understood. Orizio et al. (1989a, 1990, 1992) found a decrease in the amplitude of the soundmyogram at isometric contractions and high levels of force production compared to the beginning of the exercise. Assuming that muscle sounds and muscle vibrations have the same origin, and that the above results reflect the behavior of the amplitude of the VMG signal, a decrease in the RMS should be seen in both muscles with fatigue. However, as mentioned before, muscle tremor probably affected the results of the VMG. Muscle tremor produces vibrations of large magnitude and low frequency compared to regular muscle vibrations, and this has been related to the activation patterns of the muscle (Allum et al. 1978). The increase in the RMS seen from trial 3 to trial 4 (Figure 5.6b) may be explained by muscle tremor, which was observed at those instants in the experiments. The difference in the RMS behavior between RF and VL may be explained with the alignment of the uni-axial accelerometers. The accelerometer on RF was aligned in a predominantly anterior-posterior direction, and

the accelerometer on VL in a lateral-medial direction with respect to the thigh. Since the RMS values of VL increased and those of RF tended to decrease during the fatigue protocol, it may be presumed that the predominant direction of the tremor occurred in a medial-lateral direction.

Summary

The MDF of the EMG and VMG of RF and VL shows a decrease during sustained isometric contraction, but returns to control levels early during recovery. The RMS of the EMG signal increases during fatigue, and remains elevated after 15 minutes of recovery. The pattern of change of the RMS of the VMG signal was different for the two muscles during the process of muscle fatigue, but returned to control levels at the end of the recovery period. Further research is needed to permit interpretation of this signal, but muscle tremor may be a major factor affecting the VMG signals.

Chapter 6

Discussion

This investigation was aimed at identifying the possible mechanisms of muscle vibrations in mammalian skeletal muscle. Three different ideas have been proposed in the literature to explain the mechanisms of skeletal muscle vibrations. However, no specific theories were put forward and no testable predictions were ever proposed and systematically evaluated. In this study, we have described these theories and we have formulated testable predictions. These predictions will be discussed now within each proposed model, based on the results presented in the previous three chapters.

Cross-bridge Cycling Theory

The *cross-bridge cycling theory* (Oster and Jaffe, 1980) is based on the notion that muscle vibrations are caused by (or at least associated with) cross-bridge cycling during contractions. Using the *cross-bridge cycling theory*, the following testable predictions were formulated: (a) no vibratory signals should be observed when the muscle is at rest, (b) whereas active muscles should always produce vibrations; (c) if the force of a muscle is increased from zero to maximal, the VMG signal should increase in amplitude due to increased number of cross-bridges participating with increased voluntary effort, and if the VMG signal from each cross-bridge add algebraically, then the magnitude of the signal should be related linearly to muscular force.

Prediction (a) above was supported by our animal and human results, as no vibratory signals were observed with the muscle at rest. If cross-bridge cycling produced vibrations, there should be a vibratory signal associated with any contraction (prediction b).

This prediction was not supported by our results, as there was no observable VMG signal during fused tetanic contractions in the cat SOL (e.g. Figures 3.5, 3.7).

According to prediction (c), force increases should produce an increase in the VMG signal amplitude. If muscle vibrations were to be produced by cross-bridge cycling, increased number of cross-bridges that cycle with increasing force demands should produce an increase in the signal amplitude. Increased force production obtained by increasing the stimulation rate to the muscle produced a decrease in the signal amplitude (Figures 3.4 and 3.5). Also, increases in soleus length, which are associated with increased force production because of the force-length relationship of this muscle, were not associated with increased VMG signal amplitude (Figures 4.3 - 4.9). When soleus was activated at close to tetanic frequencies, decreases in muscle length (which are associated with decreased muscle force) were associated with increased VMG signal amplitude (Figures 4.5, 4.6 and 4.9).

Although a linear relationship was suggested to exist for the VMG-voluntary effort relationship (Barry et al. 1985, 1992; Oster and Jaffe, 1980; Stokes and Dalton, 1991b; Zwarts and Keidel, 1991; see prediction 'c' above), our results did not produce this linearity. The VMG signal amplitude increased in a stepwise manner with increasing voluntary effort (Figure 3.11c). It may be that the increase in the VMG signal amplitude with increasing force production may occur when only the initial transient VMG signal is considered (Cole and Barry, 1994). Linearity of the VMG signal magnitude with increasing force production was observed for portions of this relation in RF, for example, in the range of 40-80% MVC (Figures 3.11c and 3.14). Prediction (c) was not supported by our experimental results.

Vibrating String Theory

The *vibrating string theory* (Frangioni et al. 1987) associated VMG signals with vibrations produced by the entire muscle when stimulated, like the vibrations of a guitar string when plucked. Using the vibratory string theory, the following testable predictions were formulated: (a) when a muscle is at rest, no VMG signal should be produced, because there is no forcing function that makes the muscle vibrate, and (b) increasing muscular forces should decrease the amplitude of the VMG signal and increase its frequency content.

The results presented in chapters 3 and 4 support prediction (a), as no VMG signals were detected when the muscle was at rest.

For increasing muscular forces, and thus presumably increasing muscle stiffness, the amplitude of the VMG signal was expected to decrease and the frequency content of the signal was expected to increase (prediction b). This result was partially found for electrically elicited contractions in which increased stimulation frequencies at a constant voltage produced an increase in force (Figure 3.1a) and a decrease in the amplitude of the VMG signal (Figures 3.4, 3.5, 3.7 and 3.11b).

At stimulation frequencies of 8 and 12 Hz for animal 2 (Figures 4.5 and 4.6, respectively) and a stimulation frequency of 12 Hz in animal 3 (Figures 4.3c and 4.9), the magnitude of the VMG signal decreased with increasing muscle length. As the cat SOL works on the ascending limb of the force-length relationship, increased active and passive stiffness were assumed to increase with increasing SOL length. Increased muscle stiffness at close to fusion frequencies (12 Hz, Figure 4.12) was probably the principal factor producing the observed decrease in the VMG signal with force increases. When going from intermediate to long muscle lengths, a decrease in the VMG signal magnitude was also observed at unfused frequencies of stimulation (at 4 Hz VMG signals decreased when going from ankle angles of 100 to 80 degrees in Figure 4.7, whereas at 8 Hz VMG signals decreased when going from ankle angles of 120 to 80 degrees, Figure 4.8). This decrease in the VMG signal magnitude was probably caused by increased passive stiffness at these long lengths. The results presented in Figure 4.14 also agree with prediction (b), as there was an increase in the frequency content of the VMG signal with increasing muscle length and muscle forces.

However, at stimulation frequencies of 4 Hz (in both animals) and 8 Hz (in animal 3), the VMG signal increased when going from the shortest muscle length (ankle angles of 140 degrees in Figures 4.7 and 4.8) to intermediate muscle lengths (ankle angles corresponding to 100 degrees for 4 Hz and 120 degrees for 8 Hz in Figures 4.7 and 4.8, respectively). As discussed in Chapter 4, at 4 and 8 Hz of stimulation, the peak RMS values obtained for VMG signals at intermediate muscle lengths were assumed to be caused by two factors: (1) the increased magnitude of the force transients produced during the unfused tetani (Figures 4.10 and 4.11), combined with (2) intermediate muscle active (contractile) and passive (elastic) stiffness. During voluntary efforts, increased muscular forces were associated with increased VMG amplitudes (e.g. from 0-20% and from 40-80% MVC in Figure 3.11c), therefore not supporting prediction (b). Prediction (b) was

partially supported by our results, which suggest that muscle stiffness plays an important role in determining the VMG signal characteristics; however, it is not the only factor.

Unfused MU Theory

The *unfused MU theory* (Jorgensen and Lammert, 1976; Lammert et al. 1976; Orizio, 1993) was based on the idea that the force fluctuations produced by MUs during unfused tetani (i.e. when stimulated at frequencies below about 20-30 Hz for slow twitch MUs and 40-60 Hz for fast twitch MUs; Burke et al. 1973; McPhedran et al. 1965; Wuerker et al. 1965) was the mechanism responsible for the production of muscle vibrations. Using the *unfused MU theory*, predictions were proposed for electrically stimulated and voluntary muscular contractions.

Electrically Elicited Contractions

In experiments using electrical stimulation, it was predicted that: (a) no vibratory signals should be observed when the muscle is at rest; (b) VMG signals should be observed at all unfused frequencies of stimulation, and the signal should disappear at high frequencies of stimulation when all MUs are contracting tetanically; (c) stimulation at a constant low frequency (i.e. a frequency not producing a fused tetanic contraction in any MU) with gradual increases in the stimulating voltage should produce an increase in the VMG signal amplitude; (d) at subtetanic frequencies of stimulation, one distinct vibratory signal should be seen per stimulation pulse; (e) the VMG signal of electrically stimulated muscle should be repeatable if the same stimulation protocol is given more than once and fatigue does not occur.

The results from both the animal and the human studies supported prediction (a), as no VMG signals were observed with the muscle at rest. A distinct VMG signal was associated with each stimulus of an unfused contraction (prediction d) for both RF (Figure 3.13) and the cat SOL (Figure 3.5). For the cat SOL, distinct vibratory signals were observed for each stimulation pulse for stimulation frequencies ranging from 4-16 Hz, whereas for the human RF, distinct VMG signals were observed up to a stimulation frequency of 40 Hz.

The VMG signal disappeared for rates of stimulation exceeding 12-16 Hz in the in-situ cat SOL (Figures 3.5 and 3.7) when the contraction was a fused tetanus (prediction b). This result was not observed in the in-vivo study in which the VMG signal became smaller with increasing frequencies of stimulation, but was always larger than the signal obtained at rest (Figures 3.11b and 3.13). As RF is a muscle composed of a mixed fiber type distribution (Johnson et al. 1973), fusion frequencies were probably not obtained for the fast twitch fibers of RF, therefore explaining the existence of VMG signals at high stimulation frequencies. Similar results as those found for the human RF were observed by Orizio et al. (1993) for the cat gastrocnemius (a mixed fibered muscle; Ariano et al. 1973; Burke and Tsairis, 1973). Sound signals were recorded up to a frequency of stimulation of 50 Hz (the highest frequency used by Orizio et al. 1993).

When increasing the number of recruited MUs at given (unfused) frequencies of artificial (electrical) stimulation, VMG signals increased (prediction c), as predicted. This was correct for the animal (Figure 3.4) and human experiments (Figures 3.11a and 3.12).

Finally, for prediction (e), the VMG signal of electrically elicited contractions was expected to be repeatable if the same stimulation protocol was given more than once. This was observed to be correct as shown in Figure 3.4 (Chapter 3), in which the same stimulation protocol was applied at three different voltage levels producing VMG signals with identical behavior, although the amplitude of the signal was different due to the different voltage applied to the nerve at each level of stimulation.

Voluntary Contractions

For contractions elicited by voluntary effort it was expected that: (f) no VMG signal should be observed when the muscle was at rest; (g) for isometric contractions with increasing voluntary effort (from 0% - 100% of the maximal force), the amplitude of the VMG signal should be small initially (at low levels of force), should increase and reach a maximum at medium levels of force, and then should decrease again towards MVC.

No vibratory signal was observed when the muscle was at rest (prediction f). For isometric contractions at increasing levels of voluntary effort (from 0-100% MVC), it was expected that the signal amplitude should be small at low levels of force, should reach a peak at intermediate levels of force, and should decrease towards maximal voluntary effort (prediction g). Figure 3.14 (Chapter 3) is a clear example that prediction (f) was correct, as the amplitude of the VMG signal increased from 10-80% MVC, and decreased from 80-

100% MVC.

Voluntary Contractions During Fatigue

For contractions elicited by voluntary effort, it was expected that a decrease in the magnitude of the VMG signal should be observed during fatiguing isometric contractions, because of (1) the increased relaxation time of MUs with fatigue, which produces tetanic contractions at decreasing activation frequencies, (2) the loss of force production in specific MUs with fatigue, which will result in a decreasing number of MUs contributing to the vibratory signal, (3) a decrease in the twitch amplitude with fatigue, which will result in a decrease in the magnitude of the force transients of MUs contributing to the VMG signal, and (4) a decrease in muscle activation, which may be responsible for a decrease in the MU firing rates with fatigue (prediction h). Our results obtained in human RF support prediction (h), as there was a decrease in the RMS of the VMG signal with fatigue (Figure 5.6a); however, for VL the signal increased (Figure 5.6b). As discussed previously on pages 90-91, we believe that physiologic tremor, which is enhanced by fatigue, was responsible for the increased VMG signal amplitude in VL. Further investigation is needed to clarify this point.

One of the interesting results of the fatigue study reported in Chapter 5 was that, although the MDF of the EMG and VMG signals returned to pre-fatigue values within 15 min of termination of the fatigue protocol, the RMS of the EMG signal remained elevated during the 15 min recovery period. From these results it appears that the RMS and the MDF of the EMG signal are associated with different physiological phenomena during fatigue. While there was recovery (presumably) of the conduction velocity of the muscle fibers (as represented by the return of the MDF of the EMG signal to pre-fatigue values), complete muscle recovery was not achieved because the RMS of the EMG remained elevated throughout the recovery period. The reason for this difference in the behavior of the RMS and MDF of the EMG signal during recovery remains to be determined.

Summary and Conclusion

Different theories have been proposed to explain the mechanism of muscle vibrations: the *cross-bridge cycling theory*, the *vibrating string theory*, and the *unfused MU theory*. Specific predictions were derived from these theories and tested in a series of experiments using in-situ (cat SOL) and in-vivo (RF and VL) muscle preparations. From all the proposed theories, the *unfused MU theory* was the one that better described the mechanism of muscle vibrations than the other two theories, as all predictions proposed for this theory were supported by the results obtained.

Nevertheless, it is important to keep in mind that, although we believe that the force transients produced by the unfused contraction of MUs is the mechanism underlying muscle vibrations, the resultant VMG signal is influenced by many factors, including muscle stiffness, muscle architecture, elastic components of a muscle, and surrounding tissues to the contracting muscle.

Although our results do not represent the entire population of mammalian skeletal muscles, the similarities observed in the results obtained from cat SOL and human RF for electrically elicited contractions (i.e. increased firing rates produce a decrease in the VMG signal magnitude, whereas increased stimulation voltages are responsible for an increase in the VMG signal) suggest that the behavior of the VMG signals is similar for different mammalian muscles. The results presented for the human RF during different levels of voluntary effort will probably not represent the VMG signal behavior of other muscles, as different muscles have different recruitment strategies and different material properties that may affect the recorded signal. Nevertheless, we believe that the same principles used to explain the behavior of the VMG signal during voluntary contractions in human RF may be used to explain results obtained from different mammalian skeletal muscles. The accuracy of this statement remains to be established.

Potential Applications

VMG has been used in different types of studies, with healthy and diseased muscle preparations (e.g. Barry et al. 1990; Rhatigan et al. 1986; Rodriquez et al. 1996). However, interpretation of the results was difficult, because the studies were descriptive and lacked clear hypotheses with testable predictions. Assuming that the vibrations are produced by the force transients of MUs contracting in an unfused way, and that muscle activation and the material properties of a muscle determine the outcome of the recorded signal, VMG may be used as a basic non-invasive tool to study muscle contraction.

As previously mentioned (Chapter 3), recruitment strategies vary amongst muscles. Small muscles of the hand, for example, recruit MUs up to 50-60% MVC and depend on rate modulation for further force increases, whereas large muscles of the limbs recruit MUs up to 80-100% MVC (Basmajian and De Luca, 1985). Based on the evidence presented in Chapter 3 (i.e. that an increase in the number of active MUs is responsible for an increase in the RMS of the VMG signal, whereas increased firing rates produce a decrease in the VMG signal amplitude), VMG might be used to detect differences in MU recruitment strategies in different muscles. According to the *unfused MU theory*, the RMS of the VMG signal should reach its peak value at different levels of voluntary effort for muscles with different MU recruitment strategies. For small hand muscles, this peak should be observed around the level at which recruitment of MUs stops (i.e. around 50% MVC). For large leg muscles which recruit MUs in the full range of voluntary effort, peak VMG signals should occur at the highest levels of voluntary effort.

We have shown that increasing the number of activated MUs during electrically elicited contractions increased the amplitude of the VMG signal (Figures 3.4 and 3.11a). Therefore, the reduction in the number of recruited MUs that occurs in certain neuromuscular diseases might be observed with VMG signals. Increases in the voltage of electrical stimulation in patients with such diseases (where only part of the motor pool is activated) should produce smaller increases in the VMG signals when compared to signals obtained from healthy subjects. Therefore, the relationship between the RMS of the VMG signal and stimulation voltage (e.g. Figure 3.11a) should produce smaller RMS values at increasing voltages of stimulations in groups of diseased subjects compared to healthy subjects. Also, if rehabilitation of these patients is to occur, an increase in the VMG signal magnitude should be observed when comparing initial results with post-treatment results.

According to our previous work on muscle fatigue (Herzog et al. 1994), VMG can be used to assess the onset of fatigue during isometric voluntary contractions. The results presented in Chapter 5 also suggested that the enhancement of physiological tremor due to fatigue was probably responsible for the decreased frequency content in the VMG signals, and the increased signal amplitude. Physiologic tremor, which can be described as a shaking of the limbs that occurs during voluntary contractions and is enhanced by fatigue or by force production at high levels of voluntary effort, has been suggested to interfere with the sounds or vibrations produced by the muscle (Barry et al. 1985). The signal from tremor is added to the vibratory signal, changing the amplitude and the frequency content of the recorded signal (Herzog et al. 1994). Physiologic tremor has frequencies in the range from 6 to 12 Hz (Allum et al. 1978; Elble, 1986), and muscle vibrations frequencies have been found to range between 10-100 Hz; therefore, the addition of tremor on VMG signals causes a shift towards lower VMG frequencies.

The mechanical and physiological properties of a muscle have been shown to change if a muscle is submitted to a period of immobilization (McDonald et al. 1994; Witzmann et al. 1982). McDonald et al. (1994) showed that after 3 weeks of hindlimb suspension, the rat SOL percentage of slow twitch fibers decreased while the fast twitch type II fibers increased. As immobilization is very common with injury, and assuming that the above changes in fiber type distribution occur in a similar way with human muscles, then it may be possible to detect these changes in fiber type distribution with VMG. In Chapter 3, it was shown that the RMS of the VMG signal decreased with increasing stimulation rates. In the case of the cat SOL, a slow twitch fibered muscle, the decrease was more pronounced than in the human RF, a mixed fibered muscle. In SOL, the VMG signal disappeared when the contraction was a fused tetanus, which was achieved at stimulation frequencies between 12-16 Hz. In RF, this decrease in the RMS of the VMG signal occurred at a larger stimulation frequency range than in the cat SOL (i.e. between 30-40 Hz), although VMG signals were still observed at these high stimulation frequencies. Assuming that there is an increase in the percentage of the fast twitch fibers in muscles submitted to immobilization compared to pre-immobilization, then a shift toward higher fusion frequencies should occur in these muscles for the VMG RMS - stimulation frequency relationship. In other words, the curve for the RMS - fusion frequency relationship should increase (i.e. VMG signals should be observed at higher frequencies of stimulation in the immobilized muscle compared to pre-immobilization). Similarly, VMG might be used to study muscle function after spaceflight, as similar changes in the fiber

type distribution of muscles as the ones observed for immobilized muscles occur (Caiozzo et al. 1994).

Chapter 7

Future Directions

Some aspects of muscle vibrations were investigated in the present work; nevertheless, further research needs to be done in each one of the areas covered here (i.e. electrically elicited, voluntarily elicited, and fatiguing contractions) in order to clarify some of the unresolved issues mentioned in previous Chapters. In the following, we will try to address some of these issues and point out possible directions that the field of muscle vibrations might take.

Electromyography (EMG) has been widely used together with VMG or with acoustic myography (AMG) in order to provide the necessary information about muscle activation (Maton et al. 1990; Lee et al. 1992; Orizio et al. 1992; Rodriquez et al. 1993). The origin of the EMG signal is known, and theoretical models of the EMG signal provide good indications of the electrical state of the muscle (Basmajian and De Luca, 1985). Similarly, theoretical models about muscle vibrations could make the VMG technique more reliable by helping to establish a stronger connection between physiological phenomena and the characteristics of muscle vibrations. For example, theoretical and experimental models have been able to show that the decrease in the MDF of the EMG signal that occurs during fatigue is strongly correlated with a decrease in the conduction velocity of muscle fibers (Arendt-Nielsen and Mills, 1985, 1988; De Luca, 1981; Lindstrom et al. 1970, 1977; Merletti et al. 1984, 1990; Stulen and De Luca, 1981). We have not found any theoretical model describing the VMG signal. Therefore, the significance of the changes which occur in the MDF of the VMG signal remains to be established.

We have proposed in Chapter 6 that one of the potential applications of VMG is in studying muscles with different recruitment strategies. A detailed study attempting to establish the use of VMG in studying recruitment strategies of different muscles during voluntary contractions appears to be lacking in the field.

One of the limitations of our study using electrical stimulation was that we stimulated MUs in a synchronous way. Even when we used pseudo-random stimulation in the cat SOL, the coefficient of variation of interpulse intervals and the average stimulation rates used were delivered to the nerve synchronously. A logical (and perhaps final) step in showing that muscle vibrations are associated with the recruitment strategies of MUs during voluntary contractions, for example, would be to use asynchronous stimulation (e.g. Guimaraes et al. 1994a,b). In their study on the in-situ cat SOL, Guimaraes et al. (1994a,b) were able to use a protocol consisting of addition and rate modulation of eight to ten ventral root filaments. Although recruitment and rate modulation were not performed according to the size principle (Henneman et al. 1965), their EMG and force records were similar to those obtained during voluntary contractions. Such an asynchronous stimulation of different ventral root filaments could provide a better understanding of the influence of recruitment strategies on the VMG signal during voluntarily elicited muscle contractions.

Our preliminary results on the effects of muscle fatigue on the VMG signal, presented in Chapter 5, suggest that VMG might be useful in assessing the onset of tremor during fatigue. We have not found any report in the literature of an attempt to assess the presence and the effect of tremor on muscle vibrations during fatiguing isometric voluntary contractions. We have also suggested that the predominant direction of tremor occurred in a frontal plane because of the increased RMS values obtained for the VMG signal in VL compared to RF with fatigue. This increase in the RMS of the VMG signal in VL was explained by the different alignment of the uni-axial accelerometers which was different in the two muscles (i.e. the accelerometer on RF was aligned in a predominantly anterior-posterior direction, whereas the accelerometer on VL was aligned in a lateral-medial direction with respect to the thigh). In order to assess the existence of this directionality of tremor during fatigue, we used high speed video (100 Hz) to capture the shaking of the limb produced by tremor. Two accelerometers were placed over the RF and VL. The accelerometers were used as markers for limb and muscle movements. Two video cameras were placed perpendicular to each other to record movements in the sagittal and in the frontal plane of the thigh. If tremor occurred, the corresponding movement should be observed in both video and VMG signals. For the video data it was expected that the

increased amplitude should occur in both markers to reflect that the limb was shaking as a whole. Also, if tremor occurred predominantly in the frontal plane (as suggested by the increase in the RMS values of the VMG in VL), the VMG signal should be different for the two studied muscles (i.e. RF and VL), while the video data should reveal the same large displacement amplitude predominantly in one direction compared to the other.

Figures 7.1 and 7.2 show raw VMG signals and video records from the accelerometers in RF and VL of one subject during a knee extensor isometric contraction performed at 75% MVC. VMG signals increased in amplitude and decreased in frequency with fatigue (Chapter 5; Figure 7.1). Also, the increase in the VMG signal in VL was higher than the one in RF. In Figure 7.2, increased displacement of the markers occurred in the frontal plane (see Y3 and Y4) in both accelerometers, reflecting that the whole limb was shaking with fatigue. The resemblance of both signals (i.e. the displacement of the markers and the raw VMG signal) is remarkable, and further emphasizes the increased amplitude and decreased frequency due to tremor. Although our sample size was too small to fully support our expectations (n = 2), we believe that VMG can be used as a basic tool to assess the onset of tremor during fatigue. Nevertheless, further investigation needs to be performed with a larger sample in order to better establish the use of VMG signals in assessing physiological tremor.

Most of the fatigue studies reported in the literature using VMG were done in-vivo (e.g. Barry et al. 1985; Dalton et al. 1992; Dalton and Stokes, 1993; Orizio, 1992; Orizio et al. 1989a, 1992; Stokes and Dalton, 1991a; Zwarts and Keidel, 1991). Studying the effect of fatigue on muscle vibrations using in-situ muscle preparations may provide further understanding on the behavior of the VMG signals with fatigue. In a preliminary study (unpublished results), electrically elicited (18 Hz; periodic and pseudo-random stimulation with a coefficient of variation of the inter-pulse intervals of 0.15) isometric contractions of the cat SOL muscle produced an increase in the VMG signal magnitude from 0-15 seconds, whereas the signal decreased in magnitude from 15-120 seconds (Figure 7.3). As the nerve stimulation and the length of the muscle were constant, the initial increase and the following decrease of the VMG signal could only be produced by some physiological changes that may have occurred within the 120 seconds of our protocol. During fatigue of electrically elicited contractions (e.g. MacIntosh et al. 1994; 5 min, at 10 Hz) the half-relaxation time (i.e. the time required for relaxation to half-maximal isometric twitch tension) of the gastrocnemius muscle of the rat decreased within the first 5-10 seconds of stimulation, whereas it increased from 10-20 seconds. Decreases in the half-relaxation time

may produce an increase in the force transient responses, as the activation of a muscle following a stimulus is completed faster if the relaxation is faster, and may explain the initial increase in the VMG signal magnitude. Potentiation may also have contributed to the increased peak force of the first twitches in the study of MacIntosh et al. (1994). However, this was not the case in our preparation as potentiation has been shown to occur in fast twitch, but is virtually absent in slow twitch fibered muscles (Moore and Stull, 1984; Moore et al. 1985). Therefore, the changes in relaxation time are more likely to produce the increase in VMG signals in the cat SOL than potentiation; however, this possibility remains to be established. If the relaxation time increases, the force fluctuations may become smaller and finally disappear if the relaxation time becomes so large that the contraction becomes fused. As we did not assess the half-relaxation time of the cat SOL muscle, it would be interesting to assess the half-relaxation time of different muscles during electrically elicited isometric contractions together with the VMG signals. Establishing the relation between the relaxation time of muscle fibers and the VMG signal amplitude may help to understand the behavior of the VMG signals during fatigue in in-situ preparations in which there is no muscle tremor. Also, this information may help in the understanding of the fatigue process produced during electrically elicited contractions in paraplegic patients.

The development of muscle fatigue is one of the major issues associated with functional electrical stimulation of muscles in paraplegic patients (Levy et al. 1990). If we assume that VMG is a technique that is able to assess muscle fatigue, and may reflect the physiological properties of a contracting muscle (such as the relaxation changes that may occur following a fatigue protocol during electrically elicited contractions), then it may be possible to use VMG to assess the onset of fatigue and (perhaps) to detect the enhancement of muscle performance in patients that follow strict training programs using electrical stimulation. It would be interesting to measure VMG signals from these patients monthly, and to check for the increase/decrease behavior of the RMS of the VMG signal, and its relation to relaxation time in the muscle.

In order to assess if the effects of muscle length changes on the VMG signal were similar for electrically elicited muscle vibrations in in-situ and in-vivo muscle preparations, percutaneous electrical stimulation of the femoral nerve was performed in twenty healthy human subjects. Preliminary results (unpublished data) appear to support the findings made for the cat SOL muscle, as peak RMS amplitudes in the human RF were associated with intermediate muscle lengths at most frequencies of stimulation (Figure 7.4).

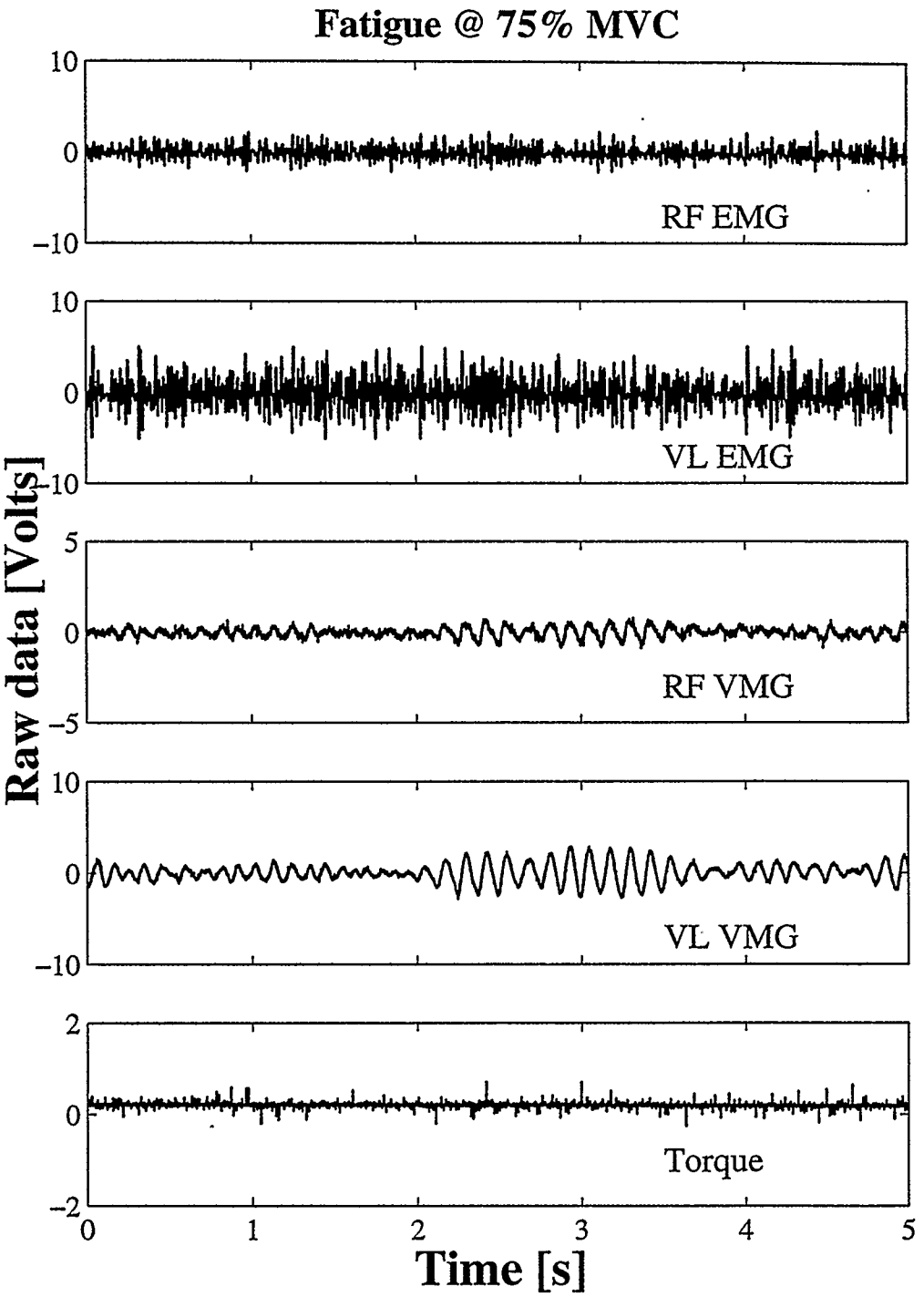

Figure 7.1. EMG and VMG signals obtained from RF and VL muscles and knee extensor moments obtained from the quadriceps of one subject during a fatigue protocol performed at 75% MVC. The data were obtained at the end of the fatigue protocol at approximately 53 seconds after the beginning of the contraction. Observe the increased amplitude and decreased frequency content in the VMG signal of VL.

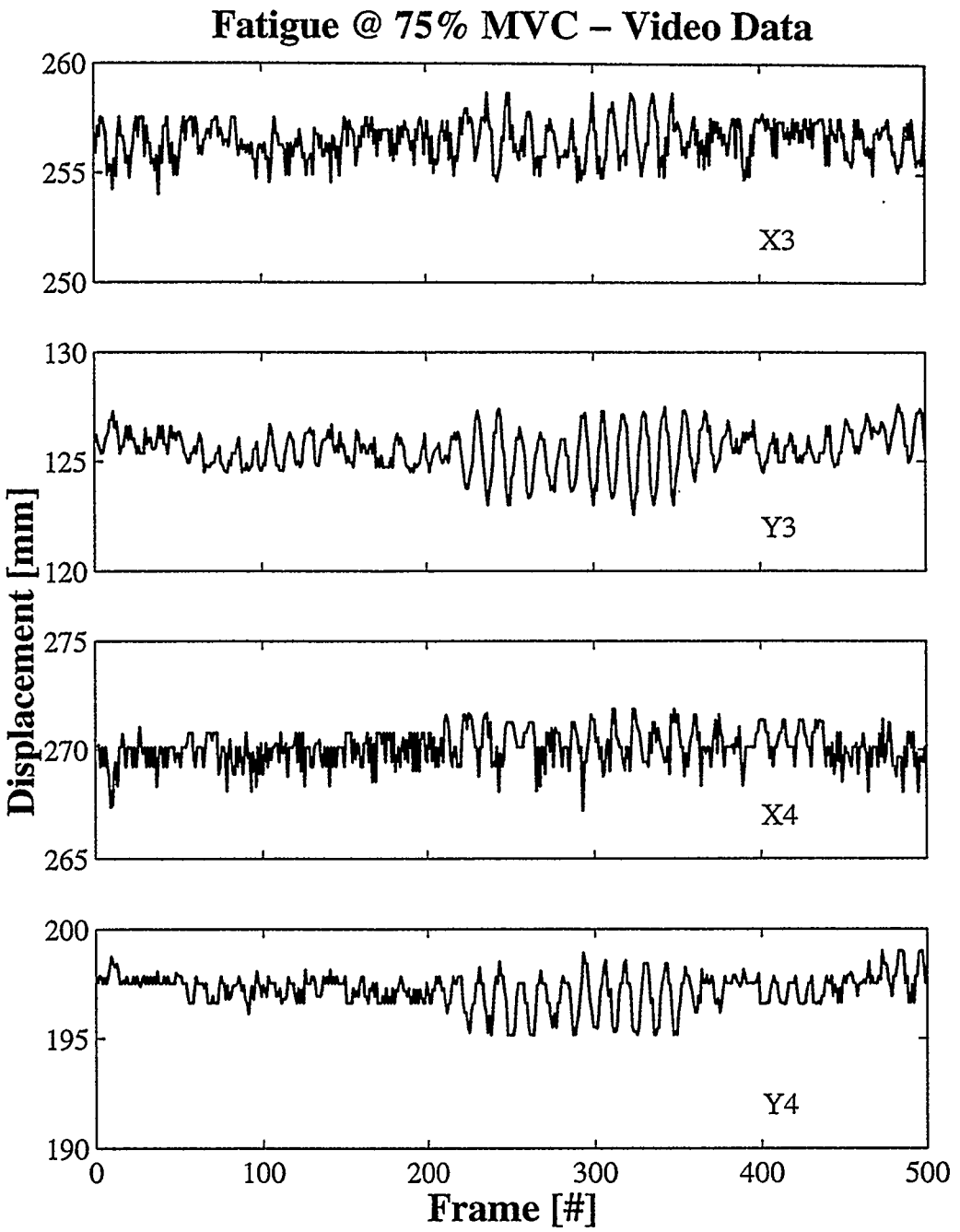

Figure 7.2. Video data obtained from the accelerometers (markers) placed in RF and VL. The data was obtained from the same subject and same time period as in Figure 7.1. Figures *X3* (RF accelerometer) and *X4* (VL accelerometer) represent displacement of the accelerometers along the axis of the limb, whereas figures *Y3* and *Y4* represent displacement in the frontal plane. Observe the same high amplitude, low frequency signal in both accelerometers in the y-coordinate, and compare with the VMG signals of VL in Figure 7.1.

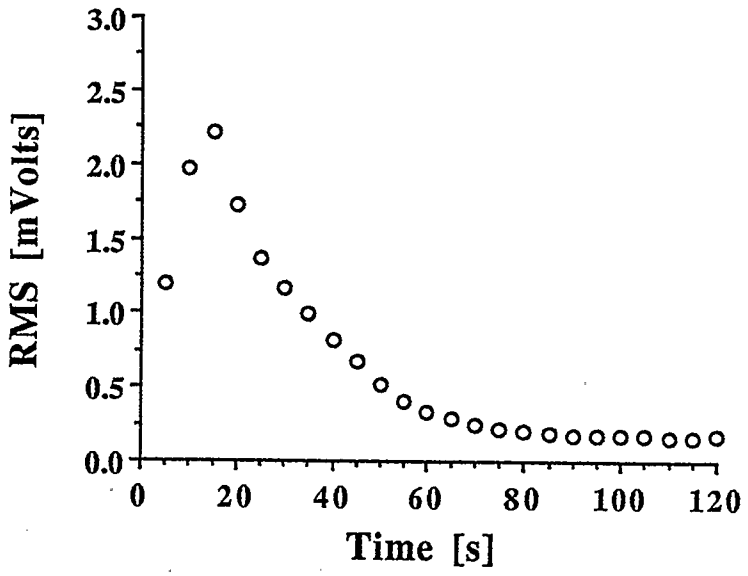

Figure 7.3. RMS of the VMG signal as a function of time during a fatigue protocol performed using pseudorandom electrical stimulation of the cat SOL nerve (18 Hz for 2 min). Observe the initial increase in the RMS values from 0-15 s and the decrease from 15-120 s.

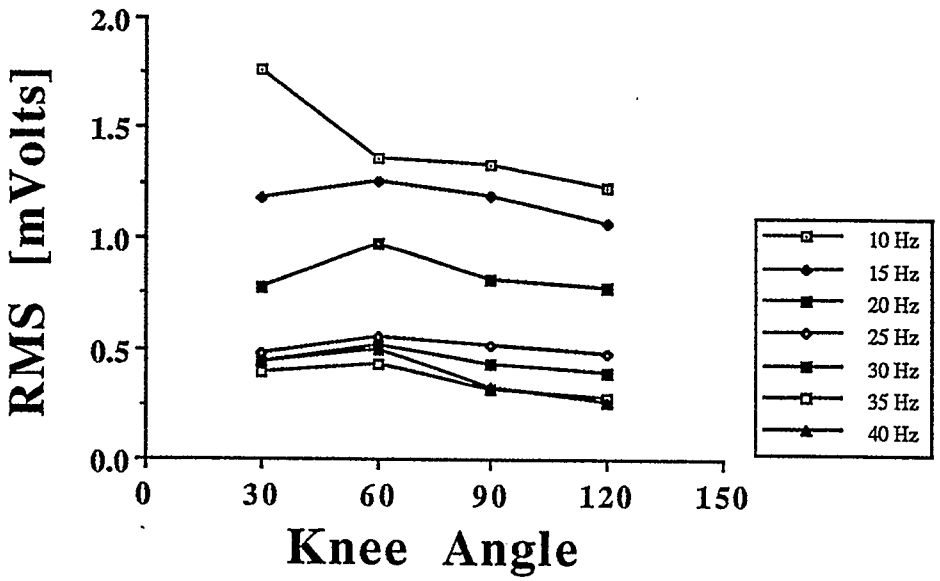

Figure 7.4. RMS of the VMG signal of RF at frequencies of stimulation of 10-40 Hz as a function of knee angle. Each value represents a mean value obtained from 20 subjects.

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