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THE EFFECTS OF DISCHARGE VARIABILITY ON THE CONTRACTILE RESPONSES GENERATED BY THE HUMAN LEG MUSCLES

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A thesis submitted to Western Sydney University in fulfillment of the requirements of Doctor of Philosophy

2016

Statement of Authentication

I, Michael Leitch, affirm that this thesis is based entirely on my own independent work, except where research has been done in collaboration with other colleagues and is stated in the journal articles listed below. I hereby declare that this thesis does not contain material previously submitted, either in full or in part, for the award of Doctor of Philosophy at Western Sydney University or any other institution.

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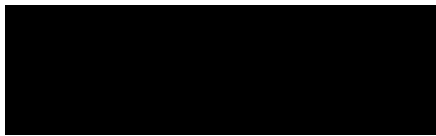
Michael Leitch

Leitch M, Macefield VG. Comparison of contractile responses of single human motor units in the toe extensors during unloaded and loaded isotonic and isometric conditions. Accepted for publication *J Neurophysiol*, 2014 (See Chapter 2)

Leitch M, Macefield VG. Microstimulation of single human motor axons in the toe extensors: Force production during long-lasting trains of irregular and regular stimuli. Accepted for publication *J Physiological reports* (See Chapter 3)

Leitch M, Macefield VG. Comparison of the ballistic contractile responses generated during microstimulation of single human motor axons with brief irregular and regular stimuli. Accepted for publication in *Muscle & Nerve* (See Chapter 4)

Leitch M, Brown R, Macefield VG. Intramuscular stimulation of tibialis anterior: The effects of discharge variability on force production and fatigue. Under review in *J Applied Physiol* (See Chapter 5)

" Life is like riding a bicycle. To keep your

balance, you must keep moving "

~Albert Einstein

Acknowledgements

First and foremost I would like to sincerely thank my supervisor, Vaughan Macefield. There have been times throughout this candidature where I have felt like giving it all up. Your continuous support, encouragement and belief in my ability have helped me to stay focused and achieve my goals. I cannot express in words my gratitude for the opportunities and endless experiences you have given me. Your astonishing career as a researcher is truly inspirational to me and I feel privileged to have worked with such an incredible scientist.

To my co-supervisor Rachael Brown, you have been such a dependable and reassuring person throughout this entire candidature. I appreciate your patience, encouragement and endless time you have spent training me. With your guidance, I have learnt essential skills that are important in becoming a successful professional. I hope this collaboration will continue for many years. Thank you so much Rachael.

To Christine Chiu, thank you for all the formatting, advice, fun times and lunches. It means a lot.

To my Uncle Steve and best friend Cam, you have supported me and kept me grounded when all my goals seem so unachievable. Thanks for everything.

To Zoe, you and I have been through a great deal together. I am privileged to have such a wonderful sister in my life. I thank you for your dependability and listening whenever I have needed it. Love you.

To my Dad, thank you for all your love and support throughout my life. I truly appreciate all the opportunities you have given me. Who would have thought, rigging to neuroscience. Thanks Dad.

To Kim, your academic achievements have inspired and driven me on more than one occasion. Without your continuous encouragement and belief in my hidden capabilities, I would not have continued this far. I am so glad that you came into our lives.

To my Mother, I am struggling to express in words my gratitude for all you have given and sacrificed throughout my entire life. Without your unconditional love, support and wisdom I would not have reached my goals. Thank you for taking Zoe and I on this amazing journey to Australia and never losing faith in me. I am forever grateful. Love you so much.

To my Grandparents, you have always given me your home, encouragement and support whenever I have needed it. Your words of wisdom and continued understanding through the toughest of times have been incredible. Without you both, this entire process would never have been achievable. Thank you for everything.

Lastly, I would like to show my appreciation to all of the Integrative team and the School of Medicine, Western Sydney University for this life changing opportunity. I will endeavor to contribute to science for many years to come.

Abstract

Both recruitment and rate coding are well-known mechanisms by which the human nervous system grades muscle force. This thesis has utilised the mechanism of rate coding to generate optimised stimulation patterns that enhance contractile responses in both a fatigued and non-fatigued state. Human motoneurones are known to fire with significant discharge variability (irregularity) during voluntary contractions. Yet many of the mechanisms as to why they fire in this manner, are still unclear. This thesis has tested the hypotheses that integrating physiological variability into trains of stimuli could offer some advantages to the human neuromuscular system that have not yet been explored. We have stimulated single motor axons and multiple motor units with long, short and continuous trains of stimuli that integrate discharge variability. The results reported in this thesis highlight the benefits that discharge irregularity offers to improving contractile responses and reducing fatigue in human leg muscles.

While the entirety of this research has been conducted in healthy human subjects, there is a potential for this to translate clinically. Functional electrical stimulation (FES) or neuromuscular stimulation is a well-known therapy utilised after stroke or spinal cord injury to assist in restoring motor function. It can help to reduce muscle atrophy, improve contractility and increase muscle strength by electrically exciting the muscles via surface electrodes directly over the muscle belly. Current FES stimulation patterns often incorporate high frequency, constant-interval stimuli that do not resemble the physiological patterns that are exhibited during normal voluntary contractions. This thesis has used novel stimulation patterns that emulate the firing of volitionally active motoneurones in an attempt to increase contractile responses and reduce the magnitude of muscular fatigue.

Acronyms and Abbreviations

α   alpha

μ micro

Ω   Ohm

Ach   Acetylcholine

ATP   Adenosine triphosphate

CFT   Constant-frequency train

CIT   Catchlike-inducing train

EDB   Extensor digitorum brevis

EDL   Extensor digitorum longus

EHL   Extensor halluces longus

EMG   Electromyography

FDI   First dorsal interosseous

FES   Functional electrical stimulation

Hz   Hertz

K^+ Potassium

mA   Milliamps

mN   Millinewtons

ms   Milliseconds

mV   Millivolts

Na^+ Sodium

SCI   Spinal cord injury

S.D   Standard deviation

SEM   Standard error mean

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CHAPTER 1

INTRODUCTION

1.1 Anatomy of the neuromuscular system

In order to appreciate the complexity of a muscle contraction, it is important to introduce the microanatomy of skeletal muscles and the motor nerves that supply them. Whole muscles are composed of muscle fibers, which, in many muscles, run the entire length. Skeletal muscles have different quantities of muscle fibers within the muscle. For example, larger powerful muscles such as gastrocnemius have more than ~ 1,000,000 muscle fibers, compared with smaller muscles such as the first dorsal interosseous of the hand ~ 40,000 fibers (Feinstein et al., 1955). Skeletal muscle cells are also multinucleated, containing important genes that regulate the production of enzymes and proteins necessary during a muscle contraction. Because they are multinucleated and multiple copies of the genes are available this allows very fast metabolic turnover, as is required with skeletal muscle. During embryological development embryonic cells known as myoblasts undergo fusion. Each nuclei in a muscle fiber is donated by a single myoblast (Okazaki & Flexner, 1966). However, not all myoblasts fuse - the unfused cells become satellite or supporting cells. After injury satellite cells then enlarge and divide which aids in the regeneration of muscle tissue.

1.2 The Motor unit

The motor unit comprises a motoneurone, its axon and terminals and all the muscle fibres it supplies. Humans have an incredible ability to control the level of force or movement

necessary to cover our wide range of behavioural repertoires. The forces generated are dependent on three main factors; 1) how many motor units are active, 2) the size of the activated motor units, and 3) how frequently those motoneurones fire an impulse. Skeletal muscles are activated by electrical impulses known as action potentials. These action potentials are delivered from the motoneurones in the anterior grey horn of the spinal cord, which can be driven through a monosynaptic connection to either corticospinal motoneurones or interneurones. The axon is surrounded by a myelin sheath composed of proteins and phospholipids, which permits propagation of nerve impulses from the neuronal cell body to the nerve terminals. When the signal travels down the axon it then divides into terminal branches that supply a group of muscle fibers within a whole muscle. One axon will supply many muscle fibers, but a single muscle fiber will only be innervated by one terminal branch. Therefore, when an action potential is propagated along a single axon, all fibers contract simultaneously, resulting in a weak yet synchronous contraction (Saladin, 2010).
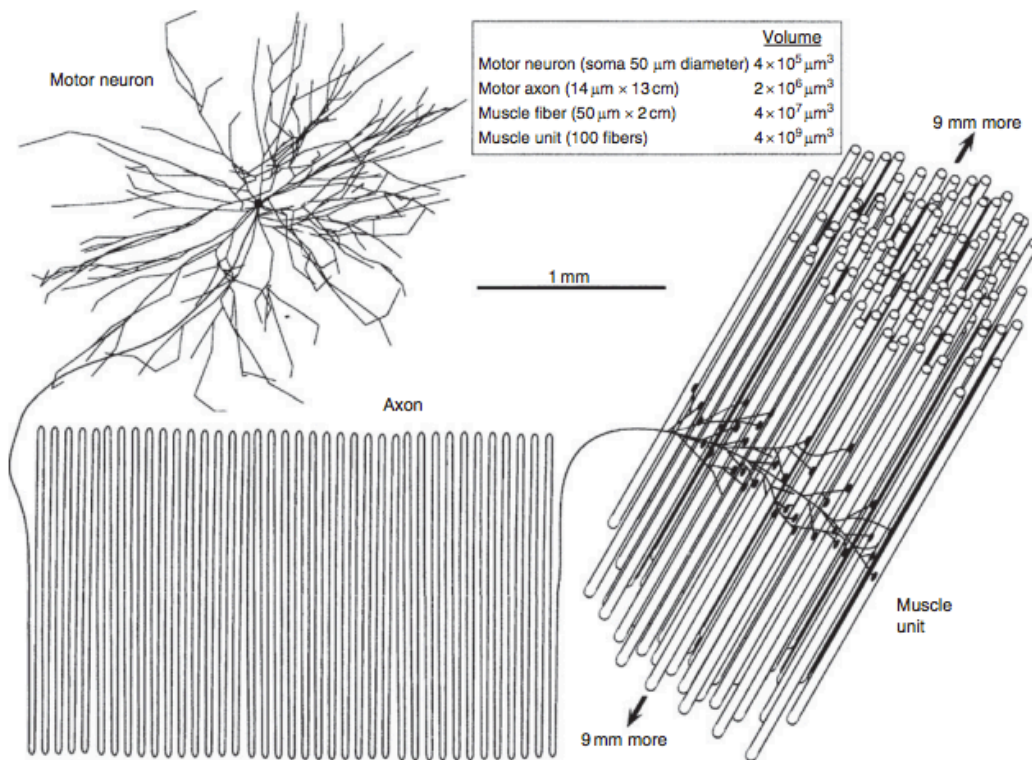
Figure 1.1: The motor unit includes the motor neuron, motor axon, terminal nerve fibers and all the muscle fibers innervated by that neuron. Note the length of the very long motor axon compared with a small neuronal cell body and large dendritic tree. One motoneurone will innervate many muscle fibers, yet one fiber will only be innervated by one terminal branch (Floeter, 2010).

1.3 Physiology of the neuromuscular system

The point at which the terminal branch of an axon meets the sarcolemma (muscle membrane) is known as the motor end plate. Essentially, this is the chemical synapse between the nervous and muscle tissue – the specialized synapse known as the neuromuscular junction. Once the nerve signal has reached the motor end plate there are a number of chemical processes, which are necessary. The terminal branch of the nerve projects onto the sarcolemma. This projection is known as the synaptic knob. However, the synaptic knob does communicate directly with the sarcolemma. There is a distinct 'gap' for chemicals to pass between the synaptic knob and sarcolemma termed, the synaptic cleft. Within the synaptic knob are an abundance of vesicles that contain a

neurotransmitter known as Acetylcholine (ACh). When an action potential arrives at the

synaptic knob there is an influx of calcium, which causes the vesicles to release their

contents through a biological process, known as exocytosis. Acetylcholine then binds to

ligand-gated channels (Ach receptor) on the post-synaptic membrane. We can consider

ACh to be the chemical messenger between the nerve terminals and the organelles of the

muscle fiber (Saladin, 2010).
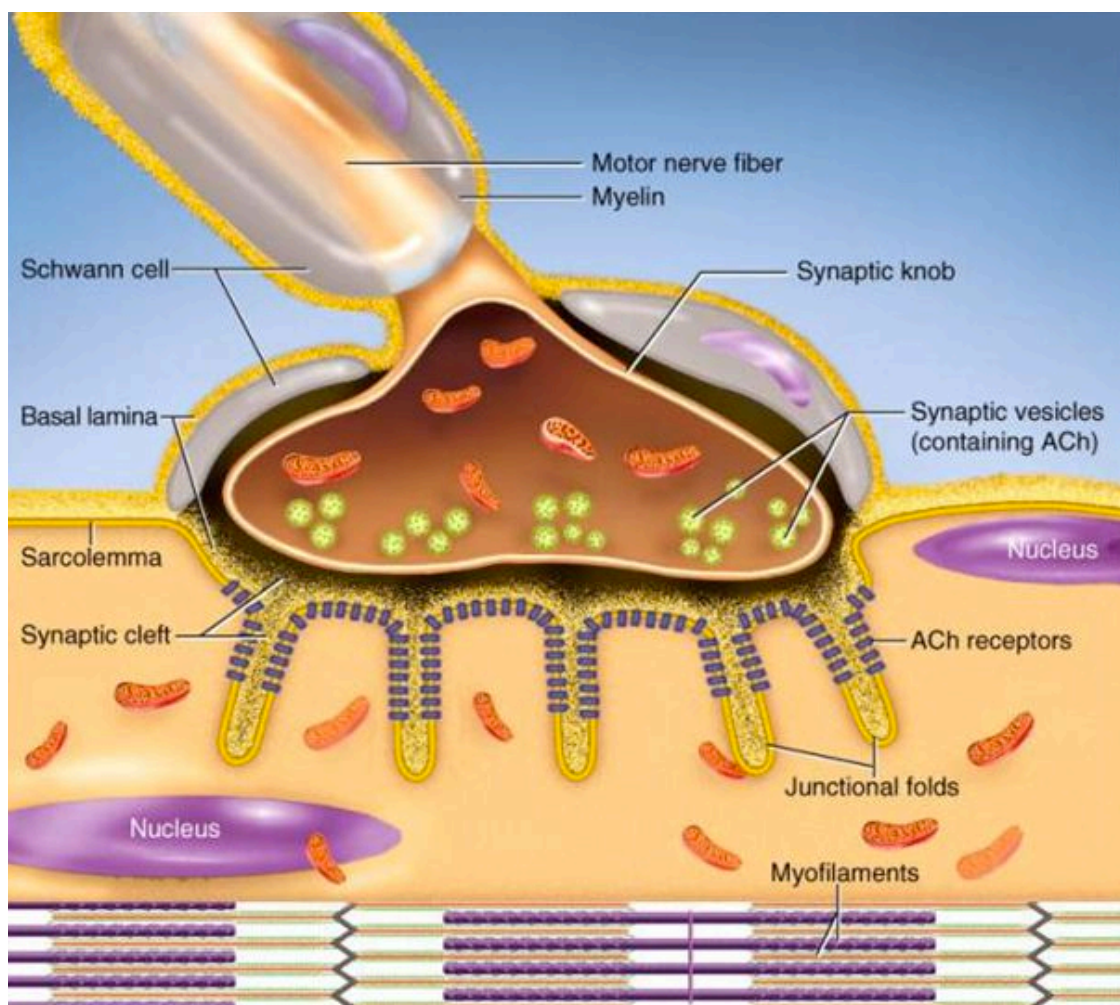
Figure 1.2: The neuromuscular junction transmits the electrical signals from the terminal branches of the motor axon across the synaptic cleft through chemical neurotransmitters to the muscle membrane (Saladin, 2010).

1.4 Excitation-contraction coupling

Once the sodium (Na^+) channel, is open there is a shift in ion concentrations from outside to inside of the cell. Sodium diffuses rapidly into the cell from its high extracellular concentration down its concentration gradient, while potassium (K^+) diffuses out. As a result of this uneven shift in ion concentration, the muscle membrane exhibits a change in polarity. Due to the high quantity of positive sodium ions, the membrane potential rises rapidly from ~ -95mV to ~ +30mV. This rapid shift in membrane potential results in the generation of an action potential, hence the sarcolemma is excited. The action potentials generated during this process are not isolated to one terminal, rather it spreads along the entire length of the muscle.

Muscle cells have many smaller organelles necessary to function successfully. Just like other cells of the body, a plasma cell membrane known as the sarcolemma surrounds the muscle fibers. And, like all cell membranes of excitable cells, the sarcolemma has a transmembrane potential, due to the uneven distribution of positive and negative ions across its membrane (Saladin, 2010). A change in this transmembrane potential is the first in many steps to elicit a muscle contraction. Skeletal muscle fibers contract simultaneously, thus, the signals must be rapidly distributed throughout the entire interior of the cell. Deep to the sarcolemma is the cytoplasm (the sarcoplasm). The sarcoplasm contains an abundance of stored glycogen necessary in muscle function and contraction. Within the sarcoplasm are all the individual muscle fibers. Just as many muscle fibers make up a whole muscle, many myofibrils also make up a single muscle fiber (Sherwood, 2004). It is important to consider the myofibril in more detail, because this is where the true complexity of muscle contraction is understood. Myofibrils are long chains of proteins, which are subdivided into smaller functional components known as

myofilaments. The myofilaments are categorised into two major classifications, associated with different contractile proteins. Firstly, thick filaments contain a contractile protein called myosin. At one end of the thick filament is a globular head projecting on a slight angle. The thin filaments are made up of another contractile protein known as actin. Actin contains the active binding site to which the head of the thick filament attaches during shortening. During a relaxed state, the active sites are covered by a further two proteins known as troponin and tropomyosin. These important proteins associated with the filaments are crucial for a muscle contraction to occur (Saladin 2010). Surrounding each myofibril is a critical organelle known as the sarcoplasmic reticulum, a specialised type of smooth endoplasmic reticulum responsible for the regulation of calcium ions. Continuous with the sarcolemma are fine tubes known as transverse tubules; these structures vary in number and size depending on muscle type. When electrical impulses (action potentials) travel along the sarcolemma this stimulates the release of calcium from the sarcoplasmic reticulum (SR) into the cytosol. While the SR contains an abundance of releasable calcium, the T-tubules are sensitive to low levels. Essentially, the sarcoplasmic reticulum is the provider of calcium, and the t-tubules signal when to release bursts of calcium into the cytosol. All of these organelles are involved in initiating muscle contraction (Jenkins et al., 2010).

As the action potential spreads along the sarcolemma it then excites the t-tubules, which penetrate the myofibrils and signal the sarcoplasmic reticulum to release calcium into the cytosol. According to the sliding-filament hypothesis, contraction occurs due to the thin filaments (Hanson et al, 1953; Huxley, 1969) over the thick filaments, generating tension in the muscle. As noted above, there is an important shift in the tropomyosin, a protein on the thin filament in order for binding of the thick filament. Calcium enters and

binds to troponin on the thin filament. This binding results in a conformational shift of the underlying tropomyosin and exposes the active binding sites. Essentially, the globular head of the thick filament attaches to the active sites, creating a myosin-actin cross-bridge, sliding the thin filament over the thick filament in a power stroke. Once nerve signals stop then ACh stops being released. Consequently, calcium is not released and the fibers relax. Of course during both contraction and subsequently relaxation, Adenosine Triphosphate (ATP) is essential, as this is the primary energy currency in the human body (Scott et al., 2001).

1.5 Isotonic vs. isometric contractions

The term contraction does not always refer to the shortening of a muscle, it often refers to the muscle producing tension with an external resistance causing it to remain the same length i.e. the tension never exceeds the resistance. For a muscle to develop tension the surface membranes must be depolarised (Thomas et al., 2006) and the cross-bridges develop sufficient tension to overcome resistance. Isotonic, by definition means 'equal tension'. Therefore, internal and external tension in the muscle rises until such time that the tension in the tendon exceeds that of the external resistance.

There are two types of isotonic contractions to consider, concentric and eccentric. During a concentric contraction external resistance is overcome by the muscle and tendon tension, such as occurs when lifting a glass off the table. The muscle tension overcomes the external resistance and the arm is flexed. Eccentric contractions are those in which the peak muscle tension developed is less than the external resistance. For instance, if you are lifting a dumbbell and the weight becomes too heavy the arm will lengthen to relieve the tension and fatigue. No matter how hard you try to pull the weight it will fall.

Therefore, contraction time or twitch duration also reflects the external resistance. For example, in the first analogy for concentric contractions, the glass is very light and thus the muscle will contract and shorten very quickly. However, with the dumbbell during eccentric contractions, while you may try to fight the resistance, eventually the external resistance will be too much. This means that during loaded isotonic conditions, contractions would be expected to be slower and twitch durations longer. Experimentally, isotonic contractions are measured in terms of linear or angular displacement. In experimental animals, where the tendon can be separated from the bone, isotonic contractions are measured as a change in length (Jewell & Wilkie, 1960), but in human subjects an isotonic contraction is typically measured in terms of angular displacement (Bosco et al., 1983; Magnusson et al., 2001; Lee et al., 1999)

During an isometric contraction there is almost a "deadlock" between the external resistance and the tension developed. During this type of biomechanical condition, the tension developed by the muscle fibers never exceeds that of the external resistance. For instance, when holding a heavy box out in front of your body the muscle itself does not change in length, however there must be an effective level of tension development, in order to retain the position of the box. During everyday life we are constantly working to maintain an upright posture. Because the weight of the head and limbs are transmitted down the vertebral column, the muscles of the back (erector spinae) are continually working to prevent the vertebral column from falling forwards (Lovejoy, 1988). Therefore, while the muscles during isometric conditions do not shorten anywhere near as much as during isotonic conditions, the muscle fibers still shorten until the tendon is tight and the tension of the muscle is equal to that of the external resistance. Unlike unloaded isotonic contractions, which are usually measured as a change in displacement,

an isometric contraction often refers to the measurement of force, as there is no change in the angle. However, during an isotonic contraction where there is addition of a load, both force and displacement could be measured concurrently.

1.6 Physiological classification of muscle fibers and motor units

Muscle fibers can be classified as fast-twitch (IIa, IIb) or slow-twitch (I) and can be described using various histochemical, biochemical, morphological or physiological techniques (Burke et al., 1971; Pette et al., 1999; Larsson et al., 1991). These different techniques do not always agree, therefore different classifications are realised on the basis of different techniques (Staron 1997). Fast twitch muscle fibers, which are rich in enzymes of the phosphogen and glycogen-lactate systems, can be subdivided into fast fatigable (IIa) and fast fatigue-resistant (IIx). It has been shown histologically that fast twitch muscle fibers contain higher amounts of myoglobin, mitochondria and blood capillaries, and have higher levels of ATPase activity which therefore leads to faster shortening than slow twitch muscle fibers (Barany 1967). Slow-twitch fibers differ from the fast-twitch fibers by displaying a much lower capacity for anaerobic glycolysis. Slow-twitch fibers depend more on aerobic glycolysis, that is, they are adapted to aerobic respiration, which does not generate lactic acid (Burke et al. 1973). Therefore, unlike fast-twitch fibers slow twitch (I) fibers do not fatigue easily. Although muscle fiber classification is essential in understanding contractile mechanisms, the functional unit of the neuromuscular system is the motor unit. The contractile properties of motor units have direct correlations to their classification, and based on their contractile properties they can be classified as fast-twitch (F) and slow-twitch (S). Research conducted in the cat has subdivided these into fast-twitch fatigue fatigable (FF) and Fast-twitch Fatigue resistant (FR) fibers by examining the physiological properties (Burke et al., 1973;

Collatos et al., 1977; Botterman et al., 1985; Jami et al., 1982). In addition, individual muscles themselves have also been classified based on function. For example soleus has been studied extensively in the cat due to its important role in locomotion and thus is considered to be comprised of mainly slow twitch (I) muscle fibers (Houk et al., 1970; Joyce et al., 1969; Rack & Westbury, 1969). Yet other muscles in the cat such as flexor digitorum longus (FDI) needed for dynamic movements have been classified as a fast twitch muscles based on their twitch and histochemical properties (Luff, 1975; Edjtehadi & Lewis, 1979). In addition to work conducted in animals, human muscles have also been classified based on their twitch properties. For example tibialis anterior is considered to be a mixed muscle (Van Custem et al., 1997) and soleus and slow twitch muscle (Bawa and Stein, 1976; Vandervoot & McComas, 1983). In addition, muscles of the human hand have been characterized based on twitch properties using either microstimulation or spike triggered averaging (Thomas et al., 1990; Calancie & Bawa, 1986; Kossev et al., 1994).

1.7   Rate coding vs. recruitment

There are two interrelated processes by which the motor system grades its output. Firstly, by varying the number of motor units that participate in a contraction. This is referred to as recruitment (Henneman et al., 1965) - whereby motor units are recruited progressively according to the size of contraction produced (Olson et al., 1968). Secondly, by modulating the rate at which action potentials drive the active motor units, which is known as rate coding. Both recruitment and rate coding are essential in activation of muscle fibres because initially there are no active units whose firing rate can be increased. However, if all the motor units in a given muscle were activate then the only way to increase the force of the contraction would be to increase the firing rate of the

active motoneurone, i.e. through the mechanism of rate coding. It has been shown that rate coding is generally more prevalent at maximal effort above 80% of MVC, and recruitment at lower levels of voluntary contraction (Milner-Brown et al., 1973a; De luca et al., 1982; Kernell & Sjoholm 1975). However, it has also been revealed that this is dependent on which muscle is being activated. For example, the deltoid (shoulder) muscle has been said to rely primarily on recruitment as a mechanism to increase force (De Luca et al., 1982), whereas the first dorsal interosseous (intrinsic muscle of the hand) relies largely on rate coding (Milner-Brown et al., 1973b). Morphologically the deltoid is classified as a large multi-pennate muscle containing more than 1000 motor units (Christensen, 1959). Functionally, it is a powerful contractor, and thus is said to utilise recruitment because each motor unit contributes only a small percentage of the total output. Conversely, the first dorsal interosseous (FDI) muscle, a small bipennate muscle that abducts the index finger contains approximately 120 motor units and acts functionally in fine motor control (Feinstein et al., 1955). Due to the low number of motor units in the FDI muscle all of the motor units must, at some point, contribute to the total force output. Therefore, the only way to increase this force is by utilising rate coding. Because the FDI muscle is involved in fine motor control of the index finger it, like other intrinsic muscles of the hand, must have the ability to produce smooth linearly varying isometric contractions and consequently relies more on rate coding to grade force output (De Luca et al., 1982). The transformation of discharge rate into force by motor units therefore represents a fundamental feature by which the nervous system controls skeletal muscles (Fuglevand et al., 1993). The use of force-frequency relationships has been widely utilised in many previous studies to understand the contribution of rate coding to increasing force production of single motor units (Adrian and Bronk, 1929,

Bigland and Lippold, 1954, Botterman et al., 1986, Fuglevand et al., 1999, Thomas et al., 1991a)

1.8   Recording from peripheral nerves

Hagbarth and Vallbo developed the invasive nerve recording technique known as microneurography in the 1960's. This involves the insertion of an insulated tungsten microelectrode through the skin directly into the peripheral nerves of awake human subjects, and allows for the direct recording of action potentials from single large-diameter sensory axons (afferents) supplying muscle spindles and Golgi tendons, from muscle fascicles and from afferents supplying mechanoreceptors in the skin from cutaneous fascicles; efferent sympathetic nerve activity can also be recorded (Vallbo & Hagbarth, 1968; Johansson & Vallbo, 1979; Johansson et al., 1982; Condon et al., 2014). The ability to record action potentials from single nerve fibers has greatly enhanced the understanding of human somatosensory and sensorimotor neurophysiology. It has allowed a detailed investigation of the firing properties of single sensory axons supplying low-threshold mechanoreceptors in the skin and properties of skeletal muscles. In the early 1980s intraneural microstimulation was developed by Torebjork and Ochoa (Torebjork and Ochoa, 1980). Using a comparable microelectrode, Torebjork and Ochoa (1980), as well as Vallbo and colleagues (1980) selectively stimulated single cutaneous afferents, giving them the ability to further understand the roles of the different tactile receptors in the human hand (Ochoa & Torebjork, 1983).

Recording from motor nerve fibers, in animals, was first developed by Adrian and Bronk in 1928 who used concentric needle electrodes to assess the discharge of motor units of the phrenic nerve in rabbits (Adrian and Bronk, 1928a; Duchateau & Enoka, 2011). In a follow up study they also assessed the discharge rates in peroneus longus and quadriceps

of the cat hindlimb (Adrian and Bronk, 1928b), during weak contractions. They concluded that the main contributor to force arises from increases in the discharge rate for those muscles. In addition to studying motor units during weak contractions, single motor unit activity has also been recorded during maximal voluntary contractions in human subjects. These studies were conducted by inserting an insulated tungsten microelectrode directly into the muscle belly of the contracting muscle (Fig.1.3). Discharge impulses could then be examined by sampling brief spike trains as the microelectrode passes through the contracting muscle (Bellemare et al., 1983; Freund et al., 1975; Thomas, 1995; Macefield et al., 2000). From this, spike trains are recorded during a sequence of contractions, which allows for the formation of a time-varying picture of changes in firing rate during maximal voluntary contractions (Milner-Brown et al., 1973). During a maximal voluntary contraction however, there is considerable movement of the electrode due to shortening of the muscle. Consequently, the microelectrode must be constantly adjusted in order to sample long-trains of action potentials (Clamann, 1970; Macefield et al., 2000; Weber et al., 2012).
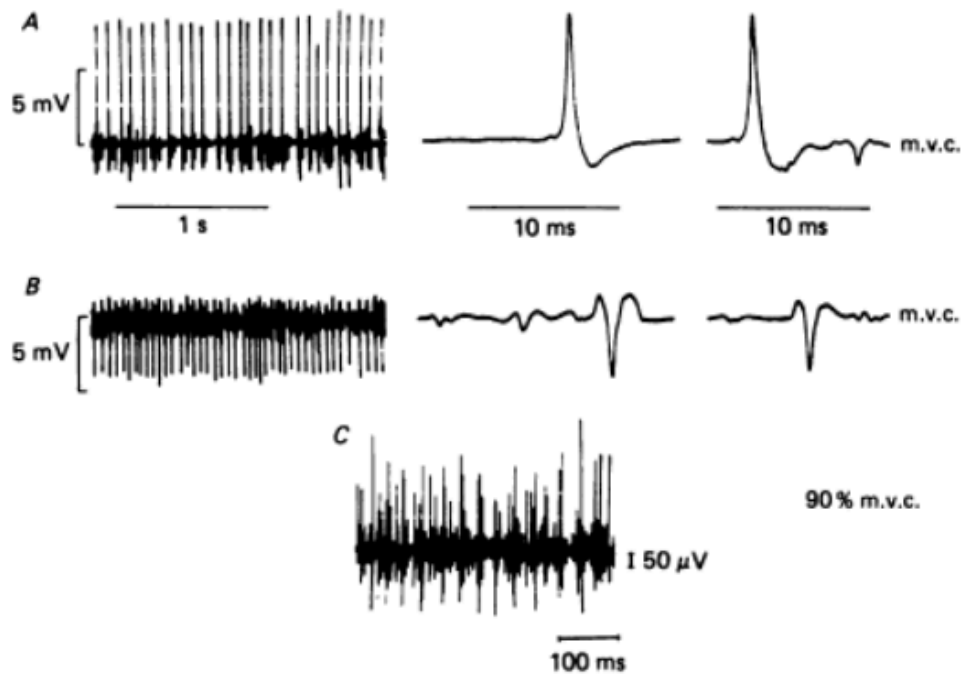
Figure 1.3: A and B represents motor unit potentials recorded from adductor pollicis during a voluntary contraction using tungsten microelectrodes. C represents raw spikes recorded from fine wire electrodes during near maximal voluntary contractions (Bigland-Ritchie et al 1983)

1.9 Intraneural microstimulation

Intraneural microstimulation of single motor axons has allowed the detailed investigation of muscles in both the upper and lower limbs (McNulty & Macefield, 2005). The first studies to examine the twitch, tetanic and fatigue properties of single motor units used intraneural microstimulation of single motor axons. They inserted tungsten microelectrodes into the median nerve of the upper arm and selectively stimulated single motor axons supplying the thenar muscles, which act on the thumb (Westling et al., 1990; Thomas et al., 1990). These are short muscles with relatively short tendons, differing from the toe extensors, which are long muscles with very long tendons. Unlike the thenar muscles, single motor units of the toe extensors generate twitches that are larger and much slower than that of the thenar units (Fuglevand et al., 1999, Macefield et al., 1996). Because of this, toe-extensor motor units require lower stimulation

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frequencies in order to generate maximal force, such that the relationship between force and frequency is shifted to the left (Macefield et al., 1996). However, like the thenar motor units, there is little association between twitch and tetanic force and contraction time for the toe-extensor motor units (extensor hallucis longus, extensor digitorum longus and extensor digitorum brevis), which are similar in this regard. While recordings from the proximal phalanx of the big toe has allowed investigators to examine force production produced by extensor digitorum longus, the force was taken from just one of the digits (Macefield et al., 1996). This is somewhat controversial because previous work has shown that multi-tendoned muscles, such as those in the cat limb, generate force that is distributed across all tendons (Fritz and Schmidt, 1992); therefore, it is reasonable to assume that the forces generated would have been greater had all four digits been measured. It is also possible that motor units within these muscles may be distributed similarly to that of the lumbricals of the cat hind foot, in which the nerve supplying the two muscles contains some motor axons that branch off and innervate each muscle (Emonet-Denand et al., 1971). Nevertheless, although both the toe extensors and longer finger flexors have long tendons, the units of the finger flexors have shown faster contraction times than the toe extensors - or thenar muscles - therefore the different muscle groups reflect different contractile properties (Fuglevand et al., 1999, McNulty et al., 2000).

The larger, yet slower responses of the toe extensors allowed for the individual twitches to fuse more readily than the thenar muscles, which have a faster twitch response. Although the mean frequency for half maximal force was lower in the toe-extensors by around 2.5 Hz this was related to the different contraction times of the different muscle groups, and it would appear that the differences in contractile properties between motor

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units of different muscles are related to differences in firing rates of individual motor units (Macefield et al., 1996). As is shown by previous studies during maximal isometric contractions, toe extensor muscles have lower maximal firing rates than the intrinsic hand muscles (Bellemare et al., 1983, Bigland-Ritchie et al., 1992, Macefield et al., 2000). These differences in firing rates could be due to their different functions within the body - i.e. holding and gripping objects vs. locomotion. While this thesis focuses on rate coding, as a major factor in force production, there is no doubt that recruitment plays a vital role in the activation of muscle fibers.

1.10   Twitch Properties of the Toe Extensors

Much of the previous research designed to characterize the contractile responses of single human motor axons have used force as the form of measurement. Macefield and colleagues (1996) examined the effects of regular trains of stimulation with increasing frequencies from 2-100Hz on the human toe extensors and illustrated that the peak force is related to the stimulus frequency in a sigmoidal fashion. In relation to raw data, their study shows longer twitch properties, with a mean rise-time of 75 ms was observed and 45 ms when measured using angular displacement. This difference in rise time has been previously explained purely on a biomechanical basis. Hill (1951) identified the significance of tendon compliance, showing that twitch forces were reduced and contraction times increased by addition of in-series compliance to muscles. This may account for some of the differences in rise-time for the toe extensors and also play a smaller role when considering isotonic contractions. Twitch tension and recruitment threshold has also been closely associated with axonal conduction velocity, with the excitability of axons being inversely related to the electrical stimulation and its diameter (Dengler et al., 1988).

1.11   Force-frequency relationships

During a voluntary contraction, the tension is proportional to the frequency of the electrical activity, under isometric (static force) and under isotonic (moving) conditions (Bigland & Lippold, 1953, Bigland & Lippold, 1954; Lippold 1952). The work conducted on humans is, however, underpinned by earlier work conducted on animals such as cats, which has provided fundamental knowledge on the response of a muscle to repetitive stimuli. In the 1930's, Cooper and colleagues showed that there is a distinct relationship between rate of stimulation and tetanus development: the response of a muscle to a repetitive stimulus was greater the shorter the inter-stimulus intervals They were the first to establish that there is in fact, a distinct relationship between frequency of stimuli and the tetanic response, at which individual twitches are fused. They concluded that higher stimulation frequencies result in greater responses from the muscle, under isometric conditions (Cooper, 1930).This relationship has been studied in further detail by others and has been pivotal in our understanding of both the mechanical and electrical properties of human motor units (Botterman & Cope, 1988; Fuglevand et al., 1999; Westling et al., 1990). Fig. 1.4 shows the force-frequency relationship for a single motor unit in flexor pollicis longus during microstimulation of a single motor axon in an awake human subject. It can be seen that the relationship is sigmoidal. Fig. 1.5 shows a steep linear portion of the sigmoidal force-frequency curve, and interestingly, volitionally activated motoneurones naturally fire within the middle of this range i.e. during ramp voluntary contractions they initiate firing at ~8Hz and stop firing at ~30Hz. Therefore, for a given increase in firing rate within this linear range there will be a predictable increase in the force produced. The stimulus frequency required to generate half-maximal force should be relatively close to the centre of the steep range, and stimulation at

frequencies above 30 Hz will not result in the production of higher forces. However, this is muscle dependent. Studies conducted by Brenda Bigland-Ritchie and colleagues (1983) reported the known minimum and mean firing rates of motor units supplying adductor pollicis, biceps brachii and soleus muscles. Minimum firing rates in soleus of 6-10 Hz and increasing to a mean of 10.7 Hz, yet for biceps brachii and adductor pollicis units began firing at 10 Hz and had a mean firing rate of 30 Hz. Importantly, the force output for all units did not increase beyond ~30Hz. They concluded that during voluntary contractions discharge rates are limited to those minimally sufficient to generate maximal force, thus, there is no useful purpose in increasing discharge rate.
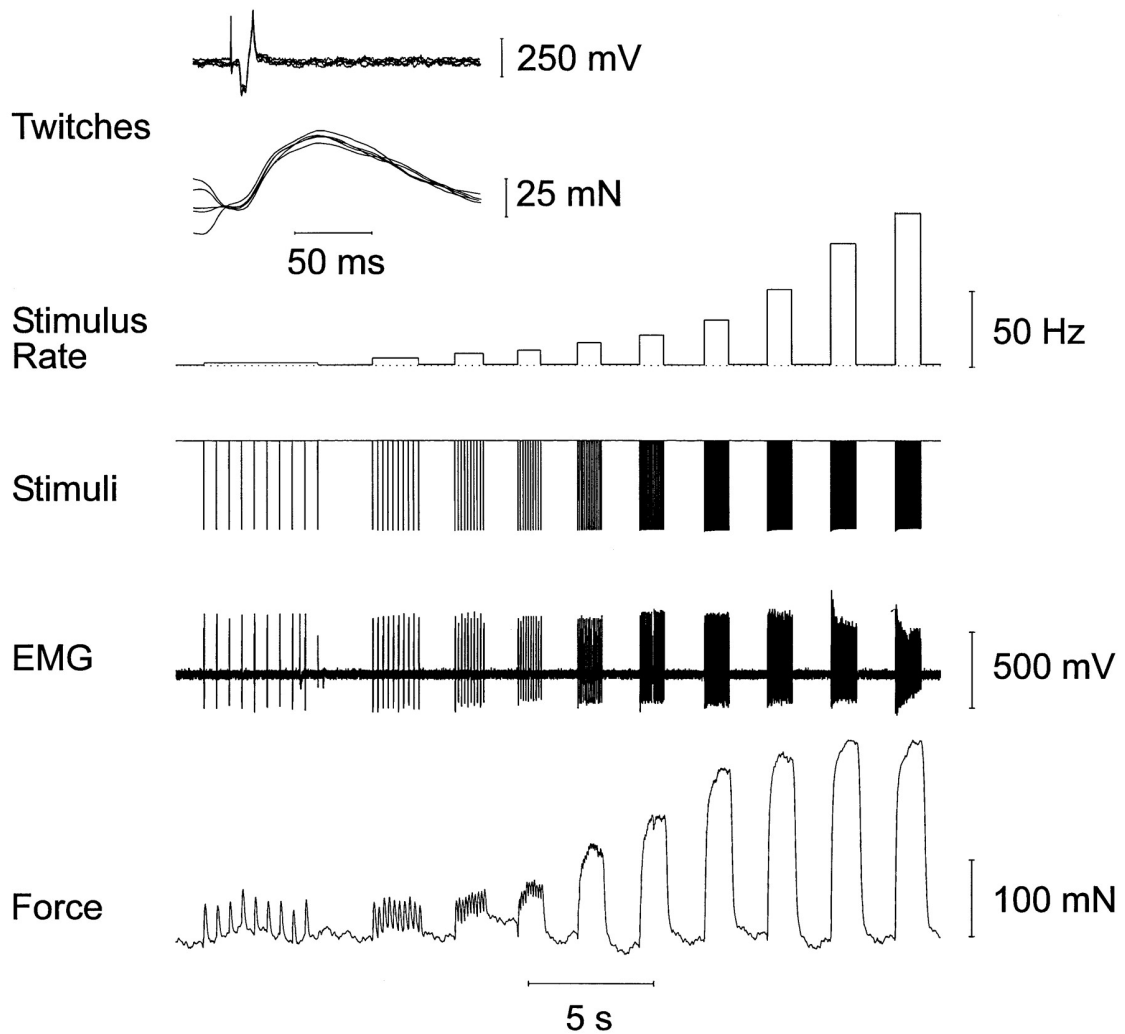
Figure. 1.4: Force-frequency relationship of a single motor unit in flexor pollicis longus produced by intraneural microstimulation of a single motor axon. The EMG response and twitch forces produced by single stimuli are shown in the top panel. It can be seen that increasing the frequency of stimulation increases the force until a plateau is reached at approximately 30 Hz (Fuglevand et al., 1999).
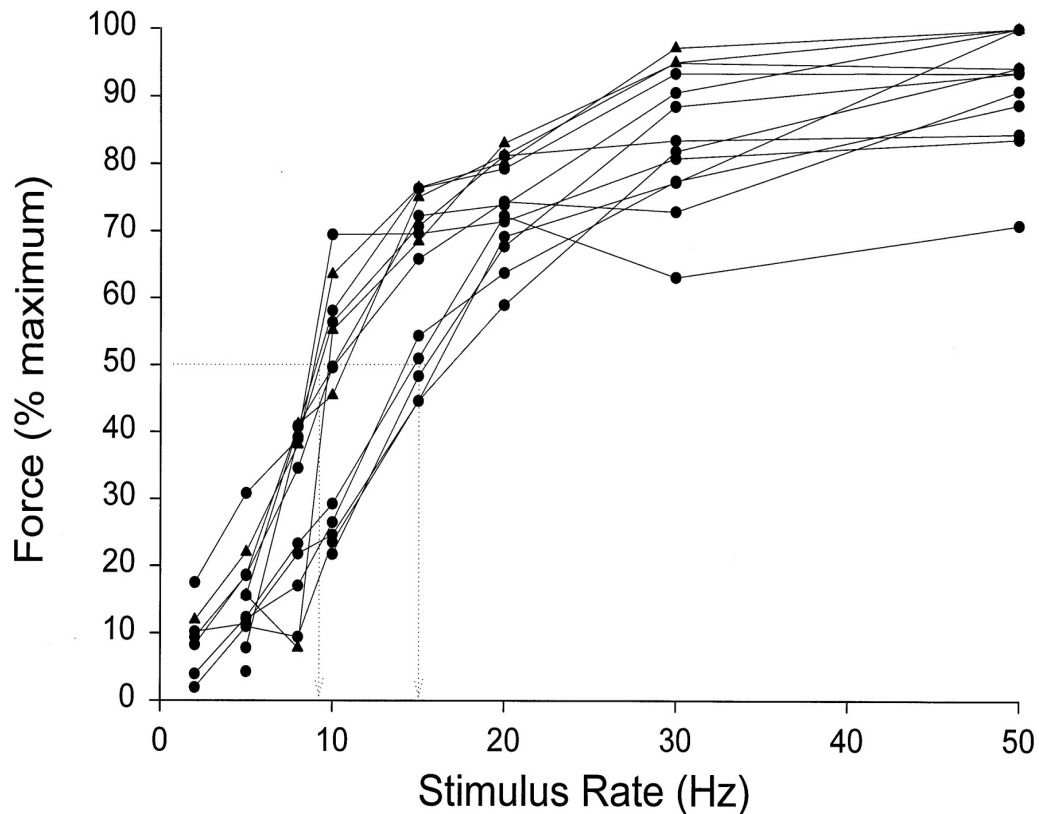
Figure 1.5: Force frequency curves for single motor units in the long finger flexor muscles. Two groups can be seen: one that requires lower stimulation frequencies in order to generate half-maximal force and one that requires higher frequencies (Fuglevand et al., 1999). This figure clearly shows the linear portion of the force-frequency curve with a clear plateau at around ~30Hz.

It has been observed that preceding a stimulus train with an "initial doublet" – two stimuli separated by 10 ms –results in a potentiation of force, as shown in Fig. 1.6. Human voluntary contractions are occasionally preceded by an initial doublet, and it has been suggested that stimuli that include high-frequency periods are more effective in generating force (Macefield et al., 1996; Bawa & Calancie, 1983; Person & Kudina, 1972; Kudina, 1974; Garland & Griffin, 1999).

extensor digitorum brevis

frequency

20 Hz

EMG

100 mV

dorsiflexion force

50 mN

0.5 s
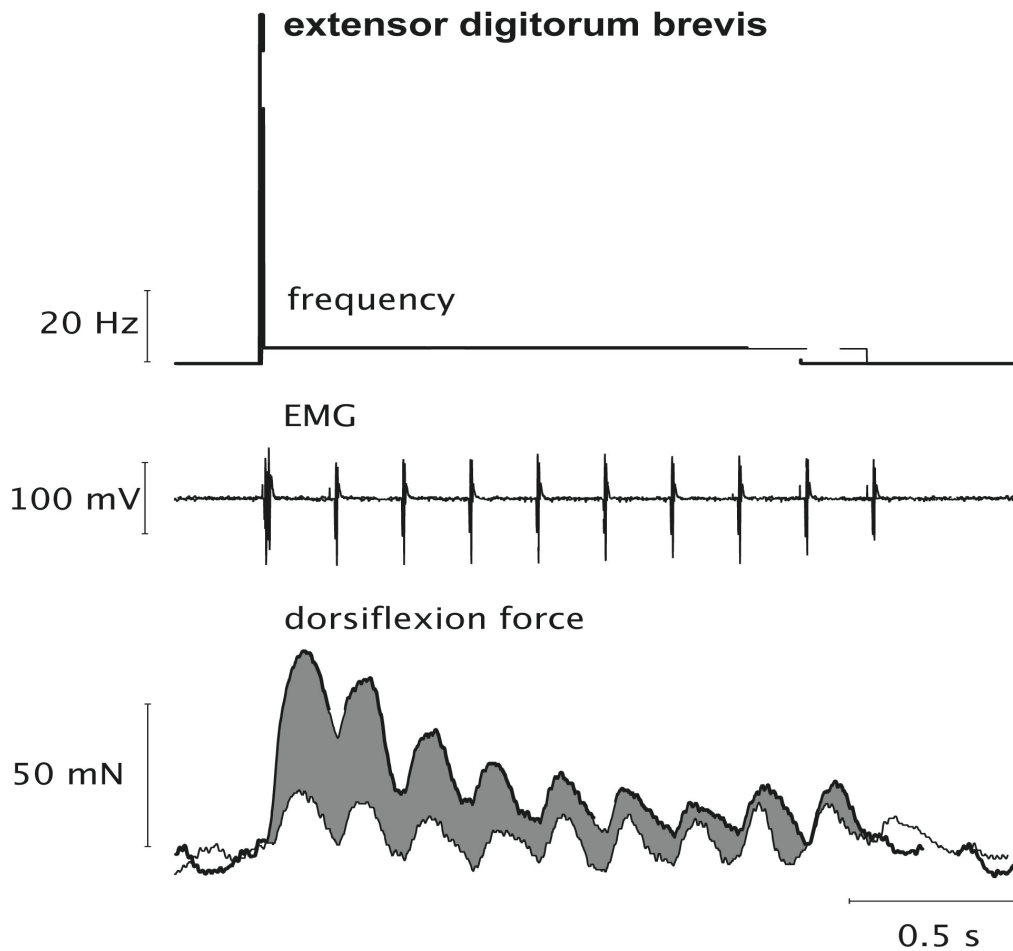
Figure 1.6: Forces generated by intraneural microstimulation of a single motor axon supplying extensor digitorum brevis (EDB) with a 5 Hz train of stimuli. Although the total number of stimulus pulses was identical, force was higher when the interval between the first two stimuli in the train was reduced to 10 ms (100 Hz), which also resulted in the forces generated by subsequent stimuli being higher (Macefield et al., 1996).

1.12 Catch-like Properties

It has long been observed that the use of initial brief high frequency pulses at the beginning of a sub-tetanic train of stimuli results in a brisk increase in force production. This has been shown in animal models (Callister et al., 2003; Celichowski et al., 1998), and in humans during microstimulation of single motor axons (Macefield et al., 1996) and whole muscle stimulation (Binder-Macleod et al., 1996 & 1999). However, this was first described by Burke and colleagues (1970), where they stimulated slow-twitch motor

units of the triceps surae muscle in the cat. They termed this physiological phenomenon the 'catch-like property'. This refers to the tension enhancement seen in skeletal muscles due to an initial brief high-frequency burst of pulses at the beginning of a sub-tetanic, constant-frequency train. When stimulating a whole nerve it has been shown that when preceding a constant interval train (CIT) with an initial inter-pulse interval of 5ms during isotonic and isometric contractions it produces a greater force-time integral, peak force and average force than a constant frequency train as can be seen in Fig. 1.7 (Binder-Macleod and Lee, 1996). More specifically Binder-Macleod and colleagues (1991; 1996; 1997) stimulated the quadriceps femoris muscle by placing surface electrodes directly over the muscle belly. They stimulated whole muscle with constant frequency trains (70 Hz) and catch-like inducing trains with initial short intervals of 5ms. They found the results as stated above were even more prominent during a fatigued state. Using initial short interval bursts more closely emulates the firing patterns of the central nervous system (CNS) during physiological conditions. It is well documented that volitionally active motor units discharge with significant variability (Moritz et al., 2004; Laidlaw et al., 2000) and is calculated by the coefficient of variation (S.D/Mean). This integrates both short and longer interspike intervals and this physiological variability is known to increase with age (Enoka et al., 2003; Christou & Carlton, 2001). When an initial short interval precedes a train of stimuli, it reduces the relaxation time and thus augments the force production.
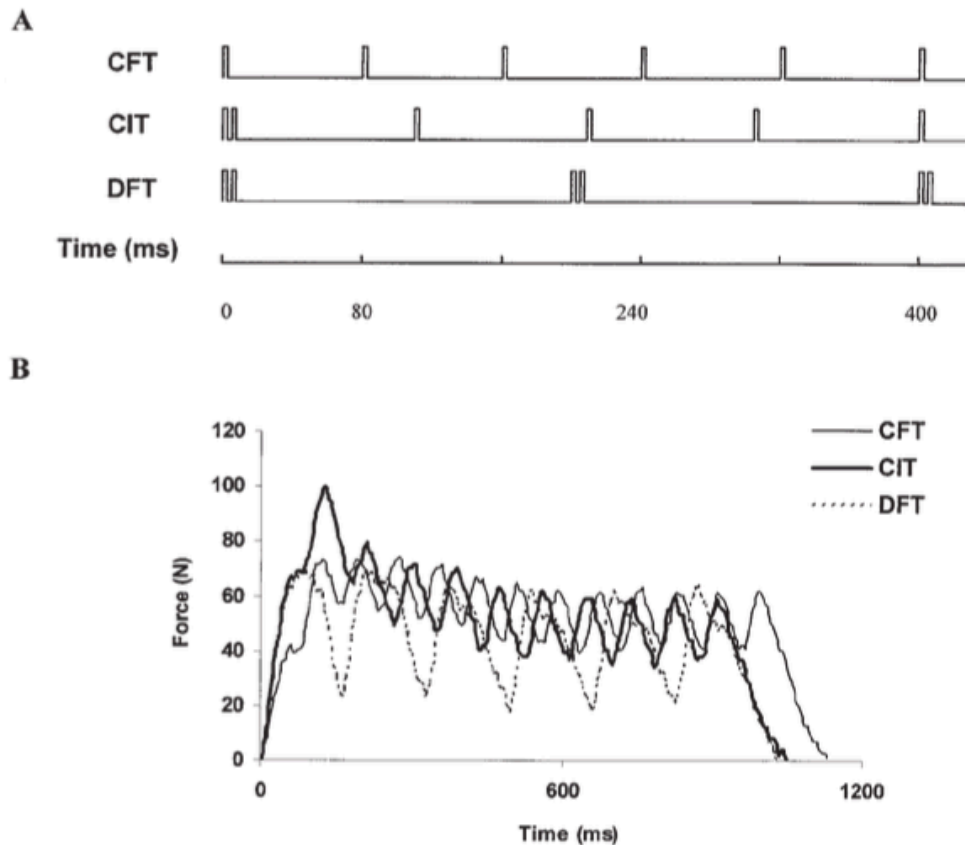
Figure 1.7: Representation of Constant frequency trains (CFT) and catch-like inducing (CIT) trains on the quadriceps femoris muscle. It can be seen that the CIT has a frequency initial doublet at the beginning of the train (Binder-Macleod and Lee, 1996)

Research conducted in the turtle hind-limb was done to validate previous concepts about catch-like properties of human muscles. It is important to show that these catch-like effects during dynamic contractions are not only present in humans, but also in animals, because in many species contractions play an important role in a variety of natural movements such as locomotion (Gillis & Biewener, 2001). Callister and colleagues (2003) undertook experiments involving the turtle hind-limb, examining the differences in force production between catch-like inducing trains and control-frequency trains as well as the effects of fatigue on the different types of trains. They found that before fatigue the catch-like inducing trains produced higher amounts of force for isometric and dynamic trains than the control-frequency trains. Following fatigue it was revealed that

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force was lower for dynamic and isometric contractions for both control frequency trains and catch-like inducing trains, yet the catch-trains still induced a greater enhancement of force after fatigue (Callister et al., 2003). This validates the importance of the stimulus pattern in force enhancement in humans, animals and non-mammalian vertebrates.

1.13 AIMS OF THESIS

Study 1: In this study I assessed the effects of load on contractile responses during microstimulation of single human motor axons (n=10) supplying the toe extensors. Specifically, we measured motor unit responses under three different biomechanical conditions: unloaded isotonic, loaded isotonic and isometric to determine what frequencies are required to generate 50% of the maximum contractile response and assess the fusion-frequency properties in varying conditions. Displacement (deg) was measured during both loaded and unloaded isotonic conditions and force (mN) during isometric conditions.

Study 2: The aim of this study was to deliver long lasting trains of stimuli during microstimulation of single motor axons supplying the toe extensor muscles. The purpose of this study was to determine whether long irregular trains of stimuli that incorporate physiological variability (irregularity) produce greater contractile responses over regular trains of identical mean frequency but zero variability lasting 45 s at a mean frequency of 18Hz.

Study 3: The purpose of this study was to stimulate single human motor axons supplying the toe extensors, to compare the isometric forces generated by high-frequency (58-69Hz) irregular and regular discharge ballistic stimuli. The purpose of this study was to

determine whether it was purely the unusually high firing rates of ballistic stimuli, shown during other studies that result in high force generation, or whether incorporating discharge variability (irregularity), into ballistic stimuli may offer advantages that have not yet been explored.

Study 4: In this study we stimulated multiple motor units in tibilais anterior by inserting a microelectrode directly into the muscle belly. Comparable to study 2, the purpose of this study was to assess whether long trains of irregular of stimuli offer similar advantages when activating multiple motor units, just as it did during study 2. However, in this study I also wanted determine whether irregular trains of stimuli reduce the magnitude of fatigue if the force begins to decline.

CHAPTER 2

COMPARISON OF THE CONTRACTILE RESPONSES OF SINGLE HUMAN MOTOR UNITS IN THE TOE EXTENSORS DURING UNLOADED AND LOADED ISOTONIC AND ISOMETRIC CONDITIONS

Published in the Journal of Neurophysiology

2.1   ABSTRACT

Much of the repertoire of muscle function performed in everyday life involves isotonic dynamic movements, either with or without an additional load, yet most studies of single motor units measure isometric forces. To assess the effects of muscle load on the contractile response we measured the contractile properties of single motor units supplying the toe extensors, assessed by intraneural microstimulation of single human motor axons, in isotonic conditions, loaded isotonic and isometric conditions. Tungsten microelectrodes were inserted into the common peroneal nerve and single motor axons (n=10) supplying the long toe extensors were electrically stimulated through the microelectrode. Displacement was measured from the distal phalanx of the toe with either, an angular displacement transducer for the unloaded (i.e. no additional load) and loaded isotonic conditions (addition of a 4 g mass), and a force transducer for the isometric conditions. Mean twitch profiles were measured at 1 Hz for all conditions: rise

time, fall time and duration were shortest for the unloaded isotonic conditions and longest for the isometric conditions. Peak displacements were lower in the loaded than unloaded isotonic conditions, and the frequency required to generate half of the maximal response in the unloaded condition was achieved at lower frequencies than in the loaded isotonic condition. We have shown that the contractile responses of single motor units supplying the human toe extensors are influenced by how they are measured: twitches are much slower when measured in loaded than unloaded isotonic conditions, and slowest when measured in isometric conditions.

2.2 INTRODUCTION

During voluntary contractions the central nervous system controls motor output by two mechanisms: (i) recruitment, whereby units are progressively recruited in order of size from smallest to largest – the smallest units generating the smallest forces or displacements - and (ii) rate-coding, by which an increase in the firing rate of active motoneurones will cause an increase in force or displacement, at least within the linear range over which the motoneurones are operating. This transformation of motoneurone discharge into force or movement is a fundamental feature by which the nervous system controls the skeletal muscles that subserve voluntary and involuntary movements (Heckman and Binder 1991). The introduction of intraneural microstimulation of single human motor axons has allowed one to assess the contractile response of single motor units to controlled trains of stimuli, specifically to examine the utility of rate-coding to control force production and the influence of fatigue on the force-frequency response. Motor axon microstimulation has been used to measure the contractile properties of single motor units in the thenar muscles acting on the thumb (Westling et al. 1990; Thomas et al. 1990, 1991a,b, 2006), the long and short extensor muscles acting on the

toes (Macefield et al. 1996; Leitch and Macefield, 2014) and the long and short flexor muscles acting on the fingers (Fuglevand et al. 1999; McNulty et al. 2000).

We have been increasingly interested in how rate-coding operates during voluntary contractions. It is known that human motoneurones do not fire in a regular fashion when volitionally activated, but rather display variability of around 25% (as measured by the coefficient of variation). This physiological variability is known to fall as mean firing rate increases (Stalberg and Thiele 1973). It has also been shown that much of this variability is derived from variations in central drive rather than variations in synaptic input from muscle afferents (Macefield et al. 1993). Physiological variability in a spike train features short and long inter-spike intervals, and - given that preceding a train of regular pulses by a short interspike interval ("initial doublet") augments force production by a single motor unit (Macefield et al. 1996) - we had reasoned that the presence of discharge variability throughout a train of stimuli would augment the contractile response. Using microstimulation of single human motor axons supplying the long toe-extensor muscles, we recently demonstrated that incorporating physiological variability into trains of stimuli produced an increase in contractile response – both before and after muscle fatigue (Leitch and Macefield 2014). Curiously, we noticed that the twitches - measured as angular displacements of a toe in isotonic conditions - were much briefer than those recorded from the same muscles in an earlier study that measured the contractile response as isometric force: the mean twitch rise time for 14 single motor units was 44.5 ± 77.6 ms in isotonic conditions (Leitch and Macefield 2014) yet 74.8 ± 3.9 ms in for 19 motor units in isometric conditions (Macefield et al 1996).

Muscular contractions can be categorized into isometric, isotonic and loaded isotonic movements. This is based on the forces generated, muscle length and dynamic shortening. Isometric contractions involve a muscle generating force without changing in length. For example, when the hand grips an object, the interphalangeal joints do not change their angles but muscles acting on the digits generate forces sufficient to maintain grip. In isotonic contractions muscle tension remains constant despite a change in muscle length, such as occurs when gesticulating with the hand. Loaded isotonic contractions occur when muscles can shorten (concentric) or lengthen (eccentric) while still generating force, such as when weight lifters are doing bicep curls; initially, the movements are concentric, however as the forces generated by the downward load exceed those that can be generated by the muscle, the contraction will become eccentric. Most studies conducted in this field have focused on isometric movements (Milner-Brown et al 1973a) and few studies have examined the biomechanical differences between isometric, isotonic and loaded isotonic conditions. While the study of muscles under isometric conditions has been pivotal in our understanding between the frequency of stimulus and forces produced, particularly with respect to microstimulation of single motor axons (Westling et al. 1990; Thomas et al. 1990, 1991a,b, 2006; Macefield et al. 1996, 1999; Fuglevand et al. 1999; McNulty et al. 2000; Leitch & Macefield 2014), with one exception all of these studies have measured force production of single motor units in isometric conditions. Indeed, ours was the only study to have measured the contractile responses of single human motor units in isotonic conditions and, as noted above, twitch durations were considerably shorter in isotonic than isometric conditions (Leitch and Macefield 2014).

Studies in experimental animals have shown that the twitch contraction time can predict the tension-frequency (t-f) relationship of a motor unit: the slower the twitch the lower the frequencies required in the steep region of the t-f curve (Kernell et al. 1975). It has also been shown that slow-twitch units summate more readily than fast-twitch units, and thus the activation rate required to produce half-maximal force is often lower (Botterman et al. 1986; Kernell et al. 1983b). Furthermore, experiments conducted by Jewell and Wilkie (1960) showed similar results. They studied the isolated whole sartorius muscle of the frog and observed shorter twitch durations during isotonic conditions than in isometric conditions. There is no doubt that the animal work has been pivotal in our understanding of the mechanical properties of muscles, but we were curious to see whether the same could be found in single human motor units, obviously studied in intact conditions. We used the long toe-extensor muscles, which have long tendons -a feature we had previously noted accounts for the longer isometric twitch durations of these muscles compared to muscles with short tendons, such as those in the intrinsic muscles of the hand (Macefield et al. 1996, 1999; Fuglevand et al. 1999; McNulty et al. 2000).

The primary purpose of the current study was to characterize the contractile responses of the same motor unit in the toe extensor muscles of humans in three conditions - unloaded isotonic, loaded isotonic and isometric conditions - to determine the frequencies required to generate half the maximal contractile response (measured as angular displacement or isometric force) and to define the frequency at which motor units are fully fused during the different conditions. We activated one and only one motor axon, which was subjected to all three recording conditions. So, while the muscle fibers supplied by a given motoneurone clearly determine the twitch properties of the motor unit, (Scott et al. 2001), we are specifically interested in how different mechanical conditions affect the

contractile responses of a single motor unit. As noted above, other studies have only examined the half maximal force during isometric conditions (Westling et al. 1990; Thomas et al. 1990, 1991a,b, 2006; Macefield et al. 1996, 1999; Fuglevand et al. 1999; McNulty et al. 2000) and, to our knowledge, no studies have compared the contractile responses of single human motor units in unloaded isotonic, loaded isotonic and isometric conditions.

2.3 METHODS

General procedures

Successful experiments were performed on one female and six male subjects, the majority of whom were 18-23 years of age; one subject was 53. Experiments were conducted under the approval of the Human Research Ethics Committee of the University of Western Sydney. Subjects provided informed written consent. The participants were seated with the knee flexed to 120 degrees. The foot was fixed onto a rigid footplate and a Velcro strap (VELCRO® Hook and Loop Fasteners) firmly attached over the tarsometatarsal joint of the foot, so as not to interfere with dorsiflexion of the relevant toes. The thigh was supported by a vacuum cast, which prevented any movement at the knee during the experimental protocol. The common peroneal nerve was located at the fibula head by delivering cathodal pulses via a 2 mm surface probe (2-10 mA, 0.2 ms, 1 Hz; Stimulus Isolator, AD Instruments, Sydney, Australia) Once the optimal site for stimulating the nerve was located a high-impedance tungsten microelectrode (Frederick Haer and Co, Maine, USA) was inserted into the skin. Constant-current cathodal pulses (0.02-1.00 mA, 0.2ms, 1 Hz) were delivered through the microelectrode and the microelectrode manually adjusted to search for a motor fascicle supplying either extensor hallucis longus (EHL), extensor digitorum longus

(EDL) or extensor digitorum brevis (EDB). EMG was recorded by attaching two surface Ag/AgCl electrodes over the following muscles; tibialis anterior, the peronei or extensor digitorum brevis. One electrode was affixed over the muscle belly and one to the proximal tendon. Signals were amplified (gain 1000 X; BioAmplifier, ADInstruments, Sydney, Australia) and filtered (10 Hz-1 kHz) and recorded (2 kHz sampling) on a computer-based data acquisition and analysis system (PowerLab 16 SP hardware and LabChart Pro 7 software, ADInstruments, Sydney, Australia). Twitch parameters were measured using the Peak Parameters feature of LabChart software. Isotonic and loaded isotonic contractile properties were measured using a highly sensitive angular displacement transducer (PanLab, Barcelona, Spain; 24 µV/V/º sensitivity) placed over the nail of the stimulated toe: either digit 1 for EHL (extensor hallucis longus) or digits 2-5 for EDL (extensor digitorum longus). Displacement was measured from the distal phalanx of the activated toe, with a bandwidth of DC-100 Hz and sampled at 200 Hz. Loaded isotonic conditions were produced by adding a 4 g mass (40 mN) to the displacement transducer. Contractile force (DC-100 Hz; 200 Hz sampling) was measured using an isometric force transducer (Model 1030, UFI, Morro Bay, USA) placed over the nail of the digit.

Recording and stimulation protocol

An appropriate intrafascular site was established when microstimulation generated a small twitch and a reproducible EMG potential, where an increase in current within a small safety margin failed to recruit an additional motor axon. These currents would typically be between 4-14 µA, depending on the diameter of the axon and the well-defined safety margin, typically 2-4 µA, over which increases in current failed to change either the twitch amplitude or EMG profile; at this point, we were stimulating a single

motor axon. The motor axon was then stimulated with a set of 10 pulses (1 Hz) at the threshold current in order to measure the twitch properties of the motor unit. Fusion properties were calculated to determine the point at which full fusion had occurred. As described previously (Macefield et al. 1996), they were calculated as the peak-trough difference (computed either from the displacement or force transducer) and were measured in each condition, starting at 2 Hz (to which the peak-trough difference was normalized) until full fusion had occurred in each of the three conditions; a peak-trough difference of zero means that the tetanic contraction was fully fused.

Following the single twitches, trains of 10 pulses were delivered at frequencies ranging from 2 Hz to 30 Hz, delivered in an ascending order at 2 Hz increments. The interval between each train was ~1.5 s and approximately 1.5 minutes elapsed between the different conditions (isotonic, loaded isotonic and isometric). This allowed sufficient time to either add load during the loaded isotonic movements or change to the force transducer for the isometric conditions. These regular trains produced a standard contractile-response curve, allowing us to compare the contractile responses produced by regular trains of stimuli at a range of frequencies (2-30 Hz). This procedure was always conducted in the same order: first, the isotonic responses, 10 pulses at 1 Hz, followed by 10 pulses from 2-30 Hz; second, loaded isotonic responses 10 pulses at 1 Hz, followed by 10 pulses from 2-30 Hz and last, the isometric responses to 10 pulses at 1 Hz, followed by 10 pulses from 2-30 Hz. All statistical analyses were performed using Prism 6 software (GraphPad Software, San Diego, USA). Values are expressed as mean ± SE.

2.4 RESULTS

Microstimulation of single motor axons was successful for 10 motor units in the long toe extensor muscles: five motor axons supplied extensor digitorum longus (EDL) and five supplied extensor hallucis longus (EHL). All motor units were studied in three conditions: unloaded isotonic (measured as angular displacement), loaded isotonic (measured as angular displacement with addition of a 4 g mass) and isometric (measured as force), with the exception of one unit that was lost before the isometric trial was run.

Table 2.1: Twitch parameters for 10 single motor units

Unit	Muscle	Rise Time (ms)			Fall Time (ms)		
		Unloaded Isotonic	Loaded Isotonic	Isometric	Unloaded Isotonic	Loaded Isotonic	Isometric
1	EDL	33	36	38	145	162	175
2	EDL	28	27	67	122	133	151
3	EDL	27	31	39	76	96	139
4	EHL	33	41	34	109	110	148
5	EHL	69	73	84	127	169	184
6	EDL	29	38	50	174	178	196
7	EHL	38	39	41	120	151	140
8	EDL	43	54	46	141	145	161
9	EHL	54	59	89	167	196	205
10	EHL	22	41	-	88	167	-
Mean ± SE		37.5±14.4	43.8±13.8	54.2±20.8	126.9±31.3	150.6±30.8	166.4±24.7

10 single motor units in two conditions (loaded and unloaded isotonic) and 9 single motor units one condition (isometric). For each unit the mean value (± SD) of 10 pulses was calculated for either extensor digitorum longus (EDL) or extensor hallucis longus (EHL).

Twitch properties

Twitch duration parameters for the individual motor units, measured at 1 Hz, are provided for the isotonic, loaded isotonic and isometric conditions in Table 2.1. Repeated-measures one-way ANOVA with Tukey's multiple comparison test was used to compare mean durations across the 9 units studied in all three conditions. Mean rise time (time to peak of twitch) was 39.3 ± 4.7 (SE) ms for the unloaded isotonic condition, increasing to 44.1 ± 4.9 ms for the loaded isotonic condition and increasing further to 54.2 ± 6.9 ms for the isometric condition ($F_{(1.080, 8.639)} = 6.566$; $P = 0.0299$). Likewise, twitch fall times increased from unloaded isotonic (131.2 ± 10.0 ms) to loaded isotonic (148.7 ± 10.7 ms) and isometric (166.4 ± 8.2 ms) conditions ($F_{(1.972, 15.77)} = 23.81$; $P < 0.0001$). Finally, as expected, total twitch durations increased progressively from unloaded isotonic (170.5 ± 12.1 ms) to loaded isotonic (192.9 ± 13.9 ms) and isometric (220.6 ± 13.8 ms) conditions ($F_{(1.520, 12.16)} = 31.38$; $P < 0.0001$). These data are shown graphically in Fig. 2.1.
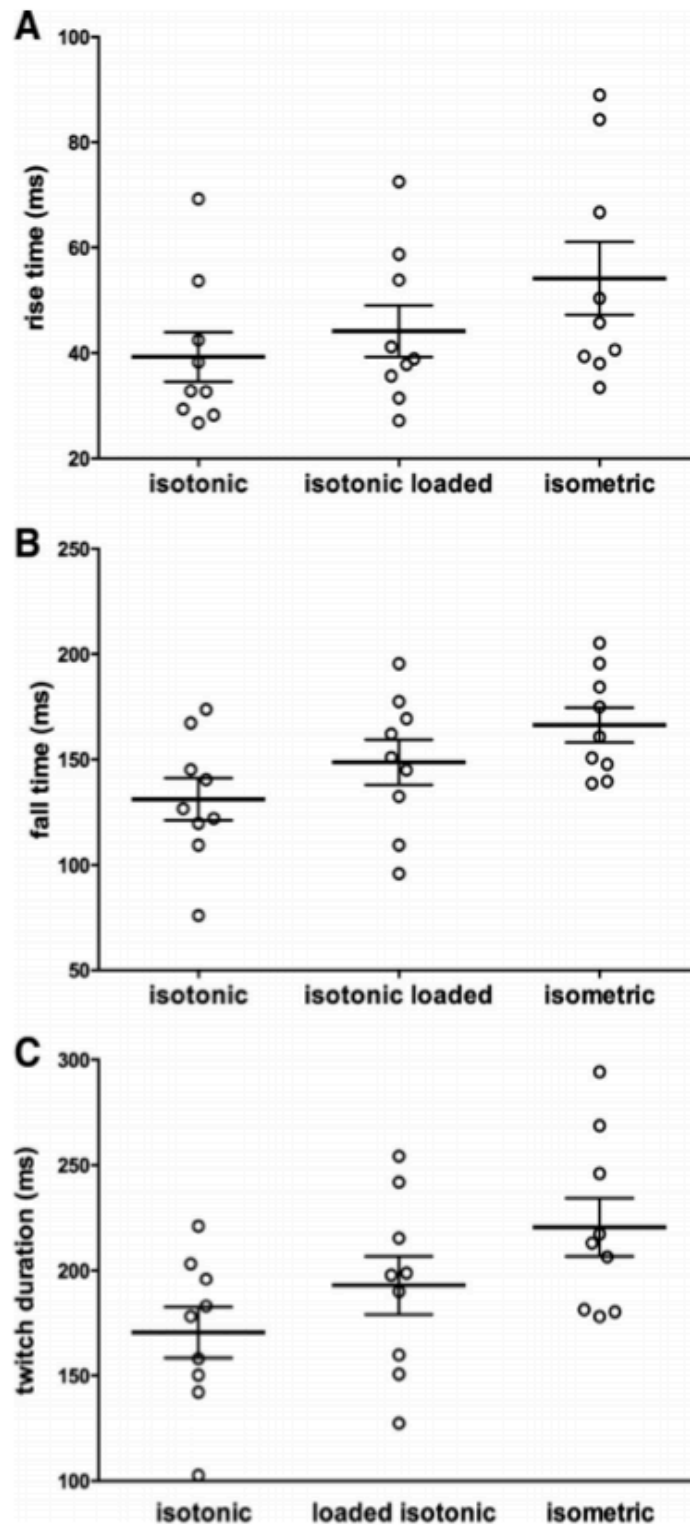
Figure 2.1: Twitch durations for 9 single motor units, studied in all three conditions. Statistical comparisons were made for rise time (**A**) fall time (**B**) and total twitch duration (**C**) across the three conditions. Rise time *p < 0.03, fall time *p< 0.0001 and twitch duration *p< 0.0001; one-way ANOVA.

Frequency-response curves

Trains of 10 pulses were delivered at frequencies ranging from 2-30 Hz. At each frequency the peak displacement was normalized to the value at 30 Hz in the unloaded condition; in the isometric conditions peak force was normalized to the force at 30 Hz. Experimental records for one motor unit, recorded in unloaded isotonic conditions, are shown in Fig. 2.2A; data from the same unit in loaded isotonic conditions are illustrated in Fig. 2.2B. Trains of 10 pulses were delivered from 2-30 Hz, in 2 Hz increments; for clarity only the sections extending from 4-16 Hz are shown. Peak displacement was seen to be significantly lower in the loaded than unloaded isotonic conditions, and the frequency required to generate half of the maximal response in the loaded condition was achieved at lower frequencies than in the unloaded isotonic condition for all 10 units. It can be seen that 50% of the maximal displacement is generated at around 11 Hz for the unloaded condition, while in the loaded condition it is closer to 13 Hz. During delivery of the trains we observed a non-linear sigmoidal relationship between stimulation frequency and normalised displacement in the isotonic conditions (Fig. 2.3A).
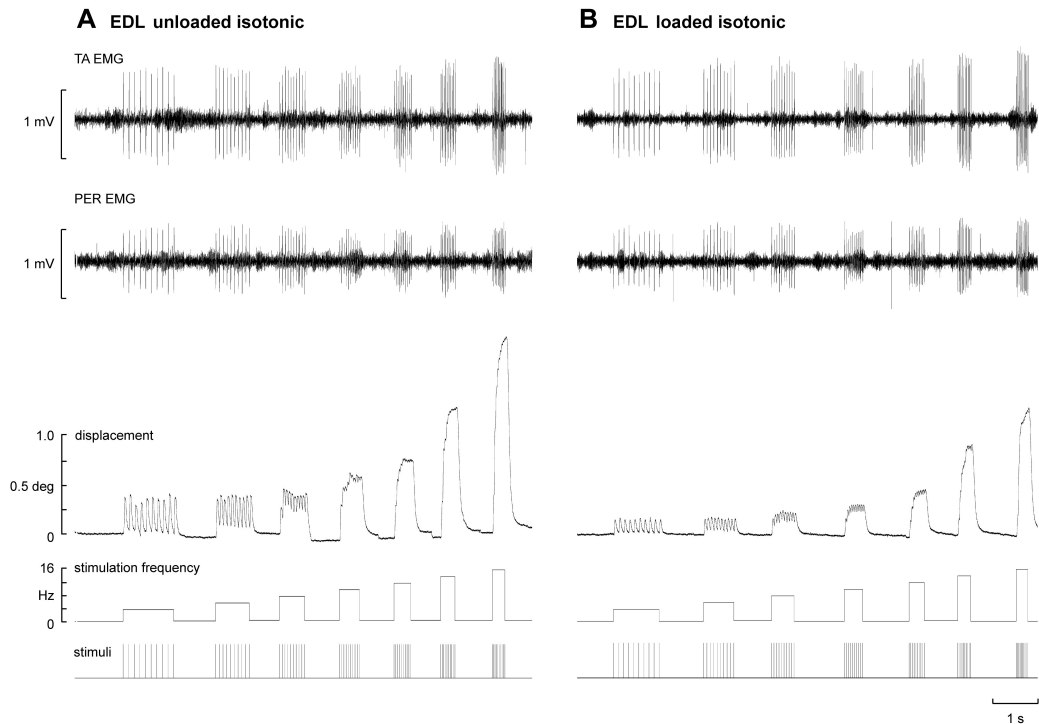
Figure 2.2: Experimental records from a single motor unit in extensor digitorum longus, during microstimulation of a single motor axon at frequencies ranging from 4-16 Hz in unloaded isotonic (**A**) and loaded isotonic (**B**) conditions.

The force-frequency curve is shown for the isometric conditions in Fig. 2.3B. Force production showed a sigmoidal relationship between force and frequency, with 85% of maximal force being generated at around 18 Hz and a very minor increase in force from 18-30Hz to reach 100% of maximal force. One of the fundamental details in Fig. 2.3B is, at what point the muscle reaches 50% of displacement in the three conditions (Fig. 2.3A).
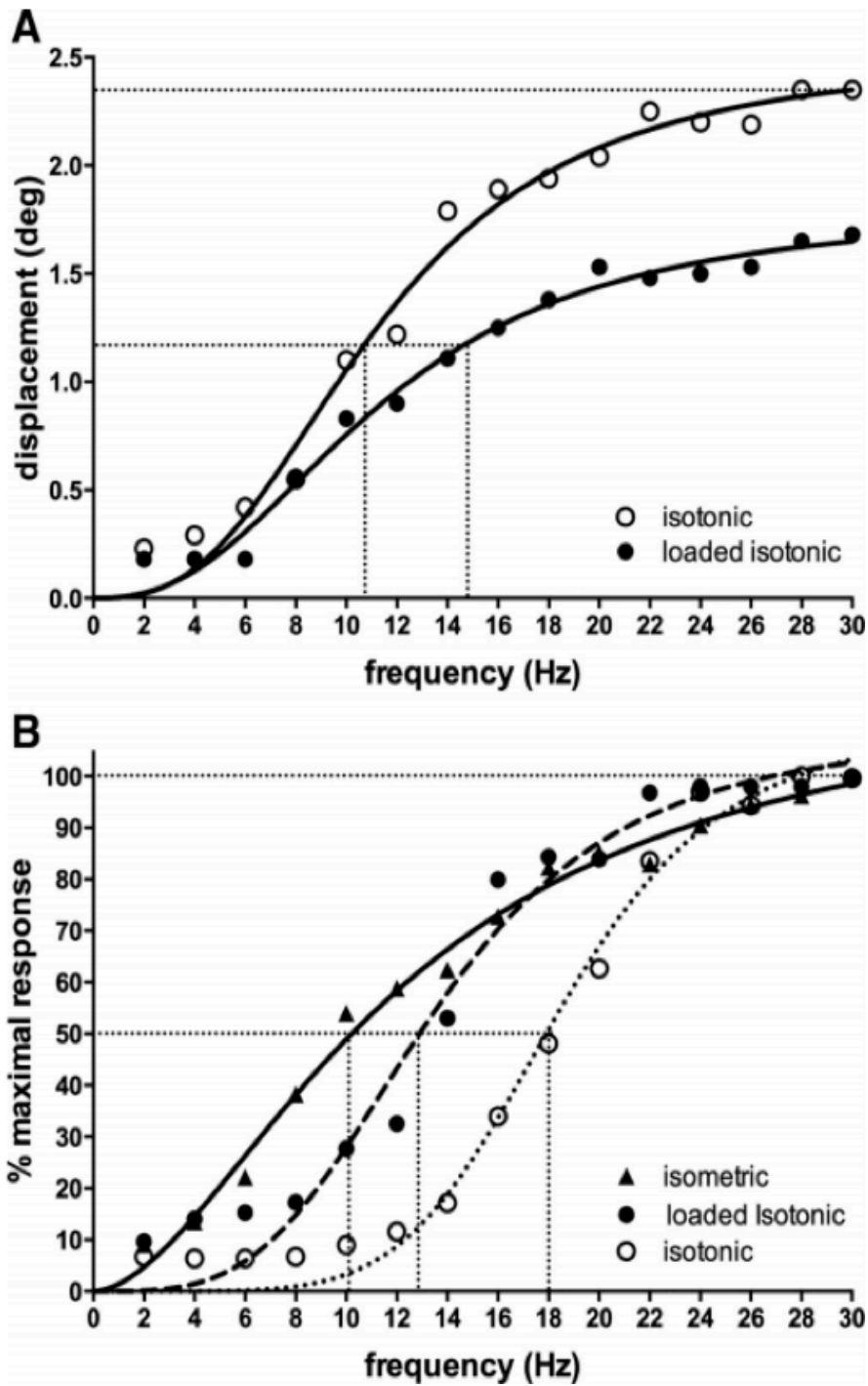
Figure 2.3: Frequency-response curve for one motor unit. Panel **A** shows the normalised angular displacements in loaded (open symbols) and unloaded (filled symbols) isotonic conditions. Panel **B** shows the normalised force for this same unit with the displacement responses in unloaded isotonic conditions shown for comparison.

Fusion-frequency relationships

Fusion properties were analysed for the three conditions. As with the 1 Hz pulses, at 2 Hz the twitches were still distinct – there was no twitch summation as the frequencies were too low. As the frequency increased, individual twitches started to superimpose and the degree of fusion increased with frequency, reaching a maximum at 14-16 Hz. At low frequencies, from 2-6 Hz, there was no significant correlation between frequency and fusion across all three conditions because at these low frequencies there was little to no twitch summation. However, from 8-16 Hz the degree of fusion increased, more so for the loaded isotonic and isometric conditions (Table 2.2). This shows that during a loaded isotonic (or isometric) condition fusion occurs at lower frequencies than in an unloaded condition. This is shown graphically in Fig. 2.4.

Table 2.2: Fusion properties of 9 single motor units

Frequency (Hz)	Unloaded isotonic	Loaded Isotonic	Isometric
4	30.6 ± 4.0	37.1 ± 4.3	30.6 ± 6.4
6	50.2 ± 4.3	52.1 ± 3.9	51.5 ± 5.9
8	60.6 ± 4.8	67.2 ± 4.1*	$80.7 \pm 6.6^{++++}$
10	69.0 ± 3.8	85.6 ± 5.3****	$100.0 \pm 0.0^{++++}$
12	87.7 ± 4.5	96.9 ± 3.1*	$100.0 \pm 0.0^{++}$
14	98.2 ± 1.8	100.0 ± 0.0	100.0 ± 0.0
16	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Mean ± SEM values for percentage of fusion in single motor units (n=9) during unloaded isotonic, loaded isotonic and isometric conditions. *=$p<0.05$, ****=$p<0.0001$ for differences between unloaded isotonic and loaded isotonic; ++=$p<0.01$, $p<0.0001$ for differences between unloaded isotonic and isometric.

In the unloaded isotonic condition the stimulation frequency required for the twitches to fully fuse was higher than in the other conditions, presumably because the twitches were shorter in the unloaded conditions. At 10 Hz, the twitches were fully fused in the isometric conditions, but in the unloaded isotonic condition the degree of fusion was only $69.0 \pm 3.8\%$ of maximum. At this same frequency, addition of the 4 g load increased the degree of fusion to $85.6 \pm 5.3\%$.
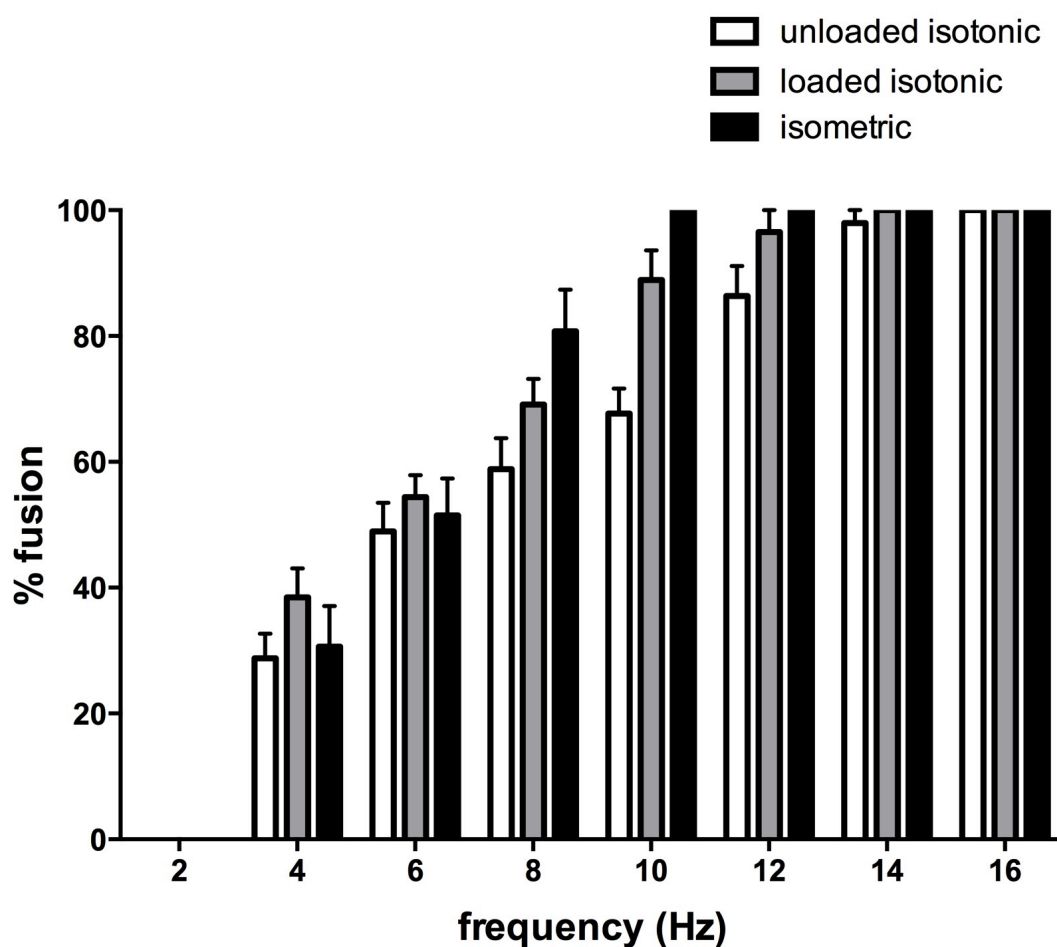
Figure 2.4: Fusion properties of all single motor units (n=10) for all three conditions. There was no fusion at 2 Hz, but fusion progressively increased with frequency.

2.5 DISCUSSION

Intraneural microstimulation of single human motor axons was employed to assess the contractile properties of individual motor units in the long toe-extensor muscles in unloaded isotonic, loaded isotonic and isometric conditions - both with respect to twitch duration and frequency response. Most studies on single motor units, in both experimental animals and humans, have measured contractile response as isometric force. Previous research has shown that there is a sigmoidal relationship between frequency of stimulation and the force produced (Bigland and Lippold 1954; Cooper and Eccles 1930), whereby an increase in frequency beyond 30 Hz fails to increase force. It has also been shown that individual motoneurones fire within the linear range of the force-frequency response curve, between 8 and 20 Hz (Macefield et al. 1996).

Twitch properties and biomechanical considerations

Dorsiflexion of the toes was measured during loaded and unloaded isotonic movements using a highly sensitive angular displacement transducer (PanLab, Barcelona, Spain; 24 μV/V/$^{\circ}$ sensitivity) placed on the nail of the activated toe. Twitch properties in unloaded conditions showed a shorter rise time and fall time, and hence a shorter total twitch duration, than in the loaded condition, which could be explained purely on a biomechanical basis. As was first identified by Hill (1951), tendon compliance affects the twitch contraction time. Due to the fact that there is no additional load associated with the isotonic condition, we would expect to see greater peak displacement and a much faster rise time and fall time, resulting in a shorter twitch duration. These faster twitch durations would agree with the fact that the tendon is able to move more readily than during loaded conditions, in which tendon compliance must be taken up before movement can occur. Addition of a load to the isotonic condition resulted in significantly

longer twitch durations, due to a combination of both increased rise time and fall time. Conversely, when we consider the biomechanics of isometric conditions, the tendon will lengthen as the muscle shortens. A way of visualizing this is to imagine the movement of a rubber band from a fixed point. If we consider the rubber band to act as the tendon and the fixed point as the toe, then when the muscle shortens during an isometric contraction the rubber band would lengthen before any build up of force at the fixed end. Clearly, the reduced displacement in the loaded condition can be explained by the downward force produced by the 4 g (40 mN) mass; the added mass would also make the twitches more sluggish, slowing both the rise time and fall time. While the mass used in these experiments would not be enough to have any effect on whole muscle, the contractile responses we are measuring are only produced by the few muscle fibers supplied by the single motor axon; we had previously shown that single motor units in the long toe-extensors produce mean twitch forces of 20 mN (Macefield et al. 2000). Isometric conditions showed a further increase in rise time, fall time and twitch duration. Both loaded isotonic and isometric conditions displayed much slower and longer twitches than during unloaded conditions.

Fusion properties of the toe extensor muscles

Between 8 and 12 Hz the degree of fusion was significantly different in the unloaded isotonic, loaded isotonic and isometric conditions, while full fusion did occur in all conditions between 10 and 16 Hz. It is well documented that human motoneurones fire within the linear range of the force-frequency curve (Macefield et al. 1996). The linear range of the force-frequency profile, by definition, is the range over which an increase in frequency produces a corresponding increase in force or movement. Depending on the muscle being studied the linear range can vary. However for the toe extensors this range

begins at approximately 8 Hz, plateauing at approximately 20 Hz. Within the linear range there is, of course, a predictable increase in contractile response for a given increase in frequency, while within the plateau region there is no increase in contractile response for a given increase in frequency. Previous studies have shown that human motor units in the toe extensors begin firing at 7-8 Hz (Hannerz 1974; Petajan 1981; Person & Kudina, 1972; Tanji and Kato, 1973). Furthermore, microstimulation of single motor axons supplying the toe extensors has shown that no further increase in contractile response occurs beyond 16-18 Hz (Macefield et al. 1996; Leitch and Macefield 2014). Therefore, we suggest that the fusion values found in the current study are physiologically relevant within the range of 8-16 Hz because this is the primary range over which motoneurones supplying the human toe extensor muscles fire during volitionally generated contractions.

Functional consequences

Many movements conducted by humans in everyday life involve isotonic movements, either unloaded or with the addition of load. While there is no doubt that isometric contractions are essential in human function, such as in stabilizing and static grip, isotonic contractions have been understudied and are clearly important. The current research has contributed significant new knowledge the displacement-frequency relationship during loaded and unloaded isotonic conditions of single motor units in human toe extensor muscles, and provided further information on the force-frequency relationships of the same motor units during isometric conditions. We have shown that twitches are slower and longer during loaded isotonic conditions, and even more so in isometric conditions. We have shown that lower frequencies are required to generate 50% of the contractile response of a muscle in isometric conditions, with significantly higher frequencies being required in isotonic conditions. By understanding how single

motor units operate in different biomechanical conditions, we can more closely emulate the patterns of neuronal activity needed to produce appropriate contractile responses in different conditions.

It is known that in the "primary range" of operation, human motoneurones fire within the linear range of the force-frequency curve; stimulating at frequencies below 8 Hz or above 18 Hz fails to result in an increase in contractile response of the activated motor unit (Macefield et al 1996; Leitch and Macefield 2014). However, it has been reported that when rapid movements occur, which require ballistic contractions - such as throwing or kicking a ball - then motoneurones can fire at greater rates when stimulated with higher-amplitude currents. This is known as the "secondary range" (Kernell 1965; Schwindt 1973). While the "secondary range" is of physiological importance, it was beyond the scope of the current study, in which we focused on stimulating single motor axons within the "primary range" of motoneurone firing.

The current research also has functional implications for therapies such as functional electrical stimulation (FES), whereby muscles weakened or paralysed by stroke or spinal cord injury are electrically activated in an attempt to improve rehabilitation. Most improvements to this technique have involved advances in technology, programming and implementation (Lee, Becker & Binder-Macleod 1999). FES involves continuous activation of the paretic or paralysed muscle with regular trains of stimulation. Our previous study (Leitch and Macefield 2014) showed that by incorporating variability into the trains of stimuli, which emulate the variability exhibited by volitionally activated motoneurones, we could generate greater contractile responses. We hypothesized that incorporating variability into the stimulation trains used in FES would optimize the

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contractile responses and overcome some of the decrements associated with fatigue. In the current study we have shown that twitch and fusion-frequency properties differ as a function of load on the muscle. Accordingly, if we are able to determine the point at which full fusion occurs in different biomechanical conditions of the muscle we can control the contractile behaviour based on the task at hand. So, if a loaded isotonic movement is required during FES we now know that lower frequencies are required to achieve full tetanic fusion, that it will reach 50% of its maximal response earlier than in an unloaded condition and that even lower stimulation rates are required to generate isometric forces. This would mean that the rate coding behaviour of human motoneurones could be optimized to the biomechanical requirements to produce the appropriate contractile response. Clearly, further research must be conducted in non-isometric conditions to further elucidate control mechanisms at the single motor unit level.

2.6 CONCLUSIONS

We have shown, for the first time, that the contractile response of single human motor units in the long toe extensor muscles – as assessed by intraneural motor axon microstimulation - are affected by the environment in which the motor unit contracts. In unloaded isotonic conditions twitches are briefer, and fusion occurs later, than in the presence of a load. Twitch durations are the longest, and fusion frequencies the lowest, in isometric conditions. We believe these differences allow the skeletomotor neurons to utilize rate coding and adjust the firing rates to the biomechanical task being conducted in everyday tasks.

2.7 ACKNOWLEDGMENTS

CHAPTER 3

MICROSTIMULATION OF SINGLE HUMAN MOTOR AXONS IN THE TOE EXTENSORS: FORCE PRODUCTION DURING LONG-LASTING TRAINS OF IRREGULAR AND REGULAR STIMULI

Published in Physiological Reports

3.1 ABSTRACT

Human motoneurones are known to discharge with a physiological variability of ~25% during voluntary contractions. Using microstimulation of single human motor axons, we have previously shown that delivering brief trains (10 pulses) of irregular stimuli at physiological rates and variability generates greater contractile responses than trains of regular stimuli with identical mean frequency but zero variability. We tested the hypothesis that longer irregular (physiological) trains would produce greater contractile responses than regular (non-physiological) trains of the same mean frequency (18 Hz) and duration (45 s). Tungsten microelectrodes were inserted into the common peroneal nerve of human subjects and single motor axons supplying the toe extensors (n=14) were isolated. Irregular trains of stimuli showed greater contractile responses over identical mean frequencies in both fatigue-resistant and fatigable motor units, but because the forces were higher the rate of fatigue was higher. Nevertheless, forces produced by the irregular trains were significantly higher than

those produced by the regular trains. We conclude that discharge irregularity augments force production during long as well as short trains of stimulation.

3.2 INTRODUCTION

Human motoneurones are known to exhibit significant discharge variability during voluntary contractions, which is known to fall as mean firing rate increases (Stalberg and Thiele 1973; Moritz et al., 2004; Laidlaw et al., 2000). Physiological discharge variability includes short and long interspike intervals and it has been shown that incorporating a short interspike interval ('doublet') at the beginning of a train of electrical stimuli augments the contractile response. This has been established in animal models (Callister, Reinking & Stuart 2003) as well as in humans - both during stimulation of whole human muscle (Binder-Macleod & Lee 1996) and during microstimulation of single human motor axons (Macefield et al. 1996).

Intraneural microstimulation allows the twitch properties and force-frequency responses of individual motor units to be examined, with no bias toward small or large motor units (Macefield et al., 1996; Fuglevand et al., 1999; McNulty et al., 2000; Thomas et al., 1991a; Westling et al., 1990). Until recently, however, all studies using intraneural microstimulation of human motor axons have measured the contractile responses to trains of regular stimuli, but we wanted to determine the influence of physiological discharge variability on the response of individual motor units. In an earlier study we stimulated single motor axons supplying the long toe-extensor muscles with short trains (10 pulses) of regular and irregular stimuli (Leitch & Macefield, 2014). Two conclusions were drawn from this study. Firstly, contractile responses (measured as angular displacements of the toe) were higher for irregular trains than regular trains over identical

mean frequencies (8-24 Hz). Secondly, when the motor unit was then subjected to a 2 min train of stimuli that led to fatigue, subsequent delivery of the short irregular trains resulted in significantly higher contractile responses than did the regular trains. The continuous train was given as a regular set of stimuli at 10 Hz, so we do not know how motor units behave during a continuous train of *irregular* stimuli. It was of interest, therefore, to test whether the same augmentation of contractile response could be exhibited when incorporating physiological discharge variability into longer trains of stimuli that closely resemble the static isometric contractions performed voluntarily. The purpose of the current study was to test this hypothesis, by delivering trains of stimuli (18 Hz for 45 s) with and without discharge variability and measuring force production by individual motor units in the long toe-extensor muscles.

3.3 METHODS

Experiments were conducted on 11 male and 2 female subjects (18-36 y) under the approval of the Human Ethics Committee of Western Sydney University. Subjects provided informed written consent. Successful microstimulation of 14 single motor axons was performed in nine subjects.

The participants were reclined in a chair with the knee flexed to approximately 120°. The foot was fixed onto a rigid footplate with the ankle at 120°. A Velcro strap was attached over the dorsum of the foot, proximal to the digits (so as not to interfere with dorsiflexion of the toes), to anchor the foot to the footplate. The leg was supported by a vacuum cast to prevent any movement during the procedure. The common peroneal nerve was located using a weak electrical stimulus (0.02-10.00 mA, 0.2 ms, 1 Hz; Stimulus Isolator, ADInstruments, Sydney, Australia) delivered by a 2 mm diameter surface probe. A high-

impedance tungsten microelectrode (Frederick Haer & Co, Maine, USA), 200 μm in diameter, was inserted into the nerve and an Ag/AgCl surface electrode on the opposite side of the knee served as the anode. Constant-current cathodal pulses (0.02-1.00 mA, 0.2ms, 1 Hz) were delivered through the microelectrode and the microelectrode manually adjusted to search for a motor fascicle supplying either extensor hallucis longus (EHL) or extensor digitorum longus (EDL). EMG was recorded with surface Ag/AgCl electrodes placed over tibialis anterior, fibularis longus/brevis and extensor digitorum brevis, amplified (BioAmplifier, ADInstruments, Sydney) and filtered (10 Hz-1 kHz) and recorded (2 kHz sampling) on a computer-based data acquisition and analysis system (PowerLab 16 SP hardware and LabChart Pro 7 software, ADInstruments, Sydney). Force (DC-100 Hz; 200 Hz sampling) was measured using a highly sensitive angular force transducer (Model 1030, UFI, Morro Bay, USA) located over the distal phalanx of the stimulated toe - either the big toe for EHL or the second or third toe for EDL. A single motor axon was activated when microstimulation generated a small twitch and a reproducible EMG potential at the lowest possible current within a well-defined safety margin, typically 3-7 uA, in which increases in current failed to modify the twitch amplitude or EMG profile over the defined safety margin. The motor axon was then stimulated with sets of 8 pulses at the threshold current in order to measure the twitch properties of the motor unit. Twitch parameters were measured using the Peak Parameters feature of LabChart. After establishing that a single motor axon had been isolated trains of 10 regular pulses from 2-18 Hz were delivered to construct a force-frequency curve. Following these trains, an irregular (physiological) long-lasting train (18 Hz, ~800 spikes) was given for 45 seconds; this allowed us to create a time varying picture of the contractile responses generated by the long-lasting trains on active motor units. In this study 45 s was used as this would be long enough to test the effects with no

significant marked fatigue. The subject then had sufficient recovery time to allow contractile responses to return to peak threshold. This could take up to 15 minutes depending on the type of motor unit stimulated (fatigable or fatigue-resistant). We intermittently gave the 10 pulse trains (2-18 Hz) in the same manner as before the irregular train. This allowed us to monitor when contractile responses had returned to normal. Once the forces were identical to those delivered before the irregular long-lasting train the regular (constant frequency) long train was delivered - with the same mean frequency, yet zero variability.

Generating the irregular long-lasting trains

The irregular long-lasting train was based on the discharge variability of single motor units recorded from tibialis anterior during a series of voluntary isometric contractions. For this purpose a tungsten microelectrode was inserted directly into the muscle belly of tibialis anterior and amplified (gain 1000x, 300 Hz-5 kHz; 10 kHz sampling; NeuroAmp EX, ADInstruments, Sydney). Spike trains from individual motor units or individual muscle fibers were recorded during dorsiflexion at the ankle and the microelectrode adjusted so as to obtain several spike trains of several seconds in duration within a given contraction. The subject was asked to sustain a ~30% maximal voluntary contraction for 45 seconds. Firing rates ranged over 145.83 with a maximum of 151.52 and minimum of 5.69. The mean frequency for this train was 18.3 ± 8.7 with discharge variability of ~25.3% (SD/mean x 100) based on the coefficient of variation. The inter-spike intervals exhibited by this train were used to construct an irregular (physiological) long lasting train lasting for 45 seconds in duration.

Regular long-lasting trains

The long-lasting regular train consisted of the same number of pulses as the irregular train ~800 and also had an identical mean frequency of 18 Hz with a duration of 45 s. This meant that we had an identical number of spikes in both trains and an identical mean frequency, but with one train without and one train with discharge variability. This allowed us to determine whether greater responses are seen when incorporating physiological variability. All statistical analyses were performed using Prism 6 software (GraphPad Software, San Diego, USA).

3.4 RESULTS

Motor unit characteristics

Successful microstimulation of a single motor axon was performed on 14 motor units supplying the long toe extensors. Once a single motor axon was isolated, twitch-properties measured and a force-frequency curve generated, a 45 s train of irregular stimuli was delivered at 18 Hz. This was followed by the generation of another set of pulses to measure twitch properties and another force-frequency curve to assess whether the unit had fatigued. After a variable recovery time this was repeated (up to three times) until the twitch properties and force-frequency relationships had normalised. Five of the 14 units were deemed fatigable units, based on their relatively long recovery time, while the other 9 units recovered very rapidly. Therefore, the second (regular) long-lasting train could be delivered almost immediately for these units, given that the twitch properties and force-frequency curve had not changed from baseline values. Fig. 3.1 shows the force-frequency curves generated before the irregular or regular continuous train; there were no significant differences between the curves at any frequency, indicating that the motor unit properties had normalised before the regular long train was given.
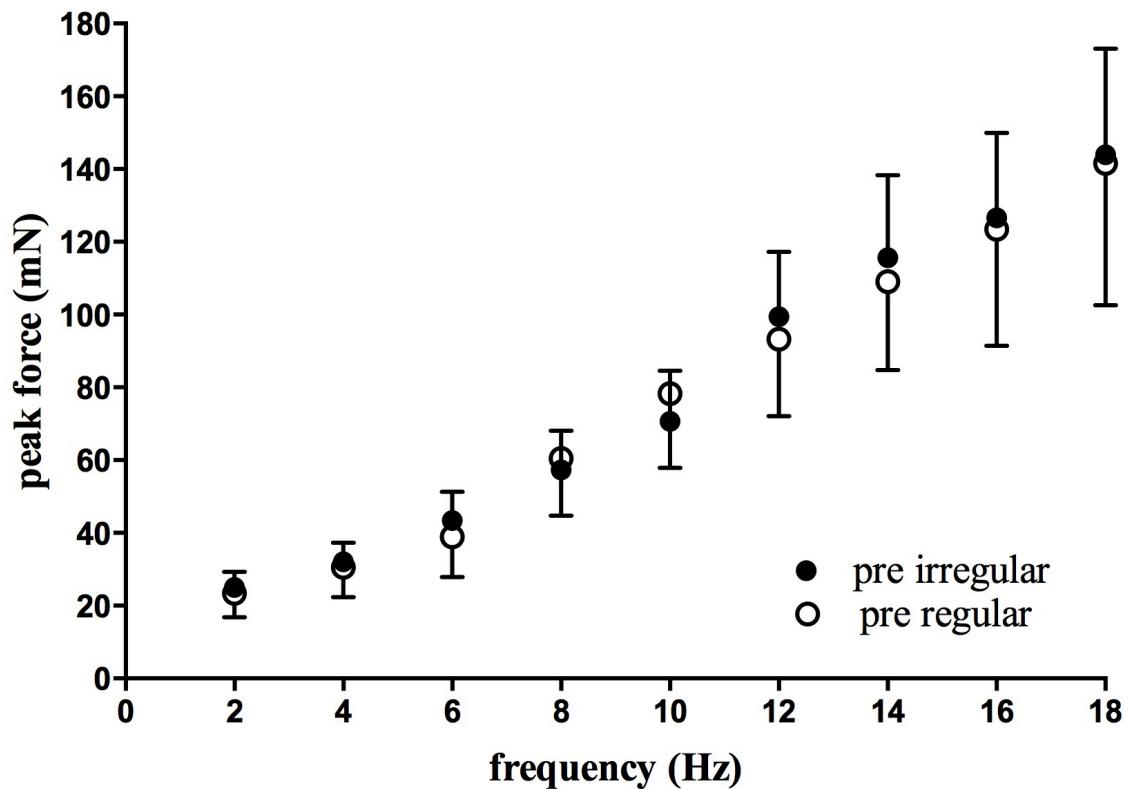
Figure 3.1: Force-frequency curves for all 14 motor units, delivered as short trains of 10 pulses at frequencies of 2-18 Hz, before delivery of the long trains of either irregular or regular stimuli. There were no significant differences in peak force (± SE) at any frequency.

Effects of discharge variability on force production

Experimental records from one motor unit are shown in Fig. 3.2. It can be seen that the irregular trains generated a much higher force at the beginning of the contraction, yet this declined in the last 20 s. Fig. 3.3 shows the time courses of the force generated during microstimulation of all 14 single motor axons with long trains of irregular or regular stimuli. The overall mean difference in force generated between the irregular and regular trains was 184.3 ± 11.5 mN in the first 5 s and 81.3 ± 22.4 mN at the conclusion of the train (45 s).

So, while there was evidence of fatigue during the course of delivery of the irregular trains, the regular trains produced a very stable force throughout the 45 s for all 14 single motor axons. However, the regular trains never generated forces that exceeded those produced by the irregular trains. As shown in Fig. 3.3, the forces generated by the irregular trains were significantly greater over the first 20 s (p<0.0001), but not over of the last 20 s.
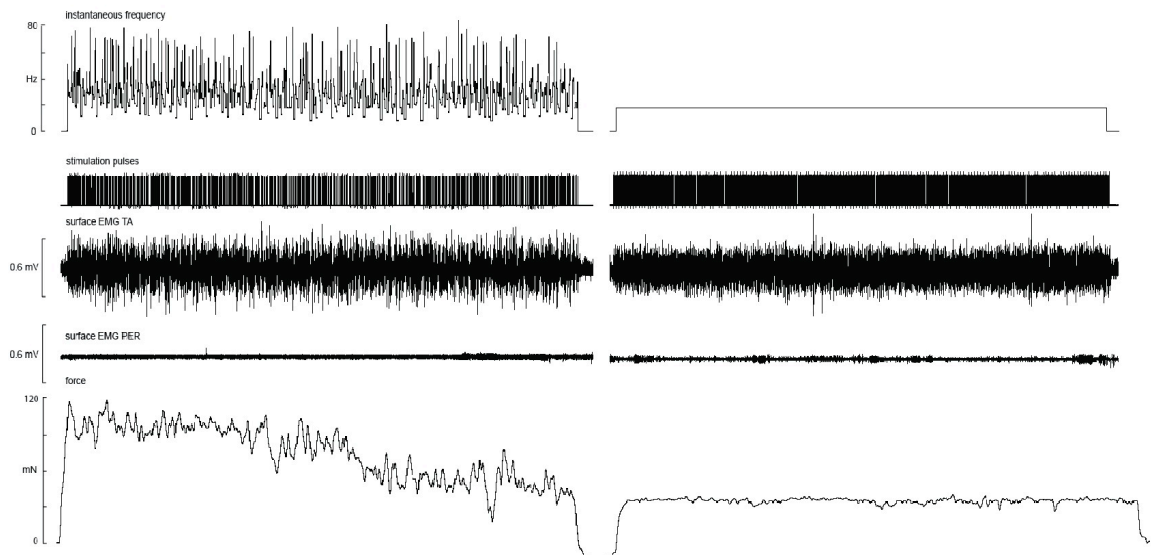
Figure 3.2: Experimental records from one subject. Microstimulation of a single motor axon supplying extensor digitorum longus with a 45 s train, delivered at a mean frequency of 18 Hz with (left panel) and without (right panel) variability.
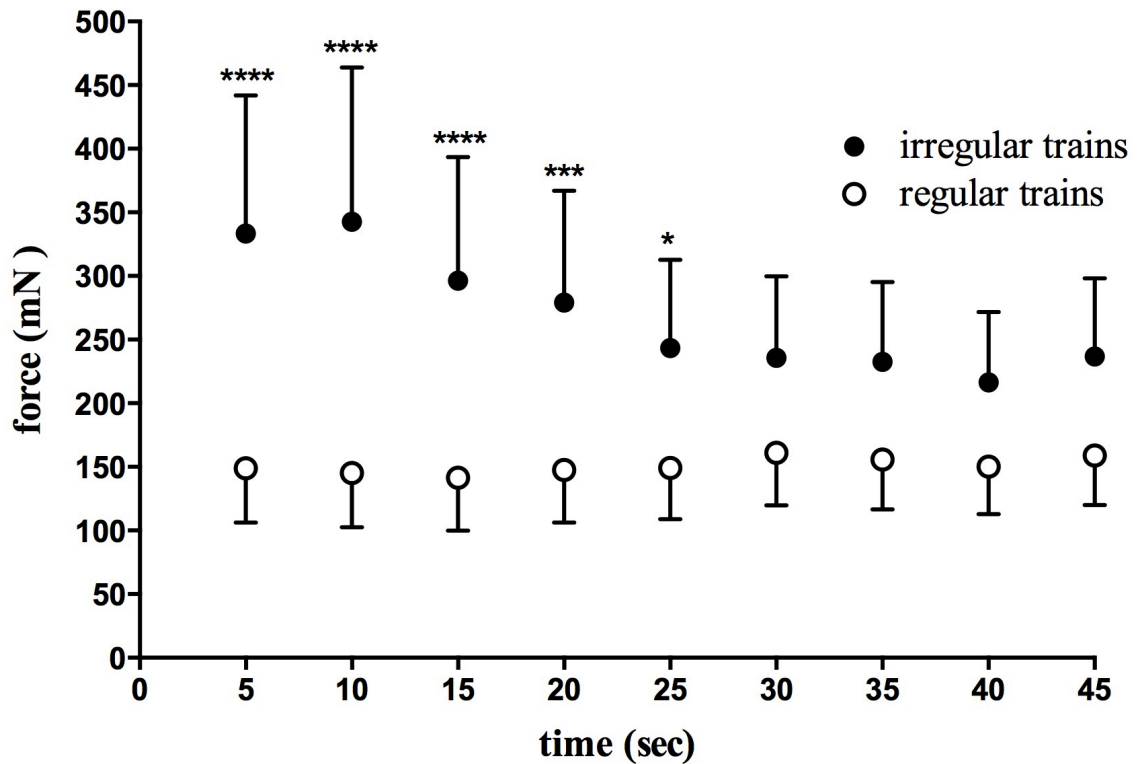
Figure 3.3: Peak force generated by 14 single motor units, comparing contractile responses produced by irregular and regular long lasting trains of stimuli. Forces generated by the irregular stimulus trains were significantly higher than those generated by the regular trains in the first 25 s. **** = $p<0.0001$; * = $p<0.05$.

Fig. 3.4 shows the total mean forces generated, when pooled over the first 20 s and over the last 20 s, for both the irregular and regular trains of stimuli. Mean forces, computed over 20 s, were significantly higher in both phases for the irregular than the regular trains, delivered with an identical mean frequency (18 Hz).
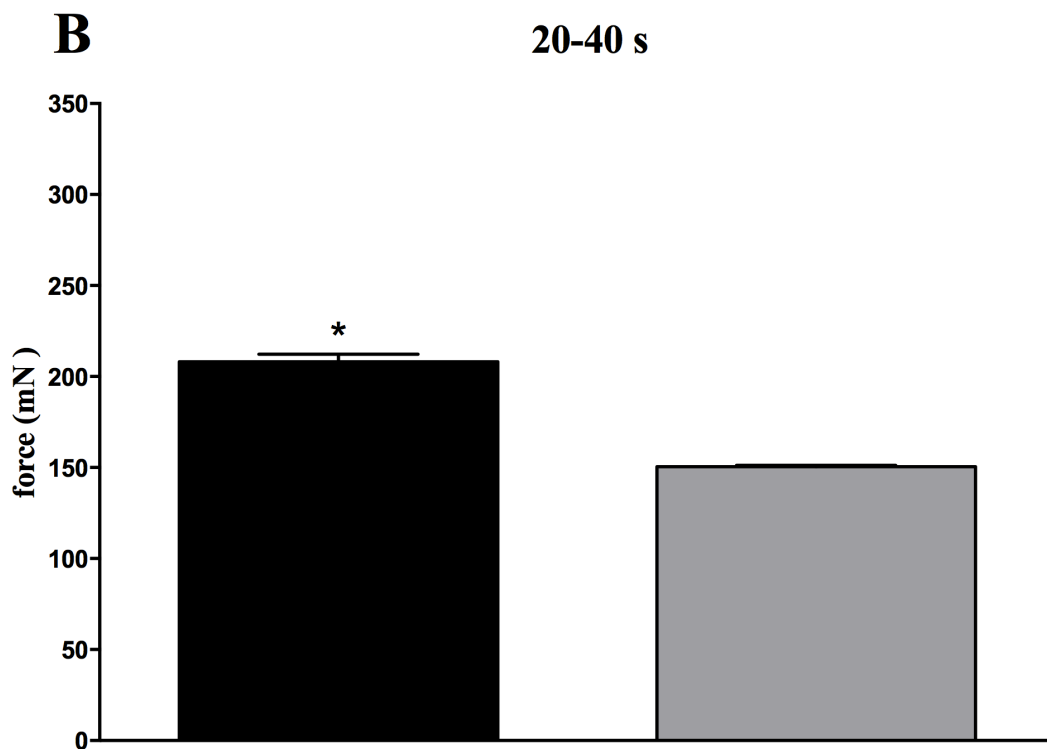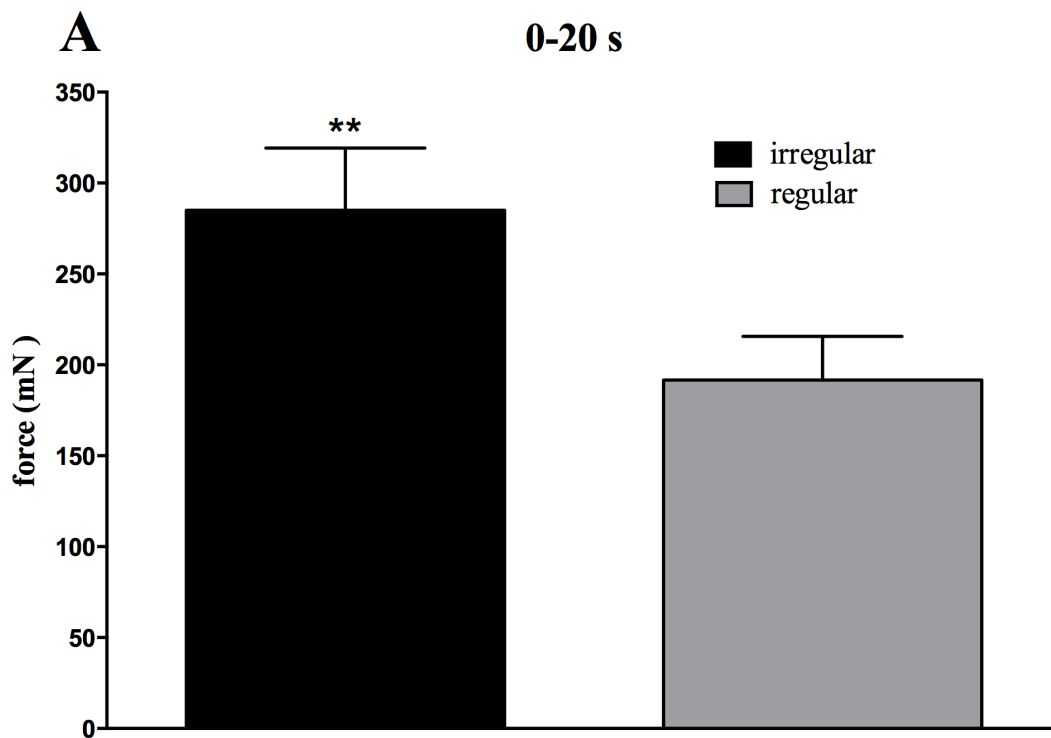
Figure 3.4: Total mean forces generated by 14 single motor axons. Mean forces generated during the first 20 s (**A**) and the last 20 s (**B**) were significantly greater for the irregular trains. ** = p<0.01; * = p<0.05.

3.5 DISCUSSION

The purpose of this study was to test the hypothesis that incorporating discharge variability into a long-lasting train of stimuli leads to augmented force production. In other words, the physiological discharge variability seen in the firing of single motor units during voluntary contractions offers advantages to the neuromuscular system. Our previous study showed that when incorporating physiological variability into short 10-pulse trains of stimuli there is an increase in peak contractile response, over mean frequencies ranging from 8-24 Hz (Leitch and Macefield 2014). This finding was the catalyst for further exploration into the potential advantages offered by incorporating physiological discharge variability into a train of stimuli. We have shown that the addition of physiological variability into long-lasting trains produces greater forces, but that this declines over time. Presumably, this decline in force production (i.e. fatigue) reflects the fact that forces were higher at the onset of the contraction produced by an irregular train, despite the mean frequency being identical to that of the regular train. In other words, the muscle itself cannot sustain the higher force generated by a train of irregular stimuli delivered at a mean frequency of 18 Hz. Nevertheless, given that the stimulation patterns we have generated emulate the firing of α motoneurones during voluntary contractions, one could expect that the nervous system takes discharge variability into account in producing force or movement.

Methodological considerations

One of the fundamental challenges in isolating a single motor unit is to selectively stimulate one and only one motor axon within a muscle fascicle of the nerve. This requires very low currents and meticulous care in order to direct the tip of the microelectrode into a fascicle supplying one of the toe extensor muscles. Very high

impedance tungsten electrodes were used, and careful monitoring of the twitch profiles and EMG potential was crucial. We were convinced that we had isolated a single motor axon when the EMG and twitch potentials were reproducible at the lowest possible current, with a safety margin usually between 3-7 uA. Within the safety margin any increases in current failed to increase the twitch and EMG of the active unit. Therefore, stimulating within this safety margin did not result in the recruitment of any additional motor axons. The trains of stimuli were delivered in the same order each time. Firstly, the irregular long lasting train was delivered, followed by the regular long-lasting train - with sufficient recovery time between the two trains. Contractile responses were monitored regularly during recovery by repeating the force-frequency curve (10 pulses, 2-18 Hz) until forces had normalised. This ensured that the muscle had fully recovered from any short-term muscle fatigue.

Mechanistic considerations

Animal work suggests that the increased contractile responses to stimulation with irregular trains could be explained purely on a biomechanical basis. Because muscle contractions are sluggish, they act as low-pass filters that limit the conversion of a motor unit action potential delivered by a motor axon to a contractile response. Accordingly, when a second stimulus is given before the motor unit has fully relaxed from the preceding stimulus, it prevents relaxation and produces augmentation of force, known as a catch-like property. This phenomenon was first observed in animals, in slow-twitch motor units in the triceps surae muscles of the cat (Burke et al., 1970) and has also been shown to produce greater tetanic responses in single motor units of the rat medial gastrocnemius (Celichowski & Grottel 1998). One possible explanation for this natural occurrence relates to calcium re-uptake. When an action potential is propagated along the

sarcolemma to the transverse tubule a signal from the t-system initiates calcium release from the sarcoplasmic reticulum into the myoplasm; if an additional stimulus were given before calcium reuptake occurred, then a decline in force would be seen.

It has been shown that calcium sensitivity of contractile proteins is unchanged during low-frequency fatigue (Westerblad et al., 1993) and that the likely cause of low-frequency fatigue is due to lack of calcium release from the sarcoplasmic reticulum (Allen et al., 1993). Therefore, a possible mechanism with regard to the current research may be that calcium does not have a chance to be released as efficiently during long lasting physiological trains due to slower reuptake of calcium caused by the shorter interspike intervals inherent in an irregular stimulus train. It is therefore not unreasonable to suggest that irregular stimuli would result in greater forces at the beginning of a long train, yet result in greater fatigue towards the end of the train, due to lack of available calcium. Irregular trains showed a greater rate of decline throughout the 45 seconds, but at the end of the train the force was still higher than that seen with the regular train. Previous studies have shown that initial doublets (10 ms interpulse interval) at the onset of stimulus train yielded an increased contractile response, both in whole muscle (Binder-Macleod 1991 & 1996; Lee et al., 1999) and in single motor units (Macefield et al., 1996). However, until now there have been no studies that have assessed the effects of adding physiological variability to a long-lasting train.

Functional Implications

Functional electrical stimulation (FES) has shown great potential as a rehabilitation therapy after injuries that affect the human motor system. It has been used after stroke and spinal cord injury (SCI) to assist in ambulation and to improve reach-to-grasp tasks

(Miller et al., 1990; Billian et al., 1992). It has also been used in stimulation of the peroneal nerve to treat foot drop (Waters et al., 1975; Voigt et al., 2000) and to help in bladder control of paraplegics (Brindley, 1973; Jonas et al., 2001). FES of whole muscle, delivered via surface electrodes, has been shown to increase muscle mass, improve contractility and increase force output (Scremin et al., 1999; Baker et al., 1979).

The patterns of stimulation applied in this therapy utilize constant-current, high-frequency, stimulation trains - typically ranging from 30-100 Hz. One of the major limitations with this therapy is muscle fatigue. It is well known that stimulating with high-frequency trains causes rapid fatigue (Bigland-Ritchie et al., 1979; Garland et al., 1988) and thus, the long-term efficacy for rehabilitation purposes may be underestimated. Because SCI patients have muscles predominately composed of fast-fatigable muscle fibers then this could be a reasonable explanation for the rapid fatigue exhibited in these patients during FES therapy (Burnham et al., 1997; Klein et al. 2006). However, another concern is the order in which the motor units are activated. Henneman's size principle states that the smallest motor units supplying the least number of fibers are recruited first (owing to their higher input resistance), through to the largest ones, supplying the most fibers, last (Henneman, 1965; Olson et al., 1968). This results in slow ramp voluntary contractions being graded in a linear fashion (Milner-Brown et al., 1973; Bigland et al., 1954). However, the smallest motoneurones have the smallest diameter axons, and hence have the highest electrical thresholds to direct electrical stimulation, while the largest motoneurones - with larger axons - have the lowest electrical thresholds. This means that when stimulating whole muscle, the largest motor axons will be preferentially activated, and these will be the most susceptible to fatigue. It is only through supramaximal stimulation that all motor axons will be excited and small,

fatigue-resistant, motor units recruited. Conversely, microstimulation of single motor axons via an intraneural microelectrode does not favor large over small axons: the axon closest to the tip of the stimulating microelectrode is the one that is recruited at the lowest currents, and we know from our own work, and that of others, that the whole range of twitch-types is sampled essentially randomly.

3.6 CONCLUSIONS

We have shown that incorporating physiological variability into long-lasting trains of stimuli offers an advantage to the neuromuscular system. Irregular trains showed significantly greater contractile responses for the first 20 seconds of the train with a greater rate of decline than regular trains over the same mean frequencies (18 Hz, 45 s). However, while irregular long-lasting trains showed a greater rate of decline the force never dropped below that produced by a regular train of identical mean frequency with zero variability.

3.7 ACKNOWLEDGEMENTS

CHAPTER 4

COMPARISON OF THE BALLISTIC CONTRACTILE RESPONSES GENERATED DURING MICROSTIMULATION OF HUMAN MOTOR AXONS WITH BRIEF IRREGULAR AND REGULAR STIMULI

Published in Muscle & Nerve

4.1 ABSTRACT

Ballistic voluntary contractions are brought about by brief, high frequency (60-100 Hz) trains of action potentials that result in maximum accelerations and forces with a very brief contraction time. During ramp voluntary contractions human motoneurones exhibit a physiological variability of ~20% and this variability has been shown to be advantageous to the neuromuscular system. We tested the hypothesis that ballistic contractions incorporating physiological variability generate greater isometric forces than ballistic stimuli of identical mean frequency but no discharge irregularity. Highly selective tungsten microelectrodes were inserted into the peroneal nerve and single motor axons stimulated with both irregular and regular ballistic stimuli with mean frequencies ranging from 57.8-68.9 Hz. Forces were measured with an isometric transducer over the distal phalanx of the relevant digit. Irregular trains of five pulses emulated the spike

trains recorded from single motor units during ballistic contractions. For an identical mean frequency irregular trains generated significantly greater peak forces than regular trains. We conclude that the high forces generated by ballistic contractions are not solely based on high frequencies, but rather on a combination of high frequencies and discharge irregularity. It appears that irregular ballistic trains take advantage of the "catch-like property" of muscle, allowing further augmentation of force.

4.2 INTRODUCTION

A slowly ramping voluntary contraction requires steady gradation of force through two primary mechanisms. Firstly, by varying the number of motor units recruited – in which small units are recruited first, according to the size principle (Henneman, 1957; Henneman et al., 1965) - and by varying the discharge rate of the active motoneurones (rate coding). In human subjects, intraneural microstimulation of single motor axons allows one to assess the contribution of rate coding to the generation of force (Thomas et al., 1991; Macefield et al., 1996; Fuglevand et al., 1999; McNulty et al., 2000; McNulty & Macefield, 2005) or displacement (Leitch & Macefield, 2014, 2015). The relationship between stimulation frequency and force or displacement follows a sigmoidal pattern: effective gradation of force or displacement occurs within the linear portion of this curve, in which for a given increase in frequency, there is a predictable increase in contraction amplitude (Copper & Eccles 1930; Bigland and Lippold 1954; Thomas et al., 1991; Fuglevand et al., 1999). However, not all movements during everyday life are slow and ramp-like in nature; many dynamic movements require extremely brief contraction times. Movements that exhibit maximum velocities and accelerations in a short period of time are termed ballistic contractions. Recordings from single motor units during ballistic contractions have revealed high firing rates, but there is no preferential activation of fast-

twitch motor units (Desmedt & Godaux 1977a,b; 1978; Zehr & Sale 1994; Duchateau & Baudry 2014). In other words, the recruitment of motor units during ballistic contractions still follows the size principle, with high firing rates determining the rapid rate of force production (Duchateau & Baudry 2014).

Recordings from single motor units supplying tibialis anterior revealed high discharge rates ranging from 60-100 Hz, with a decline in discharge rate with subsequent spikes (Desmedt & Godaux 1977a). Similar results have been observed for motor units supplying the masseter and first dorsal interosseous muscles (Desmedt & Godaux 1977b, 1979). It is well known that during static voluntary contractions individual motor units fire with considerable variability, of ~20% (Masuda and DeLuca 1991; Moritz et al. 2005). Moreover, the start of a voluntary contraction often includes an "initial doublet," in which the first two action potentials occur at a short interval of ~10 ms (Van Cutsem et al., 1998; Bawa & Calancie, 1983). It has been shown that intraneural microstimulation of individual motor axons supplying the toe extensors generates greater force when a stimulus train is preceded by a 10 ms interpulse interval that emulates these initial doublets, despite the total number of pulses in the train being identical (Macefield et al., 1996). In addition to high instantaneous firing rates, first observed by Desmedt and Godaux (1977a), motor units activated during ballistic voluntary contractions also exhibit a level of physiological variability that is yet to be explored. And while many studies have *recorded* from single motor units during ballistic contractions, no study has microstimulated motor axons with short high-frequency stimuli that emulate volitionally activated motoneurones during ballistic contractions.

The aim of the current study was to examine whether there are differences in force production between brief trains (5 pulses) of high-frequency stimuli that incorporate discharge variability and brief trains stimuli of identical mean frequency yet zero variability (i.e. regular stimulus trains of 5 pulses). Is it purely the high frequencies of ballistic stimuli that result in higher accelerations and forces, as postulated by Duchateau and Baudry (2014)? Or could incorporating physiological discharge variability into ballistic stimuli further intensify the contractile responses of single motor units?

4.3 METHODS

Successful experiments were conducted on nine male and three female subjects ranging in age from 18-52 years old. Studies were conducted under the approval of the Human Research Ethics committee of Western Sydney University. Subjects provided informed written consent to participate in the study.

Subjects were seated in a dental chair with the leg flexed to approximately 120deg and the plantar surface of the foot fixed to a rigid footplate. A Velcro strap was placed over the dorsum of the foot just below the metatarsophalangeal joint. The thigh was supported by a vacuum cushion, to prevent any movement during the experiment. The common peroneal nerve was located using weak electrical currents (0.02-10.00 mA, 0.2 ms, 1 Hz; Stimulus Isolator; ADInstruments, Sydney, Australia) delivered by a 2-mm diameter surface probe. Using the fibula head as a landmark, the course of the nerve was mapped, marking points that yielded the strongest contractile response from one of the toe extensor motor units (EHL & EDL). A High-impedance tungsten microelectrode (Frederick Haer & Co, Bowdoin, ME, USA) was guided through the skin into the nerve. An Ag/Cl surface electrode was placed on the medial surface of the knee, which served

as an anode and constant-current cathodal currents were delivered through the microelectrode (0.02-1.00 mA, 0.2ms, 1 Hz). EMG was recorded with Ag/AgCl surface electrodes placed over the anterior and lateral compartments of the leg and over the dorsum of the foot. This allowed EMG recording from EHL, EDL and EDB. Signals were amplified (gain 2,000×; Bioamplifier; ADInstruments, Sydney, Australia), and filtered (10 Hz-1 kHz) and recorded (2 kHz sampling) on a computer- based data acquisition and analysis system (Powerlab 16 SP hardware and LabChart 7 Pro software; ADinstruments). Contractile force (DC-100 Hz; 200 Hz sampling) was measured using an isometric force transducer (Model 1030, UFI, Morro Bay, USA) placed over the nail of the relevant digit.

During intraneural stimulation the microelectrode was manually adjusted to stimulate a motor fascicle supplying the dorsiflexor muscles . Meticulous precision was required to activate a single motor axon and very low currents were required, usually ranging from 7-16 μA. It was determined that a single motor axon was isolated when the twitch amplitude and EMG profiles were reproducible at very low currents. Furthermore, they displayed a well-defined safety margin, typically 2 μA, where increases in current within the safety margin, failed to modify the twitch properties or EMG profile.

Irregular ballistic stimuli were based on discharge patterns recorded from single motor units in the tibialis anterior, during voluntary contractions. Fine tungsten microelectrodes were inserted directly into the muscle belly of tibialis anterior. Once the electrode was stable the subject was asked to dorsiflex with maximum force for ~ 1 s. The microelectrode was manipulated when necessary to obtain a pool of spike trains from 23 motor units within tibialis anterior. The interspike intervals from the recorded spike

trains were used to generate short physiological trains of stimuli (5 pulses) with the maximum firing rates to emulate ballistic stimuli in normal physiological conditions. A pool of trains with mean frequencies ranging from 57.8-68.9 Hz and mean variability ranging from 42 to 54% were delivered once a single motor axon was isolated.

Regular ballistic stimuli were based on the mean firing rates of irregular ballistic stimuli. These trains were also short 5 spike trains and were delivered with the identical mean firing rate as the irregular trains but contained zero physiological variability. This allowed comparison between two types of high frequency stimuli: irregular trains that emulate the firing of volitionally active motoneurones and regular high-frequency trains with constant inter-spike intervals. All of the statistical analysis was performed using prism 6 (GraphPad Software, San Diego, CA). We tested the hypothesis that irregular ballistic stimuli produce greater contractile forces than regular ballistic stimuli over the identical mean frequencies. To assess whether greater contractile responses were produced during stimulation with irregular trains, paired one-tailed t-tests were used; differences were considered significant at $P < 0.05$.

4.4 RESULTS

Trains of motor-unit action potentials, recorded from 23 single motor units in a single subject from tibialis anterior during ballistic voluntary contractions were used to generate a set of stimulation patterns for use in intraneural microstimulation of single motor axons. Fig. 4.1 shows experimental records for one of these motor units. This unit generated up to nine spikes during a series of ballistic contractions. It can be seen that the discharge was irregular and that the rate of rise of force was greater for the contraction in which the first interspike intervals were short, leading to high instantaneous frequencies.

The particular trains shown in Fig. 4.1 were not used as model trains for the stimulation because their mean firing rates were too low, but were chosen to illustrate the effects of short interspike intervals on the rate of force production.
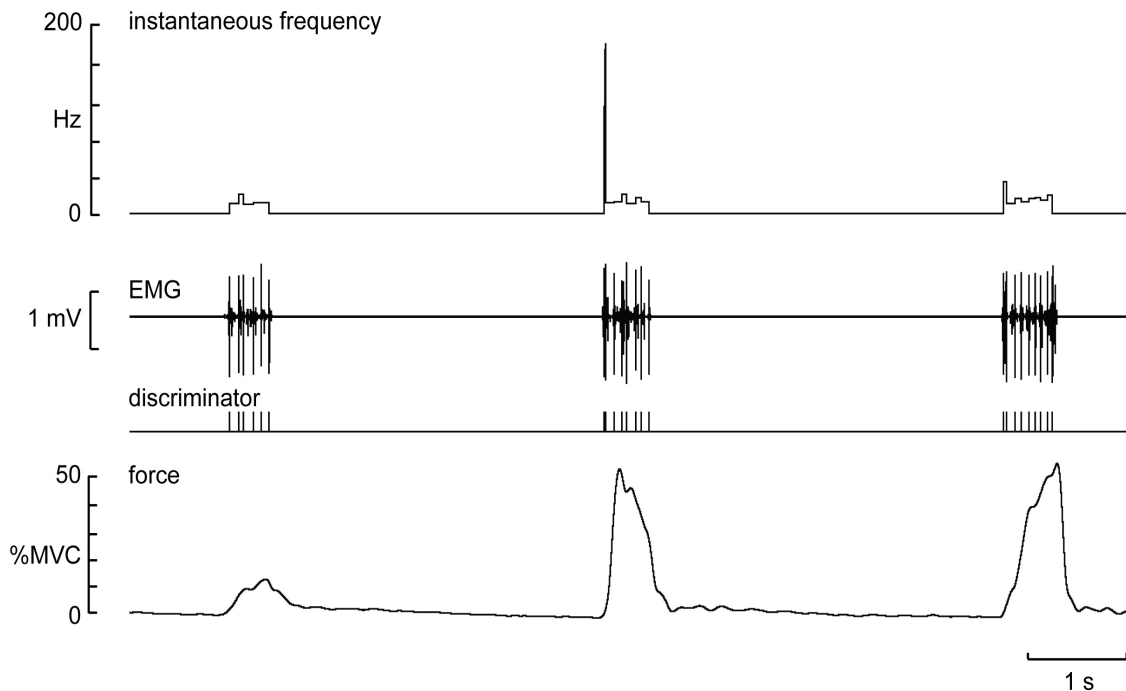
Figure 4.1: Activity of a single motor unit in tibialis anterior, recorded via an intramuscular microelectrode, during brief dorsiflexion of the ankle. This motor unit generated 5-9 spikes during a series of contractions, three of which are shown. Discriminated pulses are shown below the EMG recording.

Intraneural microstimulation was successfully performed on 12 single motor axons supplying the long toe-extensor muscles, extensor digitorum longus (EDL, n=8) and extensor hallucis longus (EHL, n=4). Ballistic stimuli were delivered using both irregular and regular trains (8-10 trains per unit). Irregular trains, emulating those found during ballistic contractions, were taken from a bank of randomly-generated stimuli and were always the first to be delivered. Mean stimulation frequencies (± S.D) of these trains was 54.0 ± 10.1 Hz, with the average maximal and minimal firing rates being 92.4 ± 23.2 Hz and 36.8 ± 4.7 Hz, respectively. Mean variability was 29.2 ± 2.1 %. Regular trains (i.e.

with zero variability) were delivered at an identical mean frequency as the set of irregular trains, both sets of trains comprising five stimulus pulses. These firing rates are shown clearly in Table 4.1.

Table 4.1: Stimulus rates for 12 single motor axons for irregular & regular stimuli

Unit	Muscle	Irregular trains			Regular trains		
		Mean stimulus rate (Hz)	Max stimulus rate (Hz)	Min stimulus rate (Hz)	Mean stimulus rate (Hz)	Max stimulus rate (Hz)	Min stimulus rate (Hz)
1	EDL	61.3	111.1	41.9	60	60	60
2	EDL	61.2	111.1	37.2	60	60	60
3	EDL	49.7	65.8	30.3	50	50	50
4	EHL	60.4	111.1	32.6	60	60	60
5	EHL	61.1	111.1	41.9	60	60	60
6	EHL	40.5	66.68	35.4	40	40	40
7	EDL	55.8	111.1	39.9	55	55	55
8	EDL	50.8	111.1	35.9	50	50	50
9	EDL	51.0	68.2	40.6	52	52	52
10	EDL	42.9	67.2	30.7	45	45	45
11	EDL	69.9	111.1	43.1	70	70	70
12	EHL	41.2	62.6	31.9	40	40	40
Mean ± SD		54.0 ± 10.1	53.8 ± 9.3	92.4±23.2	53.5 ±9.1	53.5 ± 9.1	53.5 ± 9.1

Maximum, minimum and stimulus rates for trains delivered to each subject supplying the toe extensors (n=12). 8-10 trains were given per unit and the overall means ± SD are shown in the bottom row. It can be seen that the mean firing rates are the same for each individual stimulated motor unit.
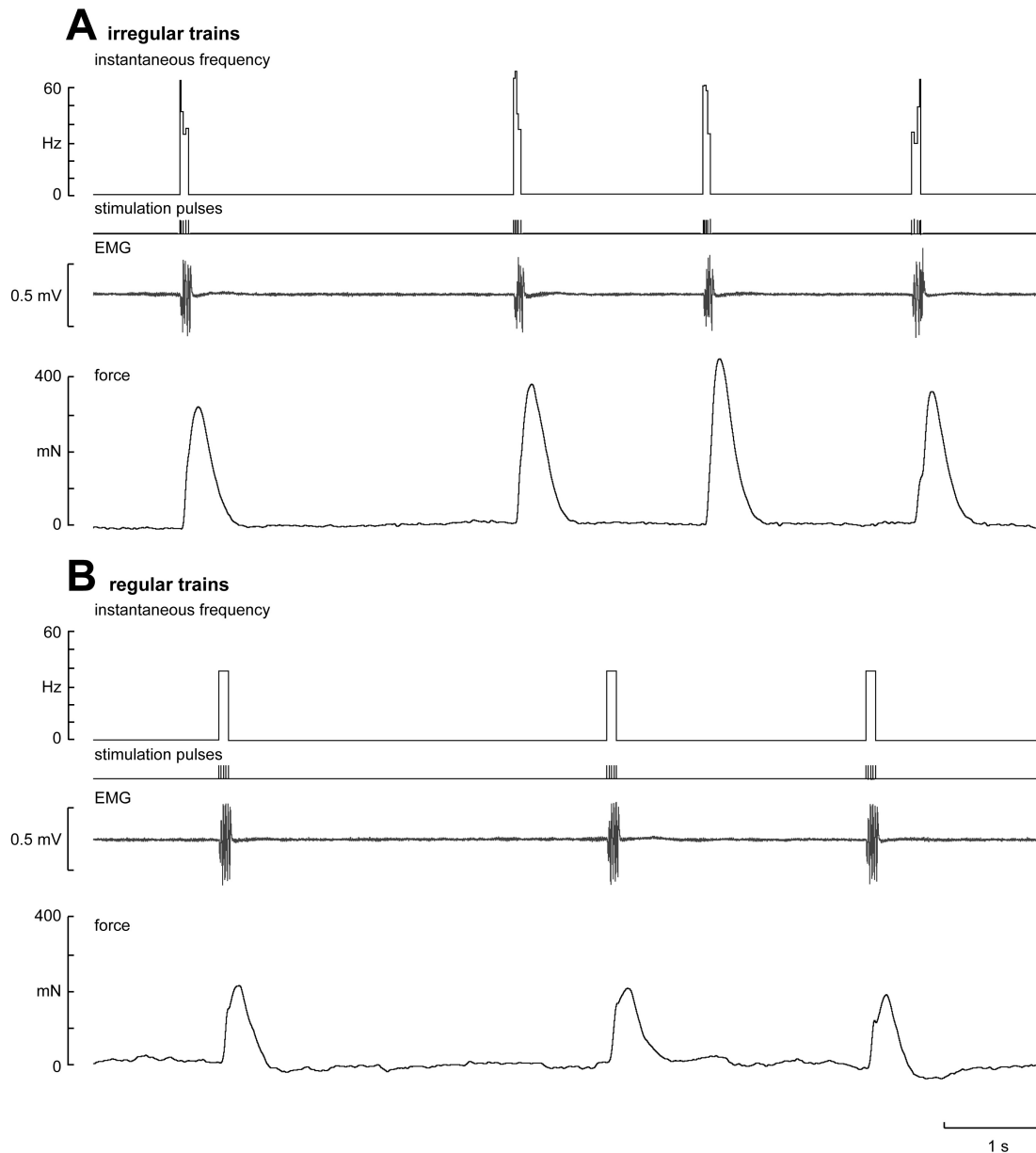
Figure 4.2: Force responses to microstimulation of a single motor axon supplying EDL with brief trains of irregular (**A**) and regular (**B**) stimuli. Four of nine stimulus trains are shown in **A**, and three of 10 stimulus trains are shown in **B**. Mean instantaneous frequency for the nine irregular trains was 40.5 ± 12.5 Hz and 40.0 ± 0 Hz for the regular trains.

Fig. 4.2 shows examples of the forces generated during microstimulation of a single motor axon supplying EHL with brief trains of irregular (A) and regular (B) stimuli. Mean instantaneous frequency and discharge variability for this motor axon was 40.5 ± 12.5 Hz and 31 % for the irregular trains, and 40.0 ± 0 Hz and 0 % for the regular trains. Despite the mean frequencies and number of pulses (n=5) comprising each train being

the same, it can be seen that the peak forces generated is greater for the irregular trains. twitch parameters for all 12 motor axons stimulated with irregular trains are shown in Table 4.2. For comparison these parameters are provided for the regular trains in Table 4.3.

Table 4.2: Twitch parameters for 12 single motor axons during irregular stimuli

Unit	Muscle	Rise time (ms)	Fall time (ms)	Peak area (mN.ms)	Peak force (mN)
1	EDL	79.2	160.5	50547	177.7
2	EDL	105.3	145.9	92487	317.1
3	EDL	72.6	141.1	11260	47.2
4	EHL	87.9	144.4	614709	2019.0
5	EHL	122.1	248.9	16266	47.4
6	EHL	83.4	199.1	89589	296.2
7	EDL	118.9	238.2	90582	233.1
8	EDL	90.1	122.4	654188	2698.0
9	EDL	66.8	282.7	36558	84.6
10	EDL	72.5	189.1	21879	76.6
11	EDL	79.0	163.1	50689	181.2
12	EHL	56.7	146.7	48005	185.3
Mean ± SE		**86.2 ± 5.8**	**181.8 ± 14.6**	**148063 ± 66125**	**530.3 ± 251.4**

Mean contractile parameters for all 12 single motor axons after stimulation with brief trains of irregular stimuli emulating ballistic contractions. For each unit 8-10 trains were given and the means calculated. Overall means ± SE are shown in the bottom row.

Table 4.3: Twitch parameters for 12 single motor axons during regular stimuli

Unit	Muscle	Rise time (ms)	Fall time (ms)	Peak area (mN.ms)	Peak force (mN)
1	EDL	41.4	175.5	32892	130.1
2	EDL	83.2	149.7	52041	199.8
3	EDL	76.2	148.5	49832	27.57
4	EHL	51.0	146.7	374919	1428.0
5	EHL	115.7	128.5	78117	26.8
6	EHL	87.3	163.6	56997	197.9
7	EDL	103.1	255.1	62500	153.6
8	EDL	89.5	120.9	505925	1973.0
9	EDL	65.5	235.7	73689	72.7
10	EDL	76.1	167.9	23617	83.5
11	EDL	66.9	179.3	31960	135.3
12	EHL	56.1	164.0	30698	113.9
Mean ± SE		**76.0 ± 6.2**	**169.6 ± 11.5**	**114432 ± 44964**	**378.5 ± 182.1**

Mean contractile parameters for all 12 single motor axons after stimulation with brief trains of regular stimuli emulating ballistic contractions. For each unit 8-10 trains were given and the means calculated. Overall means ± SE are shown in the bottom row.

Mean peak amplitude and peak area data are shown graphically in Fig. 4.3. There was a significantly greater amplitude ($p<0.001$, one-tailed t-test) and area ($p<0.01$, one-tailed t-test) with irregular trains of stimuli than regular trains of stimuli.
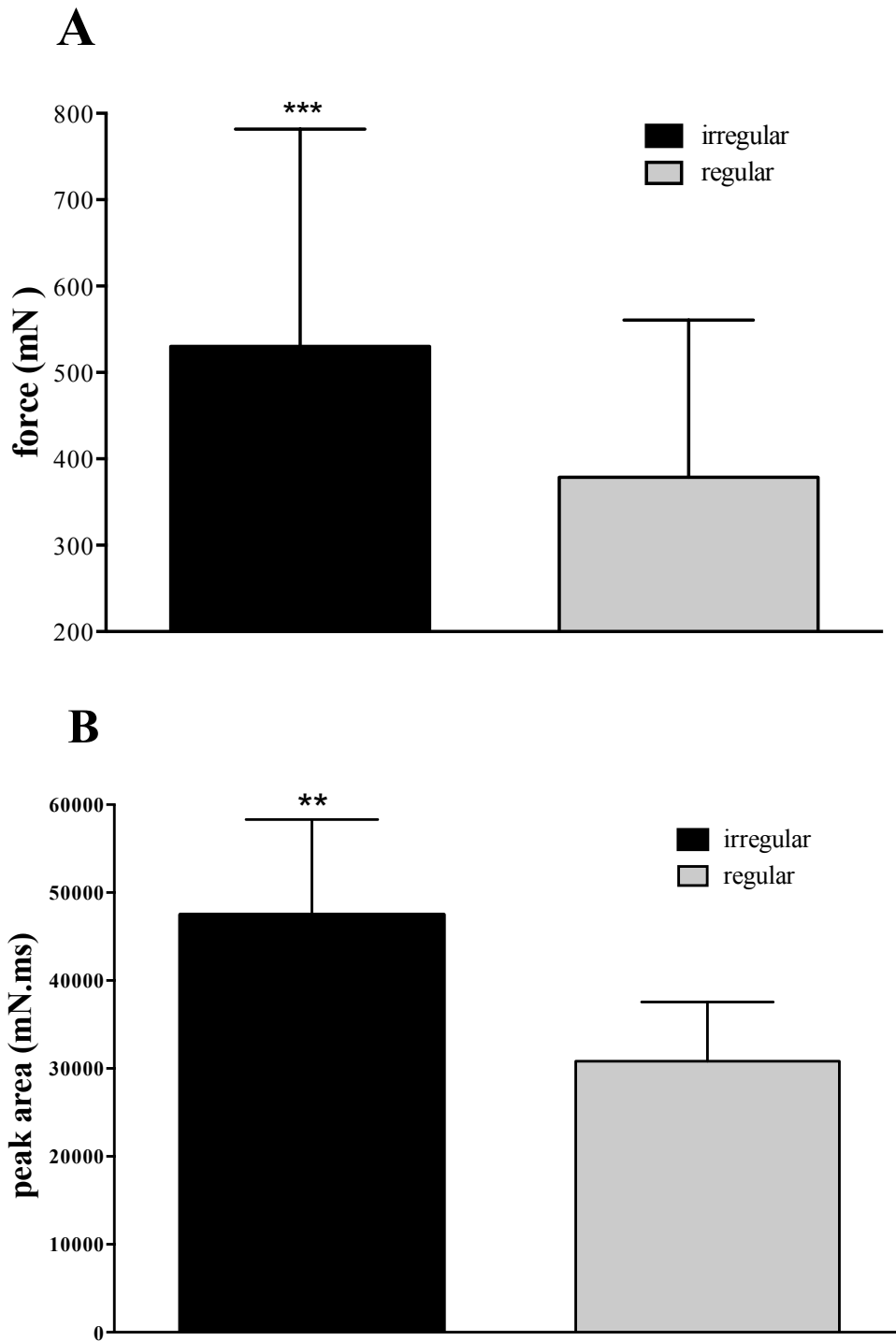
Figure 4.3: Mean peak force (**A**) and peak area (**B**) for 12 single human motor axons supplying the toe extensors, stimulated with irregular and regular stimuli emulating ballistic contractions. *** P <0.001; ** P <0.01.

4.5 DISCUSSION

The purpose of this study was to examine how motor units supplying the long toe extensors behave when stimulated with high-frequency irregular trains of stimuli that emulate the firing of real motoneurones during ballistic contractions. Until now the only data on ballistic contractions have relied on recording from single motor units during these conditions (Duchateau & Baudry 2014; Desmedt & Godaux 1977b, 1978). These studies have provided important information on the firing rates of individual motoneurones during ballistic contractions, but could not examine the influence of discharge variability on force production. We have now shown that incorporating discharge irregularity into short high frequency trains leads too much greater contractile responses than regular constant frequency trains exhibiting zero variability.

Methodological considerations

One of the greatest limitations to this technique is the low experimental yield. Single motor axon stimulation requires meticulous precision when guiding the tip of the microelectrode towards a single axon. The slightest movement of the subject or the microelectrode can result in loss of an intrafascular stimulation site. The electrode itself must have a very high-impedance (>2.6 MΩ) in order to selectively isolate a single motor axon, which makes intraneural searching within the fascicle even more difficult. In addition to diligence when manipulating the electrode, careful observation of the EMG and twitch profiles is necessary. We were convinced that the site was unitary when stimulation over a particular safety margin, usually, 2-3 μA, failed to modify the twitch or EMG profile. Thus, the EMG potentials were identical and the twitch profiles were reproducible within the safety margin. When the current was increased above the specified safety margin, additional motor axons were recruited, resulting in increases of

both EMG and twitch amplitudes. Conversely, decreasing the current below the safety margin resulted in a parallel loss of the twitch and EMG. Once we had confirmed that we were stimulating one and only one axon, the ballistic stimuli were then delivered. We always delivered the irregular ballistic trains first, followed by regular trains with the same mean frequency but zero variability. It may be postulated that giving the irregular trains first could result in some fatigue and thus the force responses elicited by the regular trains may be diminished. This is unlikely for two reasons. Firstly, there was a sufficient rest period (5-8 min) between the irregular and regular trains to allow muscle recovery. Secondly, stimulating with frequencies >50 Hz can result in low-frequency fatigue but stimulating with short high frequency trains has much less effect (Westerblad et al., 1993; Jones 1996). Thus, unlikely to result in diminished force.

Catch-like properties of muscle

It has been suggested that the high firing rates associated with ballistic contractions are responsible for the high velocity contractions in short periods of time, which results in peak forces and short contraction times (Desmedt & Godaux 1977b; 1978; Zehr & Sale 1994; Desmedt and Baudry, 2014). We set out to determine whether ballistic contractions reach maximum forces solely due to their high firing rates or whether incorporating patterns of stimuli that promote "catch-like properties" causes a further augmentation of force. It is well established that activating motor units with initial doublets at the onset of low frequency trains results in tension enhancement. This has been conducted in both animal (Bevan et al., 1992; Callister et al., 2003; Burke et al., 1970) and humans (Bawa et al., 1983; Bigland-Ritchie et al., 2000; Macefield et al., 1996; Binder-Macleod et al., 1991) models. The phenomenon is based on the original work conducted by Cooper and Eccles (1930). They first established the distinct

relationship between frequency and tetanus and concluded that higher frequencies result in greater responses from the muscle during isometric conditions. Burke and colleagues (1970) showed that incorporating an initial doublet at the onset of a train of stimuli results in augmentation of force in the cat, and termed this phenomenon the "catch-like property". The phenomenon has also been observed during microstimulation of single motor axons supplying the human toe extensors (Macefield et al., 1996).

In addition to enhancing force output, utilizing the catch-like property of muscle has been shown to reduce the magnitude of fatigue. This has been shown in the hind limb of the cat (Bevan et al., 1992), turtle (Callister et al., 2003) and human (Binder-Macleod et al., 1991 & 1999). Furthermore, a previous study conducted by our lab has shown that activating single motor axons with irregular trains of stimuli is advantageous to the neuromuscular system. We recorded physiological trains of stimuli that contained doublets throughout the train and compared those with regular trains that had zero variability. We concluded that stimulating single motor axons supplying the toe extensors with irregular trains resulted in greater force responses and reduced fatigue than regular trains over the frequency range of 8-24 Hz (Leitch and Macefield, 2014). The current study has shown that when delivering irregular and regular ballistic stimuli to motor axons supplying the toe extensors, force responses were higher for the irregular trains over identical mean frequencies. Therefore, it is not unreasonable to suggest that physiological variability in ballistic stimuli contributes to the maximum forces generated during dynamic fast contractions. While it has been shown that the " catch-like property" offers force advantages at the onset of low-frequency trains, the current study has shown that brief trains of stimuli incorporating physiological variability produce stronger

ballistic contractions than those produced by brief regular trains of identical mean frequency

Mechanistic considerations

Animal models have allowed the examination of complex cellular changes that occur from the use of doublet (short-interval) stimuli. It has been reported that the initial increases in force development from doublets is due to changes in the levels of intracellular calcium in the cell cytosol (Duchateau & Hainaut 1986b). This initial increase is most likely produced by an increase in the release of calcium from the sarcoplasmic reticulum, which is more important during a fatigued state (Westerblad & Allen 1991). Cheng and colleagues (2013) recently showed that in both fatigued and non-fatigued flexor digitorum brevis muscles of the mouse, doublets can improve isometric force production by inducing release of calcium from the sarcoplasmic reticulum (Cheng et al., 2013). Other studies have suggested that force may be increased due to excitation-contraction mechanisms, such as the persistence of attached cross bridges (Sandercock & Heckman, 1997). Irrespective of the cellular mechanisms, at the output level we clearly see an increase in peak force production with natural physiological variability, which incorporates these high-frequency doublets.

Functional implications

Many movements during everyday life are dynamic in nature and thus require different neuronal patterns to elicit particular movements. When microstimulating single motor axons we have shown higher stimulation frequencies are required to elicit 50% of the maximal contractile response during unloaded isotonic conditions, while lower frequencies are required during loaded isotonic and, especially, isometric contractions

(Leitch and Macefield, 2015). Based on the current study stimulating muscles with short irregular ballistic trains should generate augmented contractile responses during high-acceleration dynamic movements. It is not unreasonable to suggest that closely emulating the patterns of neuronal discharge patterns during the appropriate biomechanical conditions could potentially transpire to have clinical importance in the rate of recovery after motor impairments, by emulating how motoneurones behave in normal voluntary conditions

4.6 CONCLUSIONS

We have shown, for the first time, that high-frequency irregular ballistic stimuli produce greater force responses than regular trains, which exhibit zero variability. Irregular trains produced significantly greater isometric forces over mean frequencies from 57.8-68.9 Hz. Due to the complexity of this system, the cellular mechanisms are not entirely clear. However, at the integrative level it is clear that irregular trains are taking advantage of the catch-like property of mammalian muscle.

4.7 ACKNOWLEDGEMENTS

CHAPTER 5

INTRAMUSCULAR STIMULATION OF TIBIALIS ANTERIOR: THE EFFECTS OF DISCHARGE VARIABILITY ON FORCE GENERATION AND MUSCLE FATIGUE

Under review in the Journal of Applied Physiology

5.1 ABSTRACT

Continuous intramuscular electrical stimulation of tibialis anterior was used to test the hypothesis that delivering irregular (physiological) stimuli increases force production and offsets fatigue in comparison to a continuous train of regular stimuli at an identical mean frequency. A tungsten microelectrode was inserted directly into the muscle belly of tibialis anterior and stimulated at current intensities of 5-7 mA at mean frequencies of 19 or 24 Hz. Seven subjects were given a regular train of stimulation at 19 Hz for 120 s, followed by an irregular train of identical mean frequency for a further 120 s. In addition, 3 additional subjects were given a regular train at 24 Hz, followed by an irregular train at the same mean frequency. For all subjects irregular stimulation resulted in greater force production over the same mean frequencies. Interestingly, for those subjects who exhibited fatigue during regular stimulation at 24 Hz, force began to rise once the irregularity was incorporated. We conclude, that physiological discharge variability offers a significant advantage to the human neuromuscular system during fatigued and

non-fatigued states and could offer benefits to therapies such as Functional Electrical Stimulation.

5.2 INTRODUCTION

Damage to the central nervous system from stroke or spinal cord injury can result in a loss of motor function and/or somatic sensation. There is a range of therapeutic techniques that may be implemented in order to promote faster recovery. One commonly used therapy is Functional Electrical Stimulation (FES) or functional neuromuscular stimulation, in which electrical stimuli are delivered to muscles via surface electrodes. It has been shown to increase contractile strength (Carnstam et al., 1977), improve muscle tone (Gerrits et al., 2002) and improve energy efficiency during walking (Taylor et al., 1999) when volitional control has been lost. However, the major concern surrounding FES therapy is rapid fatigue of the muscle fibers (Jones et al., 1996). There are two key explanations as to why FES results in rapid muscular fatigue. Firstly, the frequencies delivered during FES therapy are often very high, exceeding stimulation frequencies at which the force-frequency relationship plateaus, previously established for human motor units (Macefield et. 1996; McNulty et al., 2000; Thomas et al., 1991a; Westling et al., 1990). Indeed, it has been shown that microstimulation of single motor axons supplying the long finger flexor muscles at frequencies above 30 Hz does not increase force production (Fuglevand et al., 1999), yet FES frequently stimulates at frequencies above 30 Hz. Secondly, it is well documented that the human nervous system fires with a significant discharge variability, ~25% (Macefield et al., 2000; Vaillancourt et al., 2002; Vaillancourt et al., 2003), yet FES utilises constant-frequency trains that exhibit *zero* physiological variability.

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Conventionally, there are two main approaches used in FES therapy to attempt to maintain force when it declines due to fatigue - increase the intensity of the current or increase the frequency of the pulses. However, fatigue is a multifactorial process and cannot always be prevented by these two methods (Binder-Macleod et al., 1991). A previous study conducted by our laboratory highlighted the advantages of incorporating physiological variability into trains of stimuli, by microstimulating single human motor axons with both short (10 pulses, 8-24Hz) and long lasting (850 pulses, 18 Hz) trains of irregular stimuli that emulated the firing of volitionally activated motor units. It was shown that discharge variability augments contractile responses and reduces the magnitude of fatigue for the toe extensors (Leitch and Macefield, 2014). The purpose of the current study was to test the hypothesis that incorporating physiological variability into a continuous frequency train of stimuli delivered to the tibialis anterior muscle would enhance force production compared to a train of identical mean frequency with zero variability. A secondary objective was to observe whether discharge variability has any effect in muscles that have exhibited fatigue.

5.3 METHODS

Successful experiments were conducted on 7 male and 3 female subjects (19-51 years) under the approval of the Human Research Ethics committee of the University of Western Sydney. Subjects provided informed written consent.

The participants were reclined in a chair, with the knee flexed to approximately 120°. The foot was fixed onto a rigid foot plate with the ankle at 120° and a Velcro strap was attached over the dorsum of the foot, proximal to the metatarsophalangeal joints, so as not to interfere with dorsiflexion of the digits. The leg was supported by a vacuum cast to

prevent any movement during the procedure. The anterior compartment of the leg was palpated to locate tibialis anterior (TA), and a 2 mm diameter surface probe was used to electrically stimulate the muscle belly to identify a motor point, defined as the site requiring the least current to evoke a twitch. An insulated tungsten microelectrode (FHC, Maine, USA) was then inserted directly into this site and manually adjusted while delivering constant current cathodal pulses (0.02-10.00 mA, 0.2ms, 1 Hz) to elicit a twitch. EMG was measured via surface Ag/AgCl electrodes placed over TA (BioAmplifier, ADInstruments, Sydney) and filtered (10 Hz-1 kHz) and recorded (2 kHz sampling) on a computer-based data acquisition and analysis system (PowerLab 16 SP hardware and LabChart Pro 7 software, ADInstruments, Sydney). Force (DC-100 Hz; 200 Hz sampling) was measured using a highly sensitive force transducer (Model 1030, UFI, Morro Bay, USA) located over the medial aspect of the foot at the first metatarsal; the transducer was oriented so as to record the optimal dorsiflexion/intorsion force vector. An appropriate intramuscular site was established when the EMG and force responses were clear and well defined. The muscle was first stimulated with a set of 8 pulses at the threshold current in order to measure the twitch properties. Twitch parameters were measured using the Peak Parameters feature of LabChart (ADInstruments, Sydney).

After the twitch parameters had been measured a regular train of stimuli was delivered for 120 s, followed by an irregular train of identical mean frequency for a further 120 s. This allowed us to compare the mean forces generated in a continuous train, which incorporates both irregular and regular stimuli. The mean forces were compared in 5 s intervals to determine any changes in force responses between the two stimuli. For seven subjects the mean frequency was 19 Hz, while for three we used a higher frequency (24

Hz) to promote fatigue. For these subjects, stimulation at 24 Hz was continued until force declined by 25% of the force at the onset of stimulation, which took between 140-210 s. At this point, the irregular train was given for 120 s to measure the effects of the irregular train on force production in a fatigued state. During delivery of all trains, there was *no relaxation time* between the regular and irregular trains of stimuli - i.e. the stimulation was continuous.

Statistical analysis was performed using Prism 6 software (GraphPad Software, San Diego, USA). To test our hypothesis that the irregular trains produced greater forces than the regular trains over the same mean frequencies, paired one-tailed t-tests were performed. We compared the total mean forces produced by the irregular and regular stimuli at 5 s intervals.

Emulating and generating the irregular trains

To create an irregular physiological train of stimuli, which integrates short and long interspike intervals, the firing patterns of single motor units were recorded. An intramuscular tungsten microelectrode was inserted into the muscle belly of tibialis anterior in a single subject performing isometric ankle dorsiflexions. The subject was asked to sustain ~30% maximal voluntary contraction for 70 seconds. The mean frequency for this train was 19.3 ± 8.7 Hz, with a discharge variability of 32.3 % (S.D/mean x 100). Firing rates ranged from 5.7 to 151.5 Hz. The inter-spike intervals exhibited by this train were used to create irregular (physiological) trains that emulated the firing of volitionally activated motoneurones.

5.4 RESULTS

This study utilised intramuscular stimulation to activate motor units in the tibialis anterior muscle of human subjects (n=10). For all stimulation sites the maximum peak forces (± SEM) for the regular trains was 188.4 ± 63.3 mN. This was significantly lower than the peak forces produced by the irregular trains (248.1 ± 81.3 mN ($p<0.0001$; paired t-test), as seen in Fig. 5.1. By incorporating discharge variability the contractile responses were either increased back to initial peak responses when the muscle exhibited a decline in force, or the contractile responses increased from a plateau when the muscle displayed no decline in the force.
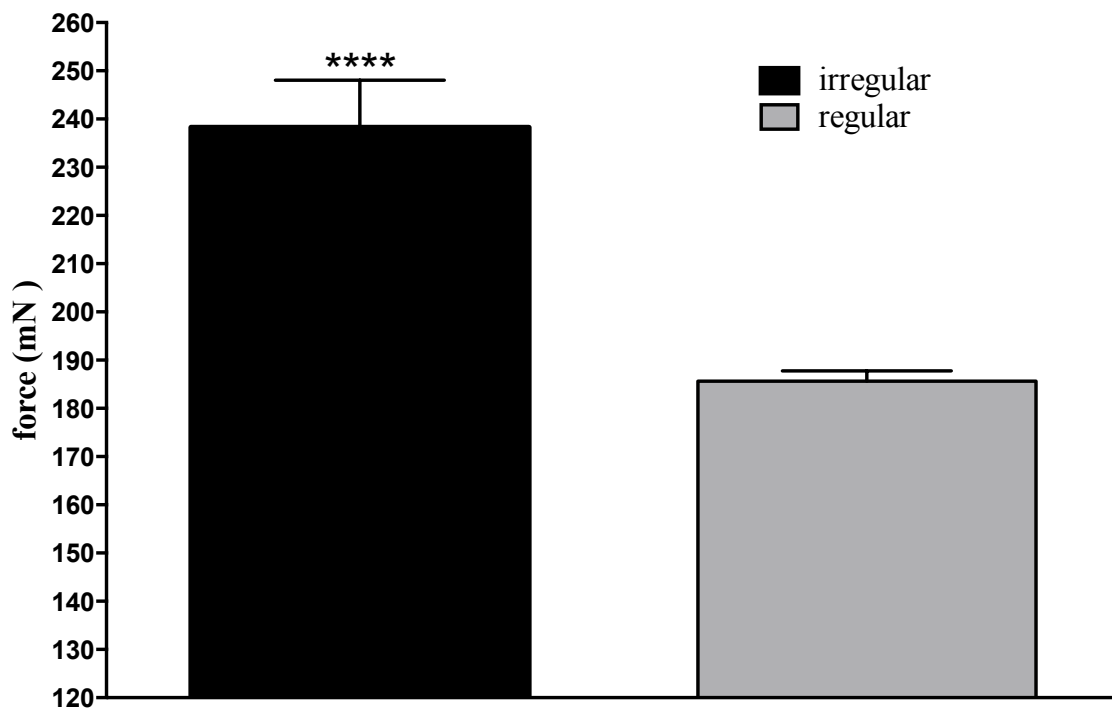
Figure 5.1: Mean peak forces generated during intramuscular stimulation of tibialis anterior (n=10). Irregular trains generated higher contractile forces than regular trains over the same mean frequencies; $p<0.0001$

A section of the experimental records from one subject are shown in Fig. 5.2. The physiological variability can be seen in the instantaneous frequency channel (top) and in the EMG channel (middle). It can be seen that when the peak force declined by ~ 25% the irregular stimulation pattern commenced. This resulted in an augmented force production over the last 85 s of stimulation. Peak force was 462 mN at the onset of the regular stimulation train, falling to 364 mN at 205 s, before reaching a mean peak force of 472 mN at the conclusion of the irregular stimuli.
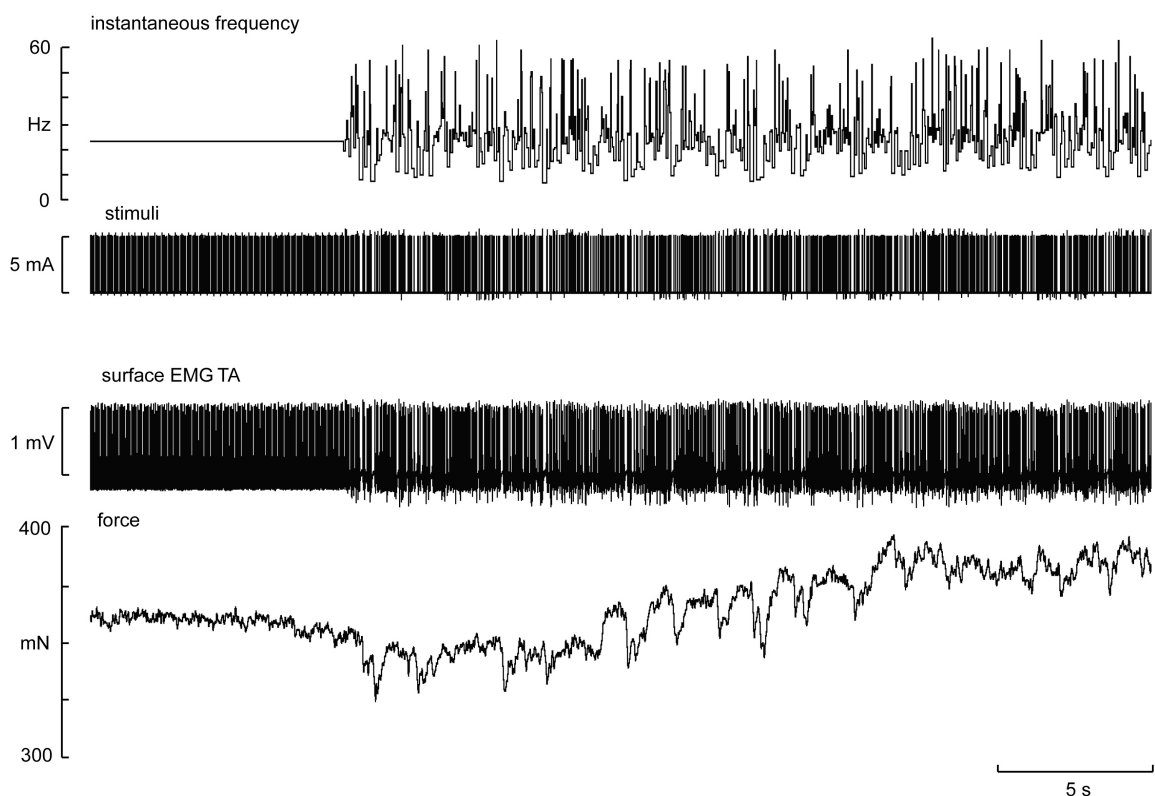
Figure 5.2: A segment of the raw data from a single subject during intramuscular stimulation of tibialis anterior at 5 mA. This subject was given the higher frequency train (24 Hz) to promote fatigue. Instantaneous frequency is shown in the top trace. The bottom trace shows the decline in force and the rapid recovery once the stimulation train becomes irregular.

Data from another subject are shown in Fig. 5.3. The peak values shown here have been averaged to obtain the mean peak forces over 5 s intervals. The initial mean peak force (±

S.D) after the first 5 s was 462.9 ± 4.6 mN, which declined by ~25% to 364.7 ± 1.5 mN

after 205 s of continuous stimulation. Once the irregular stimulation paradigm

commenced (at 210 s) the mean peak force increased from 362 ± 7.9 mN to a peak of

472.3 ± 4.9 at the conclusion of irregular stimulation. There was a statistically significant

difference between irregular and regular stimuli (p= 0.001; paired t-test).

Figure 5.3: Data from a single subject during intramuscular stimulation of tibialis anterior. A continuous train of regular stimuli was delivered at 24 Hz for 205 s, at which force had fallen by ~25%. At the vertical line physiological discharge variability was incorporated at the same mean frequency. Force rose to a peak of 472 mN at the conclusion of stimulation.

If there was no decrease in the force after 120 s of continuous regular stimulation then it

was assumed we were activating a group of fatigue-resistant units. Fig. 5.4 shows the

mean forces, sampled over 5 s, for a different subject during stimulation with both the

regular and then irregular train. We compared 60 s of force generation either side of the

transition between the two types of trains. It can be seen that force plateaued for this

subject and did not show any signs of fatigue. Mean force was 178.3 mN after 5 seconds of stimulation and 175.2 mN at 60 s. After the variability was integrated peak force increased significantly to 307.9 mN at 65 s to a maximum of 356.1 mN at the conclusion of stimulation (120 s).

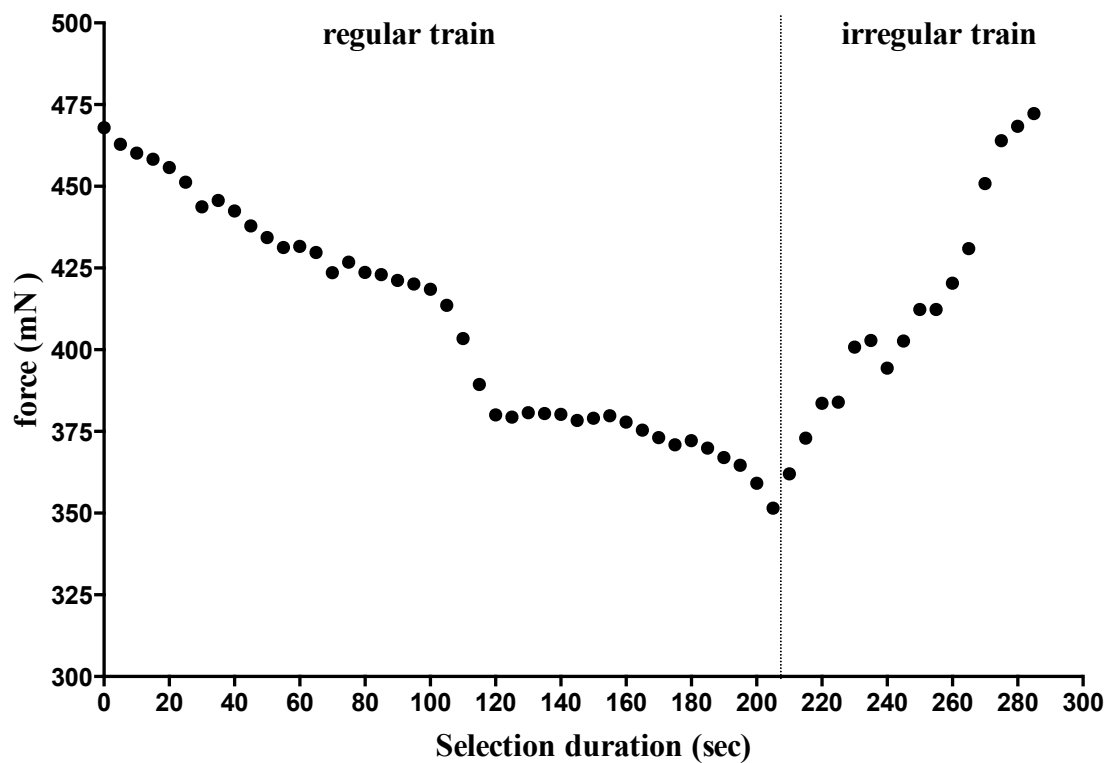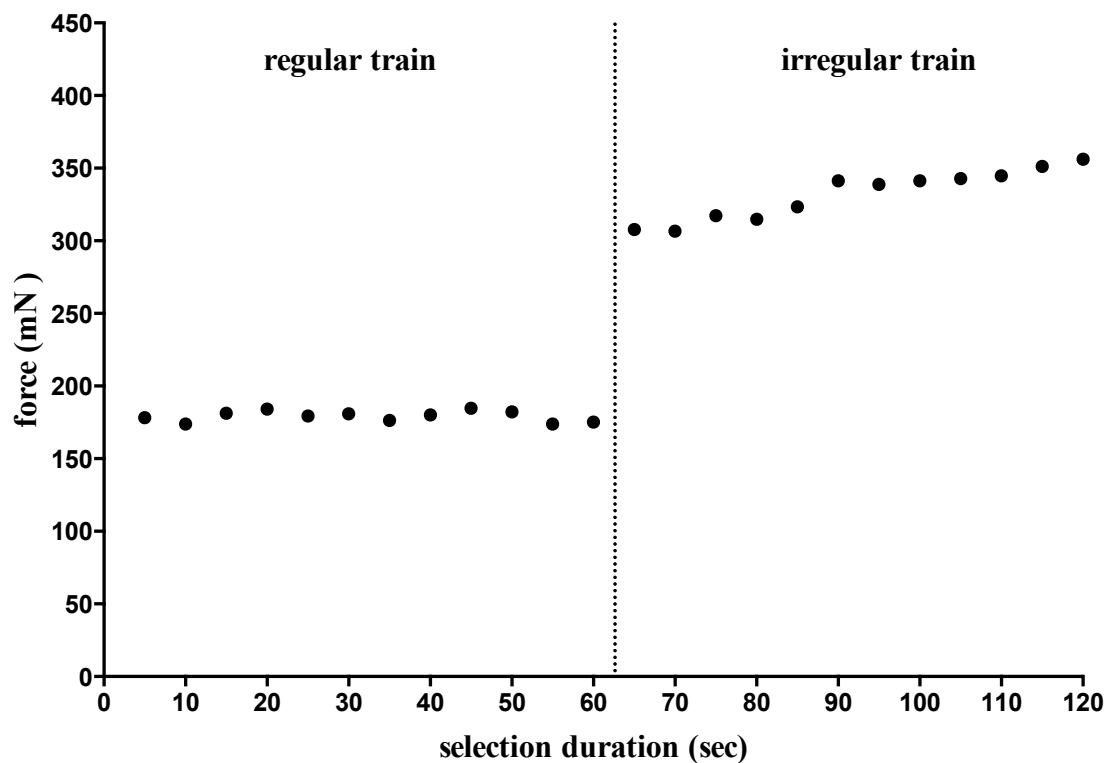**Figure 5.4:** Data from a single subject during intramuscular stimulation of tibialis anterior. A continuous train of stimulation was given at 19 Hz for 120 s; 60 s are shown on either side of the transition between regular and irregular stimulation (vertical line). Peak force was 175 mN at the conclusion of the regular train and 365 mN at the conclusion of the irregular train of stimulation.

5.5 DISCUSSION

This study has shown that continuous trains of stimuli that incorporate physiological variability produce greater forces during intramuscular stimulation of motor units in tibialis anterior compared to regular trains over the same mean frequencies, even when fatigue was present. When force began to decline, irregular trains were able to increase the force back towards the initial level of force generated at the onset of the regular train and to sustain this force until the conclusion of the stimulation. Moreover, for those motor units that did not fatigue, irregular trains increased forces above those produced for the regular trains. This emphasizes the importance of (i) the effects of irregular stimulation patterns on force production and (ii) on reducing the magnitude of fatigue for long continuous trains of stimulation.

Mechanisms for augmentation of force

Initial 'doublets' – two pulses with an interval of 10 ms – preceding a train of stimuli result in higher forces, both in whole muscle and during activation of single motor units (Macefield et al., 1996; Binder-Macleod et al., 1991). It has been suggested that the rise in force is due to extra release of calcium ions upon a short interspike interval and not prolonged release of calcium or slower reuptake (Duchateau et al., 1986). It has also been shown that during the fatigued state the available myoplasmic calcium is reduced and thus the muscle undergoes a decline in force (Westerblad et al., 1993). Therefore, a viable explanation for this phenomenon is that when calcium availability is depleted, i.e. in a fatigued state, incorporating a few short interspike intervals (irregularity), could promote the release of more calcium, therefore resulting in augmentation of force.

Another possible explanation may be due to muscle stiffness, required to build tension during contractions; it has been shown that in the fatigued state muscle stiffness decreases (Wilson et al 1994). During isotonic movements, in which the muscle is constantly shortening, there is a decrease in muscle stiffness (Curtin et al., 1994). However, when a short doublet is given in isometric conditions, where no shortening is required, the doublet is said to rapidly take up tension and force is able to build up more effectively (Binder-Macleod et al., 1996). In the current study, measurements were also taken during isometric conditions. Therefore, we suggest that incorporating short and long interspike intervals - as seen with the irregular stimulation patterns used here - increases muscle stiffness, which contributes to the buildup of tension.

Limitations of FES therapy

One limitation of FES therapy is the activation of motoneurones in the incorrect order, i.e. an order that does not follow the size principle. When stimulating whole muscle via surface electrodes there is a tendency for the larger motor units, which are known to have the lowest electrical thresholds, to be recruited first (Llewellyn et al., 2010). Larger motor units have greater axon diameters and supply more muscle fibers than smaller motor units (Henneman et al., 1965), thereby generating greater forces. However, this becomes problematic as they predominately supply fast-fatigable muscle fibers (Henneman, 1957; Burke et al., 1973). Therefore, FES results in very rapid fatigue of the active muscle. While this issue is not addressed in the current study, the use of physiological variability has still been shown to reduce the effects of fatigue and increase contractile responses when activating a group of motor units within TA. This physiological variability takes advantage of the 'catch-like property' which is known as the force augmentation produced by the inclusion of a short, high-frequency doublet, at

the onset of a subtetanic train of stimuli (Binder-Macleod et al., 1991; Callister et al., 1992; Bevan et al., 1992). Until now, studies that have examined the effects of the 'catch-like property' in whole muscle have only examined its effects at the onset of a train of stimuli (Binder-Macleod et al., 1996; 1997). Studies have examined variable trains of the thenar muscles with variable trains of stimuli using supramaximal stimulation (Indurthy 7 Griffin, 2007), but no study has incorporated physiological variability throughout trains of stimuli at the motor end plate of human leg muscles.

Advantages of integrating variability into trains of stimuli

Most advances in FES have involved improvements in technology, programming and implementation (Lee, Becker & Binder-Macleod 1999). The long-term goal, however, is to help restore motor function for those who have been injured and the biggest problem at present is rapid fatigue. Incorporating physiological trains in FES therapy may be beneficial if there is less fatigue in a stimulated muscle. The order in which the motor units are recruited is not something that is resolved in the current study, and not something that can be easily resolved in the future. However, we have previously shown that integrating physiological variability into brief trains of stimuli (10 pulses) is effective in augmenting force and reducing fatigue (Leitch et al., 2014). The current study shows that this also holds true for longer-lasting trains of stimuli.

5.6 CONCLUSIONS

Irregular trains of stimuli offer an advantage to the neuromuscular system by augmenting force production and reversing the effects of fatigue over 60 s. Current FES-therapies utilise regular non-physiological trains of stimuli that incorporate zero variability, usually at frequencies that are higher than those at which motor units discharge during voluntary contractions. Both of these factors promote muscle fatigue, which is a major limitation of FES therapies. We propose that integrating discharge irregularity may improve the current stimulation patterns utilised during FES, thereby improving the therapeutic outcomes.

CHAPTER 6

DISCUSSION AND THESIS SUMMARY

Functional electrical stimulation (FES) is a therapy used for the management and rehabilitation of patients with motor impairment. It works by electrically stimulating whole muscle either surface electrodes placed on the skin or implanted electrodes . Current stimulation patterns use constant-frequency trains of stimuli that are delivered at frequencies higher than those at which motoneurones discharge maximally and with *zero* discharge variability. FES has been shown to be effective in treating foot drop (Waters et al., 1975), and improving reach-to-grasp tasks (Miller et al., 1990; Billian et al., 1992). However, one limitation of stimulating whole muscle during FES is rapid fatigue of the muscle, and thus the effectiveness of the therapy may be compromised. It is known that many spinal cord injured patients have muscles that are comprised mainly of fast-fatigable muscle fibers, which could be one explanation for the fatigue exhibited by those patients (Burnham et al., 1997). However, fatigue is still exhibited in patients after stroke and other neuropathologies, which means that the activation order of motor units will also play a role. In natural physiological conditions, motor units are activated in order of size (Henneman, 1965; Olson et al., 1968). Small motoneurones have a higher input resistance, so for a given level of excitatory drive they will be recruited first. They also supply few muscle fibers and hence generate smaller forces. Conversely, larger motoneurones have a smaller input resistance and are recruited later; they supply more fibers and generate greater forces. This sequential pattern of recruitment results in a smooth gradation of force (Henneman et al., 1965) during normal physiological

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conditions. While large motor units produce the greatest forces, they also exhibit the lowest resistance to electrical current and supply fast-fatigable muscle fibers (Llewellyn et al., 2010). Therefore, when electrically stimulating whole muscle during FES therapy the larger motor units are preferentially activated first, inducing rapid fatigue. Though this still remains a problem, this thesis has used novel stimulation patterns that have the potential to counteract some of the weaknesses exhibited during FES. In healthy subjects, I have shown that by using stimulation patterns which emulate the firing of motoneurones during physiological conditions, contractile responses are augmented and fatigue is often reduced.

The diverse range of movements conducted in everyday life requires different mechanical conditions by the muscle groups involved. Contractions that are isotonic in nature, such as hand gesticulation, develop little tension in the muscle but active shortening. Conversely, isometric contractions build tension without changing the length, such as the stabilizing muscles activated during standing. Muscle groups that are often subject to isometric conditions, such as the back muscles, are known to be composed mainly of slow (type I) twitch fibers (Rantanen et al., 1994; Parkkola et al., 1993; Sirca et al., 1985). This is then reflected in the fusion and twitch properties exhibited by the muscle. In Chapter 3 I have shown that the fusion and thus twitch properties vary, depending on the biomechanical arrangements, and the means of measuring the contractile response to activation of a single motor unit. During microstimulation of single motor axons supplying the toe extensors I have shown that fusion occurs earlier, at ~8 Hz, during isometric conditions, and later - between 14-16 Hz - for loaded and unloaded isotonic conditions, respectively. Interestingly, this reflects the primary range of motoneurone firing already established for the toe extensors (Macefield et al., 1996).

In addition, peak angular displacement was lower and twitch durations longer for unloaded than loaded conditions. This can be explained purely on biomechanical principals. Hill (1951) showed that in-series tendon compliance affects twitch duration, such that during a loaded isotonic condition the slack in the tendon must be taken up before movement can occur. In addition, the lower peak displacements seen in loaded isotonic conditions could be explained purely by the downward force acting on the toe and thus, restricting movement. It is now known that lower frequencies of stimulation are required during isometric conditions and higher frequencies for isotonic conditions to generate full tetanic fusion (Leitch and Macefield, 2015). Therefore, during FES, if an unloaded isotonic contraction is required, e.g. finger flexion, higher frequencies must be given to generate full tetanic fusion than during loaded isotonic or (static) isometric contractions. Due to the fact that most muscles are comprised of mixed fiber types (S, FR, FF) further research is necessary to clarify these findings during whole muscle stimulation.

In addition to adjusting the firing rates for the applicable biomechanical conditions, we can also adjust the variability of a train of stimuli by incorporating short and longer interspike intervals. It is documented that human motoneurones discharge with significant discharge variability during voluntary contractions (Macefield at al., 2000; Bevan et al., 1992) and this is known to increase with fatigue (Enoka et al., 1989) and age (Tracy et al., 2005; Welsh et al., 2007). Motoneurones clearly fire in this manner for a physiological purpose, hence I hypothesized that integrating physiological variability into stimulation patterns could offer some further advantages to the motor system. My previous study (see Appendix) revealed that integrating discharge variability into short trains of stimuli (10 pulses, 8-28 Hz) augments the contractile responses in isotonic

conditions and reduces the magnitude of fatigue. The finding from this study was the catalyst for further exploration into its benefits under different conditions. In Chapter 3 I established that incorporating physiological discharge variability into longer trains of stimuli (45 seconds) offers similar advantages, when compared to trains of identical mean frequency (18 Hz) but with *zero* variability. Long-lasting physiological (irregular) trains, based on the interspike intervals recorded from single motor units within tibialis anterior, integrated short and long interspike intervals throughout. These irregular trains produced significantly greater forces at the onset of stimulation over the first 20 seconds, but showed a greater rate of decline compared with the regular long-lasting trains (Figure 3.1). The regular trains generated less force overall and plateaued from start to end (Figure 3.2). An essential point to mention is that while the irregular trains showed a rapid rate of decline, the overall force for the entire 45 seconds never dropped below that of the regular train. One proposed mechanism for this rapid rate of decline relates to the peak forces generated by the two trains. It is not unreasonable to suggest that the muscle itself cannot sustain the forces generated by the irregular train and thus fatigue is induced. Indeed, Allen and colleagues (1993) suggest that low frequency fatigue is due to a lack of calcium release from the sarcoplasmic reticulum. Therefore, in the current study calcium availability could be depleted due to the short interspike intervals displayed in irregular trains and thus, force declines. It may be, that lower mean frequencies are required to match the peak forces generated by the regular train of stimuli. For example a 14 Hz irregular train matches identical mean forces as a 20 Hz regular train. While the exact mechanisms are still unclear, this study has highlighted that during FES therapy there is no need to increase the current or frequency to generate higher forces from the muscle. Simply integrating physiological variability at the same mean frequencies is a novel method to generate greater forces.

It is well documented that during ramping voluntary contractions motoneurones begin firing at ~8 Hz (Person & Kudina, 1972; Tanji and Kato, 1973) and force plateaus in a sigmoidal fashion, with a maximal firing rate ~25 Hz; any further increase in stimulation frequency fails to increase force production (Macefield et al., 1996; Fuglevand et al., 1999; Bigland-Ritchie & Lippold, 1954). However, during ballistic contractions - where fast dynamic movements are required - the firing rates are markedly higher and maximum forces are reached in a short duration (Desmedt et al., 1977a). Until now, ballistic contractions have only been examined in terms of the firing properties of single motor units (Desmedt et al., 1978). In Chapter 4 the aim was to examine whether it was solely the high firing rates seen during these recordings that produces such high force generation or whether physiological variability could contribute, as I had shown for long-lasting trains. I found that short, high-frequency, irregular trains produced higher peak forces than regular trains over identical mean frequencies (58-69 Hz). This emphasized the observation that integrating short high-frequency interspike intervals throughout a short train offers a further force-generating mechanism not yet explored. Cheng and colleagues (2013) have shown that initial doublets can increase isometric force production by promoting extra release of calcium from the sarcoplasmic reticulum, yet others suggest that the mechanisms reside within excitation-contraction coupling, such as the tendency for cross bridges to remain attached (Sandercock et al., 1997). Nevertheless, I have documented clear increases in force production at the integrative level explored within this thesis; further research is required to reveal some of the more complex mechanisms occurring at the cellular level.

In addition to examining the benefits of discharge variability during activation of single motor axons, I have also examined the effects of discharge variability when activating multiple units. By stimulating directly within the muscle belly via a microelectrode a small group of motor units were stimulated, allowing me to further clarify the benefits of exploiting discharge variability. During these experiments groups of both fatigable and fatigue-resistant units were activated, which allowed for a detailed examination. In Chapter 5 I tested the hypothesis that integrating physiological variability into continuous regular trains of stimuli (19 or 24 Hz) could augment force production and potentially reduce the magnitude of fatigue, as reported in the single motor-axon microstimualtion studies conducted previously (see Appendix & Chapter 3). For fatigable motor units, a continuous train of regular stimulation was given until force declined by ~25%, then - without stopping the stimulation - irregularity was incorporated at an identical mean frequency. By incorporating physiological variability at the same mean frequency, forces began to rise back to the original peak levels seen at the onset of the regular continuous train (Fig 5.1). For those motor units that exhibited no decline in force (i.e. no fatigue), the stimulation irregularity was incorporated after 2 minutes to assess the differences in mean peak forces between the two types of trains. Irregular continuous trains exhibited significantly higher contractile responses than the regular continuous trains, for all units (Fig 5.4). This final study further clarified these advantages, and in many ways, superior capabilities of these irregular trains. Other studies have assessed the advantages of initial doublets at the onset of sub-tetanic contractions in whole muscle activation (Binder-Macleod et al., 2001; Karu ZZ et al., 1995; Ding et al., 2003). However, until now, no other study has assessed the advantages of physiological variability throughout a train of stimuli within a whole muscle. Not only do motor units generate greater contractile

responses, but in this case, have fatigue-reducing capabilities that have not previously been seen before.

This thesis has, by and large, utilised rate coding as a method for producing superior and efficient responses from the toe-extensor muscles in healthy subjects. I have determined the frequencies required to generate full tetanic fusion during three different biomechanical environments: isotonic, loaded isotonic and isometric conditions. Consequently, it is now known which frequencies are required to produce the optimal contractile responses, which may translate clinically for possible uses in FES stimulation patterns. By using the appropriate frequencies fatigue may be minimized, while still reaching the optimal force responses required. In addition, I have assessed the advantages of integrating physiological discharge variability into long-lasting trains of stimulation within the optimal stimulation range, as well as during the short high-frequency trains of stimuli producing ballistic contractions, and compared contractile responses over identical mean frequencies. Furthermore, these benefits have been established when activating multiple motor units within whole muscle. By emulating the firing of real motoneurones during natural physiological conditions, this shows potential for uses in clinical applications such as FES in the future.

In conclusion, I have generated the following findings from this thesis:

1. During microstimulation of single motor axons supplying the toe extensor muscles fusion occurs earlier during isometric and loaded isotonic conditions than during unloaded isotonic conditions. Peak angular displacement was higher during isotonic conditions than loaded isotonic conditions and twitch durations

were slower and more sluggish during isometric conditions than during both loaded and unloaded isotonic conditions.

2. Physiological discharge variability offers significant advantages during microstimulation of single motor axons supplying the toe extensors. The forces generated by long irregular trains of stimuli were significantly higher for both long-lasting and short high-frequency trains of stimuli.

3. This augmentation of force was also reported for long irregular continuous trains, when stimulating multiple units within tibialis anterior. In addition, irregular continuous trains were able to reverse the decline in force in groups of fatiguable units.

4. Stimulation patterns which emulate the firing of real motoneurones during voluntary contractions offer an advantage to the neuromuscular system. Using appropriate patterns of stimuli that increase contractile responses and reduce the magnitude of fatigue may be beneficial for practical uses in FES systems.

CHAPTER 7

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CHAPTER 8

APPENDIX

COMPARISON OF THE CONTRACTILE RESPONSES TO IRREGULAR AND REGULAR TRAINS OF STIMULI DURING MICROSTIMULATION OF SINGLE HUMAN MOTOR AXONS

ABSTRACT

During voluntary contractions, human motoneurones discharge with a physiological variability of ~20%. However, studies that have measured the contractile responses to microstimulation of single motor axons have used regular trains of stimuli with no variability. We tested the hypothesis that irregular (physiological) trains of stimuli produce greater contractile responses than regular (non-physiological) trains of identical mean frequency but zero variability. High-impedance tungsten microelectrodes were inserted into the common peroneal nerve and guided into fascicles supplying a toe extensor muscle. Selective microstimulation was achieved for 14 single motor axons. Contractile responses were measured via an angular displacement transducer over the relevant toe. After recording the responses to regular trains of 10 stimuli extending from

2-100 Hz, irregular trains of 10 stimuli - based on the interspike intervals recorded from single motor units during voluntary contractions - were delivered. Finally, the stimulation sequences were repeated following a 2 min period of continuous stimulation at 10 Hz to induce muscle fatigue. Regular trains of stimuli generated a linear increase in displacement with frequency, whereas irregular trains - emulating the firing of volitionally-driven motoneurones - displayed significantly greater responses over the same frequency range (8-24 Hz). This was maintained even in the presence of fatigue. We conclude that physiological discharge variability, which incorporates short and long interspike-intervals, offers an advantage to the neuromuscular system by allowing motor units to operate on a higher level of the contraction-frequency curve and taking advantage of catch-like properties in skeletal muscle.

INTRODUCTION

Volitionally activated motoneurones do not fire in a regular fashion: they exhibit significant variability in their discharge. For example, during voluntary isometric contractions of an intrinsic hand muscle - first dorsal interosseous (FDI) - the discharge variability of single motor units was 20.3 % during contractions at 5% of maximal voluntary contraction (MVC), decreasing to 13.5 % with contractions at 40% MVC (Vaillancourt et al, 2003). While discharge variability, as measured by the coefficient of variation (SD/mean), is well known to fall with increases in mean firing rate (Stalberg & Thiele, 1973; Petajan, 1981), the actual irregularity of the firing - as measured by the approximate entropy of the instantaneous frequency - shows a *positive* linear relationship to mean firing rate (Vaillancourt et al., 2002, 2003). Even during a maximal attempt to dorsiflex the big toe, discharge variability is high: single motor units recorded from extensor hallucis longus (EHL), and followed during an MVC, showed a mean

variability of 18.8 % (Macefield et al., 2000). Interestingly, variability is high even in the absence of peripheral feedback from the contracting muscles: discharge variability of deafferented motoneurones supplying the dorsiflexor muscles of the foot was 20.5 % during attempts to maximally activate the paralysed muscles (Macefield et al., 1993). This indicates that much of the variability arises from fluctuations in central drive rather than variations in reflex synaptic input.

Whether this discharge variability affects contractile output of individual motoneurones has not been specifically addressed, because all investigations using selective stimulation of individual motor axons have employed short trains of a constant frequency to examine the relationship between frequency and force (e.g. Botterman et al., 1986). It has been suggested that irregular patterns of motor unit firing may assist in maintaining force output during prolonged muscle activity (Laouris et al., 1991; Bevan et al. 1992). A greater variability in interspike intervals would increase the probability of occurrence of short interval or "doublet" discharges. Doublet discharges are known to induce prolonged enhancement of force in some types of motor units (Burke et al., 1976; Macefield et al., 1996), and the inclusion of doublets during prolonged stimulation of whole muscle (Binder-Macleod & Barker, 1992) or single motor units (Bevan et al. 1992) has been shown to reduce the magnitude of fatigue. It is not unreasonable to speculate that an increase in discharge variability during a sustained contraction may take advantage of doublet-induced enhancement of motor unit force and thereby optimise force production during fatigue.

Intraneural microstimulation of single motor axons has allowed examination of the contractile properties of individual motor units in awake human subjects; the muscles

that have been studied so far are the thenar muscles in the hand (Westling et al., 1990; Thomas et al., 1990, 1991a,b, 2006), toe extensors (Macefield et al., 1996), and the intrinsic and extrinsic finger flexors (Fuglevand et al., 1999; McNulty et al., 2000). The technique allows one to study the contractile properties of human single motor units with a level of precision comparable to that obtained in reduced animal preparations (e.g. Burke et al., 1973; Kernell et al., 1983). Using this approach, the aim of the current study was to compare the contractile properties of single human motor units when stimulated with physiological trains of stimuli (irregular trains) and with regular stimulus trains of zero variability. Until now, only regular (non-physiological) trains of stimuli have been used. While these experiments have given valuable information on the contractile responses of single motor units, it remains unknown whether these responses are the same when the normal discharge variability of human motor units is applied. In the current study, we will use a series of spike trains that cover the normal firing rates of single motoneurones during voluntary contractions and compare the contractile responses with those obtained by delivering a standard set of regular trains of frequencies extending from 2-100 Hz. By using stimulation trains that emulate the normal physiological variability of volitionally activated motoneurones, we predict that contractile responses will be higher than trains of regular stimuli with zero variability. Moreover, we predict that this augmentation during stimulation with irregular trains will persist in the presence of muscular fatigue induced by continuous electrical stimulation.

METHODS

Successful experiments were conducted on 5 male and 2 female subjects (18-29 y) under the approval of the Human Research Ethics committee of the University of Western Sydney. Subjects provided informed written consent. The participant reclined in a chair,

the knee flexed to approximately 120°. The foot was fixed onto a rigid foot plate with the ankle at 120° and a Velcro strap was attached over the dorsum of the foot, proximal to the digits, so as not to interfere with dorsiflexion of the digits. The leg was supported by a vacuum cast to prevent movement during the procedure. The common peroneal nerve was located using a weak electrical stimulus (0.02-10.00mA, 0.2ms, 1Hz; Stimulus Isolator, ADInstruments, Sydney, Australia) delivered by a 2 mm diameter surface probe. A high-impedance tungsten microelectrode (Frederick Haer & Co, Maine, USA), 200 μm in diameter, was inserted into the nerve and an Ag/AgCl surface electrode on the opposite side of the knee served as the anode. Constant-current cathodal pulses (0.02-1.00 mA, 0.2ms, 1Hz) were delivered through the microelectrode and the microelectrode manually adjusted to search for a motor fascicle supplying either extensor hallucis longus (EHL), extensor digitorum longus (EDL) or extensor digitorum brevis (EDB). EMG was recorded with surface Ag/AgCl electrodes placed over tibialis anterior, the peronei and extensor digitorum brevis, amplified (BioAmplifier, ADInstruments, Sydney) and filtered (10 Hz-1 kHz) and recorded (2 kHz sampling) on a computer-based data acquisition and analysis system (PowerLab 16 SP hardware and LabChart Pro 7 software, ADInstruments, Sydney). Displacement (DC-100 Hz; 200 Hz sampling) was measured using a highly sensitive angular displacement transducer (PanLab, Spain) located over the distal phalanx of the stimulated toe - either the big toe for EHL or the second or third toe for EDL or EDB. An appropriate intrafascular site was found when microstimulation generated a small twitch and a reproducible EMG potential at the lowest current with a well-defined safety margin, typically 2 uA, over which increases in current failed to modify the twitch amplitude or EMG profile. The single motor axon was then stimulated with sets of 8 pulses at the threshold current in order to measure the twitch properties of the motor unit. Twitch parameters were measured using the Peak

Parameters feature of LabChart. Following this trains of 10 pulses were delivered from 2-100 Hz in an ascending then descending pattern followed by trains of irregular stimuli that emulated the firing of volitionally activated motoneurones.

The irregular trains were based on the discharge of single motor units recorded from tibialis anterior during a series of voluntary isometric contractions. For this purpose a tungsten microelectrode was inserted directly into the muscle belly of tibialis anterior and amplified (gain 1000x, 300 Hz-5 kHz; 10 kHz sampling; NeuroAmp EX, ADInstruments, Sydney). Spike trains from individual motor units or individual muscle fibres were recorded during dorsiflexion of the foot and the microelectrode adjusted so as to obtain several spike trains of several seconds in duration within a given contraction. These contractions ranged from ~15% to 85% of maximal voluntary force, measured from force transducers in the foot plate, and allowed us to obtain a series of voluntary contractions at a range of frequencies, extending from ~9-25 Hz. The inter-spike intervals from these recorded trains were used to construct a set of irregular (physiological) trains of stimuli, each comprising 10 pulses. A bank of 21 of trains, with mean variability ranging from 10 – 55 %, and peak frequencies ranging from 11.5 - 49.5 Hz was delivered in a random fashion.

The regular stimulation trains consisted of 10 pulses at frequencies ranging from 2-100 Hz, incrementing by 2 Hz from 2-30 Hz and then increasing in 10 Hz increments from 30 to 100 Hz; the interval between trains ranged randomly between 1.5 and 2.5 s. This sequence was then delivered in reverse. These regular trains produced a standard displacement-frequency curve, allowing us to compare the contractile responses produced by the irregular trains of stimuli, which were always given after the regular trains. Following this sequence, the motor unit was fatigued by delivering a continuous

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train of regular stimuli at 10 Hz for 2 minutes. After the fatigue run the regular (ascending and descending sequences) then irregular trains were delivered again. This allowed us to examine the effects of fatigue on a single motor unit and to assess the effects of discharge variability on the contractile responses. All statistical analyses were performed using Prism 6 software (GraphPad Software, San Diego, USA). To test the hypothesis that the angular displacements produced by trains of irregular stimuli are higher than those produced by trains of regular stimuli, paired one-tailed t-tests were used.

RESULTS

Trains of action potentials, lasting from 3.3 to 8.7 s and comprising 37 to 128 spikes, were recorded from 22 single motor units during sustained isometric contractions of tibialis anterior. Experimental records from one unit are shown in Fig. 1. Across units, the mean ± SD firing rate was 15.7 ± 7.8 Hz (range 8.9-37.8 Hz); the corresponding mean discharge variability was 29.2 ± 2.1 % (range 10.4-55.1 %). Peak firing rates extended from 11.5-49.5 Hz. These physiological trains were used to generate irregular trains of stimuli for delivery to isolated motor axons supplying either extensor hallucis longus (EHL), extensor digitorum longus (EDL) or extensor digitorum brevis (EDB). Selective microstimulation was successful for 14 single motor axons, nine of which supplied EHL, four EDL and one EDB. Twitch parameters for each unit during delivery of single pulses are shown in Table I.
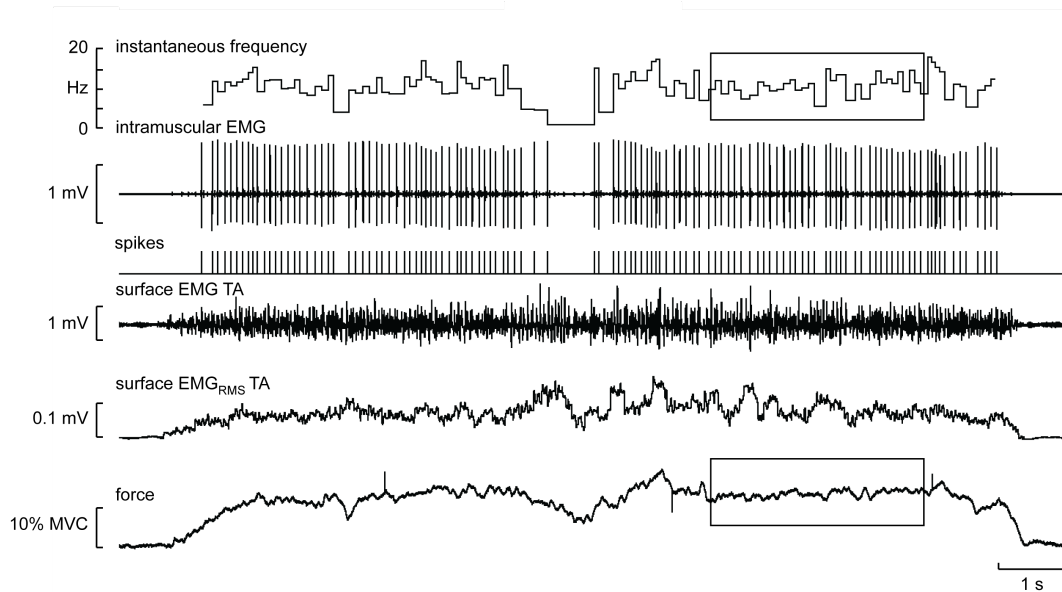
Figure 1: Intramuscular microelectrode recording from a single motor unit in tibialis anterior during a voluntary contraction of ~15 % maximal voluntary contraction (MVC). This figure illustrates the irregular discharge of human motoneurons in the instaneous frequency channel (top), the intramuscular EMG activity and surface EMG, including the discharge pattern of the spikes and the RMS (root mean square) of the surface EMG activity. The rectangle indicates a period in which force was relatively stable; discharge variability of the motor unit was measured during this period.

Unit	Muscle	Rise time (ms)	Peak amp (deg)	Width at 50% (ms)	Fall time (ms)
1	EDL	38.03	0.06	124.19	117.46
2	EHL	28.80	0.03	110.61	106.16
3	EHL	44.81	0.20	195.20	156.31
4	EHL	47.72	0.07	224.84	145.19
5	EHL	47.36	0.08	135.11	80.87
6	EDB	41.50	0.08	194.20	194.95
7	EHL	41.67	0.04	146.71	141.89
8	EHL	51.38	0.07	213.37	134.54
9	EDL	61.36	0.08	175.10	160.02
10	EHL	40.18	0.05	167.39	231.77
11	EHL	40.71	0.04	156.39	216.05
12	EDL	52.15	0.05	195.43	153.24
13	EDL	45.33	0.06	197.47	120.23
14	EHL	42.36	0.05	160.75	124.42
Mean ± SE		44.53 ± 7.61	0.07 ± 0.04	171.20 ± 34.17	148.79 ± 41.90

Table I: Twitch parameters measured from the angular displacements produced by microstimulation of 14 single motor axons. For each unit the mean value (± SE) of 8 pulses was calculated for each parameter.
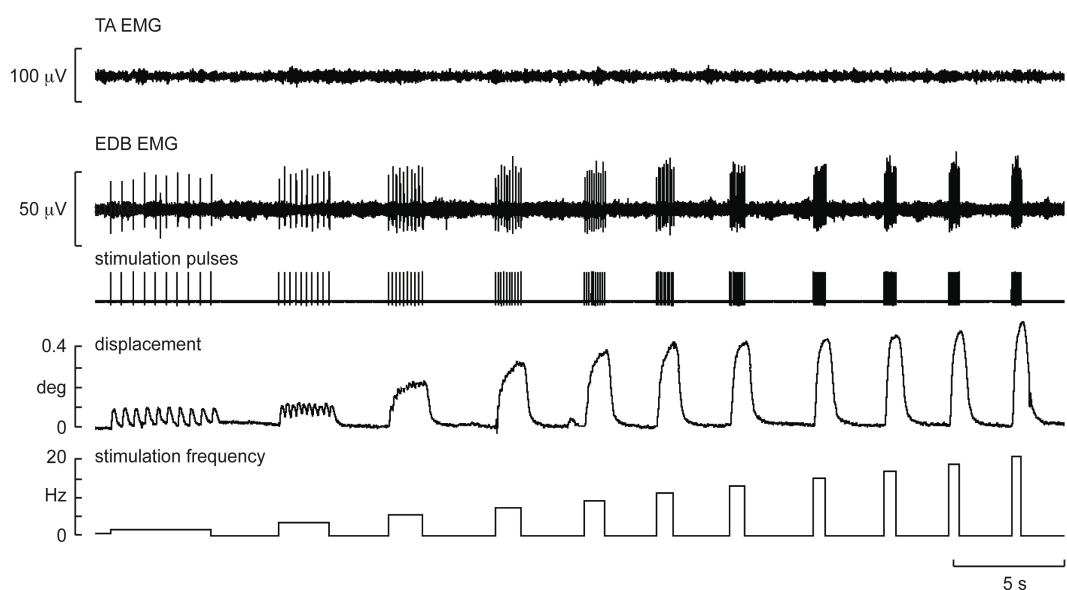
Figure 2: Experimental records from microstimulation of a single motor axon supplying extensor digitorum brevis (unit 6 in Tables I and II). Trains of 10 pulses were delivered at 2-22 Hz. Angular displacement was measured from digit II.

Comparison of contractile responses to regular and irregular trains

The regular trains comprised 10 pulses given in ascending order from 2-100 Hz. Figure 2 shows an example for one motor axon, supplying extensor digitorum brevis (EDB), during delivery of regular trains of stimuli from 2-22 Hz. It can be seen that the twitches, measured as angular displacement of the second toe, started to fuse at 4 Hz (the second train illustrated) and fusion was complete by 8 Hz. Furthermore, there was no significant increase in displacement at frequencies greater than 12 Hz.
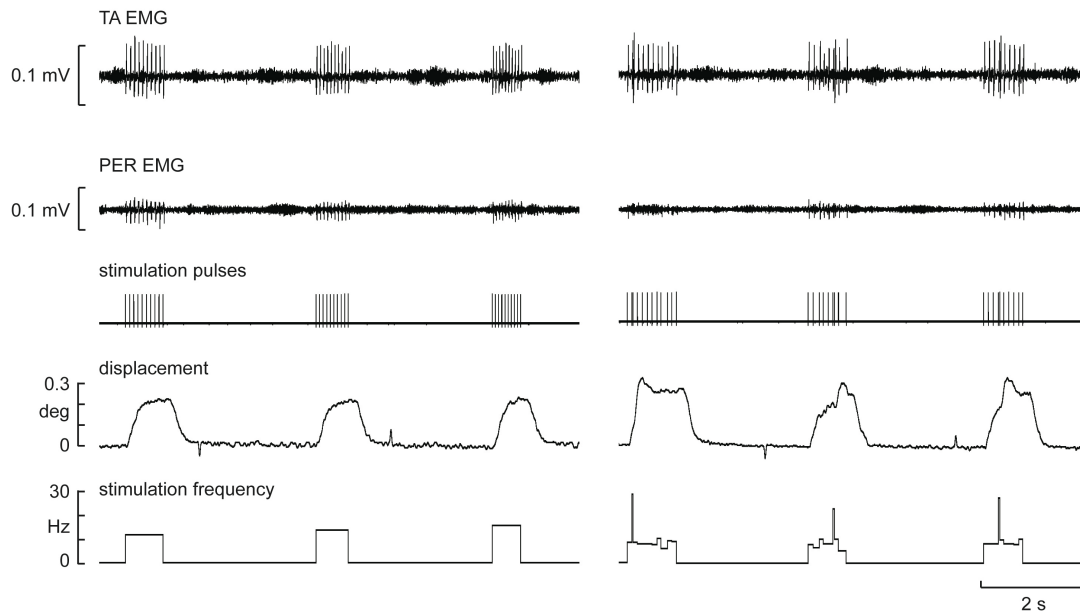
Figure 3: Experimental records from microstimulation of a single motor axon supplying extensor digitorum longus (unit 12) with regular trains of 10 pulses at 12, 14 and 16 Hz (left) and irregular trains of 10 pulses at 10.9 ± 4.7, 10.0 ± 4.8 and 11.0 ± 5.5 Hz (right). Discharge variability for the three illustrated irregular trains was 43.1, 48.0 and 50.0 %, respectively. Note that angular displacement was larger for the irregular trains, despite the lower mean frequencies.

Figure 3 compares the responses to three trains of regular and three trains of irregular stimuli for a single motor axon supplying EDL. Despite the same number of pulses comprising each train, the angular displacements produced by the irregular trains were greater than those by the regular trains. Similar records from another motor unit supplying EDL are shown in Fig. 4. Again, microstimulation of the motor axon with irregular trains caused greater angular displacements than for the regular trains. Clearly, the short inter-pulse intervals in each of the illustrated irregular trains were largely responsible for the greater displacements, but it should be pointed out that the augmented response persisted beyond the highest instantaneous frequencies, which for all units did not exceed 49.5 Hz. This can be explained by the fact that the contraction produced by a short inter-pulse interval is larger than that produced by a longer interval, such the time required for full relaxation of the twitch may not be met before another contraction

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occurs. In this way, a variable discharge pattern produces an augmented angular displacement.
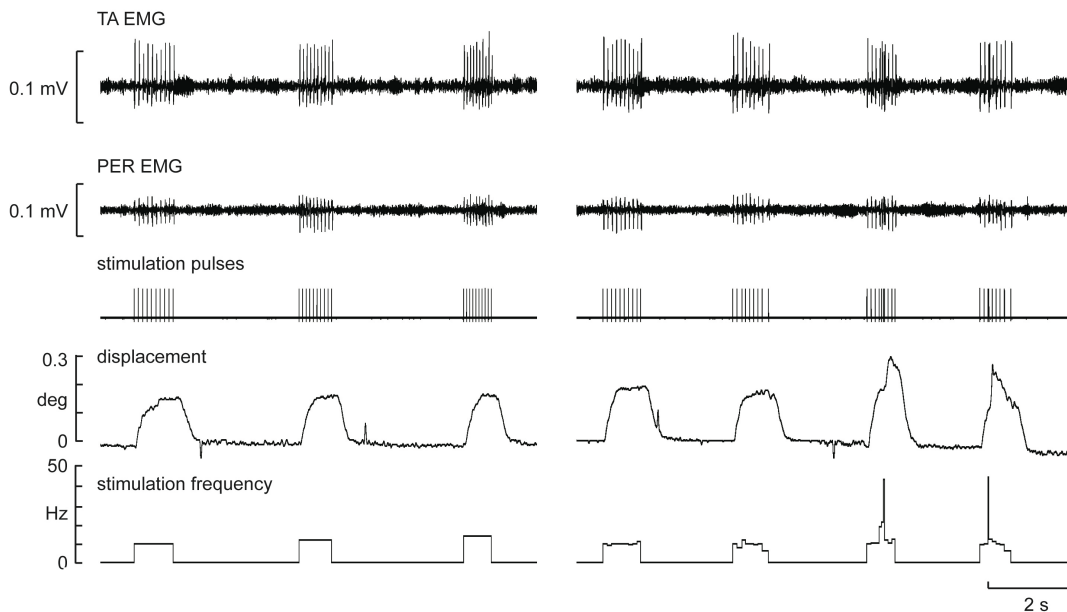

Figure 4: Experimental records from microstimulation of a single motor axon supplying extensor digitorum longus (unit 13) with regular trains of 10 pulses at 12, 14 and 16 Hz (left) and irregular trains of 10 pulses at 9.7 ± 1.9, 8.7 ± 2.7, 12.5 ± 7.1 and 10.0 ± 5.5 Hz (right). Discharge variability for the four illustrated irregular trains was 19.6, 31.0, 56.8 and 55.0 %, respectively.

The relationship between stimulation frequency and angular displacement for the unit illustrated in Fig. 2 is shown in Fig. 5A. A sigmoidal curve has been fitted to the data for the regular trains, and it can be seen that the contractile responses to the set of irregular trains lay above the standard curve. This was even more pronounced following fatigue, produced by continuous microstimulation of the motor axon at 10 Hz for two minutes, as evident in Fig. 5B. Peak amplitudes for both the regular and irregular trains were measured over the range of frequencies over which the irregular trains were given. Mean displacements before (n=14) and following fatigue (n=12) are provided in Tables II and III.

Unit	Muscle	Regular trains	Irregular trains	P-value
1	EDL	0.12	0.14	0.1295
2	EHL	0.16	0.76	0.0297
3	EHL	1.55	3.03	0.0003
4	EHL	0.53	0.76	0.0223
5	EDB	0.35	0.38	0.0116
6	EHL	0.51	0.90	0.0001
7	EHL	0.12	0.09	0.0088
8	EDL	0.23	0.35	0.0020
9	EDL	0.53	0.73	0.0186
10	EHL	0.29	0.31	0.4875
11	EHL	0.85	0.87	0.4393
12	EDL	0.18	0.24	0.0432
13	EDL	0.52	0.66	0.0264
14	EHL	0.19	0.65	0.0002
Mean ± SE		**0.44 ± 0.38**	**0.71 ± 0.72**	

Table II: Peak angular displacements produced during microstimulation of 14 single motor axons with regular and irregular trains. Mean amplitudes were calculated over 8-24 Hz. With the exception of units 1, 10 and 11, peak amplitudes were signifcantly higher during stimulation with irregular trains. Statistical signifance was based on a one-tailed t-test to determine whether displacements produced by the irregular trains were larger than those produced by the regular trains.

Unit	Muscle	Regular trains	Irregular trains	P-value
1	EDL	0.05	0.08	0.0008
2	EHL	0.13	0.18	0.0129
4	EHL	0.78	0.71	0.2062
5	EDB	0.18	0.36	<0.0001
6	EHL	0.37	0.56	0.0005
7	EHL	0.05	0.08	<0.0001
8	EDL	0.20	0.27	0.0030
9	EDL	0.25	0.41	0.0073
10	EHL	0.18	0.47	0.0023
11	EHL	0.68	0.79	0.0491
13	EDL	0.45	0.67	0.0010
14	EHL	0.10	0.18	0.0003
Mean ± SE		**0.29 ± 0.24**	**0.40 ± 0.25**	

Table III: Peak angular displacements produced during microstimulation of 12 single motor axons with regular and irregular trains, following fatigue produced by continuous stimulation at 10 Hz for two minutes. Mean amplitudes were calculated over 8-24 Hz. With the exception of unit 4, peak amplitudes were signifcantly higher during stimulation with irregular trains.
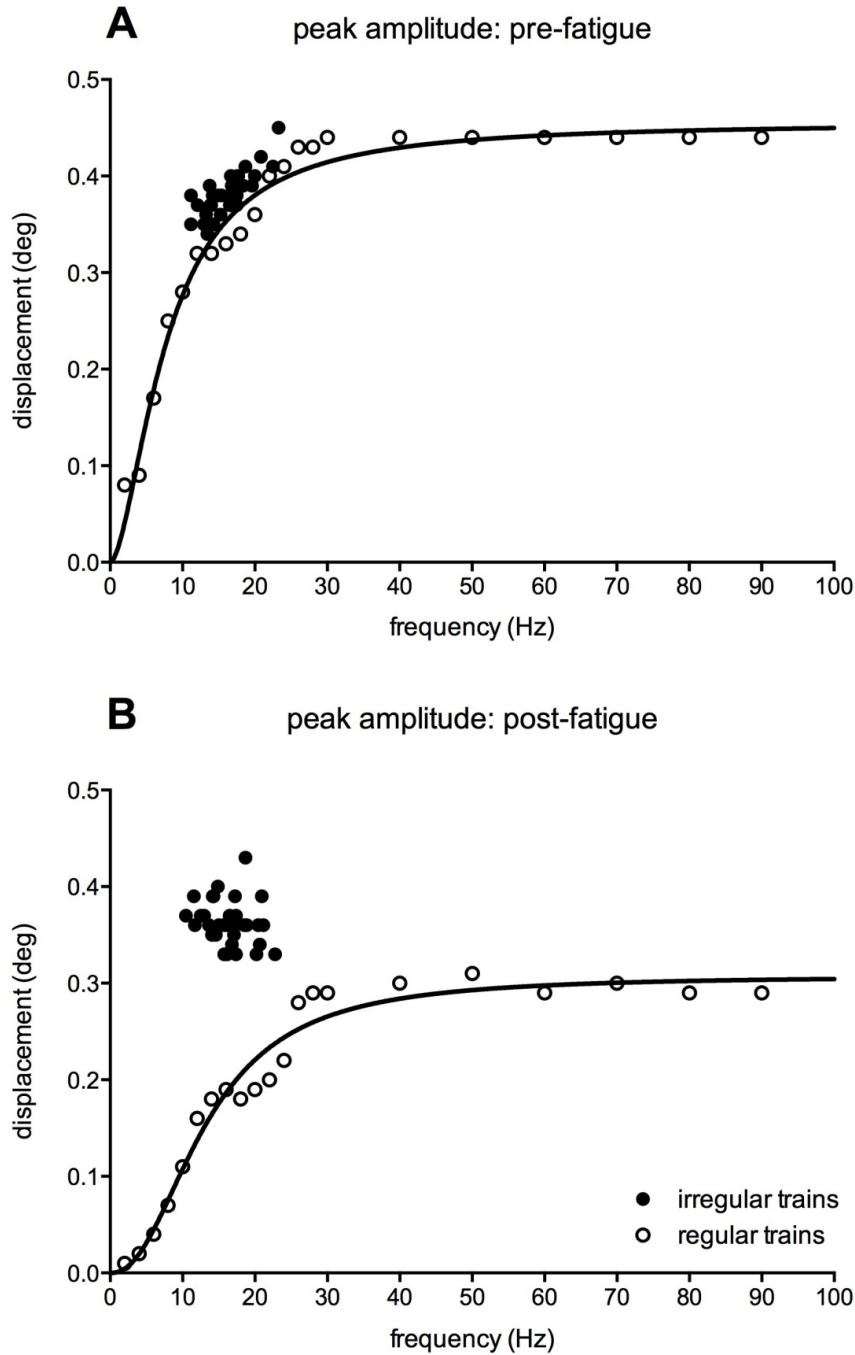
Figure 5: Relationship between stimulation frequency and angular displacement for regular and irregular trains delivered to a single motor axon innervating EDB, before (**A**) and after (**B**) two minutes of continuous stimulation to induce fatigue.

Figure 6 shows the mean amplitudes and areas of the angular displacements during stimulation of 12 motor axons with regular and irregular trains. The irregular trains produced significantly greater amplitude than the regular train; the same was true for

area. Following 2 minutes of continuous stimulation at 10 Hz the peak amplitudes were still significantly higher during stimulation with irregular trains, but this failed to reach significance for area, presumably because of slowing of twitch parameters during the fatigue.
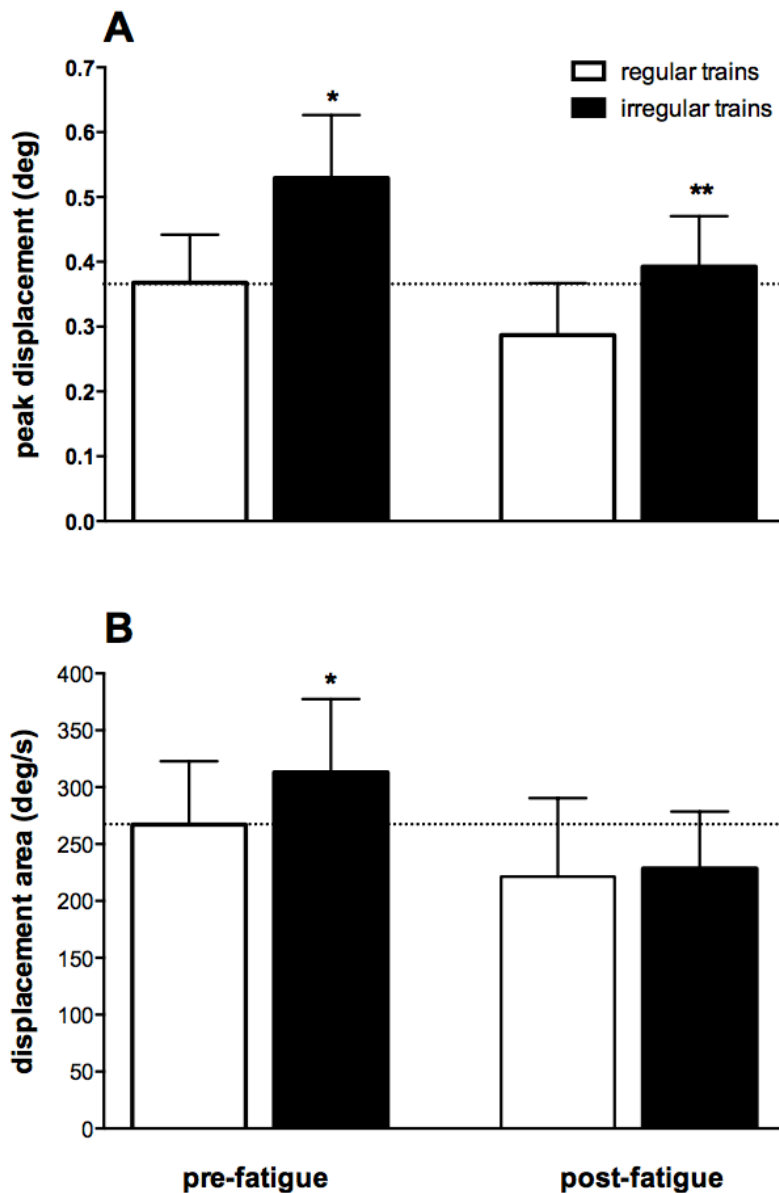

Figure 6: Peak angular displacement amplitudes (**A**) and areas (**B**) during stimulation of 12 single motor axons with regular and irregular trains of stimuli. Statistical comparisons were made between the mean values of the regular and irregular trains: * = p<0.05, ** = p<0.01; one-tailed paired t-test.

DISCUSSION

This project used intraneural microstimulation to selectively stimulate single motor axons in the common peroneal nerve with trains of regular and irregular frequencies. This allowed us to deliver controlled stimulation to single motor units within the toe extensors muscles, including EHL, EDL and EDB. We measured the contractile responses isotonically, using a sensitive angular displacement transducer applied over the distal phalanx of the relevant toe, which allowed us to further characterize mechanical features of single toe-extensor motor units in human subjects. Importantly, for the first time, we have stimulated single motor axons with trains of stimuli that emulate the firing of physiologically variable trains exhibited by actual human motoneurones during voluntary contractions. Until now, previous studies employing intraneural microstimulation have used regular trains of stimuli with zero variability. In the current study, we have shown that, over the range at which human motoneurones are volitionally activated, irregular trains of stimuli generate greater contractile responses than regular trains. Even after the induction of muscular fatigue the augmentation persists.

Methodological considerations

One of the principal challenges of this study was the difficulty in isolated single motor units, as the project required us to stimulate one and only one motor axon. To do this, it required very high-impedance electrodes, which meant that searching within the fascicle of the nerve was difficult. It required careful manipulation of the electrode in order to direct the tip into an appropriate intrafascicular stimulation site. And, because we had to ensure that only one axon was being stimulated, each experiment required careful observation of the isotonic displacement and EMG potentials being evoked by intraneural stimulation. We accepted that we were stimulating only one motor axon when

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the motor unit generated a measurable contractile response and EMG potential at the lowest electrical current, and increases in current beyond the safety margin resulted in the recruitment of an additional motor axon. Once an appropriate intrafascular site was obtained trains of stimuli were applied. The order in which the trains were given was always the same: firstly, the regular trains were given followed by a sequence of irregular trains, and then the fatigue run was given at 20 Hz for 2 minutes. Subsequently, the regular trains were given again, followed by the irregular trains. The order in which the stimuli were applied may have some importance. Since the irregular trains were delivered after the regular trains following induction of muscular fatigue, the contractile responses for the irregular trains may be an underestimated. Nevertheless, our results confirm that there was a significant difference between regular and irregular trains, not only in relation to angular displacement but contractile responses prior to and after fatigue onset.

Contractile properties of single motor units in the toe extensors

All of the previous studies designed to characterise the contractile responses of single human motor units during microstimulation of motor axons have used isometric force as the measurement. With respect to the toe extensors, Macefield and colleagues (1996) found a mean twitch rise time of 75 ms – longer than the 45 ms found here. This may be explained purely on a biomechanical basis. It has been pointed out that tendon compliance affects twitch contraction time, which is more important the longer the tendon. Indeed, Hill (1951) first identified the significance of tendon compliance, showing that twitch forces were reduced and contraction times increased by addition of in-series compliance to muscles. As described previously, this may account for some of the differences in rise-time for toe extensors, which have long tendons, and thenar motor

units, which have negligible tendons (Macefield et al., 1996). It is possible that translation of an isotonic contraction into an angular displacement may be less affected by tendon compliance than is the case for an isometric contraction, given that – in the absence of load – the tendon will be able to move freely and with it the digit(s) to which it is attached.

During delivery of regular trains of stimuli of increasing frequency we found a sigmoidal relationship between stimulation frequency and angular displacement, as had been found for the relationship between frequency and force (Macefield et al., 1996). Maximal displacements were reached at frequencies of 12-14 Hz. However, it should be pointed out that each stimulus train comprised only 10 pulses, so it is possible that greater displacements would have been produced with longer trains, but given that we did find that angular displacements reached a plateau we do not think this represents a major limitation. After all, our aim was to compare the contractile responses to the same number of pulses delivered in a regular and an irregular train. Nevertheless, our data do fit with the known maximal firing rates of single motor units in EHL (16.7 Hz; Macefield et al., 2002), which are similar to those found by microstimulating single motor axons supplying the toe extensors, the linear range of which extends from 5.5 to 16.3 Hz (Macefield et al., 1996). These maximal firing rates are lower than those for the intrinsic hand muscles - 29.9 Hz for adductor pollicis (Bellemare et al. 1983) and 30.4 Hz for first dorsal interosseus (Gandevia et al., 1990) – as well for the long finger extensors (27.4 Hz, Peters & Fuglevand 1999). Peak firing rates of TA motor units are 28.2 Hz (Bigland-Ritchie et al., 1992b), higher than those of EHL (16.7 Hz, Macefield et al., 2002) and soleus (10.7 Hz, Bellemare et al., 1983).

Contractile responses to irregular trains of stimuli

It is known that human motoneurones fire with an irregular discharge pattern. By utilizing this stimulation pattern through the use of short and long inter-spike intervals greater contractile responses can potentially be generated. It has also been demonstrated that by preceding a train of stimuli with an initial doublet (short interspike interval of, typically, 10 ms) there is an initial increase in force production. The effects of preceding a train with a short interspike interval was an increase in peak force at the onset of the contraction, but mean force at the end was not significantly different (Macefield et al., 1996). The increase in contractile response can be explained by the sluggish nature of muscular contractions: when a second stimulus is given before the motor unit has fully relaxed from the preceding stimulus there is a greater rate of rise of force, thereby providing an advantage of irregularity. This phenomenon was first observed for slow-twitch motor units in the triceps surae muscles of the cat (Burke et al., 1970); initial doublets have also been shown to produce greater tetanic responses in single motor units of the rat medial gastrocnemius (Celichowski & Grottel 1998). In the current study short intervals appeared not only at the beginning of a train, but randomly throughout the whole train. Such irregular trains produced greater angular displacements than regular trains for the same mean frequencies.

It has been observed that the use of initial brief high frequency pulses at the beginning of a sub-tetanic train of stimuli results in an increase in force: this was described as an initial doublet. A catch-like property of muscle is tension enhancement in skeletal muscles due to an initial brief high-frequency burst of pulses at the beginning of a sub-tetanic, constant-frequency, train and has been used to activate whole muscles or single motor units. Whole-nerve stimulation studies conducted in human subjects by Binder-

Macleod and colleagues (1996) showed that by using catch-like inducing trains, composed of 10 pulses with one short 5 ms interspike interval at the beginning of the train, a greater force-time integral, peak force and average force were generated than by trains of constant frequency. Later work by Binder-Macleod and colleagues (1999) showed a similar phenomenon by using 6-pulse catch-like inducing trains during isotonic contractions, producing greater increases in peak power and average power. The work conducted on humans is, however, underpinned by earlier work conducted on animals such as cats, which has provided much fundamental knowledge on the response of a muscle to repetitive stimuli. In the 1930's, Cooper and colleagues showed that there is a distinct relationship between the rate of stimulation and tetanus development: the response of a muscle to a repetitive stimulus was greater the shorter the inter-stimulus intervals (Cooper, 1930). Further work has also been done on the turtle hind-limb to validate previous concepts about catch-like properties of human muscles. It is important to show that these catch-like effects during dynamic contractions are not only present in humans, but in animals as well, because in many species contractions play an important role in a variety of natural movements such as locomotion (Gillis & Biewener, 2001). Callister and colleagues (2003) had two aims in their experiments involving the turtle hind-limb: firstly, to look at the differences in force production between catch-like inducing trains and control-frequency trains and, secondly, to examine the effects of fatigue on the different types of trains. They found that pre-fatigue the catch-like inducing trains produced higher amounts of force for isometric and dynamic trains than the control-frequency trains. Following fatigue it was revealed that fatigue had significantly lowered force production for dynamic and isometric contractions for both control frequency trains and catch-like inducing trains, yet the catch-trains induced an even greater enhancement of force after fatigue (Callister et al., 2003).

Effects of fatigue on contractile responses to regular and irregular stimuli

A secondary objective of this study was to assess the effects of fatigue on the angular displacement produced by microstimulation of a single motor axon, for both regular and irregular trains. A comparison of regular and irregular stimuli revealed that both types of stimuli showed a decrease in peak amplitude and area of the contractile response after the onset of fatigue. However, irregular trains produced significantly higher amounts of angular displacement both before and after fatigue for the same mean frequencies as the regular trains of stimuli. It may be that stimulating with irregular trains of stimuli instead of regular trains may have a functional practicality that has not yet been explored. Indeed, it may be that a long train of irregular stimuli may result in less fatigue. Functional electrical stimulation (FES) involves using electrical stimulation of skeletal muscles to assist in functional movements of individuals with damaged motor systems. FES involves continuous activation of the paralyzed muscle. Most advances in FES have involved improvements in technology, programming and implementation (Lee, Becker & Binder-Macleod 1999). One of the main issues concerned with FES is fatigue, but Binder-Macleod and colleagues (1996) showed that catch-like-inducing trains produced much greater forces also when the muscle was fatigued in comparison to constant frequency trains.

CONCLUSIONS

We have shown that irregular trains of stimulation produced significantly greater contractile responses than regular trains over the same frequency range. Regular trains showed a linear relationship in displacement with frequency, whereas irregular trains functioned on a higher portion of the displacement-frequency curve. Not only do irregular trains take advantage of catch-like properties to produce greater contractile responses but they also appeared to reduce the magnitude of fatigue.

ACKNOWLEDGEMENTS

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