H24/3168

MONASH UNIVERSITY
THESIS ACCEPTED IN SATISFACTION OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
ON..... 3 May 2002 .....
..... [redacted] .....
Sec. Research Graduate School Committee

Skeletal Muscle: Activation Strategies, Fatigue Properties and Role in Proprioception

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November 2001

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Declaration

The work presented in this thesis does not contain material that has been previously published or written by another person, except where due reference is made. The material presented has not been submitted for the award of any other degree or diploma in any other university or institution.

Andrew Wise

Acknowledgments

I would like to thank my two supervisors, Prof. Uwe Proske and Dr. David Morgan, for the opportunity to undertake my PhD and their many insightful comments and help with my thesis. I would also like to thank Dr. Ed Gregory for his experimental and intellectual input which has been most appreciated.

I would like to thank my parents for their continued support and understanding throughout my PhD. I would like to thank Nadia for her friendship and many encouraging words. Thanks also go to Camilla, James, Richard and Nick, for the many laughs we shared that helped keep things in perspective.

Preface

This thesis examined proprioception, whereby the ability of human subjects to detect small movements was compared for the passive muscle and during weak voluntary contraction. This was followed by an examination of various activation strategies of skeletal muscle and their effects on muscle mechanics and fatigue properties. A unifying theme between these two studies was the question, what are effective activation strategies for paralysed muscle (functional electrical stimulation, FES) and is there a role for proprioceptive feedback in the optimisation of the activation patterns?

The first half of the thesis was concerned with proprioception, in particular, the sense of position and movement of our limbs. The first chapter reviewed the relevant literature detailing the various sources of proprioceptive information and how this information might be used during muscle contraction. Chapter two described experiments that were carried out on human subjects in whom proprioceptive acuity was examined during and after muscle contractions. These experiments aimed to describe the effects of changes in muscle history and the influence of muscle contraction on proprioceptive acuity. Chapter three focused on a specific muscle receptor, the muscle spindle, which is known to provide information important for proprioception and motor control. Here, muscle spindle responses were examined under conditions similar to those encountered in the human proprioception experiments.

The second half of the thesis was concerned with properties of submaximally activated muscle. Experiments described a new way of using muscle tension as feedback to optimise stimulus parameters for a distributed stimulation protocol. Chapter four reviewed the relevant literature and chapter five described the stimulator, detailing the algorithm used to control stimulation parameters. Chapter six described experiments that were carried out to assess the functioning of the stimulator and to examine some mechanical properties of muscle during submaximal contractions. The seventh chapter considered a more functional purpose, with the examination of muscle fatigue when high and low rates of stimulation were used. The idea behind the experiments discussed in chapter seven was to determine whether there was a potential advantage in using optimised distributed stimulation in clinical applications where some function can be restored to paralysed muscles. Chapter nine, the general discussion, outlined some of the implications of the experimental findings and discussed some of the advantages and limitations of using distributed stimulation in FES, with potential solutions that could be explored in future experiments.

Summary

Proprioception

These experiments examined proprioceptive acuity at the elbow joint in human subjects. Movement detection thresholds for elbow extension and flexion were measured in the relaxed limb after three forms of muscle conditioning. Muscle conditioning was designed to alter the stretch sensitivity of muscle spindles located in the biceps and triceps muscles by making them either slack or taut. Thresholds were high for movements that stretched muscle spindles that were slackened by muscle conditioning whereas, thresholds were low if the movement stretched muscle spindles made taut by conditioning.

Movement detection thresholds were re-measured during co-contractions of biceps and triceps muscles. Thresholds measured during co-contractions were much higher than those measured for the relaxed limb after conditioning that had removed any slack. In other words, proprioceptive acuity was significantly reduced during contraction of biceps and triceps muscles. It was proposed that the reduced acuity was the result of fusimotor activity that is known to occur during voluntary muscle contraction.

In order to test these propositions put forward in the human proprioception experiments, recordings were made from muscle spindles in the soleus muscle of the anaesthetised cat. Stretch responses were measured after conditioning procedures similar to the human experiments. Muscle spindle stretch responses were much smaller following conditioning manoeuvres designed to introduce slack in muscle spindles compared to stretch responses measured following muscle conditioning designed to remove any slack.

A factor contributing to the decrease in proprioceptive acuity observed during muscle contraction might be a reduction in muscle spindle stretch sensitivity from fusimotor activity. To examine the hypothesis that fusimotor activity was responsible for the reduced proprioceptive acuity in humans during muscle contraction, cat muscle spindle stretch responses were measured during combinations of fusimotor and skeletomotor stimulation. All combinations of fusimotor and skeletomotor activation reduced muscle spindle stretch responses compared to the stretch responses of the passive muscle spindle.

Muscle spindle stretch responses were measured during stimulation of single dynamic fusimotor axons to examine the effects of stretch velocity and amplitude. The stretch responses of a passive muscle spindle were greater than the stretch response of the same muscle spindle during dynamic fusimotor stimulation, provided muscle stretch was of a small amplitude. For muscle stretches of a larger amplitude, dynamic fusimotor activity significantly increased muscle spindle stretch responses.

Distributed Stimulation

Distributed stimulation can be used to produce smooth muscle contractions at low rates of stimulation. In its original form, this method involved stimulation of several independent muscle portions, each generating about equal tension. Stimuli were delivered sequentially with equal time intervals between each stimulating pulse. Experiments in this thesis have used a computer algorithm to 'optimise' interpulse intervals in order to account for tension variations between muscle portions due to fatigue and/or potentiation. Using this technique, smooth contractions of a mixed muscle were generated using low stimulation rates allowing the examination of various properties of submaximally activated muscle.

Force-frequency, length-tension and force-velocity relationships were examined in rat triceps surae, cat gastrocnemius and cat soleus muscles. For a given rate of stimulation, contractions were more nearly fused and generated higher tensions with optimised distributed stimulation compared to synchronous stimulation of the whole muscle. The length at which a muscle generated maximum tension was longer when low, submaximal rates of stimulation were used. At higher stimulation rates, the force-velocity relationship of the rat triceps surae was consistent with that reported in a previous study of the fully activated cat soleus muscle. At lower rates of stimulation, the force-velocity relationship of the rat triceps surae was different to that of the cat soleus muscle. Reasons for this difference are discussed.

To optimise interpulse intervals, continual feedback of the tension ripple generated in response to stimulation is required. In theory, any signal proportional to whole muscle tension could be used as a feedback source. Experiments explored the possibility that an ensemble discharge from a number of tendon organs, or alternatively, signals from an accelerometer placed on the muscle could provide such feedback. The results indicated that the information provided by these two means were not representative of tension ripple in the whole muscle but were dominated by tension generated in certain parts of the muscle.

Fatigue properties of the cat medial gastrocnemius muscle were examined using optimised distributed stimulation and synchronous stimulation with a continuous and an intermittent stimulation protocol. The results showed that there was significantly less muscle fatigue using optimised distributed stimulation at low rates than compared to synchronous stimulation at higher rates. Contractions showed less tension sag during optimised distributed stimulation and were more reproducible when brief pauses in stimulation were introduced. These findings suggest that optimised distributed stimulation has important advantages over other methods of stimulation for applications such as FES.

Abbreviations

FES	Functional Electrical Stimulation
CNS	Central Nervous System
SREC	Short Range Elastic Component
EMG	Electromyogram
MVC	Maximum Voluntary Contraction
L_{max}	Maximum Physiological Muscle Length
pps	Pulses Per Second
FF	Fast Twitch Fast Fatigable
FI	Fast Twitch Intermediate Fatigable
FR	Fast Twitch Fatigue Resistant
S	Slow Twitch Muscle Fibre
L_{opt}	Optimal Muscle Length
ACh	Acetylcholine
EC	Excitation Contraction
T-tubule	Transverse Tubule
SR	Sarcoplasmic Reticulum
RMS	Root Mean Square
FRip	Fundamental Ripple
PP	Peak-to-Peak Tension Ripple

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Human Proprioception and Muscle Spindles

We contract our muscles in order to move our limbs and stabilise posture. To accomplish skilled movements we need to be aware of the orientation of our body and the position and movement of our limbs. We are also aware of the effort involved in, and the force produced by, the muscle contraction. The conscious perception of limb position and movement is commonly called proprioception, but is also known as kinaesthesia (Bastian 1888). An important source of proprioceptive information arises from a number of different receptors located in the periphery. This information is also important for a range of functions involved in motor control, however it is the role played by these receptors in the perception of limb position and movement that is the focus of this half of the thesis. There is ample evidence to suggest that a specific class of peripheral receptors located in skeletal muscle, the muscle spindles, provide a significant contribution to proprioception. However, this was not always the prevailing view.

The introduction to this half of the thesis will discuss some of the theories and ideas that have been proposed to explain proprioception. The introduction will discuss the contribution made by various peripheral receptors and will focus on the muscle spindle, describing the receptor, detailing its role in proprioception and its behaviour during muscle contraction. The human experiments discussed in this thesis are concerned with proprioceptive acuity in the relaxed limb and during muscle contraction. Experiments carried out on muscle spindles in the cat are used to examine the detailed behaviour of muscle spindles under conditions designed to approximate those of the human experiments.

Historical Perspective

Theories proposed by Helmholtz, Müller and Wundt in the 1800's suggested that proprioception was mediated by sensations of innervation (McCloskey 1978). When a motor command was initiated and executed, direct projections from the motor cortex to the sensory cortex signalled the intended action. However, certain observations could not be explained with this theory. For instance, human subjects were able to perceive rotations of a relaxed limb for which there was no motor command and therefore no signal from the motor cortex (Sherrington, 1900). This meant that sensations of

innervation could not be the only source of information used to perceive limb movement and position. Sherrington believed that feedback from receptors located in the periphery, in particular the muscle spindles, provided information responsible for the sensation of limb position and movement. However, this view changed over the subsequent years and until the 1960's it was thought that receptors located in the joints were the major class of receptor involved in proprioception.

The arguments against a proprioceptive role for muscle spindles were largely based on several lines of evidence that began to emerge in the 1950's. Early experiments tracing the central projections of muscle spindles failed to show a cortical representation of these afferents (Mountcastle, Covian & Harrison 1952; Gardner & Haddad 1953). It was also reported that pulling on exposed tendons during surgery did not produce sensations of muscle stretch that would be expected if muscle spindles provided perceptual signals relating to muscle length (Gelfan & Carter 1967). These findings, coupled with mounting evidence that joint receptors were able to respond to limb movements across the entire rotational range, supported the proposition that joint receptors, and not muscle spindles, provided the main source of proprioceptive information (Andrew & Dodt 1953; Boyd & Roberts 1953; Skoglund 1956). Significantly, it was argued that as muscle spindle firing rate could be altered by fusimotor activation without any change in joint angle, muscle spindles could not provide unambiguous information about limb position and movement (see McCloskey 1978). This is an important point and will be discussed in detail later.

Many of the early studies supporting the theory that joint receptors mediated proprioception were subsequently re-examined with the findings of a number of these later studies casting doubt over the ability of joint receptors to provide a major contribution to proprioception. In a study of the responses of 209 joint receptors recorded in dorsal roots in the cat, few of the receptors had slowly adapting responses in the mid-range of joint rotation (Burgess & Clark 1969). The joint receptors generally only became active at the extremes of joint extension or flexion, and therefore could not signal limb movement or position unambiguously or through the entire range (Burgess & Clark 1969; Clark & Burgess 1975). It was claimed that the previous studies, which found a significant mid-range responsiveness of joint receptors, were probably flawed in their sampling. It was suggested that for studies involving the medial articular nerve in the cat, contamination of the nerve by muscle spindles from the popliteus muscle was

responsible for the mid-range activity (Ferrell 1977; McIntyre, Proske & Tracey 1978). Additional evidence questioning the role of joint receptors in proprioception came from patients following complete joint replacement surgery (Cross & McCloskey 1973; Grigg, Finerman & Riley 1973). These patients were able to perceive limb positions even though the joint, and its complement of joint receptors, was removed. However, the ability of these subjects to detect limb movement was somewhat reduced.

While the theory that joint receptors provided the sole source of proprioceptive information was being questioned, evidence began to mount supporting a major contribution from muscle spindles. Contrary to earlier findings, it was shown that muscle spindle afferents did project to the sensory cortex (Amassian & Berlin 1958; Oscarsson & Rosen 1963; Phillips, Powell & Wiesendanger 1971). Furthermore, pulling on exposed tendons was found to produce sensations of movement (Matthews & Simmonds 1974; McCloskey, Cross, Honner & Potter 1983), contrary to earlier reports (Gelfan & Carter 1967). In these experiments the exposed tendons of anaesthetised but conscious subjects were pulled in order to stretch the muscle without causing rotation of the joint, with subjects reporting an illusion of joint rotation.

A significant finding that supported a proprioceptive role for muscle spindles was the illusions of altered limb position and movement produced by transverse vibration of muscles in human subjects (Goodwin, McCloskey & Matthews 1972). These illusions were not present when vibration was applied to the joint, and persisted when the joint and skin were anaesthetised, ruling out the contributions of vibration sensitive cutaneous and joint receptors. The primary endings of muscle spindles were thought to be responsible for the illusions due to their high sensitivity to vibration (Brown, Engberg & Matthews 1967). Subjects reported illusions in the direction that would stretch the vibrated muscle, with the illusions dependent on the amplitude and frequency of the vibratory stimulus. Increasing the frequency of vibration produced illusions of faster limb movement. Muscle vibration could produce illusions of joint position and movement beyond their anatomical range, suggesting that the muscle spindle signals could override information from joint and cutaneous receptors (Craske 1977). These findings provided evidence that muscle spindle signals are important in proprioception and that altering their response can have perceptual consequences.

Signals arising from muscle spindles located in all of the muscles acting at a joint are likely to provide proprioceptive information. Evidence supporting this

proposition has come from experiments using vibration of agonist and antagonist muscles. When both elbow extensor and flexor muscles were vibrated concurrently and at the same frequency no illusions of movement were evoked (Gilhodes, Roll & Tardy-Gervet 1986). It was only when the vibration frequency of one muscle was different to the other that movement illusions were reported, with the direction of the illusion corresponding to lengthening of the muscle vibrated at the higher frequency. The velocity of the illusion increased as the difference in vibration frequencies was increased.

Measuring Proprioceptive Acuity

Goldscheider (1889) devised a simple method to measure proprioceptive acuity in human subjects (McCloskey 1978). In his psychophysical experiments, and others like it, movements of a constant velocity were imposed on a joint and the subject reported the direction of the perceived movement. Typically the muscles acting on the limb were relaxed and the amplitude of movement required for correct detection of the movement direction was measured and expressed as a movement detection threshold (Laidlaw & Hamiliton 1937a; Laidlaw & Hamiliton 1937b; Hall & McCloskey 1983).

Movement thresholds have been measured at many joints, and Goldscheider (1889) observed that proximal joints had lower movement detection thresholds than distal joints. Hall & McCloskey (1983) later confirmed this result, however when they expressed thresholds in terms of changes in muscle fascicle length rather than in terms of degree of joint rotation, movement thresholds for the elbow and finger joints were similar. The authors concluded that muscle fascicle length, which is signalled by muscle spindles, was the important proprioceptive variable used by the CNS (Hall & McCloskey 1983; Refshauge, Taylor, McCloskey, Gianoutsos, Mathews & Fitzpatrick 1998).

Proprioception encompasses both the sense of limb position and limb movement. Separate lines of information could mediate the sense of limb position and limb movement, alternatively, limb position could be determined by integration of movement signals. It is difficult to differentiate between limb position sense and limb movement sense because it is not possible to change limb position without moving. However, there is experimental evidence that supports the theory that they are separate senses. The ability of subjects to detect changes in limb position and limb movement depends on the

velocity of the imposed movement, with faster movements detected more easily (Cleghorn & Darcus 1952; Browne, Lee & Ring 1954; Hall & McCloskey 1983). If limb movement is extremely slow subjects do not perceive an overt sense of movement, but after a while can sense that the position of their limb has changed (Horch, Clark & Burgess 1975; Clark, Burgess, Chapin & Lipscomb 1985). Additional evidence supporting the idea of separate processing of position and movement information has come from studies using low frequency vibration. In these experiments vibration produced illusions of altered position but not movement (McCloskey 1973).

Peripheral Receptors and Proprioception

By highlighting the proprioceptive role of muscle spindles it should not be implied that receptors in skin, joints and ligaments do not contribute to proprioception. These receptors are indeed well placed to provide proprioceptive signals. The skin over muscles and joints can be stretched or folded during joint rotation and changes in intracapsular pressure can activate joint receptors enabling them to signal limb movement and position. The following section describes the proprioceptive role of peripheral receptors, namely those found in skin, muscles and joints.

One experimental approach used to examine the proprioceptive role of skin, joint and muscle receptors in isolation is to selectively eliminate the contribution from one or more of these receptors and then examine the ability of subjects to detect joint rotations. This approach was used in an elegant series of experiments examining movement detection of the middle finger. For the terminal phalanx of the finger it is possible to disengage the muscles acting on the joint so that only cutaneous and joint receptors can signal joint movement. When the input from muscle receptors was eliminated with this procedure the ability of subjects to detect joint movement and position was reduced but not abolished. When muscle receptor input was restored normal proprioceptive acuity was re-established (Gandevia & McCloskey 1976).

To eliminate the contribution of joint and cutaneous receptors it is possible to anaesthetise the skin and/or the joint and then assess the ability of subjects to detect joint rotation. Using this method Clark *et al.* (1979) showed that anaesthetising the knee joint did not significantly impair movement and position sense and they suggested that cutaneous and joint receptors did not make a major contribution to proprioception for this joint. However, anaesthetising the fingers or the whole hand has been shown to

affect proprioceptive acuity but not abolish it completely (Goodwin, McCloskey & Matthews 1972; Goodwin, McCloskey *et al.* 1972; Ferrell & Smith 1987). These findings imply that signals arising from skin and joint receptors are important for some joints.

Other evidence supporting a proprioceptive role for joint and skin receptors has come from studies using stimulation of single identified skin and joints afferents in conscious human subjects. In these experiments intraneural microstimulation of afferents arising from skin and joint receptors was consciously perceived and, for some afferents, produced sensations of skin pressure and joint movement (Ochoa & Torebjork 1983; Macefield, Gandevia & Burke 1990). Perception of muscle spindle activity, unlike joint and cutaneous receptors, requires spatial summation from a number of afferents. Stimulation of single muscle spindle afferents using microelectrodes did not elicit a perceptual experience (Macefield, Gandevia *et al.* 1990). However, if the electrical stimulation recruited a number of muscle spindle afferents then illusions of movement and altered position were produced (Gandevia 1985). In these experiments stimulation of the ulnar nerve was able to elicit illusions of finger movement, the velocity of these illusions increasing at higher stimulation frequencies.

Joint and Skin Receptors

Afferent innervation of joints and ligaments consists of large diameter afferents in the Group II range, with specialised receptors including Ruffini endings, Golgi endings and Paciniform corpuscles. There are also smaller diameter afferents in the Group III and Group IV range with free nerve endings (Boyd 1954; Skoglund 1956). Electrophysiological experiments in the cat (Burgess & Clark 1969; Millar 1975) and in humans (Burke, Gandevia & Macefield 1988) have examined joint receptor responses during limb movement. These experiments showed that joint receptors were typically activated at the extremes of joint rotation in both extension and flexion, with only a small number of receptors active in the mid-range of joint rotation. These results led to the general conclusion that joint receptors alone could not provide unambiguous signals of joint position, and were therefore only likely to be of significance when combined with signals from other receptors.

Examination of joint afferent signals during muscle contraction has shown that their discharge properties are not constant and may be altered by the level of

contraction. The evidence suggests that an increase in muscle force does not increase the mid-range responsiveness of slowly adapting joint receptors in the cat elbow (Baxendale & Ferrell 1983). However, recordings of joint receptors from human subjects have shown that their response during muscle contraction may be different to their passive responses (Edin 1990). Edin (1990) concluded that joint position could not be clearly signalled by these receptors without considering the level of muscular activity.

Receptors located in skin are both rapidly adapting and slowly adapting. The rapidly adapting types included Meissner's and Pacinian corpuscles as well as hair basket endings, while the slowly adapting types are Merkel discs and Ruffini endings (Iggo 1977). Microneurographic recording of identified afferents in the median nerve of human subjects showed that all five types respond to the dynamic phase of voluntary movement with most of the rapidly adapting receptors responding to both flexion and extension (Edin & Abbs 1991). Both types of slowly adapting receptors can provide directional information (Edin & Abbs 1991), but responses to static joint position are limited to Ruffini endings (Hulliger, Nordh, Thelin & Vallbo 1979). It has therefore been suggested that the slowly adapting receptors are likely to contribute to proprioception (Vallbo, Hagbarth, Torebjork & Wallin 1979; Edin 2001). The rapidly adapting receptors respond in both directions of movement, applied either passively or actively (Burke, Gandevia *et al.* 1988; Edin & Abbs 1991). The sensations derived from maintained skin indentation fade over time and therefore cutaneous receptors may not provide static positional information (Horch, Clark *et al.* 1975). This is in contrast to the sense of joint position, which does not fade. Cutaneous receptors may provide specific proprioceptive information in addition to a facilitatory role for inputs from joint and muscle receptors (McCloskey 1978).

The possible role of Group III and Group IV small diameter afferents in proprioception cannot be ignored but it is unlikely that they contribute significantly to proprioception. These afferents represent a large proportion of all skin and muscle afferents and are responsive to mechanical, thermal, chemical and nociceptive stimuli, with many of them responding pollymodally. A small percentage of Group IV afferents are responsive to muscle contraction and stretch (Kniffki, Mense & Schmidt 1978). The exact role of these afferents in proprioception is still to be determined.

In summary, the general conclusions that can be drawn from these studies is that cutaneous, muscle and joints receptors can all provide limb position and movement information. It is likely that their relative contribution varies between different joints, and that there is a degree of redundancy in the information provided by these receptors. The current view is that muscle spindles play a particularly important role at proximal joints whilst cutaneous and joint receptors provide a significant source of information for distal joints.

At this stage it is appropriate to describe the muscle spindle in detail, but first I would like to discuss another type of muscle receptor, the Golgi tendon organ. Experiments in this thesis describe the effect of muscle contraction on the sense of limb movement. Tendon organs, whilst not implicated in the sense of limb position and movement per se, are known to be highly sensitive to muscle contraction. Signals from tendon organs are responsible for the sense of intramuscular tension (McCloskey, Ebeling & Goodwin 1974; Roland 1975). An additional motive for discussing tendon organs is that some experiments in the second half of this thesis measured tendon organ responses during muscle contraction, and some of the background literature covered in the following section is also relevant to these experiments.

The Tendon Organ

The Golgi tendon organ, first described and named by Golgi in 1880, are a class of muscle receptor typically located at the musculotendinous junction and lie in series with skeletal muscle fibres (Schoultz & Swett 1972; Barker 1974). Composed of a number of collagen strands enclosed by a connective tissue capsule, each tendon organ is innervated by a single, large diameter, myelinated afferent commonly called the Ib axon. The unmyelinated ending of a Ib axon branches and terminates on a number, but not all, of the encapsulated collagen bundles (Jami 1992). The number of muscle fibres that insert into a tendon organ ranges from 3-50 (Bridgman 1970) and can arise from motor units having different histochemical composition (Reinking, Stephens & Stuart 1975). The stimulus that tendon organs respond to is strain of the receptor terminal (Fukami & Wilkinson 1977). Tendon organs are relatively insensitive to stretch of a relaxed muscle but are much more sensitive to tension generated by muscle contraction (Matthews 1933; Houk & Henneman 1967). A large proportion of force from a passively applied stretch is not transmitted through the tendon organ directly but rather

via the surrounding connective tissue (Jansen & Rudjord 1964). If the force is applied by active contraction of a motor unit supplying the tendon organ then the tension is transmitted to the receptor terminal directly and activation thresholds are low. Tendon organ responses depend on the specific motor units activated by the stimulus (Gregory & Proske 1979). Activation of motor units that do not insert into the receptor, or activation of motor units that do insert but are not attached to innervated collagen bundles, may cause unloading of the receptor and decrease the firing rate of an active tendon organ (Zelena & Soukup 1983).

Tendon organs are sensitive to changes in muscle tension and unfused contractions modulate firing rate more than fused contractions (Jami, Petit, Proske & Zytnicki 1985; Jami 1992). During muscle contraction tendon organs can become highly sensitive to vibration and may even become more sensitive than muscle spindles for a given set of conditions. Although the response from one tendon organ can show a non-linear relationship (Gregory & Proske 1979; Gregory & Proske 1981), it is possible that the CNS monitors a signal derived from an ensemble discharge since this can provide a more representative signal of whole muscle tension (Crago, Houk & Rymer 1982; Jami 1992), a point which will be covered in more detail in the second half of the thesis.

The Muscle Spindle

Muscle spindles are a class of sensory receptor that have long been considered important for movement control and proprioception (Sherrington 1900). Muscle spindles have prominent roles in reflexes, motor learning, locomotion, and for precision movements. They are found in most skeletal muscles and lie in parallel with the muscle fibres. Muscle spindles can signal muscle length, with their response dependent on static muscle length and the velocity of the length changes (Matthews 1933).

Morphology

Muscle spindles are composed of a number of intrafusal fibres encapsulated by a fusiform shaped connective tissue sheath (Ruffini 1898). Intrafusal fibres have striated ends that are directly attached to extrafusal fibres, connective tissue or to tendinous slips. Intrafusal fibres are classified as bag or chain fibres based on histochemical, morphological and functional differences related to fibre thickness, length and the

arrangement of the nuclei within the central region of the fibre (Boyd 1962). A further distinction between intrafusal fibres can be made based on differences in their contraction speed. Two types of bag fibres have been described, with bag_1 fibres contracting more slowly than bag_2 fibres, which in turn, contract slower than chain fibres. There are typically between 2 and 12 intrafusal fibres within a muscle spindle (Ruffini 1898) and usually there are more chain fibres than bag fibres. The ends of the bag_1 and bag_2 fibres protrude outside the capsule while the chain fibres lie within the capsule limits, although there are exceptions to this rule (Bridgman, Shumpert & Eldred 1969). Different muscles contain different numbers of muscle spindles, with the soleus muscle of the cat containing approximately 50 muscle spindles (Boyd & Gladden 1985).

Sensory Innervation

Muscle spindles are supplied by two types of myelinated afferents called primary and secondary afferents (Sherrington 1894; Ruffini 1898). The primary afferents branch and form spiral terminations, the primary endings, around the equatorial region of each intrafusal fibre (Banks, Barker & Stacey 1982). The secondary afferents form spray-like secondary endings to one side of the primary ending, typically on the striated region of the bag_2 and chain fibres (Boyd 1962). The primary afferents are larger in diameter and have faster conduction velocities than the secondary afferents. Measurements of the afferent conduction velocity is a method commonly used to distinguish between the primary and secondary afferents, with the arbitrary velocity of 72m/s used as the boundary between the two populations (Hunt 1954).

Muscle spindle firing rate is related to stretch of the sensory endings. In the de-efferented cat, in response to passive shortening, afferents from both the primary and secondary endings decrease their firing rate and can fall silent (Matthews 1933; Matthews 1964). In response to passive muscle lengthening primary and secondary afferents increase their firing rate, the primary afferents doing so with greater sensitivity. The term passive, used in this context, means that the muscle and its complement of muscle spindles are not subjected to ongoing fusimotor or skeletomotor activity. The response of primary endings to passive lengthening of a relatively large amplitude is non-linear and is characterised by an initial burst at the onset of stretch (Matthews 1933). The initial burst is labile and depends upon the state of the intrafusal

fibres. For very small muscle stretches the primary endings respond linearly. The linear range is approximately 100μm (Matthews & Stein 1969) and has been attributed to the relatively high stiffness of intrafusal fibres when at rest (Matthews 1972; Morgan, Prochazka & Proske 1984). Primary endings are highly sensitive to vibration (Kuffler, Hunt & Quilliam 1951) and can respond to vibration at 100Hz with a peak-to-peak amplitude of 5μm (Brown, Engberg *et al.* 1967). The secondary endings do not have the same dynamic sensitivity as the primary endings and are less sensitive to vibration (Brown, Engberg *et al.* 1967). Their firing rate is more regular and linearly related to muscle length, making them well suited to provide information about absolute muscle length.

Muscle spindles exhibit a unique property, whereby their stretch sensitivity and resting discharge depend on the previous contraction and length history of the muscle in which they are situated. This property is an example of muscle thixotropy, meaning that the stiffness of the muscle spindle can be altered by the contraction and length history. Muscle thixotropy has been used as a tool to examine muscle spindle functioning in both human and animal experiments (Proske, Morgan & Gregory 1993). Experiments in this thesis examine the affects of contraction history on human proprioception and muscle spindle functioning, and these effects will be covered in detail shortly.

Motor Innervation

Muscle spindles receive a motor innervation from small diameter myelinated axons first identified in 1930 by (Eccles & Sherrington 1930) and called fusimotor (γ) axons. They have slower conduction velocities than skeletomotor axons (α) (Leksell 1945) and activation of fusimotor axons produces contraction of the intrafusal fibres, without producing measurable tension at the tendon (Matthews 1933; Hunt & Kuffler 1951). There are two types of fusimotor axons, static and dynamic, which act to alter the static and dynamic sensitivity of the primary endings (Matthews 1962; Emonet-Denand, Laporte, Matthews & Petit 1977). The less numerous dynamic axons exclusively innervate the bag_1 intrafusal fibres. The static axons innervate both chain and bag_2 intrafusal fibres (Barker, Emonet-Denand, Laporte, Proske & Stacey 1973; Boyd, Gladden, McWilliam & Ward 1977). There is approximately one dynamic fusimotor axon and four static fusimotor axons per muscle spindle. The unmyelinated terminal of a single fusimotor axon may innervate one or both poles of an intrafusal

fibre, with the other pole being innervated by another fusimotor axon (Banks 1991). Activation of fusimotor axons causes contraction and shortening of the polar regions of the intrafusal fibres, which acts to stretch the non-contractile equatorial region (Boyd 1966). The contraction of the bag_1 fibre is focal and slow, while the contraction of the bag_2 fibre is also focal but more rapid and forceful than the bag_1. Chain fibre contraction appears to be twitch-like and rapid, involving propagated action potentials (Bessou & Pages 1975; Boyd, Gladden *et al.* 1977). At fixed muscle lengths, fusimotor activation increases the firing rate of the primary endings (Lennerstrand & Thoden 1968).

The intrafusal fibres may receive additional efferent innervation from β motoneurons (Bessou, Emonet-Denand & Laporte 1965). These efferent axons innervate extrafusal fibres and send a branch to intrafusal muscle fibres, mainly the bag_1 fibres (Boyd, Gladden *et al.* 1977). The existence of β motoneurons could be an evolutionary trait, as it is the usual arrangement in amphibians and reptiles.

Dynamic Fusimotor System

Stimulation of dynamic fusimotor axons alters the stretch sensitivity of the primary endings and can produce a small increase in their firing rate (Emonet-Denand, Laporte *et al.* 1977). The initial experiments describing the effects of dynamic fusimotor stimulation found an increase in the stretch sensitivity to ramp-and-hold stretches of relatively large amplitude (Crowe & Matthews 1964a) and to muscle vibration (Brown, Engberg *et al.* 1967), with an increase in sensitivity of up to five times (Boyd & Gladden 1985). However, dynamic fusimotor activation does not always act to increase the sensitivity of the primary ending. Using small amplitude sinusoidal length changes within the linear range, stimulation of single dynamic fusimotor axons was found to reduce the stretch sensitivity of primary endings compared to their passive responses (Goodwin, Hulliger & Matthews 1975). Subsequent studies described a 'cross-over point' whereby dynamic fusimotor stimulation increased the dynamic sensitivity above passive responses for stretch amplitudes larger than approximately 200μm (Hulliger, Matthews & Noth 1977).

Static Fusimotor System

Activation of static fusimotor axons typically produces a large increase in the firing rate of the primary ending and can decrease the dynamic sensitivity of the primary ending by as much as 50% across all amplitudes of muscle stretch (Crowe &

Matthews 1964a; Crowe & Matthews 1964b; Chen & Poppele 1973; Hulliger, Matthews *et al.* 1977). Stimulation of static fusimotor axons may also cause 'driving', an effect whereby the firing rate is locked to the stimulation frequency of the fusimotor axon. The effect of static fusimotor stimulation on the responses of secondary endings is to raise the firing rate at a given muscle length. There are variable effects on the length sensitivity of the secondary ending with some showing an increase in sensitivity and others a decrease (Chen & Poppele 1978). For muscle shortening, even of relatively large amplitudes, activation of static fusimotor axons can prevent silencing of primary endings (Crowe & Matthews 1964b).

During combined stimulation of static and dynamic fusimotor axons the dynamic sensitivity is typically maintained for muscle stretch of relatively large amplitudes (Lennerstrand & Thoden 1968; Hulliger, Matthews & Noth 1977). The effect on the sensitivity for small amplitude length changes is similar to stimulation of static axons alone (Hulliger, Matthews *et al.* 1977).

Muscle Spindle After Effects

The ability of muscle spindles to contribute useful proprioceptive information depends on them providing consistent and repeatable signals of muscle length. However, as previously mentioned, the discharge properties of passive muscle spindles can be influenced by the muscle's contraction and length history. These history effects are commonly called muscle spindle after effects and arise as a result of a muscle property known as thixotropy. The following section will discuss the nature of muscle spindle after effects and their consequences for proprioception.

Kuffler, Hunt & Quilliam (1951) first reported that muscle spindle firing rate was influenced by its contraction history. They examined the effects of stimulating single fusimotor axons on the firing properties of muscle spindles and observed an 'overshoot' in muscle spindle firing rate lasting for some time following a period of fusimotor stimulation. They called this phenomenon 'post-excitatory facilitation' and suggested that a property of the intrafusal fibres, whereby they were slow to return to their original position owing to some 'holding' action, was the mechanism responsible. However, insight into what caused muscle spindle after effects was not gained until many years after the initial observations.

In a seemingly unrelated series of experiments on amphibian skeletal muscle, Hill (1968) observed that at the onset of muscle stretch tension rose rapidly over a short range, up to about 0.2% of the muscle length, and then continued to increase more gradually. He called this initial rise in tension a 'short range elastic component' (SREC) and suggested that the presence of a number of stable cross-bridges, which formed spontaneously between actin and myosin filaments, was responsible. The elastic behaviour was proposed to be due to the spring-like property of the stable cross-bridges which could only be stretched a short distance before detaching. However, the cross-bridge interpretation remains controversial (Mutungi & Ranatunga 1996).

The concept of stable cross-bridges was later used to explain some of the muscle history effects observed in muscle spindles. The intrafusal fibres within muscle spindles contain striated muscle, and like extrafusal muscle, they also exhibit a SREC (Hunt & Ottoson 1976). Following fusimotor activation or muscle stretch, spontaneous cross-bridges form within the polar regions of intrafusal fibres. The presence of stable cross-bridges acts to increase the stiffness of the polar regions of the intrafusal fibres. Subsequent lengthening of the muscle will stretch the intrafusal fibres and, due to the higher stiffness of the polar regions, the central sensory region will be lengthened preferentially. The primary endings wrap around the central sensory region of the intrafusal fibres and are also preferentially stretched. This can explain the initial burst often observed in primary endings in response to muscle stretch (Brown, Goodwin & Matthews 1969; Hunt & Ottoson 1976; Morgan, Prochazka *et al.* 1984). Stretching a muscle beyond the elastic limit of the stable cross-bridges causes them to detach and effectively increases the compliance of the polar regions of the intrafusal fibres. At the conclusion of stretch, with the muscle held stationary, a number of cross-bridges reform spontaneously at that particular muscle length.

The consequences of muscle thixotropy are quite different if the muscle is passively shortened following muscle contraction or stretch. During muscle shortening the compressive forces are usually insufficient to detach the stable cross-bridges. Consequently, the intrafusal fibres fall slack, reducing the resting tension on the sensory region and causing the muscle spindle to decrease its firing rate or fall silent. In addition to the low resting tension on the sensory region, the sensitivity of the muscle spindle to subsequent lengthening can be greatly reduced. Slack incorporated into the intrafusal fibres must be taken up before muscle stretch is conveyed to the sensory region. The

incorporation of slack into intrafusal fibres depends on muscle length. At long muscle lengths the relatively high passive tension produced by the structural elements of the muscle prevent slack formation. The stimulus to 're-set' the muscle spindle and remove slack from the intrafusal fibres is fusimotor activation or muscle lengthening (Proske, Morgan & Gregory 1992).

Experimental methods have been developed to control the mechanical state of muscle spindles using muscle thixotropy. These methods typically involve the use of a conditioning contraction at either a long muscle length (Hold Long) or a short muscle length (Hold Short). Following the contraction, the relaxed muscle is held at the conditioning length before being repositioned to an intermediate test length. Hold Long muscle conditioning causes muscle spindles to fall slack, while Hold Short conditioning leaves muscle spindles in a taut state. This type of muscle conditioning has provided a convenient tool to study muscle spindle responses to various stimuli and the effects of muscle conditioning have been extensively examined in the cat (Morgan, Prochazka *et al.* 1984; Gregory, Morgan & Proske 1986; Gregory, Morgan & Proske 1988) and in human muscle spindles (Edin & Vallbo 1988; Ribot-Ciscar, Tardy-Gervet, Vedel & Roll 1991). These studies have shown that muscle spindle conditioning can alter both the resting discharge and stretch sensitivity of the muscle spindle.

Experiments carried out by Gregory *et al.* (1988) examined how muscle conditioning affected limb position sense. Using Hold Long and Hold Short muscle conditioning to alter the resting discharge of muscle spindles, they found significant differences in errors of estimation of limb position. Similarly, movement detection thresholds for elbow extension and flexion were systematically altered by the same conditioning procedures (Wise, Gregory & Proske 1996). The experiments carried out in this thesis are a continuation of this study and extend these findings to include a different type of muscle conditioning, that is, a conditioning contraction at an intermediate test length (Hold Test)

Muscle spindle after effects depend on the muscle spindle remaining in a passive state. Activation of fusimotor axons will produce active cycling of cross-bridges and thus will remove slack within the muscle spindle. Therefore, although muscle conditioning can be used to study muscle spindle function, it is unlikely to be encountered in normal daily activities. Normally we voluntarily contract our muscles to maintain posture and move limbs. When we voluntarily contract our skeletal muscles

we also co-activate the fusimotor system. Co-activation of the skeletal and fusimotor system makes the interpretation of muscle spindle signals difficult. The next section will discuss some of the experimental evidence that led to the α-γ co-activation theory and discuss some of the issues relating to muscle spindle function and proprioception during muscle contraction.

α-γ Co-activation

Since the 1950's evidence has begun to mount that skeletomotor axons and fusimotor axons, tend to fire simultaneously (Hunt & Kuffler 1951; Eldred, Granit & Merton 1953). Work on the decerebrate cat, using excitation of descending motor pathways, showed evidence that skeletomotor and fusimotor axons were co-activated. The term 'α-γ linkage' was initially used to describe this observation, although it was later replaced by 'α-γ co-activation' (Matthews 1972). An early theory suggested that α-γ co-activation might be a mechanism to prevent muscle spindles from falling silent during muscle length changes (Hunt & Kuffler 1951). Another theory, the follow-up length servo theory, suggested that voluntary movement was initiated reflexively and indirectly by fusimotor activation of muscle spindles (Eldred, Granit *et al.* 1953). However, acceleration of muscle spindle firing rates was shown to follow rather than precede electromyogram (EMG) activity (Vallbo 1971), a finding that was not consistent with the follow-up length servo theory.

It is difficult to record from identified fusimotor axons during normal human activity, therefore the behaviour of the fusimotor system has often been inferred from the response of muscle spindles during muscle contraction. Recordings made from muscle spindles during voluntary contractions have provided evidence for α-γ co-activation in humans (Hagbarth & Vallbo 1968; Hagbarth & Vallbo 1969; Vallbo 1970; Vallbo 1971; Vallbo 1974; Burke, Hagbarth, Lofstedt & Wallin 1976) and in the cat (Prochazka, Westerman & Ziccone 1977). Muscle spindle firing rates were shown to increase significantly during voluntary contractions of low forces, with isometric contractions of 5-10% of a maximum voluntary contraction (MVC) reported to produce increased firing rates in 60-70% of muscle spindles (Burke 1981). Similarly, Wilson *et al.* (1997) reported acceleration in muscle spindle firing rates with contractions of 3.2% MVC. The general findings from these studies was that an increase in the strength of an isometric voluntary contraction, with muscle length held constant, produced fusimotor-

evoked increases in the firing rate of muscle spindles originating from the receptor bearing muscle.

Although there is strong evidence for α-γ co-activation in human isometric contractions, the type of fusimotor axons that are activated is still a matter of debate. There is evidence that static and dynamic axons can be activated independently (Jansen & Matthews 1962; Murphy & Martin 1993), however, it is likely that there is considerable task specificity in the manner in which the fusimotor system is activated. Some researchers have found evidence of a tonic level of α-independent static and dynamic fusimotor activity during voluntary movements in the cat (Loeb, Hoffer & Marks 1985; Prochazka, Hulliger, Zangger & Appenteng 1985), and that fusimotor activation can occur out of phase with skeletomotor activity (Loeb & Duysens 1979).

In humans, the body of evidence is consistent with co-activation of α and static fusimotor axons (Vallbo 1974; Burke, Hagbarth & Lofstedt 1978; Burke, Hagbarth & Skuse 1978). Significant static co-activation would prevent muscle spindles falling silent during muscle contraction and maintain their firing rates during shortening, but it would also act to reduce stretch sensitivity during muscle lengthening. It is possible that dynamic fusimotor axons can be co-activated with skeletomotor axons in human subjects. Recordings from human muscle spindles during voluntary contractions showed an increase in the dynamic index of 48% of primary endings to large amplitude stretches, suggesting dynamic fusimotor activity (Kakuda & Nagaoka 1998). There is not much information for the secondary endings using the technique of microneurography but the existing data suggests that their behaviour during contraction and slow voluntary shortenings is similar to that of primary endings (Vallbo 1974). The main point to consider from these studies is that during muscle contraction muscle spindle firing rate and stretch sensitivity can be influenced by the level and type of fusimotor activity.

Implications of α-γ Co-activation

An argument against a contribution from muscle spindles to proprioception is that their responses are not unambiguously related to muscle length or the velocity of length change. Muscle spindle firing rate and stretch sensitivity can be modulated by different combinations of fusimotor activation, which is likely to vary with different motor tasks, in addition to any changes in muscle length. With many factors affecting

muscle spindle behaviour, how does the CNS extract muscle length and velocity signals required for motor control and proprioception?

Merton (1961) suggested that muscle length information could be extracted from the response of a muscle spindle under fusimotor activity by 'taking into account' the level of fusimotor activation. For instance, muscle spindle firing rate normally increases during a voluntary isometric contraction, yet we do not have an illusion of limb movement. There might be some kind of central comparison, or subtraction process, whereby the fusimotor-evoked increases in muscle spindle firing rate is subtracted from the total signal leaving only muscle length related information. Only when the muscle spindle firing rate is different to that expected for a particular contraction level would limb movement be perceived. The term 'corollary discharge' (Sperry 1950) was later used to describe this process (McCloskey 1981).

There is some experimental evidence supporting the idea that an internal model of fusimotor-evoked muscle spindle activity is subtracted from actual muscle spindle signals in the CNS. Vibration induced illusions of limb movement were decreased or completely abolished by isometric muscle contractions of increasing strength (Goodwin, McCloskey *et al.* 1972; McCloskey 1973). The decreases observed in these illusions during contraction might be, in part, due to the central subtraction of the fusimotor-evoked discharge from the total muscle spindle response. The muscle spindle firing rate, already high due to the strong contraction, would not be able to increase significantly in response to muscle vibration. The high muscle spindle firing rate would be fully 'accounted for' by the fusimotor drive, therefore no movement illusion would be present. However, the decrease in muscle spindle sensitivity brought about by muscle contraction may have also contributed to the reduced illusions (Brown, Engberg *et al.* 1967). If a subtraction mechanism exists, and is used to remove the fusimotor-evoked effects, it is unlikely to be a simple computation considering that muscle spindle behaviour is influenced by the level and type of fusimotor activity.

There is evidence that central motor commands contribute to proprioception and can modify afferent signals arising from the periphery. The sense of effort is thought to be mediated by direct projections from the motor cortex to the sensory cortex. A well-used example of the sense of effort is the perceived increase in the weight of an object as a muscle fatigues. In this situation an increase in the level of motor drive is required to maintain muscle force, consequently the perceived effort associated with the motor

task increases (McCloskey, Ebeling *et al.* 1974). A similar type of connection, although not necessarily reaching consciousness, could be used to remove fusimotor-evoked muscle spindle activity during muscle contraction.

Sensory information can also be modified or 'gated' during muscle contraction. Descending motor commands can have inhibitory effects on the ascending afferent information and alter the perception of tactile stimuli. The perceived intensity of electrical stimuli is reduced during voluntary movement and contraction (Angel & Malenka 1982; Milne, Aniss, Kay & Gandevia 1988). Increases in cutaneous sensory thresholds and alterations of somatosensory evoked potentials have also been observed during muscle contraction (Lee & White 1974; Rushton, Rothwell & Craggs 1981). In addition it has been reported that information from muscle receptors might be attenuated during muscle contraction, as indicated by a reduction in the perceived amplitude of electrically elicited twitch contractions during and immediately before muscle contraction (Collins, Cameron, Gillard & Prochazka 1998).

Muscle Spindle Responses During Movement

Muscle spindles are able to signal gross changes in muscle length during natural movements in behaving animals (Prochazka, Stephens & Wand 1979; Loeb, Hoffer & Pratt 1985). By contrast, recordings from muscle spindles in human subjects during muscle contraction have shown that their responses are poorly related to muscle length changes and are more dependent on the level of muscle contraction (Vallbo 1974; Hagbarth, Wallen & Lofstedt 1975; Burke, Hagbarth *et al.* 1978; Hulliger, Nordh & Valibo 1982). The movements used in the human experiments were much slower than the natural movements of the behaving cat and it is likely that movement velocity is a significant factor in determining the response profile of a muscle spindle during movement (Prochazka, Stephens *et al.* 1979). For movements slower than 0.2 resting lengths per second muscle spindle firing rates are thought to be dominated by fusimotor activity (Prochazka 1981).

For limb movements of moderate to fast velocities, muscle spindle firing rates are more likely to be modulated by muscle length changes. Recordings from human muscle spindles in the extensor digitorum muscle revealed that during voluntary and imposed movements of a moderate velocity (20°/s) most muscle spindles exhibited a stretch response during lengthening of the non-contracting muscle and fell silent during

shortening of the contracting muscle (al-Falahe, Nagaoka & Vallbo 1990). Under these circumstances information regarding the direction and velocity of limb movement might be gained by comparing the muscle spindle firing rates from agonist and antagonist muscles (Ribot-Ciscar & Roll 1998). In addition, there is evidence that muscle spindle signals from the antagonist muscle might be a major source of proprioceptive information. In a target matching task, vibration of the antagonist muscle, but not the agonist muscle, resulted in an undershoot of the required target (Capaday & Cooke 1981; Capaday & Cooke 1983; Bullen & Brunt 1986).

Muscle spindle firing rates are likely to be more variable during limb movements involving muscle contraction. Recording from human muscle spindles during movements of a contracting muscle (5-15% of MVC) showed that muscle spindle firing rates were higher and more irregular than when the same movements were imposed on a relaxed muscle (al-Falahe, Nagaoka *et al.* 1990). During isometric contractions small variations in muscle length and unloading effects from the shortening of neighbouring motor units are likely to cause variations in muscle spindle firing rates (Burke, Hagbarth, Lofstedt & Wallin 1976; al-Falahe, Nagaoka *et al.* 1990). Conversely, the variability in firing rate of human muscle spindles in relaxed muscle is low (Burke, Skuse & Stuart 1979).

Proprioception During Muscle Contraction

One of the two major themes in the first half of this thesis concerns the effects of muscle contraction on proprioception. As previously mentioned, during voluntary muscle contraction muscle spindle firing rates and their responses to changes in muscle length are influenced by fusimotor drive. It might be expected that proprioception is altered when the muscles acting on a particular joint are contracting, compared to when these muscles are relaxed.

Studies that have measured the ability of human subjects to detect movements imposed on a joint during isometric contraction have reported an improvement in proprioceptive acuity (Gandevia & McCloskey 1976; Colebatch & McCloskey 1987; Taylor & McCloskey 1992). However, contrary to this finding, the ability of subjects to match limb position was reported to be reduced during muscle contraction (Rymer & D'Almeida 1980). Errors in matching the position of the proximal interphalangeal joint of one index finger were larger during isometric contraction than when the muscles were relaxed, with matching errors increasing in proportion with contraction force.

Therefore, the influence of voluntary muscle contraction on proprioception remains a controversial issue.

In experiments measuring movement detection thresholds of the middle finger Gandevia (1976) found that proprioceptive acuity was variable when muscles acting on that joint were relaxed. However, when thresholds were re-measured during isometric muscle contraction there was a marked improvement in acuity. One factor that might contribute to this finding relates to the mechanical linkage between the joint and muscle that might change during muscle contraction. If there is a tight coupling between joint rotation and changes in muscle length then muscle spindles would be able to signal joint angle with more precision (Rack & Ross 1984). For some joints, the link between the joint and muscle is relatively poor, so that limb rotation will not produce appreciable changes in muscle length. For example, movement detection thresholds of the big toe, measured in the mid range of joint rotation under relaxed conditions, were shown to be much larger compared to other joints under the same conditions (Refshauge, Taylor *et al.* 1998). When thresholds were re-measured with the ankle plantarflexed, to improve the mechanical linkage, movement thresholds were much lower than before, even though the muscles remained relaxed. In these circumstances, it is likely that muscle contraction would improve the mechanical transmission of joint rotation to the muscle and therefore produce lower movement thresholds.

The effect of muscle contraction on movement detection was also measured for the elbow joint during isometric contraction of biceps muscles (Colebatch & McCloskey 1987; Taylor & McCloskey 1992). In these experiments lower thresholds were found during contraction compared to thresholds measured in a separate experiment with the elbow extensors and flexors relaxed (Hall & McCloskey 1983). Taylor & McCloskey (1992) did not consider that an improvement in the mechanical link between muscle and joint was a major factor for the elbow joint. The biomechanical arrangement of the elbow, particularly when placed in the midrange of joint rotation, is such that the muscle-tendon unit is already quite stiff. Consistent with this conclusion was their finding that detection thresholds did not improve when the contraction force was increased from 4.9N to 19.6N.

One explanation proposed to account for the improved proprioceptive acuity during contraction was that muscle contraction led to the recruitment of muscle spindles that were previously silent (Taylor & McCloskey 1992). For example, with the finger

relaxed and in a comfortable resting position, fewer than 10% of muscle spindles have a resting discharge (Vallbo 1974). Taylor & McCloskey (1992) argued that the improvement in proprioceptive acuity observed during contraction might be a consequence of an increase in the number of muscle spindles providing sensory information. However, in the Hall & McCloskey (1983) and the Gandevia (1976) experiments, movement detection thresholds were measured under relaxed conditions, with the contraction and length history of the muscle unknown. Here it is possible that slack was present in a number of the muscle spindles therefore abolishing muscle spindle activity (Gregory, Morgan *et al.* 1988). The presence of intrafusal slack may increase the variability of movement detection thresholds if steps are not taken to control the muscle history. If movement detection thresholds are measured immediately following an isometric contraction, a method to control muscle spindle history, then the acuity might be improved. Most experiments have shown a prolonged enhancement of human muscle spindle firing rates in a passive muscle after a voluntary contraction (Vallbo 1970; Wilson, Gandevia & Burke 1995). Therefore, the second major theme in this half of the thesis concerns the influence of muscle spindle after effects on proprioception.

Previous experiments measured movement detection at the elbow joint during isometric muscle contraction of the biceps muscle group to generate torque in the direction of flexion (Colebatch & McCloskey 1987; Taylor & McCloskey 1992). Proprioception during co-contraction of agonist and antagonist muscles has received less attention. Muscle co-contraction is a motor strategy used to stabilise a joint and maintain a particular position. It might be expected that under these circumstances feedback of limb movement would be useful information that would allow the CNS to make adjustments in the motor command to maintain the particular posture. In light of the information presented in this introduction, which suggests that muscle spindle activity would be influenced by fusimotor activity, how is proprioception affected during voluntary contraction of agonist and antagonist muscles?

Chapter Two

Human Movement Detection

Aims

Muscle spindle stretch sensitivity is known to be affected by the length and contraction history of the muscle in which the muscle spindle is located. In the past, systematically altering muscle length and contraction history has proven to be a convenient method to examine muscle spindle properties in experimental animals. It is now well established that muscle spindles provide an important source of proprioceptive information. Could altering the contraction history of human muscles have perceptual consequences for proprioception? To examine this question, proprioceptive acuity was assessed following conditioning manoeuvres designed to alter muscle spindle stretch sensitivity. The ability of human subjects to detect movements of the elbow joint was measured by imposing small movements to the relaxed limb and asking the subject to report the direction of movement.

Movements of a relaxed limb are seldom encountered in daily activities. We move our limbs by contracting muscles. For unfamiliar or novel tasks, such as learning a new motor skill, or performing fine manipulations requiring high dexterity, the movements used are usually small and slow, commonly braced by a balance of co-contraction of antagonist muscles. In such tasks it is likely that proprioceptive feedback from both agonist and antagonist muscles are continually monitored and adjustments are made to the motor signal using this information. The experiments described here, measured human movement detection thresholds during co-contraction of elbow flexors and extensors. The experiments aimed to determine whether proprioceptive acuity is affected by ongoing fusimotor and skeletomotor activity to both agonist and antagonist muscles.

Method

Subjects

Experiments were carried out on 11 adults (aged 22-27), all but one of whom were right handed. Subjects of both genders (six male and five female) were used, none of whom had a history of neurological disorder. Informed consent was obtained from each subject and the experiments had approval from the Monash University Standing Committee on Ethics in Human Experimentation.

Experimental Equipment

Subjects were blindfolded and sat comfortably with their right forearm firmly secured with a padded cuff attached to a lever with a vertical axis of rotation. The forearm was pronated and the elbow joint was aligned with the axis of rotation. The lever was attached to a servomotor allowing small rotations of the elbow joint in the horizontal plane only. Care was taken to remove any inadvertent movement cues that might have been provided by mechanical perturbations due to friction in the pivot or electronic noise in the servomotor. The upper arm rested on a cushioned support and remained stationary during the experiment. The arm remained free of clothing and there was nothing in contact with the skin around the elbow joint to stimulate cutaneous receptors (18cm distal and 10cm proximal from the joint). The experimental set-up is depicted in figure 2.1.

The servomotor was controlled by position feedback from an LVDT (linear variable differential transformer) that provided a length signal. Control signals were generated by a ramp generator enabling controlled ramp-and-hold length changes. The servomotor, LVDT and ramp generator were custom designed and built in the Monash University Physiology Department workshop. The ramp generator signal was low pass filtered (100Hz) to remove sharp corners in the ramp signal and the length output was monitored on an oscilloscope (Nicolet 420, Nicolet Instruments, USA). Two sequencers (Digitimer D4030 and Pulsemaster A300, WPI) were used to control signal timing, and a function generator (HP 3312A) connected to a loud speaker was used to provide an audible tone as an instruction to the subject. A proving ring strain gauge, with strain gauges cemented on the inside and outside in a full bridge configuration, was attached to the servomotor in series with the lever. Servomotor position and strain gauge output

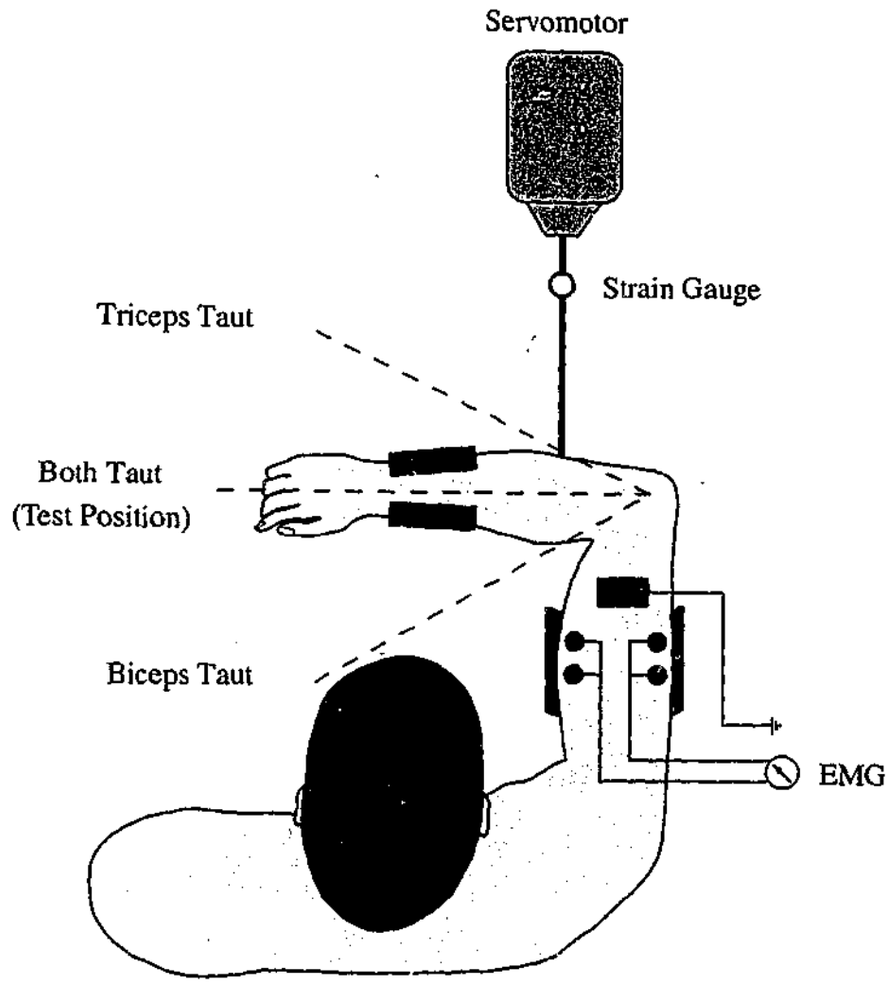


Figure 2.1. Diagram of the experimental set-up. Blindfolded subjects were seated with their right forearm attached to the lever by a cuff around the distal forearm. The upper arm rested on a padded support and two pairs of adhesive EMG recording electrodes were positioned over the biceps and triceps muscle groups. Controlled elbow rotations were provided by the servomotor that was attached to the lever. Conditioning positions are indicated. Biceps Taut and Triceps Taut were flexed and extended 30° from the Both Taut position (90°). All test movements were performed with the elbow in the test position.

were digitally recorded at 2000/s using a MacLab data acquisition system (MacLab/8s, ADInstruments, Australia) with Chart software (ADInstruments, Australia) on a PowerMac 6100/66 (Apple, USA).

Two pairs of Ag-AgCl adhesive surface electrodes (3M 'Red Dot' paediatric electrodes) were used to record EMG from the elbow extensor and flexor muscle groups. An earthed metal plate (5x3cm) coated with electrode gel was taped to the upper arm and was used as a reference electrode (see figure 2.1). The EMG signal was amplified, 100 or 1000x, and bandpass filtered (10Hz to 3kHz). EMG signals were digitally recorded at 2000Hz with the MacLab acquisition system described above and analysed offline using Igor Pro (Wavemetrics, USA). Analysis consisted of rectifying, smoothing and integrating the signal to quantify EMG activity from both biceps and triceps muscle groups.

Experimental Sequence

Movement detection thresholds were measured using four experimental conditions in three separate experimental sessions each approximately three hours in duration. In three of these conditions, thresholds were measured for the relaxed limb following 'muscle conditioning'. In the fourth condition, thresholds were measured during a maintained co-contraction of the biceps and triceps muscle groups. The experimental sequence is depicted in figure 2.2.

The sequence for movement thresholds measured for the relaxed, but conditioned arm, can be described in two parts. A muscle conditioning procedure was used to control the contraction history of biceps and triceps muscles (Triceps Taut, Biceps Taut or Both Taut). This was followed by the threshold measurement procedure in which movements in the direction of extension or flexion were imposed on the relaxed arm and movement detection thresholds were determined. In the fourth experimental condition, movement detection thresholds were measured during co-contraction of elbow extensors and flexors. The threshold measurement procedure was the same used for the relaxed, conditioned limb and is described in the following section.

Threshold Measurement Procedure

The threshold measurement procedure consisted of a number of ramp-and-hold elbow movements at a velocity of 0.2°/s. Subjects were required to detect the direction

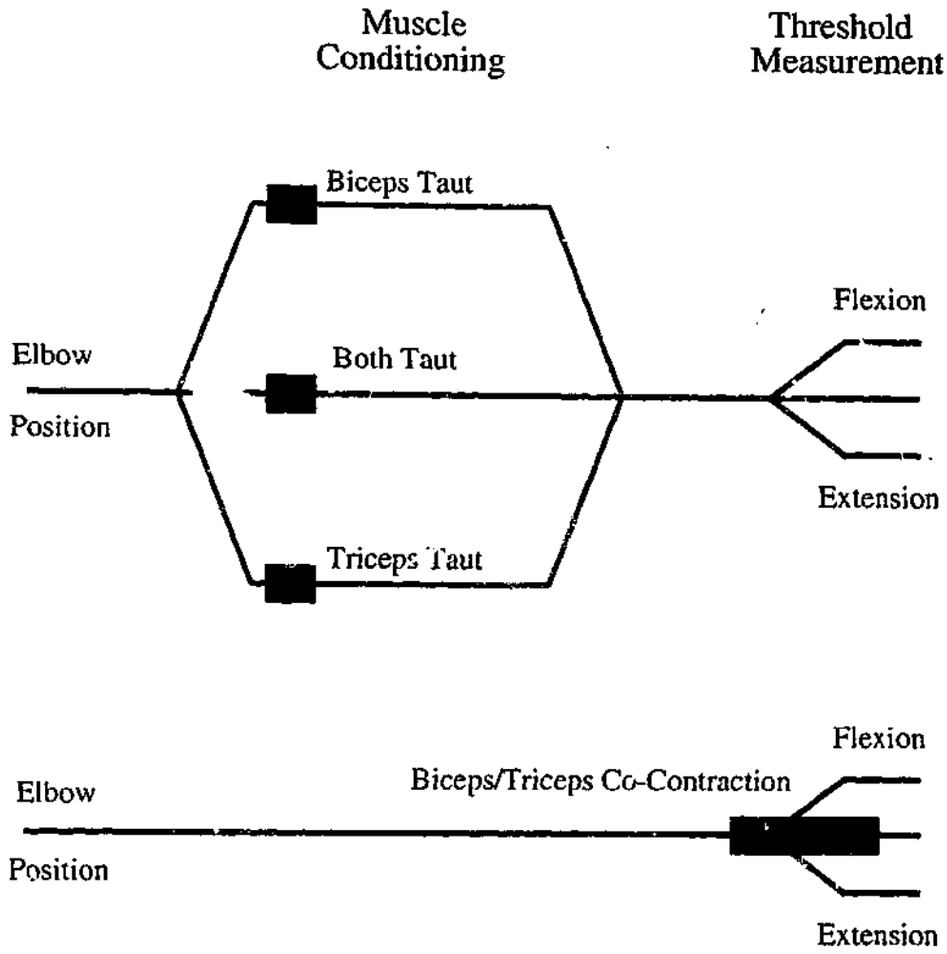


Figure 2.2. Schematic representation of muscle conditioning and movement detection threshold measurements for the relaxed elbow (*upper panel*). The forearm was placed at one of the three conditioning positions, Biceps Taut: elbow flexed 30° from test position; Both Taut: elbow at test position (90°); Triceps Taut: elbow extended 30° from test position. The subject performed a conditioning contraction of biceps and triceps muscles lasting two seconds (black rectangle) and then relaxed their biceps and triceps muscles. The elbow remained at the conditioning position for ten seconds upon which it was returned to the 90° test position by the experimenter. Movement thresholds were measured by imposing movements in the direction of extension and flexion. The subject reported the direction of the test movement. In a fourth experimental condition (*lower panel*), movement detection thresholds were measured during co-contraction of biceps and triceps muscles (black rectangle).

of the imposed movement and to respond only when they were sure of the direction. It has been argued that proprioception implies the ability to discriminate movement direction, as the conscious sensation of movement, prior to sensation of its direction, might depend on nonspecific or nonproprioceptive cues (Laidlaw & Hamiliton 1937a, 1937b; Hall & McCloskey 1983; Taylor & McCloskey 1992). Subjects were told that on some occasions they may sense movement of the elbow but not be able to ascertain the direction of the movement. In these circumstances they were instructed not to guess. Subjects were informed that movements would be both in the direction of flexion and extension and that in some trials there would be no movement (5-10% of trials). They were presented with a number of practice movements to familiarise them with the detection task and to obtain a rough estimate of their movement detection thresholds.

The method used to measure movement detection thresholds was the staircase method of limits (Cornsweet 1962) (shown in figure 2.3). The testing procedure began with an elbow movement of a magnitude that was easily detectable. The amplitude of the following movements were reduced until the subject could no longer detect the direction of the movement. At this point, called a reversal, the amplitude of the following movements were increased until the subject could again detect the direction. This process was repeated with random presentations of movements in either flexion or extension until six to eight reversal points had been measured. Movement thresholds values for elbow extension and flexion were calculated by averaging the reversal points.

Muscle Conditioning

Movement detection thresholds were measured following muscle conditioning, which consisted of placing the subject's arm at one of three different conditioning positions and the subject performing a voluntary contraction of biceps and triceps muscles lasting two seconds (see figure 2.2). The elbow remained at the conditioning position for ten seconds, after which the arm was returned to the test position by the experimenter. Subjects were instructed that after the conditioning contraction, their elbow muscles must remain completely relaxed until the conclusion of that particular trial. EMG was monitored during each trial and there was no evidence that subjects had difficulty in producing conditioning contractions or maintaining fully relaxed biceps and triceps muscles.

The muscle conditioning was designed to alter the mechanical state of muscle spindles located within biceps and triceps muscles, leaving them either slack or taut.

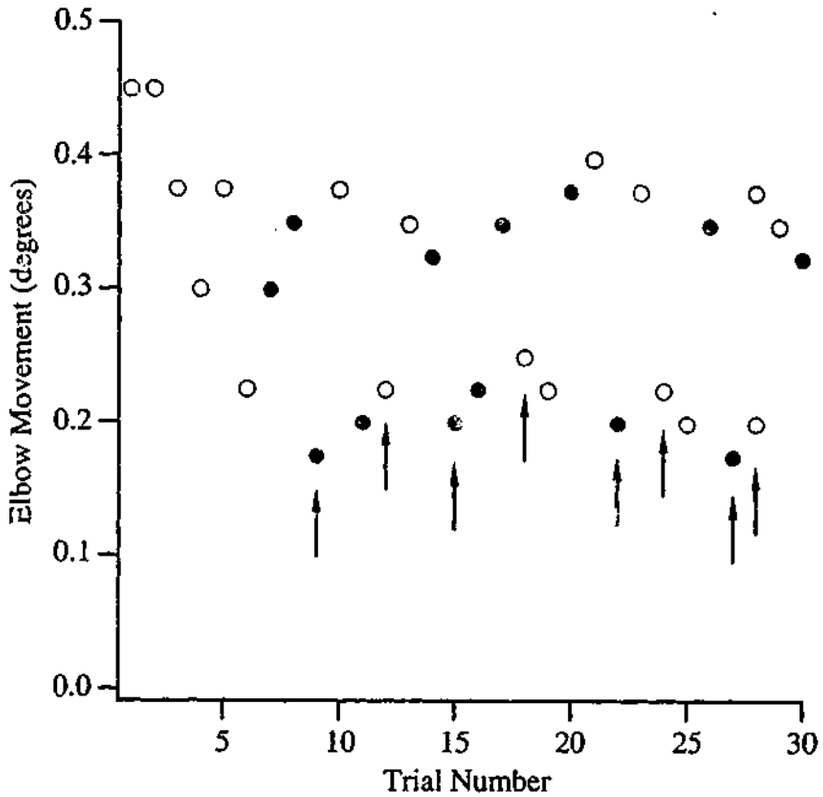


Figure 2.3. Staircase method used to measure movement detection thresholds. Movements into extension (red circles) and into flexion (blue circles) were presented randomly. Initially, movements of a large amplitude were presented. When the subject reported the direction of movement correctly (open circles) the amplitude of subsequent movements were decreased until the subject could no longer detect the movement direction (closed circles). This sequence was repeated until 6-8 reversal points (black arrows) were measured. The movement detection threshold was calculated by the average of the movement amplitudes for each of the reversal points, in this example the thresholds were 0.21° for extension and 0.35° for flexion.

When a muscle contracts at a length longer than the test length, and is then passively shortened back to the test length, slack develops in the intrafusal fibres of the muscle spindles. The hypothesis under examination here was the possibility that muscle conditioning would alter the mechanical state of muscle spindles within the biceps and triceps muscle groups and that this would effect the ability of subjects to detect elbow movements. The predicted effects of muscle conditioning on biceps and triceps muscle spindles are shown in figure 2.4.

For Biceps Taut conditioning the biceps and triceps muscles were conditioned with the elbow flexed. When the elbow was re-positioned at the intermediate test position, slack developed within muscle spindles located in triceps, whereas biceps muscle spindles remained taut. Triceps Taut conditioning had the opposite effect. Here, the triceps muscle spindles were taut and biceps muscle spindles slack. For the Both Taut muscle conditioning, there was no change in muscle length after the conditioning contraction, therefore muscle spindles in both biceps and triceps muscles were taut.

Muscle Co-Contraction

For the three experimental conditions discussed above movement detection thresholds were measured for the relaxed limb. In the fourth experimental condition detection thresholds were measured during voluntary co-contraction of biceps and triceps muscles. These experiments consisted of a training procedure followed by the experimental procedure in which movement thresholds were measured. Seven subjects participated in these experiments, all of whom had participated in the relaxed limb experiments.

The training procedure commenced with the measurement of EMG during maximal co-contractions. Subjects were instructed to produce five maximal co-contractions of biceps and triceps, each approximately five seconds in duration. EMG was measured and analysed offline to quantify the EMG during maximal co-contractions. Subjects then practised producing repeatable submaximal co-contractions (15-20% maximal EMG) of biceps and triceps using visual feedback of EMG displayed on an oscilloscope. Subjects were instructed to try to produce co-contractions that did not generate any net torque. Once subjects could generate reproducible co-contractions without visual feedback, the experimental sequence began.

Subjects were instructed to produce a co-contraction at the sound of a tone and maintain the co-contraction during the threshold measurement procedure. Movement

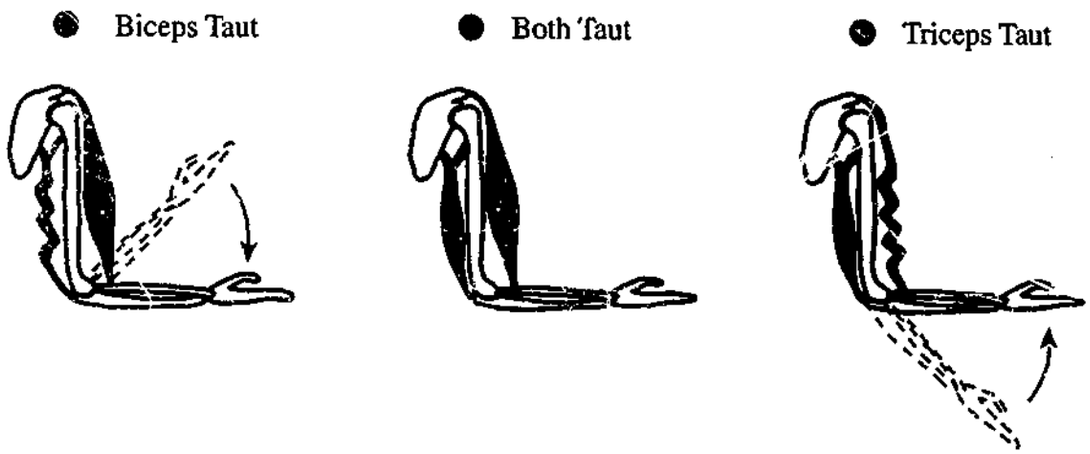


Figure 2.4. Muscle conditioning procedures used to alter the mechanical state of muscle spindles. After a conditioning isometric co-contractions of biceps and triceps with the elbow flexed by 30° (Biceps Taut), on return to the 90° test position slack developed within triceps muscle spindles but muscle spindles located in biceps remained taut and stretch sensitive. For the Both Taut condition biceps and triceps muscles were conditioned at the intermediate test position so that muscle spindles within both muscles remained taut. Conditioning with the elbow extended (Triceps Taut) introduced slack into muscle spindles within biceps while those in triceps were taut and stretch sensitive.

detection thresholds were measured as for the relaxed limb and EMG levels were monitored over the course of the experiment. An example the test procedure is shown in figure 2.5.

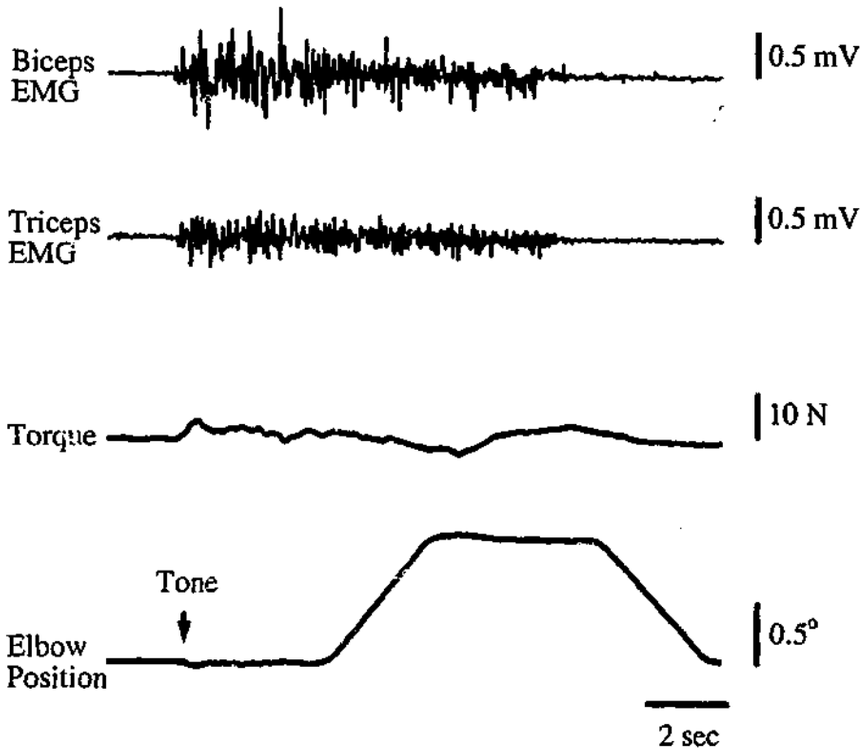


Figure 2.5. An example of the procedure used to measure movement detection thresholds during co-contraction of biceps and triceps muscles. An audible tone (0.5sec duration) was the signal for the subject to produce a co-contraction of biceps and triceps (*upper two traces*). The net torque generated by the co-contraction was measured and an upwards deflection in the trace indicates torque in the direction of elbow flexion. During the co-contraction ramp movements of the forearm were imposed by the servomotor, either in the direction of extension or flexion (*lower trace*). Subjects reported the direction of the imposed movement.

Results

Movement detection thresholds were measured for the relaxed, conditioned arm following Biceps Taut and Triceps Taut muscle conditioning. Measurements were not made on unconditioned arms since it was argued that these would have an unknown muscle contraction and length history which would result in an increased variability in the movement detection thresholds. The upper panel of figure 2.6 shows each reversal point and the movement detection thresholds measured for one subject. In this example, when movements were imposed following Biceps Taut conditioning, the detection threshold for movements in the direction of extension was lower (0.12°) than the threshold for movements into flexion (0.39°). After Triceps Taut conditioning, the threshold for flexion movements (0.22°) was lower than for elbow extensions (0.32°).

The lower panel of figure 2.6 shows the movement detection thresholds for extension plotted against the flexion thresholds for all eleven subjects. For most subjects, thresholds measured following Biceps Taut muscle conditioning produced values above the line of unity, indicating that extension thresholds were lower than flexion thresholds. Conversely, when Triceps Taut muscle condition was used, movement detection thresholds were lower for flexion for all subjects. These results indicate that movement detection thresholds were low for movements that stretched the taut muscle (see elbow diagrams in figure 2.6). That is, extension following Biceps Taut conditioning and flexion following Triceps Taut conditioning.

An example of movement detection thresholds measured for the relaxed limb following Both Taut muscle conditioning and during voluntary co-contraction of biceps and triceps for one subject (upper panel) and the movement detection thresholds for all seven subjects (lower panel), are shown in figure 2.7. For Both Taut muscle conditioning, thresholds were low for movements in either direction. For the subject shown in the upper panel of figure 2.7 the threshold for extension movements was (0.10°) and the flexion threshold was (0.13°). Using Both Taut muscle conditioning, movements in either direction would stretch a taut muscle (see elbow diagrams in figure 2.7). Movement detection thresholds measured during co-contraction of elbow flexors and extensors yielded values substantially higher than in the passive limb for flexion (0.42°) and extension (0.52°).

When comparing the movement threshold data from all seven subjects (lower panel figure 2.7), most values lie close to the line of unity indicating that extension and

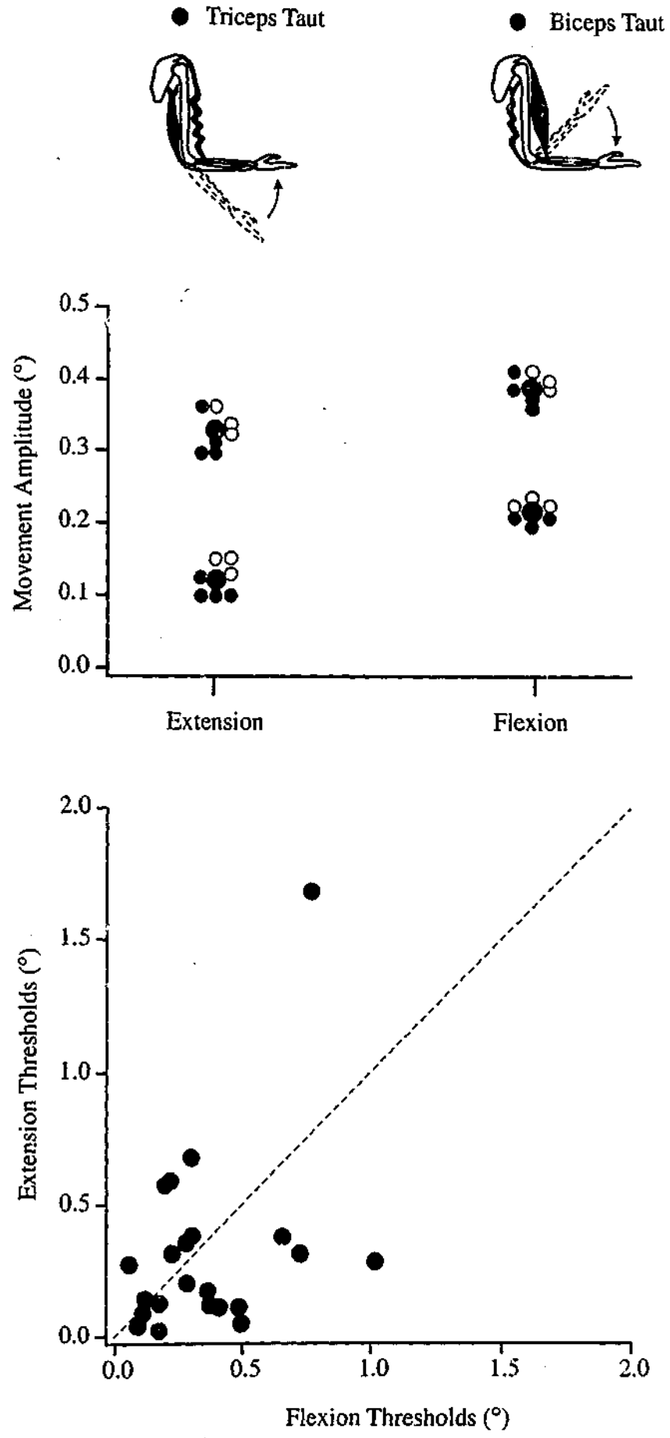


Figure 2.6. Movement detection thresholds measured following Triceps Taut (blue) and Biceps Taut (red) muscle conditioning. The elbow diagrams at the top of the figure illustrate muscle conditioning. *Upper panel* shows the data from one subject. Using the staircase method, elbow movements were imposed and a number (6-8) of reversal points were measured. The reversal points for movements that were correctly detected by the subject are shown as open circles and the reversal points for undetected movements as filled circles. The movement detection threshold represents the average of these reversal points (black ringed circles). *Lower panel* shows the movement detection thresholds measured for all eleven subjects. Extension thresholds are plotted against flexion thresholds. Flexion thresholds were lower than extension thresholds after Triceps Taut conditioning, whereas extension thresholds were lower than flexion thresholds following Biceps Taut conditioning. The dashed line indicates the line of unity.

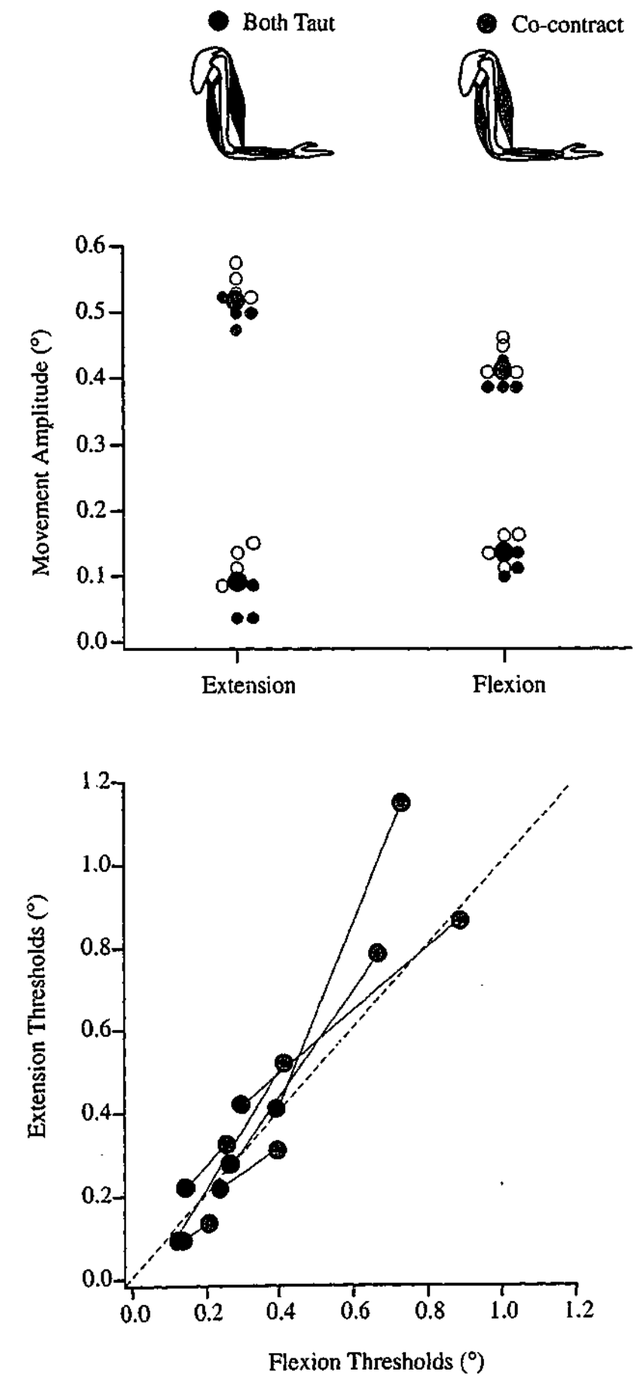


Figure 2.7. Movement detection thresholds measured for Both Taut (green) and Co-contract (orange) conditions. The elbow diagrams at the top of the figure show muscle conditioning. *Upper panel* illustrates the data from one subject showing the reversal points for correctly detected movement (open circles) and reversal points for undetected movements (filled circles). Average detection thresholds for extension and flexion (black ringed circles) are shown for each muscle condition. *Lower panel* shows the movement detection thresholds measured for all seven subjects. Extension thresholds are plotted against flexion thresholds. Flexion and extension thresholds were similar for Both Taut and Co-contract conditions, lying on or near the line or unity. However, thresholds measured for Both Taut conditioning were consistently much lower than thresholds measured during muscle co-contraction. For each subject the threshold values measured for the Both Taut and Co-contract conditions are joined by a line.

flexion movements were detected equally well. The main finding of this experiment was that movement detection thresholds were larger when measured during a co-contraction of elbow extensors and flexors than in the passive limb. In other words, subjects with relaxed, but taut elbow muscles were better able to detect small imposed movements than when they were voluntarily contracting biceps and triceps muscles.

Analysis of rectified and smoothed EMG data indicated that, on average, EMG levels during a co-contraction were 21% of the MVC in biceps and 13.5% of the MVC level in triceps, implying that the co-contractions were biased in the direction of flexion. Analysis of the torque recorded during co-contraction in these experiments confirmed this. At the onset of contraction, torque would typically overshoot in the direction of flexion and then gradually fall as the co-contraction became more even. However, torque levels typically had not fallen to zero net torque before the test movement occurred, but were still slightly biased in the direction of flexion. This lead to a small displacement of the elbow in the direction of flexion amounting to 1.5% (±2.0% SEM) of detection threshold. Additionally, during the co-contraction there were small fluctuations in the arm position. The peak-to-peak amplitude of these fluctuations in elbow position was 1.4% (±1.4% SEM) of the detection threshold. It was thought that these effects were not significant given the large change in threshold caused by muscle co-contraction.

There was a considerable range in the ability of different subjects to detect arm movements (up to a ten-fold difference in detection thresholds), therefore movement thresholds were normalised for each subject. Thresholds were expressed as the average of flexion and extension thresholds measured for the Both Taut condition and figure 2.8 shows the average normalised threshold data for all experimental conditions. When movement detection thresholds were measured during co-contraction of flexor and extensor muscles, thresholds were always high. The thresholds measured were in a similar range to the threshold values obtained with movements into extension after Triceps Taut conditioning and into flexion after Biceps Taut conditioning (where the movement stretched a slackened muscle). Movement detection thresholds measured after Both Taut conditioning were always low and were similar to thresholds measured following conditioning that stretched a taut muscle. That is, extension following Biceps Taut and flexion following Triceps Taut. Threshold averages (±SEM), in degree of elbow rotation, are shown in table 2.1.

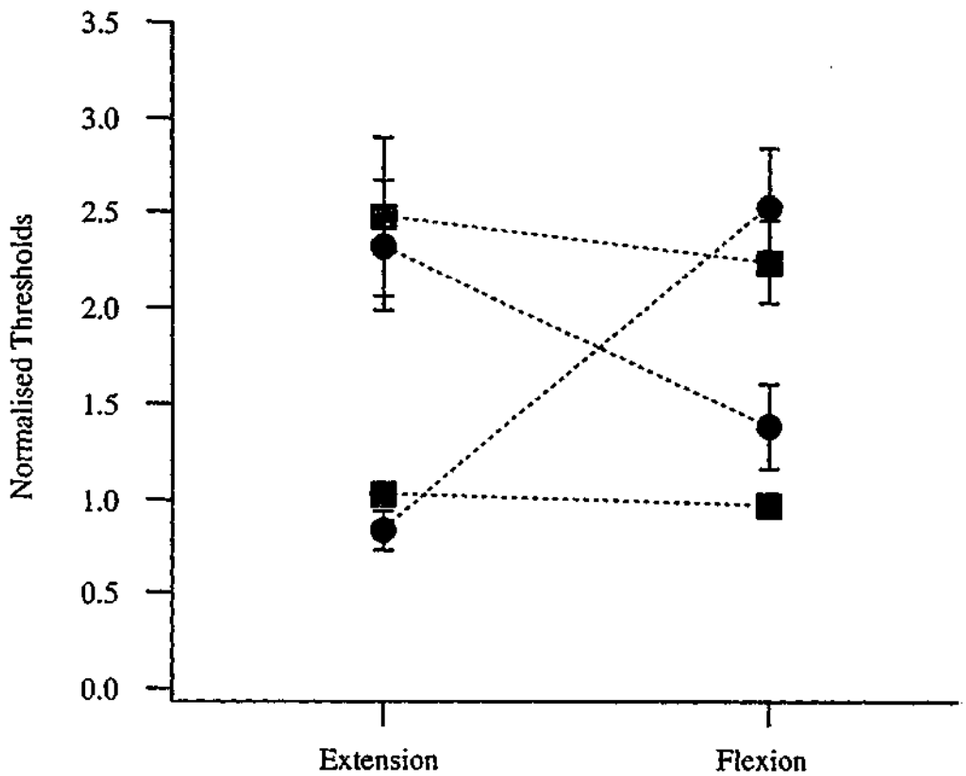


Figure 2.8. Average (±SEM) normalised movement detection thresholds for all muscle conditions and all subjects. Biceps Taut (red circle), Triceps Taut (blue circle), Both Taut (green square) and Co-contract (orange square). Thresholds for each condition and direction of movement were normalised to the average of the extension and flexion thresholds measured during Both Taut muscle conditioning.

	Extension		Flexion	
	Average (°)	SEM	Average (°)	SEM
Biceps Taut	0.16	0.04	0.46	0.08
Triceps Taut	0.47	0.13	0.25	0.06
Both Taut	0.21	0.04	0.18	0.03
Co-contract	0.58	0.14	0.51	0.10

Table 2.1. Average (±SEM) movement detection thresholds for all subjects represented in terms of angular rotation of the forearm. There was considerable range in the ability of different subjects to detect elbow movements.

Data Analysis

Statistical analysis of the raw data using a repeated measures ANOVA (n=11) indicated a significant interaction between movement direction (extension and flexion) and muscle conditioning (Biceps Taut, Triceps Taut and Both Taut) ($p<0.001$). Post hoc analysis (LSD) showed that extension thresholds after Biceps Taut and Both Taut conditioning were significantly lower than when Triceps Taut conditioning was used ($p<0.01$). For movements into flexion, thresholds after Triceps Taut and Both Taut conditioning were significantly lower than when Biceps Taut conditioning was used ($p<0.01$).

A repeated measures ANOVA (n=7) revealed that thresholds measured during co-contraction of elbow extensors and flexors were significantly higher than those obtained following Both Taut conditioning ($p<0.01$). Post hoc analysis (LSD) showed that thresholds for the Co-contract condition were no different to those obtained for extension following Triceps Taut and flexion following Biceps Taut conditioning. However, as expected, Co-contract thresholds were significantly higher than those following Biceps Taut extension ($p<0.01$) and Triceps Taut flexion ($p<0.01$).

The number of errors made by each subject during the experiments was monitored. An error was recorded when a subject incorrectly reported the direction of the imposed movement or reported directional displacement to trials in which no movement was imposed. The average error for movements in the direction of flexion

was 6.6% and for extension movements 5.5% of the total number of trials. There was no change in the average number of errors for each of the four conditions used.

To explore the possibility that producing a co-contraction may have distracted the subjects from the movement detection task a control experiment was carried out on three subjects. In these experiments subjects were required to produce a co-contraction of the biceps and triceps muscles in the left (non-test) arm, while movement detection thresholds were measured on the relaxed right arm that had been conditioned with Both Taut. The average of the extension and flexion thresholds for subjects co-contracting the contralateral arm was 0.25° (±0.05 SEM), whereas, when the standard Both Taut condition was used, with both arm relaxed, the average was 0.23° (±0.03 SEM). These differences were not significant (repeated measures t-test).

Discussion

Detection thresholds for slow elbow movements were measured following muscle conditioning at different elbow angles. When the elbow was conditioned while extended (Triceps Taut), thresholds were low for movements into flexion, whereas, after conditioning in the flexed position (Biceps Taut), thresholds were low for extension. It is proposed that these two forms of muscle conditioning altered the mechanical state of muscle spindles located within biceps and triceps muscles. These results provide additional evidence in support of the case for a role of muscle receptors in proprioception.

Two additional findings were reported. When the biceps and triceps muscles were conditioned with the elbow positioned at the test length (Both Taut), detection thresholds were low and movements into extension and flexion were detected equally well. However, detection thresholds measured during co-contraction of the elbow muscles were significantly higher compared to thresholds measured for Both Taut conditioning. In other words, proprioceptive acuity was superior for the relaxed, appropriately conditioned limb, compared to acuity during co-contraction of elbow extensors and flexors.

Muscle Contraction History and Movement Detection

Muscle conditioning left muscle spindles in one of two extreme states, taut and stretch sensitive, or slack and insensitive. Detection thresholds were low for movements that stretched taut muscle spindles but were high when the movement stretched slackened muscle spindles. It is proposed that the mechanism responsible for changes in movement detection thresholds is a change in muscle spindle sensitivity mediated by contraction history effects.

Muscle thixotropy is a property of both extrafusal and intrafusal muscle and because of the complicated links between intrafusal fibres and their connective tissue attachments, muscle spindles show rather pronounced thixotropic effects. Following a conditioning contraction at a short length and stretch of the muscle to an intermediate test length, a number of cross-bridges in the striated polar regions of intrafusal fibres will spontaneously form at the test length (Hill 1968). This provides the polar regions of the intrafusal fibres with a relatively high stiffness. In this situation, muscle spindles are taut and highly sensitive to muscle stretch. They are also likely to have a high level of background activity (Wilson, Gandevia & Burke 1997). Lengthening the muscle will

preferentially stretch the sensory region of the intrafusal fibres, producing an increase in muscle spindle firing rate. This was evident in the proprioception experiments, as movement detection thresholds were low when the direction of movement stretched the muscle containing taut, sensitive muscle spindles.

If a muscle is passively shortened following a contraction, the intrafusal fibres may fall slack. The stable cross-bridges formed after the conditioning contraction remain attached at the longer sarcomere length (Herbst 1976; Lakie, Walsh & Wright 1984; Morgan, Prochazka *et al.* 1984), as the compressive forces on shortening are insufficient to detach any cross-bridges. Therefore, sarcomeres are unable to passively shorten and slack is incorporated into the intrafusal fibres (Proske, Morgan *et al.* 1993). The shorter fibre length is achieved by shortening of the sensory region and the compliant attachment points at the ends of the intrafusal fibres, where they anchor to the extrafusal endomyosin, with some intrafusal fibres actually kinking (Boyd & Ward 1975). Consequently, the tension on the sensory region is low and the muscle spindles typically fall silent. In addition, the presence of slack within intrafusal fibres means that the muscle spindles are relatively insensitive to subsequent movements, as the slack must first be removed before the full sensitivity of the muscle spindle is restored (Gregory, Morgan *et al.* 1988).

The effect of contraction history alterations on the stretch sensitivity of muscle spindles is the only satisfactory explanation for the observed changes in movement detection thresholds. It is unlikely that the sensitivity of other receptors involved in proprioception would be affected in such a way by contraction history. That is, the muscle history dependence of thresholds demonstrated here is a property unique to muscle and therefore points unambiguously to muscle spindle as the source of the alteration in proprioceptive acuity.

To extend this concept, if the limb is conditioned by an isometric contraction at the test position, cross-bridges would reform at the test length in both muscles, so in theory, muscle spindles in both triceps and biceps would be taut and stretch sensitive. Therefore, movements of the elbow joint in either direction would stretch taut muscle spindles and movement detection thresholds should be low for both extension and flexion. The results for Both Taut conditioning were consistent with this explanation, as thresholds were equally low for both extension and flexion.

Proprioceptive Signals from the Shortening Muscle

An additional factor to consider with Both Taut muscle conditioning is the potential contribution from muscle spindles within the shortening muscle. Muscle spindles within both biceps and triceps muscles would be expected to have a maintained discharge after the Both Taut conditioning contraction. Therefore, movement of the elbow joint would cause an increase in the firing rate of muscle spindles stretched by the movement, whereas muscle spindles in the shortening muscle would be expected to show a fall in firing rate. Therefore, the CNS has two potential sources of information, the decrease in muscle spindle firing rate from the shortening muscle and an increase from the lengthening muscle. This source of information would not be available when muscle spindles of one muscle were silent because their intrafusal fibres were slack (i.e. Biceps Taut and Triceps Taut).

Movement detection thresholds measured for Both Taut conditioning were not significantly different from thresholds measured with Biceps Taut or Triceps Taut conditioning in which muscle spindles in the muscle shortened by the movement lay slack. This suggests that with Both Taut conditioning, a decrease in firing rate from muscle spindles located within the shortening muscle was not a significant cue for movement detection. This implies that movement information coming from a relaxed limb arises predominantly from the muscles undergoing stretch. However, this conclusion is based on the assumption that the stretch sensitivity of taut muscle spindles, after Biceps Taut and Triceps Taut conditioning, is the same as that after Both Taut conditioning. It would be expected that for each form of conditioning, muscle spindles located in the taut muscle should be stretch sensitive and therefore their response to slow muscle stretches should be the same. This issue will be discussed in chapter 3.

Muscle Co-Contraction and Movement Detection

Movement detection thresholds were measured during a voluntary co-contraction of biceps and triceps muscles. A co-contraction was chosen because it is likely to occur more regularly in daily activity than passive movement of a relaxed limb and therefore has more physiological significance. A co-contraction of elbow extensors and flexors is also a strategy used by the CNS to stabilise the elbow joint and maintain a particular posture. Presumably, proprioceptive feedback provides important information in such a task, allowing the CNS to monitor and control limb position and movement.

A significant finding in these experiments was the increase in movement detection thresholds observed during co-contraction of elbow extensors and flexors when compared to thresholds measured for the relaxed limb following Both Taut conditioning (figure 2.7). The average threshold for extension (0.58°) and flexion (0.51°) measured during co-contraction was almost three times higher than the average threshold measured for extension (0.21°) and flexion (0.18°) of the relaxed limb following Both Taut conditioning. A major difference between the Co-contract condition and the relaxed Both Taut condition is α-γ co-activation that occurs during muscle contraction. Could factors associated with the co-activation of the fusimotor system contribute to the reduced proprioceptive acuity observed during co-contraction?

There is evidence for co-activation of the fusimotor and skeletomotor systems during voluntary muscle contractions producing as little as 3% of MVC (Wilson, Gandevia *et al.* 1997). The voluntary co-contractions of biceps and triceps used in the experiments in this thesis produced EMG levels approximately 13.5% to 21% of their maximum. It is reasonable to assume that there was significant α-γ co-activation in both elbow extensors and flexors. Any pre-existing slack would have been removed, therefore, the existence of slack in intrafusal fibres cannot be used to explain the higher thresholds observed during co-contraction.

There are two relevant issues in relation to α-γ co-activation during measurements of human movement detection thresholds. Firstly, how does the CNS extract movement related signals from a muscle spindle that is under fusimotor control? Secondly, what is the influence of fusimotor activity on muscle spindle sensitivity?

Signals arising from muscle spindles within a contracting muscle are not only modulated by changes in muscle length by also by fusimotor activation. One theory suggests that the CNS may extract muscle length information by cancelling out the fusimotor-evoked response from the total signal using an efferent copy (McCloskey 1981). According to this theory a subtraction process can account for any change in muscle spindle discharge produced by fusimotor activity, with only movement-related responses reaching consciousness. However, the subtraction process required would need to be quite complex and would need to take into account not only the level of fusimotor activation, but also the type of fusimotor axon activated. Consequently, the subtraction process might be relatively inefficient in computing movement information leading to the increase in movement thresholds observed during muscle contraction. The situation is different for relaxed muscles as there is no confounding influence from

fusimotor activity, so any change in muscle spindle firing rate can be attributed to changes in muscle length. Therefore, movement signals arising from a relaxed, taut muscle spindle might be easier to interpret than signals from a muscle spindle under fusimotor control.

Another factor that might have contributed to the increased movement detection thresholds seen during co-contraction is the contribution from the agonist and antagonist muscle spindles. There is evidence that the human subjects compare sensory information from agonist and antagonist muscles when making decisions about the direction of a movement. Studies examining the effects of vibration of agonist and antagonist muscles showed that illusions of movement were prevalent only when there was a mismatch in the vibration-evoked increase in muscle spindle firing rate (Gilhodes, Roll *et al.* 1986). It was suggested that movement direction and velocity could be derived by comparing the difference in firing rate of muscle spindles in the agonist and antagonist muscles (Ribot-Ciscar & Roll 1998).

In the experiments in this thesis the voluntary co-contraction of biceps and triceps meant that there was likely to be ongoing fusimotor-evoked muscle spindle activity in both muscles. Supporting this suggestion are the findings of Nielsen (1994) that reported increased firing rates of muscle spindles located in antagonist and agonist muscles during co-contraction. If comparisons of signals arising from elbow flexors and extensors are used to determine the direction of elbow movement then co-contraction of these muscles might have caused a problem for this strategy. The CNS might use an increase in firing rate from muscle spindles in one muscle group in combination with a decrease in rate, or complete silence, from muscle spindles in the other muscle group to obtain an unambiguous indication of movement direction. It is likely that ongoing fusimotor activity combined with the slow velocity of the imposed movement meant that the firing rate of muscle spindles within the muscle shortened by the movement did not decrease significantly. In other words, the muscle spindle firing rate might be maintained at a relatively high level by the fusimotor activity and be relatively insensitive to muscle shortening. The velocity of movements used in the human movement detection experiments was approximately 0.001 resting length/s. Recordings from muscle spindles during fusimotor stimulation have shown that firing rate is dominated by fusimotor activation during slow muscle shortening (<0.2 resting length/s) (Lennerstrand & Thoden 1968; Prochazka, Stephens *et al.* 1979). Therefore, it

is likely that as a result of fusimotor activity the firing rate of muscle spindles in the shortening muscle would not fall appreciably.

A maintained firing rate from muscle spindles in the shortening muscle was not a factor for movement detection of the relaxed limb following Both Taut conditioning. In the Both Taut conditioned experiments, muscle spindles in both muscles were expected to be taut, and have a resting discharge but no α-γ co-activation. When movement is imposed, muscle spindles in the agonist will decrease their rate in response to muscle shortening (Crowe & Matthews 1964b; Hagbarth & Vallbo 1968), and those in the antagonist will increase when lengthened. The combination of an increase and decrease from agonist and antagonist muscle spindles could explain the smaller thresholds observed here.

Central Attenuation of Proprioceptive Signals

Transmission of sensory information may be modified by motor activity. This phenomenon is known as 'gating' and can occur at many different levels in the sensory pathway. Attenuation of electrical potentials measured from the somatosensory region of the cortex was observed during voluntary movement (Tapia, Cohen & Starr 1987). There is evidence that somatosensory gating occurs before movement onset, which implies an action at premotor levels within the cerebral cortex, or cortical pathways which inhibit afferent input at the level of the thalamus (Coulter 1974; Starr & Cohen 1985). In experiments examining the perception of electrically evoked muscle twitches in human subjects it was found that the perceived twitch amplitude was reduced during voluntary movements compared to control perceptions (Collins, Cameron *et al.* 1998). There was a reduction in the perceived amplitude immediately (up to 100ms) before movement and also a reduction evoked by stretch of skin on the dorsum of the hand. The authors concluded that during movement there was a general attenuation of sensory information. Perception of a muscle twitch is quite different, of course, to the detection of imposed movements but it is possible that similar gating mechanisms contributed to the increased movement threshold observed during co-contractions.

It is possible that the requirement for subjects to maintain a co-contraction of biceps and triceps muscles during movement detection trials provided a distraction. Subjects may have not been fully attentive to detecting imposed movements, but were more concerned with maintaining the required level of co-contraction. This possibility was examined for three subjects in which they were required to produce co-contractions

of one arm (left arm) while thresholds were being measured with the other arm (right) which had been conditioned with Both Taut conditioning. There was no difference in thresholds measured in these experiments with those for the standard Both Taut condition. This result suggested that failure of the subjects to pay attention to the movement detection task during co-contraction was not a significant factor for the observed increase in detection thresholds observed during co-contraction. It implies that the mechanisms responsible for the higher thresholds was associated with co-contraction of the test arm.

Comparison with Previous Studies

Two previous studies have measured proprioceptive acuity at the elbow joint during muscle contraction (Colebatch & McCloskey 1987; Taylor & McCloskey 1992). These studies reported lower movement detection thresholds during contraction of elbow flexors, when compared to the relaxed limb, measured in a separate series of experiments (Hall & McCloskey 1983). There are several possible explanations why these studies found seemingly different results to those reported here. Firstly, it is necessary to consider the movement thresholds measured for the relaxed limb by Hall & McCloskey (1983).

In the experiments carried out in this thesis movement thresholds measured for the relaxed limb, following Both Taut conditioning, were approximately 0.2°. This value is much lower than the thresholds measured for the relaxed elbow (approximately 1.6°) reported by Hall & McCloskey (1983). A number of factors could contribute to this difference. Firstly, the measurements of Hall & McCloskey (1983) were made without controlling for the contraction history of the elbow extensors and flexors. In the experiments in this thesis, detection thresholds were higher after conditioning designed to slacken muscle spindles. Therefore, it is possible that the relatively high detection thresholds measured in the passive limb by Hall & McCloskey (1983) were, in part, due to the presence of some slack within muscle spindles of the extensors and flexors. If slack was present, with all other factors being equal, thresholds might be expected to lie somewhere within the 0.2° to 0.6° range. However, even taking into consideration the presence of slack, the thresholds measured by Hall & McCloskey (1983) were still outside the range found here.

Another factor that should be considered when comparing threshold data between different experiments is acceleration of the limb from rest. In designing the

protocol for these experiments it became apparent that if ramp onset was sudden, even though the same constant velocity was reached, subjects were able to detect smaller movements than if there was a more gradual onset of the movement. In these experiments the ramp signal was filtered to produce a gradual increase in movement velocity. Therefore, it is possible that there were differences in the ramp acceleration responses for the two sets of experiments. The effect of limb acceleration and the perceptual implications of varying this factor deserve closer examination in future experiments.

Taylor & McCloskey (1992) and Colebatch & McCloskey (1987) measured movement detection thresholds measured during contraction of elbow flexors and found values of approximately 0.1°. This is in contrast to the movement detection thresholds measured during co-contraction that were found to range from 0.5° to 0.6°. A significant difference between these experiments is the use of a co-contraction compared to an active flexion. In the experiments using an active flexion, one might expect significant fusimotor drive only to the contracting muscle, with the elbow extensors presumably inactive. Indeed, the findings of Taylor & McCloskey (1992) that thresholds for extension were significantly lower than for flexion supports the idea that proprioceptive signals from muscles undergoing a co-contraction or an active flexion are not the same. Colebatch & McCloskey (1987) made an interesting observation; for two subjects they reported that the ability to detect elbow movement was markedly reduced if they produced a co-contraction of elbow extensors and flexors. This finding implies that activation of the antagonist muscle during co-contraction in some way decreased the ability of the subject to detect limb movement.

Peripheral Mechanisms

Some subjects in this study reported that they felt there was more 'noise in the system' during muscle co-contraction. It is possible that small changes in muscle length as a result of contraction and muscle tremor may have increased the variability in afferent discharge. Analysis of limb position immediately before the onset of the imposed movement showed that the arm was not quite steady and there was a peak-to-peak fluctuation in limb position of 1.4% (±1.0 SEM) of detection threshold. While it was felt that this variation in muscle length would not significantly affect detection thresholds, it may have contributed to the 'noisiness' of the system.

A source of cutaneous input could have come from skin receptors responding to directional pressure from the cuff around the forearm. The cuff was well padded and fitted tightly around the wrist to provide a constant pressure dispersed over a wide area. It was felt that this arrangement would minimise any possible cues from skin receptors, particularly with the slow movement velocity used. However, a contribution from these receptors cannot be ruled out as changes in the ensemble firing rates from a number of skin receptor may provide information on the direction of limb movement. Although the sensitivity of cutaneous receptors is not affected by muscle contraction it is possible that central gating of ensemble discharges from these receptors could have occurred during muscle contraction. Taylor & McCloskey (1992) found that anaesthetising the skin around the wrist did not alter movement detection thresholds providing evidence that contraction mediated changes to signals arising from cutaneous receptors do not affect proprioceptive thresholds. However, Taylor & McCloskey (1992) used an ischaemic block to induce skin anaesthesia. This procedure can cause localised pain and discomfort and therefore change the balance of proprioceptive information between skin and muscle receptors. A more convincing control would be to apply a local anaesthesia to the skin and remeasure proprioceptive thresholds.

Muscle contraction may have increased the joint capsular tension and therefore altered the contribution from joint receptors, possibly increasing the angular range over which they responded (Millar 1973). However, other studies have reported no effect of increased muscle tone on the midrange responsiveness of joint receptors (Baxendale & Ferrell 1983). Therefore, it was felt that changes in joint receptor firing rate was not likely to be responsible for the increase thresholds found during co-contraction. Tendon organs would be relatively unresponsive to movements of the relaxed limb but would increase their firing rate in response to voluntary contraction. Tendon organs signal muscle tension and it is difficult to implicate them with the increase in movement detection thresholds observed during co-contraction.

Of all the peripheral receptors involved in movement and position sense muscle spindles are the most likely candidates to be affected by muscle contraction. Muscle spindle stretch sensitivity is known to change during α-γ co-activation. Since the initial observations of fusimotor effects on muscle spindle properties were made it has been reported that the stretch sensitivity of muscle spindles can be decreased during fusimotor stimulation (Crowe & Matthews 1964a). It is possible that, for the small, slow muscle length changes used in these experiments, there was an attenuation of the muscle spindle stretch sensitivity as a direct result of α-γ co-activation. Therefore, a

reduction in the stretch sensitivity of muscle spindles may have contributed to the higher thresholds observed during co-contraction. This possibility was explored in animal experiments described in the next chapter.

Chapter Three

Muscle Spindle Stretch Response

Aims

In human proprioception experiments, after appropriately conditioning elbow extensors and flexors, subjects were able to detect slow movements of their elbow joint as small as one fifth of a degree. Muscle conditioning altered proprioceptive acuity, with the results suggesting that little improvement was obtained when information from the shortening muscle was made available to the CNS after the removal of slack. Significantly, movement detection thresholds were found to increase during co-contraction of the elbow flexors and extensors when compared to the appropriately conditioned relaxed limb. The conclusions drawn from these experiments included a number of assumptions about the effect of muscle conditioning on the stretch sensitivity of muscle spindles located in biceps and triceps muscles. In addition, it was proposed that a possible mechanism for the increase in movement detection thresholds seen during muscle contraction was a reduced stretch sensitivity of muscle spindles brought about by α-γ co-activation.

In the following series of experiments, direct measurements were made from cat muscle spindles in order to explore these issues. These experiments aimed to answer a number of questions. Are muscle spindles indeed sensitive enough to respond to the small, slow movements used in the human experiments? What is the effect of muscle conditioning on muscle spindle sensitivity? Finally, what is the effect of fusimotor activation on the stretch response of muscle spindles? In the following experiments the stretch sensitivity of muscle spindles was measured after muscle conditioning and also under combinations of fusimotor and skeletomotor stimulation, conditions designed to simulate those in the human experiments. The aim, therefore, of this series of parallel animal experiments was to confirm by direct recordings, some of the proposals made in the proprioception experiments.

Method

Cat Dissection

The experiments were carried out on a total of 14 cats of both sexes weighing between 4kg and 7.9kg. The animals were housed in the Physiology Animal House at Monash University and all experiments were performed with approval from the Monash University Standing Committee on Ethics in Animal Experimentation.

The cats were anaesthetised with an intraperitoneal injection of sodium pentobarbitone (40mg/kg, Nembutal, Boehringer Ingelheim, Australia). The right cephalic vein was cannulated and supplementary doses of 0.5ml to 1ml of anaesthetic (diluted 5x in physiological saline to 12.5mg/ml) were administered through the cannula as required. The trachea was intubated with a glass tube and expired CO_2 levels were monitored with an infrared capnograph (Normocap, Datex, Finland). Body temperature was measured with a rectal thermometer and was maintained at 38±1°C with a feedback-controlled electric blanket. End tidal CO_2 levels and the presence of pinna, toe-pinch and corneal reflexes determined the depth of anaesthesia.

The animal was placed on a metal base plate and fixed into position with a clamp to support the head and a pair of hip pins inserted into the iliac crest. The ankle and knee were fixed to the base plate and a laminectomy was carried out to expose spinal roots L6-S2. An incision was made along the midline of the back and several back muscles (dorsi communis, longissimus dorsi medialis and multifidus spinae) were removed to expose the dorsal vertebral column. The spinal segments L7 to L4 were removed with bone rongeurs and the spinous process L3 was clamped with a crossbar supported by uprights fixed to the base plate to ensure stability of the spinal cord. Skin flaps were fashioned into a bath in which warmed (37°C), and oxygenated (95% O_2 and 5% CO_2), mineral paraffin was poured. An incision in the dura allowed the spinal roots to be exposed and cut at their point of entry into the spinal cord. The L7 and S1 dorsal and ventral spinal roots were set aside for later dissection.

Experiments were performed on the soleus muscle of the left hind-limb. An incision was made at the popliteal fossa and extended to the heel exposing the biceps femoris muscle that was subsequently deflected to allow access to the underlying triceps surae. The popliteal fat was removed and the sural, common peroneal and tibial nerves were cut. The hip was extensively denervated and all other nerves were cut leaving intact only the soleus nerve. This involved exposing the sciatic nerve to the level of the

hip by deflecting the gluteus maximus and caudofemoralis muscles and by removing the pyriformis muscle. Nerve branches to these muscles including gluteus superior and gluteus inferior as well as the pudendal nerve and nerves to tail muscles were then cut or crushed.

Maximal physiological muscle length (L_{max}) was measured by referencing a mark placed on the tibial bone to a marker placed on the muscle tendon during full ankle dorsiflexion. For all experiments the test length used was L_{max}–10mm. Medial gastrocnemius, lateral gastrocnemius and plantaris muscles were removed leaving the soleus tendon attached to the calcaneum. The calcaneum was detached from the ankle and a small hole was drilled through the bone. A threaded rod was passed through the hole and the calcaneum was clamped between a pair of nuts and washers. The rod was attached to the electromagnetic muscle stretcher in series with a U-type tension transducer, with two semiconductor strain gauges attached that formed one half of a Wheatstone bridge. The compliance of the system was 5µm/N. Muscle length and tension were amplified and recorded digitally on a MacLab 8/s data acquisition system using Chart software (ADI, Sydney Australia) on a power Macintosh 6100/66 or G3 computer (Apple, USA). Event timing was controlled with two sequencers (D4030, Digitimer Ltd. and Pulsemaster, A300, WPI).

Dorsal and ventral roots were viewed through a microscope (M8, Wild, Switzerland) and were dissected on a glass plate with a black backing. Watchmakers' forceps were used to tease apart the dorsal and ventral roots until a filament contained a functionally single afferent or fusimotor axon. A stimulating electrode on the soleus nerve enabled identification of the origin of the axons.

Afferent Recording

Monophasic recordings of action potentials from muscle afferents were made by placing split dorsal root filaments containing functionally single afferents on one pole of a platinum bipolar recording electrode. A section of dorsal root filament (that did not contain functioning afferent axons) was placed on the other pole of the recording electrode to act as a reference. The input lead was shielded and the neural signals pre-amplified 1,000x and bandpass filtered (80-3,000Hz). Action potentials were identified with a window discriminator (Bak Electronics, USA) using spike width and height as selection criteria. Action potentials were converted to transistor-transistor logic pulses and then displayed as instantaneous rate by a custom designed ratemeter (Monash

University, Physiology Workshop). The output of the ratemeter was viewed on an oscilloscope (Nicolet 420, Nicolet Instruments, USA) and recorded digitally at 2000/s on the MacLab acquisition system. Once stored, data were analysed off-line using the software package Igor Pro (WaveMetrics, Lake Oswego, Oregon, USA) and custom designed analysis procedures.

Muscle afferents were identified based on their response to ramp lengthening of the muscle, their response to muscle twitch contractions and their conduction velocity. Conduction velocity was measured by stimulating the leg nerve and recording the delay at the level of the dorsal root. Afferents were classified as muscle spindles if they decreased their firing rate as tension rose and increased their firing rate during relaxation of a muscle twitch contraction. Measurement of conduction velocity enabled muscle spindle afferents to be categorised as primary endings (greater than 72m/s) or secondary endings (less than 72m/s). Muscle afferents were categorised as tendon organs if they increased their firing rate during a twitch contraction and had conduction velocities in the Group I range. In all experiments only muscle spindle primary endings were studied.

Efferent Stimulation

Platinum bipolar stimulating electrodes were used to deliver square wave pulses of 0.1ms duration to ventral roots and the peripheral nerve. Pulses were constant voltage (0.2 to 5V), isolated through a radio-frequency transformer (Stimulus Isolation Unit SIU5, Grass Instrument, USA) and were adjusted to be supramaximal. Stimulation was delivered to both skeletomotor axons and functionally single fusimotor axons. Fusimotor axons were isolated in filaments of ventral root and were identified by the excitatory effect of their stimulation on muscle spindle primary endings in the absence of extrafusal tension. They were classified as static or dynamic by their effect on the response of the muscle spindle to a ramp-and-hold muscle stretch of 5mm at 10mm/s (Emonet-Denand, Laporte *et al.* 1977) (see figure 3.1). Fusimotor axons were classified as dynamic if there was an increase in the 'dynamic index' during stimulation. The 'dynamic index' is a measure of the difference between the firing rate just before the completion of the dynamic phase of stretch and the firing rate at the final muscle length, 0.5sec after the completion of the dynamic phase of stretch (Crowe & Matthews 1964a). The fusimotor axon was defined as static if there was an increase in the static length

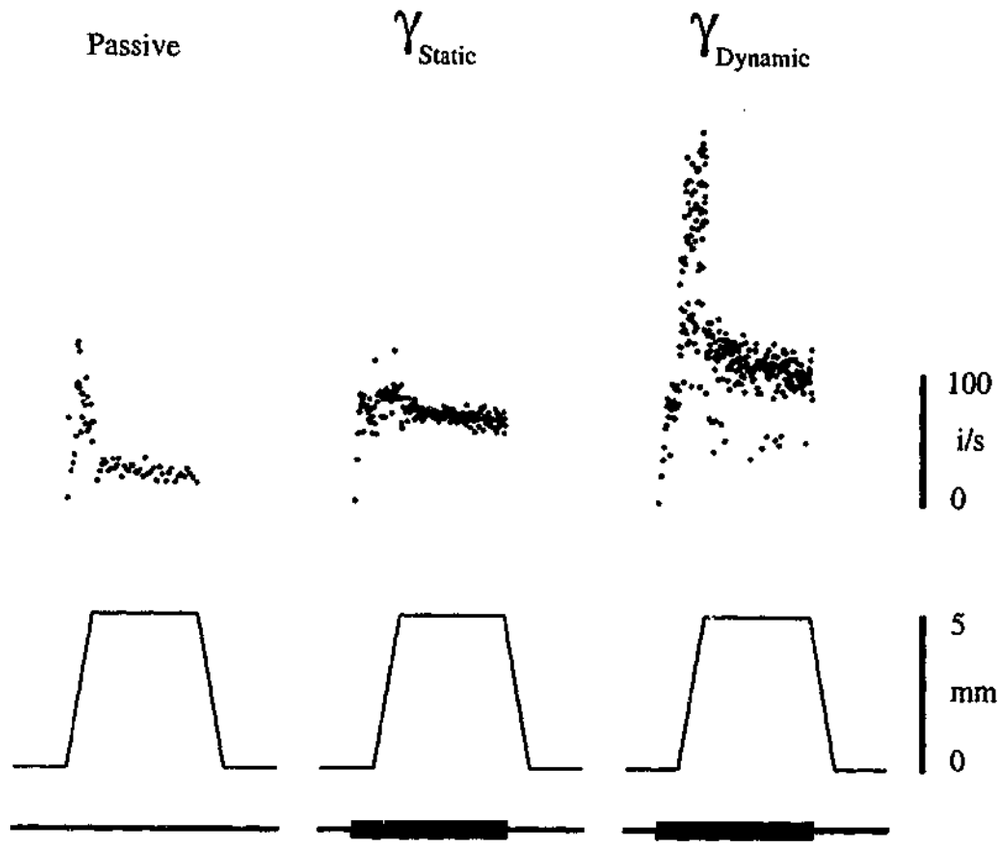


Figure 3.1. Classification of fusimotor axons. The *upper traces* show the instantaneous firing rate of a muscle spindle primary ending in response to muscle stretch (*middle trace*). Responses are shown for the Passive condition (without fusimotor stimulation) and during static and dynamic fusimotor stimulation (100pps). A thickening of the line in the *lower traces* indicates the timing of fusimotor stimulation. The response of the muscle spindle during the dynamic phase of muscle stretch was reduced by static fusimotor stimulation and increased by dynamic fusimotor stimulation compared to the response for the passive muscle spindle.

response of the primary ending without an accompanying increase in dynamic sensitivity.

Comparison with Human Data

These experiments examined muscle spindles responses using movements similar to those used in the human experiments. In order to compare movement detection thresholds in the human experiments to the responses of muscle spindles in the cat soleus muscle it was necessary to express detection thresholds in terms of the magnitude and rate of change of muscle fascicle length. The calculation for converting degrees of rotation about the human elbow joint to fascicle length changes in the soleus muscle of the cat were based on published measurements of muscle fascicle lengths.

With the human elbow joint positioned at 90°, biceps muscle fascicles are approximately 170mm in length. A one degree movements of the elbow produces a 0.6mm, or 0.3% change in muscle fascicle length (Hall & McCloskey 1983). The movement velocity used in the human movement detection threshold experiments was 0.2°/s, which equates to a 0.12mm/s change in muscle fascicle length of the biceps muscle. For the cat soleus muscle, with the ankle joint positioned at 90°, the average muscle fascicle length is 30mm (Rack & Westbury 1969). A length change of 0.3% equates to 0.106mm with the equivalent velocity equating to 0.02mm/s. Therefore, in an approximate comparison with the human experiments, muscle spindles in the cat soleus muscle would be required to respond to fascicle length changes at a velocity of 20μm/s and an amplitude of less than 110μm.

Experimental Protocols

Three experimental protocols were used to measure muscle spindle responses in the following experiments:

1. Muscle spindle stretch thresholds following muscle conditioning.
2. Muscle spindle stretch responses during combinations of fusimotor and skeletomotor activity were compared to their passive stretch responses.
3. The effects of changing stretch velocity and amplitude on muscle spindle sensitivity during stimulation of dynamic fusimotor axons.

Movement Thresholds

These experiments were designed to evaluate the stretch sensitivity of muscle spindles following conditioning procedures similar to those used in the human proprioception experiments. Thresholds to muscle stretch of 22 muscle spindles were measured after Hold Test and Hold Long muscle conditioning.

The conditioning sequence is depicted in figure 3.2. A conditioning contraction was elicited by stimulating the ventral root or muscle nerve at 100 pulses per second (pps) for one second at a strength sufficient to engage fusimotor axons. The conditioning contraction was carried out at the test length (Hold Test) or at a length 5mm longer (Hold Long). The muscle was held at the longer length for five seconds after the contraction before it was returned to the test length. Ramp-and-hold length changes of amplitudes ranging from 5μm to 110μm were applied nine seconds after the end of conditioning stimulation. In one experiment muscle spindle responses were measured following conditioning at a length 5mm shorter than the test length (Hold Short) (see figure 3.2). Theoretically, Hold Short should leave muscle spindles in a taut and responsive state, a condition that should be similar to the Hold Test condition. This experiments aimed to test this idea.

To measure muscle spindle thresholds a number of muscle stretches were imposed on the conditioned muscle. The amplitude of each ramp stretch was reduced until the muscle spindle was no longer able to respond to the stretch with a significant increase in firing rate. To determine the significance of the stretch response, the instantaneous rate for all the impulses within the five second period immediately before the stretch and the instantaneous rate for all the impulses during the stretch were measured. A t-test between these two populations determined whether the means were significantly different. This provided a simple measure that took into account the different variability of firing rates for different muscle spindles, and did not require any arbitrary decisions, such as what degree of smoothing to use. A muscle spindle with a relatively large variability in instantaneous rate needed a larger increase to become significant, but that is likely to be true in the CNS as well.

Muscle Spindle Responses During Fusimotor Stimulation

Muscle spindle stretch responses were measured during combinations of static fusimotor and dynamic fusimotor stimulation. For some trials (n=3), fusimotor stimulation was combined with stimulation of skeletomotor axons generating 0.5% to

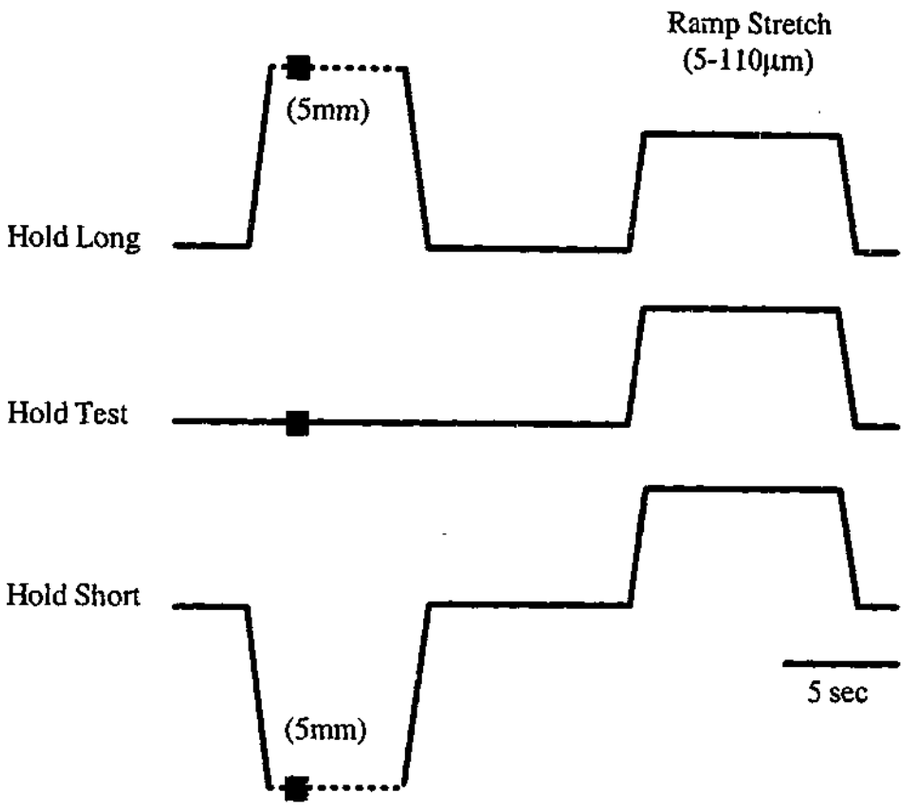


Figure 3.2. Schematic diagram showing muscle length during the experimental sequence. The muscle was conditioned with a one-second fusimotor-strength stimulus at 100ps (black square) at one of three muscle lengths. Hold Long: muscle length 5mm longer than the test length; Hold Test: conditioning at the test length, and in one experiment, the muscle was conditioned at a length 5mm shorter than the test length (Hold Short). Ramp-and-hold muscle length changes, with a velocity of 20μm/s and amplitudes ranging from 5μm to 110μm, were imposed and muscle spindle responses recorded.

9% of maximum whole-muscle tetanic tension. The muscle spindle stretch response during stimulation, called the Active condition, was compared to the passive stretch response, called the Passive condition. The term passive used here describes the experimental condition in which there was no fusimotor or skeletomotor stimulation during muscle stretch. The aim of these experiments was to compare the stretch response of muscle spindles under conditions that simulated the Co-contract and Both Taut conditioning procedures used in the human proprioception experiments.

The experimental sequence is depicted in figure 3.3. The muscle was conditioned at the test length by stimulating the whole muscle at a rate of 100pps. Before and during muscle stretch a single fusimotor axon was stimulated at a rate of 70pps. On some occasions two fusimotor axons were stimulated together at the same rate (70pps). A rate of 70pps was chosen in order to reduce the likelihood of the muscle spindle firing rate becoming locked to the rate of stimulation. To calculate an Active stretch response and a Passive stretch response four measurements were made for each muscle spindle (table 3.1).

	Ramp Stretch	No Stretch	Cumulative Count
Passive Spindle (without fusimotor)	A	B	A-B
Active Spindle (with fusimotor)	C	D	C-D

Table 3.1. For each muscle spindle responses were recorded for the Passive condition, with (A) and without (B) a 110μm ramp, and for the Active condition, with (C) and without (D) the ramp. A cumulative count measure of the stretch response minus the no stretch response was calculated for the Passive (A-B) and Active (C-D) conditions.

A cumulative count between two impulse trains A and B was calculated as follows (figure 3.4). A new wave A-B was constructed that increased by one whenever an impulse occurred in train A, and decreased by one whenever an impulse occurred in train B. Thus, it represented a progressive count of the excess number of impulses in trace A over trace B. In other words, a count of the number of impulses generated by the muscle spindle in response to stretch. The total impulse count obtained at the end of

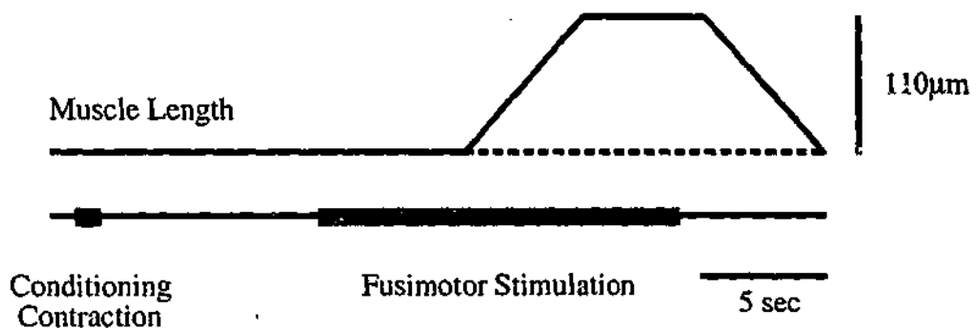


Figure 3.3. Schematic illustration showing muscle length (*upper trace*) and the timing of fusimotor stimulation (*lower trace*). Each test measurement was preceded by a conditioning contraction at the test length (black square). For measurements made during fusimotor stimulation (red bar), a fusimotor axon was stimulated repetitively (70pps), for 15 seconds beginning nine seconds after the conditioning contraction. In some recordings, fusimotor stimulation was combined with stimulation of skeletomotor axons. Four experimental conditions were tested. For the Active condition, that is with fusimotor stimulation, measurements were made with (solid line), and without (dashed line) the ramp stretch. For the Passive condition, with no fusimotor stimulation, measurements were also made with and without the ramp stretch. The ramp stretch was 110μm in amplitude with a velocity of 20μm/s.

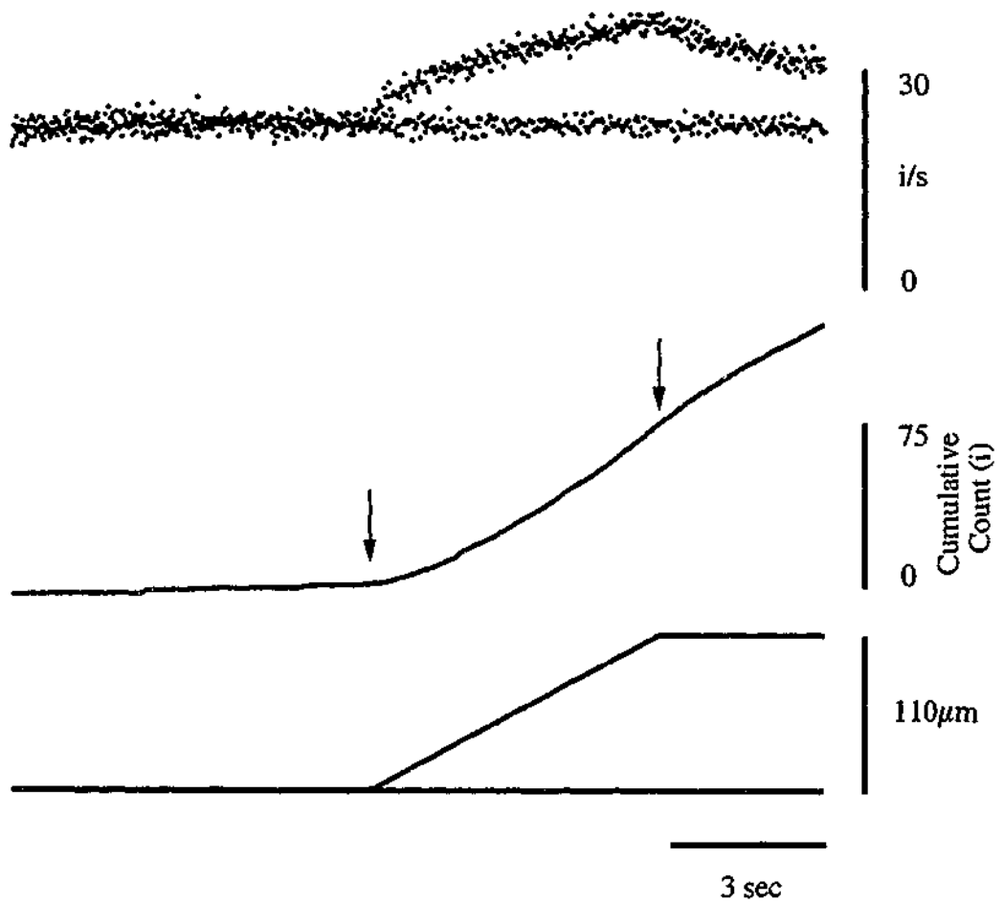


Figure 3.4. An example of the data used to calculate the cumulative count. The *upper traces* show the instantaneous firing rate of a passive muscle spindle with stretch (red) and without stretch (blue). The *middle trace* shows the cumulative count (green), which measured the number of impulses generated by the muscle spindle in response to the ramp stretch. The difference in count at the start and end of the ramp (shown by the arrows) was used in the statistical analysis. In this example the cumulative count value at the end of the ramp was 75 impulses. The *lower traces* indicate muscle length.

the ramp was compared with and without fusimotor stimulation i.e. (A-B) compared to (C-D). A paired t-test was carried out to determine the significance of any differences between cumulative counts for the Active (C-D) and the Passive (A-B) conditions. An example of the data used to construct the cumulative count measure is shown for the passive muscle spindle in figure 3.4.

Since the central nervous system may detect movements, not by integrating muscle spindle impulses over time, but by responding to changes in instantaneous rate, a measure of the peak rate of increase in cumulative count was calculated. The derivative of the cumulative count, after appropriate smoothing, would represent the rate of increase of impulse counts. Cumulative counts were averaged over 500ms periods during the stretch phase of the ramp and the peak rate of increase was measured between the 500ms bins. Statistical significance was evaluated using a paired t-test.

Effect of Stretch Amplitude and Velocity

These experiments examined the effects of stretch velocity and amplitude on the responses of eight muscle spindle primary endings during dynamic fusimotor stimulation in comparison to their responses under Passive conditions. Muscle stretches with velocities of 10, 20, 40, 80 and 160μm/s were imposed with the stretch amplitude ranging from 0.3mm for the slowest velocity to 0.8mm for the fastest velocity. The duration of dynamic fusimotor stimulation was adjusted for different stretch velocities. For instance, the longest stimulation period was 35 seconds for the 10μm/s stretch and the shortest period was 12 seconds for the 160μm/s stretch. Smaller stretches were used for the slowest velocity to prevent long durations of fusimotor stimulation and thus limit intrafusal fatigue. Dynamic fusimotor axons were stimulated at 70pps with the procedure shown in figure 3.3. The muscle was conditioned at the test length with a fusimotor-strength stimulus (100pps) following which a ramp stretch was imposed. Four recordings were made for each of the five stretch velocities: the Passive response with and without stretch and the Active response (during dynamic fusimotor stimulation), with and without stretch. Therefore, a total of 20 records were collected for each of the eight muscle spindles.

As before, cumulative counts were calculated in order to determine the number of impulses generated by the muscle spindle in response to muscle stretch. However, to enable comparisons between different stretch velocities, an additional analysis procedure was used. A cumulative count difference was calculated by subtracting the

cumulative count for the Passive condition from the cumulative count for the Active condition. For instance, referring to figure 3.5, an Active – Passive difference was calculated by (A-B)-(C-D). The resulting cumulative count difference was averaged over 10msec time periods (lower left graph in figure 3.5). The cumulative count difference was then differentiated to express the measure in terms of impulse rate (Difference Rate). This procedure was repeated for all five ramp velocities and each of the eight muscle spindles. The average Difference Rate (±SEM, n=8) for each stretch velocity was calculated, and the statistical significance of the effect of velocity and stretch amplitude on the Difference Rate was determined using an ANOVA.

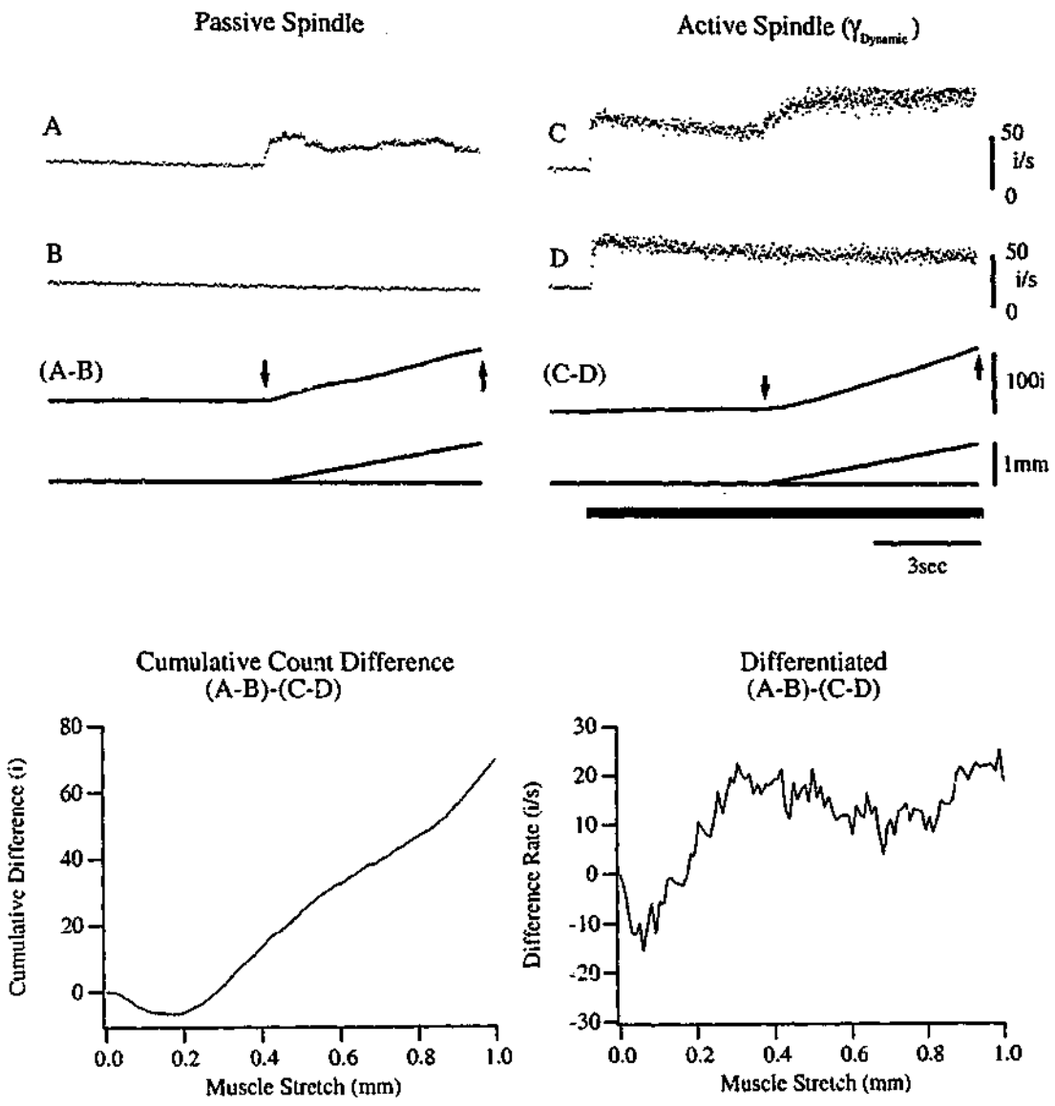


Figure 3.5. *Upper left panel* shows the instantaneous firing rate of a muscle spindle under Passive conditions with (A) and without (B) muscle stretch. *Upper right panel* shows instantaneous rate for the Active condition (dynamic fusimotor stimulation) with stretch (C) and without stretch (D). The traces below the afferent records show the cumulative counts (green), for the Passive condition (A-B), and for the Active condition (C-D). Muscle length is shown in red and blue. Stretch responses were measured over a range of stretch velocities, 10, 20, 40, 80 and 160 μm/s. The black bar indicates the period of fusimotor stimulation. *Lower left panel* shows the cumulative count difference, i.e. (A-B)-(C-D), measured between the arrows and plotted against muscle length. The *lower right panel* shows the cumulative difference expressed as a rate (Difference Rate) and plotted against muscle length.

Results

Muscle Spindle Stretch Thresholds

Muscle spindle stretch thresholds were measured following Hold Test and Hold Long muscle conditioning. Typical stretch responses from one muscle spindle following Hold Test muscle conditioning are shown in figure 3.6. This muscle spindle was responsive to ramp-and-hold stretches as small as 5μm. It was possible that the true threshold was lower still, but below 5μm, movements of the servomotor could not be reproduced reliably. Although the analysis was only concerned with the stretch phase of the ramp it is also interesting to observe that there was a reduction in the muscle spindle firing rate during the hold phase of the 40μm ramp compared to the pre-ramp levels.

Stretch thresholds were measured after Hold Test conditioning for a total of 22 muscle spindles. For 15 of these muscle spindles, thresholds were also measured after Hold Long conditioning. The distribution of muscle spindle thresholds is shown in figure 3.7. For each muscle spindle, a number of ramp stretches were imposed and the amplitude of each stretch was reduced until the muscle spindle no longer showed a detectable stretch response. Each muscle spindle was categorised into a threshold range in which the larger ramp produced a significant increase in firing rate but the smaller ramp did not. For instance, following Hold Test conditioning, 15 muscle spindles responded with a significant increase in firing rate for the 10μm ramp but not the 5μm ramp (see figure 3.7).

After Hold Test conditioning all muscle spindles had thresholds lower than 15μm. Thresholds increased dramatically when Hold Long was used. Eleven of the 15 muscle spindles had thresholds above the maximum 110μm ramp used. Two had thresholds in the range of 80μm to 110μm and two had thresholds below 80μm. Most muscle spindles fell silent after Hold Long conditioning and remained silent until after the onset of the ramp. The few muscle spindles that regained their resting discharge before the onset of the ramp were those that had thresholds below 110μm, suggesting that those muscle spindles had not been fully slackened or that slack was removed before the onset of the ramp.

In one experiment muscle spindle responses following Hold Short and Hold Test conditioned were measured in order to make direct comparisons of these two forms of conditioning. Examples of muscle spindle stretch responses following Hold Test and Hold Short muscle conditioning for two muscle spindles (muscle spindles A and B) are

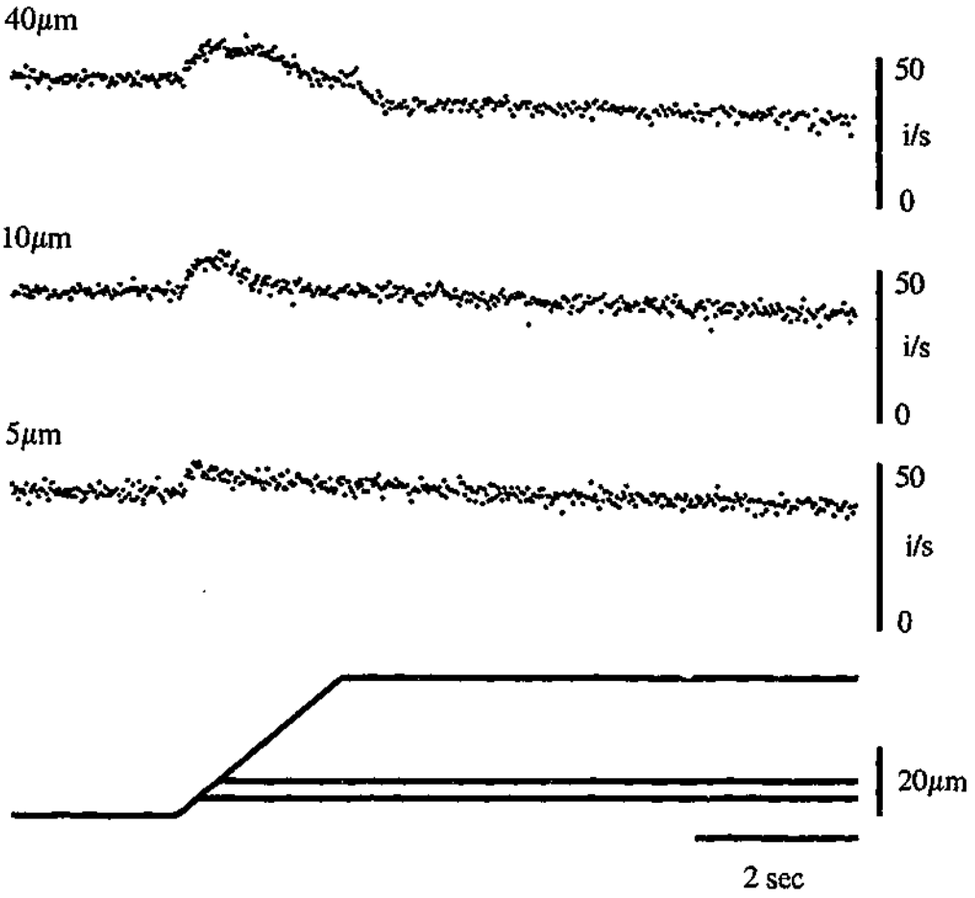


Figure 3.6. Recordings from a muscle spindle showing its response to muscle stretch of different amplitudes, 5μm, 10μm and 40μm at a velocity of 20μm/s. Measurements were made following Hold Test muscle conditioning (conditioning not shown) at a muscle length of L_{max} –10mm. The three *upper traces* show the instantaneous firing rate of the muscle spindle. The *lower trace* shows the three superimposed length records.

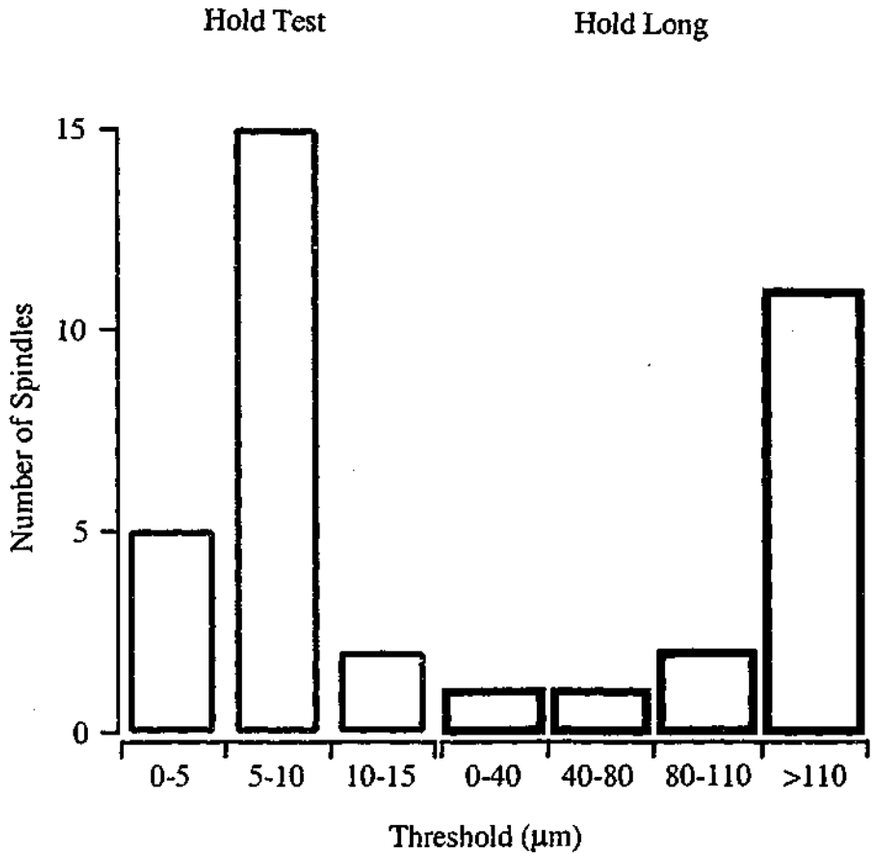


Figure 3.7. Distribution of muscle spindle stretch thresholds after Hold Test conditioning (red bars, n=22) and after Hold Long conditioning (blue bars, n=15). Note the different bin widths for the Hold Long and Hold Test conditioning. Following Hold Test conditioning all muscle spindles were able to respond to muscle stretches smaller than 15μm whereas, when Hold Long conditioning was used, most muscle spindles did not respond to the 110μm stretch. The maximum excursion of the servomotor was 110μm and the velocity used was 20μm/s.

shown in figure 3.8. The muscle spindle responses to ramp stretches were similar following Hold Short and Hold Test conditioning. Although this comparison was made for only five muscle spindles in one experiment, these results support the assumption made in the human experiments that muscle spindle stretch sensitivity was similar following muscle conditioning at the test length and conditioning at a length shorter than the test length.

Stretch Responses During Fusimotor Stimulation

Responses of muscle spindles to 110μm ramp length changes of the whole muscle at 20μm/s were examined during static, dynamic, combined static and dynamic, and combined fusimotor and skeletomotor stimulation. Figure 3.9 shows an example of the data collected for one muscle spindle. Muscle spindle firing rate, represented as instantaneous frequency, is shown for the passive muscle spindle and during static and dynamic fusimotor stimulation. The panel on the left shows the muscle spindle responses to stretch. Static fusimotor stimulation had the effect of raising the muscle spindle firing rate and abolishing the stretch response when compared to the passive muscle spindle. The dynamic stimulation also had the effect of increasing the muscle spindle firing rate and slightly reducing the size of the stretch response.

The combined data from all muscle spindles illustrated in figure 3.10 shows the cumulative count for the muscle spindle during fusimotor stimulation, (C-D) and (E-F), plotted against the cumulative count for the passive muscle spindle (A-B). The upper panel is a plot of the data obtained during static fusimotor stimulation and measurements from 12 muscle spindles are shown. Here, all the points lie below the line of unity. Statistical analysis indicated that the impulse count during static fusimotor activation was significantly lower than the count for the passive muscle spindle ($p<0.0001$, paired t-test, $n=10$). Responses for two muscle spindles were excluded from the analysis because they showed stimulus locked driving. The peak rate of increase in impulse number during the ramp was 10.0±1.4 i/s in the presence of static fusimotor stimulation. This was also significantly lower than for the Passive condition where the peak rate was 19.5±1.6 i/s ($p<0.0004$, paired t-test, $n=10$).

Dynamic fusimotor stimulation had a similar but less profound effect (lower panel figure 3.10). Of the six muscle spindles tested, three provided approximately equal cumulative counts during dynamic fusimotor stimulation compared to the Passive condition, that is their counts lay on the line of unity. For the other three muscle

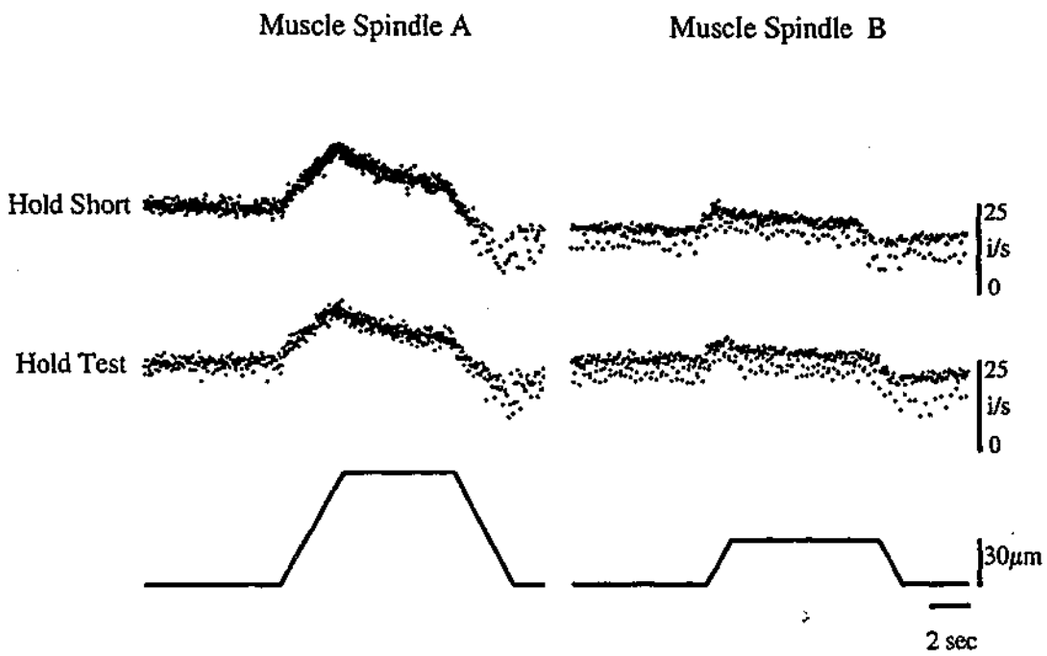


Figure 3.8. Example of muscle spindle responses to ramp length changes following Hold Short conditioning (black) and Hold Test conditioning (red) for two different muscle spindles (A and B). The muscle spindle stretch responses following Hold Test and Hold Short conditioning were similar for muscle spindle A (75μm ramp) and muscle spindle B (30μm ramp).

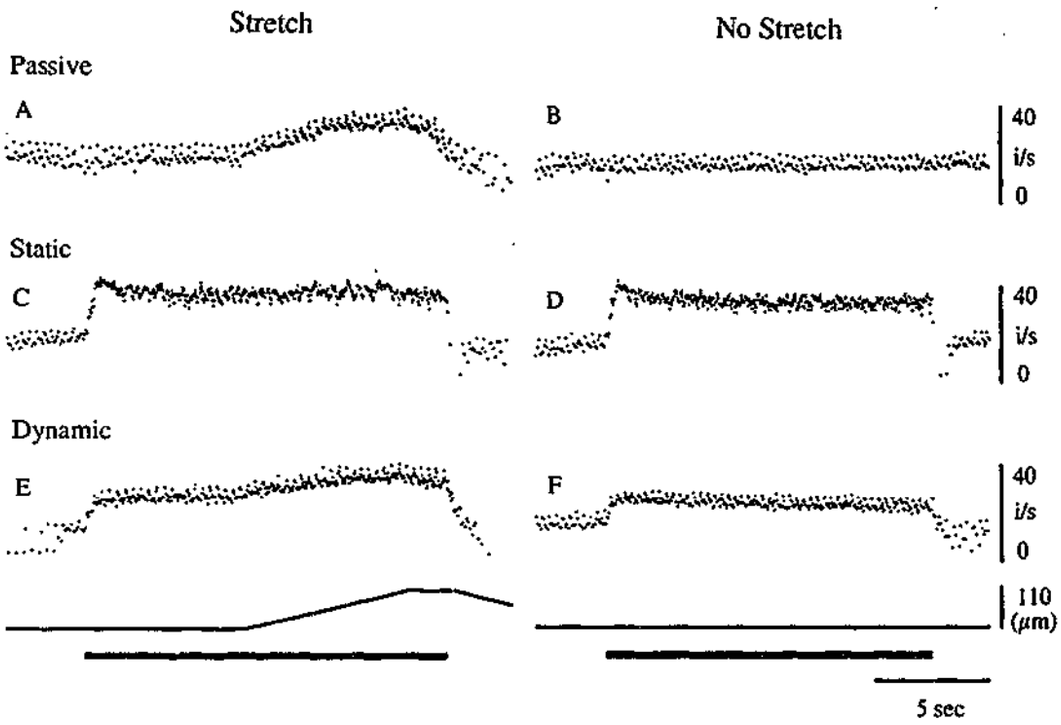
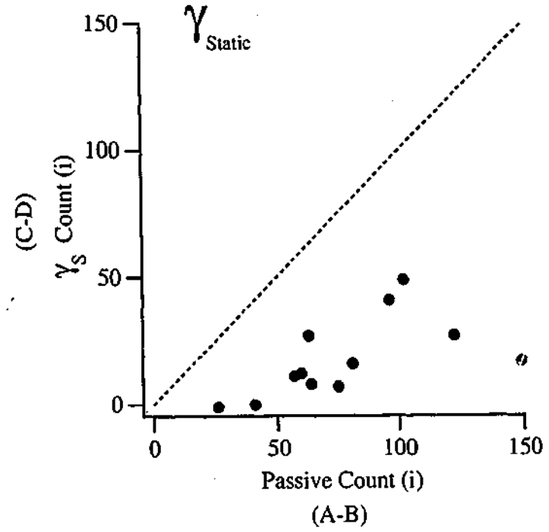


Figure 3.9. Instantaneous firing rate of a muscle spindle under Passive conditions (blue) and during either static or dynamic fusimotor stimulation (red). Before each recording the muscle was conditioned with a contraction at the test length (conditioning not shown). The *left panel* shows the response to a 110μm ramp muscle stretch at a velocity of 20μm/s. The *right panel* shows the response of the same muscle spindle in the absence of muscle stretch. Muscle length is depicted by the black trace, and the red bar at the bottom of the figure indicates the timing and duration of fusimotor stimulation (70pps). Notice that the discharge of this muscle spindle is modulated by the cat's arterial pulse.



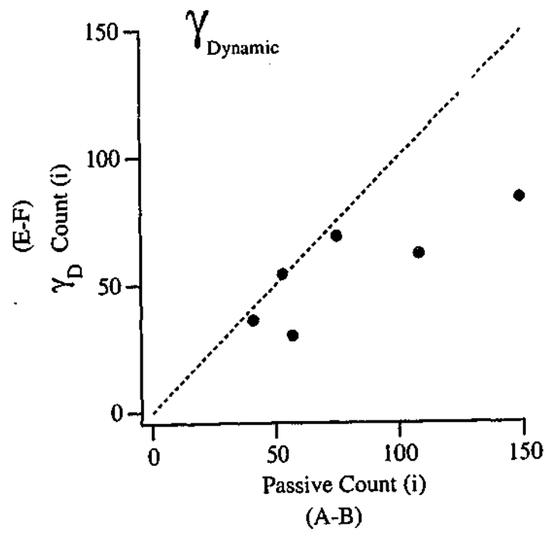


Figure 3.10. Cumulative count measured for the Active and Passive conditions. The cumulative count is the number of impulses generated by a muscle spindle, above the resting rate, in response to muscle stretch of 110μm. Counts were measured with and without fusimotor stimulation. *Upper panel* shows the cumulative count generated during static fusimotor stimulation (C-D from figure 3.9) plotted against the cumulative count for the muscle spindle under Passive conditions (A-B, from figure 3.9). *Lower panel* shows the cumulative count measured during dynamic fusimotor stimulation (E-F, from figure 3.9) and is plotted against the cumulative count for the Passive condition (A-B, from figure 3.9).

spindles, their responses during dynamic fusimotor stimulation were lower than their passive responses. Overall, differences between the Passive impulse count and the Active impulse count (during dynamic fusimotor stimulation) were not significantly different (p=0.07, paired t-test, n=6). The peak rate of increase in impulse number during combined stretch and dynamic fusimotor stimulation (15.5±1.7 i/s) was also not significantly different to the peak rate for the passive muscle spindle (20.0±2.6 i/s) (p=0.98, paired t-test, n=6).

The results from these experiments showed that the sensitivity of muscle spindles to small slow stretches was significantly reduced during static fusimotor stimulation. Dynamic stimulation, on average, also tended to reduce the stretch response compared to the passive muscle spindle. These results were obtained with fusimotor stimulation at a rate of 70pps. For static fusimotor stimulation two lower rates 40pps and 50pps were also used. The result was qualitatively the same although the differences between passive responses and responses during static stimulation became smaller for the lower frequencies. For dynamic fusimotor stimulation responses were also measured using a stimulation rate of 100pps. There was no observable difference in responses when the higher stimulation rate was used compared to the 70pps result.

The effect of combined fusimotor and skeletomotor stimulation on muscle spindle stretch sensitivity was examined. Skeletomotor axons were stimulated at 70pps using ventral root filaments and produced tension ranging from 0.5% to 9% of whole-muscle tetanic tension. Three muscle spindles were tested under these conditions. In addition, four muscle spindles were tested during combined static and dynamic fusimotor stimulation, in the absence of skeletomotor activation. For both of these conditions the cumulative counts were lower than the counts for the passive muscle spindle, figure 3.11 shows the averages from these measurements. Also included are the average cumulative counts of the Active conditions during static fusimotor stimulation and dynamic fusimotor stimulation from figure 3.10, plotted against their values for the Passive condition.

It can be seen from the data shown in figure 3.11 that, under the conditions of these experiments, all fusimotor activity tended to decrease the stretch responsiveness of the muscle spindle when compared to the passive response after appropriate conditioning. When combined static and dynamic fusimotor stimulation was used muscle spindle responses appeared to be dominated by the effect of static fusimotor

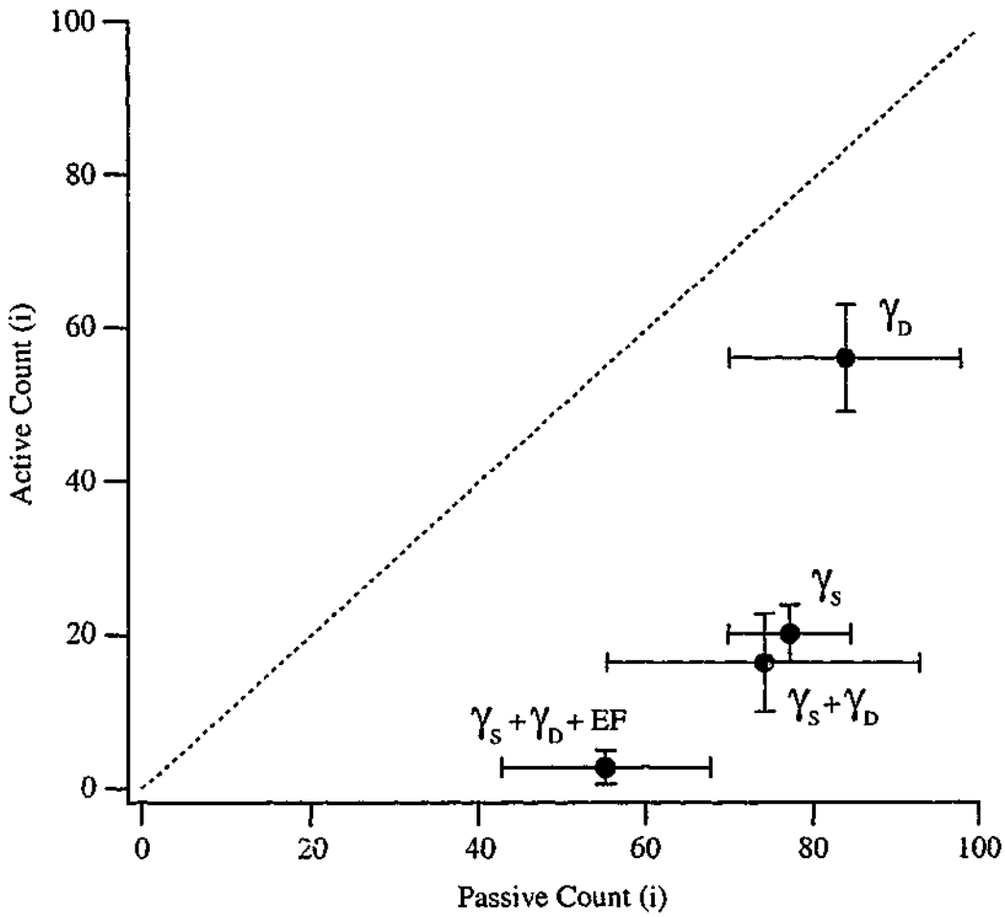


Figure 3.11. Average (±SEM) of the cumulative counts for all four Active conditions plotted against their Passive values. The values for dynamic fusimotor stimulation (red, n=6) and static fusimotor stimulation (blue, n=10) represents the average of the data points shown in figure 3.10. Muscle spindle responses obtained during combined stimulation of dynamic and static fusimotor axons (green, n=4) were similar to the responses measured during static fusimotor stimulation alone. If fusimotor stimulation was combined with extrafusal tension (black, n=3) then the stretch related increase in muscle spindle firing rate was reduced further.

stimulation. In the presence of skeletomotor activity muscle spindle sensitivity was similarly reduced.

Effects of Stretch Amplitude and Velocity During γ_D Stimulation

In the previous experiments muscle spindle stretch sensitivity during dynamic fusimotor stimulation could, under conditions of these experiments, be lower than that of the passive muscle spindle (figure 3.10 lower panel). This result was somewhat unexpected and it raised the question of whether stretch velocity and amplitude were important factors. The next series of experiments examined the effects of stretch velocity and amplitude on muscle spindle sensitivity during dynamic fusimotor stimulation compared to the sensitivity of the passive muscle spindle. The Difference Rate measure was calculated (see figure 3.5) so that muscle spindle responses could be compared between different stretch velocities. The average (±SEM) of the Difference Rate calculated for each stretch velocity (n=8) is shown in figure 3.12.

Over the initial stages of muscle stretch the Difference Rate fell below zero for all stretch velocities indicating that the stretch-evoked increase in muscle spindle firing rate was greater for the passive muscle spindle compared to the response of the same muscle spindle under dynamic fusimotor stimulation. This effect was maximal for a stretch of approximately 100μm.

As the amplitude of the stretch increased beyond 150μm, stretch velocity became a significant factor in determining the difference between the response of the passive muscle spindle and the response during dynamic fusimotor stimulation. An ANOVA revealed a significant main effect of stretch velocity ($p<0.0001$, n=8) for amplitudes greater than 150μm. For the three faster velocities (40μm/s, 80μm/s and 160μm/s) there was a cross-over point in the range of 125μm to 175μm. At the cross-over point the stretch response of the passive muscle spindle was equal to the response of the muscle spindle during dynamic stimulation. For stretch velocities of 80μm/s and 160μm/s the stretch response for amplitudes beyond the cross-over point were larger during dynamic fusimotor stimulation. For stretches with a velocity of 160μm/s the average Difference Rate increased to about 10i/s at a stretch amplitude of approximately 350μm, where it reached a plateau over the remainder of the stretch. For the 80μm/s stretch velocity the average Difference Rate reached a plateau at approximately 300μm with a value of about 5i/s. For the two slower ramp velocities (10μm/s and 20μm/s), the Difference Rate remained negative, that is, the passive muscle spindle had a greater stretch

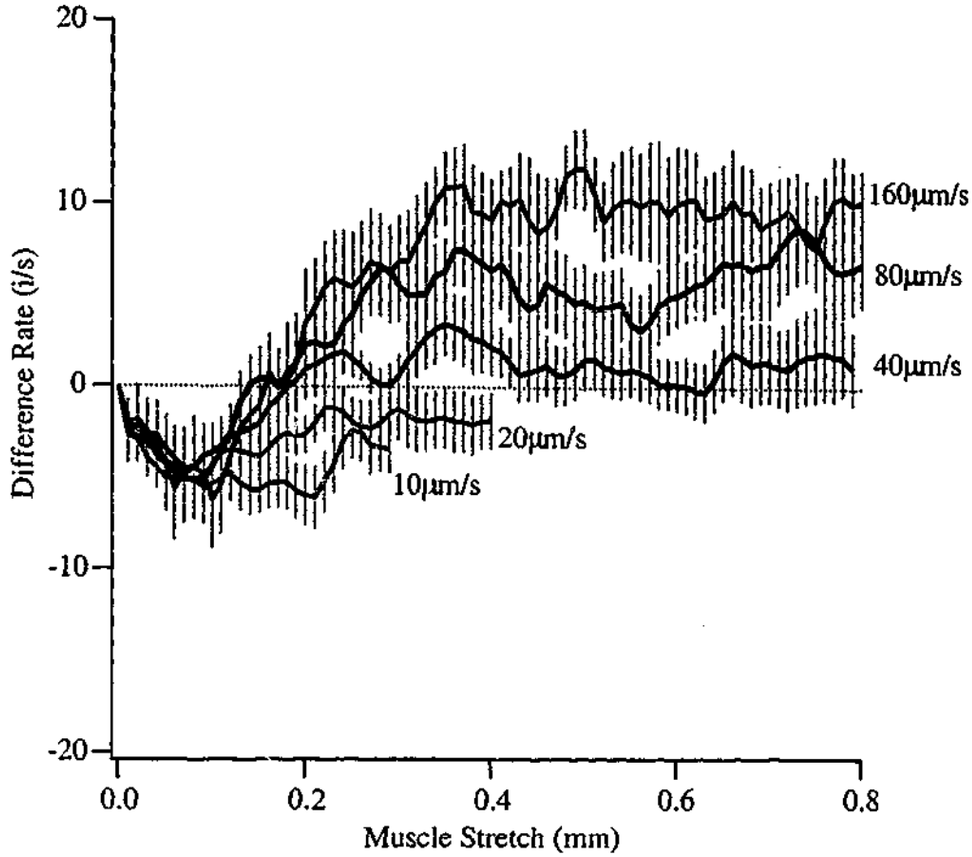


Figure 3.12. Average (±SEM, n=8) of the Difference Rate measure plotted against stretch amplitude for each stretch velocity (10μm/s red, 20μm/s green, 40μm/s blue, 80μm/s black, and 160μm/s purple). At the start of muscle stretch the Difference Rate fell below zero indicating a greater muscle spindle response under Passive conditions. The effect was maximal at a stretch amplitude of approximately 100μm with the Passive response, on average, 5i/s greater than the response during dynamic fusimotor stimulation for all stretch velocities. For slow stretches (10μm/s and 20μm/s) values during dynamic fusimotor stimulation remained below the Passive values. When faster stretches were used (80μm/s and 160μm/s), the stretch response during dynamic fusimotor stimulation became greater than the response for the muscle spindle under Passive conditions for stretch amplitudes larger than approximately 200μm.

sensitivity for slow ramp velocities than the muscle spindle during dynamic fusimotor stimulation.

The responses varied for different muscle spindles. This can be seen in the relatively large standard error bars (figure 3.12). Statistical analysis revealed a significant interaction between velocity and muscle spindle and an interaction between amplitude and muscle spindle ($p<0.0001$, $n=8$). This indicated that the velocity and length effects were not consistent for all muscle spindles tested.

In summary, for small stretches over the range of 0μm to 150μm the passive muscle spindle was more sensitive than the muscle spindle during dynamic fusimotor stimulation. As muscle stretch increased beyond 100μm, stretch velocity became a significant factor. A cross-over region, 125μm to 175μm, was observed for the stretch velocities 40μm/s, 80μm/s and 160μm/s. For the two slower velocities, 10μm/s and 20μm/s, the passive muscle spindle remained more sensitive than during dynamic fusimotor stimulation.

Discussion

One aim of these experiments was to determine whether muscle spindles in the cat were able to signal muscle length changes comparable to those detected by human subjects under passive conditions. These experiments also aimed to examine the effects of contraction history and the effects of fusimotor and skeletomotor activity on the stretch response of muscle spindles under conditions designed to approximate those of the human experiments. In addition, these experiments examined the paradoxical effect whereby the sensitivity of muscle spindles to large, but not small amplitude stretches can be enhanced during dynamic fusimotor stimulation (Goodwin, Hulliger *et al.* 1975). The rationale behind these later experiments was to determine whether this effect was influenced not only by the amplitude but also the velocity of muscle stretch.

One methodological issue that should be considered is the calculations used to convert degrees of human forearm rotation to changes in the length of the cat soleus muscle. The calculations are based on the muscle fascicle length data of the short head of biceps published in Hall & McCloskey (1983). There are a number of muscles acting at the human elbow joint that provide proprioceptive information about elbow rotation. Each muscle has a different size, structure and tendon compliance compared to that of the cat soleus muscle, which has relatively long fibres with a rather small pennation angle. Movement imposed on the muscle can produce changes in pennation angle and tendon compliance without producing an appreciable change in muscle fascicle length. These extrapolations therefore represent only a rough approximation of the conditions of the human experiments and, in any comparison, these differences should be kept in mind.

Muscle Spindle Stretch Thresholds

The stretch sensitivity of 22 muscle spindles was measured after muscle conditioning. Following a conditioning contraction at the test length (Hold Test), there was a significant increase in the firing rate of all muscle spindles in response to stretches of 15μm. Five muscle spindles were capable of responding to a muscle stretch of 5μm. These results are consistent with previously reported muscle spindle thresholds of 5μm observed for tendon vibration (Brown, Engberg *et al.* 1967). The stretch thresholds measured for cat muscle spindles were comparable to the movement detection thresholds observed in human subjects. A 15μm change in the length of the cat soleus muscle, when expressed in terms of angular movement of the human biceps

muscle, equates to a movement of approximately 0.14°. The average human movement thresholds following Both Taut conditioning were approximately 0.2°. The similarity of these values supports the claim that muscle spindles are sensitive enough to respond to the small elbow movements used in the human experiments.

It was interesting to observe that during the hold phase of a small amplitude ramp stretch (eg. 40μm in figure 3.6), the firing rate of the muscle spindle was actually lower than the rate observed at the shorter test length, before stretch onset. This implies that following muscle conditioning the intrafusal fibres can become quite strained such that a small stretch of the muscle can actually cause a decrease in the tension on the sensory region of the intrafusal fibre, possibly due to intrafusal creep. This would suggests that the role of the primary endings in proprioception is mainly concerned with limb movement and less with absolute limb position. Primary endings are more sensitive to the velocity component of stretch than to static muscle length (Hulliger, Matthews *et al.* 1977) and absolute muscle length is more likely to be signalled by the secondary endings.

The finding that muscle spindle stretch sensitivity was dramatically reduced when Hold Long conditioning was used highlighted the importance of contraction history. For 11 of the 15 muscle spindles examined, the muscle spindles fell silent following Hold Long conditioning. A ramp stretch of 110μm was unable to elicit any response from these muscle spindles indicating a decrease in stretch sensitivity of ten times or more when compared to their Hold Test thresholds. The mechanism thought to be responsible for the decrease in muscle spindle stretch sensitivity seen with Hold Long conditioning is the presence of slack within the muscle spindle (Morgan, Prochazka *et al.* 1984). As discussed in the previous chapter, it is postulated that the intrafusal fibres become slack due to the presence of stable cross-bridges between actin and myosin filaments that form when the muscle contracts at a length longer than the test length (Hold Long). Any subsequent stretch must first remove the slack before the tension on the sensory region of the muscle spindle increases sufficiently to raise the firing rate. The important point in this finding is that slack within muscle spindles can affect stretch thresholds and therefore the muscle contraction and length history should be taken into account whenever thresholds are measured. This result is consistent with the human movement detection experiments in which increases in thresholds were observed when the muscle that was stretched by the imposed movement had been previously slackened by muscle conditioning at a longer length.

Muscle spindle resting discharge was typically abolished after Hold Long conditioning. The presence of slack within the polar regions of intrafusal fibres would decrease the resting tension on the sensory region of the muscle spindle causing the spiral terminals to close and the muscle spindle to fall silent. However, this effect did not appear to be consistent for all muscle spindles examined. In four muscle spindles, after the Hold Long conditioning, there was a recovery in the resting discharge before movement onset. Stretch thresholds for these muscle spindles, although below 110μm, were still considerably higher than after Hold Test conditioning indicating that the stretch response was still diminished. It might be that for these muscle spindles the spiral terminations around the sensory region are not affected by the presence of slack within the intrafusal fibres, possibly by the action of elastic elements between the spiral terminations and the muscle spindle capsule that prevent the spiral terminals from closing (Gregory, Morgan & Proske 1991).

For the Hold Short condition, the muscle is stretched to the test length following the conditioning contraction whereas, for the Hold Test condition, the muscle is already at the test length. Theory would suggest that following either form of conditioning muscle spindles should be taut and equally stretch sensitive. To examine this point muscle spindle stretch sensitivity was measured after Hold Test and Hold Short conditioning. The results of this experiment showed that muscle spindles were equally sensitive to small slow stretches after these conditioning manoeuvres. This finding supports the suggestion made in the previous chapter that Both Taut and Biceps Taut conditioning had similar effects on the sensitivity of muscle spindles within biceps, and likewise, Both Taut and Triceps Taut conditioning was similar for triceps muscle spindles. However, these findings were from five muscle spindles in only one experimental animal and therefore additional data is required before any firm conclusions can be drawn.

Stretch Responses During Fusimotor Stimulation

Human movement detection thresholds were higher during co-contraction of elbow flexors and extensors compared to when these muscles were relaxed and appropriately conditioned. There are a number of mechanisms that may contribute to the larger movement detection thresholds seen in human subjects during co-contraction. The experiments in this chapter have examined one possible mechanism, specifically, an attenuation of the muscle spindle stretch response due to fusimotor activity that is

known to occur during voluntary contraction. The experiments described here examined the stretch responses of cat muscle spindles under combinations of fusimotor and skeletomotor activity. Stretch responses were measured during fusimotor stimulation and compared to the stretch response of the passive, conditioned muscle spindle.

During static fusimotor stimulation there was a pronounced decrease in the stretch-evoked response of the muscle spindle when compared to the passive muscle spindle conditioned at the test length. Previous studies examining muscle spindle stretch sensitivity during static fusimotor stimulation have shown similar results (Matthews 1962; Crowe & Matthews 1964a; Crowe & Matthews 1964b). The mechanism thought to be responsible for the decrease in stretch sensitivity seen during static fusimotor stimulation relates to the contraction speed of the chain and bag_2 fibres that are innervated by static fusimotor axons. The chain and bag_2 fibres have relatively fast twitch times (Bessou & Pages 1975; Boyd, Gladden *et al.* 1977). This means that, although there is more tension on the sensory region of the muscle spindle during stimulation, hence a biasing of the firing rate, the stiffness of the chain and bag_2 intrafusal fibres is relatively low due to the rapidly cycling cross-bridges. Consequently, there is less extension of the sensory region during muscle stretch and the stretch-evoked increase in firing rate is relatively low compared to the passive muscle spindle.

The effect of combined dynamic and static fusimotor stimulation on the stretch sensitivity of the muscle spindle was similar to the sensitivity during static fusimotor stimulation alone (figure 3.11). This result was consistent with previous findings that showed muscle spindle firing rate, in response to small amplitude sinusoidal stretch during combined static and dynamic stimulation, was dominated by static fusimotor effects (Hulliger, Matthews *et al.* 1977). The authors suggested that the dynamic fusimotor action was suppressed, or occluded, by the action of the static fusimotor stimulation. When a small amount of skeletomotor activity, ranging from 0.5-9% of maximum tension, was incorporated during combined fusimotor stimulation the muscle spindle was unloaded and the stretch response was dramatically attenuated. Although the cat soleus muscle contractions were lower than that achieved in the human proprioception experiments (co-contractions of 15-20% MVC) it was likely that increases in contraction force above these levels would lead to further reductions in muscle spindle stretch responses. The results confirm that, for small, slow muscle stretch similar to that used in the human proprioception experiments, activation of static fusimotor axons alone or combined with dynamic fusimotor stimulation, with or

without some extrafusal muscle tension, lead to a decrease in muscle spindle stretch sensitivity. It is proposed that these effects contributed to the increase in movement detection thresholds observed during voluntary contraction.

The initial measurements of the stretch-evoked response of muscle spindles during dynamic fusimotor stimulation, compared to their passive response, were less conclusive (lower panel figure 3.10). Of the six muscle spindles tested, three were equally sensitive for the Active and Passive conditions. The other three muscle spindles showed a reduced sensitivity to stretch during dynamic fusimotor stimulation. On average there was a decrease in response for muscle stretch (figure 3.11), but this was not significant. To examine this effect more closely a series of experiments were carried out to measure the response of muscle spindles to stretch over a range of velocities. The aim of these experiments was to define in detail the properties of the stretch stimulus, both its amplitude and velocity, which were factors in determining whether the stretch response of a muscle spindle during dynamic fusimotor stimulation was greater than the passive muscle spindle.

Effects of Stretch Amplitude and Velocity During γ_D Stimulation

It is well known that dynamic fusimotor stimulation acts to increase muscle spindle sensitivity for stretches of relatively large amplitude, however its effect on muscle spindle sensitivity, when the amplitude of stretch is much smaller, can be more variable. As before, cumulative impulse counts were made for the Active and Passive conditions, but here, in order to compare data for different stretch velocities, the Difference Rate measure was calculated (see figure 3.5).

For stretch amplitudes below approximately 150μm the stretch-evoked response was greater for the passive muscle spindle than during dynamic fusimotor stimulation (see figure 3.12). This was indicated by a negative value in the average Difference Rate, the effect being maximal for a stretch amplitude of 100μm. The significance of 100μm is that it lies within the linear range of the passive muscle spindle (Matthews & Stein 1969). For the passive muscle spindle, following conditioning at the test length, the polar regions of intrafusal fibres are quite stiff. During muscle stretch the stiffened polar regions do not stretch appreciably so that most of the length change is transferred to the sensory region of the intrafusal fibres. This property of the intrafusal fibres provides the passive muscle spindle with a relatively high stretch sensitivity for small amplitude stretches. Once a muscle spindle is stretched beyond its linear range the stable cross-

bridges within the polar regions of the passive muscle spindle detach and slide past one another reducing the intrafusal stiffness. At any instant in time there are fewer attached cross-bridges allowing the intrafusal fibres to lengthen, reducing the stretch of the sensory region and therefore reducing the stretch response of the muscle spindle.

The situation is different during dynamic fusimotor stimulation. Here, the active cycling of cross-bridges within the bag_1 intrafusal fibre means that there is an increase in both the cross-bridge attachment and detachment rates. Relatively speaking, the stiffness of the polar regions of intrafusal fibres is lower during dynamic fusimotor stimulation compared to the passive muscle spindle within the linear range. The net result is less tension on the sensory region of the intrafusal fibre during stretch and the resultant stretch response is diminished. Therefore, the finding that the short range sensitivity of the passive muscle spindle was greater than that of the muscle spindle during dynamic fusimotor stimulation for all stretch velocities is indicative of the high short range sensitivity of the muscle spindle under passive conditions.

For muscle stretch beyond the linear range stretch velocity became a significant factor in determining the muscle spindle stretch response during dynamic fusimotor stimulation. For the three faster stretch velocities 40μm/s, 80μm/s and 160μm/s muscle spindle responses during dynamic fusimotor stimulation increased above their passive values. There was a cross-over point in the amplitude range of 125μm to 175μm where the response during dynamic fusimotor stimulation was equal to the passive response. For stretch amplitudes larger than the cross-over point the response from the muscle spindle was greater during dynamic fusimotor stimulation. Examinations of these effects by other researchers have found a cross-over point of 200μm using sinusoidal length changes (Hulliger, Matthews *et al.* 1977).

However, for slower velocities (10μm/s, 20μm/s) the stretch-evoked response for the passive muscle spindle remained higher than the response during dynamic fusimotor stimulation. The explanation for this finding relates to the slow cross-bridge cycling rate of the bag_1 fibres during dynamic fusimotor stimulation (Boyd 1966). The bag_1 intrafusal fibres contain cross-bridges that cycle slowly, thus providing the bag_1 fibre with a relatively high dynamic stiffness for relatively fast stretches. However, when the stretch is very slow the active cycling of cross-bridges gives a higher turnover rate than for the passive muscle spindle. The fibre is progressively 'giving way' to the slow stretch.

The effect of velocity was variable for the different muscle spindles examined. There was a significant interaction between different muscle spindles and velocity, indicating that the effect of stretch velocity was not consistent. Others have reported varying effects of dynamic fusimotor stimulation on muscle spindle stretch sensitivity. Chen & Poppele (1978) found the muscle spindle stretch sensitivity to sinusoidal length changes could be unchanged, increased or decreased during dynamic fusimotor stimulation. The variability of the result may be due to the 'strength' of the dynamic fusimotor stimulation on the muscle spindle. For 'strong' dynamic fusimotor axons, those that caused a larger increase in modulation as tested with a 1mm stretch at 1Hz, the cross-over amplitudes were smaller than those when the dynamic action was defined as weak (Hulliger, Matthews *et al.* 1977). Therefore, the variation in muscle spindle responses found here maybe a result of this mechanism.

The conclusions that can be drawn from these experiments are that when appropriately conditioned, muscle spindles in the cat soleus muscle are sensitive enough to respond to movements comparable to the movement detection thresholds reported in the human movement detection experiments. Muscle spindle stretch sensitivity was reduced after muscle conditioning designed to slacken intrafusal fibres, a finding consistent with the increase in movement detection thresholds following similar conditioning procedures in the human experiments.

All combinations of fusimotor and skeletomotor stimulation reduced muscle spindle responses to small muscle stretches when compared to the response of the passive muscle spindle. This finding provides evidence that the decrease in proprioceptive acuity found during co-contraction of elbow extensors and flexors, can, at least in part, be attributed to a decrease in the ability of muscle spindles to signal length changes. These results emphasise the high sensitivity of the passive muscle spindle when conditioned appropriately.

Although there is experimental evidence that both peripheral and central factors can provide a separate gain-control mechanism during human movement the findings presented in this chapter provide strong evidence that peripheral factors make a significant contribution to the attenuation of proprioceptive signals during movement.

Chapter Four

Submaximal Activation of Skeletal Muscle

We activate our muscles to produce forces that move our limbs and maintain our body posture. Most of the time the forces required for daily activities are submaximal, that is, muscles typically generate forces much lower than their maximum. In day-to-day activities our muscles lengthen and shorten at different speeds depending on muscle properties, the level of activation and the load. However, much of the existing literature on the fundamental properties of muscle has examined these properties during maximal or near maximal contractions. Very few studies have examined muscle properties when the muscles are stimulated at the submaximal levels at which they normally operate. This is partly due to the difficulty in achieving smooth submaximal contractions with the methods of stimulation that are currently available.

One method of stimulation that can produce smooth muscle contractions at physiological rates of stimulation is called distributed stimulation. Rack & Westbury (1969) developed this method and, in their landmark experiments, they showed that the properties of submaximally activated muscle could not be predicted in any simple way from properties measured with maximal activation. However, due to certain limitations with this method that will be discussed shortly, the suitability of distributed stimulation was limited to muscles containing one type of muscle fibre.

A new way of using distributed stimulation has been developed so that it can be used on muscles containing a mixture of fibre types. This half of the thesis describes experiments designed to evaluate this new method, and to determine whether smooth submaximal activation of mixed muscle can be achieved. Using this technique, experiments were carried out to examine the mechanical and fatigue properties of mixed muscle when activated at submaximal rates of stimulation.

This introduction will briefly review properties of skeletal muscle and the activation strategies used by the CNS. It will examine the way in which muscle is typically activated in the laboratory and in rehabilitative medicine, focussing on some of the limitations of these methods. The introduction will examine the method of distributed stimulation highlighting some of its limitations and how these limitations can be overcome. Finally, the mechanical and fatigue properties of submaximally activated muscle and how they compare to the maximally activated muscle will be discussed.

Muscle Fibres and Motor Units

Muscles are made up of numerous muscle fibres that can be categorised in terms of contraction speed and force, fatigue properties and energy source. Burke and colleagues defined four different categories of muscle fibres based on their contraction speed and fatigue index (Burke, Levine & Zajac 1971; Burke, Levine, Tsairis & Zajac 1973; Burke, Rudomin & Zajac 1976). These categories are fast fatigable (FF), fast intermediate (FI), fast fatigue resistant (FR) and slow (S). The slow twitch fibres had long twitch contraction times and were much more resistant to fatigue. The fast fibres had shorter twitch contraction times and differed from each other in their susceptibility to fatigue. However, muscle fibres exhibit a range of fatigability, such that it is sometimes difficult to place some muscle fibres in one category or the other (Burke, Levine *et al.* 1973; Proske & Waite 1974; Reinking, Stephens & Stuart 1975). An important characteristic of skeletal muscle is that its ability to produce force depends on what has previously happened to the muscle. For instance, during or after a period of stimulation the force generating capabilities of a muscle can either decrease, as a result of fatigue, or increase, due to muscle potentiation (Brown & Euler 1938). Potentiation is thought to arise from an increase in sensitivity of the contractile apparatus to Ca^{2+} (Grange, Vandenboom & Houston 1993; Sweeney, Bowman & Stull 1993; Vandenboom, Grange & Houston 1993), or an increased magnitude of Ca^{2+} transients in response to each efferent volley (Close & Hoh 1968). Potentiation effects are proposed to be more pronounced at low rates of stimulation that are required to produce submaximal muscle contractions (Vandenboom, Grange *et al.* 1993; MacIntosh & Willis 2000).

Some muscles contain a mixture of muscle fibres types, for example the cat medial gastrocnemius muscle contains fast and slow twitch fibres. Other muscles contain predominantly one type of muscle fibre, such as the cat soleus muscle, which contains slow twitch fibres (Ariano, Armstrong & Edgerton 1973). The stimulation frequency required to produce tetanic contractions (fusion frequency) is related to the contraction speed of the muscle. Muscles that contain fast twitch fibres have a higher fusion frequency because the twitches that are generated by these fibres are briefer, so a higher stimulation frequency is required for complete twitch summation (McPhedran, Wuerker & Henneman 1965). Slow twitch muscle has slower twitch times, therefore

fused contractions can be produce at lower stimulation rates (Wuerker, McPhedran & Henneman 1965).

A number of muscle fibres are innervated by one motoneuron and together they are called a motor unit (Edstrom & Kugelberg 1968; Burke & Tsairis 1973). There is a close relationship between the number and type of muscle fibres and the properties of the motoneuron that innervate them. It is generally assumed that all muscle fibres belonging to a single motor unit have the same properties. Motoneurons that are small and slowly conducting, typically innervate only a few slow twitch muscle fibres, whereas larger diameter, rapidly conducting motoneurons typically have larger innervation ratios and innervate fast twitch muscle fibres. The CNS employs activation strategies that take advantage of the differences in motor unit properties during graded muscle contraction.

Physiological Activation of Muscle

One important feature of a voluntary contraction is that it is characterised by asynchronous activation of motor units (Bigland & Lippold 1954), meaning that individual motor units contract independently and seldom contract at the same time (Milner-Brown, Stein & Yemm 1973). Asynchronous activation of muscle allows smooth, low force contractions to be generated, even when only a small number of motor units are active. The contraction is smoothed at the tendon by summation of the tension developed from each active motor unit. The overlapping tension profiles of individual motor units mean that the tendon does not have to be fully re-stretched by each twitch, as tension is maintained in the muscle-tendon complex (Lind & Petrofsky 1978). Smooth contractions ensure that there is minimal internal motion within the muscle, thus optimising cross-bridge functioning (Joyce, Rack & Westbury 1969). Under most conditions motor units fire asynchronously, although there are some conditions where motor units are synchronously activated, for instance after fatigue, where there is noticeable muscle tremor (Lippold 1981).

There are two basic strategies that the CNS employs to grade contraction strength; motor unit recruitment and rate modulation (Adrian & Bronk 1929; Smith 1934; Lindsley 1935; Denny-Brown 1949; Bigland & Lippold 1954). Motor units are recruited in order of increasing diameter of their motoneurons (Henneman, Somjen & Carpenter 1965a; Henneman, Somjen & Carpenter 1965b). As the contraction becomes stronger, progressively larger motor units of the fast twitch variety are activated, with

the most fatigable units recruited as the contraction approaches maximal strength. Generally there is a linear relationship between motor unit size and the force level at which units are recruited (Milner-Brown, Stein *et al.* 1973; Monster & Chan 1977; Yemm 1977), although in some forms of muscle activity the fast motor units can be recruited first, for example in ballistic movements requiring rapid muscle contractions (Grimby 1984; Zehr & Sale 1994).

An increase in contraction strength can also be achieved by increasing the motor unit firing rate. Monster & Chan (1977) recorded from motor units with intramuscular microelectrodes and showed that as the force of a voluntary contraction increased so did the firing rate of the active motor units. The initial firing rate for most motor units was 8pps and this increased to 20-25pps with higher contraction forces. It has been suggested that motor units can discharge steadily at rates as low as 5-12pps during sustained contractions (Smith 1934; Clamann 1970; Monster & Chan 1977; Freund 1983). The highest rates of human motor unit firing are somewhat more controversial and values range from 70-90pps (Marsden, Meadows & Merton 1971) to 140pps (Norris & Gasteiger 1955). The most likely range of discharge frequencies during a steady contraction is 8-40pps (Bigland & Lippold 1954; Milner-Brown, Stein *et al.* 1973). Firing rates also depend on the type of muscle activated with lower rates observed during steady maximal contractions of the slow twitch soleus muscle (11pps) compared to the mixed biceps brachii muscle (31pps) (Bellemare, Woods, Johansson & Bigland-Ritchie 1983).

Motor unit firing rates are known to vary over the duration of a contraction. It is thought that the variation in firing rate enables the motor system to optimise force by utilising certain properties of the muscle. At the onset of a contraction, motor units fire with a brief high frequency burst (Denslow 1948; Garnet & Maton 1989; Maton & Gamet 1989) that has been shown to enhance muscle force during a contraction. The effect has been called the 'catch-like property', whereby greater forces can be developed by a muscle if an extra stimulus, or 'doublet', is incorporated at the start of activation (Burke, Rudomin & Zajac 1970; Burke, Rudomin *et al.* 1976; Binder-Macleod & Clamann 1989; Binder-Macleod & Lee 1996). Although motor unit firing rates are initially high, during the course of a strong contraction they tend to decline (Marsden, Meadows *et al.* 1971). This reduction in firing rate was called 'muscular wisdom', and it was suggested that the decline helped to optimise contraction force. As twitch duration increased, a fully fused contraction could be maintained at a lower

activation rate (Marsden, Meadows & Merton 1976; Dietz 1978; Bigland-Ritchie, Johansson, Lippold, Smith & Woods 1983; Marsden, Meadows & Merton 1983).

In summary, motor units fire at low rates during submaximal contractions. The CNS can achieve smooth low force contractions by asynchronous activation of motor units, even though the firing rates of the individual motor units are low. When stronger contractions are required, the CNS can increase the firing rate of the active motor units and recruit larger motor units. When we artificially activate muscle in the laboratory or in the clinic it has proven difficult, if not impossible, to precisely emulate the CNS.

Artificial Activation of Muscle

Muscle can be activated by electrically stimulating motoneurons. This can be achieved at the level of the spinal cord, in the peripheral nerve trunk, or by intramuscular stimulation of preterminal endings. There are several important differences between electrical stimulation and physiological activation of muscle that make electrical stimulation a less than ideal way to activate muscle.

Muscle is most commonly activated electrically by synchronously stimulating all of the recruited motor units with each stimulating pulse. This is quite different to the asynchronous activation used by the CNS, where a number of motor units are activated independently. When muscle is stimulated synchronously using low rates of stimulation, the contractions are unfused. The muscle responds to each stimulating pulse producing contractions with tension ripple. In order to achieve fused contractions high stimulation rates are required, particularly for fast twitch muscle (Cooper & Eccles 1930).

The CNS can grade contraction strength by recruiting additional motor units. Some techniques of electrical stimulation have tried to employ this strategy by adjusting the intensity of the electrical stimulus to alter the number of motor units activated by each stimulating pulse. However, the steep non-linear relationship between stimulus intensity and level of recruitment makes this an unreliable method of grading contraction strength. Recruitment characteristics also vary with changes in muscle length and electrode placement (Crago, Peckham & Thrope 1980; Boom, Mulder & Veltink 1993). Additionally, in whole nerve stimulation, there is a reversal of the recruitment order (Gorman & Mortimer 1983). The largest and most fatigable motor units, recruited last during voluntary activation, are activated first by electrical stimulation. As the stimulus intensity increases, progressively smaller motor units are

recruited. Various methods of shaping the stimulus pulse or providing high frequency anodal blocking stimulation have been tried in order to achieve a physiological recruitment order but the reliability of such methods remains debatable (Petrofsky 1978). Therefore, the most reliable way to grade contraction strength during electrical stimulation is to use a supramaximal stimulus and adjust the stimulation rate.

Distributed Stimulation Using Equal Intervals

An experimental preparation, developed by Rack & Westbury (1969), enables muscle to be stimulated in a more physiological manner. The preparation used was the soleus muscle of the anaesthetised cat. Rack & Westbury (1969) divided the muscle's ventral root supply into several functionally separate portions and then stimulated each portion in rotation, with equal time intervals between each stimulating pulse. By asynchronously activating the separate muscle portions in a sequential fashion, they were able to produced smooth contractions over a wide range of muscle force using physiological rates of stimulation (3pps to 35pps). The experiments carried out using this method of muscle stimulation were the first to examine the properties of a slow twitch muscle when activated submaximally using low stimulation rates.

There were technical limitations with this method, as it required separation of the ventral roots into portions that produced near equal twitch tension (Peckham, Van Der Meulen & Reswick 1970). If the tension contribution from each portion became uneven then contractions generated tension ripple at the base stimulation frequency. The method of distributed stimulation was well suited for use with slow twitch muscle because the ventral root portions could be arranged so that they developed equal tension, and importantly, the fatigue resistant property of the muscle ensured that tension remained equal during long periods of stimulation (Burke, Levine *et al.* 1973).

The reliance on equal tension contributions from each portion meant that the method of distributed stimulation was less suitable for stimulating muscle containing a mixture of muscle fibre types, such as the cat medial gastrocnemius. Due to the influence of stimulation history on muscle tension, most noticeably the competing effects of potentiation and fatigue, what started off as equal portions could quickly become unequal. If tension contributions became unequal, then the contraction developed tension ripple at the stimulation frequency.

Distributed Stimulation Using Optimised Intervals

A stimulator has been developed that has released the method of distributed stimulation from the limitation that muscle portions must maintain equal tension (Brown, Huang, Morgan, Proske & Wise 1999). The stimulator was designed to continually adjust the pattern of stimuli to account for any changes in the tension contribution from each muscle portion. The Rack & Westbury (1969) method of distributed stimulation used equal intervals between stimulation of each muscle portion, whereas the method described in this thesis 'optimises' interpulse intervals. It is hypothesised that by continuously adjusting the interval between stimulation of successive muscle portions, smooth, low force contractions can be generated and maintained even though the tension contribution from each portion may change over the period of stimulation. A description of the stimulator and the optimisation process is presented in chapter five.

One of the aims of this half of the thesis was to test the functioning of the stimulator and determine whether it could produce and maintain smooth submaximal contractions of a muscle containing a mixture of muscle fibre types. Once this had been established, experiments were carried out to examine some basic properties of mixed muscles when activated at low stimulation rates. The properties measured include force-frequency, length-tension, and force-velocity relationships. The following section discusses some of the background literature relating to these muscle properties.

Mechanical Properties of Submaximally Activated Muscle

Much of the existing knowledge of muscle properties has come from studies involving maximally activated muscle and the behaviour of submaximally activated muscle has often been inferred from scaled models. However, muscle exhibits different mechanical properties depending upon the level of activation, properties that in some respects are unexpected and which are not adequately described by scaled versions of the fully activated muscle (Guimaraes, Herzog, Hulliger, Zhang & Day 1994; Huijing 1998).

Rack & Westbury (1969) examined the mechanical properties of slow twitch muscle when stimulated at low rates using distributed stimulation. They found that muscle contractions produced during distributed stimulation were more fused and developed greater tension than the unfused contractions generated by synchronous

stimulation at the same rate. They concluded that muscle was best able to generate tension when there was little or no change in muscle fibre length and the contraction approached a truly isometric condition. It is believed that the internal motion present in an unfused contraction decreases the number of attached cross-bridges, so that the force generating capacity of the muscle contraction is reduced. In addition, for fused contractions, motor units are able to produce tension without having to re-stretch the tendon during each period of activation (Lind & Petrofsky 1978).

Length-Tension Relationship

There is a well defined relationship between the length of a muscle and the maximum tension that it can generate (Ramsey & Street 1940). The sliding filament theory can explain the length-tension properties of fully activated muscle (Huxley & Niedergerke 1954; Huxley & Hanson 1954; Huxley 1957). The theory was based on the observation that muscle fibres contain a number of units arranged in series, called sarcomeres. The sarcomeres contain thick myosin and thin actin filaments that slide past each other, essentially without changing length. According to the theory, force is generated by the formation of independent force generating structures between myosin and actin filaments called cross-bridges. The maximum isometric force that a sarcomere can generate is related to the overlap of the myosin and actin filaments, which determines the number of available binding sites for cross-bridge formation (Gordon, Huxley & Julian 1966). When overlap is optimal, a maximum number of cross-bridge sites are available to generate force. The muscle length that produces maximum force is commonly called the optimal length (L_{opt}). If the muscle is stretched beyond the optimal length then there is less filament overlap, reducing the number of potential attachment sites, and therefore the force produced by an isometric contraction is lower. If the muscle is shortened from the optimal length, then there are fewer sites available due to compression of the myosin filaments against Z-lines.

When muscle is activated submaximally the effects of filament overlap alone cannot fully describe the muscle length-tension relationship. The length-tension relationship was shown to shift its peak towards longer lengths for submaximal activation of the cat soleus muscle (Rack & Westbury 1969), the cat medial gastrocnemius muscle (Heckman, Weytjens & Loeb 1992), and the rat medial gastrocnemius muscle (Roszek, Baan & Huijing 1994). It was suggested that the processes involved in muscle activation exhibit a length dependence that becomes

apparent during submaximal activation. At short muscle lengths the activation processes were less effective than at long muscle lengths (Rack & Westbury 1969). Two mechanisms have been observed that are likely to contribute to this activation length dependence. Endo (1972) showed in skinned frog and toad muscle fibres that a given submaximal concentration of Ca^{2+} is more effective at long lengths than short lengths. In the skinned muscle fibre preparation the sarcolemma is made permeable with glycerol allowing the intracellular ionic environment to be manipulated. By adjusting the intracellular pCa levels, the sensitivity of the contractile mechanisms to Ca^{2+} was found to be greater at longer muscle lengths (Endo 1972; Stephenson & Williams 1981; Stephenson & Williams 1982; Stephenson & Wendt 1984; Stienen, Blange & Treijtel 1985).

Rudel & Taylor (1971) showed that the twitch potentiators caffeine and Zn^{2+} could potentiate tetani at short muscle lengths, when using a stimulus rate that was maximal at long lengths (Rudel & Taylor 1971). It was suggested that Ca^{2+} induced activation is normally less than maximal along the ascending and plateau region of the length-tension curve (Lopez, Wanek & Taylor 1981). These effects were dependent on the level of muscle activation and disappeared when the muscle was fully activated (Taylor, Lopez, Griffiths, Trube & Cecchi 1982).

Force-Velocity Relationship

Whether a muscle shortens or lengthens during a contraction depends on the load and the task being performed. There is a well defined relationship between the force that a muscle can produce during a constant velocity length change and the velocity of that length change. While examining the heat produced during muscle contractions, Hill (1938) showed that the velocity at which frog sartorius muscle could shorten decreased as the load increased. He described the relationship between force (load) and shortening velocity with a rectangular hyperbola, having a maximum shortening velocity at zero load. He also noted that for loads exceeding isometric tension, that is, where the muscle was forcibly lengthened, the force-velocity relationship became more complicated (Hill 1938).

Tension exerted by a fully activated muscle during lengthening rises above isometric tension and during shortening falls below isometric tension (Katz 1939; Abbott & Aubert 1952). Early models of muscle force-velocity relationships have based their values on the data obtained from fully activated muscle and inferred the behaviour

of submaximally activated muscle from scaled versions of the models (Huxley 1957). However, alterations to the force-velocity relationship were observed when muscle was submaximally activated. The experiments performed by Joyce, Rack & Westbury (1969) on the cat soleus muscle showed that tension developed at low stimulation rates in response to constant velocity muscle lengthening fell below the isometric levels at the same muscle length (see figure 6.23). Models of the force-velocity relationships of the slow twitch soleus muscle have incorporated the activation level of the muscle by making the rate constant for cross-bridge attachment dependent on the level of activation (Zahalak 1986) (see the appendix). However the behaviour of submaximally activated mixed muscle, containing fast as well as slow muscle fibres, has received relatively less attention, partly due to the difficulties in producing in such muscles, smooth graded contractions that are submaximal. Does a muscle containing a mixture of fibre types behave in the same way as the cat soleus muscle when submaximally activated?

Functional Electrical Stimulation

The second aim of this half of the thesis was motivated by more functional purposes and aimed to explore the potential use of optimised distributed stimulation in rehabilitative medicine. Skeletal muscle can be electrically stimulated to restore some function to the paralysed limbs of paraplegics and hemiplegics. This method, called Functional Electrical Stimulation (FES), involves stimulating muscles via implanted or surface electrodes. Although the activation of paralysed limbs with electrical stimulation was tried more than 200 years ago (Franklin, 1757) it is only relatively recently that the method was used successfully to restore function to paralysed limbs (Stein, Peckham & Popovic 1992). The first successful implementation of FES came from the work of Liberson (1961) in hemiplegic patients with footdrop. Stimulation of the peroneal nerve activated the tibialis anterior muscle to dorsiflex the ankle during locomotion (Stein, Peckham *et al.* 1992). Since then the use of FES has been extended to restore muscle function for activities such as standing, hand grasp and cycling (Peckham, Keith & Crago 1988).

In most FES applications muscle is stimulated synchronously at high rates in order to produce fused contractions (Peckham, Van Der Meulen *et al.* 1970; Bigland-Ritchie, Jones & Woods 1979). With this type of stimulation the muscle fatigues rapidly and tension falls soon after the onset of stimulation (Kugelberg & Edstrom 1968). A

common method to improve the fatigability of the paralysed muscle is to exercise the muscle with a regime of low frequency electrical stimulation. This converts fast twitch fibres into slow twitch, fatigue resistant fibres and shifts the fusion-frequency relationship to lower rates (Salmons & Vrbova 1969; Peckham, Mortimer & Marsolais 1976; Lenman, Tulley, Vrbova, Dimitrijevic & Towle 1989). Although this improves the fatigability of the muscle, the maximal force and the velocity at which the muscle can contract is dramatically reduced compared to a normal subject.

An additional consequence of synchronous stimulation is that the ability to grade contraction strength is limited. High stimulation rates are required to produce fused contractions and therefore contractions typically generate near maximal force. However, it is commonly desirable to produce contractions of low force for slow movements or the adoption of a given posture to support light loads. With synchronous stimulation at low rates muscle contractions become unfused leading to jerky movements that are not useful from a functional point of view.

Rapid muscle fatigue is a major problem in FES (Peckham, Mortimer *et al.* 1976; Isakov, Mizrahi & Najenson 1986; Marsolais & Edwards 1988; Boom, Mulder *et al.* 1993; Franken, Veltink, Van Harn & Boom 1994; Binder-Macleod & Russ 1999). If optimised distributed stimulation could be used in FES then this would enable the use of lower stimulation rates which would presumably lead to less muscle fatigue and provide a significant improvement over the current techniques of muscle stimulation. Experiments carried out in this thesis aimed to examine the advantage, in terms of muscle fatigue, provided by optimised distributed stimulation at low rates compared to the conventional method of muscle activation using synchronous stimulation at high rates. The following section is a brief discussion of the processes involved in muscle activation and the points at which fatigue can occur during low frequency and high frequency stimulation.

Muscle Fatigue

Muscle fatigue can be broadly defined as a decrease in muscle contraction force during or after a period of activity. Changes that can occur as a result of fatigue include changes in contraction speed, power and relaxation time (Fitts 1994). Factors that determine the fatigability of a muscle include the muscle fibre type, the level of activation, metabolic factors related to energy supply and substrate build-up, as well as task dependent factors such as the duty cycle. Normally the motor system is activated in

a task specific way in order to derive maximum performance at minimal energy expenditure. For instance, slow twitch fatigue resistant motor units are used in the maintenance of posture, where long lasting, low force contractions are required. When faster more forceful contractions are needed, for tasks like jumping or locomotion, then larger fast twitch motor units are recruited (De Luca, LeFever, McCue & Xenakis 1982). However, as discussed previously, it is difficult to imitate the physiological activation of muscle that occurs during voluntary contractions. When we artificially activated muscles with electrical stimulation we do so in a way that can lead to rapid fatigue.

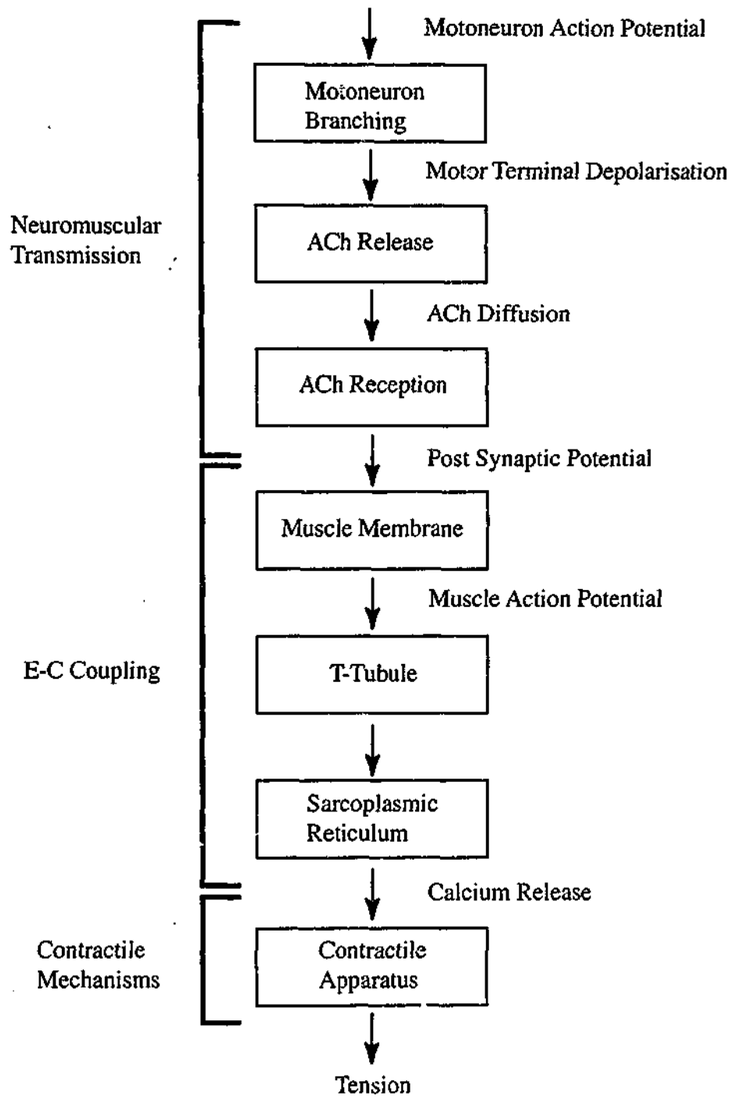
There are several sites along the motor pathway extending from cortex to muscle that can contribute to muscle fatigue. This introduction will focus on the peripheral mechanisms responsible for fatigue, that is, sites that are distal to the spinal cord. Effects related to 'central fatigue' will not be discussed as the experiments described in this thesis used electrical stimulation of muscle and therefore were not influenced by cortical or spinal reflex mediated mechanisms. To simplify the discussion the processes involved in muscle activation and the potential sites of fatigue have been separated into three areas that are summarised in figure 4.1.

Neuromuscular Transmission

When muscle is activated with high stimulation rates it fatigues rapidly. A significant factor contributing to high frequency fatigue is neuromuscular transmission failure (see figure 4.1). Neuromuscular transmission involves the propagation of motoneuron action potentials, the release and diffusion of Acetylcholine (ACh), and the initiation of the muscle action potential. Under certain conditions the neuromuscular system is susceptible to transmission failure, that is, failure of motoneuron action potentials to fully excite the muscle membrane. There is a relationship between the rate of neural stimulation and the extent of neuromuscular fatigue (Krnjevic & Miledi 1958; Clamann & Robinson 1985; Sandercock, Faulkner, Albers & Abbrecht 1985; Kuei, Shadmehr & Sieck 1990).

An early study on neuromuscular transmission failure showed a rapid decrease in muscle tension when the medial gastrocnemius muscle of the cat was activated via its motor nerve at high rates of stimulation (40-200pps). If the stimulation was then switched to directly activate the muscle, effectively bypassing the motoneuron and neuromuscular junction, there was significant recovery in tension (Brown & Burns

Figure 4.1. Summary of the processes involved in the activation of skeletal muscle and potential sites of muscle fatigue (shown in box). A motoneuron action potential depolarises the motoneuron terminal. ACh is released onto the post synaptic membrane. Depolarisation of the muscle membrane produces a muscle action potential that travels along the sarcolemma and into the T-tubules, leading to excitation of the sarcoplasmic reticulum. Calcium is released into the intracellular environment and interacts with actin filaments. Links form between the actin and myosin filaments and tension is generated. Muscle fatigue can occur at any site along this pathway.



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1949). This finding indicated that failure of neuromuscular transmission was a significant factor in the reduction in force during high frequency stimulation. Additional evidence supporting this idea came from in-vitro experiments using caffeine to produce contracture of the fatigued muscle. The muscle was fatigued with high rates of stimulation and caffeine was then added to the organ bath. Caffeine at high concentrations directly activates the sarcoplasmic reticulum causing the release of Ca^{2+} and muscle contracture, a process that normally depends on electrical excitation. There was almost full recovery of the force suggesting that the contractile mechanisms and Ca^{2+} supply were relatively unfatigued by the higher stimulation rates (Lannergren & Westerblad 1989; Edman & Lou 1992).

Fast fatigable motor units are particularly susceptible to neuromuscular transmission failure (Stephens & Taylor 1972; Bodine, Roy, Eldred & Edgerton 1987). Evidence supporting this claim has come from experiments that showed less depletion of glycogen stores when muscle fibres in the rat diaphragm were stimulated via the phrenic nerve compared to stimulation of the muscle fibres directly (Johnson & Sieck 1993). The authors concluded that all fibre types, particularly the FF, are susceptible to neuromuscular transmission failure and that the extent of neuromuscular transmission failure was greatest at the higher rates of stimulation.

Neuromuscular transmission failure can occur at several different locations. For example, the blocking of motoneuron action potentials at branch points in the intramuscular neuron (Smith 1980). The branching points of a motoneuron, where the neuron bifurcates to innervate muscle fibres, have been identified as regions of low safety for neural conduction and have been implicated as a contributing factor in the failure of neuromuscular transmission during fatigue for stimulation rates of 10pps or more (Krnjevic & Miledi 1958). The fine nerve terminals in the most distal branches were the first to fail (Hatt & Smith 1976), and the mechanism thought to be responsible for this failure was depolarisation of the motoneuron membrane due to K^+ accumulation that caused inactivation of the Na^+ channels (Hodgkin & Huxley 1952b; Smith 1980).

ACh release and uptake from the terminal of the motoneuron and the sensitivity of the post synaptic membrane to ACh have also been implicated in neuromuscular transmission failure. Experiments have shown that after a period of stimulation, depolarisation of the motoneuron terminal failed to initiate muscle action potentials. The mechanisms thought responsible in this case were a pre-junctional deficiency in the

release of ACh and a decreased sensitivity of the post synaptic membrane to ACh (Katz & Thesleff 1957; Krnjevic & Miledi 1958; Thesleff 1959).

Excitation-Contraction Coupling

Excitation-contraction (EC) coupling involves the depolarisation of the muscle membrane, the propagation of the muscle action potential along the sarcolemma and into the T-tubule, the release and uptake of Ca^{2+}, and Ca^{2+} activation of the contractile machinery. For muscle activation at low stimulation rates, fatigue is likely to be associated with E-C coupling failure and/or a reduced force generating capacity of the contractile mechanisms (Edwards, Hill, Jones & Merton 1977) (see figure 4.1).

If a muscle is repetitively activated the ionic balance of the muscle membrane can be affected. Usually the Na^+/K^+ pump acts to restore ion concentrations but under some fatigue conditions it is not able to maintain ionic equilibrium. K^+ ions can accumulate in the extracellular environment, altering sarcolemma function, reducing the resting membrane potential and therefore preventing membrane depolarisation and propagation of the muscle action potential (Sjogaard 1991; Edman & Lou 1992; Fitts 1994).

The rises in extracellular K^+ concentrations would particularly effect conduction within the T-tubular system. The narrow T-tubule lumen restricts ion diffusion (Hodgkin & Horowicz 1959), and in combination with the higher permeability to K^+ and lower density of the Na^+/K^+ pump, compared to the surface membrane (Eisenberg & Gage 1969), would promote K^+ build-up and membrane depolarisation (Fambrough, Wolitzky, Tamkun & Takeyasu 1987). As the T-tubules are particularly susceptible to ionic build-up, the inward spread of current into the muscle can be affected, thus preventing activation of the innermost muscle fibres (Stalberg 1966; Gydikov, Kostov, Kossev & Kosarov 1984; Fitts 1994).

The release of Ca^{2+} from the sarcoplasmic reticulum (SR) can be compromised by impaired coupling between the T-tubule and the SR membrane. In addition, reduced levels of Ca^{2+} stores and alterations in Ca^{2+} channel functioning can decrease the Ca^{2+} release from the SR in response to electrical stimuli. Application of caffeine, to produce muscle contracture, has been shown to reverse the loss of force production from fatigued muscle fibres (Lannergren & Westerblad 1989). Raised intracellular concentrations of Ca^{2+} have also been shown to affect the processes of E-C coupling (Lamb, Junankar & Stephenson 1995). Altered sarcolemma functioning can decrease

the conduction velocity and increase the duration of the muscle action potential measured during continuous electrical stimulation of single motor units (Stalberg 1966) and in the entire muscle (Lindstrom, Kadefors & Petersen 1977; Gydikov, Kostov *et al.* 1984).

Contractile Mechanisms

Muscle fatigue can be associated with altered cross-bridge functioning. Experiments measuring muscle stiffness after fatigue showed that the decrease in force (25%) was not accompanied by a decrease in stiffness of the same magnitude (9%). This suggested that fatigue was not entirely due to fewer numbers of attached cross-bridges, but rather, due to alterations in cross-bridge kinetics that caused a reduction in force output from the cross-bridges themselves (Edman & Lou 1992). It has been suggested that cross-bridge kinetics can be affected by changes in intracellular substrate concentrations brought about by adenosine triphosphate hydrolysis, increases in adenosine diphosphate, inorganic phosphate from the breakdown of phosphocreatine and H^+ from lactic acid (Fitts 1994). For instance, in skinned muscle fibre preparations, increases in inorganic phosphate concentration were shown to decrease the isometric force (Altringham & Johnston 1985; Cooke, Franks, Luciani & Pate 1988). The authors suggested that the high inorganic phosphate levels increased the proportion of cross-bridges that were in a low-force state. Analysis of the intracellular Ca^{2+} concentrations with fura 2 showed that moderate fatigue was not associated with a decrease in Ca^{2+} transients, supporting the claim that the muscle was fully activated. However, in more extreme fatigue a decrease in Ca^{2+} transients was observed (Lee, Westerblad & Allen 1991).

The Electromyogram

Insight into the electrical activation of muscle can be gained by measuring the EMG from surface electrodes or by means of implanted intramuscular wires. When a muscle is activated with electrical stimulation the EMG represent the summed activity of action potentials from active motor units in response to each electrical stimulus. EMG can be quantified by measuring the spike duration and peak-to-peak amplitude, which are indicative of the temporal summation of muscle action potentials. EMG area, or integral, is a compound measure that depends on the timing and amplitude of muscle action potentials (Enoka, Rankin, Stuart & Volz 1989). Relationships between muscle

force and EMG have been studied extensively for both voluntarily activation and electrical stimulation of skeletal muscle. Using distributed stimulation of cat soleus muscle Guimaraes *et al.* (1994) showed a linear relationship between rectified, integrated EMG and muscle force for isometric contractions producing between 5% and 88% of maximum force. Similarly, in the unfatigued state, there was a linear relationship between force and EMG for voluntary contractions (Bigland & Lippold 1954; Stephens & Taylor 1972).

EMG has been used as an indicator of muscle fatigue and correlates well with muscle force during high frequency stimulation but it is less reliable when the muscle is fatigued using low stimulation rates (Sandercock, Faulkner *et al.* 1985). Changes in the EMG elicited with electrical stimulation, with factors like muscle length and recruitment level kept constant, may indicate alterations in neuromuscular transmission and propagation of the muscle action potential. A reduction in EMG levels would indicate that the electrical activation of the muscle has decreased, suggesting altered functioning of the muscle action potential or processes upstream from it (see figure 4.1). If there is no change in the EMG then this would suggest that any decline in muscle force can be attributed to fatigue at sites beyond the muscle action potential, such as E-C coupling or contractile mechanisms.

In summary, the material presented indicates that the sites of muscle fatigue are quite different for high frequency and low frequency stimulation. Muscle fatigue from high frequency stimulation is likely to be associated with neuromuscular transmission failure whereas low frequency fatigue is likely to be caused by factors relating to EC coupling and cross-bridge functioning. As previously mentioned, strategies that are used to activate skeletal muscle, in applications like FES, generally use high frequency stimulation delivered synchronously to the entire muscle. Stimulation at high frequencies initially produces contractions that generate high forces but are susceptible to rapid muscle fatigue. If paralysed muscle could be stimulated in a way that minimises muscle fatigue then this would significantly improve the functional effectiveness of this rehabilitative technique. A system that enables the user to generate reproducible muscle contractions, which can be graded over a wide range of force levels, would provide a great advantage over the methods that are currently available.

Chapter Five

The Distributed Stimulator

Hardware

The stimulator used for optimised distributed stimulation was developed by Dr. Ying Huang as part of her PhD thesis and has been described in Brown *et al.* (1999). The stimulator was a custom made eight channel stimulator incorporating a CS-64180 miniature microprocessor with software and two CS-AIO16/4 analog input/output cards (figure 5.1). One analog input was used to record tension from a strain gauge and up to eight analog output channels were used to deliver constant voltage (2-10V) square wave pulses (100μsec). Each stimulating channel was isolated inductively with a stimulus isolation unit (SIU5, Grass Instrument, USA) and delivered pulses to bundles of motoneurons via custom made bipolar platinum electrodes. The stimulation parameters were controlled by the commercial software package Igor Pro (Wavemetrics, USA) run on a 6100/66 Macintosh computer that was connected to the stimulator via the serial port.

Software

The operating software was programmed on a Macintosh using Igor Pro (Wavemetrics, USA). It consisted of a custom designed user interface and an optimising algorithm. The user interface enabled the operator to set the various stimulation parameters, which included the number of stimulating channels, pulse width, stimulus duration, sampling interval and the stimulus intensity for each channel. The stimulator could be set to provide continuous or intermittent stimulation. Once the stimulation parameters were determined they were passed via the serial port to the stimulator where the stimulating pulses were generated. Muscle tension was measured and the signal relayed back to the operating software through the serial port.

The stimulator provided three modes of stimulation. In the synchronous mode, all n filaments were stimulated together at p pulses per second, the fundamental frequency. In the uniform distributed mode, the filaments were stimulated in sequence, each still at p pulses per second. The interpulse intervals, the times between stimulating a filament and stimulating the next filament, were all $1/np$ seconds. In the optimised mode, these interpulse intervals were adjusted to minimise the component of tension at the fundamental rate.

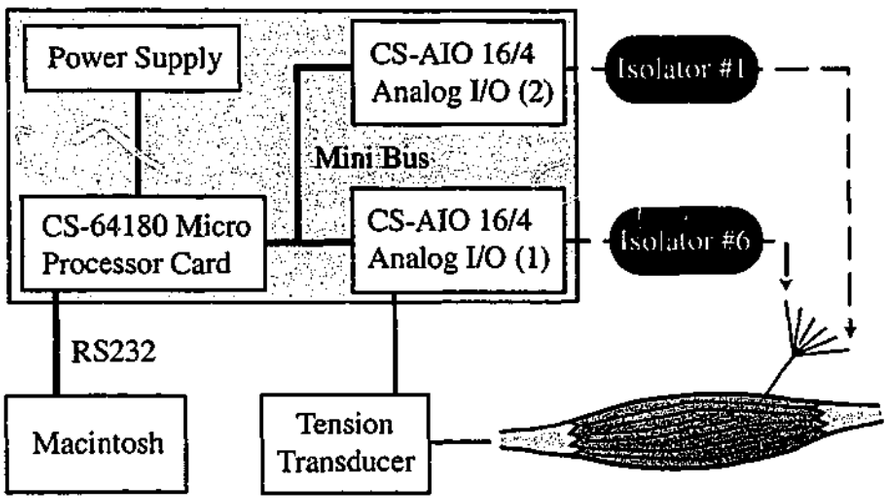


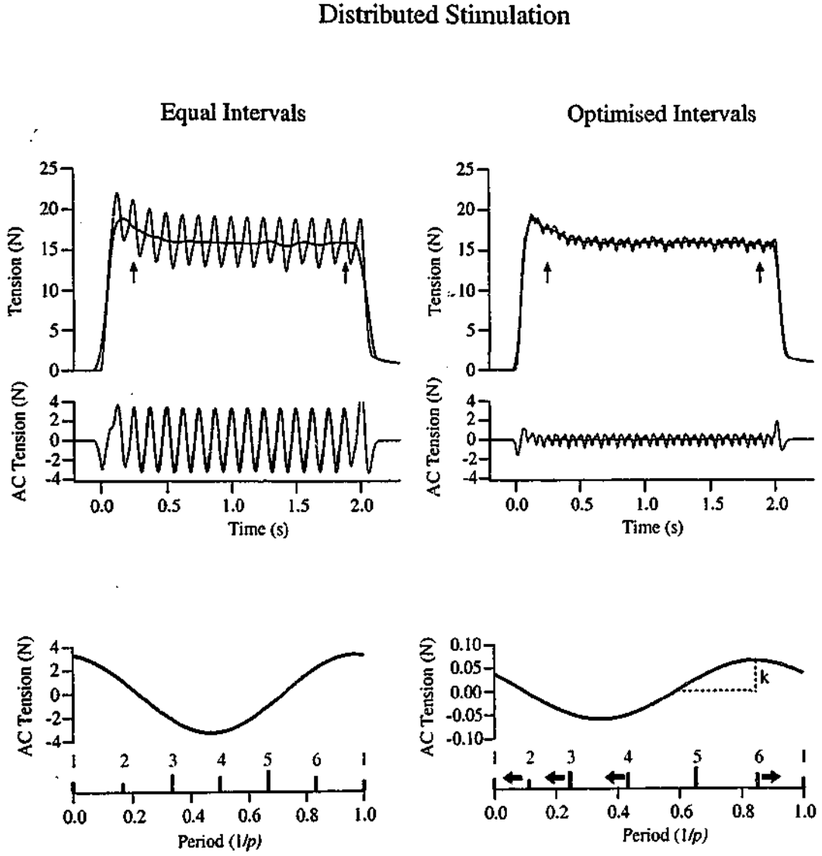
Figure 5.1. Diagrammatic illustration of the distributed stimulator and muscle preparation. The stimulator was connected via the serial port (RS232) to a Macintosh computer that controlled the stimulus parameters and optimising algorithm. The nerve supply to the muscle was dissected into *n* separate portions, usually five or six. Each stimulating channel was used to deliver stimulating pulses to individual nerve bundles to activate separate parts of the muscle. Muscle tension was transmitted back, via the serial port, to the computer (Brown *et al.* 1999).

The optimisation procedure aimed to reduce the fundamental component of the tension ripple, that is, by optimally adjusting interpulse intervals, tension fluctuations during stimulation were minimised. The tension signal of the preceding contraction was fed back to the optimisation algorithm and used to calculate a new set of interpulse intervals. This process was repeated iteratively until the fundamental ripple (FRip) was reduced to levels less than 1% of average tension.

The optimisation process involved measurement of the tension ripple by subtracting a smoothed version of the tension record (average tension over $1/p$) from the tension record to remove the steady component of tension (figure 5.2). A sinusoid with the period $1/p$ was fitted to a section of the ripple trace by adjusting amplitude and phase. To get the timing for the next contraction, each stimulus train was delayed by an amount proportional to the amplitude of the fitted sinusoid at the time at which stimulus pulses in that train occurred. The end result was that small twitches were effectively brought closer together while larger twitches were spread further apart. This iterative systematic shuffling of interpulse intervals reduced the amplitude of the fitted sinusoid. Three measurements of tension ripple were calculated and each were expressed as a percentage of average tension (AvTen), where k is the amplitude of the fitted sinusoid;

- RMS % fundamental ripple = 70.7*k/AvTen (with sinusoid period = $1/p$)
- RMS % second harmonic = 70.7*k/AvTen (with sinusoid period = $1/(2p)$)
- Peak-to-peak tension ripple = (Maximum – Minimum)/AvTen*100

Figure 5.2. Optimisation process showing tension during distributed stimulation using equal interpulse intervals (*left panel*) and optimised interpulse interval (*right panel*). In the *upper panel*, a smoothed tension trace (black) was subtracted from the raw tension trace (red). In the *middle panel*, a sinusoid (blue trace T =1/*p*), was fitted to a section of the ripple trace (red trace). The RMS fundamental ripple (FRip) was calculated using the fitted sinusoid. For the example using equal interpulse intervals (*left panel*), the FRip= 70.7*3.3/16 = 14.6% of average tension. The peak-to-peak ripple (PP) was calculated from the tension ripple trace (*middle trace* - red) and here the PP = (8.9/16)*100 = 55.6% of average tension. The *lower panel* shows the fitted sinusoid over one period (1/*p*) of stimulation. The timing of each stimulation pulse is shown at the bottom of the graph (numbered 1-6) and the twitch amplitudes of each muscle portion are represented by the height of each line. When the interpulse intervals were optimised (*right panel*) pulses were delayed by an amount proportional to the fitted sinusoid at the time of the pulse. Pulses were closer together when tension was falling and further apart when tension was rising. Notice that the amplitude of the fitted sinusoid is smaller after the optimisation process (note different scale). The FRip of the optimised trace is now 0.27% of average tension. The PP values have also been reduced and are now 9.4% of average tension.



Chapter Six

Properties of Submaximally Activated Skeletal Muscle

Aims

Much of the existing knowledge of the mechanical properties of skeletal muscle has come from experiments that used synchronously activated muscle to produce contractions generating maximal or near maximal force. Very few studies have examined the mechanical properties of muscle during contractions that generate submaximal forces. This is especially true for muscle containing a mixture of fibre types and is partly due to the difficulty in generating smooth muscle contractions at low stimulation rates. Experiments in this chapter were carried out to evaluate a new method of using distributed stimulation to asynchronously activate muscle. By optimising interpulse intervals, to adjust for tension variations due to fatigue and potentiation, smooth contractions at low stimulation rates were produced and a number of muscle properties were examined.

The specific aims of these experiments were to evaluate the optimisation algorithm and determine whether optimisation of interpulse intervals can eliminate tension ripple and generate smooth submaximal muscle contractions of slow and mixed muscles. These experiments also aimed to examine muscle properties during submaximal asynchronous activation at low stimulation rates. The specific muscle properties examined were the force-frequency and length-tension relationship in the cat medial gastrocnemius muscle, the cat soleus muscle and the rat triceps surae muscle group, in addition to the force-velocity relationship of the rat triceps surae muscle group.

Muscle tension was fed back to the analysis software to obtain a measure of fundamental ripple that was required to optimise interpulse intervals. However, any signal that can provide a reliable indication of the fundamental tension ripple should, theoretically, be useable. Two alternative sources of feedback signals were monitored to determine whether they could be used as an alternative to whole muscle tension. Here, the longer-term aim was kept in mind, to use optimised distributed stimulation in human applications, where it would be difficult to get a direct measure of whole muscle tension.

Method

Two animal species were used in these experiments, the rat (*Rattus norvegicus*) and the cat (*Felis domesticus*). For the rat preparation, the triceps surae muscle group was stimulated via split portions of the sciatic nerve. In the cat experiments two different muscles were used, the soleus muscle and the medial gastrocnemius muscle. In these experiments stimulation was delivered to subdivided portions of the ventral root.

Rat Experiments

A total of 31 adult male Sprague-Dawley rats were used with an average weight of 615g (±11 SEM). The rats were obtained from the Monash University Central Animal House and kept in the Physiology Animal House. All experiments were carried out with approval from the Monash University Standing Committee on Ethics in Animal Experimentation. Rats were anaesthetised with an intraperitoneal injection of Urethane (1g/kg; ethyl carbamate, Sigma Chemicals, Australia) in isotonic saline. The ventral side of the neck was shaved and the jugular vein was cannulated. Forelimb withdrawal and corneal reflexes were tested regularly and additional doses (0.05-0.1g urethane in saline solution) were given via the jugular vein when required. These were seldom required as urethane has long lasting effects. A tracheotomy was performed and a tracheal cannula was inserted to ensure that the trachea was not obstructed during the course of the experiment. A rectal thermometer measured core body temperature that was maintained at 37±1°C via a water heated blanket (K-20-D, Baxter, USA) with an infrared heating lamp providing additional heat when required. The left hind-limb was shaved and dissected to access the sciatic nerve and the triceps surae muscle was attached to a servomotor by the Achilles tendon. The dissection is illustrated in figure 6.1.

After the initial dissection the hind-limb was fixed to a metal base with pins inserted into the head of the tibia. The calcaneum was cut and the Achilles tendon with its bony fragment was attached to a T shaped rod that was connected to a proving ring that had four strain gauges cemented on the inside and outside in a full bridge configuration. The T shaped rod was attached to the servomotor and the compliance of the system was 20μm/N. The experimental set-up is depicted in figure 6.2.

The proximal end of the sciatic nerve was placed on a mirrored dissection plate in the paraffin bath. The epineurium and connective tissue surrounding the sciatic nerve

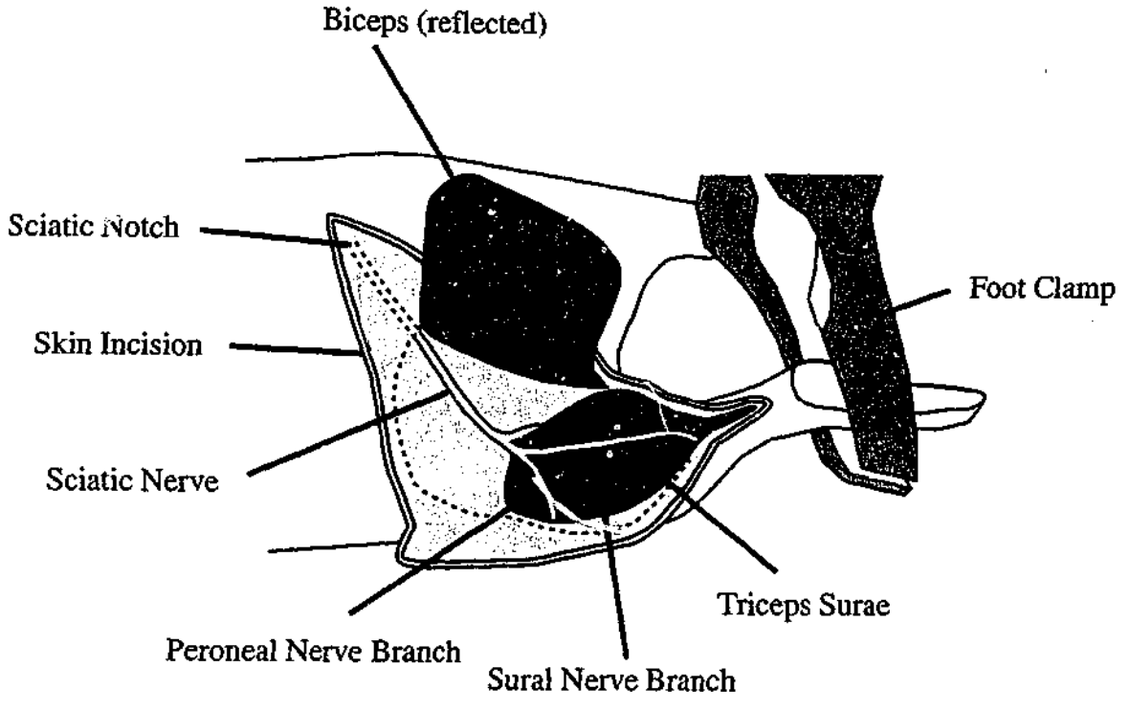


Figure 6.1. Diagrammatic representation of the rat triceps surae dissection. The rat lay prone on a metal base with the left foot secured in a foot clamp. The biceps femoris muscle was detached from the lateral side of the tibia and folded back revealing the sciatic nerve and lateral and medial gastrocnemius muscles. Nerve and blood supply to biceps was cut without interference to the blood supply of triceps surae. The sciatic nerve was traced back to the sciatic notch and was cut as high as possible to ensure a long length of nerve to work with. The popliteal fat was removed and the sural and peroneal nerve trunks were cut. The maximum physiological muscle length was determined by relating a cauterised mark on the tibia to a silk suture tied to the Achilles tendon during full ankle dorsiflexion. The plantaris tendon was cut at the level of the calcaneum leaving gastrocnemius (lateral and medial) and soleus muscles remaining attached.

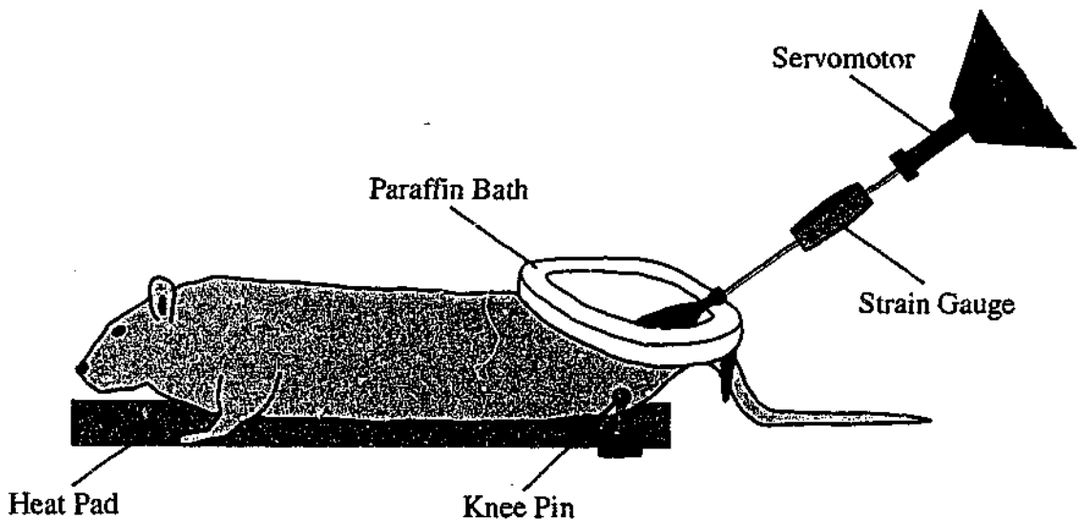


Figure 6.2. The servomotor was aligned with the longitudinal axis of the muscle. Skin flaps were tied to a circular support bar and fashioned to form a paraffin bath. Strips of gauze soaked in a warm agar solution (4% agar in saline) were applied to the paraffin bath to prevent leakage and ensure that the muscle and tendon remained covered throughout the experiment. Bath temperature was monitored and maintained at approximately 35°C (±2°C) with an infrared heat lamp.

was carefully removed using fine scissors and watchmakers' forceps. The sciatic nerve was divided into several large portions each approximately 30mm in length. Each nerve portion was placed onto separate bipolar stimulating electrodes and stimulated with supramaximal pulses. Tension was monitored to determine whether triceps surae motoneurons were stimulated and only the nerve portions that contained functional triceps surae motoneurons were used and they were arranged into five portions that produced twitches of similar tension (approximately ±30%). To minimise stimulus spread between fragments they were kept separate and distal end was earthed to the dissection plate by a thin layer of saline that acted as a current shunt. Evidence of stimulus spread was sought by comparing the twitch of all five portions with the sum of the twitches of one portion and the remaining four portions. If the size of the twitch of all five portions was less than linear summation of the one portion twitch and the four portion twitch then this indicated stimulus spread. In practice, stimulus spread could always be kept to a minimum. Muscle tension was amplified (x100) using a custom made amplifier (Physiology Electronics Workshop, Monash University), and along with muscle length was digitised and recorded at 2000/s with a MacLab data acquisition system (MacLab/8s, ADInstruments, Australia) using Chart software (MacLab, ADInstruments, Australia).

During electrical stimulation at submaximal rates there was significant movement in the muscle. This raised the possibility that a sensor responding to movement, like an accelerometer, could provide a signal related to tension ripple with sufficient fidelity to drive the stimulator. In two experiments an accelerometer (PKS, ShockSensor, muRata, Japan) was sutured to connective tissue on the muscle and the output recorded during distributed stimulation. The accelerometer was positioned so that its axis of maximum sensitivity was longitudinally in line with the muscle.

Cat Experiments

These experiments were performed on a total of five cats weighing between 2.1kg and 4.2kg, prepared as detailed in Chapter Two (page 52). The cats were obtained from the Monash University Central Animal House and kept in the Physiology Animal House. All experiments were carried out with approval from the Monash University Standing Committee on Ethics in Animal Experimentation.

The cats were deeply anaesthetised with pentobarbitone (40mg/kg, Nembutal, Boehringer Ingelheim, Australia) and a laminectomy was carried out to expose the

ventral roots L6-S2. In experiments using the soleus muscle, the left hind-limb was dissected according to the procedure described in Chapter Two (page 52). In experiments using the medial gastrocnemius muscle the procedure was similar but here the medial gastrocnemius muscle was separated from the lateral gastrocnemius and soleus muscles. The tendons of lateral gastrocnemius muscle and soleus muscle were cut from the Achilles tendon leaving the medial gastrocnemius tendon attached to the calcaneum. The calcaneum was detached from the ankle and attached in series with a U-shaped strain gauge to an electromagnetic servomotor. The compliance of the system was 5μm/N. All hind-limb nerves, other than the medial gastrocnemius nerve, were cut including those to the hip. A bath containing mineral paraffin oil, created from the skin flaps of the hind-limb, covered the exposed muscle. Muscle tension and length was digitised and recorded at 1000/s with the MacLab data acquisition system (MacLab/8s, ADInstruments, Australia) using Chart software (MacLab, ADInstruments, Australia) on a Macintosh G3 computer.

Tendon organs provide the CNS with a signal related to muscle tension. It has been suggested that the average signal from several tendon organs, an ensemble, can accurately signal whole muscle tension (Prochazka & Gorassini 1998). In one experiment recordings of single, identified tendon organs from the soleus muscle of the cat were monitored during distributed stimulation using six portions of ventral root filaments. Units were defined as tendon organs based on their conduction velocity (≥75m/s) and their response to a muscle twitch, showing an increase in discharge during the rising phase of muscle tension (figure 6.3). The tendon organs were mounted on monopolar recording electrodes. The recorded signal was high and low pass filtered (300Hz and 8kHz) and header stage pre-amplified (x1000), amplified (x20) and notch filtered (50Hz) and then digitally recorded (10k/s) on a G3 Macintosh computer using custom software programmed in Igor Pro (Wavemetrics, USA). Tendon organ activity was stored as a series of action potential time intervals and displayed as instantaneous rate. Analysis was performed by backfilling the instantaneous firing rate for each of six tendon organs and then calculating an ensemble response by averaging the backfilled data (see figure 6.3). Backfilling was necessary to convert the discrete instantaneous rate data into continuous data thus enabling firing rates from all afferents to be averaged.

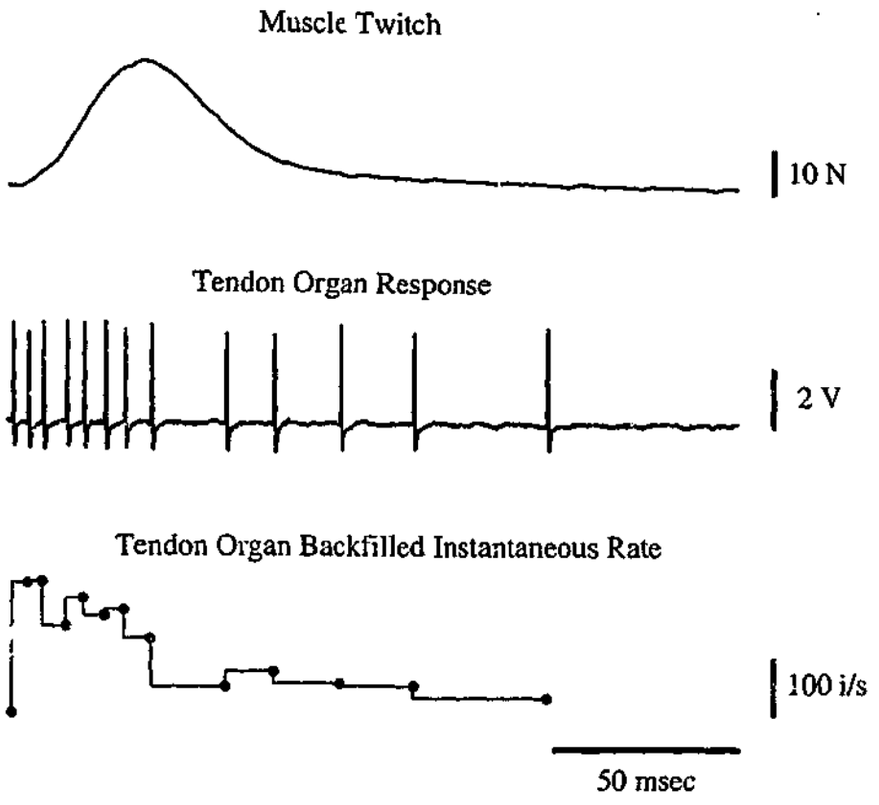


Figure 6.3. An example of the response from a tendon organ (*middle trace*) to muscle twitch (*upper trace*). The tendon organ responded to the rising phase of the muscle twitch with a burst of impulses at a high rate (>200i/s). The instantaneous rate is shown in the *lower trace* (black circles). Instantaneous rate was backfilled (red line) to transform the discrete instantaneous rate data into continuous rate data thus enabling the average response from a number of tendon organs to be calculated. Backfilling assumed a constant firing rate to the preceding impulse.

Experimental Procedures

Optimisation of Interpulse Intervals

The procedure used to optimise interpulse intervals consisted of a number of isometric contractions at low stimulation rates (4-8pps), each one-second in duration. Beginning with equal interpulse intervals, the intervals were optimised iteratively over a series of contractions and were considered optimised when the fundamental ripple was reduced to less than 1% of average tension. The effect of stimulation rate on tension ripple was examined using distributed stimulation with equal and optimised interpulse intervals and using synchronous stimulation.

Length-Tension

Twitch length-tension curves were constructed for all rat triceps surae muscles using whole muscle twitches. Optimal length was defined as the muscle length that gave maximum twitch tension. Tetanic length-tension curves were also measured for the cat soleus muscle and the rat triceps surae muscles using tetanic contractions (average steady tension) for various rates of optimised distributed stimulation (4-32pps). All length-tension curves were constructed starting from short muscle lengths and extending to maximum physiological length, or just beyond, in 0.5mm steps.

Force-Velocity

The force-velocity relationship of the rat triceps surae muscle group was examined using three rates of optimised distributed stimulation (8pps, 16pps and 32pps) delivered to each of five muscle portions. The tension developed at the optimal muscle length during isometric contractions was compared to tension, measured at the same length, during 2mm ramp muscle length changes. Ramps using a range of different velocities (1-64mm/s) were used to lengthen and shorten the contracting muscle. For lengthening ramps, muscle length was initially set at L_{opt}-1mm and for shortening ramps it was initially set at L_{opt}+1mm, with the mid point of the 2mm ramp corresponded to L_{opt}.

Stimulation began 500msec before the start of the ramp and it lasted no less than 900msec (for the fastest velocity) and no longer than three seconds (for the slowest velocity). The duration of stimulation was kept to a minimum to minimise the effects of fatigue. Each ramp was repeated without stimulation to measure the muscle's passive

tension, which was later subtracted from the total tension. All of the tension data presented shows active tension only. A rest period between muscle contractions was used to reduce fatigue effects, the rest period being 90 seconds for 8pps and 120 seconds for 16pps and 32pps. A ratio of the tension measured at L_{opt} during the ramp length change (P) and during isometric contractions (Po) was determined for each ramp velocity. Force-velocity curves were calculated from the (P/Po) ratio measurements for each stimulation rate with the procedure described in figure 6.4.

To control for tension variations between contractions, over the course of the experiment, isometric contractions were regularly repeated every fourth or fifth contraction during the experimental sequence. The isometric (Po) value used in the P/Po ratio was calculated by interpolating an expected value between the two isometric records. This procedure is illustrated in figure 6.5.

During optimised distributed stimulation, different portions of the muscle were stimulated sequentially. In order to obtain a measure of whole muscle tension the tension trace was smoothed (averaged) over one stimulation period ($T=1/p$). For instance, when a stimulation rate of 8pps was used the stimulus period was 125msec, the tension trace was smoothed over this time period. This was acceptable for the relatively slow ramp velocities, where the ramp duration was longer than or equal to two periods, but for the faster velocities it posed a problem. Here the faster ramps were completed within one period of stimulation. To account for the shorter ramp duration, tension was smoothed over 1/2 or 1/4 of a period, depending on the ramp velocity. Measurements were then repeated with the ramp onset delayed by the 1/2 or 1/4 period (figure 6.6). This ensured that contributions from all five muscle portions were included in the calculations but also limited the ramp velocities to 32mm/s for a stimulation rate of 8pps and 64mm/s for 16pps.

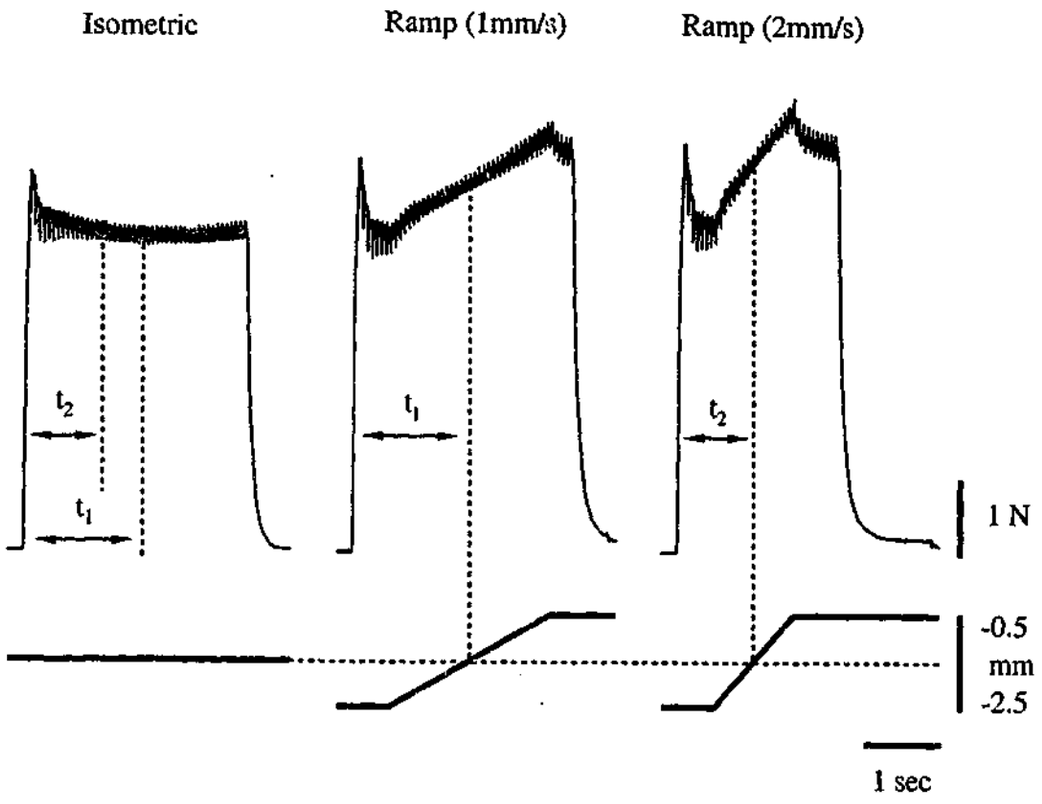


Figure 6.4. Isometric tension (*upper left panel*) and tension during ramp lengthening of the muscle at 1mm/s (*upper middle panel*) and at 2mm/s (*upper right panel*). Muscle length is shown in the *lower panel*. The optimal length was L_{max} −1.5mm and corresponds to the middle of each ramp. To control for tension variations during the period of stimulation, tension for isometric and ramp contractions was measured at the same point in time, corresponding to L_{opt}. For instance, tension measured at t_1 during the 1mm/s ramp was compared to tension measured at t_1 for the isometric contraction (green circle). For the 2mm/s ramp, tension at t_2 was compared to the isometric tension at t_2 (green circle). For each velocity a P/Po ratio was measured. The same procedure was used to measure force-velocity relationship for the shortening ramps.

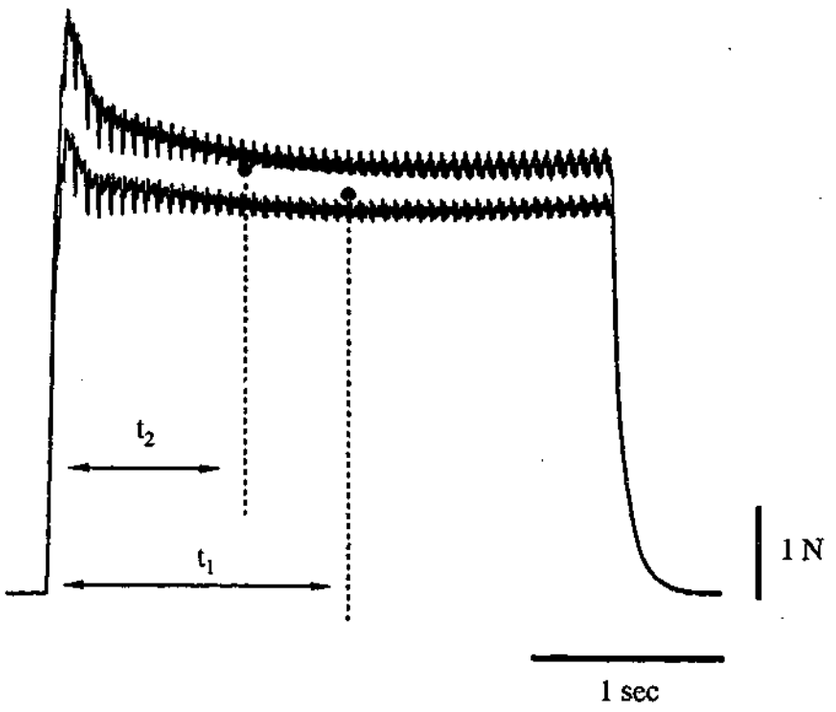


Figure 6.5. Linear interpolation of isometric value Po (green circle) used in the ratio measurement. Two isometric records, the first contraction (red), and the fourth contraction (blue) in the experimental sequence (shown in figure 6.4) were used to determine the Po measurement. The interpolated values (green circle) represent a predicted tension value for a second isometric contraction (t_1) and a third isometric contraction (t_2). This procedure was used to minimise effects of fatigue or potentiation over the course of the experiment and assumes that tension varied linearly between isometric contractions.

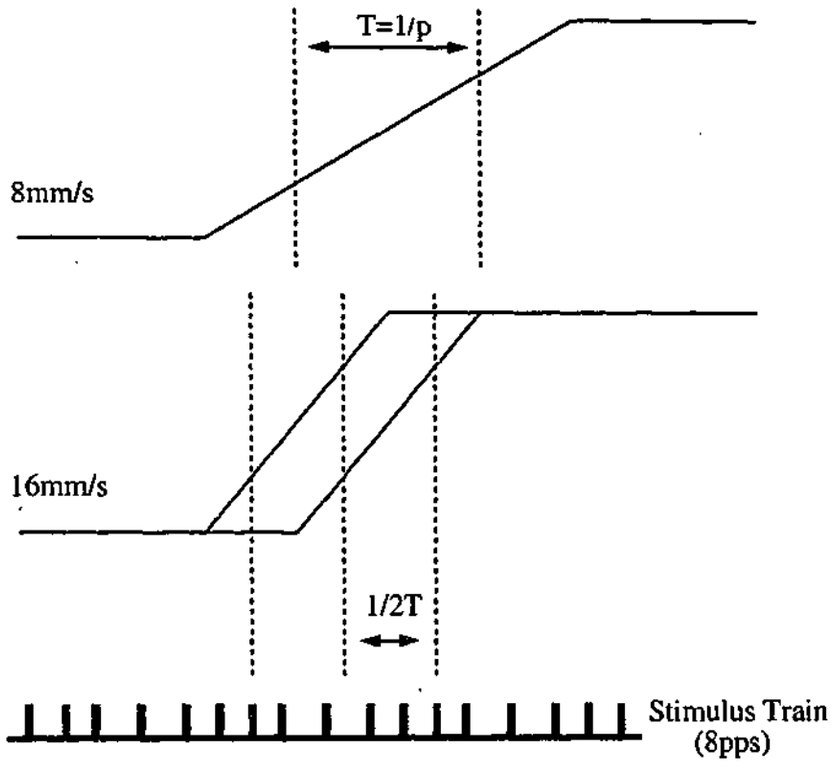


Figure 6.6. Tension smoothing procedure used for different ramp velocities, 8mm/s ramp shown in the *upper trace* and 16mm/s ramp shown in the *middle trace*. The stimulus train (8pps) is shown in the *bottom trace*, each bar represents a stimulus delivered to each of the five muscle portions (red bar – stimulus to first portion). Tension traces were smoothed over one stimulation period (T) for ramp length changes in which the duration was 2T or greater, for example the 8mm/s ramp. However, for the 16mm/s ramp, the duration was less than 2T, therefore the tension trace was smoothed over 1/2T. The contraction sequence was repeated, but this time the ramp onset was delayed by 1/2T. For the two 16mm/s ramps, two ratios were calculated over different parts of the stimulus cycle. These two values were then averaged to provide a single P/Po ratio for the 16mm/s ramp.

Results

Distributed stimulation using optimised interpulse intervals enabled smooth contractions to be generated and maintained in the face of changing tension contributions from individual muscle portions. The method of distributed stimulation using equal interpulse intervals (Rack & Westbury 1969), had previously been restricted to slow twitch muscle because of the requirement that tension contributions from each portion should be equal and stable over time.

The cat medial gastrocnemius muscle contains a mixture of muscle fibres types each having different fatigue characteristics. If the ventral root supply to the medial gastrocnemius muscle is divided into six portions it is possible that individual portions will have different potentiation and fatigue properties. Evidence for this is shown in figure 6.7. The main point to note here is that individual portions of the muscle did not fatigue by equal amounts. If portions were initially equal at the start of the experiment, they became unequal during stimulation due to differences in muscle fibre composition. If distributed stimulation using equal intervals was used then the muscle contractions would develop increasingly more ripple as the twitch sizes became more dissimilar. Optimisation of interpulse intervals removes this restriction enabling the method of distributed stimulation to be used with muscle containing a mixture of fibre types that exhibit various potentiation and fatigue properties.

Optimisation of Interpulse Intervals

The stimulator was designed to deliver stimuli, distributed in time across several muscle portions, to produce smooth muscle contractions. Experiments typically began with a series of optimising contractions, each one-second in duration, in which interpulse intervals were iteratively adjusted. Typically, 10 to 15 contractions were required to optimise interpulse intervals. This number varied and depended on differences in tension generated by each portion. If the tension varied considerably between contractions then a larger number of optimisation contractions were required. The effect of muscle length on optimisation was examined and it was found that interpulse intervals calculated at short muscle lengths also produced optimised contractions at longer muscle lengths. Optimisation of interpulse intervals was carried out continuously to counteract the effects of changes in the tension contribution of the individual muscle portions. Two examples of optimisation over a 10 minute period of

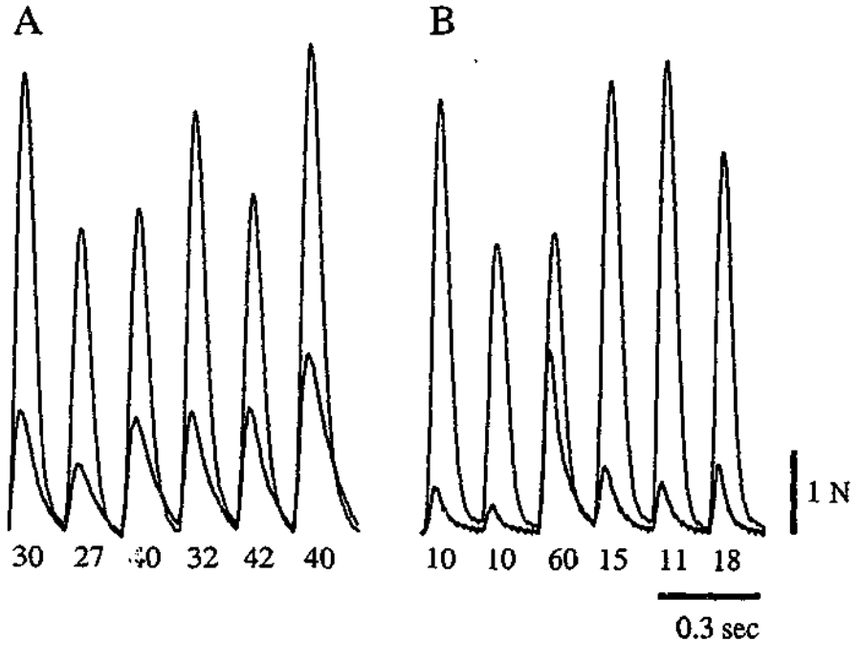


Figure 6.7. Medial gastrocnemius twitch tension profiles from two different experiments (A and B). Twitch tension is shown for each muscle portion before (red) and after (blue) a fatiguing contraction. The numbers below each twitch represents the tension remaining as a percentage of its pre-fatigue level. This data shows that the decline in twitch tension was not uniform across the six muscle portions.

continuous stimulation of the cat medial gastrocnemius muscle at 6pps are shown in figure 6.8. Although the interpulse intervals were optimised before the continuous contraction began, there was ongoing readjustment of the intervals during the contraction.

The optimisation process reduced the fundamental ripple, the tension fluctuations with the same frequency as the stimulation rate, to minimal levels. Intervals were classified as optimised when the FRip was reduced to levels less than 1% of average tension. The peak-to-peak (PP) tension ripple was measured and is defined as the maximum minus the minimum level of tension ripple as a percentage of average tension. The PP ripple is a measure of the absolute variation in tension during the contraction and is not specific to any frequency component whereas the FRip is a measure of tension ripple at the stimulation rate. The data presented in the following section for the cat medial gastrocnemius muscle shows that both FRip and PP ripple were reduced by optimisation of interpulse intervals.

Optimised and Equal Interpulse Intervals

In comparison to equal intervals, optimised intervals produced clear advantages in terms of generating and maintaining contractions with almost no FRip and less PP ripple. It should be kept in mind that direct comparisons of distributed stimulation using optimised interpulse intervals and equal intervals depends on the equality of tension between muscle portions. If the tension in all portions were similar then optimised interpulse intervals did not provide a significant advantage in terms of tension ripple. It was only when tension contributions from each portion became different that clear advantages of optimising interpulse intervals became apparent. Two examples of contractions of the cat medial gastrocnemius muscle using optimised and equal interpulse intervals are shown in figure 6.9.

The relationship between stimulation rate and tension ripple was measured during distributed stimulation with equal and optimised interpulse intervals. At higher stimulation rates contractions became more fused. For stimulation rates higher than 20pps to 25pps, there was no difference in tension ripple between equal and optimised intervals. The advantage of optimised intervals, in terms of maintaining minimal tension ripple, was apparent at low stimulation frequencies. An example of this relationship in one experiment on the cat medial gastrocnemius muscle is shown in figure 6.10.

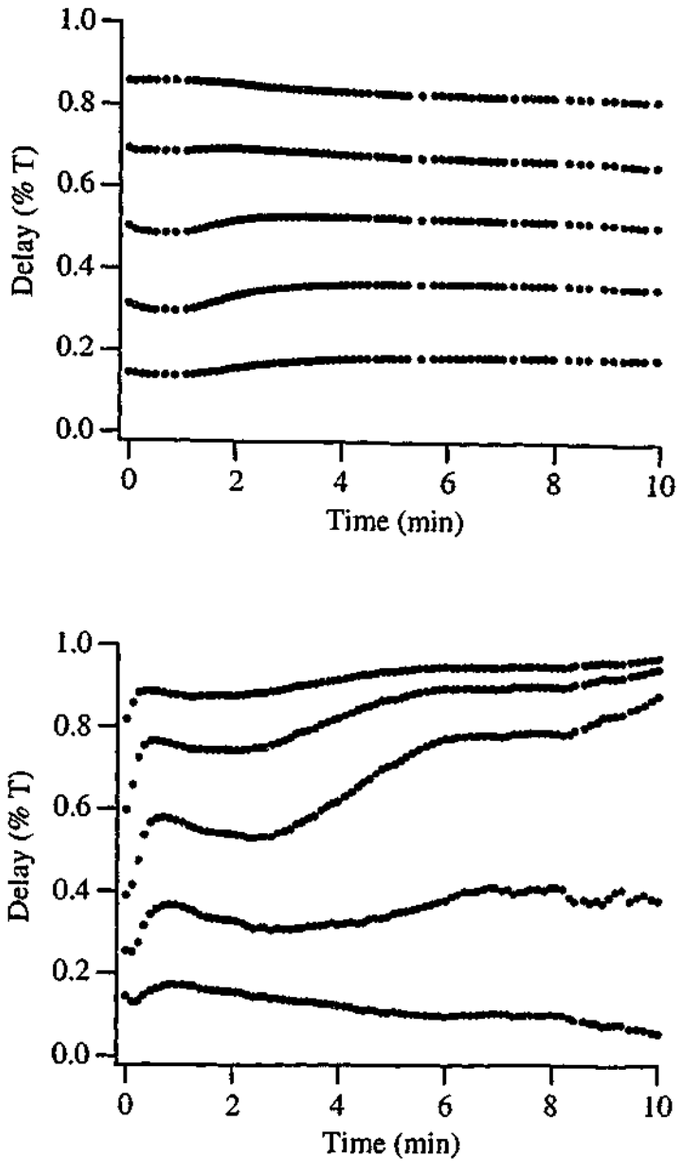


Figure 6.8. Changes in the interpulse delays during continuous stimulation of cat medial gastrocnemius at 6pps in two experiments. The interpulse delay is represented as the delay from muscle portion number one (zero delay) as a proportion of one period of stimulation (T=1/*p*). There were six muscle portions so five sets of interpulse intervals are shown. Each dot on the trace represents an optimisation of the intervals. In the *upper panel* there were 73 optimisations during the ten-minute stimulation period. After approximately two minutes of stimulation tension became more stable and the intervals remained relatively constant. In the *lower panel* there were 85 optimisations during the ten-minute stimulation period. In this example there was significant adjustment of interpulse intervals over the period of stimulation.

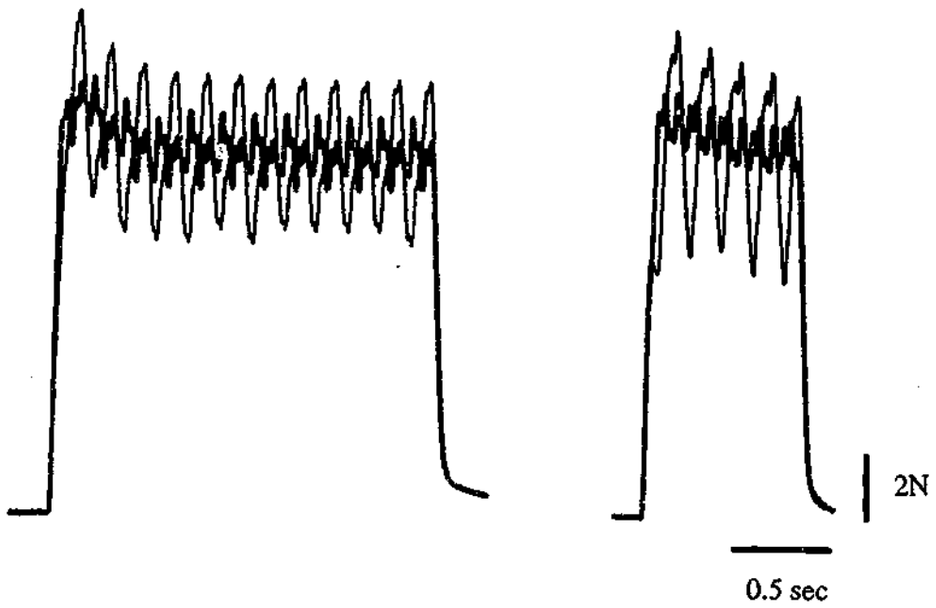


Figure 6.9. Examples of isometric contraction using distributed stimulation with optimised (red) and with equal (black) interpulse intervals. Data taken from the cat medial gastrocnemius showing that the tension ripple generated during distributed stimulation of six muscle portions using equal intervals can vary between experiments. The PP ripple using equal intervals for the *left trace* was 55% while in the *right trace* it was larger at 69% of average tension. The similarity in tension between individual muscle portions determined how smooth contractions were using equal intervals.

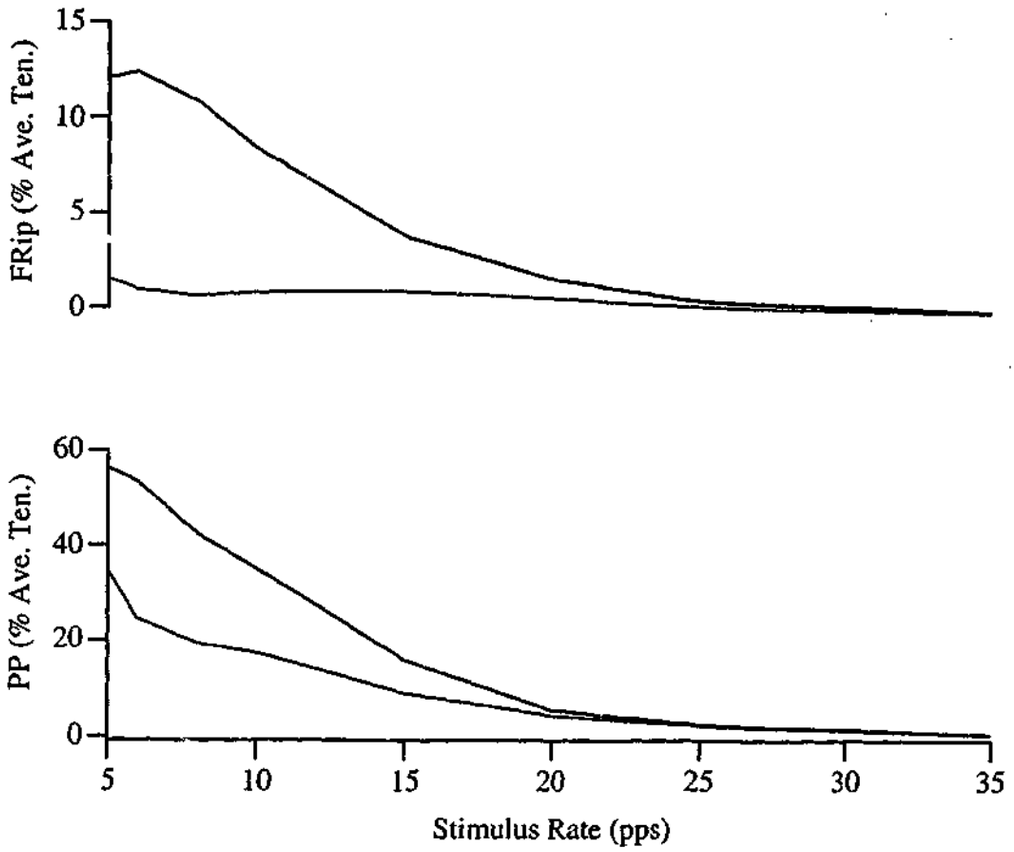


Figure 6.10. The effects of stimulation rate on FRip (*upper panel*) and PP ripple (*lower panel*) for optimised intervals (red) and equal intervals (black). Six muscle portions of the cat medial gastrocnemius muscle were used. Muscle contractions using optimised intervals had less FRip and PP ripple for rates of stimulation less than 20-25pps. The dominant frequency component of tension ripple using equal interpulse intervals was at the stimulation frequency.

Distributed and Synchronous Stimulation

Optimised distributed stimulation had clear advantages over synchronous stimulation in terms of generating contractions that were more fused and generated greater tension at low stimulation rates. An example of the contractions generated by optimised distributed stimulation and synchronous stimulation is shown for the cat medial gastrocnemius muscle in the upper panel of figure 6.11. Synchronous stimulation of the muscle at 6pps produced an unfused contraction consisting of a series of muscle twitches in response to each stimulating pulse. When optimised distributed stimulation was used, contractions were smoother and produced more tension than during synchronous stimulation at the same rate. This was observed for stimulation rates below 35pps and the relationship between stimulation rate and tension is shown in the lower panel of figure 6.11.

Stimulation of Different Muscles

To examine whether the muscle stimulator was equally effective in producing smooth contractions at submaximal stimulation rates for different types of muscle, experiments were also carried out on the cat soleus muscle and the rat triceps surae muscle group. In the experiments discussed here the soleus muscle was used so that direct comparisons could be made with the cat medial gastrocnemius muscle and the rat triceps surae muscles. For the rat triceps surae preparation a different way of accessing separate muscle portions was undertaken. Instead of subdividing the ventral roots, here the sciatic nerve was subdivided and stimulation was delivered in the periphery. Example contractions using optimised distributed stimulation of the rat triceps surae muscles and cat soleus muscle are shown in figure 6.12.

Stimulation Frequency and Tension Ripple

Tension ripple during optimised distributed stimulation was examined in all three muscle preparations for different stimulation rates. As before comparisons of tension ripple were made between synchronous and optimised distributed stimulation. The results indicated that optimised distributed stimulation reduced tension ripple for all three muscle preparations (figure 6.13).

Although optimised distributed stimulation reduced FRip to levels less than 1% of average tension, there was still some degree of higher frequency tension ripple

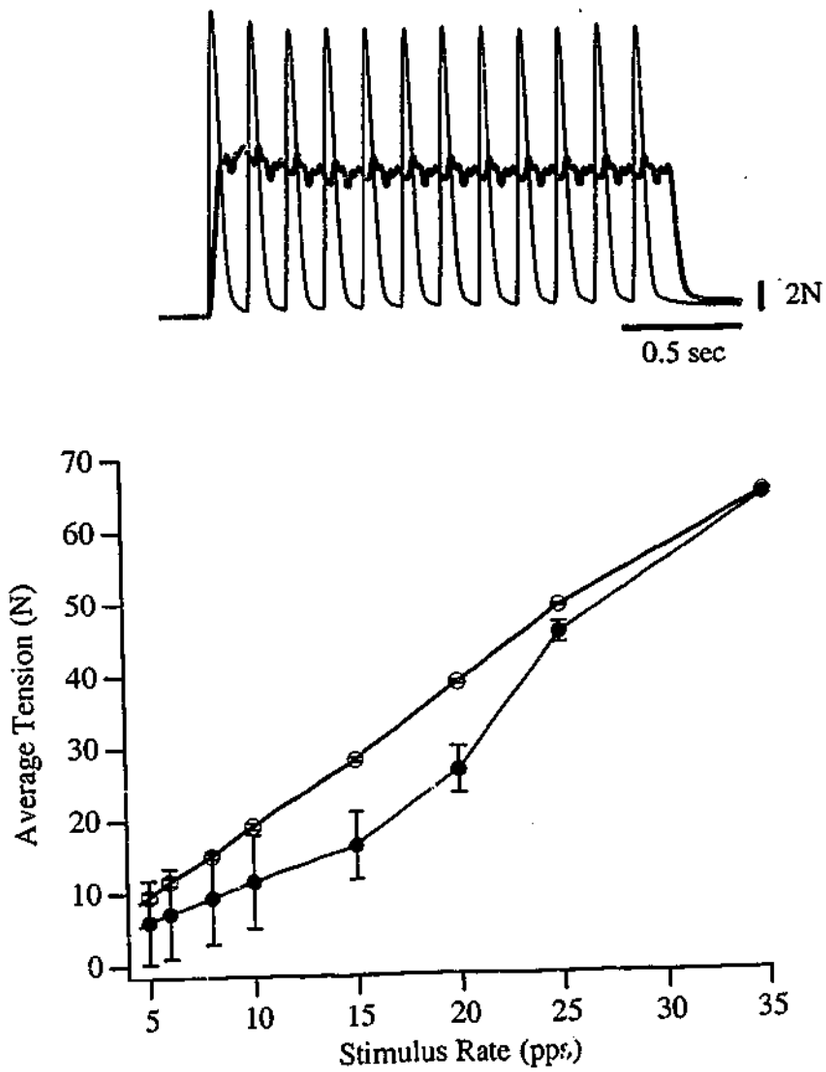


Figure 6.11. *Upper panel* shows an example of a contraction of a cat medial gastrocnemius muscle using synchronous (blue – 6pps) and optimised distributed stimulation (red) using six muscle portions each stimulated at 6pps. *Lower panel* illustrates the relationship between mean tension and stimulation rate for optimised distributed stimulation and synchronous stimulation. Error bars indicate the mean peak-to-peak tension ripple measured for each contraction.

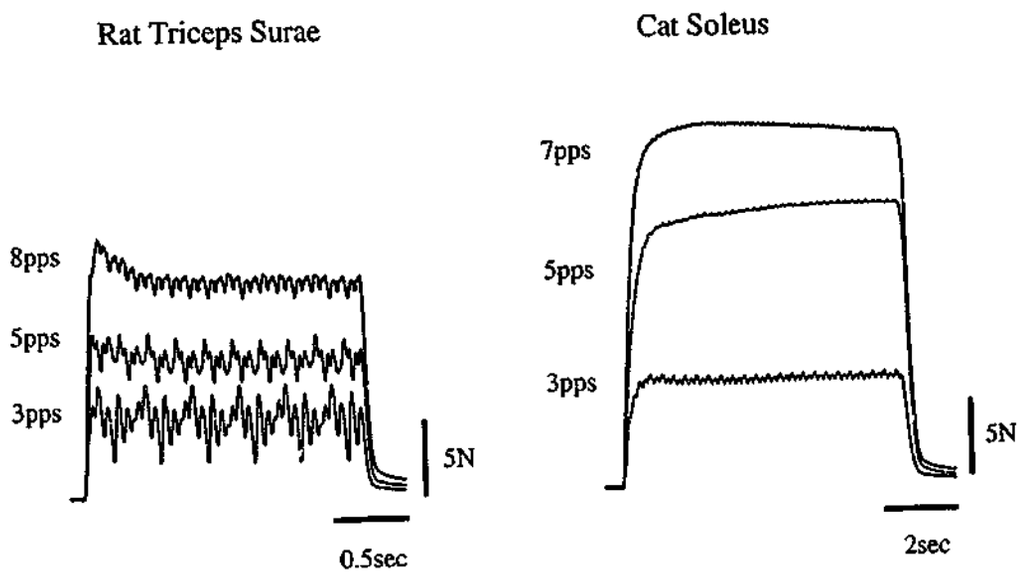


Figure 6.12. Examples of contractions using low rates of optimised distributed stimulation for the rat triceps surae muscle group using five muscle portions (*left panel*) and the cat soleus muscle using six portions (*right panel*). Contractions of the cat soleus muscle were fused at lower rates of stimulation.

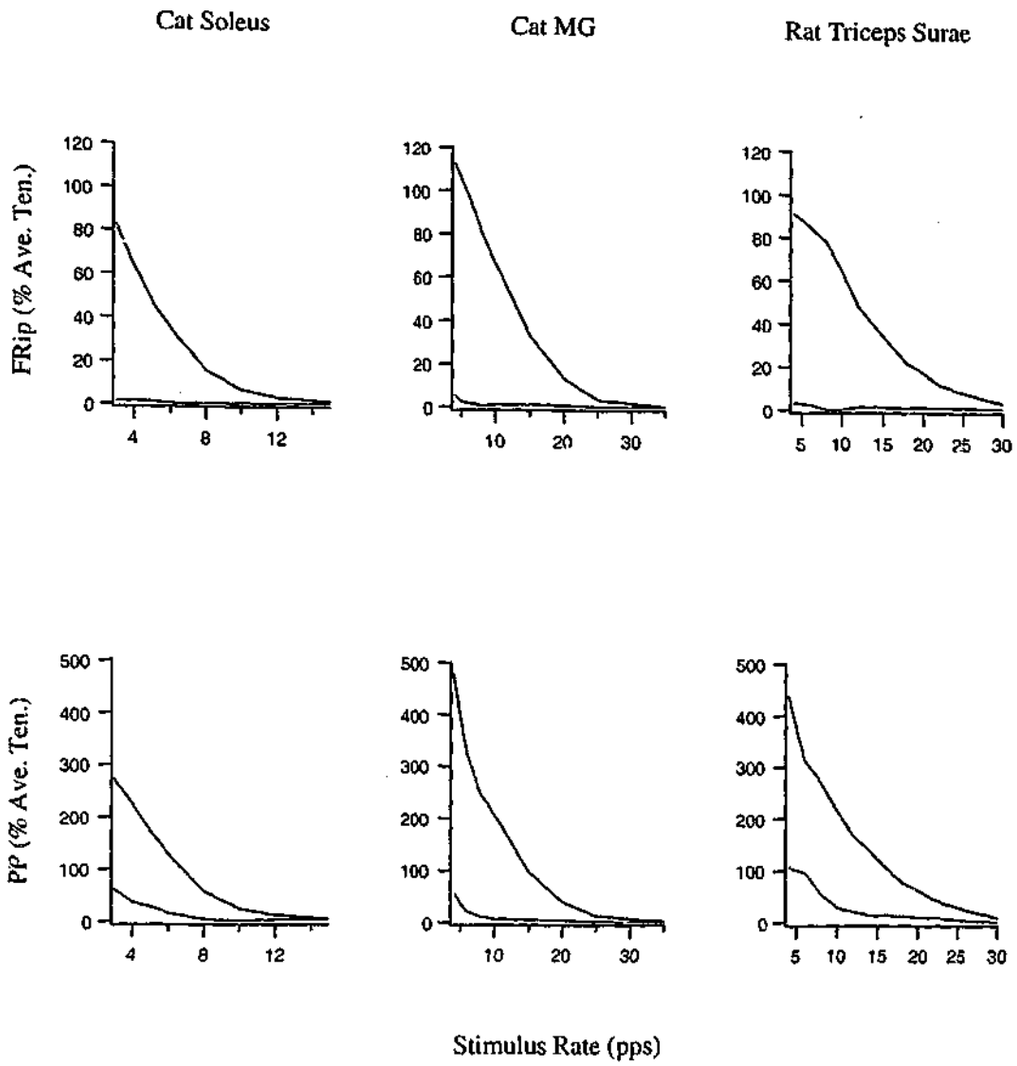


Figure 6.13. Tension ripple plotted against stimulation frequency for each preparation using optimised distributed stimulation (red) and synchronous stimulation (blue). Fundamental ripple is shown in the *upper panel* and peak-to-peak ripple in the *lower panel*. Six muscle portions were used for optimised distributed stimulation of the cat soleus muscle and medial gastrocnemius muscle and five muscle portions were used for the rat triceps surae muscle group. Optimised distributed stimulation was effective in reducing tension ripple in all three preparations.

present, particularly for the lowest stimulation rates. The results from figure 6.13 suggest that smoother contractions were generated for cat medial gastrocnemius muscle (10% PP at 8pps) than for rat triceps surae muscle group (29% at 8pps). It should be noted that five stimulating channels were used with the rat triceps surae muscle group and six channels for the cat medial gastrocnemius muscle. Although the relationship between tension ripple and number of muscle portions used was not systematically studied it was observed that a larger number of portions produced smoother contractions.

Optimisation of interpulse intervals was effective in reducing tension ripple at the fundamental frequency. However, for the lower rates of stimulation (<10pps) tension ripple was present, having components at harmonics of the fundamental frequency. The tension ripple at the second harmonic was measured and data is shown in figure 6.14. The data from the cat soleus muscle and the rat triceps surae muscle group indicated that, for low stimulation rates, the second harmonic was relatively large in comparison to the fundamental ripple. As the stimulation rate increased the contractions became more fused and the level of second harmonic ripple decreased. By contrast, the data from the medial gastrocnemius muscle indicated that the second harmonic ripple was relatively low. The level of second harmonic tension ripple varied between different experiments. This was not due to species or muscle differences but was dependent on the pattern of the twitch amplitudes of muscle portions. For instance, if a few of the muscle portions produced larger twitch tensions than the other portions then the second harmonic ripple was usually more pronounced.

Mechanical Properties of Submaximally Activated Muscle

Examinations of the mechanical properties of the cat soleus muscle using distributed stimulation have described changes in the length-tension and force-velocity relationships when submaximal stimulation rates were used instead of maximal activation (Joyce, Rack *et al.* 1969; Rack & Westbury 1969). The experiments described in this section of the thesis measured the length-tension relationship of the cat soleus muscle and the rat triceps surae muscle group in addition to the force-velocity relationship of the rat triceps surae muscle group using different rates of stimulation.

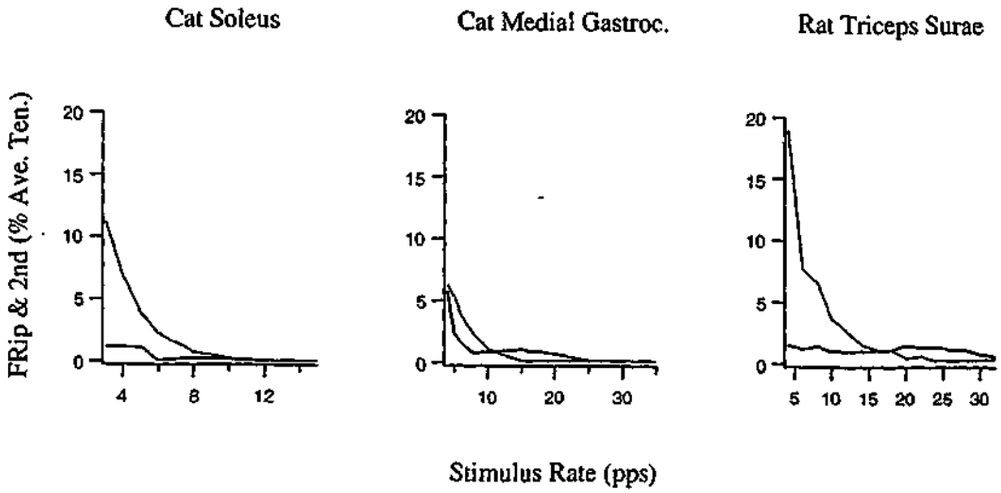


Figure 6.14. Tension ripple generated at the stimulation frequency (FRip) (black) and second harmonic (red) for distributed stimulation with optimised intervals. The data presented is from one cat soleus muscle (*left panel*), one cat medial gastrocnemius muscle (*middle panel*) and the average results from six rat triceps surae muscle groups (*right panel*). While the fundamental component of the ripple had been reduced to approximately 1%, the tension ripple remaining was at higher frequencies, including twice the stimulation frequency.

Length-Tension Relationship

Cat Soleus Muscle

When the cat soleus muscle was stimulated at submaximal rates the muscle length that produced maximal tension was longer than when higher rates of stimulation were used. There was a stimulation rate-dependent shift in the optimal length to longer muscle lengths. When the rate of stimulation was decreased from 30pps to 3pps the optimal length shifted 6.5mm to a longer length. This result is shown in figure 6.15.

Rat Triceps Surae Muscle

The effect of stimulation rate on the length-tension relationship was examined for the rat triceps surae and the results from two experiments are shown figure 6.16. The optimal length for the rat triceps surae muscle was relatively long even at high stimulation rates (L_{max} -2mm to L_{max} –1mm). When low rates of stimulation were used, the curves were much flatter and it was often not possible to extend the muscle onto the descending limb of the length-tension curve without going to lengths beyond L_{max}.

Force-Velocity Relationship

Muscle tension from the rat triceps surae was recorded in response to ramp muscle length changes during stimulation at three different rates, 8pps, 16pps, and 32pps. Ramps, 2mm in amplitude with velocities ranging from 1-64mm/s, were employed to stretch and shorten the contracting muscle through its optimal length (see figures 6.4, 6.5 and 6.6 for a description of the method). An example of a complete data set collected during one experiment is shown in figure 6.17. Tension was normalised to its value immediately before the onset of the ramp and has a value of one at this point. For muscle lengthening, the starting length was L_{max} –2.5mm and the muscle was stretched to L_{max} –0.5mm during stimulation. It is evident, particularly at the higher stimulation rates, that tension initially increased rapidly over the first 0.5mm and then more gradually. For muscle shortening, the same procedure was used but this time the starting length was L_{max} –0.5mm and the muscle was shortened to L_{max} –2.5mm during stimulation. Tension decreased to lower levels for the fastest ramp shortenings.

To calculate the force-velocity relationship from this data, muscle tension measured at the optimal length during the ramp (P) was expressed as a ratio of the

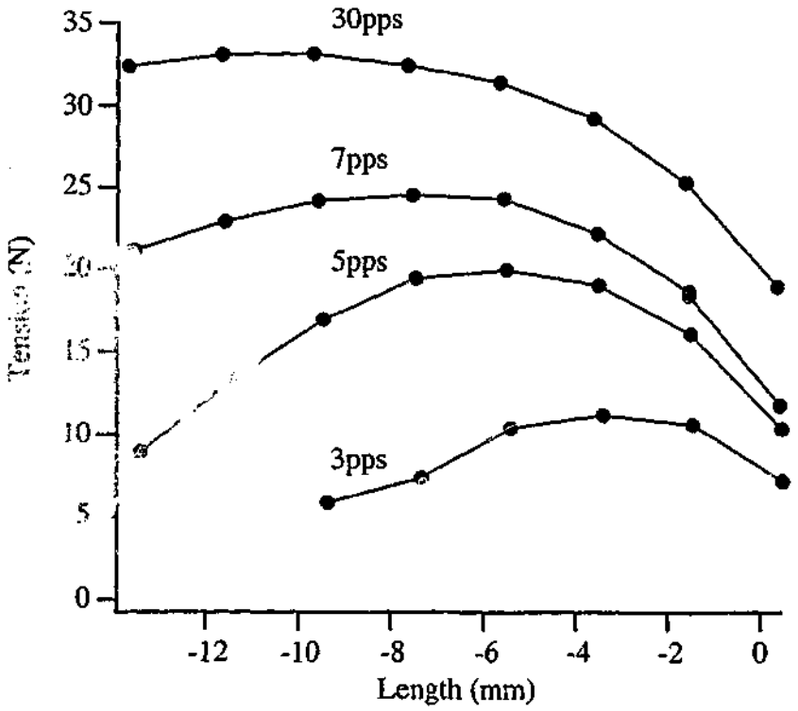


Figure 6.15. Tetanic length-tension relationship for cat soleus muscle using different rates of optimised distributed stimulation. Muscle length plotted relative to maximal physiological length (L_{max}=0). The optimal length was shifted to longer muscle lengths as the rate of stimulation was decreased from 30pps to 3pps.

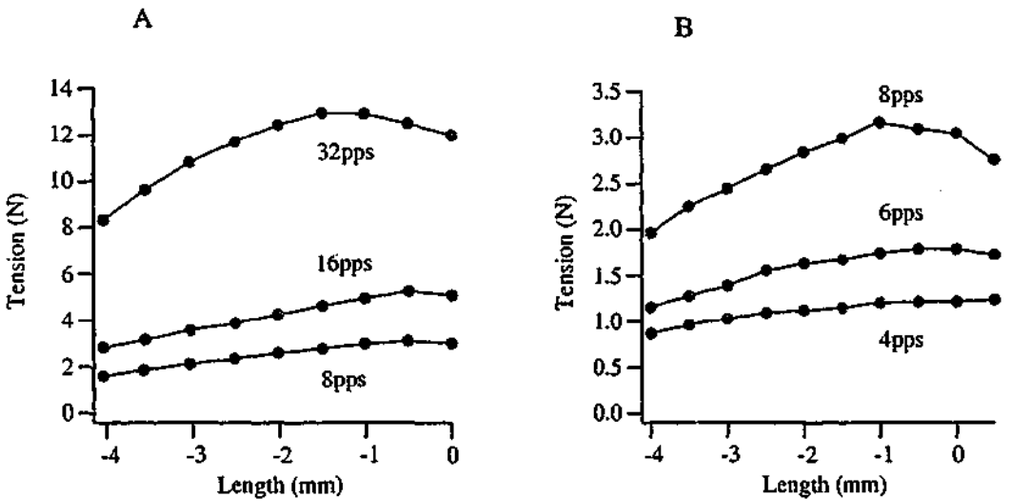


Figure 6.16. Tetanic length-tension relationship for rat triceps surae muscle using different rates of optimised distributed stimulation. Data for graphs A and B were collected in two separate experiments. Muscle length plotted relative to maximum physiological muscle length (L_{max}=0). The optimal muscle length shifted to longer muscle lengths with lower stimulation rates.

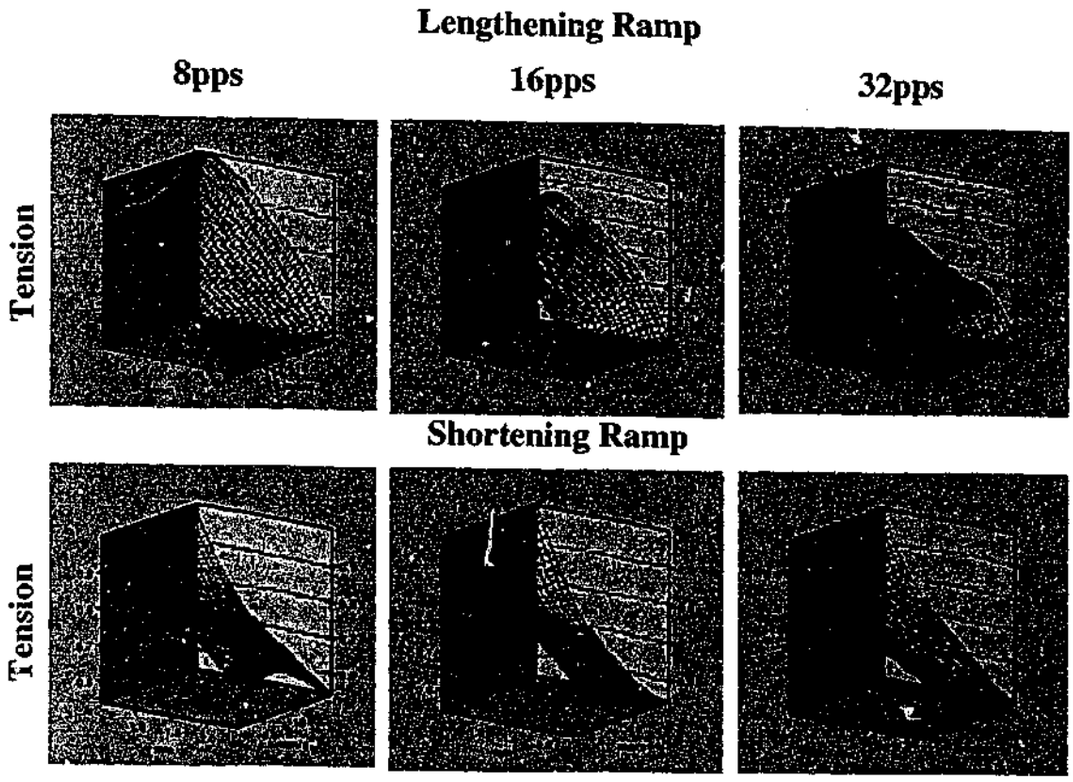


Figure 6.17. Three dimensional surface plots of tension, length and velocity. Passive tension was subtracted and the active tension normalised to its value just before the onset of the ramp. The optimal muscle length in this experiment was –1.5mm. The *upper panel* shows plots of muscle tension during lengthening ramps. The muscle was initially positioned at –2.5mm and the 2mm ramp stretched the muscle to –0.5mm during stimulation. For 8pps, tension increased gradually in a linear fashion during the ramp. When higher stimulation rates were used, 16pps and 32pps, tension increased in a biphasic manner, increasing rapidly over the first 0.5mm and then more gradually as the ramp continued. The *lower panel* shows data from the same experiment but for ramps that shortened the contracting muscle. The muscle was positioned at –0.5mm and shortened during stimulation to –2.5mm. Tension declined at a greater rate for the faster shortening velocities.

isometric tension (Po) at the same length. For each velocity and stimulation rate P/Po ratios were calculated. For example, in the experiment shown in figure 6.17 muscle tension measured at L_{max} –1.5mm during the ramp was expressed as a ratio to the isometric tension measured at L_{max} –1.5mm. The results from eight experiments are shown in figure 6.18.

The force-velocity relationship was similar for 16pps and 32pps. Tension during muscle shortening declined in a hyperbolic manner. During muscle lengthening, the P/Po ratio increased to about 1.2 for the slower ramp stretches, and remained approximately stable or increased a little, as the lengthening ramp velocity was increased. When the muscle was stimulated at 8pps the results were different. The force-velocity relationship had a more linear shape across the range of velocities used. The P/Po ratios gradually increased as the lengthening velocity increased. The decline in tension during muscle shortening was also more gradual and linear during 8pps stimulation. For all three rates of stimulation the P/Po ratios did not decrease below values of one during lengthening of the contracting muscle.

To enable a direct comparison of the data collected in these experiments to the results of Joyce *et al.* (1969) (see figure 6.23), the ratio averages from figure 6.18 have been rescaled and replotted in figure 6.19. Here, each ratio value has been rescaled to the average isometric tension generated at each stimulation rate. The average isometric tension measured during muscle stimulation at 8pps was 2.6N (±0.31 SEM). For stimulation rates of 16pps the average isometric tension was 5.6N (±0.91 SEM) and for stimulation rates of 32pps the average was 11.1N (±1.91 SEM). There was a consistent effect whereby doubling the stimulation rate produced isometric contractions of twice the tension. The rescaled ratio data is shown in figure 6.19.

Control Signal

The optimisation procedure described so far used a signal of whole muscle tension to determine the fundamental component of tension ripple. However, any signal that can provide a reliable measure of the amplitude and phase of the fundamental ripple could, theoretically, be used instead of whole muscle tension. Two alternative feedback signals were monitored to determine whether they could signal the fundamental component of tension ripple.

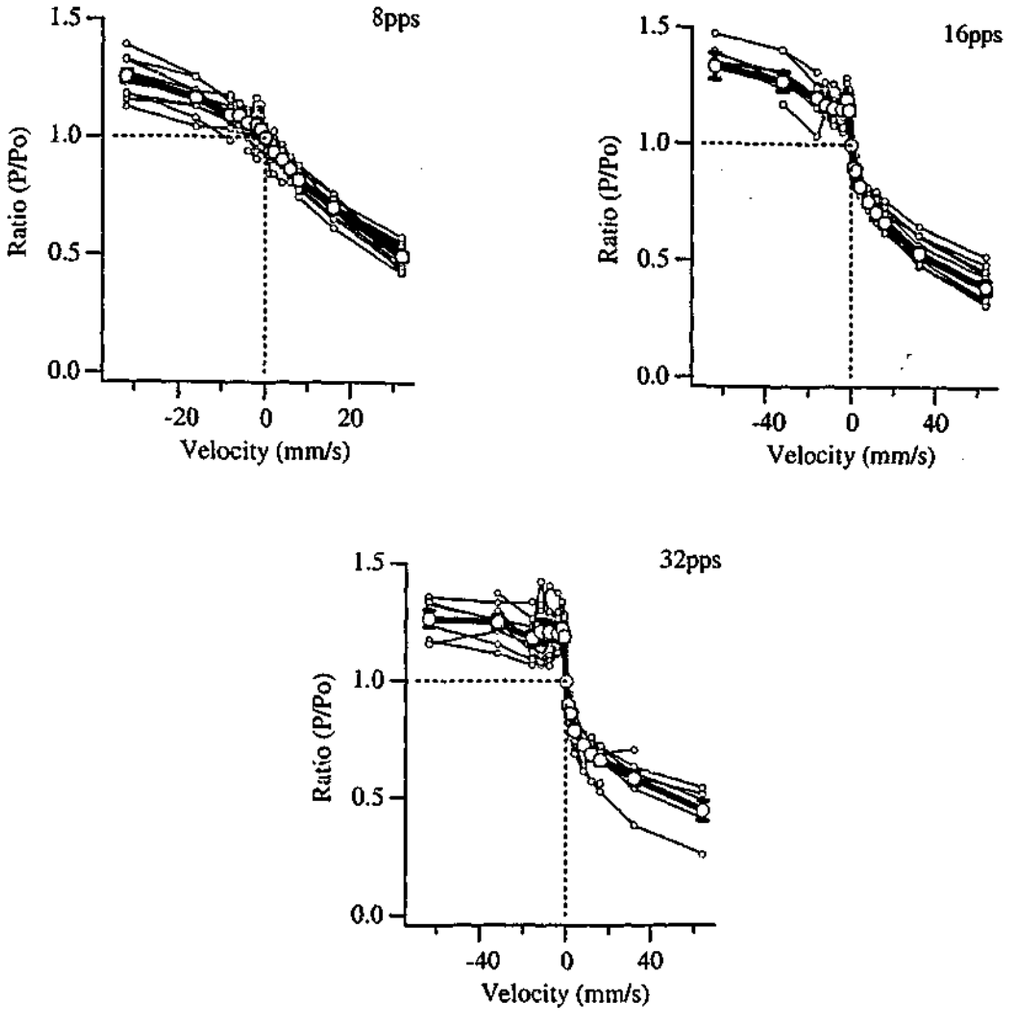


Figure 6.18. Force-velocity data represented as a ratio of tension measured at optimal muscle lengths during the ramp and during isometric contractions. Measurements were made using three different stimulation rates (8pps, 16pps and 32pps). Data from all eight experiments (green) and their averages (open circles) (±SEM) are plotted for all ramp velocities and stimulation rates. Negative velocities indicate muscle lengthening.

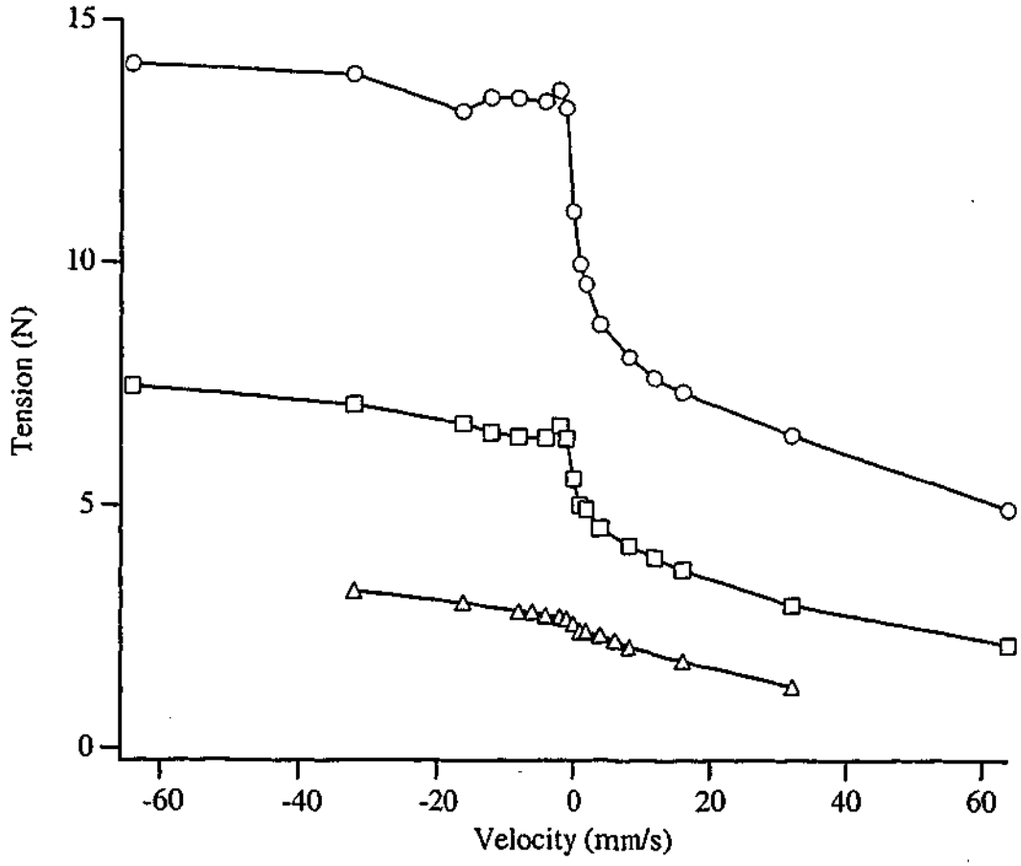


Figure 6.19. The force-velocity ratio data rescaled to the average isometric tension measured for each rate of stimulation, red (32pps), blue (16pps) and black (8pps). For stimulation rates of 32pps and 16pps the tension during muscle shortening declined in a hyperbolic manner. The decline in tension during muscle shortening was more gradual and linear during stimulation at a rate of 8pps. The average P/Po ratios did not decrease below values of one during muscle lengthening.

Tendon Organ Ensemble

Tendon organs provide signals related to muscle tension and are particularly sensitive to changes in muscle tension. However, a single tendon organ responds only to a small number of muscle fibres, and so does not provide a useful measure of whole muscle tension. It was hypothesised that an ensemble response measured from a number tendon organs would provide a signal proportional to the tension generated by stimulation of each muscle portion.

In this experiment, recordings were made from dorsal root filaments containing single identified tendon organs afferents from the soleus muscle of the anaesthetised cat. The responses from six tendon organs were recorded during distributed stimulation using equal and optimised interpulse intervals with the muscle positioned at L_{max} –10mm. An ensemble response during distributed stimulation was calculated by averaging the firing rates from all six tendon organs. The aim here was to determine whether this ensemble response could signal fundamental ripple of the whole muscle tension. An example showing the firing rate of two tendon organs in response to distributed stimulation using equal interpulse intervals can be seen in figure 6.20. These tendon organs were most responsive to a particular phase of the stimulation cycle. When a muscle portion containing motor units innervating the particular tendon organ was stimulated, the tendon organ increased its firing rate. Some tendon organs were excited when only one or two muscle portions were stimulated, while other tendon organs were excited by stimulation of all muscle portions.

Instantaneous rates from six tendon organs were backfilled before being normalised and averaged to obtain an ensemble signal in response to distributed stimulation. The ensemble response was measured during distributed stimulation at different stimulation rates when intervals were equal and when intervals were optimised. The aim was to monitor the tendon organ ensemble output and determine whether the fundamental ripple in this signal was also minimised when the fundamental ripple in the tension signal was reduced to levels below 1% of average tension. This comparison is shown in Figure 6.21.

The ripple in the tendon organ ensemble response did not decrease even though the tension FRip was significantly reduced, FRip was 7% for equal intervals and was reduced to less than 1% for optimised intervals. In fact there was an increase in the amplitude of ripple seen in the tendon organ ensemble discharge. This result indicated that, at least in this particular case, the ensemble discharge did not provide a reliable

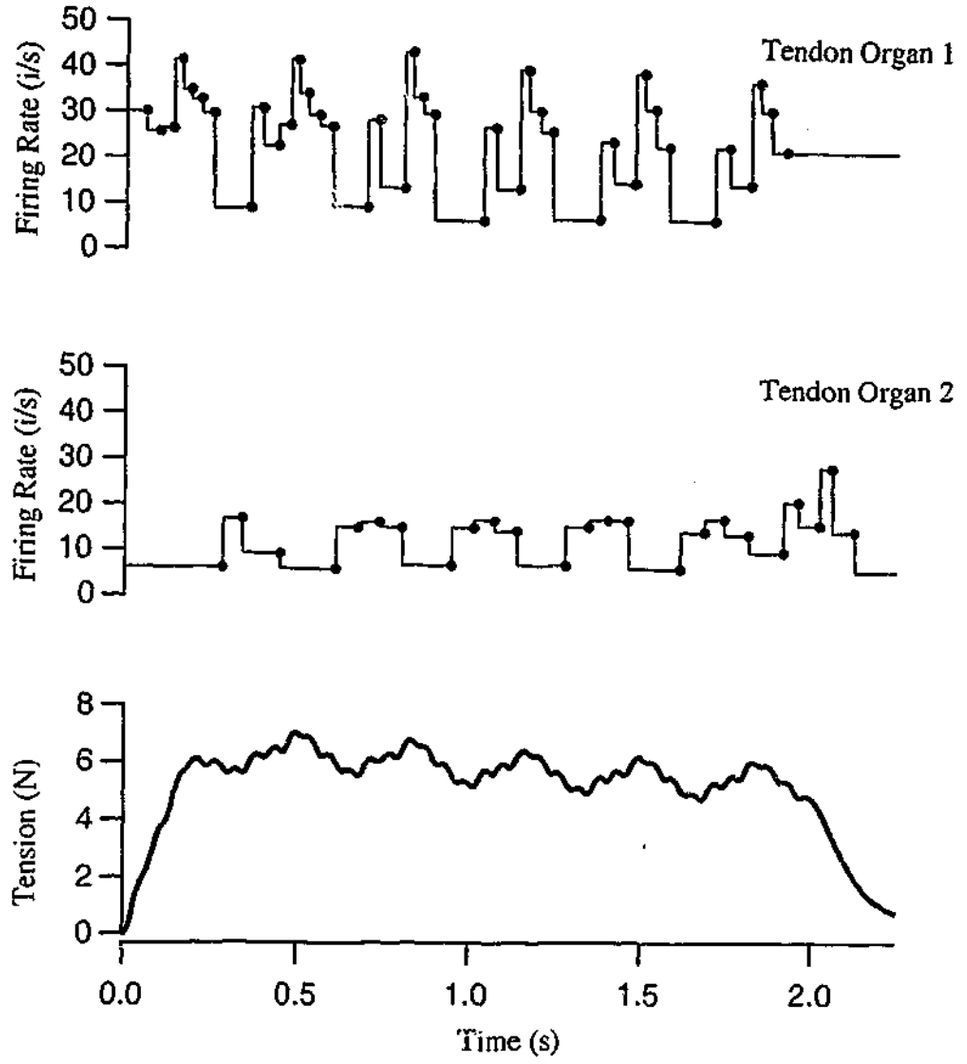


Figure 6.20. *Upper traces* show the backfilled firing rates from two tendon organs located in the cat soleus muscle in response to distributed stimulation at 3pps using equal intervals. Muscle tension shown in the *lower trace*. The black circles indicate instantaneous firing rate (i/s) which was 'backfilled' (red line) to transform the discrete instantaneous rate data into a continuous trace. This was necessary so that an average response, or ensemble, from six tendon organs could be calculated. Backfilling assumed a constant rate from the preceding impulse to the current impulse.

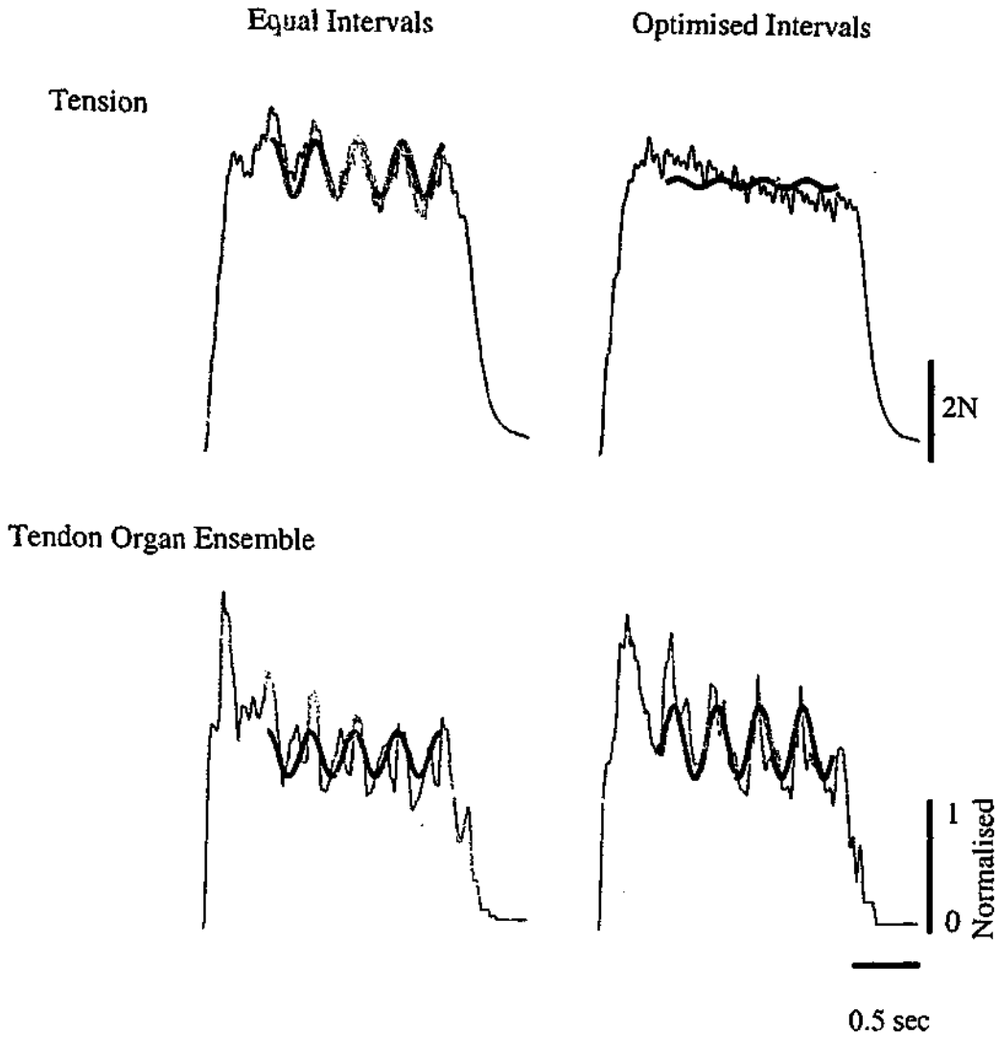


Figure 6.21. Tension (*upper panel*) and ensemble response from six tendon organs (*lower panel*) for equal (*left panel*) and optimised (*right panel*) interpulse intervals. The stimulation rate was 3pps. The ensemble response was calculated by backfilling each tendon organ record, normalising this to the minimum firing rate during stimulation, and then averaging this data for six tendon organs. The fitted sine waves indicate the fundamental component of tension ripple (black) and tendon organ firing rate ripple (red). It is evident that when the FRip of the tension trace was reduced below 1% of average tension (*upper right panel*) there was still considerable ripple present in the ensemble tendon organ response (*lower right panel*).

measure of fundamental tension ripple. On closer inspection of the tendon organ ensemble discharge in responses to twitch contractions of each muscle portion it became clear that the discharge did not correlate well with twitch tension.

Accelerometer

During a submaximal contraction there was always some movement of the muscle as different fibres contracted and relaxed. This raised the possibility that muscle movement might provide a signal proportional to tension ripple. The idea under investigation was that stimulation of muscle portions producing larger muscle twitches might cause greater movement of the muscle through greater stretch of the tendon. In this situation, could an accelerometer attached to the muscle provide signals related to fundamental tension ripple?

To determine whether relevant ripple information could be recorded from muscle movement, an accelerometer was attached to the muscle belly of the rat triceps surae muscle group and its output recorded during distributed stimulation. As in the previous experiment using tendon organ output, the accelerometer output was monitored during distributed stimulation using equal and optimised interpulse intervals. Like the previous result, there was no decrease in the fundamental component of ripple in the accelerometer output, even when the tension trace was relatively smooth and fundamental tension ripple was reduced to less than 1%.

On closer inspection the relationship between twitch tension and accelerometer output was relatively poor. Muscle portions producing relatively large twitch tension could, on occasion, produce small accelerometer signals. An example of this is shown in figure 6.22. This result indicated that an accelerometer placed on the muscle belly was not able to provide output proportional to twitch amplitude and therefore could not provide an alternative source of fundamental tension ripple.

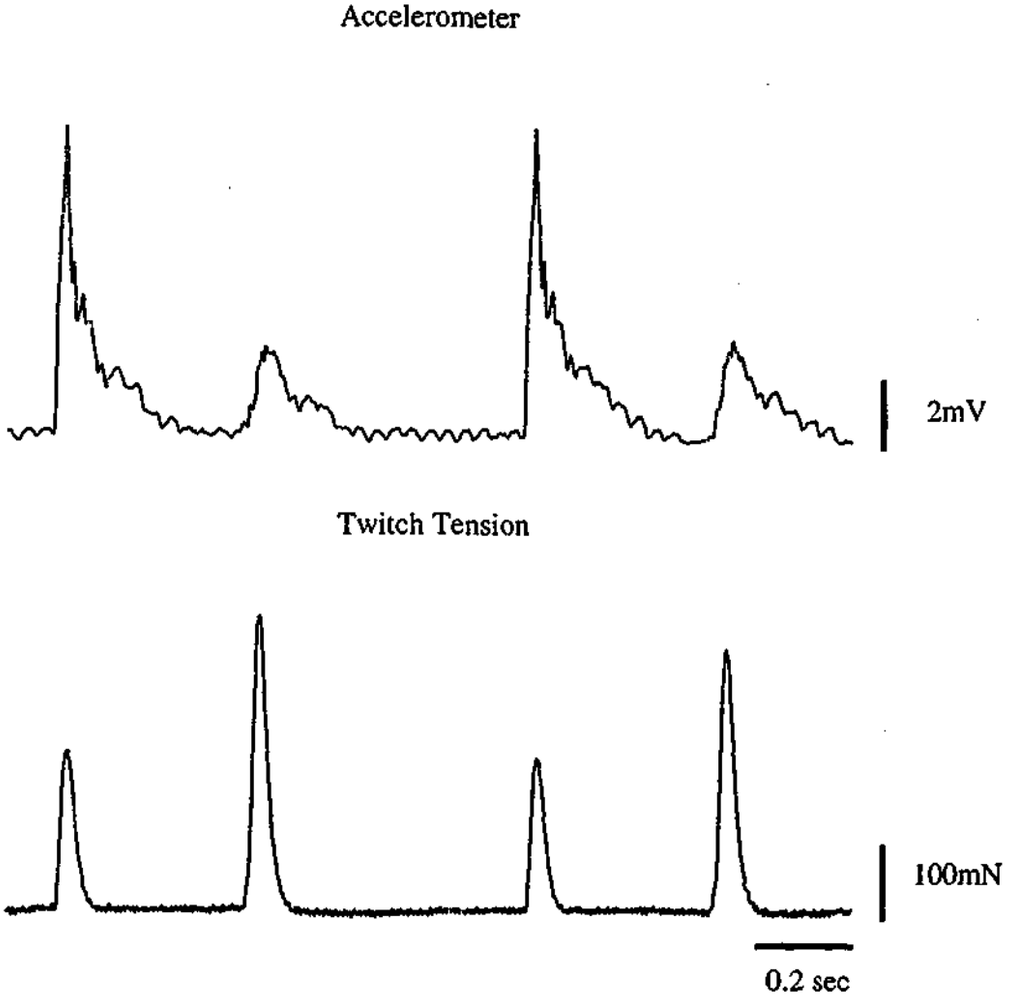


Figure 6.22. *Upper panel* shows the rectified and smoothed output of the accelerometer in response to muscle twitch. *Lower panel* shows the muscle twitch tension during stimulation of two separate portions of the rat triceps surae. There was no correlation between twitch amplitude and accelerometer output. In this example there was less movement on the surface of the muscle when the muscle portion generating the larger twitch tension was stimulated.

Discussion

Rack & Westbury (1969) developed the method of distributed stimulation in order to produce smooth contractions of the cat soleus muscle with low stimulation rates. A limiting factor with this method was a requirement that tension contributions from muscle portions were equal and remained equal over time. If tension inequalities developed between muscle portions then the contraction would no longer be smooth but would oscillate at the stimulation frequency. This requirement meant that distributed stimulation was not particularly suitable for stimulation of muscle containing a mixture of fibre types. With mixed muscle, stimulation history effects, most noticeably muscle potentiation and fatigue, can change the tension of muscle portions in a non-uniform way (see figure 6.7). A stimulator was used that could adjust for these tension inequalities by adjusting, or optimising, the interpulse intervals. With this new method of optimising intervals, tension inequalities could be accommodated and smooth contractions could be generated and maintained. The functioning of the stimulator was examined using the cat medial gastrocnemius, cat soleus and rat triceps surae muscles.

Smooth contractions were produced and maintained during distributed stimulation using optimised interpulse intervals. Compared to equal intervals, stimulation using optimised intervals produced contractions with less peak-to-peak tension ripple and virtually no fundamental ripple, less than 1% of average tension (see figure 6.10). The difference between tension ripple for equal compared to optimised intervals depended on the relative twitch amplitudes of the muscle portions. If twitch amplitudes of all muscle portions were approximately the same then there was no advantage in optimising the stimulus intervals. The benefits of optimised intervals became apparent as the twitch amplitudes became unequal with fatigue or potentiation.

There were pronounced differences between synchronous stimulation and optimised distributed stimulation (see figure 6.11). Synchronous stimulation of the cat medial gastrocnemius muscle at rates lower than approximately 25pps produced unfused contractions, with lower average tension than that generated during optimised distributed stimulation at the same rate. When contractions are unfused, muscle fibres will shorten and lengthen thereby stretching and releasing the elastic component of the tendinous structures. Internal motion within the muscle will lead to some mechanical detachment of cross-bridges reducing the force generating capabilities of the muscle. This effect has been observed during muscle vibration where tension is known to fall

(Joyce, Rack *et al.* 1969). For optimised distributed stimulation there was less internal motion due to the asynchronous activation of different muscle portions. Tension was maintained within the muscle so elastic elements could not passively shorten therefore producing contractions that were more nearly isometric (Lind & Petrofsky 1978). It is well known that muscle produces greater tension when contracting isometrically than when shortening (Hill 1938).

Contractions from three different muscles were compared using low rates of optimised distributed stimulation. For a given rate of stimulation, smoother contractions were obtained from the cat soleus muscle, a slow twitch muscle, than the rat triceps surae muscle group or the cat medial gastrocnemius muscle. Muscle fibre type and muscle length are known to influence twitch contraction time, which is longer for slow twitch muscle. The rate of synchronous stimulation required to produce smooth contractions was shown to be about double that required when optimised distributed stimulation was used (see figure 6.13).

Optimisation of interpulse intervals reduced the fundamental component of tension ripple to levels below 1% of average tension. However, at low rates the contractions were not completely fused and there was some degree of peak-to-peak tension ripple. The remaining ripple was at frequencies higher than the fundamental frequency having ripple components at harmonics of the stimulation frequency. The second harmonic varied between experiments and it appeared to be influenced by the pattern of tension generated by each muscle portion. If a few muscle portions produced relatively large twitch tension, then typically the second harmonic ripple was more pronounced even though the fundamental ripple had been reduced to minimal levels. In future experiments, one possible way to reduce the second harmonic ripple would be to re-program the optimisation algorithm to calculate both fundamental and second harmonic components, and then optimise interpulse intervals according to which component was the larger. For instance, if the second harmonic ripple was larger as a percentage of average tension, then the intervals would be optimised to reduce the second harmonic component. From a functional point of view, the existence of tension ripple at higher frequencies is not so much of a problem in FES, as the inertia of the limb would reduce the movement for higher frequency components of tension. Therefore, the primary concern is always to reduce the tension ripple at the stimulation frequency.

Length-Tension Relationship

The length-tension relationship was examined in the cat soleus muscle and the rat triceps surae muscle group using optimised distributed stimulation with rates ranging from 3pps to 32pps. The results indicated that the optimal length shifted towards longer muscle lengths when lower rates of stimulation were used. For the rat triceps surae, optimal length could be shifted up to or beyond the maximum physiological length during low frequency optimised distributed stimulation (3pps-8pps). Possible mechanisms for this finding were discussed in Chapter Four on page 77. These results are consistent with previous findings for the cat soleus muscle using 3pps to 35pps (Rack & Westbury 1969; Guimaraes, Herzog *et al.* 1994), for cat the medial gastrocnemius muscle using stimulation rates of 10pps to 100pps (Heckman, Weytjens *et al.* 1992) and for the rat medial gastrocnemius muscle using stimulation rates of 15pps to 100pps. However, the two studies on the cat and rat medial gastrocnemius muscles used synchronous stimulation of the entire muscle, therefore the contractions at the lower frequencies were relatively unfused.

For fully activated muscle the sarcomere length-tension relationship can be explained in terms of the overlap of actin and myosin filaments in sarcomeres. It is hypothesised that when high rates of stimulation were used, 32pps for the rat triceps surae muscles and 35pps for the cat soleus muscle, filament overlap was the primary mechanism responsible for the length-tension relationship. When the muscle was not fully activated (3pps to 16pps), factors other than filament overlap influence the length-tension relationship. It was suggested that length dependent changes in Ca^{2+} release and sensitivity was a mechanism contributing to the shift of the optimal length to longer muscle lengths (Rudel & Taylor 1971; Endo 1972; Stephenson & Williams 1982). It is also true that the duration of a muscle twitch is longer at longer muscle lengths, which leads to more effective fusion of the contraction. The reduced internal motion at longer muscle lengths could contribute to the shift in the optimal length observed during submaximal contractions.

There are differences in Ca^{2+} sensitivity between mammalian fibre type with a greater shift in the force-pCa relationship observed for slow twitch rat soleus muscle than fast twitch rat EDL muscle (Stephenson & Williams 1982; Stephenson & Williams 1983). The effect was not due to altered Ca^{2+} concentrations at different lengths but was most likely attributable to the sensitivity of the contractile mechanism to Ca^{2+} (Balnave & Allen 1996). This finding is relevant to the experiments using the rat triceps surae

muscle group as muscle fibres located in the rat soleus muscle, the medial gastrocnemius muscle and the lateral gastrocnemius muscle could show different changes in optimal lengths during submaximal stimulation.

Force-Velocity Relationship

The aim of this experiment was to examine the force-velocity properties of a mixed muscle group, the rat triceps surae, at various levels of activation. The results are explained in terms of the sliding filament theory of muscle contraction and are compared to the force-velocity data obtained by Joyce *et al.* (1969) for the cat soleus muscle.

According to the 'independent generator sliding filament theory' of Huxley (1957), muscle generates tension by the formation of links called cross-bridges. Cross-bridges form between actin and myosin filaments within each sarcomere and act to slide these filaments past one another. The number of cross-bridges attached at any one time depends on the rate of attachment and the rate of detachment. Attachment can only occur over a limited range of cross-bridge distortion, and the rate of attachment is related to the activation level of the contractile mechanisms. Cross-bridges are only slowly detached if they remain in the position in which they were formed but the rate of detachment increases when the cross-bridges are distorted by movement of the actin and myosin filaments, such as occurs when a muscle shortens or is obliged to lengthen during contraction.

When a contracting muscle shortens at a constant velocity, the force generated is lower than its isometric level. This is due to the combined effects of an increase in the average rate of detachment, and hence the number of attached cross-bridges, and a decrease in the force exerted by each cross-bridge. When a muscle lengthens during contraction the cross-bridges are stretched and the force that they exert increases. Counteracting this effect is an increase in the average detachment rate. For a given level of activation the tension generated when a contracting muscle lengthens is a balance between the extra force developed by each attached cross-bridge and the decrease in the number of attached cross-bridges. If a muscle is activated maximally then the rate of attachment is sufficient to ensure rapid reattachment of detached cross-bridges, therefore the tension is likely to stay above isometric levels. However, if the level of activation is insufficient to overcome the higher detachment rate then the tension

generated during muscle lengthening might decrease below isometric levels (Joyce, Rack *et al.* 1969).

Active tension during lengthening of a maximally activated muscle is biphasic in appearance. There is an initial increase in tension over a short range due to an increase in the force of each attached cross-bridge before they detached. Beyond this, the force is relatively constant for a given velocity, independent of how far the muscle has been stretched. This observation led to the idea of the force-velocity curve. In a Huxley (1957) model, this corresponds to a steady state cross-bridge distribution. While the force during constant velocity stretch is relatively constant for maximally activated muscle fibres on the plateau of their length-tension curve, greater variations with length are seen when muscle is submaximally activated. Different levels of activation can alter the nature of these tension transients. Tension may initially increase and then drop, sometimes below isometric values (Joyce, Rack *et al.* 1969). The data from the rat triceps surae experiments using maximal activation showed that tension initially increased rapidly, and then continued to increase gradually over the remainder of the stretch (see figure 6.17). One possible reason for the gradual increase in tension is that different muscle fibres within the rat triceps surae muscle group may have different optimal lengths. Tension from muscle fibres on the ascending limb of their length-tension curve would increase during stretch, whereas tension from the fibres on the descending limb would be nearly constant (Morgan 1990). Measurements were always made 1mm into the ramp length change in order to minimise the effect of increasing tension during constant velocity muscle lengthening.

Tension responses to constant velocity lengthening and shortening ramps were measured during optimised distributed stimulation of the rat triceps surae. Ratios (P/Po) were calculated and then rescaled to enable comparisons of this data to the results of Joyce *et al.* (1969), which is shown in figure 6.23. When comparing the two sets of data, the force-velocity relationship of rat triceps surae muscle, when stimulated at 16pps and 32pps (refer to figure 6.19), was similar to the cat soleus muscle data for 35pps (figure 6.23). Tension ratios during lengthening contractions rose above isometric levels and during shortening, ratios showed a clear velocity dependence and decreased exponentially with increasing velocity. The force-velocity relationship of rat triceps surae was different when the lowest stimulation rate was used (8pps, figure 6.19). Here, the relationship was more linear and was similar in appearance to the force-velocity relationship of the cat soleus muscle for 3pps (figure 6.23). One noticeable difference in

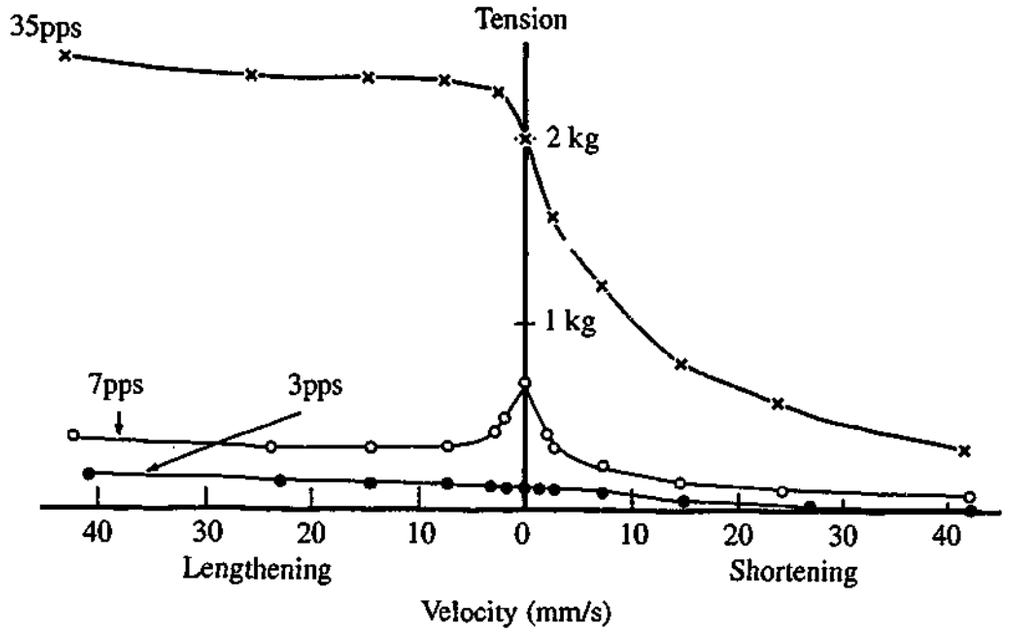


Figure 6.23. Experimental data from Joyce *et al,* (1969) showing muscle tension measured during ramp lengthening and shortening movements at different velocities. Distributed stimulation of the cat soleus muscle was used to produce contractions using three different stimulation rates (3pps, 7pps and 35pps). When 35pps was used tension increased above isometric levels during lengthening. When 7pps was used and the muscle was lengthened, tension decreased below isometric levels. A stimulation rate of 3pps produced a force-velocity curve that was relatively flat and linear in appearance (reproduced from Joyce *et al,* (1969) with permission).

the two sets of data was that, for the rat triceps surae, tension remained above isometric levels during muscle lengthening for all stimulation rates. However, the results for the cat soleus muscle showed that for stimulation rates of 7pps, tension decreased below isometric values. This finding might reflect differences in the length over which measurements were made. Tension ratios for the rat triceps surae were determined at L_{opt} (usually L_{max}-1mm to L_{max}-2mm) whereas measurements by Joyce *et al.* (1969) were made with the ankle fixed at 70°, a position corresponding to a muscle length of approximately L_{max}-7mm, on the ascending limb of the length-tension curve.

Another possible mechanism responsible for this difference relates to movement within the muscle. At low stimulation rates isometric contractions may not be truly isometric because of movement within the muscle. As mentioned previously, internal motion will increase the average rate of cross-bridge detachment. For a given velocity of shortening, this would reduce the number of attached cross-bridges and so decrease muscle tension. Supporting this suggestion is the finding that when contractions were generated by synchronous stimulation, average tension was lower than when optimised distributed stimulation was used, where contractions were much smoother (see figure 6.11). Optimised distributed stimulation of the cat soleus muscle produced smoother contractions compared to the rat triceps surae muscle group at the same rate (see figure 6.12). Therefore, one possible reason accounting for the difference between the force-velocity relationships of the rat triceps surae muscle group (8pps) and the cat soleus muscle (7pps) could relate to how fused, or smooth, the respective contractions were. The presence of some internal motion within the rat triceps surae during stimulation at 8pps would mean that the isometric tension levels were lower than if the contraction was fully fused. When a higher rate of stimulation was used (16pps), contractions were more fused but the stimulation rate might have been too high to observe the effects of submaximal stimulation on the force-velocity relationship. This was not a problem for the 7pps result of Joyce *et al.* (1969) as presumably the contractions were sufficiently smooth and submaximal to observe these effects.

The effects of fused and unfused submaximal contraction can be modelled as an increased rate of cross-bridge detachment in the range 0-h (see appendix, figure 9.2). To examine the effect of a high detachment rate on the force-velocity relationship a simulation was carried out using Huxley's model of molecular contraction dynamics (Huxley 1957) with detachment rate constants from Zahalak's modelling of Rack & Westbury (1969) (Zahalak 1986) (see appendix for a detailed description). Two

conditions were examined, one using the attachment and detachment rates defined by Zahalak (1986) that produced a close approximation of the Joyce *et al.* (1969) result for cat soleus muscle stimulated at 7pps. In the other condition, the detachment rate was increased to simulate an increase in internal motion. The results are shown in figure 6.24.

For submaximal activation levels using the attachment and detachment rate constants defined by Zahalak (1986) the force-velocity curve (red) was similar to the cat soleus muscle experimental data (7pps) obtained by Joyce *et al.* (1969), which Zahalak was modelling. However, when the simulation was repeated, this time using a higher detachment rate constant, the isometric force decreased and the force-velocity curve (blue) was much flatter. This result is consistent with the experimental data from the rat triceps surae muscle group for 8pps and the cat soleus muscle for 3pps reported by Joyce *et al.* (1969).

Would an intermediate stimulation rate, for instance 12pps, produce force-velocity curves similar to the curve found when the cat soleus muscle was stimulated at 7pps? The stimulation rates of 8pps, 16pps and 32pps used in these experiments were chosen because they generated isometric contractions over a similar force range, relatively speaking, to those used by Joyce *et al.* (1969). A stimulation rate of 16pps might not be low enough and a rate of 8pps not fused enough to observe the effects of submaximal activation on muscle lengthening.

Two approaches could be used in future experiments to investigate this possibility. The rate of stimulation could be increased within the range 8pps to 16pps. Alternatively, the number of portions used in optimised distributed stimulation could be increased so that smoother contractions at low stimulation rates could be produced. Combinations of both of these approaches might be the best method to examine this point. It would be interesting to define the relationship between smoothness and stimulation rate to determine how smooth and how submaximal must a contraction be to observe a decrease in tension during muscle lengthening?

Control Signal

To optimise interpulse intervals during distributed stimulation a feedback signal representing tension ripple was provided by a force transducer placed in series with the muscle. However, if optimised distributed stimulation is to be used clinically then alternative feedback methods will need to be explored. The control system does not

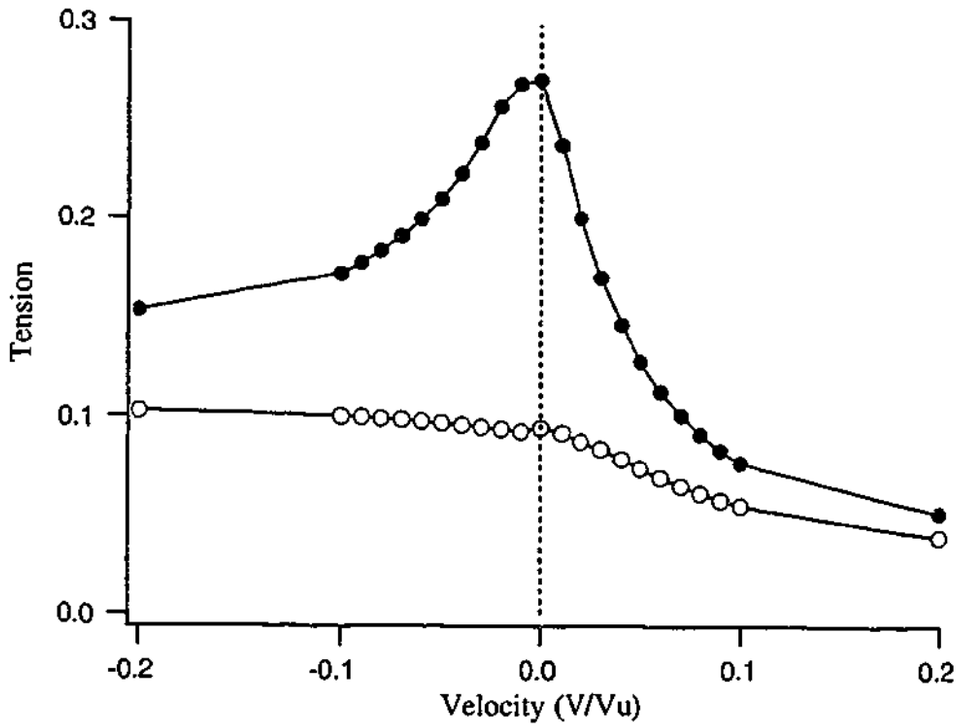


Figure 6.24. Simulated force-velocity data during submaximal activation using Huxley's model of molecular contraction dynamics (Huxley, 1957). V_u is maximum unloaded velocity and positive values represent muscle shortening. Shown in red is the relationship between force and velocity for a model of the submaximally activated cat soleus muscle using attachment rate constants and detachment rate constants chosen to represent the cat soleus muscle (Zahalak, 1986) (see appendix for detailed description). If the detachment rate constant within the attachment region was increased (blue), to simulate an unfused contraction, the force-velocity relationships became more linear, being similar to the rat triceps surae (8pps) and cat soleus (3pps) data.

need to access absolute muscle force but just the fundamental component of tension ripple. In this study the suitability of using two different sources of signals was explored, the output from tendon organs and from an accelerometer.

Tendon Organ Ensemble

It has been suggested that an ensemble discharge from several tendon organs might signal changes in whole muscle tension more accurately than just one receptor (Crago, Houk *et al.* 1982; Appenteng & Prochazka 1984). This raised the possibility that an alternative feedback signal of tension ripple could be provided by the average response from a number of tendon organs. The aim of the experiment was to determine whether it might be possible to use signals from tendon organs to optimise interpulse intervals.

An ensemble discharge was calculated from the responses of six tendon organs during optimised distributed stimulation. The fundament component of ripple in the tendon organ ensemble discharge was calculated during contractions using equal and optimised interpulse intervals. The results from this experiment were largely negative and showed that the fundamental ripple in the ensemble discharge actually increased when the fundamental ripple in muscle tension was minimised (figure 6.21). Therefore, it was concluded that under these conditions the ensemble discharge does not provide a measure that could be used to reduce fundamental tension ripple.

The ensemble discharge was not well correlated with twitch tension generated by each muscle portion, it was relatively high for some muscle portions but not others. There are approximately 45 tendon organs in cat soleus muscle each innervated by muscle fibres from up to ten motor units (Crago, Houk *et al.* 1982). Some muscle portions contained motor units that did not innervate the selected tendon organs and therefore were under represented in the sample. The ensemble discharge was dependent on which motor units were stimulated and was not altered significantly by adjustment of interpulse interval. In future experiments, the ventral roots could be subdivided making sure that each muscle portion was represented in the sample of tendon organs. Recent experiments by Morgan, Proske & Gregory (personal communication) have taken a different approach. They were able to optimise the firing rate of one tendon organ by adjusting the interpulse intervals between muscle portions based the tendon organ firing rate. In these experiments the fundamental component of ripple in the tendon organ was reduced to low levels but the fundamental ripple in tension remained high.

Accelerometer

In two experiments an accelerometer was attached to the belly of the rat triceps surae muscle and the output was monitored during distributed stimulation. Similar to the tendon organ experiment, the fundamental component of the accelerometer output was measured during stimulation using equal and optimised interpulse intervals. The results showed that there was no reduction in the fundamental component of the accelerometer output when the contractions were optimised. On closer examination of the twitch profile of the muscle portions, it became apparent that the magnitude of the accelerometer output was not matched to twitch size. Presumably the most superficial motor units, those lying closest to the sensor, had the greatest influence on the accelerometer output. Motor units located in the soleus muscle of the rat triceps surae, while not producing much movement on the surface of the muscle, would have contributed to the developed tension.

In these experiments the accelerometer was attached to the muscle itself. In future experiments, for example, using distributed stimulation of human muscle with surface electrodes, the accelerometer could be placed on the limb itself. Movement of the limb in response to muscle stimulation might enable the accelerometer to provide a signal related to fundamental tension ripple. This idea could be explored in future experiments.

Chapter Seven

Muscle Fatigue

Aims

It is well known that muscle activated at high rates of stimulation fatigues more rapidly than if it is stimulated at low rates. The rationale behind these experiments was not to re-examine this phenomenon directly, but rather, to explore the fatigue properties of muscle when activated using optimised distributed stimulation. In applications like FES, muscle is normally activated synchronously using high stimulation rates to produce fused contractions. The use of optimised distributed stimulation as a strategy to activate paralysed muscle might provide benefits in terms of minimising muscle fatigue and generating stable contractions that can be graded over a wide range of force. Experiments in this chapter were carried out to explore this possibility.

This series of experiments examined the fatigue properties of the cat medial gastrocnemius muscle using optimised distributed stimulation at low rates (6pps and 10pps) and synchronous stimulation at high rates (36pps and 60pps). Two different protocols were examined, intermittent and continuous stimulation, reflecting two types of muscle contractions that would be required of an FES system. For example, generating continual muscle force to maintain a posture or intermittent contractions for cyclical tasks such as locomotion.

Method

The experiments were carried out on a total of 10 cats (*Felis domesticus*) of both sexes weighing between 4kg and 7.9kg, prepared as detailed in Chapter Two (page 52). The cats were obtained from the Monash University Central Animal House and kept in the Physiology Animal House. All experiments were carried out with approval from the Monash University Standing Committee on Ethics in Animal Experimentation.

The cats were deeply anaesthetised with pentobarbitone (40mg/kg, Nembutal, Boehringer Ingelheim, Australia) and a laminectomy was carried out to expose the ventral roots L6-S2. The left hind-limb was dissected and the medial gastrocnemius muscle was separated from the lateral gastrocnemius muscle and the soleus muscle. The tendons of the lateral gastrocnemius muscle and the soleus muscle were cut from the Achilles tendon leaving the medial gastrocnemius tendon attached to the calcaneum. The calcaneum was detached from the ankle and attached in series with a U-shaped strain gauge transducer to an electromagnetic servomotor. The compliance of the system was 5μm/N. All hind-limb nerves, other than the medial gastrocnemius nerve, were cut including those to the hip. A bath containing mineral paraffin oil, created from the skin flaps of the hind-limb, covered the exposed muscle.

The ventral root supply to the medial gastrocnemius muscle was divided into two parts which, when stimulated, produced approximately 70% and 30% of whole muscle twitch tension. The 30% part was used for synchronous stimulation and the 70% part was further divided into six portions and used in optimised distributed stimulation. Ventral root portions were mounted onto an array of platinum bipolar stimulating electrodes and stimulus strength was adjusted to be supramaximal. Evidence of stimulus spread was sought by comparing the twitch size of all portions with the sum of the twitches of one portion and the remaining portions. If the size of the twitch of all portions was less than linear summation then this indicated stimulus spread. In practice, stimulus spread could always be kept to a minimum. In each experiment fatigue was measured for optimised distributed stimulation and synchronous stimulation. It was assumed that fatigue was restricted to the motor units activated by the electrical stimulation and that the unstimulated motor units were unfatigued. Muscle tension was digitised and recorded at 2000/s with the MacLab data acquisition system (MacLab/8s, ADInstruments, Australia) using Chart software (MacLab, ADInstruments, Australia) on a Macintosh G3 computer.

In each experiment the optimal muscle length was determined using 0.5sec duration 50pps tetanic stimulation. All experiments were carried out with the muscle fixed at optimal length, typically 8mm to 10mm shorter than maximum physiological length. For the experiments using optimised distributed stimulation interpulse intervals were initially optimised (FRip < 1% of average tension) during a number of one-second contractions, and then continually optimised over the course of the experiment.

It was possible that muscle fatigue was not only due to mechanical factors but also from alterations in muscle excitation during stimulation. Muscle EMG was measured to monitor changes in the electrical excitation of the muscle. Intramuscular EMG was measured with a pair of flexible platinum wires, bared at their tips and inserted into the belly of the muscle with a hypodermic needle. The EMG signal was filtered with a pass band from 10 to 1000Hz, suitably amplified and recorded digitally at 2000/s with a MacLab data acquisition system (MacLab/8s, ADInstruments, Australia) using Chart software (MacLab, ADInstruments, Australia) on a Macintosh G3 computer. Analysis was carried out using Igor Pro (Wavemetrics, USA).

The width and the amplitude of the each compound muscle action potential were measured (figure 7.1). The EMG was then rectified and integrated. EMG amplitude and integral provided qualitatively similar results so only the integral data will be presented. EMG was normalised, relative to its value during the last minute of stimulation, to account for recording differences between muscle portions. The last minute of stimulation was chosen to normalise EMG as here the EMG levels were stable over time. For optimised distributed stimulation, EMG from six muscle portions was averaged and all data is represented as averaged normalised EMG.

Fatigue was examined using two stimulation protocols, continuous (n=5) and intermittent stimulation (n=5). In each experiment synchronous stimulation of 30% of the muscle was compared to optimised distributed stimulation of 70% of the muscle. The intermittent protocol consisted of a three-second contraction every ten seconds for 40 minutes. The average tension and integrated EMG was calculated for each three-second contraction. In the continuous stimulation protocol the muscle received continual stimulation without rest over a 20 minute period for optimised distributed stimulation, and for five to eight minutes during synchronous stimulation. The shorter period of stimulation reflects the severe fatigue produced with this protocol. Two stimulation rates (6pps and 10pps) were used for optimised distributed stimulation using six muscle portions. This meant that the muscle effectively received a total stimulation

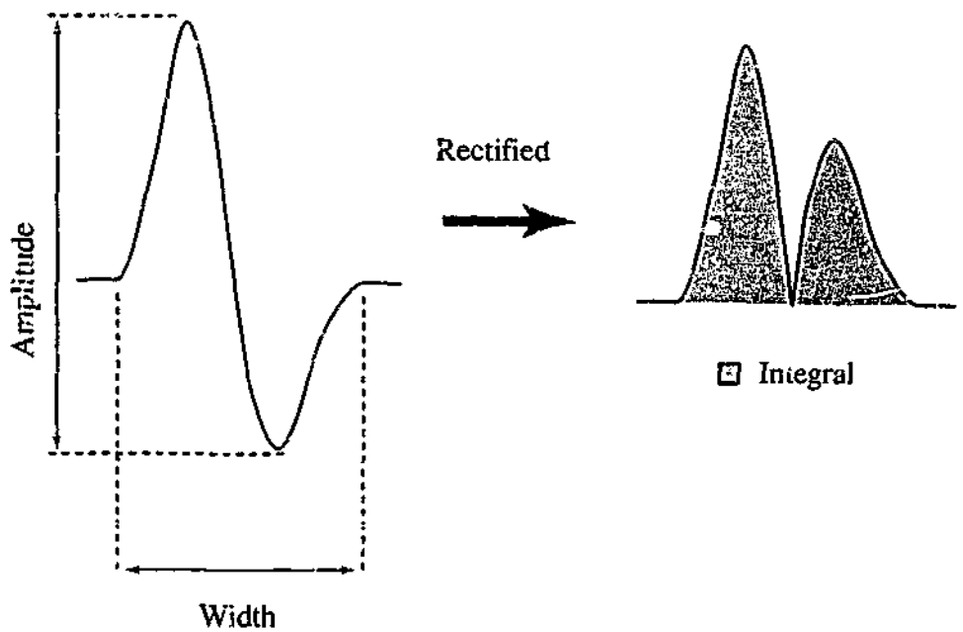


Figure 7.1. EMG analysis showing spike width (duration) and amplitude in the *left panel*. The EMG was then rectified and the integral was calculated (*right panel*). EMG integral was then normalised to its level during the last minute of stimulation. For optimised distributed stimulation, the normalised EMG from all six muscle portion was then averaged.

rate of 36pps and 60pps. For synchronous stimulation of one muscle portion (30% of muscle) 36pps and 60pps were used. Table 1 shows a summary of the eight experimental conditions.

	Distributed	Synchronous
Continuous	6pps	36pps
	10pps	60pps
Intermittent	6pps	36pps
	10pps	60pps

Table 7.1. Summary of the experimental conditions. In each experiment optimised distributed stimulation and synchronous stimulation was used to fatigue the medial gastrocnemius muscle using either a continuous or intermittent stimulation protocol.

A fatigue index (muscle tension after five minutes of stimulation relative to initial tension measured over the first three seconds) and a potentiation index (maximum tension relative to initial tension) were calculated for each condition. A repeated measures ANOVA was used to tests for the statistical significance of the fatigue and potentiation index.

Results

Muscle tension and EMG were measured during optimised distributed stimulation using six muscle portions and during synchronous stimulation at six times the rate of optimised distributed stimulation. Results for continuous stimulation are presented first followed by the results for intermittent stimulation. All results are summarised in table 7.2 on page 121.

Continuous Stimulation

Muscle tension during continuous distributed stimulation at 6pps was compared to synchronous stimulation at 36pps. The results of one experiment are shown in figure 7.2. Both methods of stimulation were characterised by tension potentiation at the start of stimulation. Potentiation was more gradual and pronounced during optimised distributed stimulation and in this example it peaked at 132% of initial tension after 20 seconds of stimulation. This was consistent with the average potentiation from three experiments, 133% (±2.8% SEM) (see table 7.2 page 121). For synchronous stimulation peak tension was 106% of initial tension following seven seconds of stimulation. The average potentiation in all three experiments was 106% (±0.6% SEM). Although only 30% of the muscle was activated during synchronous stimulation (36pps), in the example in figure 7.2, tension reached a value of 47N compared to 25N for optimised distributed stimulation (6pps and 70% of the muscle).

Differences between synchronous stimulation and optimised distributed stimulation were observed in the time course and the magnitude of muscle fatigue. Tension during synchronous stimulation declined rapidly following 15 seconds of stimulation until tension, after five minutes, was only 4.6% of its initial value (figure 7.2). The average was a little higher for all three experiments, 11.6% (±3.7% SEM). For optimised distributed stimulation muscle tension declined to approximately 60% of initial levels after two minutes, and then more gradually to 52% of initial levels after five minutes of continuous stimulation, averaging 56% (±3.7% SEM) for the three experiments.

In two experiments, higher rates of stimulation were used, 10pps for optimised distributed stimulation and 60pps for synchronous stimulation. The results were qualitatively similar but there was a greater degree of tension potentiation for optimised distributed stimulation, 163% (±6.1% SEM) and a fatigue index of 61.7% (±8.0%

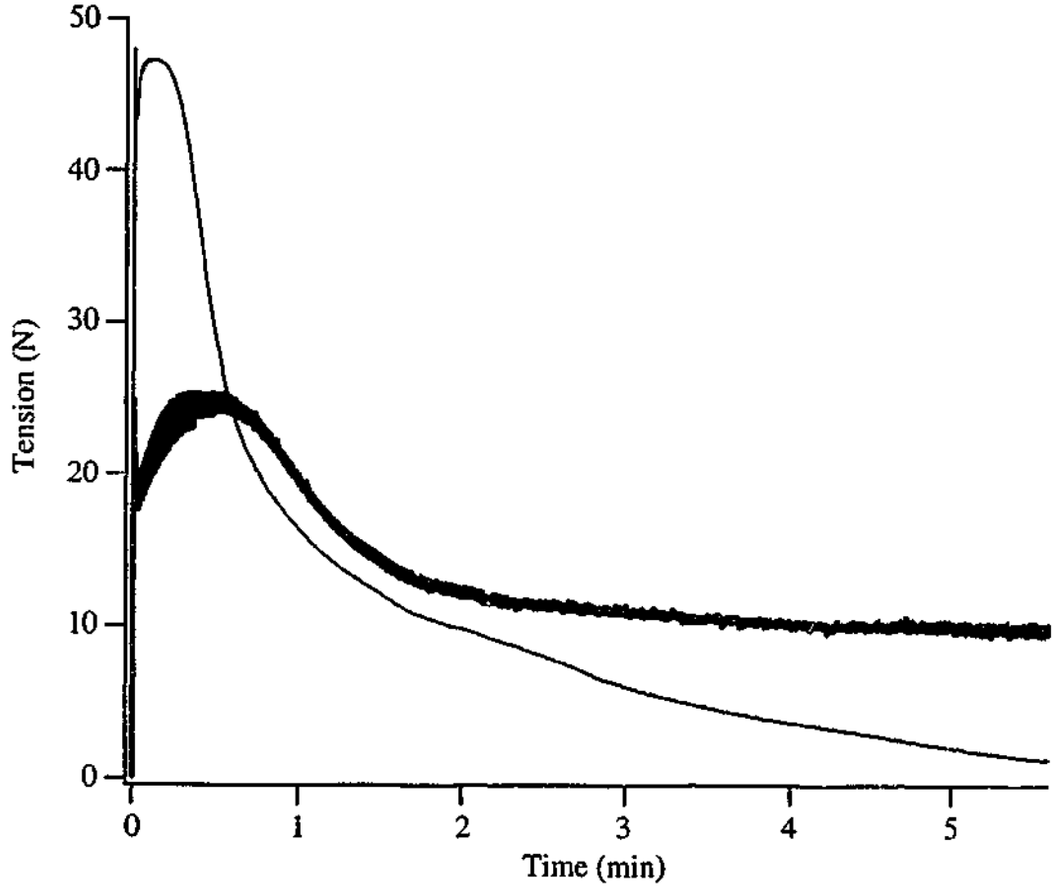


Figure 7.2. Comparison of tension for one experiment using optimised distributed stimulation at 6pps (red trace) and synchronous stimulation at 36pps (blue trace) for five minutes of continuous stimulation. Although the tension during synchronous stimulation reached a higher peak it fell more rapidly and to lower levels than tension during optimised distributed stimulation.

SEM). For synchronous stimulation, on the other hand, tension potentiation was lower at 103% (±1.8% SEM) and, as might be expected with the higher stimulation rate, the muscle fatigued more rapidly with an average of 4% (±0.9% SEM) of initial tension remaining after five minutes (see Table 7.2).

It was possible that alterations in the electrical excitation of the muscle may have contributed to the changes in muscle tension observed during continuous stimulation. In order to examine this possibility intramuscular EMG was measured and examples of tension and EMG using synchronous stimulation at 36pps and optimised distributed stimulation at 6pps are shown for one experiment in figure 7.3. In this experiment, it was evident that after a brief increase, both tension and EMG declined rapidly over the first minute of synchronous stimulation. During the period between 40 seconds and two minutes EMG was better sustained than tension, and from three minutes onwards EMG and tension declined at a similar rate. The spike width during the first three minutes of synchronous stimulation increased from 6-7msec to approximately 10msec, and then decreased to about 4-6msec by the end of stimulation.

The relationship between EMG and tension was not well correlated for optimised distributed stimulation (figure 7.3). Over the first minute of stimulation, tension initially increased more rapidly than EMG, and then began to decline by approximately 50% while EMG levels remained high. From two to ten minutes there was a gradual decline in both tension and EMG. After approximately ten minutes of stimulation EMG declined rapidly but this was not reflected in the tension trace. By the end of stimulation the EMG had decreased by 50%. On average, spike width increased over the first five to ten minutes of stimulation from about 6msec to 10msec where it remained reasonably constant. It became increasingly more difficult to measure the width because the tail of the spike became less discernible above baseline noise.

Pausing Stimulation

Muscle tension and EMG, in response to brief pauses in continuous stimulation, were examined after a period of muscle fatigue. After 5.5 minutes of synchronous and 19.5 minutes of optimised distributed stimulation, pauses lasting 1, 2, 5, and 10 seconds were imposed and figure 7.4 shows the results from one experiment.

Following a pause in synchronous stimulation tension and EMG rose sharply but, within five seconds of the recommencement of stimulation, decreased rapidly to pre-pause levels. The results were different for optimised distributed stimulation. After

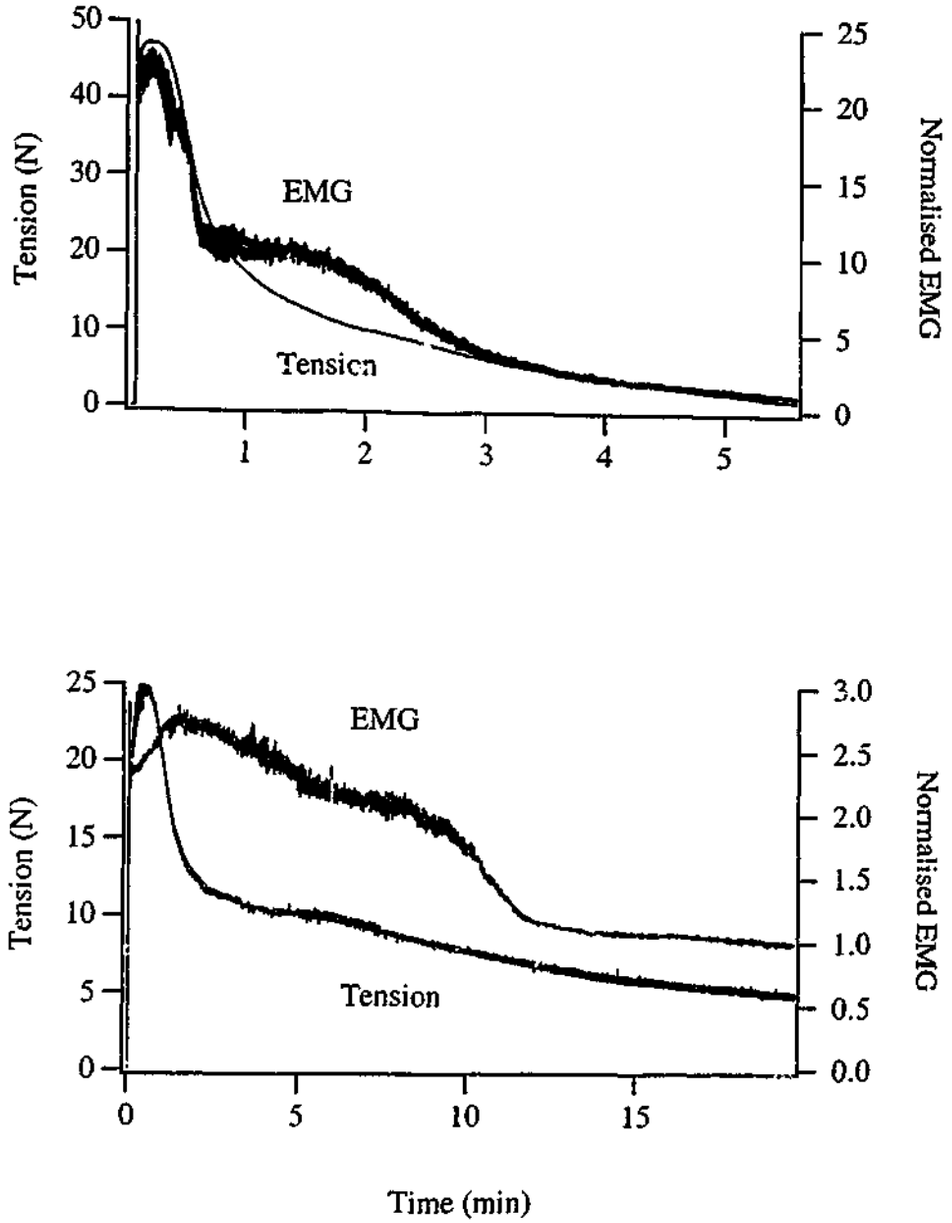


Figure 7.3. *Upper panel.* Muscle tension (thin blue trace) and integrated EMG (thick blue trace) for synchronous stimulation (36pps). After a brief period of potentiation both EMG and tension decreased to a fraction of their initial levels as muscle fatigue set in. *Lower panel.* Muscle tension (thin red trace) and integrated EMG (thick red trace) for optimised distributed stimulation (6pps) over a longer time period. Again there was potentiation in both the tension and EMG followed by a decline in tension that was not well correlated with the decline in EMG.

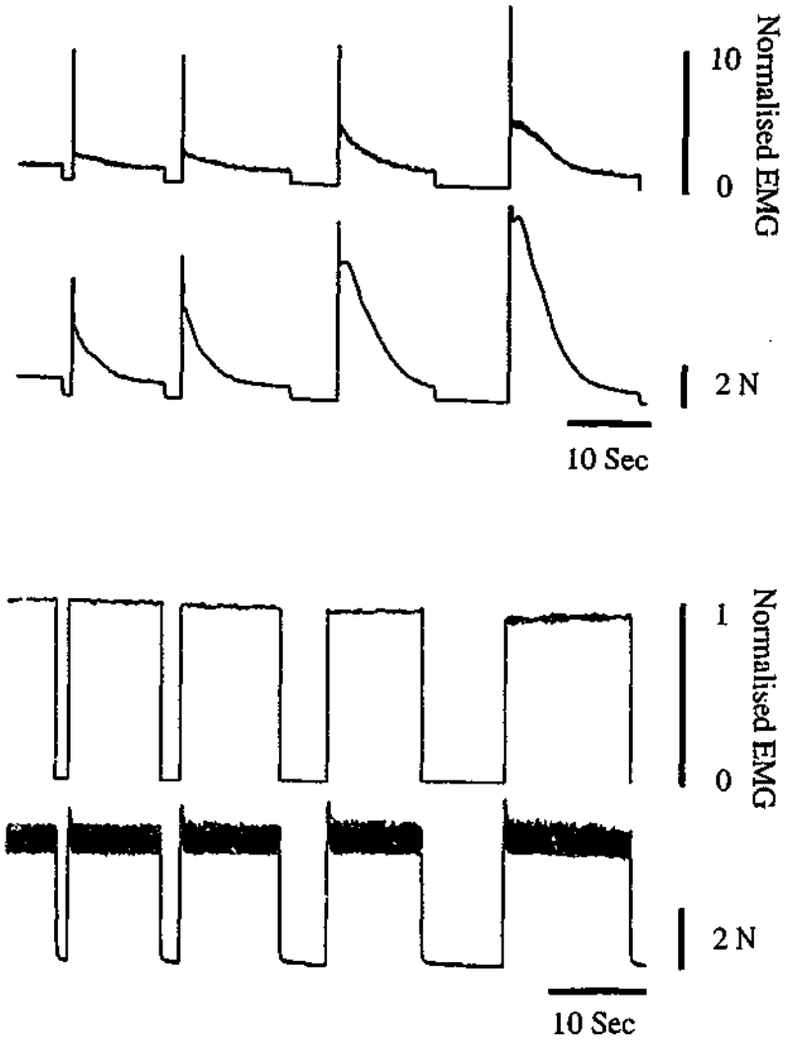


Figure 7.4. Interruptions of 1,2,5, and 10 seconds to continual stimulation following a period of fatigue (data from figure 7.3). *Upper panel.* Tension and EMG during synchronous stimulation (36pps). After 5.5 minutes of continual stimulation brief pauses in stimulation were presented. Both tension and EMG recovered substantially, but then declined rapidly to their previous levels. *Lower panel.* Tension and EMG during optimised distributed stimulation (6pps for each of six portions). Following pauses in stimulation, tension and EMG recovered and remained stable at their pre-interruption levels.

pauses in stimulation both tension and EMG recovered to pre-pause levels. Unlike synchronous stimulation, tension and EMG did not decline rapidly but remained stable.

Intermittent Stimulation

The intermittent stimulation protocol consisted of a three-second contraction every ten seconds. Muscle tension changes were more gradual using intermittent stimulation so the period of stimulation was increased to 40 minutes. Average tension and EMG for each three-second contraction were calculated for synchronous stimulation (36pps) and optimised distributed stimulation (6pps) and the results from one experiment are shown in figure 7.5.

In this experiment the most dramatic difference between synchronous stimulation and optimised distributed stimulation was evident during the first five minutes. For synchronous stimulation, there was no potentiation in muscle tension and tension decreased rapidly during the first five minutes to 52.5% of its initial level. This result was consistent for the two experiments, with an average 53% (±0.8% SEM) of initial tension remaining after five minutes. Conversely, EMG levels did not show a similar decline. Integrated EMG doubled over the first five minutes of stimulation, while at the same time, muscle tension declined rapidly. EMG then decreased to levels approximately one third of its initial value after 40 minutes of stimulation. The spike width increased over the first ten minutes of stimulation from 7-8msec to 12-14msec before it returned to approximately 8msec over the next ten minutes. It remained relatively stable for the remainder of stimulation.

When optimised distributed stimulation was used tension increased over the first few minutes to 110% (see figure 7.5). Tension then gradually decreased as fatigue set in. After five minutes of stimulation the tension had returned to its initial level and, by 40 minutes, it had dropped to 63%. The integrated EMG and the spike width (6-7msec duration) did not change significantly over the 40-minute period of stimulation but remained similar to its levels at the start of stimulation. This result was consistent for the two experiments, the average tension potentiation being 111.2% (±1.0% SEM) and the average muscle tension after five minutes was 99.6% (±1.3% SEM) relative to initial levels.

Optimised distributed stimulation at 10pps and synchronous stimulation at 60pps were used in three experiments (see table 7.2). On average potentiation levels were larger with optimised distributed stimulation at 10pps than when 6pps was used,

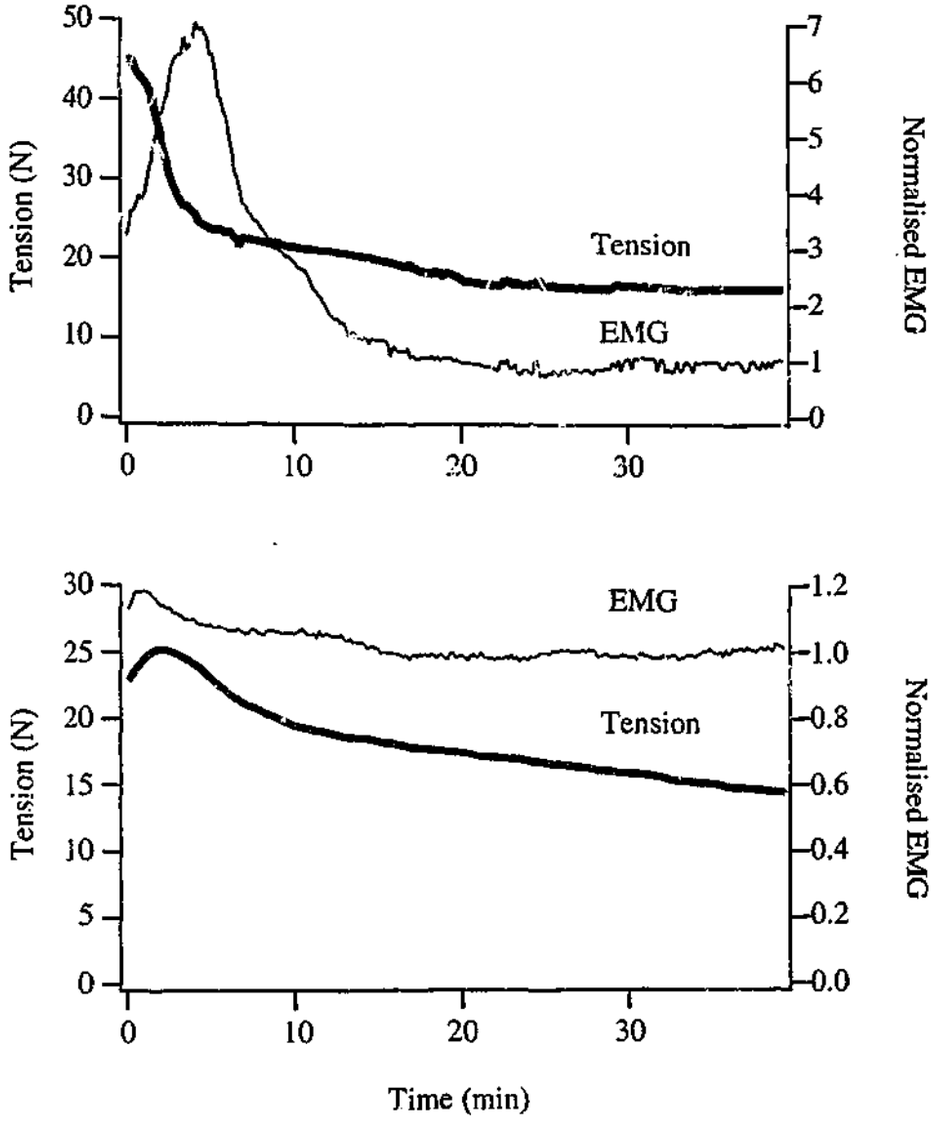


Figure 7.5. Average tension and normalised integrated EMG over a 40–minute period of intermittent stimulation. This protocol consisted of a three–second contraction every ten seconds. Average tension and EMG was measured during each contraction. *Upper panel.* Tension and EMG for synchronous stimulation at 36pps. Tension declined rapidly at first and then more gradually. EMG doubled over the first five minutes of stimulation and then decreased to approximately one third of its initial level. For the remainder of stimulation EMG was relatively stable. *Lower panel.* Tension and EMG for optimised distributed stimulation over six muscle portions at 6pps. Tension initially rose but then declined gradually over 40 minutes of stimulation. EMG remained relatively stable and did not fall appreciably.

115.9% (±6.0 SEM). The decline in tension after five minutes was also greater with tension decreasing to 75.5% (±4.8 SEM) of its initial level. When synchronous stimulation (60pps) was used, tension decreased to 46.9% (±3.2 SEM) of initial levels after five minutes of stimulation, and again there was no tension potentiation.

Tension Sag

The results discussed thus far have been concerned with the average tension for each three-second contraction and monitored its change over the stimulation period. However, when the tension decline within each three-second contraction, called tension sag, was examined then other differences between optimised distributed stimulation and synchronous stimulation became apparent. Sag was defined as the maximum minus the minimum tension as a percentage of average tension for each three-second contraction. Figure 7.6 shows a plot of tension sag for the experiment shown previously in figure 7.5.

Over the first 15-20 minutes of stimulation there was considerably more sag during synchronous stimulation than during optimised distributed stimulation. After 20 minutes of stimulation the synchronous contractions became more steady and the level of sag decreased. There was considerable variation in tension sag between experiments with some muscles showing a maintained difference in sag between synchronous and optimised distributed stimulation and others showing a time dependent difference such as that seen in figure 7.6. The general finding from the analysis of tension sag was that during intermittent stimulation sag was more pronounced for synchronous stimulation than when optimised distributed stimulation was used.

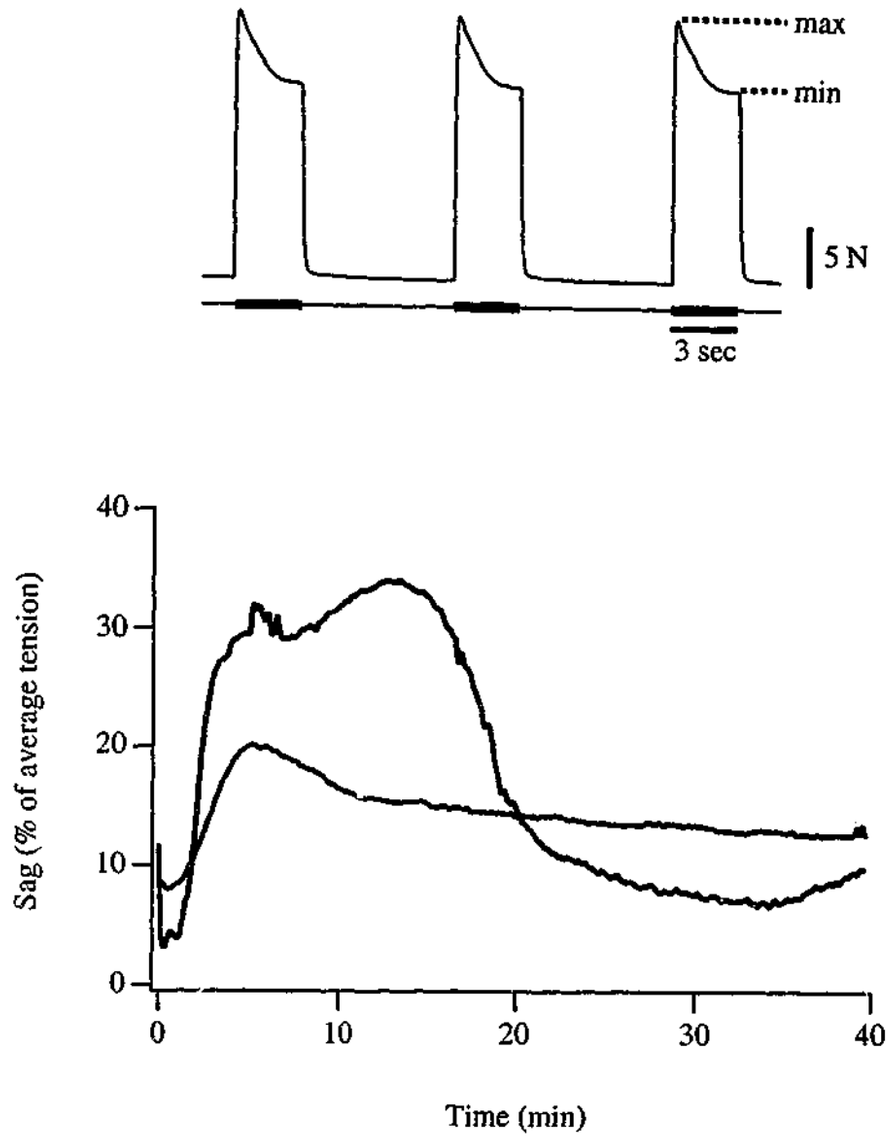


Figure 7.6. Tension sag during each three–second contraction for optimised distributed stimulation (red) and synchronous stimulation (blue) using the intermittent stimulation protocol. *Upper Panel.* An example of three contractions during synchronous stimulation. Sag was a measure of the maximum minus the minimum tension as a percentage of the average tension for that contraction. *Lower Panel.* Sag measured for synchronous and optimised distributed stimulation. There was an initial increase in sag over the first five minutes of optimised distributed stimulation but as the contractions continued the level of sag gradually decreased. For synchronous stimulation sag initially decreased but then increased rapidly only to fall again to levels below those observed with optimised distributed stimulation.

Data Summary and Statistical Analysis

	Distributed				Synchronous				No.
	Stim. Rate	Potentiation (%)		Fatigue 5 Min. (%)	Stim. Rate	Potentiation (%)		Fatigue 5 Min. (%)	Exp.
Continuous	6 pps	133.26 ±2.80		55.94 ±3.67	36	106.56 ±0.58		11.61 ±3.66	3
	10 pps	163.05 ±6.14		61.74 ±7.96	60	103.25 ±1.78		4.23 ±2.90	2
Intermittent	6 pps	111.24 ±1.04		99.55 ±1.30	36	100.00 ±0.00		53.16 ±0.84	2
	10 pps	115.88 ±5.66		75.35 ±4.84	60	100.66 ±0.66		46.92 ±3.22	3

Table 7.2. Summary of data for fatigue and potentiation expressed as a percentage of initial tension (measured at the start of stimulation). Values shown as means and ±SEM for a total of ten experiments.

An ANOVA was used for statistical analysis of the fatigue and potentiation data (table 7.2). Analysis of the fatigue data (tension remaining after five minutes of stimulation) showed that optimised distributed stimulation produced significantly less fatigue than synchronous stimulation ($p<0.0001$). There was significantly less fatigue with intermittent stimulation than with continuous stimulation ($p<0.0001$) and significantly less fatigue with the lower stimulation rates 6pps/36pps compared to 10pps/60pps ($p<0.05$).

Analysis of potentiation (peak tension above initial tension) showed that the level of potentiation was significantly higher using optimised distributed stimulation ($p<0.0001$). There was only a small amount of potentiation during synchronous stimulation. Continuous stimulation produced significantly more potentiation than intermittent stimulation ($p<0.0001$) and there was also a small but significant effect of stimulation rate ($p<0.05$). Two significant interactions were observed; stimulation method (distributed or synchronous) interacted with stimulation rate ($p<0.01$) and with stimulation protocol (continuous or intermittent) ($p<0.001$). These interactions were driven by the high potentiation observed during continuous distributed stimulation with stimulation at 10pps.

Discussion

These experiments examined fatigue properties of the cat medial gastrocnemius muscle when activated asynchronously and sequentially with low stimulation rates and during synchronous activation using high stimulation rates. The main finding was that muscle fatigue was significantly lower using optimised distributed stimulation compared to synchronous stimulation. The fatigue advantage of optimised distributed stimulation was observed with both the continuous and intermittent protocols but the differences were more pronounced during continuous stimulation. It is proposed that different fatigue mechanisms were responsible for fatigue at high and low rates of stimulation using the intermittent and continuous protocols.

Continuous Stimulation

During continuous synchronous stimulation of the cat medial gastrocnemius muscle at high rates (36pps and 60pps), muscle tension declined rapidly over a five-minute period. It is well established that muscle fatigue using high rates of stimulation is associated with a decrease in the electrical excitation of the muscle, in particular, neuromuscular fatigue. There was evidence that failure of neuromuscular transmission was a major cause of fatigue during continuous synchronous stimulation. EMG levels declined to a fraction of their initial value during the course of stimulation (see figure 7.3). The decline in tension was well matched to the decline in EMG suggesting that the mechanism responsible for muscle fatigue was a reduction in muscle excitation. Muscle EMG was previously reported to provide a good estimate of muscle fatigue in the cat medial gastrocnemius muscle during high frequency stimulation (Sandercock, Faulkner *et al.* 1985).

When the stimulation was paused for brief periods there was a transient recovery of both EMG and muscle tension that declined rapidly (see figure 7.4). The rapid recovery of both EMG and tension after pauses in stimulation was consistent with neuromuscular failure, which can recover over a few seconds. The mechanisms most likely responsible for neuromuscular failure are, branch point failure in the motoneurons (Smith 1980), and/or ACh factors such as transmitter depletion from the pre-terminal membrane or ACh desensitisation of the post synaptic membrane (Thesleff 1959). However, the methods employed in this study are unable to pinpoint the relative

contribution of each mechanism responsible for the failure of neuromuscular transmission seen here.

Changes in the EMG spike width were observed during continuous synchronous stimulation. The spike width was shown to increase as stimulation continued. Presumably, this indicates slowing of the conduction velocity of muscle action potentials during stimulation (Stalberg 1966; Gydikov, Kostov *et al.* 1984). This finding is consistent with previous data where slowing of the muscle action potential was observed during continuous activity in single motor units of the cat medial gastrocnemius muscle (Stalberg 1966).

There was less muscle fatigue and greater tension potentiation with optimised distributed stimulation using low stimulation rates (see table 7.2). Twitch potentiation of single motor units following periods of tetanic stimulation was previously shown in the cat medial gastrocnemius muscle (Burke, Levine *et al.* 1973). Presumably, the increase in tension observed during the first minute of stimulation reflects post-tetanic potentiation of muscle tension. The intracellular mechanisms most likely responsible for the tension potentiation are, increased phosphorylation (Moore & Stull 1984), and enhanced calcium kinetics (Krarup 1981; Allen, Lee & Westerblad 1989). Fast twitch muscle fibres, in particular fatigue resistant fibres, have been shown to potentiate strongly in the cat medial gastrocnemius muscle (Stephens & Stuart 1975).

After tension had reached maximum levels during optimised distributed stimulation, it then decreased. However, during this period, integrated EMG did not mirror the decrease in muscle tension but increased to maximal levels (see figure 7.3). This finding suggested that the major decline in tension observed during continuous stimulation was associated with E-C coupling or factors affecting the contractile mechanisms directly. There was no evidence of a decrease in the level of muscle activation over this period. Here, EMG did not correlate well with tension, an observation that has been made previously for low frequency fatigue of motor units in the cat medial gastrocnemius muscle (Sandercock, Faulkner *et al.* 1985).

Over the remainder of stimulation, from two minutes onwards, tension and EMG declined with relatively slow time courses. It is likely that a decrease in the activation level of the muscle (50% decrease in EMG) may have contributed to the decline in tension observed here. The working hypothesis for the decrease in muscle activation during continuous distributed stimulation was an altered functioning of the sarcolemma, most likely including the inward spread of muscle action potentials into the T-tubules

(Edman & Lou 1992). The mechanism thought to be responsible is an increase in the extracellular K^+ concentration, causing membrane depolarisation (Sjogaard 1991; Fitts 1994), that would trigger slow inactivation of Na^+ channels (Ruff & Whittlesey 1992) and slow the conduction velocity of the muscle action potential (Jones 1996). The rate of removal of K^+ from the extracellular environment will depend on the Na^+/K^+ pump and blood supply, processes that would have a relatively slow time course.

There was no evidence of neuromuscular transmission failure during continuous optimised distributed stimulation. When the stimulation was interrupted, tension and EMG returned to their previous values and remained stable at these levels. This result was consistent with previous findings showing that with stimulation rates between 5pps (Steiman 1943) and 10pps (Krnjevic & Miledi 1958; Sandercock, Faulkner *et al.* 1985) transmission failure was not present.

Intermittent Stimulation

During intermittent synchronous stimulation there was a rapid decrease in tension over the first five minutes of stimulation but a two-fold increase in integrated EMG (figure 7.5). The increase in EMG was not due to the recruitment of additional muscle fibres but was probably a result of changes in the ionic balance of the sarcolemma.

Reports of an enlargement of compound muscle action potentials have been made in previous experiments using intermittent stimulation at comparable stimulation rates (Hicks & McComas 1989). The term 'pseudofacilitation' has been used to describe the increase in EMG observed in these experiments (McComas, Galea & Einhorn 1994). The authors suggested that sarcolemma hyperpolarisation, from activation of the electrogenic Na^+/K^+ pump in response to K^+ accumulation, was the mechanism responsible for the muscle action potential enlargement. The increase in EMG was most often observed in fast twitch muscle fibres (Enoka, Rankin *et al.* 1989; Enoka, Trayanova, Laouris, Bevan, Reinking & Stuart 1992) although a contribution from slow twitch muscle could not be ruled out (Everts, Retterstol & Clausen 1988). For the period of stimulation from 20 minutes to 40 minutes EMG decreased to approximately one third of its initial level. The decrease in EMG below its initial level suggested that the muscle was not fully activated during this period.

When intermittent distributed stimulation was used, the tension decline was gradual over the 40-minute stimulation period. There was no decrease in the integrated

EMG suggesting that the muscle was fully activated over the period of stimulation. The seven-second rest period between contractions was long enough to prevent a reduction in EMG similar to that observed with continuous stimulation. This suggests that the potential factors contributing to the muscle fatigue seen here were associated with E-C coupling and contractile mechanisms although depolarisation of the T-tubule due to K^+ accumulation cannot be ruled out (Edwards, Hill *et al.* 1977).

After five minutes of intermittent distributed stimulation at 10pps the average tension was 75% of initial levels. Experiments by Edman & Lou (1990) produced similar levels of muscle fatigue (tension 75% of initial) in an isolated frog muscle with an intermittent stimulation protocol. When caffeine was added to potentiate the muscle contraction, there was no increase in tension indicating that the contractile system was fully activated and not limited by Ca^{2+} release. They concluded that the fatigue observed using the intermittent stimulation protocol was a result of alterations to cross-bridge functioning, most likely due to the acidification of the intracellular environment and also the accumulation of inorganic phosphates. Although there is no direct evidence of altered cross-bridge functioning in the experiments using optimised distributed stimulation, it seems likely that these factors contributed to the tension decline observed.

Tension Sag

Tension sag during stimulation of isolated motor units in the cat medial gastrocnemius muscle has been observed in previous experiments using an intermittent stimulation protocol with a duty cycle of 0.33 (330ms stimulation every second) (Burke, Levine *et al.* 1973). In these experiments sag was present during contractions using high and low frequency stimulation, with the authors concluding that the mechanism responsible was a 'subtle change in the active state of the muscle fibre'. For the experiments carried out in this thesis, sag was observed during optimised distributed and synchronous stimulation. Sag levels were larger and more variable during synchronous stimulation with evidence of a decrease in the activation level of the muscle as indicated by a decline in EMG over this period. The level of sag varied over the course of the experiment possibly reflecting fatigue of fast twitch motor units. Although there was evidence of decreased muscle activation, it is possible that there were contributions of other fatigue mechanisms involved here such as E-C coupling and

cross-bridge functioning. However, the exact contribution of these mechanisms could not be determined with the methodology used in these experiments.

Blood Flow and Muscle Fatigue

During muscle contraction rises in intramuscular pressure can cause mechanical compression of blood vessels and limit blood flow to the muscle (Barcroft & Millen 1939; Humphreys & Lind 1963) thus restricting energy supply and the removal of metabolites (Kugelberg & Edstrom 1968; Sadamoto, Bonde-Petersen & Suzuki 1983; Sjogaard, Kiens, Jorgensen & Saltin 1986). Neuromuscular transmission can become more variable or totally blocked by ischaemia (Dahlback, Ekstedt & Stalberg 1970), with the neuromuscular junction of FF fibres being susceptible (Romanoul 1965). It is possible that blood flow occlusion may have been a contributing factor in the fatigue observed during optimised distributed and synchronous stimulation, being most pronounced during continuous stimulation. However, the seven-second rest period in intermittent protocol might have been sufficient to offset any occlusion effects during this type of stimulation.

There is some evidence that blood flow is different during synchronous and distributed stimulation. Experiments using intramuscular electrodes to sequentially stimulate different regions of the gracilis muscles in dogs, showed that muscle blood flow was significantly greater using distributed stimulation at lower rates compared to synchronous stimulation of the entire muscle at higher rates (Zonnevijlle, Somia, Stremel, Maldonado, Werker, Kon & Barker 2000). However, in these experiments four separate regions of the muscle were activated with intramuscular electrodes, therefore only one region was active at any one point in time. Within each stimulation cycle it might be expected that the intramuscular pressure in different regions of the muscle was low, so promoting blood flow into those regions. For the experiments discussed in this chapter, portions of ventral roots were stimulated. Here, due to intermingling of motor units within the muscle, optimised distributed stimulation did not activate separate regions of the muscle per se, but the active motor units would be more uniformly distributed throughout the entire muscle. Each motor unit activates many muscle fibres, and these are on average distributed over about one fifth of the total muscle cross section (Buchthal & Schmalbruch 1980). Therefore, in these experiments intramuscular pressure would be more even through the entire muscle. In future experiments, blood flow could be measured during optimised distributed stimulation at different levels to

ascertain whether there are direct benefits of maintained blood flow during submaximal stimulation.

Methodological Issues

The method of stimulating split ventral roots to compare fatigue during synchronous and optimised distributed stimulation assumes that the distribution of motor units was uniform between the ventral root subdivisions used for each method. The ventral root supply to the medial gastrocnemius muscle was separated into two subdivisions, 70% used for optimised distributed stimulation and 30% used for synchronous stimulation. If one subdivision contained a larger number of fatigue resistant units than the other, then misleading estimates of fatigue might have resulted. Motoneurons to the medial gastrocnemius muscle are found in the ventral horn of spinal segments L7 and S1. There is substantial intermingling of motoneurons of different sizes in the medial gastrocnemius cell column. However, the most rostral third of the medial gastrocnemius nucleus contained a higher ratio of large to small cells than expected by random distribution (Burke, Strick, Kanda, Kim & Walmsley 1977). Here, a further confounding effect is the variation between experimental animals, where segmental outflow is a little different for different animals. Therefore, care was taken to ensure that ventral root portions selected for synchronous and optimised distributed stimulation were not always from the same part of the ventral roots. The results showed that there was always a large amount of fatigue seen with synchronous stimulation, and that this varied little from one experiment to the next as indicated by the relatively low standard error values. Therefore, it was concluded that the two muscle subdivisions had similar fatigue properties.

Conclusion

Optimised distributed stimulation at 6pps and 10pps produced contractions of low force that were significantly more resistant to fatigue than synchronous stimulation at 36pps and 60pps. This was observed for both continuous and intermittent protocols. There was a 5 to 15 fold difference in fatigue during continuous stimulation and a two-fold difference for intermittent stimulation. There was no evidence of neuromuscular transmission failure when optimised distributed stimulation was used whereas, for synchronous stimulation at high rates, particularly with continuous protocol, evidence for neuromuscular failure was found. It was proposed that fatigue during optimised

distributed stimulation at low rates was associated with mechanisms relating to E-C coupling and the contractile machinery.

For fatigue associated with failure of neuromuscular transmission, tension increased but then rapidly declined again after pauses in stimulation. This was observed during synchronous stimulation for both the continuous and intermittent protocols. For optimised distributed stimulation, contractions were more stable and repeatable when brief pauses in stimulation were introduced. This was also evident with less tension sag observed during intermittent stimulation. Sag represents a change in the muscle transfer function and becomes a complicating factor for a control system that is required to adapt during each contraction as well as between contractions. Successful closed-loop control of muscle stimulation depends on reliable and stable open-loop response properties (Crago, Mortimer & Peckham 1980). The reduced level of fatigue and sag found during optimised distributed stimulation suggests that this method is suitable for FES.

Chapter Eight

General Discussion

This thesis covered a broad range of research areas relating to proprioception and muscle function during submaximal contractions. Proprioceptive acuity was examined during and after voluntary contractions of agonist and antagonist muscles and experiments in the cat showed that the ability of muscle spindles to provide muscle length information was affected by muscle conditioning and fusimotor activity. In the second half of the thesis, experiments showed that the technique of distributed stimulation using optimised interpulse intervals could produce smooth submaximal contractions of a muscle containing a mixture of fibres types. This stimulation technique was then used to evaluate the mechanical and fatigue properties of muscle when activated at low rates of stimulation. The following discussion will focus on the significance of the experimental observations and discuss some of the implications and future directions of this work. Particular reference is made to the potential benefits and limitations of using optimised distributed stimulation in FES. On a more speculative level, ideas of how sensory feedback and muscle stimulation can be integrated in a control system for FES are discussed.

The manner in which muscle is activated in the clinic and in the laboratory is radically different to the activation strategies used by the CNS. The CNS has access to a large number of independent motor units that are activated in a size dependent order. Motor units are asynchronously activated and contractions are smooth even though the firing rates of individual motoneurons are low. The current methods available to activate muscle with electrical stimulation cannot emulate the physiological activation and control strategies used by the CNS. Distributed stimulation using optimised intervals is a method that can be used to produce muscle contractions in a manner that approximates the asynchronous activation employed by the CNS.

The CNS receives continual proprioceptive feedback providing a conscious representation of body movement and position and also enabling automatic motor adjustments, for instance, the maintenance of balance or switching between different phases of the locomotion cycle. Continual feedback from the periphery enables fine motor adjustments to be made on an ongoing basis and ensures optimal motor functioning. This thesis examined how proprioception can be affected during and after muscle contraction.

Proprioception

Experiments in this thesis measured movement detection thresholds after muscle conditioning that was designed to alter the mechanical state of muscle spindles by making them either taut or slack. Thresholds were high for movements that stretched slackened muscle spindles. The functional significance of muscle conditioning is relevant to any discussion of their physiological effects. Is slack likely to be encountered in normal day-to-day activity, or is it only found after specific forms of muscle conditioning, carried out in a controlled experimental environment? It is likely that in normal motor activity, co-activation of the fusimotor system can prevent muscle spindles from becoming slack. To introduce slack into a muscle spindle a muscle must be passively shortened. Usually when a muscle undergoes shortening, for instance when a muscle contracts to move a limb, fusimotor activity will cause active cross-bridge cycling within intrafusal fibres and therefore prevent the formation of intrafusal slack. One situation in normal motor functioning where slack could possibly be incorporated into muscle spindles is when a muscle actively lengthens, for example, biceps lowering a weight. Assuming that there is no co-contraction of the passively shortening triceps muscle group, then muscle spindles within triceps might become slack. More generally, whenever our limbs are moved passively, slack may develop in some muscles.

Muscle length and contraction history was shown to have dramatic implications for muscle spindle stretch sensitivity and also perceptual consequences for human proprioception. This finding provides additional evidence that supports a role of muscle spindles in proprioception and highlights the importance of controlling muscle history when dealing with muscle spindle recordings and human proprioception.

When the elbow was conditioned so that no slack was present in muscle spindles located in biceps or triceps muscles, thresholds were equally low for extension and flexion. Muscle spindles within biceps and triceps would be expected to have a maintained firing rate following the conditioning contraction. Here, there are two potential sources of information, a decrease in muscle spindle firing rate from the shortening muscle and an increase from the lengthening muscle. The decrease in firing rate would not be available when muscle spindles of one muscle were already silent because their intrafusal fibres were slack. The experimental results suggested that the

availability of this cue from the antagonist did not improve proprioceptive acuity significantly.

Passive limb movements seldom occur in daily activities, therefore it could be argued that testing proprioceptive acuity under passive conditions has limited functional significance. Movement detection thresholds were measured during co-contraction of elbow flexors and extensors. When compared to thresholds measured for the relaxed and appropriately conditioned elbow, thresholds during co-contraction were significantly higher, that is, proprioceptive acuity deteriorated during co-contraction. Several mechanisms might contribute to this finding.

One mechanism considered was a decrease in the stretch sensitivity of muscle spindles brought about by co-activation of the fusimotor system. This possibility was explored in a series of experiments that examined muscle spindle stretch responses during combinations of fusimotor and skeletomotor stimulation. The results showed that all combinations of fusimotor and skeletomotor activation reduced the muscle spindle stretch response. It became apparent that a muscle spindle is most sensitive to small stretches when it is passive and intrafusal fibres are taut.

The functional significance of the finding that proprioceptive acuity was diminished during co-contraction of agonist and antagonist muscles remains an intriguing question. On a speculative level, this finding might have relevance to motor learning and the performance of skilled motor tasks. For example, elite athletes and musicians generate precise motor patterns after hours of practise by learning, not only which muscles to activate, but also which muscles to keep relaxed. In this situation, proprioceptive feedback from the relaxed muscles may be particularly important in signalling limb movement and position. However, for the novice, who is without these finely tuned motor skills, movements are likely to be executed by significant motor drive to both synergist and antagonist muscles. Muscle spindle feedback is likely to be complicated with fusimotor-evoked activity and possibly compromised by a reduction in the sensitivity to small movements. All of this raises the frequently discussed question of how the CNS is able to access muscle spindle length and velocity information, in the presence of the potentially confounding effects of fusimotor activity.

Optimised Distributed Stimulation

The results from experiments in this thesis suggest that optimised distributed stimulation may provide a significant advantage over the conventional mode of synchronous muscle activation that is commonly used in FES. Stimulation of multiple channels at low rates was shown to generate repeatable and stable contractions that were less prone to fatigue. With advances in computer miniaturisation and electrode development multiple channel closed-loop stimulation of muscle controlled by afferent feedback from natural or manufactured sensors would be the preferred method used in rehabilitative medicine. However, a number of technical difficulties must be overcome before optimised distributed stimulation can be implemented clinically.

Activation Strategies

One limitation of optimised distributed stimulation is the requirement that each stimulating channel should access independent parts of the target muscle. In the experiments in this thesis five to six separate muscle portions were accessed via splitting the ventral roots or the peripheral nerve. These methods are suitable for acute preparations however, they cannot be used in a clinical situation as the integrity of the neural supply must be maintained. There are a few methods that could be used to access separate muscle portions that might be suitable for a clinical application. For example, nerve cuff electrodes (Goodall, de Breij & Holsheimer 1996; Loeb & Peck 1996) or multiple electrode arrays, like the Utah Array (Branner & Normann 2000), could be examined to determine whether they can access different motoneuron populations in a peripheral nerve. Alternatively, small intramuscular electrodes, like the Bion (Loeb, Peck, Moore & Hood 2001), could be implanted into a muscle to selectively activate independent muscle portions. The possibility of using spinal cord microstimulation could be explored (Mushahwar & Horch 1997; Mushahwar, Collins & Prochazka 2000), although it is unclear whether separate populations of motoneurons from the one muscle can be accessed using this method. An alternative to accessing independent parts of the one muscle with selective electrodes is to use a muscle with several separate motor points and attach separate electrodes to each point (Lau, Liu, Pereira, Kumar & Pho 1995). Alternatively, different synergist muscles having common tendon insertions could be independently stimulated (Pournezam, Andrews, Baxendale, Phillips & Paul

1988). A combination of these different methods may also be tried in future experiments.

Initially, optimised distributed stimulation should be used with surface stimulation. Previous experiments have shown that graded contractions of triceps surae, over the range of 0-25% of MVC, could be generated in human subjects using three surface electrodes (Gregory, Wise, Wood, Prochazka & Proske 1998). One of the major limitations of surface stimulation is that it is virtually impossible to get stimulation of independent muscle portions. Normally there is considerable overlap of the stimulation channels so that a certain percentage of the muscle will be activated by more than one stimulating channel. This poses a problem because some muscle fibres will be effectively activated at a higher rate of stimulation and therefore will be more susceptible to fatigue. In addition, it is likely that 'hot spots' would be found over the muscle such that tension generated from one stimulating channel would be significantly greater than the other channels. However, the continual optimisation of intervals can help to reduce the affects associated with fatiguing muscle fibres and muscle 'hot spots'. Finally, surface stimulation will, inevitably excite some afferent fibres that may, in certain circumstances, lead to unwanted reflex effects.

Another obstacle for the optimisation system is the requirement for a signal providing feedback of the fundamental component of the tension ripple. In the experiments reported here whole muscle tension measured by a transducer place in series with the muscle was used to provide tension feedback. However, it is difficult to measure muscle tension from an intact muscle in a clinical setting. If optimised distributed stimulation was to be used clinically then an alternative source of tension ripple feedback would be required. Experiments in this thesis explored the possibility of using two different sources of feedback for closed-loop control of stimulus intervals. Feedback from a number of tendon organs and from an accelerometer was examined during distributed stimulation. The results suggested that signals from these sources could not provide reliable feedback of tension from all of the stimulation channels. Nerve cuff electrodes might be suitable to record compound afferent responses in a clinical situation (Hoffer, Stein, Haugland, Sinkjær, Durfee, Schwartz, Loeb & Kantor 1996), but the suitability of these signals for the optimisation process would need to be explored in future experiments. Therefore, the use of external electronic sensors might be the method most likely to be successful in the immediate future. Further examination of the use of accelerometers is required, for instance the use of accelerometers attached

to the limb instead of the muscle belly may provide a more reliable signal of tension ripple.

Control Strategies

The CNS is likely to have an internal representation, a working model, of the input/output characteristics of the motor system that would be continually adjusted by feedback from the periphery. Most FES control systems are open-loop systems in which prespecified stimulation parameters are determined by trial and error (Abbas & Chizeck 1995). If FES is to be used to successfully restore function to paralysed limbs then one of the requirements is that the control system has a basic frame of reference from which to operate and an indication of how a muscle behaves under a given set of conditions. One limitation of controllers for FES is the poor models of stimulated muscle (Durfee 1993). This is partly due to the non-linear property of muscle and the difficulty in obtaining predictive models of muscle properties under various conditions. Experiments in this thesis examined the mechanical properties of muscle when activated at submaximal rates. The properties under examination were fatigue, force-velocity, length-tension and force-frequency relationships. The results suggest that the mechanical properties of a muscle activated at submaximal levels are not easily predictable from simple scaled models of the fully activated muscle. If optimised distributed stimulation is to be implemented clinically then more information about the properties of submaximally activated muscle is required.

A control system not only requires a basic model of muscle kinematics but also must be adaptable to varying circumstances and changing environmental conditions. An adaptive neural network could be implemented to optimise muscle stimulation parameters based on continual feedback from the muscle (Abbas & Chizeck 1995). Typically, signals used in FES control systems are supplied by external sensors that have various problems such as calibration time and mechanical vulnerability (Hoffer, Stein *et al.* 1996). Some control systems have used EMG measured from the stimulated muscle to control stimulation parameters (Graupe & Kordylewski 1995). However, as shown in the fatigue experiments, EMG can vary depending on how the muscle is stimulated and does not reliably correlate with muscle force under all circumstances. Models of muscle fatigue are difficult to construct because of the variability of fatigue properties between muscles and the manner in which the muscle recovers from fatigue.

Is there potential for the use of signals from muscle spindles to control muscle stimulation in applications like FES? One of the experimental findings suggested that signals from the muscle passively lengthened by an imposed movement were an important source of proprioceptive information. Recording from muscle spindles in a passively lengthening muscle could provide limb movement and position feedback used in a closed-loop control system. Neural recording from the passively lengthening muscle would reduce problems associated with interference from EMG and other noise sources originating from the electrically activated muscle. Using this approach Yoshida & Horch (1996) were able to control the position and movement of a cat ankle joint during stimulation of the medial gastrocnemius muscle using signals from muscle spindles in an agonist/antagonist pair, the tibialis anterior and the lateral gastrocnemius muscles. It is conceivable that multiple channel stimulation and recording systems could be used to stimulate an agonist muscle in a distributed fashion and simultaneously record multiunit muscle spindle activity from the antagonist muscle. In addition, length changes of the antagonist muscle might provide a convenient method to measure the fundamental ripple, therefore enabling the optimisation of interpulse intervals during distributed stimulation. However, any control system that uses muscle spindle output as a feedback source would need to account for muscle spindle non-linearities, such as the dynamic burst that is particularly prevalent in taut muscle spindles, contraction and length history effects, such as intrafusal slack, and the presence or absence of fusimotor activity. The real challenge for the implementation of muscle stimulation in rehabilitative medicine is to stimulate muscle in a manner that approximates the physiological activation strategies of the CNS using afferent feedback to make continual automatic adjustments by optimising stimulus parameters and also to provide the patient with feedback related to limb position and movement.

Appendix

The Huxley model of molecular contraction dynamics was used to simulate the data obtained for the rat triceps surae muscle group and the cat soleus muscle at low stimulation rates. Software for the model was adapted from software written by Dr. David Morgan in Igor Pro (Wavemetrics, USA). Here, the question was, could the rat triceps surae force-velocity relationship for 8pps be simulated by increasing the detachment rate (in the attachment range) using the Huxley model?

The mechanical response of a contracting muscle to imposed length changes depends upon a combination of factors that can be described by the sliding filament theory of muscle contraction (Huxley & Niedergerke 1954; Huxley & Hanson 1954) (see figure 9.1). When a muscle contracts isometrically there is, after a long time, an even distribution of attached cross bridges in the range 0-h (see figure 9.2). When a muscle is lengthened cross-bridges can be stretched to values larger than h. The force per cross-bridge for these cross-bridges is relatively high but counteracting this is the reduced lifetime of cross-bridges that are stretched into a region of high detachment rate constant. This leads to fewer attached cross-bridges with a higher average force per cross-bridge. For maximally activated muscle the rate of attachment is high enough to keep the number of attached cross-bridges near maximal. Here, the balance is shifted in favour of greater force generation for lengthening contractions compared to isometric contractions. If however, the muscle is submaximally activated the attachment rate is reduced and the balance now swings in the other direction. Isometric tension is now greater than tension during lengthening. This condition, seen in the cat soleus muscle by Rack & Westbury (1969), was modelled by Zahalak (1986) with the rate constants $g_1=7s^{-1}$, $g_2=200s^{-1}$, $g_3=30s^{-1}$ and $f_1=7s^{-1}$. The result of this simulation was shown in figure 6.24 (red) and is consistent with the data for the submaximally activated cat soleus muscle (7pps) Joyce *et al.* (1969) (see figure 6.23). If the detachment rates are altered so that there is an increased rate constant of detachment, as would be expected when there is internal movement within the muscle, the simulated force-velocity relationship is altered. In this simulation, the detachment rates were $g_1=30s^{-1}$, $g_2=200s^{-1}$, $g_3=30s^{-1}$ with the attachment rate remaining the same $f_1=7s^{-1}$. The results are consistent with the rat triceps surae data for 8pps figure 6.24 (blue).

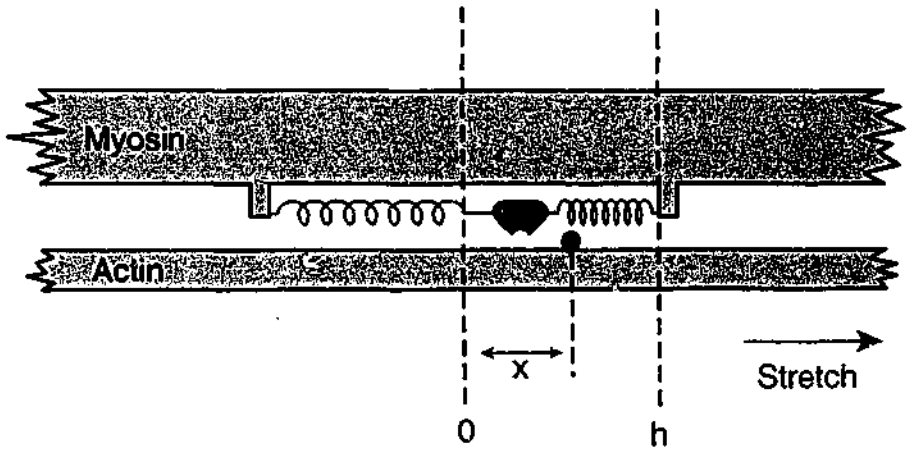


Figure 9.1. Schematic representation of the sliding filament theory of muscle contraction. The model suggests that cross-bridges (depicted in blue) move back and forth in the range 0-h and can form links, or attachments, with the active site on the actin filaments (red). Once a cross-bridge has attached it produces a force which acts to slide the actin filament past the myosin filament towards the equilibrium point (0). The tension produced is proportional to the number n(x) and displacement from rest position of attached cross-bridges. A cross-bridge can only form links between 0-h and any link formed at the equilibrium point (0) will generate zero force. The number of cross-bridges attached at any one point depends on the rate of attachment and the rate of detachment. The Huxley model defines attachment and detachment rates, shown in figure 9.2.

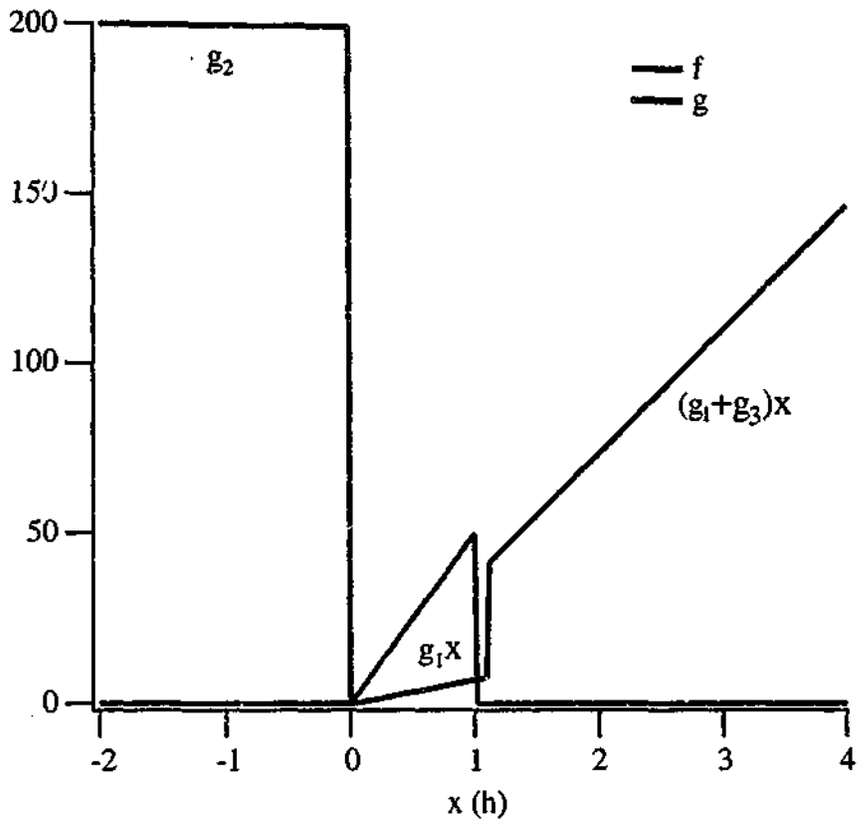


Figure 9.2. Cross-bridge attachment and detachment rate functions f(x) and g(x). The rate of attachment is f(x)(1-n(x)) and the rate of detachment is g(x)n(x), where n(x) is defined as the fraction of attached cross-bridges that have, or would have if they were attached, distortion x. There are three regions for the detachment rate constant g. When $x<0$, $g(x)=g_2$ (a constant); when $0\leq x\leq 1$, $g(x)=g_1(x)$ and when $x>h$, $g(x)=(g_1+g_3)x$ (Zahalak, 1986).

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