In the Name of God

Reflexes Elicited by Per-Cutaneous Stimulation of the Medial and Lateral Ligaments of the Knee

A Thesis Submitted for Degree of Doctor of Philosophy

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Dedication

To my Family.

Declaration

I hereby declare that this thesis embodies the result of my own special hard work that the work of which it is a record has been done by myself except where assistance has been acknowledged, that it has not been submitted in any previous application for a higher degree. All sources of information have been specially acknowledged by means of references.

Dr. Seyed Mohsen Rahimi

Summary

Joint disease is common in adults and in adolescents. It particularly affects the knee joints. In young people, joint disease has a number of causes including: genetic factors, defects in joint cartilage and sports related injuries. In particular, overtraining and traumatic injuries are common in many sports Participation in sports has expanded during the last two decades in many parts of the world. This has led to an increased number of injuries. Our knowledge of the role of ligaments in the control of movements and how they should be managed after injuries needs to expand as fast as participation rates.

The aims of the current study were firstly, to investigate if reflexes can be elicited by electrical stimulation of ligaments. Secondly, to investigate if different muscles are affected differently by these reflexes. The final aim was to investigate if these reflexes can be modulated by posture or muscle activity.

A total of 44 volunteers participated in a series of experiments. These experiments were designed to elicit reflexes following electrical stimulation of the collateral knee ligaments during sitting, standing and walking on treadmill. The reflexes were observed in averaged rectified electromyograms from Rectus Femoris, Vastus Medialis, Vastus Lateralis, Lateral and Medial Gastrocnemius and Soleus. Muscle activity was essential if reflexes were to be elicited. No reflexes were elicited in relaxed muscles.

During the first series of experiments reflexes in Rectus Femoris, Vastus Lateralis and Vastus Medialis were investigated while the

subjects sat on a chair with their hip joint at 100° and the knee at 180°. During the experiments the subjects maintained sustained contractions at 5 to 20% of their maximum voluntary contraction (MVC) for 60 seconds. A train of electrical pulses was applied to the ligaments. The experiments used stimulating currents of up to 45 milliamps. Subsequently, similar tests were conducted to investigate reflexes while the volunteers were standing. A final set of experiments investigated the reflexes as the volunteers walked on the treadmill.

Reflexes were identified as changes in the averaged rectified electromyograms (EMG). The EMG in the immediate post stimulus period was compared with the pre-stimulus control. Inhibitory and excitatory reflexes were elicited following ligament stimulation in all three sets of experiments. The mean latency in quadriceps for early excitation was 57 $\pm$ 6 msec and 67 $\pm$ 10 msec for early inhibitions. The equivalent means were 70 $\pm$ 6 msec and 77 $\pm$ 6 msec for triceps. The shortest latency recorded in quadriceps was 46 msec. Longer latency reflexes were frequently observed. The mean latency in quadriceps for late excitation was 102 $\pm$ 6 msec and 113 $\pm$ 11 msec for late inhibitions. The equivalent means were 110 $\pm$ 6 msec and 119 $\pm$ 11 msec for triceps. There was no significant difference in the latencies of reflexes from MCL and LCL. The latencies in triceps were approximately 10 msec longer (90 msec for quadriceps a 100 msec for triceps) than those in quadriceps and this can be attributed to their longer conduction pathway. The reflexes were also recorded during gait. During walking, the latencies of both excitations and inhibitions were significantly longer than they were during sitting and standing. The mean latency of excitatory reflexes in Vastus Lateralis after LCL stimulation were 71 $\pm$ 5

msec. For inhibitory reflexes the mean latency was 87 ± 1 msec. The mean latency for excitation reflexes in Lateral Gastrocnemus was 82 ± 2 msec and for inhibition reflexes was 94 + 3 msec.

In each set of experiments the shortest latencies were consistent with slow group II or group III afferents excited by relatively strong stimulation. Control experiments using topical cutaneous anaesthesia minimised the possibility of cutaneous contributions to the observed reflexes. It is also possible that the electrical stimulation excited capsular afferents located close to the ligaments. This cannot be settled by the experiment reported in this thesis and the observed reflexes are best described as ligamento-muscular reflexes.

These observed effects are consistent with recent results already published by Kim et al in 1995.

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Abbreviations

ACL	Anterior Cruciate Ligament
CNS	Central Nervous System
EMG	Electromyogram
FBLS	Faculty of Biomedical and Life Sciences
GTO	Golgi Tendon Organ
LCL	Lateral Collateral Ligament
LG	Lateral Gastrocnemius
MCL	Medial Collateral Ligament
milliamps	Milliamperes
msec	Millisecond
m/sec	meter per second
MG	Medial Gastrocnemius
MVC	Maximum Voluntary Contraction
PST	Peri Stimulus Time
PT	Perceptual Threshold
RF	Rectus Femoris
Sol	Soleus
VL	Vastus Lateralis
VM	Vastus Medialis

Chapter 1

General Introduction and Literature Review

The principal purpose of this study is to extend our knowledge of reflexes associated with the medial and lateral collateral ligaments of the knee. Disease and injury of ligaments, muscles and joints are major causes of pain and disability in the population, especially in athletes.

Joint disease is common in adolescents and adults, both men and women. In the lower limb it particularly affects the knee joints (Felson 1988). Osteoarthritis, or degenerative joint disease, is a chronic disease of joints which has many causes. It most commonly affects middle-aged and elderly people. However, in young people joint disease is associated with genetic factors, defects in joint cartilage, overtraining and traumatic joint injury in sports (Kuipers and Keizer (1988), Felson (1990), National Institute of Arthritis and Musculoskeletal and Skin Disease (2006)). Osteoarthritis affects all the components of the joint: the joint capsule, the ligaments and the tendons. Athletes participating in sports may be exposed to sudden and forceful movements, quick changes of direction and unpredictable physical contact with other athletes. All of these can all lead to joint injuries.

In addition, osteoarthritis occurs in many people due to advancing age. Joint deformity and exposure of joints to repetitive stress cause degenerative changes in the joints (Felson and Chaisson 1997). Increasingly, obesity has been identified as an important factor in the development of osteoarthritis. This is especially common in women and most commonly affects the knee joints (Felson, Zhang, Anthony, Naimark and Anderson (1997), Felson and Zhang (1998), Hart,

Doyle and Spector (1999)). In the UK, hip and knee replacement rates are higher in women than in men (Liu, Balkwill, Banks, Cooper, Green and Beral 2007).

1.1. Ligament Injury

Knee ligament injuries are common in sports (Woo, Chan and Yamaji (1997), Woo, Abramowitch, Kilger and Liang (2006), Liden, Ejerhed, Sernert, Laxdal and Kartus (2007)). The mobility and stability of the knee will be affected following rupture of the knee ligaments (Abramowitch, Yagi, Tsuda and Woo 2003). In sports which require running, twisting and jumping, as well sudden acceleration and deceleration, players are at high risk of injury. These movements expose the limbs to large forces. It has been found that 68% to 88% of all football injuries occur in the lower extremities. (Fried and Lloyd (1992), Witvrouw, Donneels, Asselman, D'Have and Cambier (2003)). About 25% of football injuries are musculoskeletal lesions mainly located in the thigh (17%) and the groin (8%) (Albert 1983). The American Academy of Orthopaedic Surgery (AAOS 1997) has reported that 5 million people visit offices of orthopaedic surgeons each year because of knee problems, 1.4 million people go to hospital emergency room for knee problem; 80 percent of the visits are due to injuries.

In 1994, 7000 hospital visits were recorded for patients with a torn quadriceps tendons; 39.8 percent of the patients were under 18 years old; 24.7 percent were 18-44 years old; 25.7 percent were 45-64 years old and 9.8 percent were 65 or older (AAOS 1997). Thus, problems are mostly found in younger, more active people.

Female athletes are 3 to 11 times more vulnerable to injury than their male counterparts (Lindenfeld, Schmitt, Hendy, Mangine and Noyes (1994), Arendt and Dick (1995), Hewett, Myer and Ford (2005), Zazulak, Hewett, Reeves, Goldberg and Cholewicki (2007)). In particular, injuries to the anterior cruciate ligament are more frequent in women than men (Hewett et al (2005), Chaudhari, Lindenfeld, Andriacchi, Hewett, Riccobene, Myer and Noyes (2007)). At first sight this is surprising since women have a lower body weight than men and tend to participate in sports which have less physical contact.

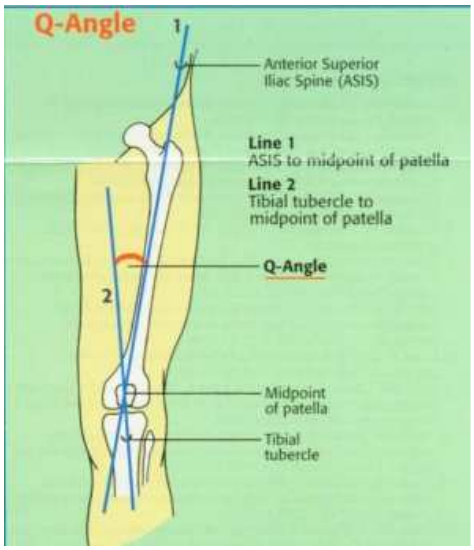
There are relevant anatomical differences between men and women. The differences in anatomical alignment and geometry of the pelvis, femur and tibia may partly explain the higher incidence of ligament injury in women (Arendt and Dick (1995), Hewett (1998), Huston, Greenfield and Wojtys (2000), Noyes, Barber-Westin, Fleckenstein, Walsh and West (2005), Yu, McClure, Onate, Guskiewicz, Kirkendall and Garrett (2005) Dick (2007)).The pelvis is wider in women than men and this difference causes the thigh bones to come down to the tibial tubercle at a wider angle. This increases the Q angle and subsequently causes the knee to bend inward. The quadriceps femoris muscle angle (Q angle) is the angle formed by a line drawn from the anterior superior iliac spine to the central patella and a second line drawn from the central patella to the tibial tubercle. An increased Q angle is a risk factor for patellar subluxation and injury to the knee ligaments. Normally, the Q angle is 14 degree for males and 17 degree for females. (Agliettis, Insall and Cerulli (1983), Evans (2001)).

The Q angle is shown in figure 1.1.

As the Q angle increases, more force will be applied through the medial aspect of the knee. During sports, particularly when landing after jumps, the medial ligaments of the knee are exposed to high forces (Chaudhari and Andriacchi 2006). In men, the limb tends to be straighter on landing and the lateral forces are lower. In addition, many studies have reported that during the female menstrual cycle, ligaments can be influenced cyclically by sex hormones. There may be an association between menstrual cycle phase and the frequency of injury to the anterior cruciate ligament (ACL) (Wojtys, Huston, Lindenfeld, Hewett and Greenfield (1998), Yu, Liu, Hatch, Panossian and Finerman (1999)).

Wojtys (1998) has reported that the most non-contact injuries of the ACL of female athletes happen during the ovulatory phase, when oestrogen concentrations are raised. Oestrogen is known to reduce the total collagen content of tendons in rats (Dyer, Sodek &Heersche 1980) and may weaken other connective tissues. This explanation of the distribution of injuries needs to be treated with some caution of neuromuscular performance varies changes during the menstrual cycle (Davies, Elford and Jamieson 1991). However, the idea has been supported by other authors (Heiz, Eisenman, Beck and Walker (1999), Adachi, Nawata, Maeta and Kurozawa (2008)).

A



B

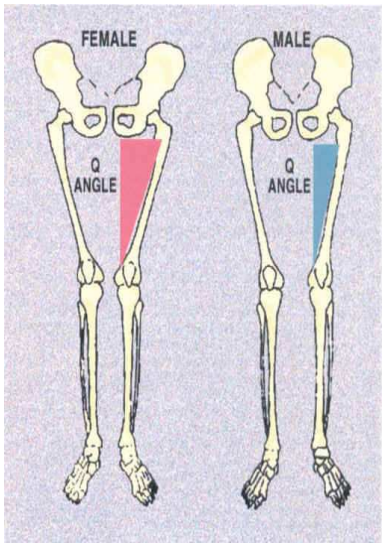


Figure 1.1
Panel A of the figure shows the definition of the Q angle. Panel B shows the Q angle in women is wider than men.
www.drtimmaggs.com/images/picture2.jpg
(Accessed 9th March 2008)

In contrast, testosterone produces an increase in the muscle mass in males and this protects the knee if the muscle activity is used appropriately to stabilise the joint. This in turn may reduce the injury rates during activity (Hewett, Lindenfeld, Riccobene and Noyes (1999), Myklebust, Engebresten, Braekken, Skjolberg, Olsen and Bahr (2003), Mandelbaum, Silvers and Watanabe (2005)).

There is a well-recognised historical trend for athletes, particularly professional athletes, to be taller and heavier in recent years. Part of this can be attributed to a general improvement in nutrition and health, generation by generation. However, athletes also develop the size and strength of their muscles by specialised training and possibly by the use of special diets. There are also many recent cases of athletes using illegal pharmaceutical techniques to further enhance their physique or performance (Sheehan 2002). The anthropometric data for some sports is well documented. In the 1900s the average baseball player weighed 174 pounds. This rose to 186 pounds in the 1970s. In the 1990s the average player weighed 198 pounds. This increase is over 5% per decade (Neyer 2000). During the last decade some athletes have abused a variety of the banned drugs to enhance their performance (National Institute on Drug Abuse 2007). One reason for the increased number of knee injuries is likely to be the fact that players are bigger and faster than ever before (Morrall and Sullivan 1969). Knee ligaments may be more likely to be injured when they are exposed to the forces from overdeveloped muscles.

In the normal population during normal movements, the joints are not exposed to extreme forces. Several factors may contribute to stabilising a joint. Muscles, ligaments and joint

capsules can contribute forces to stabilising the knee (Shelburne, Torry and Pandy 2005).

The passive visco-elastic properties of tissues provide some forces which will stabilise the joint position by resisting displacement. These will act almost immediately since they are not delayed by reflex latencies or muscle contraction times. Stretching of the connective tissues will provide the most immediate resistance to displacement of the structures. Experiments in isolated tissues from laboratory animals have shown that connective tissues in tendons are known to be surprisingly stiff when subjected to small displacements (Proske and Rack 1976). The stiffness increases when the velocity of stretch increases and when even small muscle contractions are present. Capsular laxity, perhaps as a result of previous injury or due to joint hyper-mobility syndrome has been suggested as a risk factor for joint injury and is associated with increased prevalence of osteoarthritis (Hall, Baxendale, Ferrell and Hamblen (1995), Ferrell, Tennant, Baxendale, Kusel and Sturrock (2007)).

Unlike connective tissues, muscles can change their mechanical properties actively by developing force or changing their length or stiffness. Thus muscle contraction can resist joint loading or excessive joint displacement to maintain knee stability. The knee is stabilised by the hamstrings and quadriceps muscles (Shoemaker and Markolf (1982), Louie and Mote (1987), Solomonow, Baratta, Zhou, Shoji, Bose, Beck and D'Ambrosia (1987), Buchanan and Lloyd (1997)). Their precise roles depend on the biomechanics of the particular movement. The muscle contraction may be brought about by reflexes initiated by stretch reflexes or by joint displacement (Nashner 1977). In these cases the muscle force must be delayed by tens or hundreds of milliseconds due to

conduction delays and the time needed for force development. It is also possible that muscle contractions are initiated predictively i.e. before any potentially damaging movement has taken place. This would be especially valuable in protecting the joint during high speed movements or collisions as could occur during sports participation. The quadriceps muscles have been extensively investigated and are thought to have a strong role in stabilising the joint (Dyhre-Poulsen and Krogasgaard 2000).

1.2. Quadriceps Muscle

The quadriceps is subdivided into four separate portions. One of them, Rectus Femoris (RF) is in the middle of the thigh and connects the ilium to the patella. The other three muscles: Vastus Lateralis (VL), Vastus Medialis (VM) and Vastus Intermedius have their origins on the femur and insert into the patellar ligament. Vastus Lateralis, has its origin on the upper part of the intertrochanteric line, the anterior and inferior borders of the greater trochanter, the lateral lip of the gluteal tuberosity and the upper half of the lateral lip of the linea aspera. Its insertion is on the lateral border of the patella. Vastus Medialis arises from the lower half of the inter-trochanteric lines, the medial lip of the lina aspera, the upper part of the medial supracondylar line, the tendons of the Adductor Longus and the Adductor Magnus and the medial intermuscular septum. The insertion of the muscle is along the medial border of the patella by the ligamentum patella into the tibia tuberosity. The origin of Vastus Intermedius is on the front and lateral surfaces of the body of the femur and insertions are the superior border of the patella and the tibial tuberosity (Martini 2006). These are illustrated in figure 1.2. It is relatively simple to record electromyograms from the skin over the superficial components of quadriceps: Vastus

Lateralis, Vastus Medialis and Rectus Femoris. It is impractical to make surface recordings from Vastus Intermedius as it lies deep to the vastus muscles. No recordings from this muscle are shown in this thesis.

The quadriceps muscle is supplied by femoral nerve and artery.

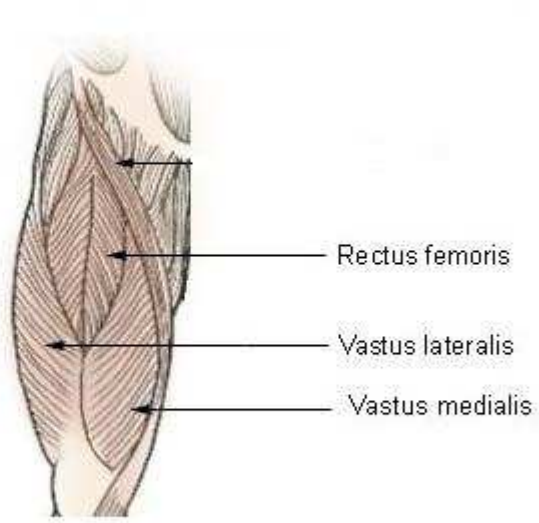


Figure 1.2
The figure shows the components of the quadriceps muscles, from which EMG was recorded. Modified from: http://training.seer.cancer.gov/module_anatomy/unit4_4_muscle_grp4_lower_extremity.

1.3. Triceps Muscle

The most superficial of the calf muscles is Gastrocnemius and it forms the greater part of the calf. The muscle has two different parts: medial and lateral. They have two different origins. The origin of the medial head is posterior to the medial condyle of femur. Lateral Gastrocnemius arises from the posterior surface of the lateral part of the femoral condyle. Both heads also arise from the inferior of the capsule of the knee. The muscle inserts into the calcaneus via the calcaneal tendon. The muscle is innervated by the tibial nerve (Martini 2006).

The details of the origins and insertions of the muscle are shown in figure 1.3.

The Soleus is a flat muscle situated immediately anterior to Gastrocnemius. Its origin is on the posterior of the head of the fibula, the upper third of the posterior surface of the body of the bone; the popliteal line, and the middle third of the medial border of the tibia. Some fibres also arise between the tibial and fibular origins of the muscle. The insertion of the muscle is an aponeurosis which covers the posterior surface of the muscle and gradually becoming thicker and narrower before it joins with the tendon of the Gastrocnemius to form the tendo-calcaneus (Martini 2006).

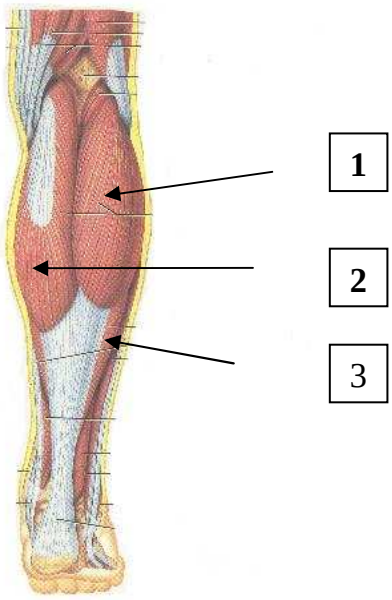


Figure 1.3
The figure shows the lower leg muscles. Number 1 is the lateral head and number 2 is the medial head of the Gastrocnemius muscle. Number 3 is Soleus muscle. Modified figure from Tortora and Grabowski (1993).

1.4. Knee Ligaments and Menisci

Ligaments consist of many minor ligaments which run in different directions and layers. There are two separate ligaments located on the medial and lateral side of the knee joint. They are the medial collateral ligament (MCL) and the lateral collateral ligament (LCL). The MCL is attached above to the femur and inserts into the tibia. The LCL is attached above to the femur and inserts into the fibula. In addition, the ACL connects the femur and the tibia. ACL injury is more common in females than men. The reasons for this are addressed in section 1.1. The ligaments act together to provide the knee with stability and flexibility.

The ligaments may be injured directly by mechanical trauma as described earlier in section 1.1. Medial collateral ligament injury is more common than lateral collateral ligament in men and women (Abbott, Saunders, Dec, Bost and Anderson (1944), Naik, Rao and Rao (2007)).

The menisci are two cartilaginous elements within the knee joint which protect the ends of the bones from rubbing on each other. They also play a role in shock absorption. They are shown in figure 1.4. The upper surfaces of the menisci are concave and this deepens the tibial sockets into which the femur attaches. The menisci are in contact with the condyles of the femur. The lower surfaces are flat and rest upon the head of the tibia. Both surfaces are smooth and are covered by the synovial membrane. The menisci can be cracked or torn when the knee is flexed and forcefully rotated.

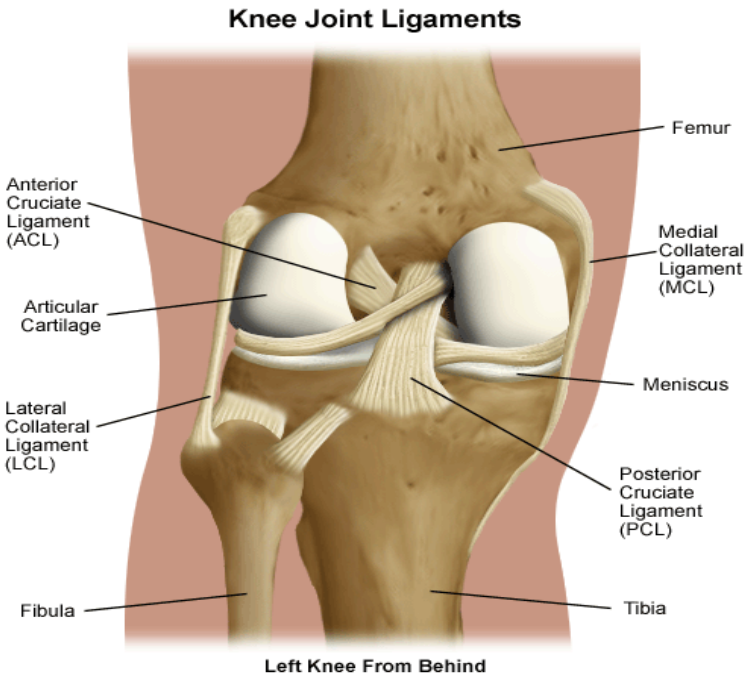


Figure 1.4
The figure shows the knee ligaments and menisci of the left knee seen from behind.
www.nationalsportsmed.com/knee.html

1.5. The Role of Reflexes

The role of reflexes in preventing damage to joints by over-load or over-range movements has been widely discussed. In a recent paper, Solomonow and Krogsgaard (2001) reported that the idea of protective reflexes, elicited by mechanical stimulation of ligaments, was first proposed by Payr, Derheutige and Gelenkchirugie in 1900. This was the first description of the "Kinetic chain". In this theory Payr discussed ligamento-muscular protective reflexes and believed that ligaments with bones, muscles and receptors act to provide safe, stable motion of the joint. Payr had little experimental evidence to support his theory. Subsequently, many researchers have investigated such protective reflexes (Partridge (1924), Palmer (1958), Stener (1959), Petersen and Stener (1959), Ferrell, Gandevia and McCloskey (1987), Baxendale, Ferrell and Wood (1988), Johansson, Sjolander and Sojka (1991b)).

The fundamental suggestion is that sensory endings in the ligaments form part of a negative feedback system that controls the muscle activity stabilising the knee to prevent abnormal movements of the joint (Andersson and Stener (1959), Johansson et al (1991b)). There have been two main experimental approaches to investigating this suggestion: some studies have used surgically invasive techniques to study reflexes in laboratory animals. These animals have generally been under the influence of anaesthesia or made insensible by decerebration. The other common experimental approach uses human volunteers.

The animal studies have been more invasive in their approach using reduced preparations, denervations and long term surgical interventions. Thus, there are sufficient experimental

data to confirm the existence of ligamento-muscular reflexes, probably involving low threshold mechanoreceptors. They influence muscle activity via the γ-muscle spindle system, whereas high-threshold mechanoreceptors may exert effects directly onto the skeleto-motoneurons (Appelberg, Hulliger, Johansson and Sojka (1983), Johansson, Sjolander and Sojka (1991b)).

Sherrington (1903, 1910) really provided the scientific foundations of the experimental investigation of reflexes in laboratory animals. Later Matthews (1933), Gardner (1944), Gardner, Latimer and Stilwell (1949) published studies of the behaviour of the mechanoreceptors associated with joints. Boyd and Roberts (1953) investigated the afferent discharge in the posterior articular nerve of the knee joint of the cat. They categorised the responses into two different types, slowly and rapidly adapting. Also they concluded that the sense-endings are stretch receptors responding to extension in a particular direction and they may play an important part in the control of movement. Later, Wyke (1981) and Newton (1982) classified the nerve endings into four categories: Ruffini endings, Pacinian corpuscles, Golgi tendon organ-like endings and free nerve endings. Subsequently, several authors have reviewed knee joint function and have identified that the mechanoreceptors named above are distributed in the collateral ligaments, cruciate ligaments and the menisci of several laboratory species and humans (Grigg and Hoffman (1982), Schultz, Miller, Kerr and Micheli (1984), Halata, Grim and Christ (1990)).

The experimental studies in human volunteers have been more restricted. The ACL, MCL and LCL of human knee ligaments have been investigated (Palmer (1958), Petersen and Stener (1959), Kim et al (1995), Buchannan and LIoyd (1997), Dyhre-

Poulsen and Krogsgaard (2000)). The studies have been explained in page 22.

1.6. Articular Mechanoreceptors in the Knee Joint

Several different types of mechanoreceptors have been identified in human and animal knee cruciate ligaments, collateral ligaments and the menisci (Grigg and Hoffman (1982), Schultz et al (1984)). Many authors have proposed that mechanoreceptors of the cruciate and collateral ligaments in knee, ankle and shoulder joints have important roles in eliciting reflexes (Boyd and Roberts (1953), Skoglund (1956), Palmer (1958), Andersson and Stener (1959), Petersen and Stener (1959), Ekholm, Eklund and Skoglund (1960), Freeman and Wyke (1967), Grigg, Harrigan and Fogarty (1978), Ferrell (1980), Grigg and Hoffman (1982), Wood and Ferrell (1984), Johansson and Sojka (1985), Baxendale et al (1988), Pope, Cole and Brand (1990), Johansson, Sjolander and Sojka (1991a), Kim, Rosen, Brander and Buchanan (1995), Solomonow and Krogsgaard (2001)).

Skoglund (1956) found that the discharge of slowly adapting receptors in the cat knee joint could signal the steady-state angle of the joint as well as the direction and velocity of movement. These observations were extended by Ferrell (1980) who found that a significant number of slowly adapting joint afferent fired across the mid-range of joint positions.

Four types of mechanoreceptors are found in capsular ligaments and menisci: Ruffini endings, Pacinian corpuscles, Golgi tendon organs and free nerve endings. The properties of the different kinds of mechanoreceptors are shown in table 1.1.

Articular mechano-receptors Type	Location	Sensitive To	Adaptation Rate	Freeman & Wyke Classific-ation	Afferent Fibre Group
Ruffini Endings	Joint Capsule Ligaments and Menisci	Mechanical Pressure	Slowly Adapting	I	II
Pacinian Corpuscles	Fibrous Capsules	Mechanical Stress	Rapidly Adapting	II	II
Golgi Tendon Organs	Ligaments Tendons and Menisci	Mechanical Force	Slowly Adapting	III	Ib
Free Nerve Endings	Ligaments, Capsules and Menisci	Stretch	Non Adapting	IV	III/IV

Table 1.1
The table shows the properties of the different types of articular mechanoreceptors.

Ruffini endings are encapsulated, slowly-adapting, stretch sensitive mechanoreceptors. They are located in the collateral and cruciate ligaments, capsule, and menisci (Polacek (1966), Freeman and Wyke (1967), Zimny, Schutte and Dabezies (1986), Solomonow and Krogsgaard (2001)).

Pacinian corpuscles are encapsulated, rapidly adapting, force sensitive mechanoreceptors. They are located in the joint capsule, cruciate ligaments and menisci in cats and humans (Boyd (1954), Freeman and Wyke (1967), Halata, Rettig and Schulze (1985)). Pacinian corpuscles are active in acceleration and deceleration of joints and they are inactive in immobile joints (Freeman and Wyke (1967), Zimny (1988), Johansson et al (1991b)).

Golgi tendon organs (GTOs) are encapsulated, slowly adapting mechanoreceptors. They have a higher force threshold for activation than the Ruffini endings (type I receptors) when the forces are applied passively. They are found in the collateral and cruciate ligaments, capsule and menisci (Matthews (1933), Skoglund (1956), Grigg, Hofman and Fogarty (1982), Schultz et al (1984), Zimny et al (1986)). Golgi tendon organs are most commonly located at the junctions between the muscle fibres and the collagen strands composing tendons and aponeuroses (Milana, Mileusnic, Gerald and Loeb 2006). Because of the nature of their highly transient response, they are involved in accelerations, quick movements, and vibrations (Freeman and Wyke (1967), Halata (1977), Grigg (1984)). Golgi tendon organs are arranged in series with extrafusal muscle fibres because of their location at the junction of muscle and tendon. Some authors believe that activation of the Golgi tendon organs inhibits muscular contraction to protect muscles from injury (Houk and Henneman (1966), Houk and Simon (1967)). In other circumstances activation of GTOs leads to increases

in muscle force (Conway, Hultborn and Kiehn (1987), Pratt (1995), Prochazka, Gillard and Bennett (1997), Pearson, Misiaszek and Fouad (1998), McCrea (1998), Stephens and Yang (1999)).

Free nerve endings are not encapsulated and have no complex sensory structures. Most have polymodal responses and they respond to a range of mechanical and chemical stimuli. Free nerve endings are located in muscles, ligaments and cutaneous tissue layers (Freeman and Wyke (1967), Zimny (1988), Solomonow and Krogsgaard (2001)). They are the most common type of nerve ending.

1.7. Ligament Reflex

In chapter 3 (sections 3.3.1, 3.3.2. and 3.3.3.) details of reflexes elicited by electrical stimulation of ligaments will be described. In all of these experiments, there several important questions which need to be considered:

1. Which type of articular mechanoreceptors will be activated?

2. Will only one group of fibres be activated?

3. Will several groups be activated simultaneously?

4. Can specific reflex actions be attributed to particular types of afferents?

Many studies in animal preparations have found that reflexes can be elicited by electrical or physiological stimulation of the knee joint receptors. Cohen and Cohen (1956) and Eccles and Lundberg (1959) demonstrated that stimulation of the high threshold afferents of the cat knee joint activated α-motoneurons via reflex connections.

Experimental data gathered from animal studies confirm the existence of ligament muscular reflexes, probably through low threshold mechanoreceptors that influence muscle activity via the γ-muscle spindle system. Hongo, Jankowska and Lundberg (1969) found that electrical stimulation of low-threshold joint afferents evoked excitatory and inhibitory potentials in alpha motor neurons. Baxendale, Davey, Ellaway and Ferrell (1992) used restricted mechanical stimulation to stimulate group II joint mechanoreceptor and elicited inhibitory and excitatory responses in γ-motoneurons supplying the cat hindlimb. Scott, Ferrell and Baxendale (1994) stimulated group II/III afferents in the Posterior Articular Nerve of the cat knee joint. They could elicit excitatory responses in lateral gastrocnemius and soleus γ-motoneurons.

At low stimulation intensities, joint afferents and ligamentous afferents have more potent effects on the γ-motoneurones rather than on the α-motor neurons. It has been suggested that joint mechanoreceptor reflexes operating via the γ-motor neurone loop (γ-motor neurons- intrafusal fibres in muscle spindles-primary muscle spindle afferents-α-motor neurons) may contribute to the pre-programming of stiffness of muscles around the joint and thereby to the regulation of joint stiffness and joint stability (Johansson et al (1991b), Sjolander, Johansson and Djupsjobacka (2002)).

There are some fundamental studies in reduced animal preparations indicating that articular mechanoreceptors have short latency reflex actions on spinal alpha motor neurones (Baxendale, Ferrell and Wood (1987), Johansson et al (1991b)). Solomonow and Krogsgaard (2001) have proved that direct mechanical stimulation of the anterior cruciate ligaments

in cat knees can result in increased activation of the hamstrings and inhibition of quadriceps.

Sjolander, Djupsjobacka, Johansson, Sojka and Lorentzon (1994) showed that fusimotor effects are elicited after electrical stimulation of ligaments or application of low to moderate changes in the tension of the medial and lateral collateral ligaments. There was no concomitant activation of skeleto-motoneurons.

In parallel with the animal studies, other researchers have attempted to elicit reflexes in humans following mechanical or electrical stimulation of the knee ligaments. These reflexes were first examined in the late 1950s. Palmer (1958) suggested that afferent signals from the MCL of the knee were able to modify the activity in the muscles around the knee. Petersen and Stener (1959) tried to elicit reflexes from several muscles in the limb such as Sartorius, Semimembranosus and Vastus Medialis muscles following mechanical stimulation of the MCL by flexing the limb. The surface EMG was investigated in 30 healthy volunteers. But, however strongly they applied tension to the MCL they could not record any reflexes.

Other experiments performed in humans have shown the existence of reflexes (Kim et al (1995), Dyhre-Poulsen and Krogsgaard (2000)). They elicited reflexes following electrical stimulation of the ligaments of the human knee joint. Some of the researchers have used invasive methods (Kim et al (1995), Buchanan and Lloyd (1997), Dyhre-Poulsen and Krogsgaard (2000)). In 1995, Kim et al placed fine wire electrodes directly in the ligaments using percutaneous needles. They observed that the muscles on the medial side of the joint were activated following electrical stimulation of the medial collateral ligament

and that lateral muscles were activated following stimulation of the lateral collateral ligament. Subsequently, Dyhre-Poulsen and Krogsgaard (2000) placed a stimulation electrode in the proximal and mid part of the ACL during arthroscopy. They elicited reflexes in quadriceps and hamstring muscles. These tended to be inhibitions. The reflex latency was 65 $\pm$ 20 msec.

Others have used a less or non-invasive approach and stimulated the ligaments through or over the skin (Jenner and Stephens (1982), Gandevia, Miller, Aniss and Burke (1986), Gibbs, Harrison and Stephens (1995), Bagheri and Baxendale (1995), Priori, Berardelli, Inghilleri, Pedace, Giovannelli and Manfredi (1998), Grey, Ladouceur, Andersen, Nielsen and Sinkjaer (2001), Kalantari (2002), Haridas and Zehr (2003), Dhaher, Tsoumanis and Rymer (2003)).

The advantage of the invasive method is that the stimulation is more accurately focussed on a particular structure than it is in the non-invasive method. Less current is required for stimulation and the EMG is less affected by artefacts. The disadvantages of this method include difficulty in recruiting volunteers because of the more invasive nature of the experiment, problems in identifying the correct placement of the electrodes and the risk of infection.

The advantages of the non-invasive method are that the volunteers are easier to recruit and they return for repeat tests. The main disadvantages of non-invasive method is controlling the stimulation and recording in the presence of large stimulation artefacts.

The experiments described above provide evidence that reflexes can be elicited by electrical stimulation of knee ligaments. The overall picture resembles that of the reflexes

associated with Golgi tendon organs. Electrical stimulation of Ib sensory afferents from extensor muscles in laboratory animals like the cat elicits a complex pattern of depolarization of Ib terminals from various muscles. The afferents from Golgi tendon organs located in extensor muscles are an important source of positive force feedback signal during locomotion (Conway et al (1987), Dietz, Gollhofer, Klieber and Trippel (1992), Pratt (1995), Prochazka et al (1997), Pearson, Misiaszek and Fouad (1998), McCrea (1998), Stephens and Yang (1999)).

Many studies have investigated the role of Ib afferents during locomotion in the cat and human (Nichols and Houk (1976), Conway et al (1987), Yang, Stein and James (1991), Pearson and Collins (1993), Sinkjaer, Andersen, Nielsen and Hansen (1999)).

This is a clear example of the modulation of reflexes depending on the context in which they are elicited. To date, there has been no similar investigation of the modulation of ligamentous reflexes during different motor tasks.

Theoretical and experimental evidence indicate that ligament afferents, together with afferents from other joint structures, muscles and the skin provide the CNS with information during movements and posture through ensemble coding mechanisms, rather than via modality specific private pathways (Sjolander et al 2002). It means that reflexes travel via pathways other than those projecting directly to the skeletomotor system. In addition, during gait, the effects may feed forward to modify future movements rather than feedback to modify ongoing movements in the classical manner. Stretch reflexes in the leg muscles are certainly activated during locomotion and they appear to help launch the body into the

next step (Funase, Higashi, Sakakibara, Imanaka, Nishihira and Miles 2001).

Aims

In conclusion, classical electrophysiological experimentation in laboratory animals has demonstrated that short latency reflexes can be elicited by stimulation of mechanoreceptors in the joint capsule ligaments and tendons. The reflexes are complex and may be fed-back into ongoing movements or fed-forward in future movements. The situation is less clear in humans. Early attempts to elicit reflexes by mechanical stimulation of ligaments were unsuccessful but direct electrical stimulation of ligaments via per-cutaneous wire electrodes has shown that the reflexes can be elicited. The main aim of this project was to investigate if less invasive stimulation techniques could also be used to study ligamento-muscular reflexes. It was of particular interest to investigate the effects of muscle force, changes in posture and on-going movement on these reflexes.

Consequently, the aims of the experiments reported in this thesis are:

1. To investigate if reflexes can be elicited by electrical stimulation of ligaments.

2. To investigate if different muscles are affected differently by these reflexes.

3. To investigate if these reflexes can be modulated by posture or muscle activity.

Chapter 2

Materials and Methods

This chapter provides details of the materials and methods that were common to all experiments. Materials and methods specific to only one experiment will be discussed in the appropriate chapter.

2.1. Subjects

Forty-four volunteers aged between 21 and 49 years participated in four series of experiments. All were healthy asymptomatic individuals with no history of any neurological, musculo-skeletal or cardiovascular problems. All volunteers were recruited from the University of Glasgow staff and students. The Glasgow University Research Ethics Committee approved the experimental protocol. All subjects had given informed consent and they were free to withdraw from the test at any stage. Subjects had different tolerances for stimulation. Some of them tolerated relatively intense stimulation, up to 45 milliamps. Some had a much lower limit of tolerance. This is illustrated for 17 of the subjects in table 3.2. None of the volunteers withdrew from the experiments. All reached their individual limit.

2.2. Experimental Set-up

The subjects sat on a chair with their hip joint at 100° and the knee joint at 180°. Figure 2.1 shows the position of the subject.

The maximal voluntary contraction (MVC) of the quadriceps was measured at 180° of knee extension. The protocol for measuring the MVC consisted of asking the volunteer to make 3 maximal contractions of their knee extensor muscles (quadriceps). Each contraction lasted for 2 to 3 seconds and was separated by about the same period. A typical set of contractions can be seen in figure 2.2.

The experiments described in chapter 3 and 4 used a Neurolog 106 EMG Integrator unit (Digitimer Ltd, Welwyn Garden City, UK) with a time constant of 100 msec to process the EMG. Effectively the integration is performed using a 'leaky resistor/capacitor' circuit. The initial EMG data in chapter 5 was recorded in the same way. The NL106 Integrator was replaced by using the 'channel process' commands in Spike 2 version 5.03 (Cambridge electronic Design, Cambridge UK). The software performed the full wave rectification function before the signal was subjected to a 'smoothing period' of 60 msec. This is a running average of a 60 msec period of data (personal communication from CED engineer). In both cases the analysis was performed on-line during the experiment. The integration and subsequent signal processing would have the effect of time shifting the signal so that artefacts appear slightly delayed from their true position. This was indicated by the position of the trigger pulse issued to the stimulator.

The time scales of the published records have been adjusted so that the zero position is coincident with the onset of the

artefact. The repositioning is applied to the whole record so that there is no effect on the measured reflex latency since both artefact and reflex are exposed to the same delay.

The MVC was identified from the largest amplitude EMG in the set of three contractions. This was used to set the magnitude of subsequent sub-maximal contractions at 5, 10 and 20% of MVC. The 20% MVC value previously selected was marked on the screen and the subjects were asked to maintain the contraction at this level.

Bipolar stimulation electrodes were positioned on the skin over the collateral knee ligaments. The position was identified by physical examination with reference to anatomical landmarks such as muscles like Vastus Medialis, the knee joint and patella. This process was aided by reference to anatomy texts such as Fundamentals of Anatomy and Physiology by Martini (2006). The location of the stimulating electrodes is shown in figure 2.1.

The ligaments were electrically stimulated by a train of three pulses of 1 msec duration given at 100 Hz. Before each experimental run, during relaxation, the perceptual threshold (PT) was determined for each subject. Experiments started with the lowest current and on subsequent runs the current was increased. This process was repeated until the current limit at which it became too painful to continue was reached. The experiment was ended at that point. Obviously, the maximum intensity was not the same in all subjects. The greatest current ever used was 45 milliamps. The lowest current ever used to elicit these reflexes was 20 milliamps.

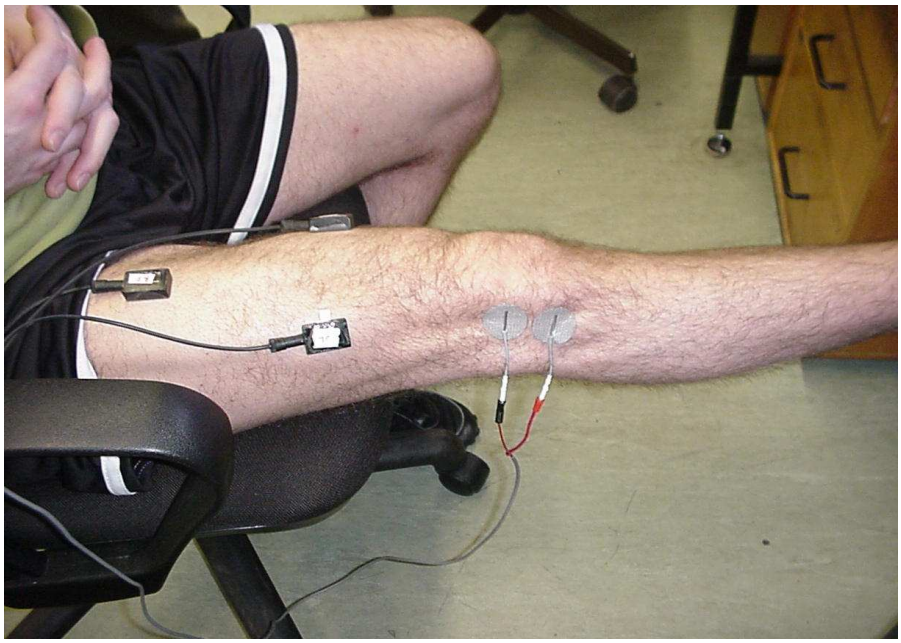


Figure 2.1
The figure shows a subject during the experiment. Three recording electrodes were placed on the RF, VM and VL. Two stimulating electrodes were positioned on lateral knee ligaments.

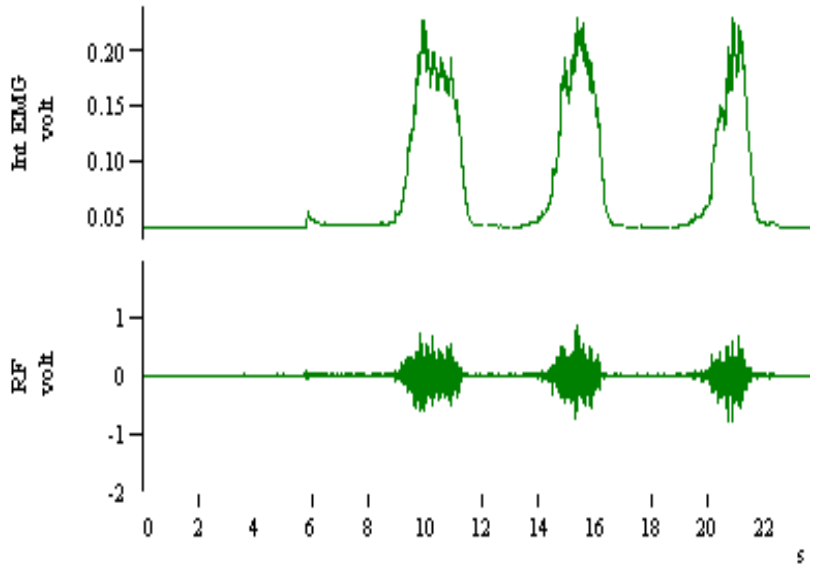


Figure 2.2
This figure shows recordings of EMG during three maximum contractions of quadriceps. The lower trace shows the surface EMG recorded over RF. The upper trace shows the full-wave rectified smoothed EMG.

2.3. Electrical Stimulation

A constant current stimulator, DS7A (Digitimer Ltd, Welwyn Garden City, England) was used in all the experiments. Rectangular stimulus pulses of 0.1msec duration were given through surface electrodes. The maximum voltage of the stimulator was set at 400 Volts. The collateral knee ligaments were stimulated by placing a round cathode, 3.2 cm in diameter, on the skin at the level of the lateral side of patella. A photograph of the electrodes can be seen in figure 2.3.

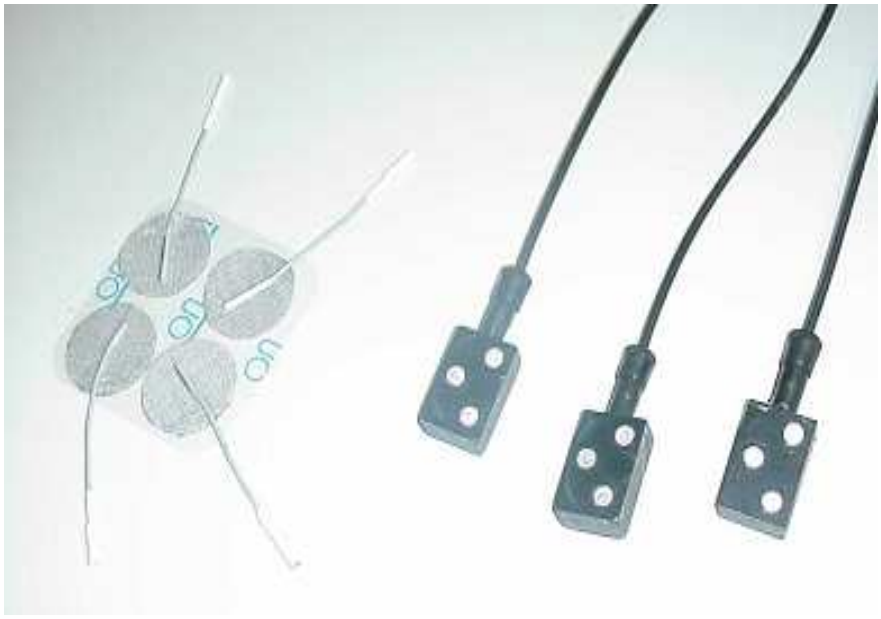


Figure 2.3
The figure shows the examples of the electrodes used. On the left four PALS self-adhesive stimulating electrodes can be seen. Each is 3.2 cm in diameter. On the right, three EMG recording electrodes can be seen. Each has a common electrode in the middle and two recording electrodes. Each electrode is silver and 0.5cm in diameter. The distance between the recording electrodes was 2cm. The block also encased an amplifier unit.

2.3.1. Electromyography Recording

Action potentials from active motor units were recorded and displayed by using conventional techniques. The electromyogram (EMG) is the electrical signal recorded by needle or surface electrodes during a muscular contraction. Surface EMGs were recorded from Rectus Femoris, Vastus Lateralis and Vastus Medialis of the right and left legs of the subjects. The skin at the recording sites was prepared very carefully before attaching the electrodes. The area was shaved and cleaned with alcohol. An electrode gel was used under the electrodes to decrease the contact resistance (Signa Creme, Parker Laboratories Inc, Orange, NJ, USA). The EMG signals were amplified 5000× by a head stage amplifier mounted on the skin surface. The bandwidth of the amplifiers was 10Hz to 1 KHz (-3dB at these frequencies) and their input impedance was 10 Mohms. Their common mode rejection ratio was 100 dB a 50Hz. Neurolog 106 amplifiers were used when additional amplification was needed. Their bandwidth was set at 3 Hz to 1 KHz.

The recording electrodes were placed near the middle of the muscles. This is shown in the figure 2.1. The electrodes were aligned along the long axis of the muscle. The diameter of each electrode was 5mm and the distance between the electrodes was 20mm.

All signals were digitised by a C.E.D. 1401 Micro interface (C.E.D. Ltd, Cambridge, England) at a sampling rate of 5000 Hz and stored in a PC. The data in chapters 3 and 4 were recorded and processed using Spike2 version 3.5 (C.E.D. Ltd, Cambridge, England). Version 5.03 was used for the data in chapter 5.

After digitisation the EMG filters were processed using the digital filters available in Spike 2. A high pass filter set at 10 Hz was used to remove any offset in the signal before the rectification was done. When necessary a band stop filter (-3dB at 46.5 and 53.9 Hz) was used to reduce 50Hz mains corruption of the EMG.

2.4. Averaging of EMGs

The reflexes elicited by ligament stimulation were identified by calculating peri-stimulus time (PST) averages of the rectified EMG. The surface EMGs of three muscles were recorded continuously using Spike 2. Each channel was sampled at a rate of 5000 Hz. The analysis was performed after the experiment was completed.

Each channel was full wave rectified and smoothed using the channel process functions of Spike 2. A period of 10 msec was used to smooth the rectified EMG. This calculates a running average for each time point for the interval $\pm$ the smoothing period. The first trigger pulse in each stimulus sequence was then used to create a peri-stimulus time (PST) average of each channel. The period before and after each trigger could be set using Spike 2. Typically, 400 msec before the onset of stimulation and 600 msec after stimulation were averaged.

A typical data collection run lasted one minute and the stimuli were delivered each second. Consequently, sixty responses were averaged to create a single PST average. This was ultimately limited by the desire to avoid fatigue in long duration contractions and to minimise the number of strong shocks delivered to the volunteer.

Increasing the number of repetitions leads to a better ratio of signal to noise. The signal/noise ratio improves with the square root of the number of sweeps averaged. Initially, the improvement will be substantial e.g. 16 sweeps gives an improvement in the ratio of x4 but an additional 20 to give a total of 36 sweeps gives a smaller benefit as the ratio improves from 4 to 6.

The choice of 60 repetitions in these experiments gives an improvement of 7.75. Lengthening the period of data capture was considered but could not be justified because of the problems of fatigue developing during sustained contractions. In addition, at the higher stimulus current intensities used, some volunteers approached their limits of tolerance. The choice of 60 repetitions is a compromise since it gives a reasonable signal/noise ratio without causing problems with muscle fatigue or volunteer withdrawal.

2.5. Analysis of pre-stimulus EMG

The EMG recorded in the immediate pre-stimulus period represents asynchronous activity of a number of motor units. Reflex responses were identified by comparing the pre-stimulus background with the post stimulus period.

The rectified smoothed averaged EMG data were translated into a text file and saved. The subsequent analysis was done using Excel to perform the calculations.

The time scales of the published records have been adjusted by the relative movement of the time points and the voltage points so that zero position is coincident with the onset of the artefact. The repositioning is applied to the whole record.

The mean background EMG was estimated by calculating the mean of the voltage values for the 400 msec before the onset of the stimulation artefact. This background period was selected to avoid any inclusion of the artefact. These could be large compared to the magnitude of the EMG and so it was important make an appropriate selection. Inappropriate choices of background period led to calculations of means which did not fit the data or to large confidence intervals which contained the whole of the pre-stimulus EMG. On the rare occasions when this happened, the period was reviewed or the data were rejected as unsuitable for analysis.

The standard deviation of the EMG during this period was also calculated. The mean plus and minus two standard deviations were used to calculate a 95% confidence interval for the background signal. These values were extrapolated into the post stimulus period to assist in identifying reflex responses. Responses were treated as significant if the mean EMG rose above or fell below the confidence interval for periods of longer than 5 msec. This is similar to the criteria used by other authors (van der Glas, de Laat and van Steenberghe (1985), van der Glas, Cadden and Abbink, (1999)). The former paper used a minimum duration of 5.8 msec and the latter included a minimum period of 4.7 msec outside the confidence interval in their list of criteria for identifying responses. The variation in durations is due to the number of sweeps averaged. Increasing the number of sweeps reduces the chances of random fluctuations. Both papers report that these periods excluded 95% of chance excursions outside the interval.

An example is given in figure 2.4.

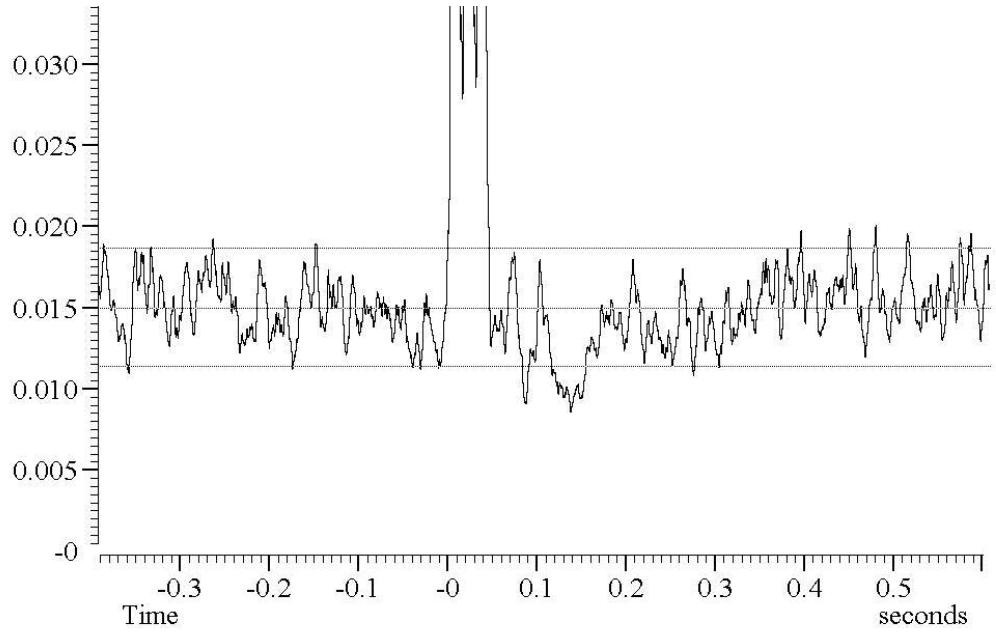


Figure 2.4
The figure shows the peri-stimulus time average of the rectified EMG recorded from RF after MCL stimulation. The stimulus current was 40 mamps and the volunteer sustained a background contraction of 20% of MVC. The vertical axis shows the EMG signal in volts. The horizontal axis shows time in seconds.
It shows 400 msec before the stimulation artefact and 600 msec after the stimulation. The mean of the pre-stimulation EMG is shown and extrapolated into the post stimulation period. The 95% confidence intervals are also shown as the upper and lower horizontal lines.
The EMG goes outside the lower confidence interval twice. The first inhibition happen 76 msec after stimulation and the signal is outside the interval for 6 msec. The second period of inhibition started after 108 msec and was outside the interval for 39 msec. The EMG signal goes outside the confidence interval on a number of other occasions, for example between 350 and 500 msec after stimulation but the period outside the interval is too short to be significant.

The latency of the reflex was measured as the time between the first stimulus pulse and the time at which the integrated EMG moved outside the confidence interval.

Chapter 3

Reflexes Elicited by per-cutaneous Stimulation of the Medial and Lateral Ligament of the Knee during Sitting and Standing.

3.1. Introduction

The earliest experiments investigating reflexes from joints were carried out in laboratory animals such as the cat (Stener (1959), Andersson and Stener (1959), Johansson, Sjolander and Sojka (1986), Baxendale et al (1987)). This literature was reviewed in sections 1.5 and 1.6 of the general introduction.

Stener (1959), Andersson and Stener (1959) elicited reflexes in the Rectus Femoris, Vastus Lateralis, Vastus Medialis, Sartorius, Gracilis, Semitendinosus, Semimembranosus and the medial head of Gastrocnemius of decerebrate cats by stretching the MCL. In a third paper in this series Petersen and Stener (1959) attempted to elicit reflexes in conscious humans by mechanical stretching of the MCL. They tested 35 volunteers, but they never observed any signs of reflexes. Other researchers have tried to investigate reflexes in humans using electrical stimulation of the knee ligaments. Palmer (1958) succeeded in demonstrating ligamento-muscular reflexes in Sartorius, Semimembranosus and Vastus medialis after electrical stimulation of the medial collateral ligaments of the knee joint in man.

Kim et al (1995) used fine-wire electrodes implanted in the MCL and LCL of eleven healthy volunteers during arthroscopy to stimulate the collateral knee ligaments. They used fine-wire intramuscular electrodes for EMG recording to study: Sartorius, Gracilis, Vastus Medialis, Vastus Lateralis,

Semitendinosus, Biceps Femoris long head and Tensor Fascia Latae. This technique did elicit reflexes and they believed that the muscles on the medial side of the joint were activated following electrical stimulation of the MCL and the lateral muscles were activated following stimulation of the LCL. The latencies of reflexes that they observed were 69 to 144 msec.

Dyhre-Poulsen and Krogsgaard (2000) used similar techniques to insert fine-wire electrodes into the anterior cruciate ligament of eight patients during arthroscopy. They could elicit inhibitory reflexes in Rectus Femoris and Semitendinosus following ACL stimulation with latencies ranged between 45 to 85 msec.

The aims of this current study were to extend earlier investigations by using transcutaneous stimulation of lateral and medial knee ligaments in humans. The intra-ligamentous stimulation technique is invasive and it is unlikely that it could be used to study reflexes during movement. The longer term aim of this project is to use this technique to investigate ligamentous reflexes during movement in athletes.

3.2. Materials and Methods

The general methods were described earlier in chapter 2. Information specific to these experiments is given below.

3.2.1. Subjects

Twenty-six volunteers (1 woman, 25 men), who were healthy and had no history of injury, participated in two series of experiments. 17 volunteers participated in the first series and 9 in the second series. Their ages ranged from 21 to 49 years. The Faculty of Biomedical and Life Sciences Ethics Committee for Non Clinical Research Involving Human Subjects approved the experimental protocols. All subjects gave informed consent and they were free to withdraw from the test at any stage.

3.2.2. First Series of Experiments

The aim was to investigate if reflexes can be elicited by stimulation of the ligaments. The subjects sat on a chair with their hip joint at 100° and the knee at 180°. The position of the subject is shown in figure 2.1.

The volunteer was encouraged to make maximal voluntary contractions of their quadriceps. The protocol for measuring the maximal voluntary contraction consisted of asking the volunteer to make 3 maximal contractions. Each contraction lasted for 2 to 3 seconds and they were separated by about the same period. A typical set of contractions can be seen in figure 2.2

Stimulation electrode pairs were placed in two locations on the MCL and LCL, which were localized by palpation and

knowledge of the bony landmarks. The ligaments were electrically stimulated by a train of three pulses with an inter-stimulus interval of 10 msec.

Before each experimental run, the perceptual threshold was determined for each subject. This was done by gradually increasing the intensity of the stimulation until the volunteer reported the first signs of sensation. This was typically below 2 milliamps. Volunteers had different perceptual thresholds and the current intensities required were between 0.75 -1.75 milliamps.

When testing for reflexes, the experiment started with a low current, typically at 10 milliamps, and on subsequent runs the current was increased. This process was repeated until it reached the current limit at which it became too painful to continue. The experiment was ended at that point. Obviously, the maximum intensity was not the same in all subjects. The duration of intensity was 10 to 45 milliamps.

3.2.3. Second Series of Experiments

Nine healthy subjects (all men) aged between 22 to 47 years participated in this experiment. After cleaning the site of the electrodes, recording electrodes were placed over the Medial Gastrocnemius, Lateral Gastrocnemius, and Soleus muscles. The postural EMG in the muscles was increased by asking the volunteers to raise their heels clear of the ground and to balance on their toes. The ligaments were electrically stimulated by a train of three pulses of 1 msec duration given at 100 Hz. Before each experimental run, when the volunteers were relaxed, the perceptual threshold was determined for each subject. Most of this experiment was the same as the

first experiment, which has been explained in chapter 2 sections 2.1 and 2.2.

Figure 3.1.shows a subject during the test.

3.2.4. Electromyography

Surface electromyograms were recorded from the following muscles: Rectus Femoris, Vastus Lateralis, Vastus Medialis, Soleus, Medial Gastrocnemius and Lateral Gastrocnemius. The technique of electromyography has been explained in section 2.3.1. All signals were digitised by C.E.D. 1401 Micro interface (C.E.D. Ltd, Cambridge, England).The data were recorded and processed using Spike2 version 3.5 (C.E.D. Ltd, Cambridge, England). A sample of EMG recording is shown in figure 3.2.

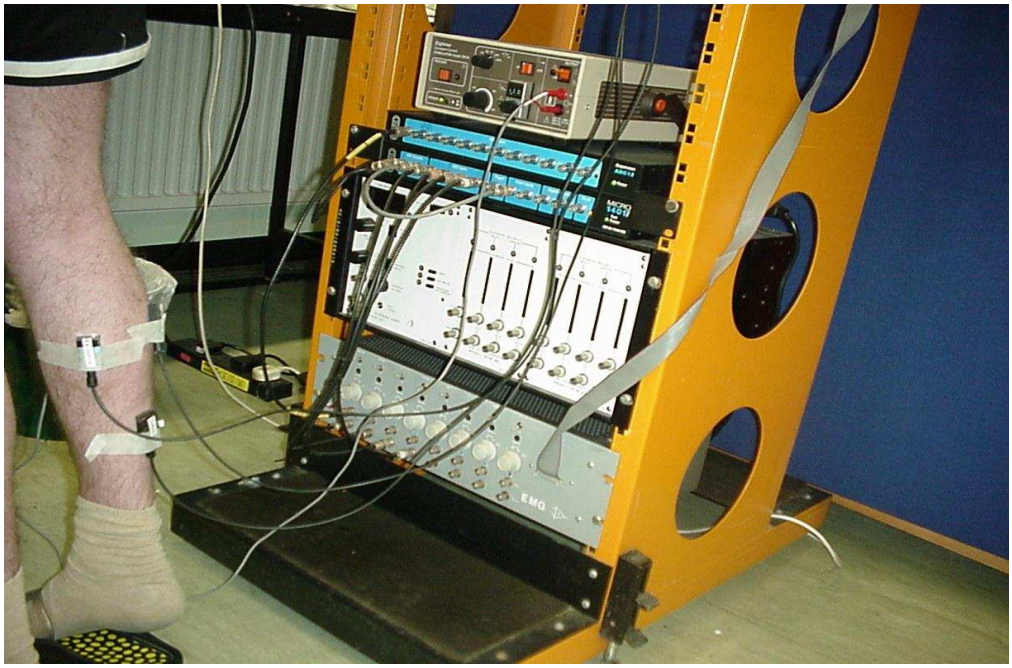


Figure 3.1
The figure shows a subject during the test. The volunteer is standing on his toes and the three recording electrodes are placed over the MG, LG and Sol muscles. The CED 1401 Micro Interface and the DS7 stimulator are also shown.

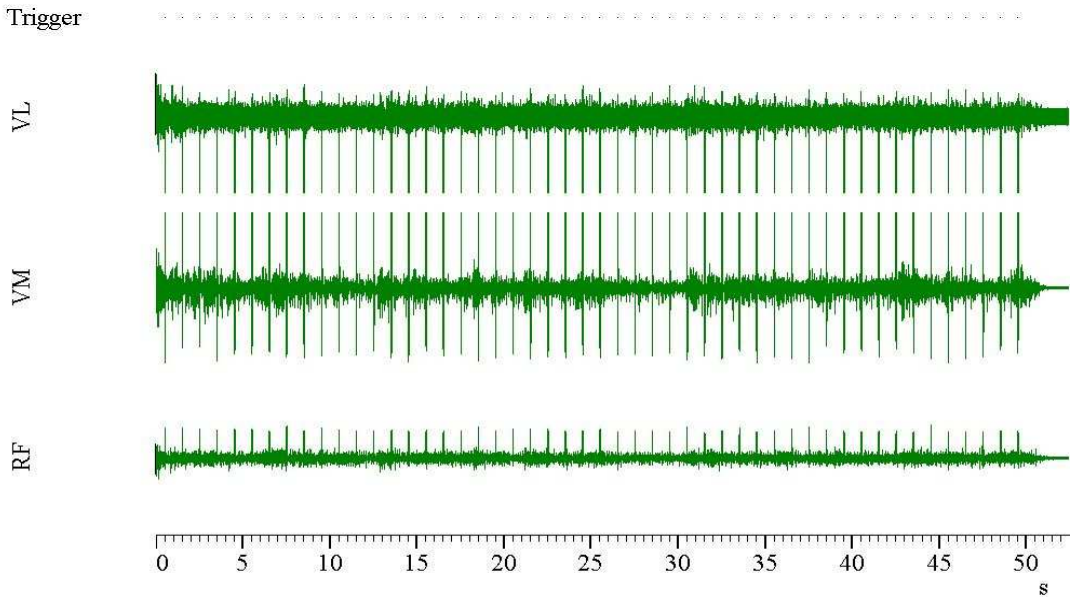


Figure 3.2
The figure shows a sample of unprocessed EMG recorded during an experiment. The top trace shows the trigger pulses used to initiate stimulation over the ligament. EMG from VL, VM and RF is shown and each channel clearly shows the stimulation artefacts.

3.3. Results

3.3.1. Ligamento-muscular Reflexes

Firstly, no excitatory reflexes were ever observed in relaxed muscles in any volunteers. It is clearly impossible for inhibitions to be recorded in a silent EMG. However, stimulation applied over the MCL and LCL did not elicit muscle contractions in Rectus Femoris, Vastus Lateralis, Vastus Medialis, Lateral Gastrocnemius, Medial Gastrocnemius or Soleus. In contrast, inhibitory and excitatory reflexes were frequently observed in active muscles even when the contractions were as small as 5% of MVC. An example of reflexes in Vastus Medialis after stimulation of MCL is shown in figure 3.3. The averaged rectified EMG signal is relatively flat in the 400 msec before the artefacts between 0 and 30 msec. This signal continues almost unchanged until it goes below the confidence interval at 75 msec for a period between 20 to 70 msec. The signal subsequently rises above the upper confidence interval for a period of 12 msec at 440 msec after the stimulation. This later event is too long after the stimulation to be classified as a simple reflex.

The recordings in figure 3.4 show three rectified averaged electromyograms from Vastus Lateralis in the same volunteer. As it can be seen the background EMG increases with increasing the percentage of MVC. With the contraction held at 5% of MVC the post stimulus EMG is modulated and it crosses the upper confidence interval at 64, 65 and 58 msec. As the background contraction is increased to 10 and then 20% of MVC the reflexes become more obvious and significant.

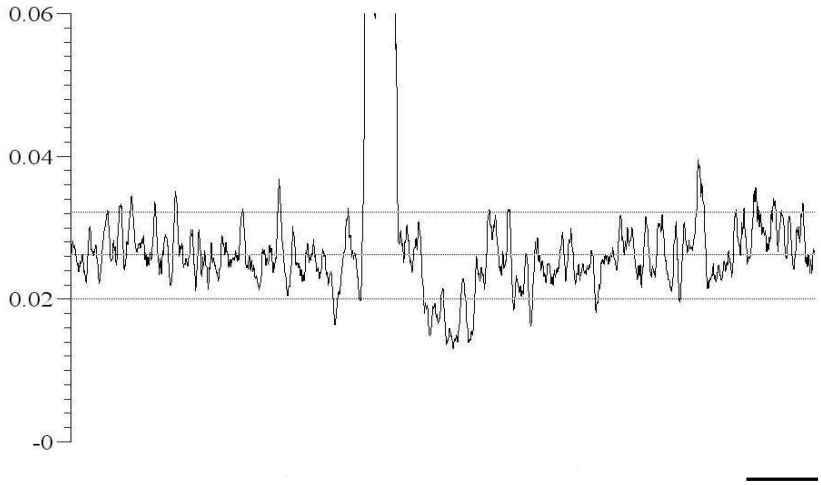


Figure 3.3
The figure shows rectified averaged EMG recording from VM after LCL stimulation. The stimulus current was 40 mamps and the volunteer sustained a background contraction of 20% of MVC. The vertical axis shows the EMG in volts. The horizontal axis is time The horizontal line indicates 100 msec
There is an early inhibitory reflex recorded after a latency of 75 msec. The signal is below the confidence interval for a least 20 msec. There are later periods where the signal rises above the confidence interval but these are too short to be significant.

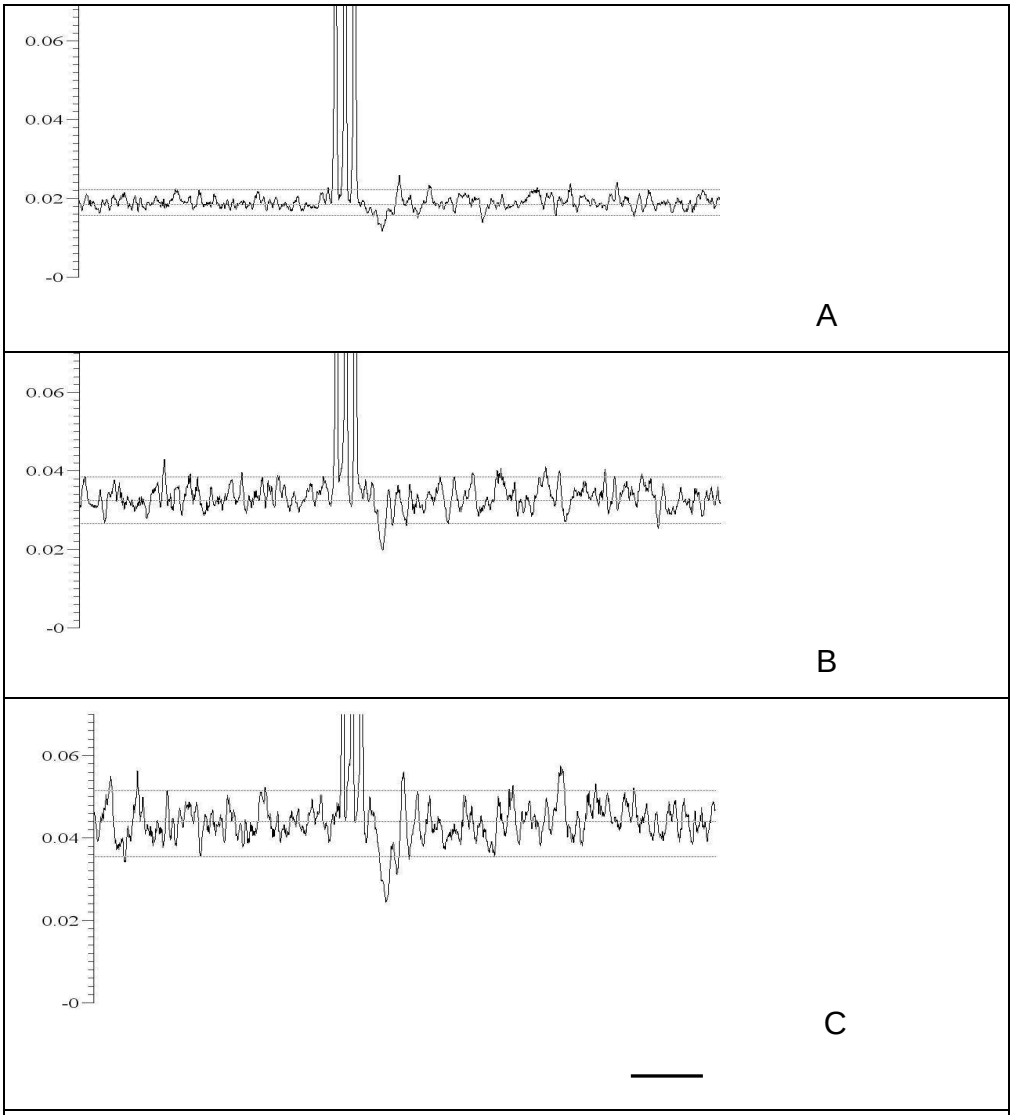
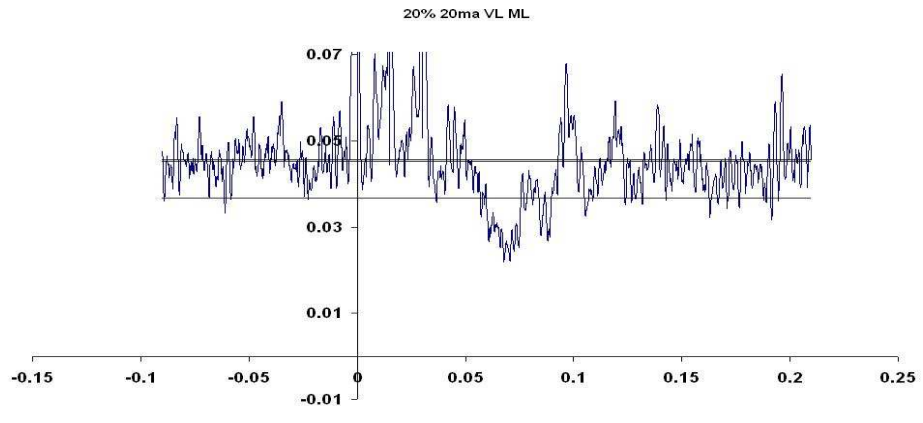
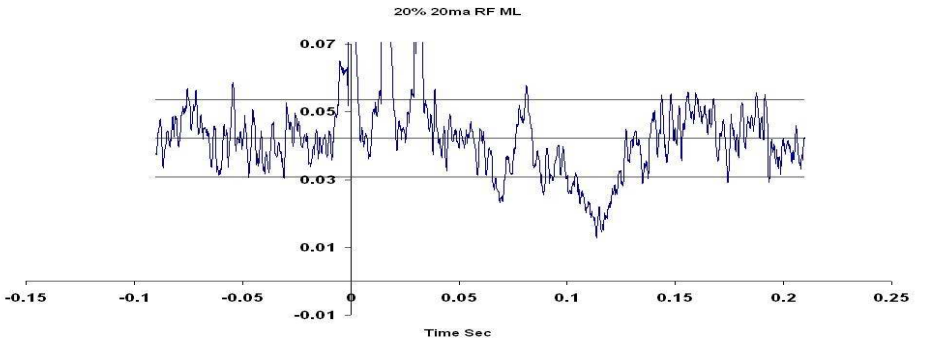


Figure 3.4
The figure shows rectified averaged EMG during three sustained contractions recorded from VL in one volunteer. The maintained contractions were 5, 10 and 20% of MVC. The vertical axis shows the EMG in volts. The horizontal axis is time The horizontal line indicates 100 msec
Each trace shows a period of background EMG before the stimulation artefacts. In each case the MCL was stimulated with the same current intensity.
Shortly after the artefact the EMG decreases in each case. The magnitude of the inhibition increases as the force of contraction increases.

In other volunteers the inhibition was combined with a second response. This could be a later excitation or a second wave of inhibition. Examples of these responses are shown in figure 3.5. The trace A shows a short latency inhibition response which starts at 61 msec then a long latency excitation which starts 99 msec after the stimulation artefact. Trace B shows a similar short latency inhibition followed by a long latency inhibition starting 103 msec after the stimulation artefact. The latencies of the later responses ranged between 91-150 msec.



A



B

Figure 3.5
The figure shows two examples of rectified averaged EMG showing longer latency responses. The vertical axis shows the EMG in volts. The horizontal axis is time in seconds.
The upper trace (A) shows a shorter latency inhibition at about 62 msec followed by a longer latency excitation at 99 msec after stimulation artefact.
The lower trace (B) shows a shorter latency inhibition followed by a longer latency inhibition. The reflex latencies are 62 and 103 msec respectively.

3.3.2. The Frequency with which Ligamento-muscular Reflexes were Observed

The results for all 17 volunteers are summarised in table 3.1. This shows the frequency with which reflex responses, either inhibitory or excitatory, were observed in Rectus Femoris, Vastus Medialis, Vastus Lateralis, Medial Gastrocnemius, Lateral Gastrocnemius and Soleus after LCL stimulation. For example, reflexes were observed in the Rectus Femoris in 12 of the 17 volunteers. Of these 12 volunteers, 8 showed inhibitions and 6 showed excitations. The frequency of reflexes was similar in the other heads of quadriceps.

When muscles in the lower segment of the limb were tested, reflexes were observed in Lateral Gastrocnemius, Medial Gastrocnemius and Soleus in 6 of 9 volunteers. The pattern was similar to that in quadriceps. Inhibitions were observed more frequently than excitations. In summary, it is clear that reflexes can be elicited by repetitive stimulation over LCL in the majority of volunteers.

One might wonder why some volunteers did not show any signs of reflexes. This question is addressed by the data shown in table 3.2. It shows the maximum current tolerated by each volunteer and the presence or absence of reflexes. It can be seen that the volunteers who did not display reflexes tended to have a low tolerance of electrical stimulation. The experiment allowed volunteers to limit the intensity of stimulus current. It may be that in a number of cases the low tolerance of the volunteer kept the stimulation below threshold for ligament afferent activation.

Muscle tested	Number of volunteers showing reflexes	Number of volunteers showing inhibitions	Number of volunteers showing excitations
Rectus Femoris	12/17	8/12	6/12
Vastus Lateralis	12/17	8/12	4/12
Vastus Medialis	11/17	7/11	4/11
Lateral Gastrocnemius	6/9	5/9	2/9
Medial Gastrocnemius	6/9	6/9	3/9
Soleus	6/9	5/9	4/9

Table 3.1
The table shows the number of subjects who had reflexes following electrical stimulation of the LCL. For example, in the first row of data, 12 of 17 volunteers had reflexes in RF. Of those 12, 8 showed inhibitions, 6 showed excitations and 2 had both excitations and inhibitions. The most commonly observed reflex effect was inhibition.

Volunteers	Max Stimulation Intensity (milliamps)	Reflex Present
Subject 7	10	No
Subject 17	15	No
Subject 3	15	No
Subject 1	20	No
Subject 5	20	No
Subject 8	20	Yes
Subject 12	20	Yes
Subject 14	20	Yes
Subject 16	25	Yes
Subject 4	25	Yes
Subject 6	25	Yes
Subject 10	25	Yes
Subject 2	30	Yes
Subject 13	30	Yes
Subject 11	35	Yes
Subject 15	35	Yes
Subject 9	45	Yes

Table 3.2
The table shows a list of volunteers with the maximum stimulation intensity they received. It also shows if this stimulation elicited reflexes.

The maximum current intensity tolerated varied considerably between subjects. The highest intensity recorded was for subject 9 with 45 milliamps and the lowest current is for subject 7 with 10 milliamps.

12 out of 17 of subjects displayed a reflex. No reflexes were observed in experiments where the volunteer limited the maximum current to 10 or 15 milliamps. 5 volunteers limited the maximum current to 20 milliamps and 2 showed no reflexes whilst 3 did. All the volunteers who tolerated currents stronger than 25 milliamps exhibited reflexes.

3.3.3. Reflexes Elicited at Different Percentages of Maximum Voluntary Contraction

The aim of these experiments was to investigate if the reflexes elicited by ligament stimulation were affected by the intensity of the muscle contraction. The maximal voluntary contractions of the quadriceps were measured at 180° of knee extension. This was then used to set the magnitude of subsequent sub-maximal contractions at 5, 10 and 20% of MVC. An identical stimulation sequence was delivered during sustained contractions.

Figure 3.6 shows another example of increasing the background contraction in a different subject. As the background contraction is increased from 5% to 10% of MVC the reflexes become more obvious and significant. The reflex is recorded in Rectus Femoris after stimulation of the LCL. The upper panel shows a barely noticeable reflex excitation at 116 msec elicited when the background contraction was 5% of MVC. Increasing the background contraction to 10% elicits stronger reflexes.

The effect of posture on the reflexes was investigated. In these experiments the same stimulation was delivered with the volunteer sitting and standing. The EMG recorded during standing was closely matched by adjusting the intensity of an isometric voluntary contraction whilst sitting. Figure 3.7 shows examples of reflexes elicited in Vastus Medialis and Soleus by stimulation of 20 milliamps in these two postures.

Only very minor changes in reflexes occurred when the postures were changed. These small effects can attributed to small changes in background EMG rather than to any effects of posture.

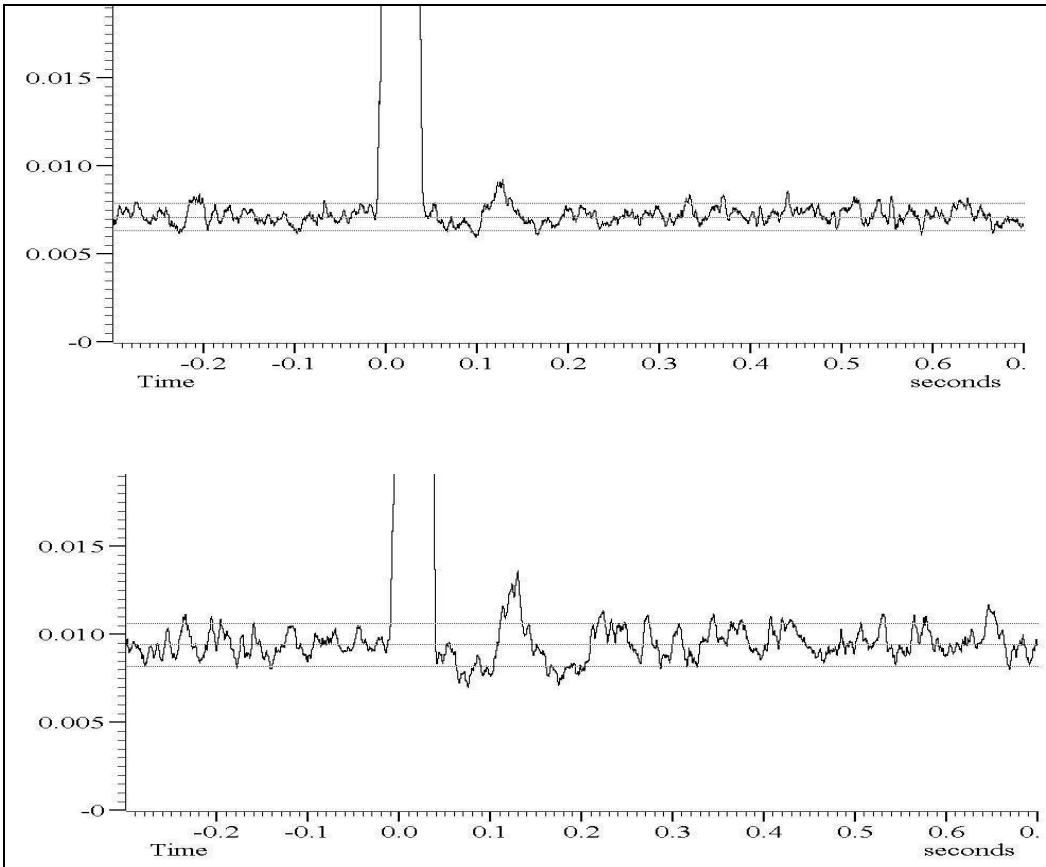


Figure 3.6
The figure shows the reflex responses in the rectified averaged EMG of RF in volts during contractions at different forces. The background contractions are 5 and 10% of MVC in traces A and B respectively. Trace A shows an excitatory reflex with a duration of 15 msec recorded 116 msec after the stimulation artefact. By increasing the background contraction (trace B) stronger reflexes were elicited. An inhibitory reflex was recorded at 62 msec with a duration of 16 msec. A larger excitatory reflex was recorded at 112 msec with a duration of 21 msec.

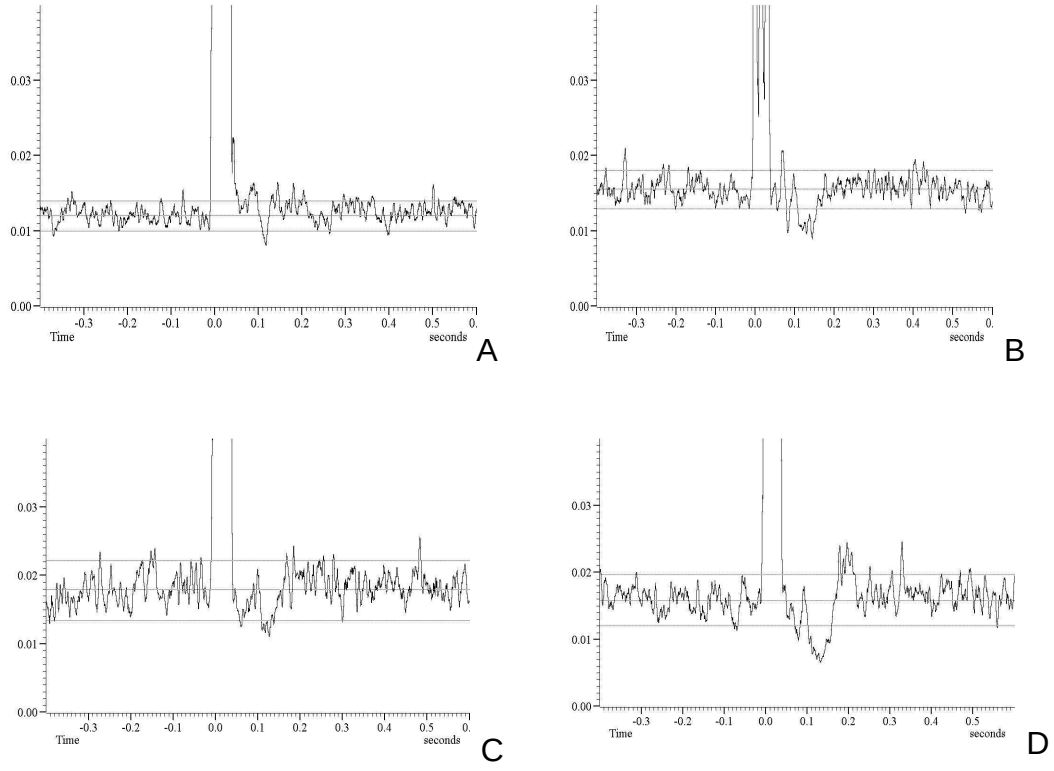


Figure 3.7
The figure shows rectified averaged EMG recordings in the same subject during two recording sessions. The LCL was stimulated with currents of 20 milliamps on all occasions. The upper traces are EMG recordings from Sol during standing (A) and sitting (B). The lower traces are recordings from VM during standing (C) and sitting (D).

The form of the reflex responses is similar in both postures.

3.3.4 Reflex Latencies

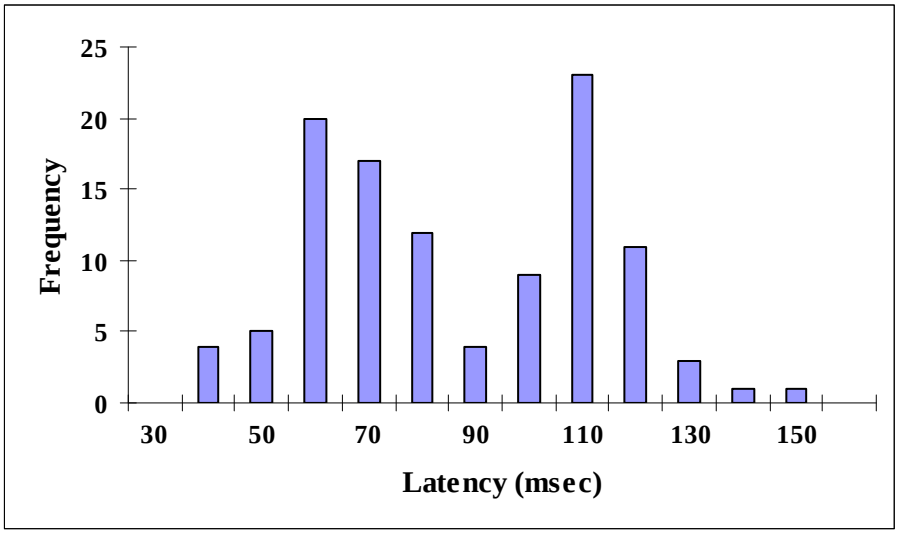
The aim of this section was to investigate if the excitations and inhibitions following stimulation are similar. Tables 3.3 and 3.4 show the latencies of inhibitions and excitations observed in experiments.

The distribution of reflex latencies was initially investigated by pooling all the reflex latencies, both excitations and inhibitions in the muscles tested. The distributions are shown in figure 3.8. It is clear than the excitations and inhibition must come from different reflex pathways and could use different afferent fibres. The aim in pooling the latencies was to investigate if a simple division of responses into shorter and longer latency groups could be achieved.

The latencies range from 46-150 msec. Two peaks can be seen in the distributions in both muscle groups. There is no clear separation of these. For quadriceps reflexes of 90 msec was used as an arbitrary division between the short and long latency reflexes. The triceps data also show two peaks representing the earlier and later reflexes. The peaks and the cut-off for short/long latency components lie about 10-msec after those in figure 3.8A. This probably reflects the longer reflex pathway to triceps.

These arbitrary times were used to separate the reflexes into long and short latency groups for subsequent analysis.

A



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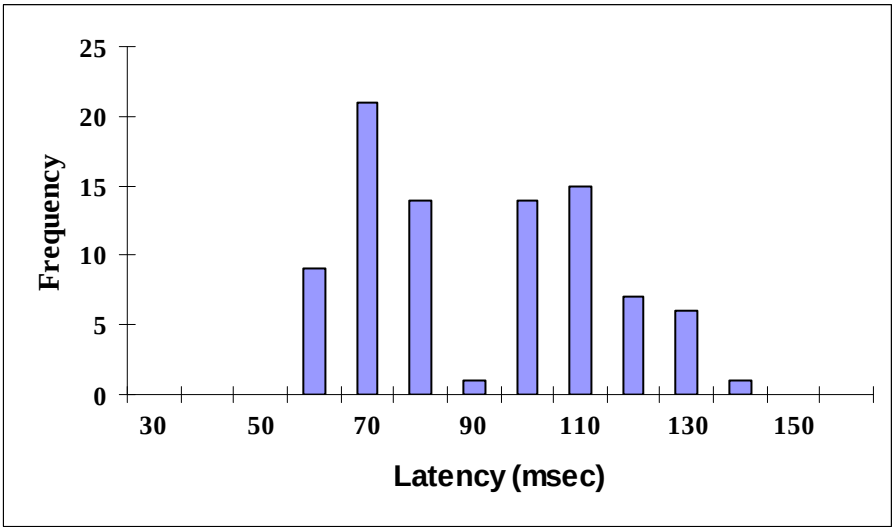


Figure 3.8
The figure shows the distribution of pooled data of the reflexes latencies in quadriceps (A) and triceps (B) after stimulation of both MCL and LCL.
The most common latency for earlier latency reflex in quadriceps is 65 msec and for longer latency is 110 msec, but the mean latency for all reflexes is 90 msec. In comparison, the equivalent latencies in triceps are 75 msec, and 110 msec. The mean latency for reflexes in triceps is 100 msec.

The latencies of the reflexes are tabulated below in tables 3.3 and 3.4. The first summary table 3.3 shows the effects separated into long and short latency excitations and inhibitions in quadriceps. The latencies following stimulation of the LCL and MCL are shown. The following table 3.4 shows the data from measurements made on triceps.

	Quadriceps Inhibition (msec)					Quadriceps Excitation (msec)			
	LCL	LCL	MCL	MCL		LCL	LCL	MCL	MCL
	Short	Long	Short	Long		Short	Long	Short	Long
	68	103	54	107		55	98	57	105
	71	130	74	101		54	96	60	98
	73	138	72	150*		46	98	61	93
	82	108	47	118		57	109	55	109
	59	116	55	98		59	109	50	105
	64	120	50	118		57	107	70	104
	62	109	65	103		62	105	57	92
	65	112	90	119		68		46	98
	60	117	85	105		72		58	103
	63	110	78	103					
	77	117	61	94					
	51	118	68	105					
	66	121	59	110					
	58	112	57	105					
	59		65	108					
	70		76	121					
	72		77	116					
	68		72	107					
	62		78						
			85						
			56						
			77						
Mean	**66**	**117**	**68**	**110**		**57**	**103**	**57**	**101**
SD	**7**	**9**	**12**	**12**		**6**	**6**	**6**	**6**

Table 3.3
The table shows the latencies of inhibitory and excitatory reflex responses in quadriceps following electrical stimulation of MCL and LCL. * This value omitted from statistical analysis. It is an outlier and it compromises the normal distribution of values. See Figure 3.13.

	Triceps Inhibition (msec)					Triceps Excitation (msec)			
	LCL		MCL			LCL		MCL	
	Short	Long	Short	Long		Short	Long	Short	Long
	81	138	81	118		75	119	74	109
	82	143	70	116		82	116	75	108
	85	135	74	114		74	109	71	114
	73	125	79	108		66	108	62	113
	78	105	73	106		70	112	66	
	79	132	71	113		66	99	68	
	69	124	68	108		63	108	64	
	86	119	71	110			102		
	87	131	78	110					
	80	123	80	117					
	65	108	86	137					
	82	109	78	130					
	72	105	66	125					
	79	104	82	121					
	78	123	66						
	82	126	82						
	80	117							
	78								
Mean	**79**	**122**	**75**	**117**		**71**	**109**	**69**	**111**
SD	**6**	**12**	**6**	**9**		**7**	**7**	**5**	**3**

Table 3.4
The table shows the latencies of inhibitory and excitatory reflex responses in triceps following electrical stimulation of MCL and LCL.

3.3.5. A Comparison of Reflex Latencies after MCL and LCL Stimulation

The aim of this section was to investigate if there is a difference in reflex latency after stimulation of the two ligaments.

3.3.5.1. Statistical Analysis

All statistical analyses were performed using Minitab 14 (Copyright ©2008 Minitab Inc). The first step in the statistical analysis of latencies was to test if the data were normally distributed. Ryan Joiner tests were used for this purpose. If the data were normally distributed then summary statistics such as means and standard deviations were calculated. One way ANOVA tests were used to search for difference. If significant differences were found post hoc t tests were used to search for differences in means.

3.3.5.2. Short Latency Inhibitions in Quadriceps. The first comparison was made using the short latency inhibitions in quadriceps. The first step in the statistical analysis of latencies was to confirm that the data were normally distributed. A Ryan Joiner test confirmed that the values in tables 3.3 and 3.4 were normally distributed ($P>0.1$). The mean latency of early inhibition in quadriceps muscles after LCL stimulation was 66 $\pm$ 7 msec and after MCL stimulation was 68 $\pm$ 12 msec. The difference was not significant when tested with a one way ANOVA. ($P=0.451$). No post hoc t test was needed.

These data are illustrated in figure 3.9.

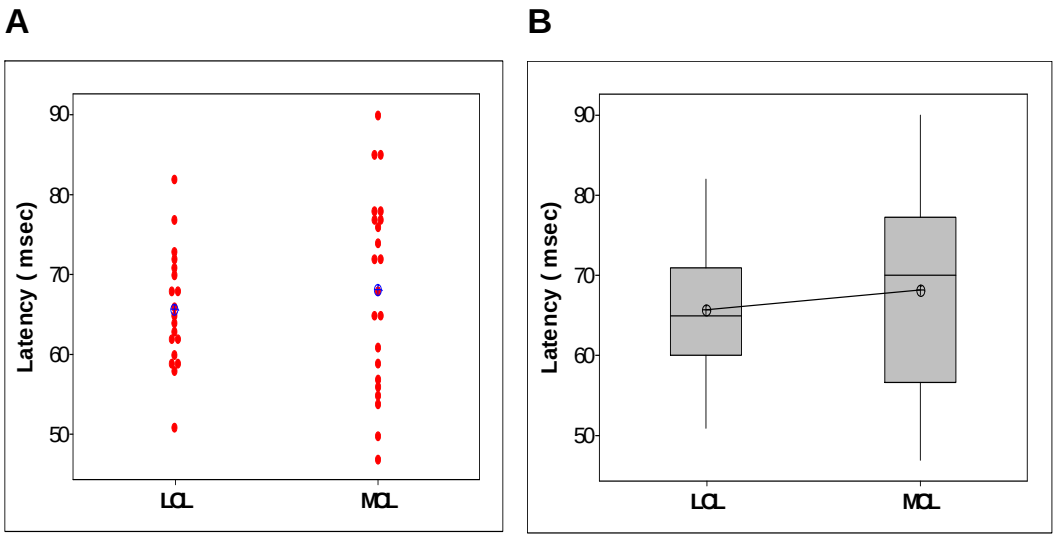


Figure 3.9

Panel A shows the short latencies of reflex inhibitions in quadriceps following stimulation of LCL and MCL.
Panel B shows the box and whisker plots of the same data. Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1). A one way ANOVA showed no significant differences P=0.451.

A similar analysis was performed with the short latency excitations in quadriceps. The data are summarised in figure 3.10. The normality of the distribution of data was confirmed using a Ryan Joiner test ($P>0.1$). The mean latency of early excitation after LCL stimulation was 57 $\pm$ 6 msec and for the MCL stimulation was not significantly different. A one way ANOVA showed no significant differences $P=0.611$.

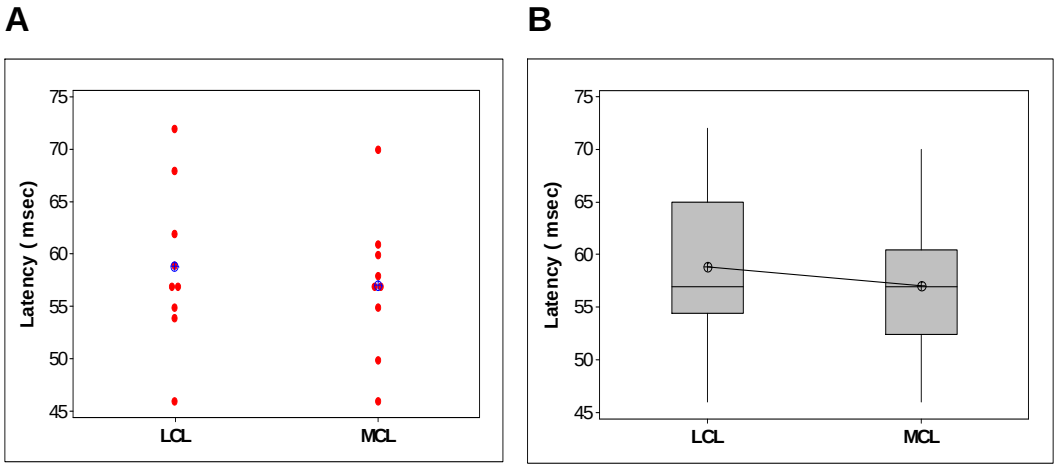


Figure 3.10

Panel A shows the short latencies of reflex excitations in quadriceps following stimulation of LCL and MCL.
Panel B shows the box and whisker plots of the same data. Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1). A one way ANOVA showed no significant differences P=0.611.

Similar data for the reflexes in triceps are shown table 3.4. Statistical tests confirmed that the distributions of latencies of inhibitions and excitations were normally distributed (Ryan Joiner, P>0.1).The mean latency of early inhibition after LCL stimulation was 79 $\pm$ 6 msec and for MCL it was 75 $\pm$ 6 msec. A one way ANOVA showed no significant differences (P=0.113).

The mean latency of early excitation after LCL stimulation was 71 $\pm$ 7 msec and for MCL it was 69 $\pm$ 5 msec. A one way ANOVA showed no significant differences (P=0.478).

The latencies of the inhibitions in triceps are shown in figure 3.11 and the excitations are shown in figures 3.12.

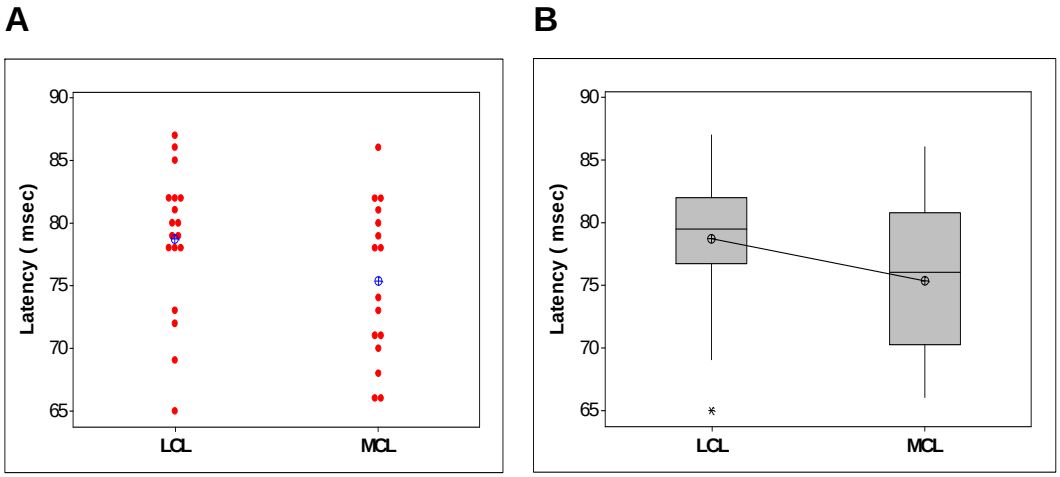


Figure 3.11

Panel A shows short the latencies of reflex inhibitions in triceps following stimulation of LCL and MCL.
Panel B shows the box and whisker plots of the same data.
Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1).
A one way ANOVA showed no significant differences (P=0.113).

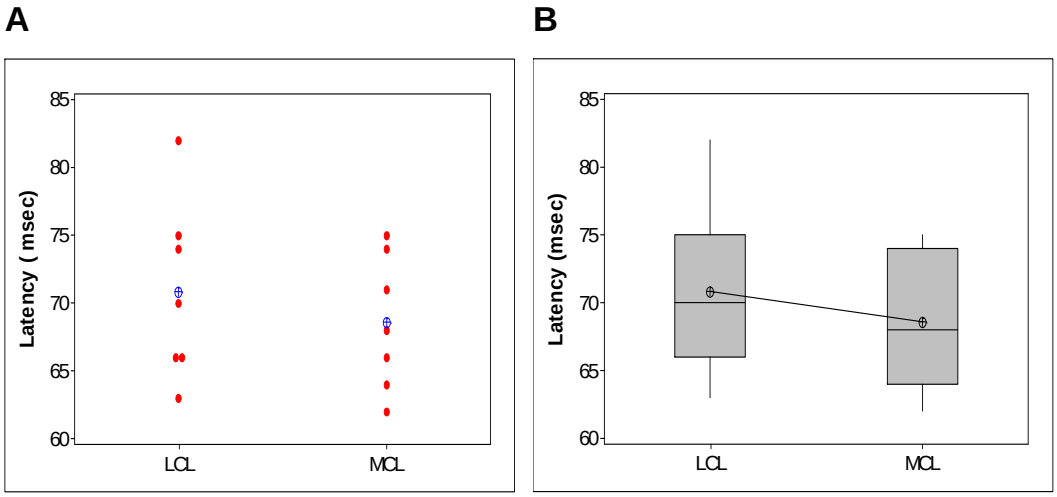


Figure 3.12

Panel A shows the short latencies of reflex excitations in triceps following stimulation of LCL and MCL.
Panel B shows the box and whisker plots of the same data.
Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1).
A one way ANOVA showed no significant differences (P=0.478 ANOVA)

3.3.5.3. Longer Latency Reflexes

These analyses were extended to the longer latency inhibitions and excitations.

Statistical tests showed that the distribution of excitation latencies was normally distributed (Ryan Joiner, $P>0.1$) and a one way ANOVA showed no significant differences ($P=0.137$). The latencies for inhibitions after LCL stimulation were normally distributed but those after MCL stimulation were not normally distributed (Ryan Joiner, $P<0.01$). Inspection of the data in table 3.3 shows that there is a single outlying value. It is clearly different from the other values and it lies almost four standard deviations from the mean. It is very unlikely to be part of the same distribution and is most likely a chance observation. When the point was omitted, the remaining population was normally distributed (Ryan Joiner, $P>0.1$). Tests were performed on the remaining points.

These data are plotted in figure 3.13

The mean latencies for longer inhibition responses in quadriceps after LCL stimulation was 117 $\pm$ 9 msec and after MCL stimulation it was 110 $\pm$ 12 msec for all values and 108 $\pm$ 8 msec with the outlying point removed. These values were significantly different when tested with an ANOVA ($P=0.01$). A subsequent post hoc t test showed that the means were significantly different ($P=0.005$ unpaired t test).

The mean latencies for longer excitation responses in quadriceps after LCL and MCL stimulation were 103 $\pm$ 6 and 101 $\pm$ 6 msec respectively. Statistical tests confirmed that the data were normally distributed (Ryan Joiner, $P>0.1$).

A one way ANOVA showed no significant differences (P=0.428 ANOVA). These are shown in figure 3.14.

The distributions of the longer latency excitations and inhibitions in triceps after stimulation of MCL and LCL are compared in figures 3.15 and 3.16. Statistical tests confirmed that the distributions of latencies of both inhibitions and excitations after LCL and MCL stimulation were normally distributed (Ryan Joiner, P>0.1).

The mean latencies for longer latency inhibition responses in triceps after LCL and MCL stimulation were 122 $\pm$ 12 and 117 $\pm$ 9 msec respectively. A one way ANOVA showed no significant differences (P=0.608 ANOVA).

The mean latencies for longer latency excitation responses in triceps after LCL and MCL stimulation were 109 $\pm$7 and 111 $\pm$ 3 msec respectively. A one way ANOVA showed no significant differences (P=0.218 ANOVA).

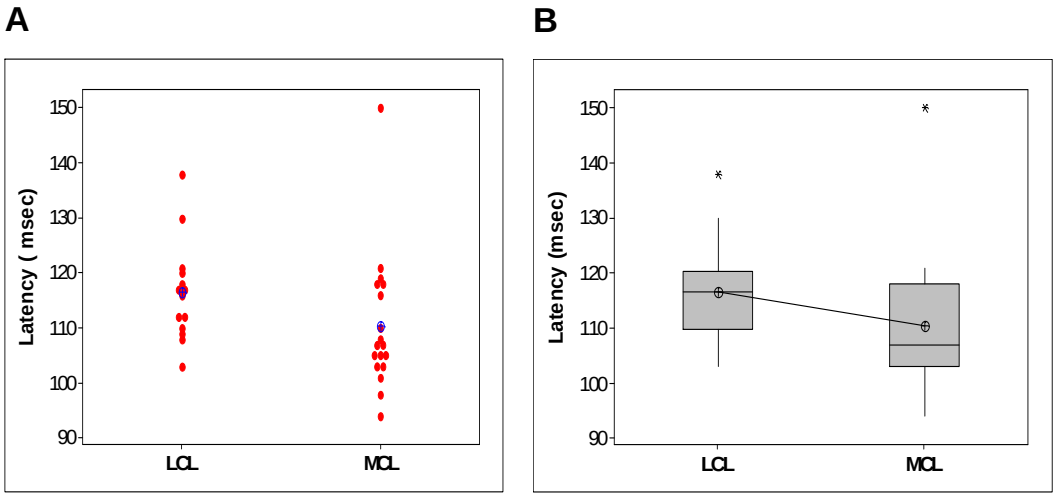
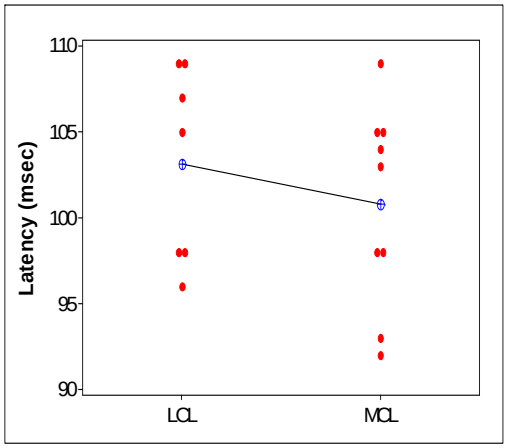


Figure 3.13
Panel A shows the distribution of longer latency reflex inhibitions in quadriceps following stimulation of LCL and MCL.
Panel B shows the box and whisker plots of the same data. The value of 150 msec in the MCL column lies far from the other values. When the data were tested for normality, it was found that the population was not normally distributed (Ryan Joiner, $P<0.01$). When this point was omitted the remaining population was normally distributed ($P>0.1$).

A one way ANOVA showed that the latencies are significantly different ($P=0.01$).

Unpaired t test $P=0.005$

A



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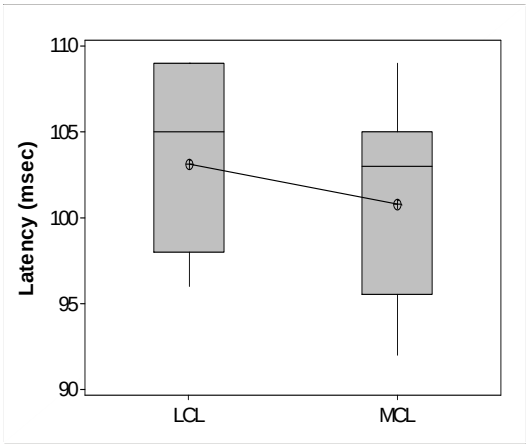


Figure 3.14
Panel A shows the distribution of longer latency reflex excitations in quadriceps following stimulation of LCL and MCL.
Panel B shows the box and whisker plots of the same data.
Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1).
A one way ANOVA showed no significant differences (P=0.428 ANOVA).

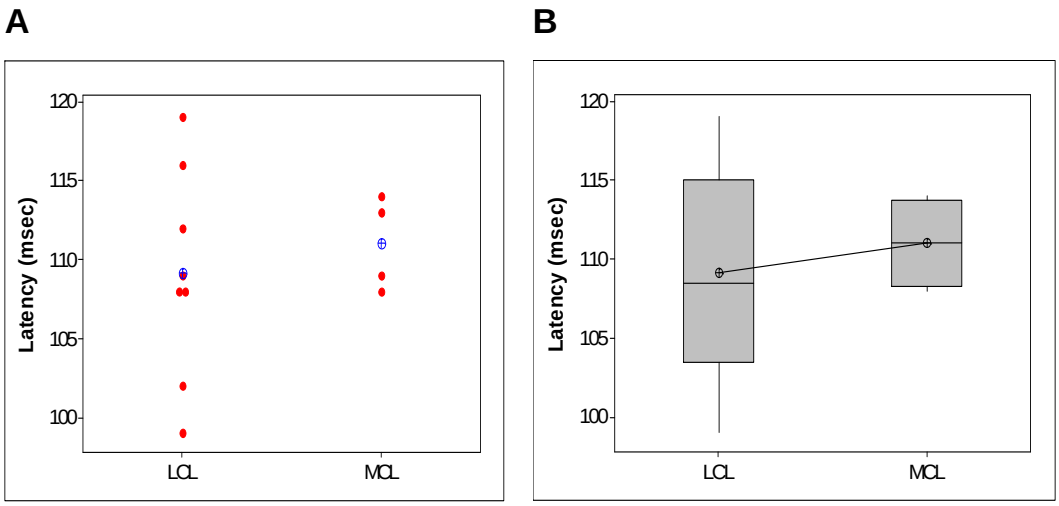


Figure 3.15

Panel A shows the distribution of longer latency reflex excitations in triceps following stimulation of LCL and MCL. Panel B shows the box and whisker plots of the same data. Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1).
A one way ANOVA showed no significant differences (P=0.608 ANOVA).

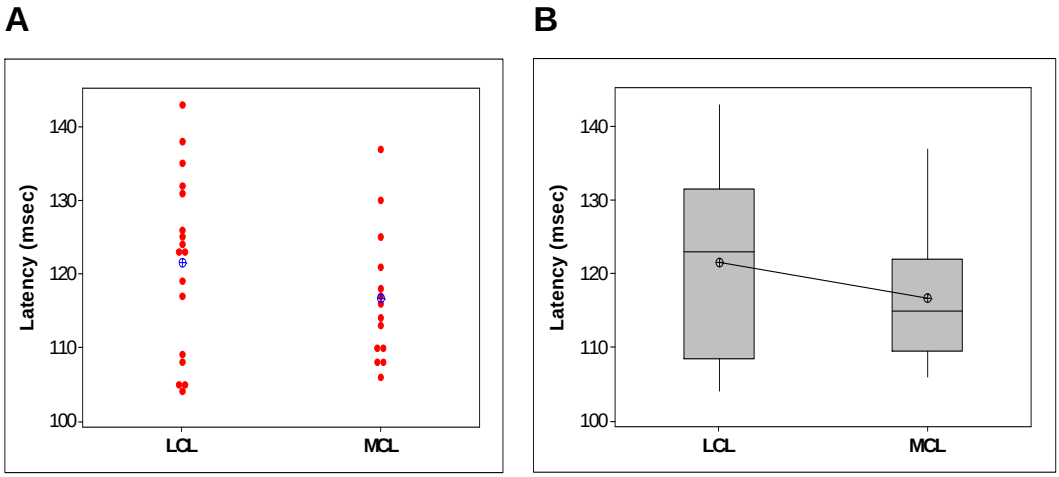


Figure 3.16

Panel A shows the distribution of longer latency reflex inhibitions in triceps following stimulation of LCL and MCL. Panel B shows the box and whisker plots of the same data. Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1).
A one way ANOVA showed no significant differences (P=0.218 ANOVA).

In summary, these results show that the mean latencies of the short latency excitations and inhibitions elicited by stimulation LCL and MCL were not statistically different in quadriceps and triceps.

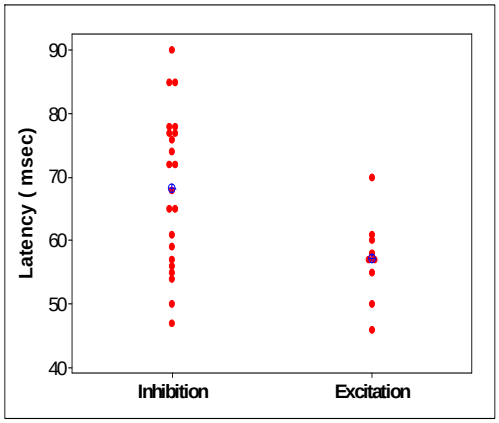
The long latency inhibitions and excitations in triceps are not significantly different after LCL and MCL stimulation. The long latency excitations in quadriceps are not significantly different after LCL and MCL stimulation. The only significant difference is that after MCL stimulation the long latency inhibitions in quadriceps are slower than after LCL stimulation.

3.3.5.4. The Response Latency of Excitations and Inhibitions.

Since no significant differences were found between the mean latencies of short latency responses after LCL and MCL stimulation the data were pooled and then re-plotted to examine the relative latencies of the excitations and inhibitions. The data for reflexes observed in quadriceps are illustrated in figure 3.17 and for triceps in 3.18. Statistical tests confirmed that the data were normally distributed for both excitations and inhibitions (Ryan Joiner, $P>0.1$). A one way ANOVA showed that there is a significant difference between the latencies of excitations and inhibitions in quadriceps ($P = 0.031$). An unpaired t test confirmed a significant difference in means ($P=0.016$). There was a similar difference in the data for triceps. An ANOVA showed a significant difference ($P=0.02$) and an unpaired t test showed a significant difference in means ($P=0.01$).

The mean latency of the excitations was 59 msec for quadriceps and 71 msec for triceps. The equivalent values for the inhibitions were 66 and 79 msec.

A



B

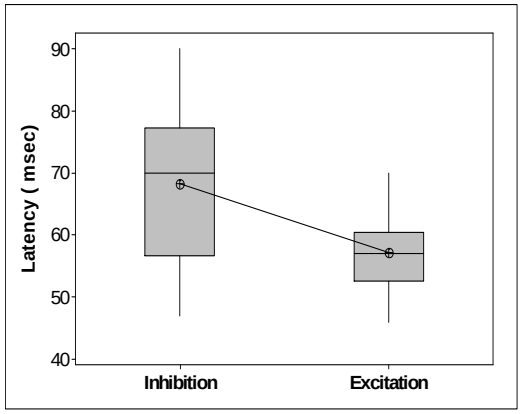


Figure 3.17

This shows a comparison of the distributions of short latency excitations and inhibitions in quadriceps after stimulation of MCL.
Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1).
A one way ANOVA showed significant differences (P = 0.031 ANOVA, P=0.016 unpaired t test).

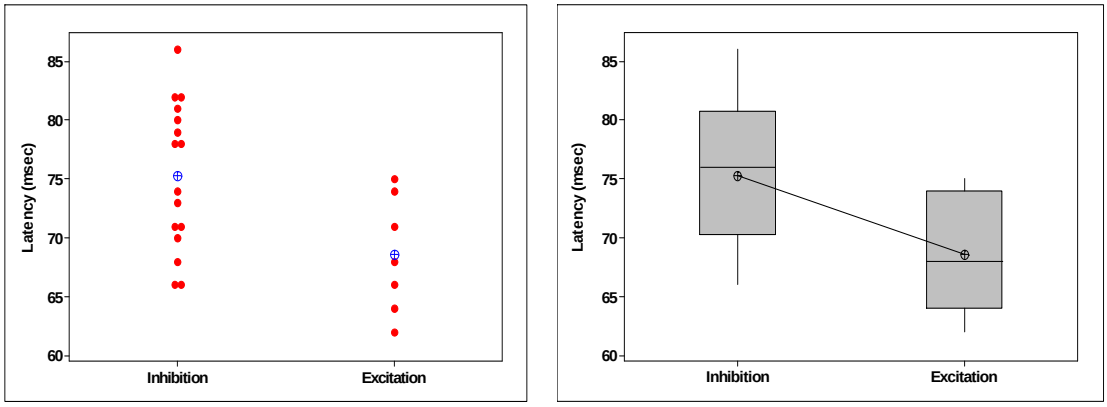


Figure 3.18

This shows a comparison of the distributions of short latency excitations and inhibitions in triceps after stimulation of MCL. Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1).
A one way ANOVA showed significant differences (P= 0.02 ANOVA, P=0.01 unpaired t test).

The longer latency reflexes were analysed in a similar way and the data are plotted in figures 3.19 and 3.20.

Statistical tests had already confirmed that the data for triceps latencies were normal as were the longer latency excitations in quadriceps. The distribution of longer latency inhibitions was corrected by the exclusion of single outlying point.

A one way ANOVA showed that the mean of the longer latency excitations and inhibitions are significantly different in quadriceps but not in triceps. The values were (P=0.038 ANOVA for quadriceps and P=0.244 ANOVA for triceps). A subsequent t test (P=0.019 unpaired t test) showed that the mean latency of excitations was shorter in quadriceps. The mean latency of excitations was also shorter in triceps but this was not statistically significant.

In both quadriceps and triceps the longer latency excitations had shorter latencies than the longer latency inhibitions. The numbers of long latency excitations observed in triceps were very small (n=4) and this may have compromised the test.

In conclusion, it is clear that the mean latency for early inhibitions is longer than the mean latency for early excitations. The difference is 9 msec in quadriceps and 8 msec in triceps. The difference was statistically significant in quadriceps but not in triceps. A similar result is found when the longer latency reflexes are tested. Again the inhibitions occur significantly later than the excitations.

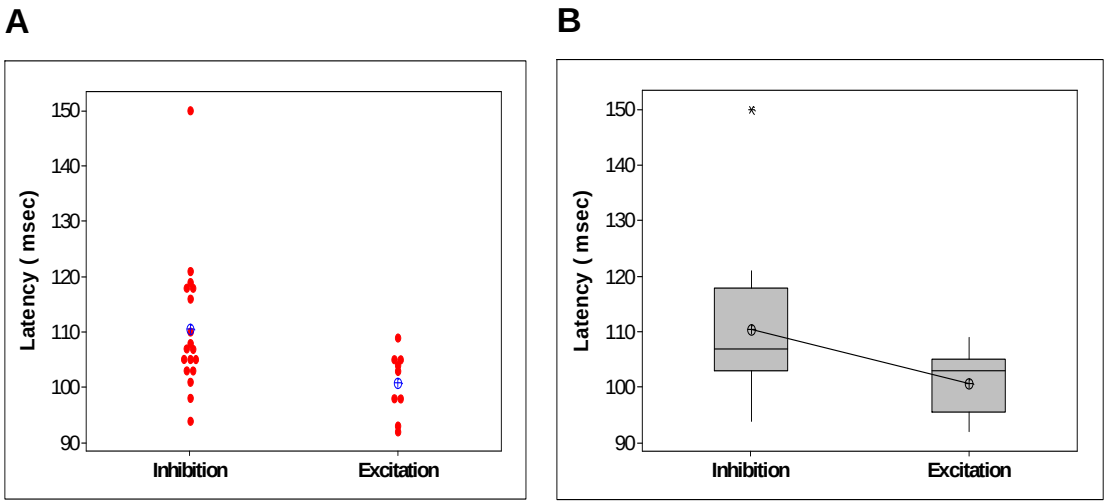


Figure 3.19

This shows a comparison of the distributions of long latency excitations and inhibitions in quadriceps after stimulation of MCL. The value of 150 msec in the inhibition column lies far from the other values. When the data were tested for normality, it was found that the population was not normally distributed (Ryan Joiner, P<0.01). When this point was omitted the remaining population normally distributed (P>0.1). A one way ANOVA showed significant differences (P=0.038 ANOVA, P=0.019 unpaired t test).

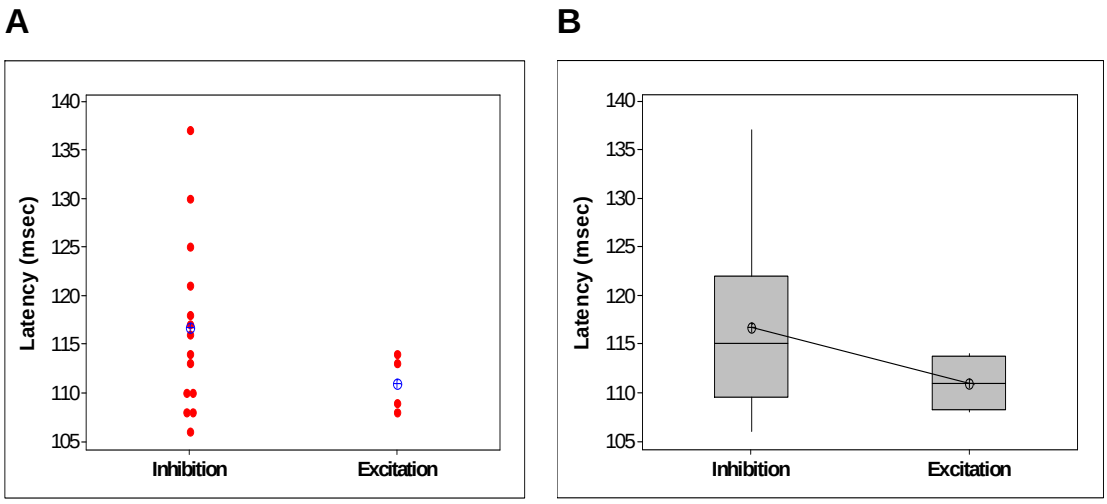


Figure 3.20

This shows a comparison of the distributions of long latency excitations and inhibitions in triceps after stimulation of MCL. Statistical tests confirmed that the data were normally distributed (Ryan Joiner, P>0.1).
A one way ANOVA showed no significant differences (P=0.244 ANOVA).

3.4. Discussion

The main aim of the current experiments was to investigate if reflexes can be elicited by electrical stimulation of the medial and lateral collateral knee ligaments. It is clear from the data shown in figures 3.9 to 3.20 that reflexes can be elicited following electrical stimulation of the MCL and LCL provided that the stimulation intensity was strong enough i.e. ≥20 milliamps. Also the muscles must have developed a sustained contraction. The results in this chapter agree in most respects with those of Kim et al (1995). They also found ligamentous reflexes with the shortest latency components starting between 69 and 144 msec. One significant difference is that Kim et al in 1995 found evidence that the reflexes following medial ligament stimulation were strongest in muscles like Vastus Medialis. The data in this chapter did not support any topographical organisation of the reflexes.

The next aim of these experiments was to investigate if these reflexes can be modulated by posture or muscle activity. The amplitude of the reflexes was not significantly changed. There is no evidence to suggest that the reflexes are changed by movement from a seated to the standing posture.

The final aim of the research was to compare the inhibition and excitation latencies in the quadriceps and triceps muscles. It can be seen from data in tables 3.3 and 3.4 that reflexes were frequently observed in both muscle groups. Inhibitions tend to be seen more frequently than excitations in both groups. Thus stimulation of MCL and LCL seems to elicit reflexes widely in the limb and to affect muscles acting above and below the knee. The pattern of the reflexes from MCL and LCL was similar.

When the distributions of short latency excitations and inhibitions were compared, it was found that there were no significant differences between the reflexes elicited by LCL and MCL stimulation. There is no evidence of topographical organisation of ligamento-muscular reflexes in terms of differences in latencies of response.

The short latency excitatory reflexes were consistently quicker than short latency inhibitory reflexes. This was seen in both quadriceps and triceps after stimulation of MCL and LCL. One possibility is that the excitations and inhibitions are associated with different populations of afferents. The afferent pathway length must be the same for both and so we might speculate that the afferents which mediate excitations have higher conduction velocities. Alternatively, the inhibitions could be the result of a longer of more complex spinal pathway. There is no experimental evidence here to suggest which of these possibilities is more important. There was certainly so sign that two groups of afferents could be separated by stimulation thresholds.

The mean latency for the earliest reflexes was consistently shorter in quadriceps than it was in triceps. Tables 3.3 and 3.4 show the earliest inhibitions are 66 msec for LCL, 68 msec for MCL and for excitations both LCL and MCL are 57 msec in quadriceps. The equivalent values in triceps are 79, 75 and 71 and 69 msec respectively.

The afferent delay will be identical for these reflexes and the later responses in triceps can be largely explained by the longer efferent distance. There is little evidence to support the suggestion of a more complex organisation of postural reflexes of the sort investigated by Nashner in 1977. In his experiments rapid joint rotations elicited the shortest latency response in

the most distal muscles tested. He interpreted these data to show delays in the central pathways that maintained posture by stabilising the ankle first, then the knee then the trunk. These muscle stretch reflex systems seem distinct from ligament reflexes.

Chapter 4

Control Experiments to Investigate the Possible Contribution of Cutaneous Afferents

4.1. Introduction

The data shown in the previous chapter illustrate that it is possible to elicit reflexes following electrical stimulation of the collateral knee ligaments. These reflexes are associated with relatively intense stimulation, typically above 20 milliamps. These currents are well above the perceptual thresholds for cutaneous sensation and it is sensible to ask: do the reflexes come from ligament mechanoreceptors or possibly from cutaneous receptors?

There is an extensive literature describing cutaneo-muscular reflexes in normal individuals and in pathological cases. To take a few examples: Jenner and Stephens (1982) elicited cutaneo-muscular reflexes in thirty-six healthy volunteers. Reflexes were recorded in the averaged EMG from the First Dorsal Interosseous and Extensor Digitorum Brevis muscles following electrical stimulation of the digital nerves of the index finger and second toe respectively. The reflex responses were triphasic: an initial short latency excitation (E1) was followed by an inhibition (I1) and finally a long latency excitation (E2). The reflex latencies were 34 msec for E1, 40-45 msec for I1 and 65 msec for E2. A similar triphasic pattern was seen in Extensor Digitorum Brevis. The latencies were longer, 51 msec for E1, 60 msec for I1 and 81 msec for E2, probably as a result of the longer conduction distances.

Becker, Hayashi, Lee and White (1987) attempted to elicit cutaneo-muscular reflexes in Flexor Carpi Radialis and Extensor Carpi Radialis by stimulation of digital nerves. They could elicit reflexes with a pattern similar to those described earlier when they stimulated at 2-3 times the perceptual threshold. The reflex latencies were 38.5 $\pm$ 5.3 msec for early inhibition and 54.2 $\pm$ 3.3 msec for late inhibition. The early excitation latency was 30 msec and the longer excitation latency was 60 msec.

The next investigation of cutaneo-muscular reflexes examined the effects in the lower limb. Gibbs et al (1995) succeeded in eliciting reflexes in Extensor Digitorum Brevis, Tibialis Anterior, Soleus, Quadriceps Femoris and Erector Spinae in ten healthy subjects. The responses were recorded following electrical stimulation of the digital nerves of the second toe. Again polyphasic reflexes were found with an initial excitation, followed by inhibition and followed by a second excitation. The reflex latencies ranged between 43-78 msec for early excitation, 48-81 msec for inhibition and 60-103 msec for late excitation.

Bagheri and Baxendale (1995) found similar results in lower limb muscles of 62 healthy volunteers. They elicited cutaneo-muscular reflexes in Tibialis Anterior, Gastrocnemius, Quadriceps, Hamstring and Abductor Hallucis muscles following electrical stimulation at three times perceptual threshold of the skin of: the hallux, the heel, the lateral border of the foot, the plantar surface of the foot and the shank. The pattern of the reflexes in Bagheri's work is similar to those in the earlier papers.

The earliest studies of cutaneomuscular reflexes during walking were conducted in cats (Duysens and Stein (1978),

Grillner and Rossignol (1978), Forssberg (1979), Duysens and Loeb (1980), Abraham, Marks and Loeb (1985)). Subsequently, very similar procedures were used in human volunteers. Duysens, Tax, Murrer and Dietz (1996) conducted a similar investigation of cutaneo-muscular reflexes in Semitendinosus, Biceps Femoris, Rectus Femoris and Tibialis Anterior. They elicited reflexes after low-intensity stimulation from 10 volunteers during walking on treadmill. The stimulation electrodes were positioned on the left leg on the mid point of muscle between the external malleolus and the Achilles tendon. The reflex latencies they observed were between 70 to 80 msec. Crenna and Frigo (1987) and Belanger and Patla (1987) stimulated cutaneous afferents of the human foot and they elicited reflexes with medium latency within the step cycle during walking.

In summary, cutaneo-muscular reflexes can be elicited with relatively low intensity stimulation of some areas of skin or digital nerves. The immediate aim of the experiments reported here is to compare the reflexes elicited by stimulation over the LCL before and after cutaneous anaesthesia.

4.2. Materials and Methods

4.2.1. Subjects

The FBLS Ethics Committee for Non-Clinical Research Involving Human Subjects approved the experimental protocols. All subjects gave informed consent and they were free to withdraw from the test at any stage.

The five volunteers who participated in these experiments had already participated in those experiments described in

chapter 3. Thus the characteristics of their reflexes were already known. The subjects were invited to give separate consent for this second experiment.

4.2.2. Experiment Procedure

The experimental protocol described in chapter 3 was repeated to establish the presence of a reflex in Vastus Lateralis, Rectus Femoris and Lateral Gastrocnemius.

The 5% anaesthetic cream containing a mixture of lidocaine and prilocaine at 25milligrams/gram (Emla, AstraZeneca UK Limited) was applied to the area. After 20 to 30 minutes, the cutaneous sensation was tested by pricking the area with a sharp pin or light brushing. If the sensation persisted, the Emla cream was reapplied and then the sensation was re-tested after a further 5 or 10 minutes. This process was repeated until the cutaneous sensation was abolished.

When the cutaneous anaesthesia had been achieved as assessed by repeating the pin prick and light touch tests, the stimulating electrodes were re-applied. The minimum current needed to elicit barely perceptible sensations was not reassessed. The stimulation over the ligament was repeated at the intensity which had formerly elicited reflexes was repeated and where possible higher intensities were also used.

4.3. Results

The minimum current needed to elicit a sensation was measured several times in each volunteer. This perceptual threshold current ranged between 0.85 and 1.2 milliamps in

the five volunteers. It was consistent in any one volunteer. The results for all 5 volunteers are summarised in table 4.1.

Stimulation at perceptual threshold did not elicit any reflexes in the volunteers. An example is shown in figure 4.1. The left panel shows the averaged electromyogram following stimulation at the perceptual threshold. No change is seen. The right panel shows the same volunteer when the stimulation current has been increased to 25 milliamps. A clear reflex can now be observed. The same pattern occurred in all five volunteers (see table 4.2).

The volunteers did not report any sensation when the perceptual stimulation was repeated after the application of the Emla cream. The maximum tolerated current increased by up to three times after application of Emla. These observations support the hypothesis that the perceptual threshold is determined by low threshold cutaneous afferent which can be silenced by topical anaesthesia. It also suggests that the tolerance for current is limited by cutaneous nociceptors.

The data in table 4.1 also shows that the minimum current needed to produce ligamento-muscular reflexes is at least 18 times greater than that needed to evoke a perception of stimulation.

Subjects	Perceptual Threshold (milliamps)	Minimum Stimulation Current needed to elicit a reflex (milliamps)
1	0.85	20
2	1.1	20
3	1.2	25
4	0.95	20
5	0.90	25

Table 4.1
The table shows the minimum stimulation current needed to elicit a barely perceptible sensation (perceptual threshold) in different subjects. None of the volunteers showed any reflexes when stimulated with perceptual threshold currents. Reflexes could be elicited by increasing the intensity of stimulation. The minimum current needed to produce ligamento-muscular reflexes was at least 18 times greater than that needed to evoke a perception of stimulation.

It should be noted that however, the minimum stimulation current needed to elicit a reflex was 20 milliamps. As shown in table 3.2, of the 5 subjects who limited the stimulation current to 20 milliamps, 3 showed reflexes and two did not. (See table 3.2 subjects 1 and 5).

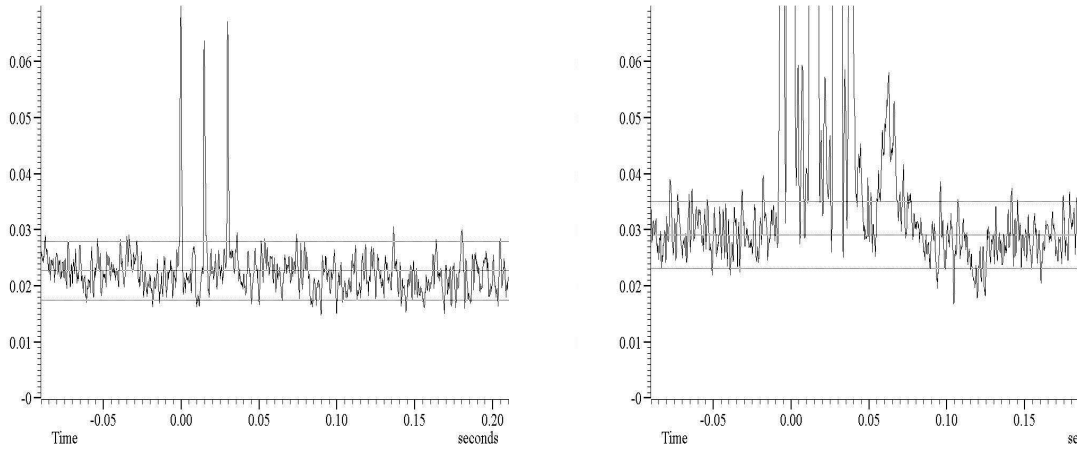


Figure 4.1
The left trace shows the rectified averaged EMG in RF after stimulation at perceptual threshold, in this case 0.90 milliamps. The vertical axis shows the EMG in volts. The horizontal axis is time in seconds.
No signs of any reflex can be seen.
The right trace shows data from the same volunteer after the intensity of stimulation had been increased to 25 milliamps. Significant excitation and inhibition reflexes are now clear.

Subjects	Maximum Current before Emla (milliamps)	Maximum Current after Emla (milliamps)
a	20	30
b	20	35
c	25	30
d	25	40
e	35	45

Table 4.2
The table shows maximum current tolerated before and after topical anaesthesia. The volunteers had different tolerances for maximum current. The current was always greater after anaesthesia.

The experimental protocol repeated the stimulation intensity needed to elicit a reflex after the application of Emla. Specimen recordings are shown in figures 4.2 and 4.3. The upper panel of figure 4.2 shows a short latency excitatory reflex in Lateral Gastrocnemius elicited at 58 msec after stimulation. The lower panel shows recordings from the same volunteer with the same stimulus protocol repeated after topical anaesthesia of the skin under the stimulating electrodes. The reflexes have a very similar form.

The data in figure 4.3 show that the increased stimulation current, which can be tolerated after topical anaesthesia, has increased the amplitude of the observed reflex. In the upper panel before the application of Emla, a reflex cannot be seen. The signal does cross the upper confidence interval but its time outside the confidence interval is too short to characterise the event as a reflex. After topical anaesthesia the current rose to 30 milliamps and the more intense stimulation elicited a clear reflex is seen to start after a latency of 77msec. The signal is above the confidence interval for 8 msec.

In some experiments, after ligamento-muscular reflexes had been elicited by stimulation over the LCL, the stimulating electrodes were removed and placed elsewhere on the leg. They were positioned over the patella and the tibia at sites where no muscle contraction could be elicited when the stimulus was repeated. When the stimulation protocol was repeated at these sites, no reflexes were elicited.

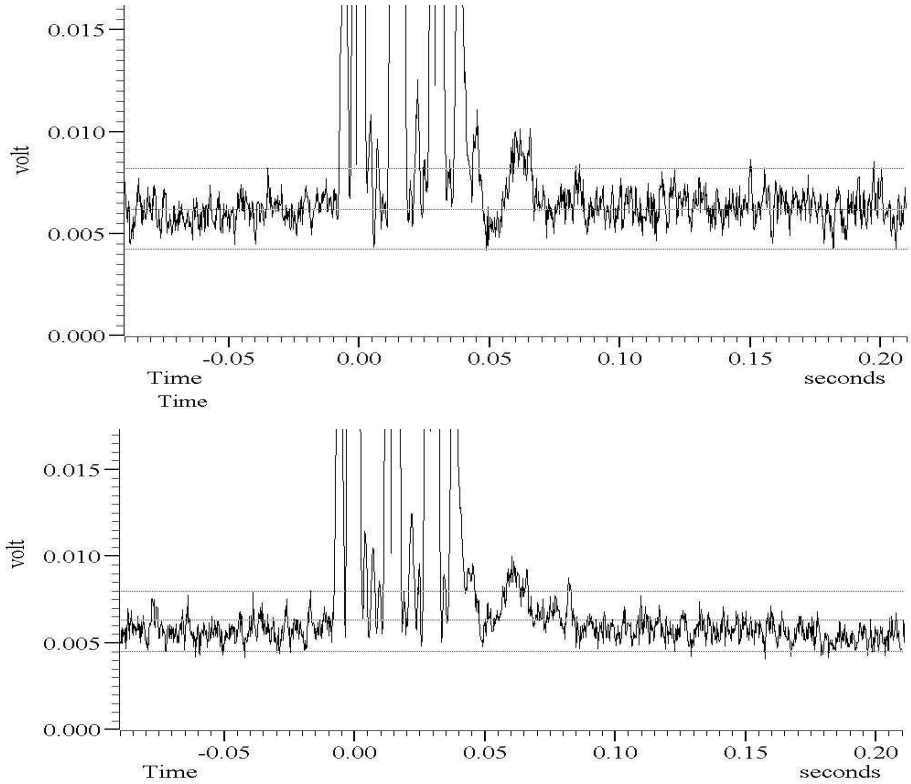


Figure 4.2
The upper trace shows a short latency reflex excitation in LG with a latency of 58 msec following stimulation of LCL. The stimulation current was 25 milliamps.
The lower trace shows result of the experiment repeated after topical anaesthesia of the skin at the stimulation site. The reflexes are essentially unchanged.

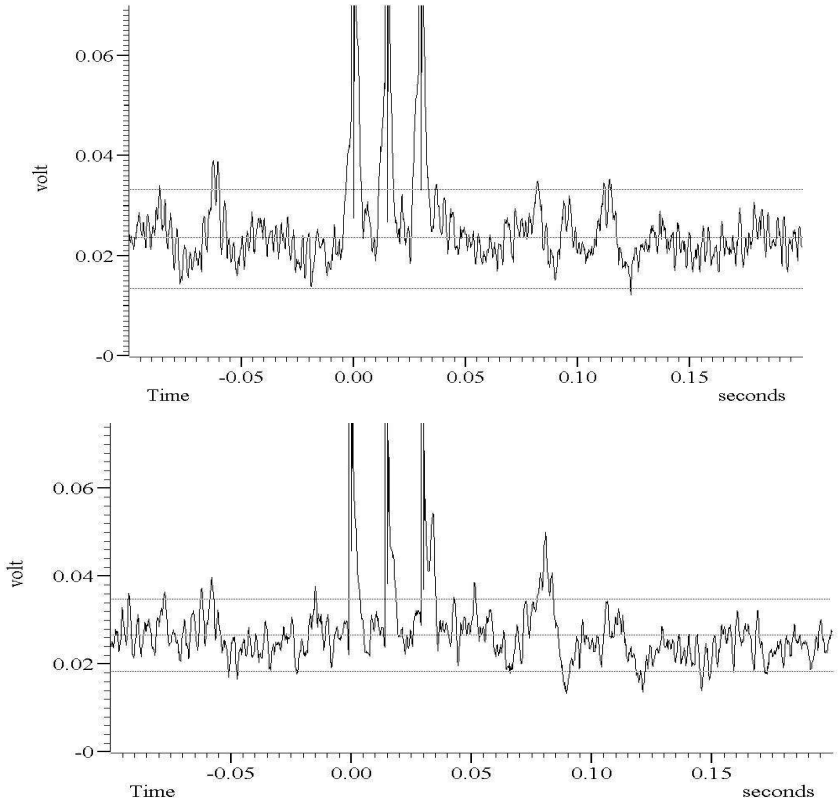


Figure 4.3
These traces show rectified averaged EMG in the RF following stimulation of LCL. Both recordings were made in a volunteer before and after the cutaneous anaesthesia of the skin under the stimulation electrodes.
The upper trace, before topical anaesthesia, shows no signs of a significant reflex when stimulated at 20 milliamps. The lower trace, after topical anaesthesia, shows the stimulation repeated at a higher intensity (30 milliamps). The result was an excitation reflex with a latency of 77 msec with duration of 8msec.

4.4. Discussion

The immediate aim of these experiments was to compare reflexes elicited by stimulation over the LCL before and after cutaneous anaesthesia. It is clear that reflexes can be elicited by stimulation of the ligaments after cutaneous anaesthesia. This can be seen in figures 4.2 and 4.3. A second interesting observation is that the volunteer's tolerance of high intensity stimulation is increased by the topical anaesthesia immediately underneath the site of stimulation. This can be seen in the data in table 4.2.

These observations address the question of the nature of the afferents which give rise to the reflexes observed in chapters 3 and 4. The stimulation in all these experiments clearly excites cutaneous afferents under the electrodes. However, stimulation at perceptual threshold never elicited any reflexes. An example of this is shown in figure 4.1. This observation was originally made by Bagheri and Baxendale (1995). Cutaneo-muscular reflexes could be elicited in their experiments when the stimulation was increased to three times perceptual threshold. However, in those experiments reflexes from the skin covering the lower part of the limb below the knee and the foot were examined. Gibbs et al (1995) could also elicit cutaneo-muscular reflexes by stimulation of the digital nerve of the second toe at 2 times the perceptual threshold. Becker et al (1987) stimulated the second, third and fourth digits while maintaining a steady contraction of the wrist flexors. They could elicit short latency inhibition reflexes at 2-3 times the perceptual threshold.

Willer, Boureau and Albe-Fessard (1978) performed a detailed electrophysiological study of the role of cutaneous

afferent groups in the development of nociceptive reflexes in humans. Their experiment allowed good control of the stimulation of the sural nerve and they were able to record the sensory evoked potentials and reflex excitations in Biceps Femoris. They described this as an RIII reflex. It is clear in their experiment, and likely in these experiments, that cutaneous A-delta fibres were stimulated. Their stimulus conditions fell in the range of 4-6 shocks of 0.1–0.5 msec duration at 100 Hz and intensities up to 40 milliamps and so their conditions were very close to those used in the experiments described in this thesis. However, there are also very significant differences. Firstly, the results shown in this chapter suggest that cutaneous afferents play little part in the observed reflexes. Secondly, the RIII reflexes are associated with nociception and are particularly clear when 4 or more pulses are delivered in the train. The latency of the RIII reflexes is over 100 msec in Biceps. The shorter latency reflexes described in this thesis have latencies of less than 100 msec in latency; the shortest latency observed was 46 msec. In addition, the reflexes reported here are often excitations in extensors, see figures 4.2 and 4.3, rather than excitations in flexors. Lastly, the short latency reflexes are only observed when stimulation is applied to skin over the LCL or MCL and are not observed when identical stimulation is applied to other adjacent areas of skin. It seems unlikely that the observed short latency reflexes are RIII reflexes.

In summary, stimulation of the lowest threshold cutaneous receptors does not elicit reflexes. Stimulation of higher threshold cutaneous receptors can elicit reflexes as described by Willer et al (1978). However, in this study topical cutaneous anaesthesia does not alter the reflexes elicited by intense stimulation over the lateral collateral ligaments. The stimulation used in these experiments is 20

to 25 times greater than perceptual threshold. Stimulation at lower intensities, say 10 times perceptual threshold, did not elicit ligamentous reflexes. Thus, currents far greater than that used by previous studies to investigate cutaneous reflexes failed to initiate reflexes and so it can be concluded that there is no significant cutaneo-muscular reflex contribution to the observed reflexes.

After applying the topical anaesthetic cream the volunteers reported decreased touch and pinprick sensations and increased tolerance of stimulus intensity. This is illustrated in table 4.2. It appears that the unpleasant sensations associated with intense stimulation have a cutaneous component but these sensations are distinct and do not contribute to the observed reflexes.

Because the site of electrical stimulation was over the collateral knee ligaments and not near any muscle it was improbable that muscle spindle sensory afferents played any role in the responses recorded. Toft, Sinkjaer, Anderassen and Larsen (1991) showed that muscle afferent responses are much shorter latency than the reflexes observed in this study. They found typical latencies of 40 msec for responses after muscle afferent stimulation whereas those in chapter 3 are in the range of 46 to 150 msec as shown in table 3.3.

In addition, no muscle twitches or H reflexes were ever observed in the averaged electromyogram during experiments. If the stimulus current had spread to excite muscle afferents or low threshold motor axons, there would have been short latency synchronised activity in the electromyogram. These M response or H reflexes are typically much larger than the observed reflexes.

There is one final other possible afferent contribution to the observed reflexes; there could be activation of capsular joint receptors. Their reflex actions were discussed in chapter 1 section 1.6. Indeed, during experiments in experimental animals it is often difficult to be certain of the exact location of receptors in ligaments, tendons or the capsule even when the structure are exposed and can be activated by direct pressure or stretch (Baxendale, personal communication). It will be even harder to be sure that activation is confined to the ligament receptors when electrical stimulation is used because of uncertainties about the precise current path. It should be noted that reflexes were not observed when the stimulating electrodes were moved from the skin over the ligaments to adjacent areas even though they would still have been over the capsule.

The final conclusion which can be drawn from the experiments in this chapter is that the reflexes identified as ligamento-muscular in chapters 3 and 4 did not come from the skin or muscle afferents, but that they can be provisionally attributed to afferents in the ligaments with a possible contribution from capsular joint afferents. For clarity and convenience they will be described as ‘ligamento-muscular’ reflexes for the remainder of this thesis.

Chapter 5

Reflexes Elicited after Lateral Knee Ligament Stimulation during Walking

5.1. Introduction and Literature Review

Electrophysiological recording techniques capable of investigating responses during locomotion were developed and demonstrated first in cats by Engberg and Lundberg (1969). Their techniques can be slightly modified and used to analyse reflex contributions to human locomotion.

There are clearly some common features shared between the underlying neuronal mechanisms which control quadripedal locomotion in cats and those active during bipedal gait in humans. However, there are distinct differences between locomotion in cats and humans (Forssberg, Grillner and Rossignol (1975), Forssberg and Nashner (1982), Armstrong (1988)).

5.2. Human Gait Cycle

Repetitive features occurring during each gait cycle can be used to characterise human gait. A gait cycle is defined as the time interval between two successive occurrences of one of the repetitive events during walking. The instant of heel strike is often used to identify the initial event of gait cycle. The cycle is divided into stance and swing phases. The former (stance) is the period when the foot is in contact with the ground and it accounts for about 60% of the cycle. The latter phase (swing) is the period that the foot is moving forward in the air and it accounts for about 40% (Whittle 1996). However, the relative durations vary with the speed of walking and the

swing phase becomes proportionately longer as the speed of walking increases (Murray 1967).

The stance phase can be divided into 4 sub-phases (Rose and Gamble, 1994):

1. Loading response: starts from heel strike until the time when the other foot is lifted from the floor (toe-off of the opposite foot).

2. Midstance: starts from the toe off of the opposite foot until the body is positioned directly over the stance foot.

3. Terminal stance: starts from the end of midstance until the heel strike of the opposite foot.

4. Pre-swing: starts from the heel strike of the opposite foot until the foot is lifted from the floor.

Different parts of stance phase are shown in figure 5.1

5.3. Reflex Modulation during the Gait Cycle

The Hoffmann reflex (H-reflex) is perhaps the most extensively studied reflex in the literature on human and mammalian neurophysiology (Misiaszek, 2003). The H-reflex can be elicited in different muscles (Day, Marsden, Obeso and Rothwell (1984), Dietz, Faist and Pierrot-Deseilligny (1990)). In humans, the Soleus H-reflex amplitude is deeply modulated during locomotion (Capaday and Stein 1986). These authors also confirmed that the amplitude of the H-reflex in Soleus during walking is lower than standing at the same level of muscle activity. Similar observations have been made on the H-reflex in Tibialis Anterior (Schneider, Lavoie and Capaday 2000).

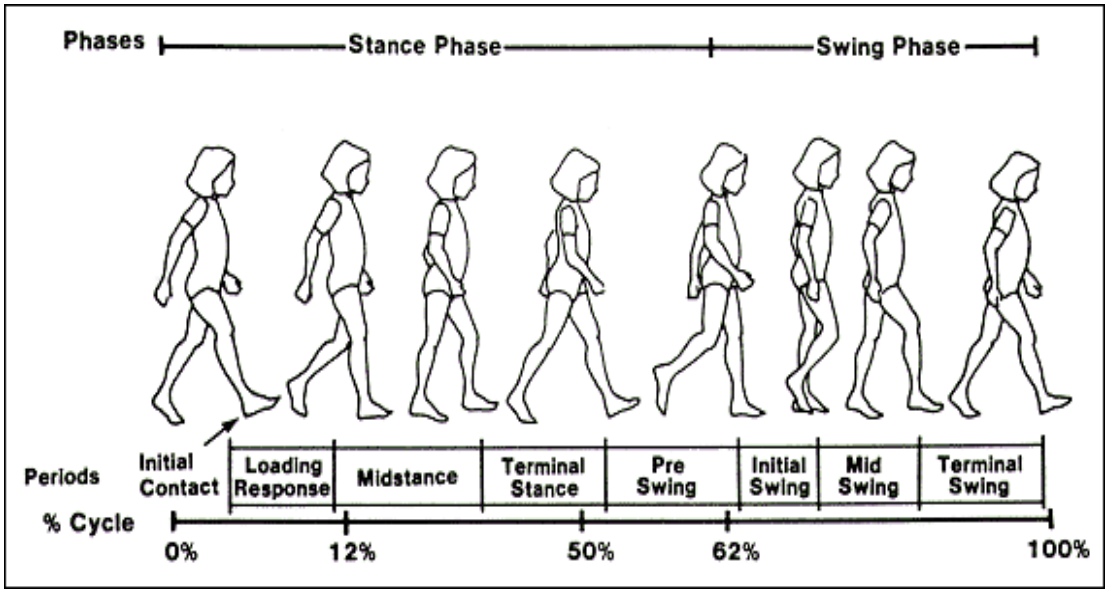


Figure 5.1
The figure shows stance and swing phase of the gait cycle. The stance phase is the period when the foot is in contact with the ground covering about 60% of the gait cycle and the latter phase is the period that the foot is moving forward in the air covering about 40%.
Figure is from Sutherland, Olshen, Biden and Wyatt (1988) The Development of Mature Walking, Cambridge University Press.

5.3.1.Ib Reflexes during Locomotion in Humans

The Golgi tendon organ afferents from extensor muscles are an important source of the positive force feedback signals during the stance phase of gait cycle (Pratt 1995). Many studies have demonstrated the role of Ib afferents during rest and locomotion in cats and humans (Nichols and Houk (1976), Conway et al (1987), Yang et al (1991), Pearson and Collins (1993), Sinkjaer et al (1999)). It has been argued that the Ib afferents affect both static and locomotion positions. In several subsequent studies, it became clear that Ib afferent activity has a positive feedback effect during the locomotion situation (Conway et al (1987), Pratt (1995), Prochazka et al (1997b), Pearson et al (1998), McCrea (1998), Stephens and Yang (1999)).

Muscle afferent reflexes can be modulated during gait and it remains an open question if the ligamentous reflexes can be modulated in a similar way. One might speculate that since ligaments play an important role in force transmission through the limb and that the ligament innervation may be important in protecting joints for force overload, the ligamentous reflexes may be more prominent during gait. The aim of the experiments performed in this the section was to investigate the effects of ligament stimulation during normal walking on a treadmill.

5.4. Materials and Methods

5.4.1. Subjects

Thirteen healthy subjects (4 women, 9 men), without any history of injury, participated in this experiment. The age range of the volunteers was from 21 to 45 years. The FBLS Research Ethics Committee approved the experiment. All subjects had given informed consent and they were free to withdraw from the test at any stage.

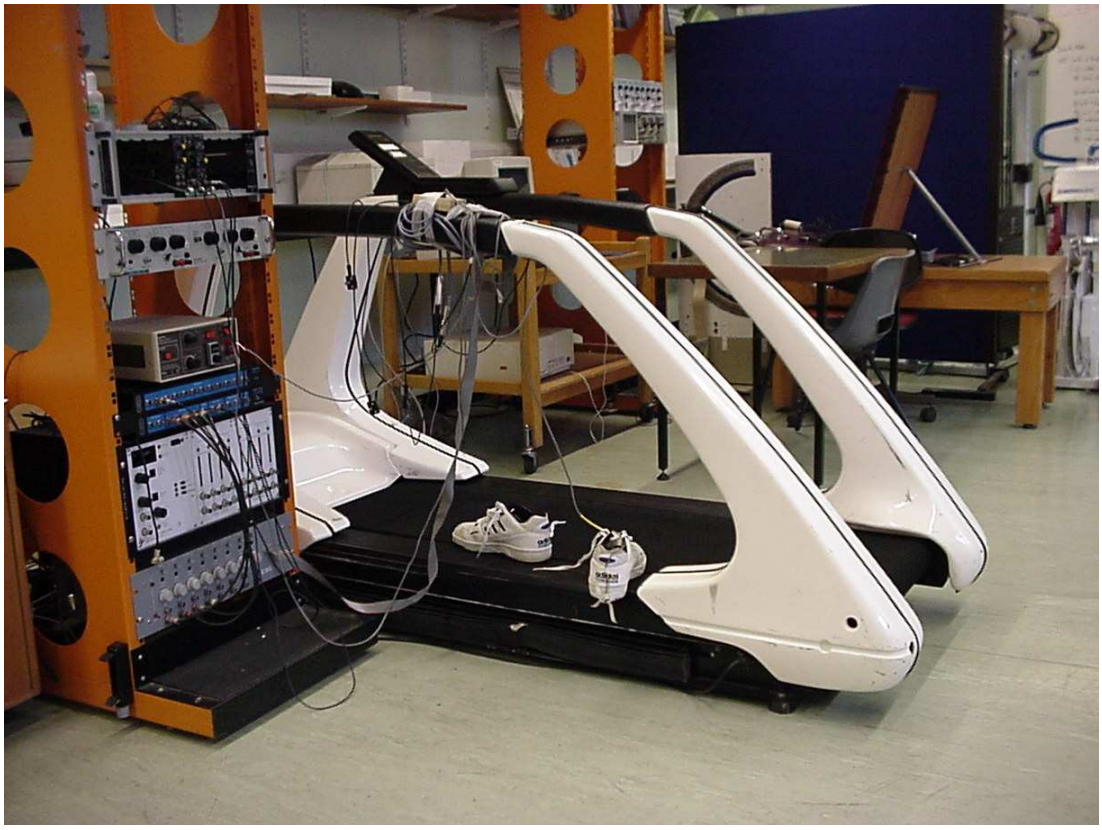
5.4.2. Experimental Procedure

The site of the electrodes was shaved then cleaned by alcohol wipe. Stimulation electrodes were placed over the lateral collateral knee ligament. EMG recording electrodes were positioned over the Vastus Lateralis and lateral head of the Gastrocnemius muscles of the left leg of the subjects. The volunteers walked on the treadmill for periods of one minute at 4 kmph. Figure 5.2 shows the equipment. Before each experimental run, during relaxation, the perceptual threshold was determined for each subject. The experiment started with the lowest current and on subsequent runs the current was increased. An electrode gel was used under the electrodes to decrease the contact resistance.

Figure 5.3 shows the volunteer during the experiment.

5.4.3. Electrical Stimulation

The electrical stimulation equipment was the same for all of the experiments. The electrical stimulation procedure has been explained on chapter 2.



Figurer 5.2

The figure shows the equipment used in the experiment.

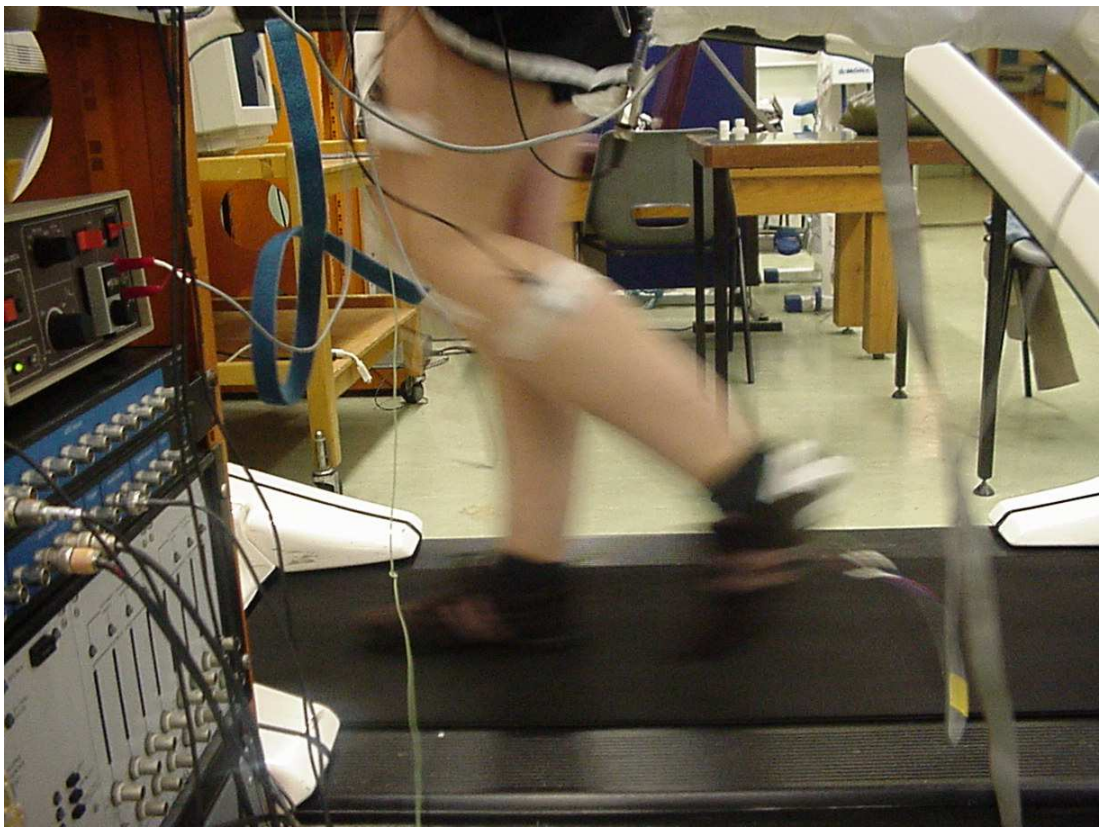


Figure 5.3
The figure shows a volunteer during walking on treadmill. The apparatus is described in chapter 2 section 2.3.1 and figure 5.3.

5.4.4. Triggering the Stimulation on Heel strike

A small pressure switch (1cm×2cm) was located on the heel of the sole of the volunteer's left shoe. The switch was secured in place by strong adhesive tape. The position of the heel switch was selected to close at heel strike when the foot makes contact with the floor. This identified the start of the stance phase of the gait cycle.

Closure of the switch provided a trigger for the stimulation circuit. The output of the DS7A could be delayed by fixed periods between 0 and 400 msec after heel strike by a Neurolog NL-403 delay-width module. The switch closure also provided a synchronisation signal for the 1401. The time of heel strike and the time of stimulation was stored as an event channel and recorded in the computer along with the EMG recordings. These were subsequently used to trigger peri-stimulus averages of the EMG recording.

5.4.5. Electromyography Recordings

Surface EMGs were recorded from Vastus Lateralis and Lateral Gastrocnemus muscles. The process of the EMG recording was the same in the three experiments and has been explained in chapter 2 section 2.3.1. The EMG was recorded by the Spike 2 system. The EMG was rectified and averaged. The data were exported as text files from Spike 2 and transferred to Excel spreadsheets for further analysis.

Figure 5.4. shows a sample of raw EMG from Vastus Lateralis and Lateral Gastrocnemius muscles recorded during walking.

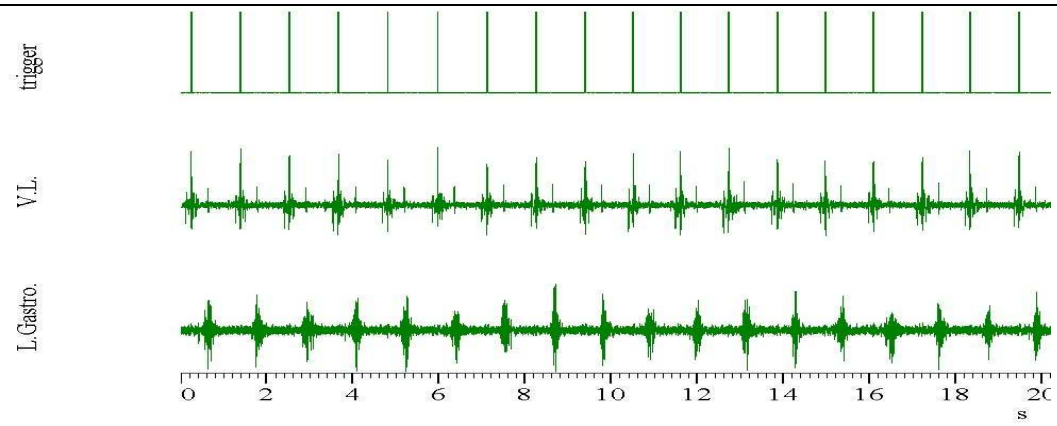


Figure 5.4
A sample of raw data of EMG activity from a volunteer recorded during walking.
The upper trace shows the trigger signals derived from the heel switch at heel strike. The middle and the lower traces are EMG recordings from VL and LG muscles, which were recorded simultaneously.

5.5. Results

Thirteen volunteers participated in these experiments. Ten of the volunteers had already participated in the experiments detailed in chapter 3 and so the characteristics of their 'ligamento-muscular' reflexes were well known. The three novice volunteers all had clear 'ligamento-muscular' reflexes during walking.

In total, 'ligamento-muscular' reflexes were observed in eleven of the thirteen volunteers. All of these volunteers were able to tolerate stimulus currents of about 20 milliamps and so the failure to record reflexes in two volunteers cannot be attributed to inadequate stimulation. The major difficulty in these cases lay in the variability of the gait cycles even though the volunteers walked at the same average speed. The averaged EMG records did not allow unambiguous identification of any peri-stimulus changes. The reflex could be elicited at the very end of the swing phase and reached its peak shortly after heel strike. The period from midstance to terminal swing phase was absolutely areflexic.

Eleven out of thirteen of the volunteers had a reflex in one muscle or in both Vastus Lateralis and Lateral Gastrocnemius.

5.5.1. Ligamento-muscular Reflexes in Vastus Lateralis.

Figure 5.5 shows averaged EMG recordings from Vastus Lateralis in one of the volunteers during walking. The right panel (B) shows the EMG during walking without stimulation. The EMG profile is exactly as expected from classical gait studies. The EMG begins to rise before heel strike, shown as 0 msec. It reaches a peak at 150 msec before heel strike and then falls silent at 250 msec after heel strike. There is a

second wave of EMG about 700 msec after heel strike. The other panel in this figure (A) shows the similar recordings made during walking at the same speed but with the ligament stimulation applied at zero msec after heel strike. The EMG waves start and stop at very similar points on the gait cycle. However, it is obvious that the large stimulation artefacts occur during the main period of EMG activity. It is not clear by simple visual inspection of these recordings if any reflex is elicited. In the examples shown earlier in chapters 3 and 4, it is easy to identify reflexes because confidence intervals can be calculated from the pre-stimulus period and then extrapolated. Reflexes are identified when they cross these confidence intervals. It is clear that EMG changes during gait and so it is not sensible to calculate a mean EMG and confidence intervals. No clear examples of reflexes were seen by simple visual inspection. The natural variability in the step-by-step EMG probably obscured any reflexes.

A new approach was adopted to search for reflexes. This is illustrated in figure 5.6. The figure shows subtraction result from Vastus Lateralis. Panels A and B show the averaged EMG with and without ligament stimulation. Again, there are no obvious reflexes in the averaged EMG. Panel C shows the difference in these two signals. The two recordings were synchronised by the heel strikes and the averaged EMG signals were subtracted. When the two signals are similar the difference signal is close to zero. This is obvious from about 300 msec to just about heel strike. The signal clearly identifies the artefact as a very large deviation. The difference signal returns to near zero by about 75 msec and this is followed by a second deviation at about 85 msec before the signal returns to close to zero where it stays until the end of the recording. Thus the two EMG signals differ at the time of the stimulus artefact and about 66 msec later the second deviation occurs

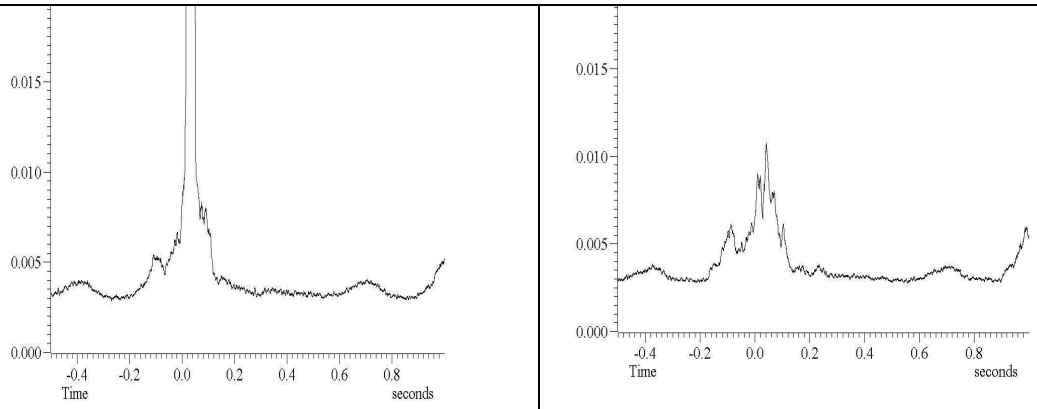


Figure 5.5
The figure shows rectified averaged EMG recordings from VL in one of the volunteers during walking. The vertical axis shows the EMG in volts. The horizontal axis is time.
The averaged EMG data on the left shows walking with stimulation applied at heel strike indicated as 0 msec. The large stimulation artefact is clear. The data on the right shows the averaged EMG of the same volunteer walking at the same speed without stimulation.

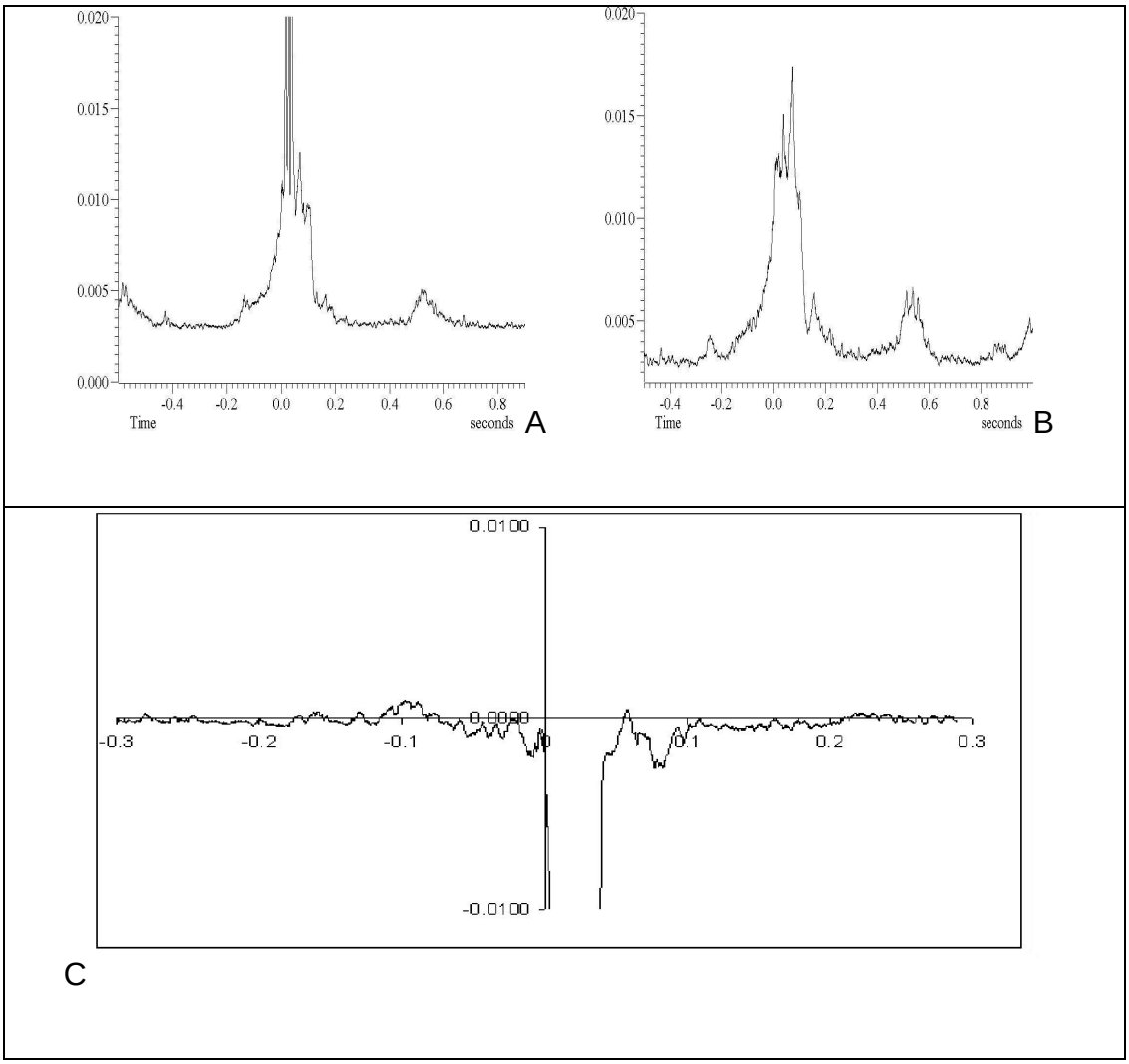


Figure 5.6
The figure shows result of EMG subtraction for VL. The upper traces (panel A and B) show the rectified averaged EMG with (A) and without (B) stimulation of LCL. The vertical axis is the EMG in volts. In panel B, EMG begins to rise before heel strike, shown as 0 msec. It reaches a peak at 80 msec after heel strike and then falls silent by 200 msec after heel strike. There is a second wave of EMG about 500 msec after strike. The panel A shows the similar recordings made during walking at the same speed but with the ligament stimulation applied at 0 msec after heel strike. The EMG waves start and stop at very similar points on the gait cycle. Panel C shows the difference in these two signals. The vertical axis shows the EMG in volts. The horizontal axis is time The difference signal is close to 0 up to 100 msec before heel strike. The large negative difference between 0 and 0.05 sec is due to the artefacts. The difference returns to zero before a sharp negative difference, indicating a reflex excitation is seen about 66 msec after stimulation.

at just the expected latency of the excitation ‘ligamento-muscular’ reflexes observed in chapter 3 table 3.3 and table 3.4.

Figure 5.7.shows a second example of a clear difference in the two EMG signals in Vastus Lateralis. In this case the stimulation was delivered at heel strike and the artefact causes a sharp change in the difference signal at time zero. This returns to zero and is followed by two short periods of positive difference and then a longer lasting negative difference about 74 msec after the stimulation artefact. The difference then returns close to zero for the later part of the step cycle.

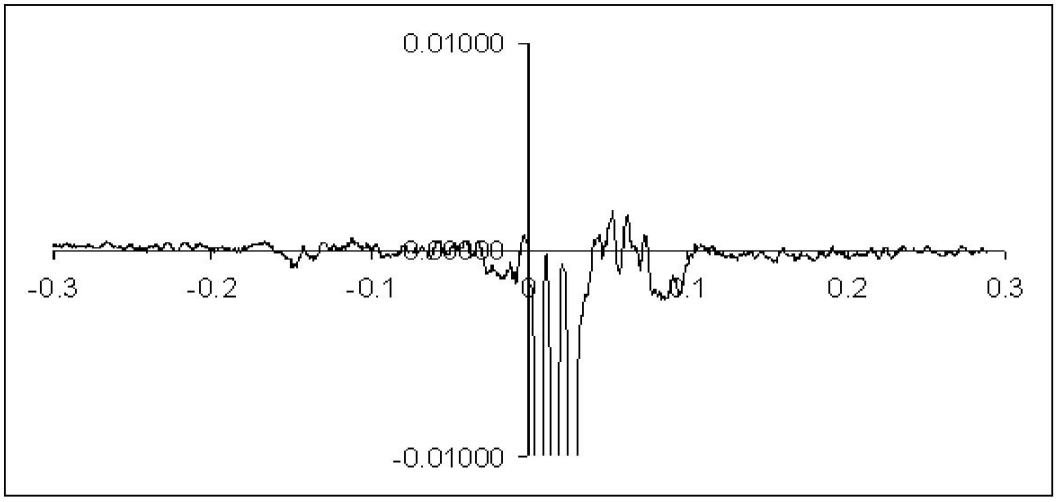


Figure 5.7
The figure shows difference between rectified averaged EMG recorded from VL during gait with and without stimulation. The vertical axis is volts.
Heel strike occurs at 0 msec. The differences are small during the 300 msec before heel strike. There is a big difference during the stimulation artefact. Short lasting positive differences are seen between 60 and 71 msec before a larger longer lasting negative difference also occurs after 74 msec after heel strike.

In two of the volunteers no ‘ligamento-muscular’ reflexes could be detected in the EMG difference signal. Figure 5.8 shows an example of this. The EMG shown was recorded from Vastus Lateralis. In this case the stimulation was delivered with a 50 msec delay after heel strike. The figure shows the small differences in EMG until the time of the artefact. The difference also stays small and close to zero in the period after the artefact suggesting that the EMG profile is almost identical in the stimulation and no stimulation walking i.e. there is no difference due to any ‘ligamento-muscular’ reflex in the period 50 to 150 msec after stimulation.

Table 5.1 shows the latencies of excitations and inhibitions detected using the EMG difference method. The recordings were made in VL and the LCL was stimulated. Ryan Joiner tests showed that both sets of data were normally distributed. ANOVA and a post hoc t test confirmed that the latencies of excitations were significantly shorter than inhibitions ($P>0.1$, ANOVA $P=0.001$, t test $P=0.0002$). The latencies of excitations were also shorter when the volunteers were sitting or standing. See tables 3.3 and 3.4. However, the latencies during walking are significantly longer that those recorded in chapter 3. The mean latency for excitations in VL after LCL stimulation was 57 ± 6 msec when sitting and 71 ± 5 msec during walking. ANOVA tests calculate $P= 0.008$. The mean latency for inhibitions in VL following LCL stimulation was 66 ± 7 msec when sitting and 87 ± 1 msec during walking. ANOVA tests calculate $P<0.001$.

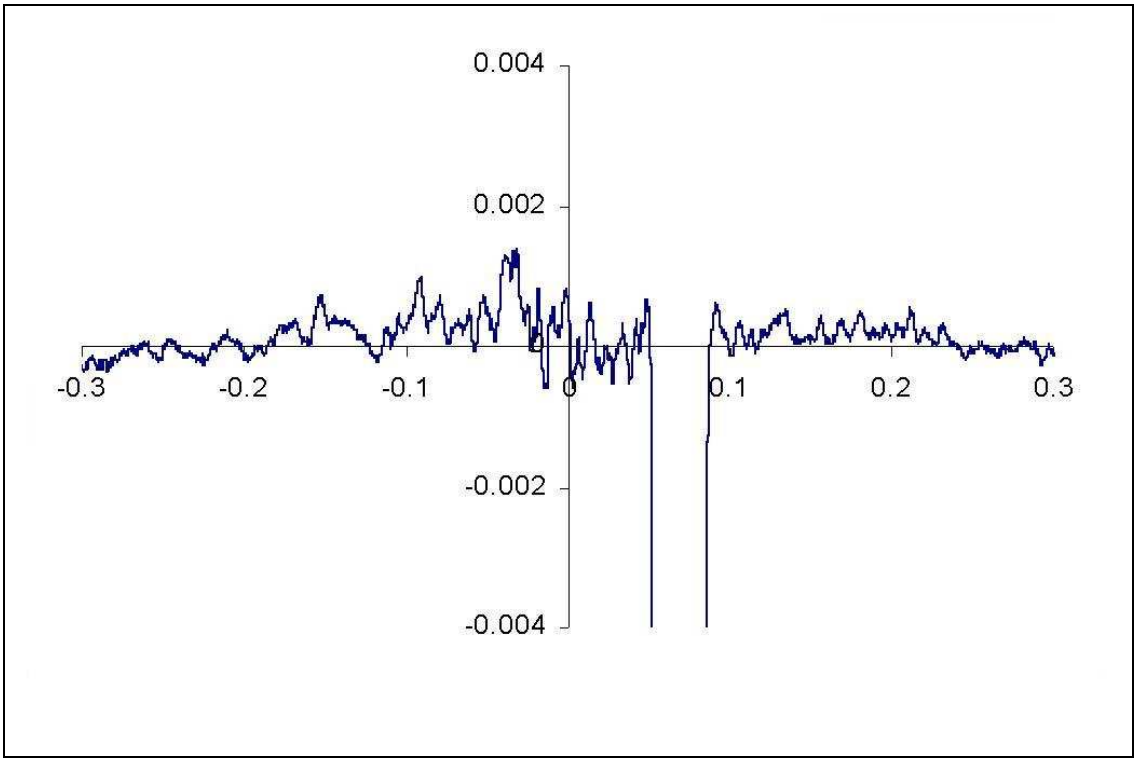


Figure 5.8
A sample of EMG differences from VL showing no indication of 'ligamento-muscular' reflex during walking. The vertical axis shows the EMG in volts. The horizontal axis is time.
In this case the stimulation is delayed to 50 msec after heel strike. The difference in EMG signals before the stimulation is close to zero until just before heelstrike. The stimulation artefacts are clear between 50 msec and 80 msec. The difference signal stays close to zero for the rest of the record.

	VL Latency (msec)	
	Excitation	**Inhibition**
	66	85
	69	87
	69	87
	74	88
	78	
Mean	**71**	**87**
SD	**5**	**1**

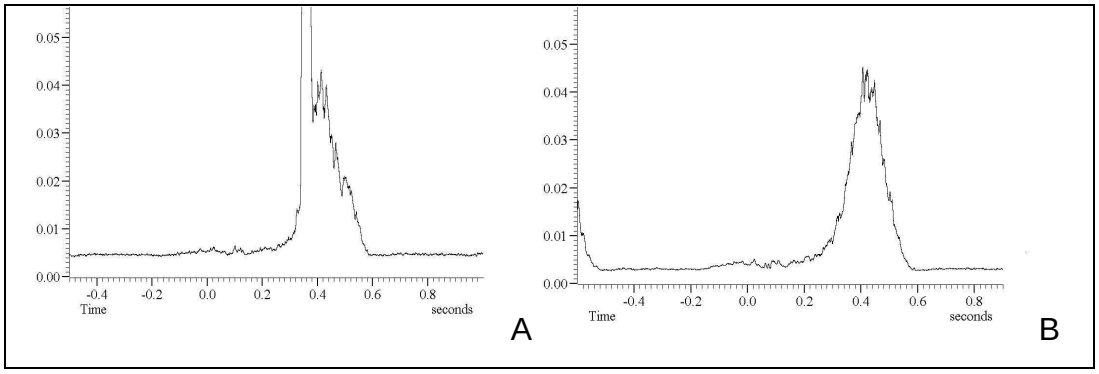
Table 5.1
This shows the latencies of excitations and inhibitions detected using the EMG difference method. The recordings were made in VL and the LCL was stimulated. Both sets of data were normally distributed. ANOVA and a post hoc t test confirmed that the latencies of excitations were significantly shorter than inhibitions (Ryan Joiner P>0.1, ANOVA P=0.001, t test P=0.0002).

5.5.2. ‘Ligamento-muscular’ Reflexes in Lateral Gastrocnemius

Similar methods were used to investigate the Lateral Gastrocnemius EMG for signs of ‘ligamento-muscular’ reflexes elicited by ligament stimulation. The recordings of Lateral Gastrocnemius EMG were made concurrently with the recordings of Vastus Lateralis shown earlier in this chapter.

Figure 5.9 shows the averaged rectified integrated EMG recorded from Lateral Gastrocnemius during walking. The right panel B shows the EMG during walking without stimulation. There is a low level of EMG activity which begins before heel strike. The main peak of activity starts at about 250 msec after heel strike. It reaches a peak at about 400 msec and then falls silent by 600 msec after heel strike. Panel A in this figure shows similar recordings made during walking at the same speed but with ligament stimulation applied at 350 msec after heel strike. The EMG waves start and stop at very similar points on the gait cycle.

Panel C shows the EMG difference signals when the stimulus is delivered 350 msec after heel strike. Before heel strike there is no sign of a significant difference in the EMG. The two signals differ substantially just after heel strike and so it can be concluded that the gait cycles are not entirely similar at this time. However, the signal returns to close to zero between 250 and 350 msec after heel strike. This is just before the delivery of the stimulation at 350 msec. After a delay of 100 msec, a sharp inhibition reflex started. There is a clear difference in the EMG showing that the EMG is bigger after stimulation than it is the control condition.



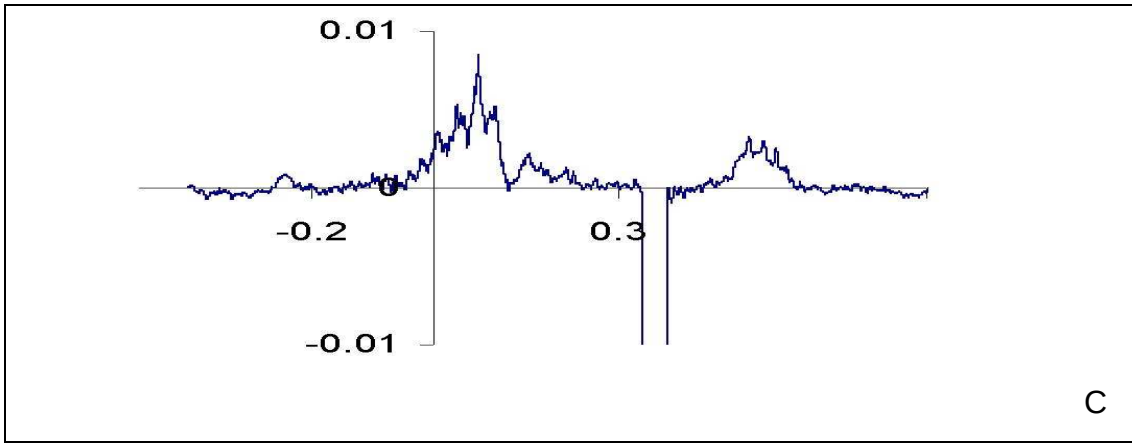


Figure 5.9
The figure shows EMG recording from LG during walking. The right panel (B) shows the EMG during walking without stimulation. The EMG begins to rise before heel strike, shown as 350 msec. It reaches a peak at 400 msec before strike and then falls silent by after heel strike. The left panel (A) shows the similar recordings made during walking at the same speed but with the ligament stimulation applied at 350 msec after heel strike EMG from Lateral LG. The lower trace (C) is the differences between panel A and B.
Heel strike is at zero msec, where the axes cross. The stimulation artefact delivered 350 msec after heel strike.
A sharp inhibition reflex is indicated by the rising difference signal which starts about 98 msec after stimulation artefact.

Table 5.2 shows the latencies of excitations and inhibitions detected in LG after LCL was stimulated. The EMG difference method was used as described earlier. Both sets of data were normally distributed (Ryan Joiner P>0.1 for both sets). The latencies of excitations were significantly shorter than inhibitions (ANOVA P=0.001, post hoc t test P<0.001). The mean latency for the excitation in LG was 82 $\pm$ 2 msec. The excitations seen during walking are of longer latency than the excitations during standing still (mean 71 $\pm$ 7 msec, see table 3.4). When these data were compared using a one way ANOVA, P=0.001. The mean latency of inhibitions in LG during standing was 79 $\pm$ 6 msec. The mean latency of inhibition during walking was 94 $\pm$ 3 msec. Again this difference was significant P < 0.001.

Figure 5.10 shows the difference signal in EMG recorded from LG with and without stimulation of LCL. The stimulation was at heel strike. The difference signal was almost zero before and after the artefact. A sharp excitation reflex is indicated by the rapid fall in the difference signal which starts about 85 msec after stimulation artefact.

	LG Latency (msec)	
	Excitation	**Inhibition**
	80	90
	80	92
	80	95
	82	96
	82	98
	83	
	85	
Mean	**82**	**94**
SD	**2**	**3**

Table 5.2
This shows the latencies of excitations and inhibitions detected using the EMG difference method. The recordings were made in LG and the LCL was stimulated. Both sets of data were normally distributed. ANOVA and a post hoc t test confirmed that the latencies of excitations were significantly shorter than inhibitions (Ryan Joiner P>0.1, ANOVA P=0.001, t test P=0.001)

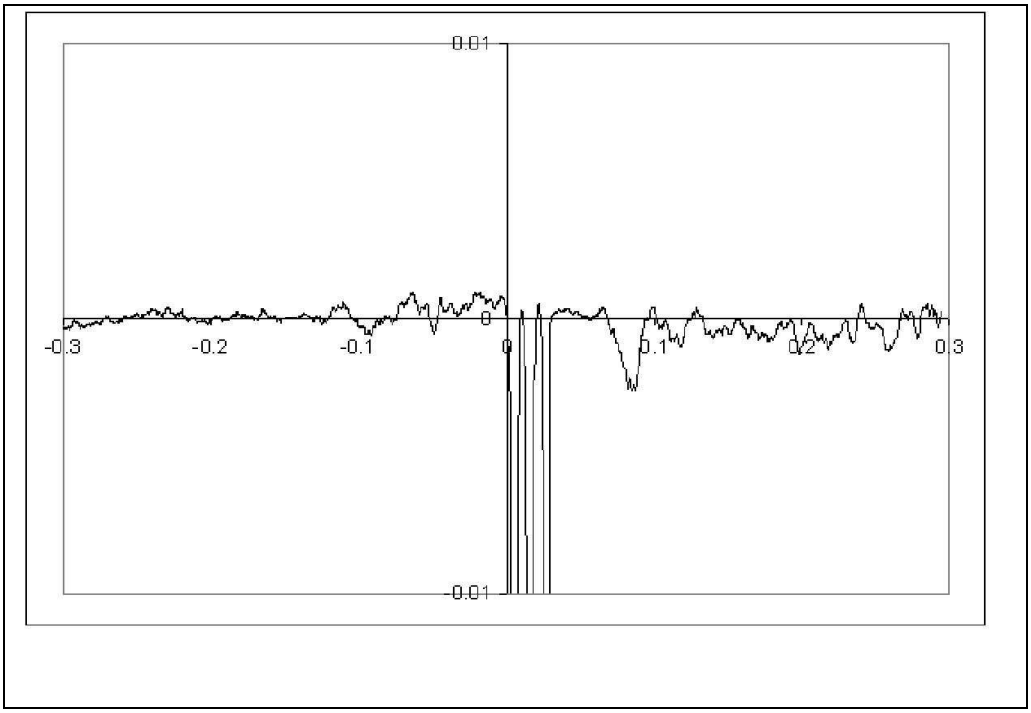


Figure 5.10
The figure shows the difference signal in EMG recorded from LG during walking with and without stimulation of LCL. Stimulation was at zero time i.e. at heel strike. The EMG difference before and after the stimulation artefact is close to zero.
A sharp increase in difference, indicating an excitation reflex, starts about 85 msec after the stimulation artefact.

Figure 5.11 shows the effect of changing the stimulation delay to 50 msec after heel strike. At this point the Lateral Gastrocnemius EMG is almost silent. As before, the upper two panels show the averaged Lateral Gastrocnemius EMG in the two conditions: with and without stimulation. The lower panel shows the difference signal. Like the data in figure 5.13. the difference signal is close to zero before heel strike, except for period at about 100 msec before heel strike. The difference returns to about zero before the stimulation is applied. In this case the signal difference stays very small after stimulation and no signs of a ‘ligamento-muscular’ reflex can be detected. This is rather similar to what was seen previously in figure 5.8. In both Vastus Lateralis and Lateral Gastrocnemius it is not possible to elicit a reflex if the muscle EMG is silent because there is no EMG activity to modulate.

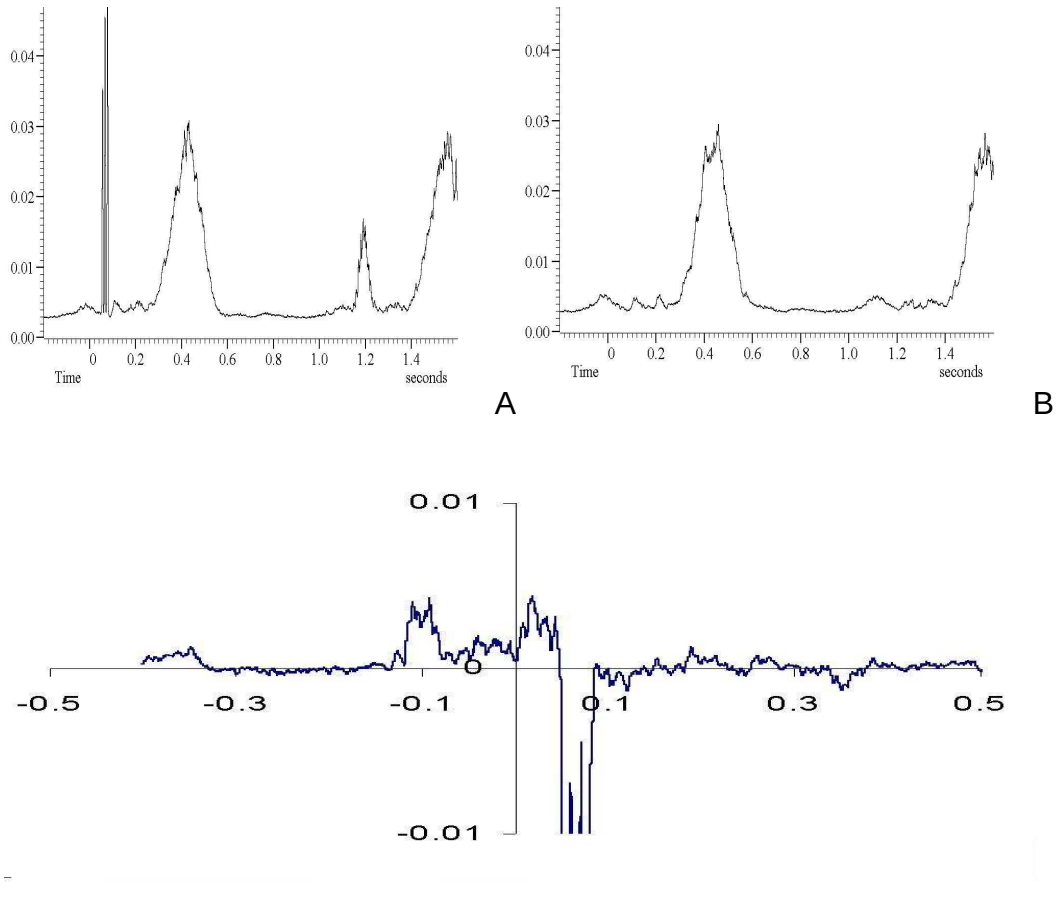


Figure 5.11
The figure shows the effect of changing the stimulation delay to 50 msec after heel strike. At this point the LG EMG is almost silent. The traces A and B show the averaged LG EMG in the two conditions: with (A) and without (B) stimulation. The lower panel shows the difference signal between two panels. In this case the difference signal stays very small after stimulation and no signs of a reflex can be detected.

5.5.3. Effect of Ligament Stimulation at Various Points in the Gait Cycle

The stimulation of the ligaments was repeated at various points in the step cycle by delaying the time of stimulation with respect to heel strike. The previous figures have shown the effects of stimulation at heel strike. This section will describe the effects of stimulation at 0, 50, and 350 msec after heel strike i.e. at two different times during the stance phase and then in the swing phase.

Figure 5.12 shows examples of the EMG difference signals in two volunteers when the stimuli are delivered with delays of 0 and 50 msec after heel strike. Volunteer A shows 'ligamento-muscular' reflexes with a latency of 90 msec when the stimulation is delivered at heel strike and when it is delivered 50 msec after heel strike. Volunteer B shows similar reflex excitations when the delays are 0 and 50 msec. Figure 5.13 shows a difference in EMG signals. In this case the stimulation of LCL was delayed by 350 msec after heel strike. There is no sign of a 'ligamento-muscular' reflex in the EMG difference signal.

This places the stimuli in a period of relative EMG silence as can be seen in figure 5.6 A, B. Inhibitory reflexes should be difficult or impossible to detect at this time but it should theoretically be possible to detect in excitations.

Volunteer A Volunteer B

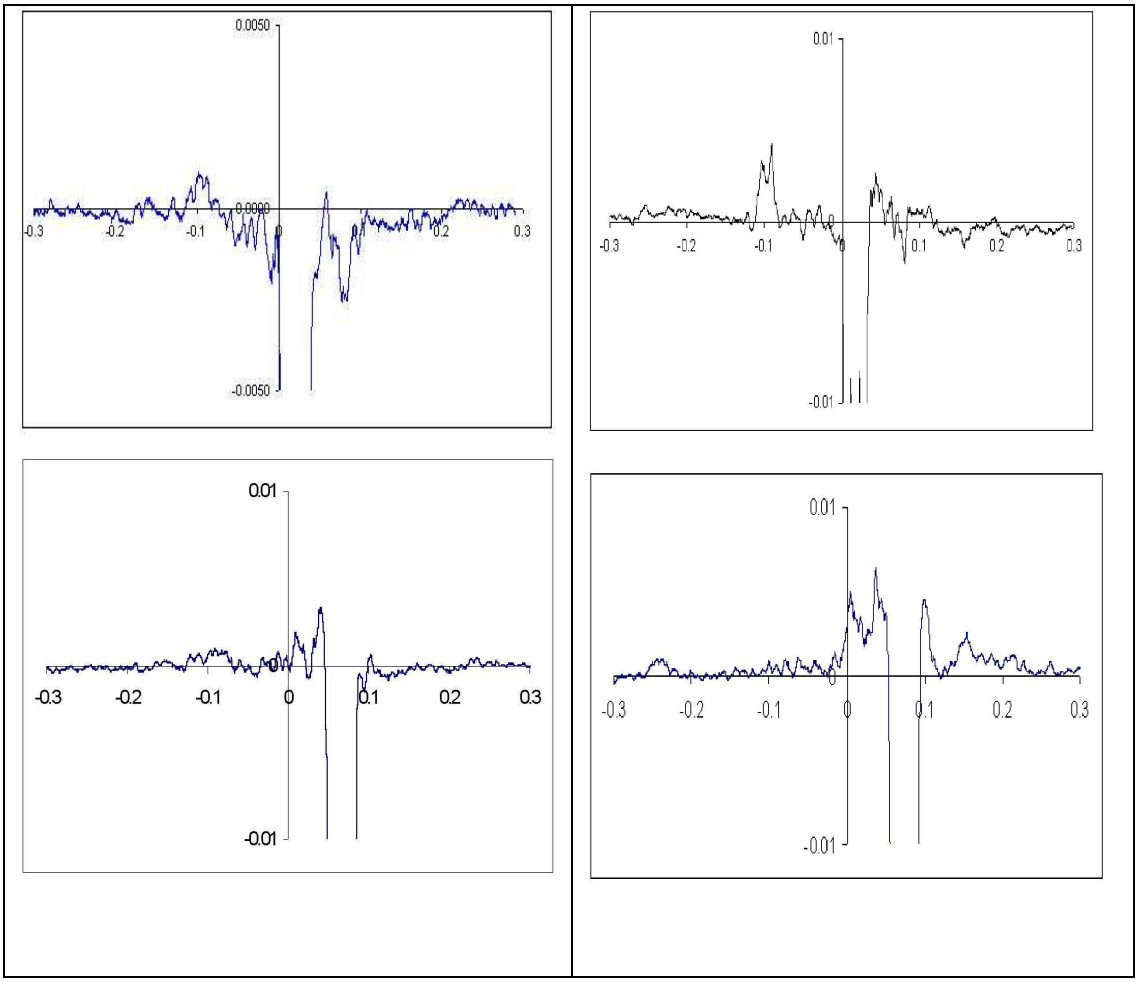


Figure 5.12
The traces above have been recorded from VL in two subjects during walking. The left traces come form one volunteer the right traces from the other. In the top pair of traces the stimulation is delivered at heel strike. The traces have the stimulation delayed by 50 msec after heel strike.
The excitation reflexes can be seen in both volunteers when the stimuli are delivered at heel strike. The excitation is present but reduced in volunteer B when the stimuli are delayed. The reflex excitation is less clear with delayed stimulation in volunteer A.
In the right panels the data show large fluctuations in the control period about 100 msec before the artefact. The appearance of these events means that the interpretation of results has to be treated with caution.

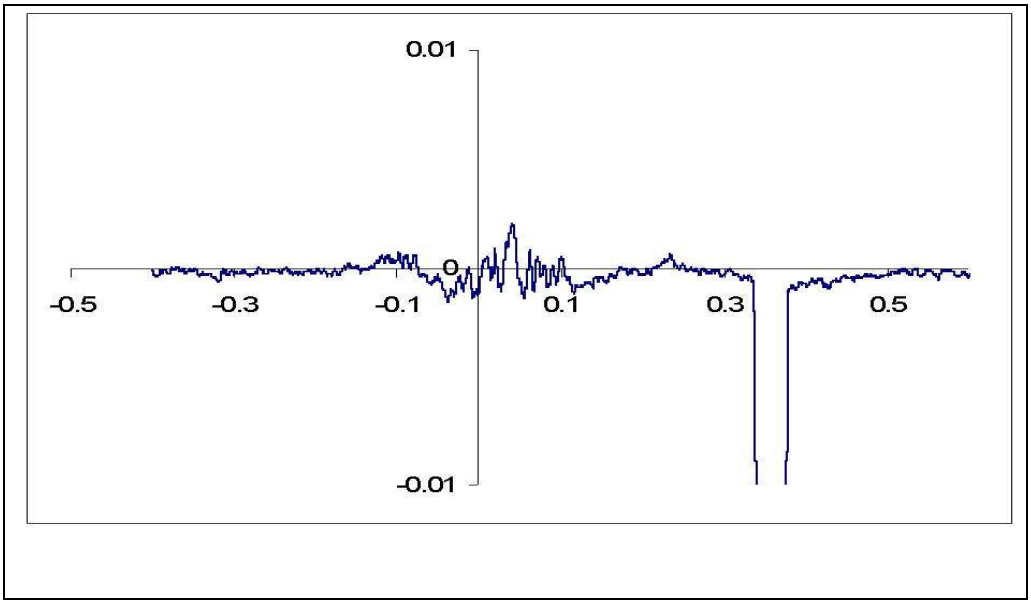


Figure 5.13
The figure shows EMG difference signal. VL activity was compared with and without stimulation of LCL. The stimulation was delayed to 350 msec after heel strike. Heel strike occurs at zero time.
The data show relatively large fluctuations at about heel strike. The appearance of these events means that the interpretation of results has to be treated with caution. However, the signal returns close to zero before the time when a reflex might be expected. There is no sign of a 'ligamento-muscular' reflex in the EMG difference signal.

5.5.4. The Relative Frequencies of Excitations and Inhibitions in 'Ligamento-muscular' Reflexes

The data in the tables of reflex latencies during standing or sitting shown in chapter 3 and those for latencies during walking shown in chapter 5 indicate that inhibition are more common when the volunteer is still and excitations are more common when the volunteer is walking. The relative frequencies are shown in table 5.3.

A chi squared test was used to test these data to investigate is the differences were statistically significant. The differences were not significant when the two muscles were tested separately. The Chi-Squared value was 2.862, and P-Value was 0.09 for the VL data. The Chi-Squared value was 3.172 and P-Value was 0.075 for the LG data. The low number of counts created problems in both analyses. However when the data was pooled and test repeated as significant result was obtained (Chi-Sq = 6.012, P = 0.014).

Vastus Lateralis	**Standing/Sitting**	**Walking**
Number of excitations observed	8	6
Number of inhibitions observed	19	4
Lateral Gastrocnemius	**Standing/Sitting**	**Walking**
Number of excitations observed	7	7
Number of inhibitions observed	18	5

Table 5.3
The relative frequencies of excitations and inhibitions seen during experiments when the volunteer was moving and still.

5.6. Discussion

The aims of these experiments were to investigate: if 'ligamento-muscular' reflexes can be elicited during the gait cycle, if the pattern of responses were similar to those seen during sitting and standing and if the latencies of the reflexes were changed.

The data shown in the figures and tables in this chapter confirm that 'ligamento-muscular' reflexes can be elicited regularly in both Vastus Lateralis and Lateral Gastrocnemius during walking. The latencies of excitation and inhibitions are 15-20 msec longer during walking than when the volunteer in not moving. This difference is significant. The excitations have shorter latencies than the inhibitions in both situations.

The most common 'ligamento-muscular' reflexes are inhibitory when the volunteer is not moving and this changes to excitations being more common when the volunteer is walking. The relatively small number of volunteers compromises the test for individual muscles but a significant result is obtained when the data for VL and LG are combined.

Whilst it is possible to elicit ligamento-muscular reflexes when the volunteer is stationary and moving, it is not clear if the same pathways are active in both states. The afferent and efferent pathways are almost certainly the same and the longer latency suggests a longer or more complex central path. The change in the relative frequency of excitations and inhibitions also supports this suggestion. However, in both states the magnitude of the reflexes remain relatively modest.

The main problem encountered in these experiments lay in inconsistent walking style by the volunteers. They were all experienced treadmill walkers and they all walked at the same

average speed of 4 kmph. However, the natural variation in stride length and small variations in speed stride by stride resulted in averaged EMG which resembled published patterns of activity but which were subtly different on two walks. These were clear when the difference between the two signals was calculated. For the most part these differences are close to zero, as shown in figures 5.6, 5.7, 5.10 and 5.12 but at times close to heel strike, noticeable differences occur.

It was not possible to detect evoked 'ligamento-muscular' reflexes in the raw EMG signals. There was simply too much variation in the period after stimulation. However, 'ligamento-muscular' reflexes were relatively easy to detect in the difference signals, as can be seen in figures 5.6 to 5.13. It is not possible to make definitive statements about the relative amplitudes of 'ligamento-muscular' reflexes during standing and walking due to differences in the baseline EMG. This was constant during the experiments in the sitting condition but constantly changing during walking. Thus there is no absolute baseline to compare with each reflex amplitude. In favourable circumstances the EMG signals in the two conditions (with and without stimulation) are well matched specially in the 'control period' just before the delivery of stimuli and should return close to zero between the artefact and the appearance of any putative reflex. Examples of this can be seen in figures 5.7, 5.9 and 5.10. Other figures show examples where these criteria are not met so clearly. For example in figure 5.12 and 5.13 there are large deviations in the signal before the stimuli artefact. These could be chance events or the residual effect of single atypical step during the walking. The appearance of these events means that the interpretation of results has to be treated with caution. The figures are included to illustrate the range of results.

The EMG difference technique helped in the identification of ‘ligamento-muscular’ reflexes. The ‘ligamento-muscular’ reflexes could be elicited in Rectus Femoris and Lateral Gastrocnemius at the very end of the swing phase and reached their peak shortly after heel strike. The period from midstance to terminal swing phase was absolutely areflexic. One possibility is that no reflex inhibitions are observed at this time because there is no EMG activity to modulate. However, reflex excitations were also observed, see figures 5.6, 5.7, 510 and excitations can be detected against a silent EMG if they were sufficiently intense to raise motor neurones above threshold.

Chapter 6

General Discussion

The aims of the experiments reported in the present study were: to extend our knowledge of reflexes elicited by stimulation of the medial and lateral collateral knee ligaments in humans. The second aim was to investigate if different muscles are affected differently by these reflexes. The third aim was to investigate if these reflexes can be modulated by posture, muscle activity or movement.

The results of the experiments described in chapters 3, 4 and 5 show that stimulation applied over the collateral knee ligaments produce inhibitory and excitatory reflex responses in several muscles of the lower limb. Forty-four volunteers participated in four series of experiments. Reflexes were elicited in 34 of the 44 volunteers. The volunteers without reflexes had a low tolerance for stimulation, on the other hand any volunteer who tolerated 25 milliamps or greater showed reflexes. Data are shown in table 3.2.

The experiments reported in chapter 4 specifically addressed the role of cutaneous afferents. Stimulation with low intensity currents between 0.85 and 1.2 milliamps was capable of eliciting unambiguous sensations in the volunteers. There can be no doubt these currents excited the lowest threshold cutaneous afferents. The volunteers were all aware of the stimulation. However, this low intensity stimulation never elicited any signs of reflexes in the muscles tested. Increasing the stimulus intensity up to 10 times the perceptual threshold was similarly ineffective in eliciting reflexes. The minimum intensity of stimulation, which successfully elicited reflexes, was about twenty times

greater than perceptual threshold. The maximum intensity of stimulation used was about 45 times greater than perceptual threshold. These observations strongly suggest that low threshold cutaneous afferents did not elicit these reflex effects and that even stimulation of group II/III cutaneous afferent are ineffective. When these afferents are anaesthetised by topical application of Emla cream there is no change in the reflexes observed following stimulation.

The stimulus conditions, the intensity, pulse duration and pulse frequency resemble those used by Willer et al (1978) to excite the sural nerve and elicit nociceptive flexor reflexes in human volunteers. Willer's experiment used up to 6 shocks in the stimulus train compared with the three used in these experiments. The volunteers tested in the experiments reported here did not show flexion reflexes and the short latency excitation observed in extensor muscles indicate that the reflexes described here are not the RIII reflexes reported by Willer et al (1978).

The experiments described in chapter 3 repeat the stimuli at intervals of 1 second. This was a compromise between the need to collect a number of repetitions for averaging and the need to keep the total recording time relatively short to minimise fatigue. Finally, this interval is close to the normal stride time as seen in the experiments in chapter 5. One possibility is that this repetition interval could cause habituation of the reflexes and decrease the magnitude of responses as described by Desmedt and Godeaux (1976). However, other authors have used stimuli frequency linked to stride durations without significant problems (Capaday and Stein 1986).

Stimulation over the LCL and MCL is effective in eliciting reflexes but when the site of stimulation is moved away to sites on the patella or elsewhere, and the currents are reapplied, it is impossible to elicit reflexes. The requirement for the electrodes to be placed over the MCL or LCL strongly suggests that the afferents responsible are located in or near the ligaments and that they have a relatively high threshold for electrical stimulation. Group II and group III afferents arising from the ligament are one obvious source of these reflexes. It is not possible to eliminate activation of capsular afferent by the same stimulus current. However, since the joint capsule extends well beyond the ligament area it seems likely that moving the electrodes away from the ligaments should continue to activate the capsular receptors. Yet no reflexes were observed when this was done. On balance, the author believes that stimulation of ligament receptors is the primary cause of the observed reflexes. Any capsular contribution will probably be small but to be fair the reflexes are best described as "Ligamento-muscular" reflexes to avoid implying certainty of the causes.

The latencies of the reflexes from quadriceps and triceps muscles after MCL and LCL stimulation were between 46 to 150 msec. Both sites of stimulation elicited reflexes with the same latencies. However, the short latency excitations were significantly shorter that the short latency inhibitions in both quadriceps and triceps (see figures 3.17 and 3.18). The latencies of excitations were also significantly shorter than those of inhibitions when elicited during walking. In this case both excitations and inhibitions had longer latencies than was seen when the subject was at rest (see tables 5.1 and 5.2). During sitting and during walking the difference between excitation and inhibition latencies was less than 10 msec and could be attributed to the excitations being

associated with faster afferent fibres or with simpler central connections.

The conduction distance from the spinal cord to Rectus Femoris is typically about 50 cm, giving a conduction delay for efferent fibres of 10 msec. The shortest reflex latency observed in the current study was 46 msec. If an allowance of 5 msec is made for the central delay, the afferent delay must be a minimum of 31 msec. The afferent distance is typically 65 cm and this suggests an afferent velocity of about 21 m/sec i.e. compatible with the slower group II or faster group III fibres. The shortest latency inhibition was 47 msec and this suggests almost identical afferent times and velocities. It supports the suggestion that inhibition latencies are longer because of additional interneurones in the pathway.

The mean latency for excitatory reflexes in quadriceps was 57 msec. In this the afferent velocity must be about 12 m/sec. This is in the middle of the range of human group III conduction velocities, 7-15 m/sec reported in textbooks (Jennett 1989). This does nothing to resolve the discussion about the source of the afferents since both ligament and the capsule give rise to group III afferent fibres.

Similar calculations can be done for the reflexes in triceps. The efferent conduction distance to Lateral Gastrocnemius is about 75 cm, giving a conduction time for efferent fibres of 15 msec. The shortest latency reflex observed in the current study was 62 msec. This suggests an afferent delay of 42 msec and an afferent velocity of 12 m/sec. Thus the reflex effects described are likely to have been mediated by group III joint afferents.

The difference in mean latencies of reflexes in quadriceps and triceps is almost 10 msec and this is almost certainly due to the longer efferent pathway to triceps than quadriceps.

In the initial experiments, the volunteers were sitting relaxed, and the background EMG was silent. Ligament stimulation never elicited any signs of reflex activity in relaxed muscles. This was shown in figure 3.5. Reflexes were observed during sitting, standing and walking in conditions when there was muscle activity. However, there were significant differences when reflexes were compared during walking and when the subject was stationary. The latencies of excitations and inhibitions were significantly longer during walking than when the subject was sitting. The increase was between 15 and 20 msec. In addition at rest the frequently observed response was inhibition whilst excitations were more common during walking. No comparable changes were seen when the force of contraction was changed whilst the subject was sitting. It can be speculated that the ligamento-muscular reflexes are mediated by two different pathways in these two states. Probably the effects mediated by longer or more complex paths during movement.

The experiments described in chapter 5 were performed in the hope that the reflexes would be enhanced during walking, possibly to aid protecting the joint during the higher force loading. However, no observations were made which would directly support this suggestion. The reflexes are of longer latency and shift in sign from mostly inhibitory to mostly excitatory in VL and LG. The ultimate biomechanical action of these reflexes is no clear. If reflexes from the ligaments do act to protect the joint, they must be active in

circumstances requiring higher forces and greater velocities of movement than those used in normal walking.

The general experiment methods were described in chapter 2. The techniques and equipment used were mostly well established and usually worked without problems.

All volunteers were healthy and they co-operated well during the experiments. In addition, most of the volunteers were male; (4 females and 40 males). It was noted in section 1.1 of the introduction that female athletes have a higher risk of knee injury than male athletes. Time constraints prevented the recruitment of more female volunteers. Most volunteers tolerated the intense stimulation over the ligaments with only minor complaints. There was a range of individual tolerance to the discomfort caused by stimulation. 10 of the 44 volunteers had a limit of tolerance that lay between 10 and 20 milliamps. They terminated the experiment with stimulation currents too low to elicit reflexes. However, they were at their personal limit. It was clear from the protocol submitted to the Glasgow University Research Ethics Committee that volunteers could terminate the experiment. This can be viewed as a success of the experimental design; volunteers were recruited and understood the protocol and operated it to protect themselves. However, it did reduce the total number of individuals in whom reflexes could be elicited. It is likely that the unpleasant sensations which caused the 10 volunteers to terminate the experiment arose from cutaneous afferents. The experiments described in chapter 4 showed that the tolerance for currents increased after topical anaesthesia of the skin under the stimulating electrodes.

34 of the 44 volunteers tolerated the high intensity stimulation and reflexes were elicited in all of them. The biggest current used was 45milliamps. There was no obvious characteristic identifying whether the volunteer had a high or low toleration for stimulation.

Surface EMG recording was used in all the experiments. There were no serious problems with this during the experiments in chapter 3 and 4 because the volunteers developed constant isometric contractions. However, during the experiments in chapter 5, where volunteers walked on a treadmill, there were sometimes problems with movement artefacts. These could be remedied by reducing the cable movements and appropriate use of high-pass filters.

During the experiments described in chapter 5 reflexes were elicited while the volunteers walked on a treadmill at an average speed of 4 km/h. The Vastus Lateralis activity consisted of two prominent bursts. The first burst was associated with the knee extension, which occurs late in the swing phase. The second burst is the time which the heel contacted the ground (the early part of the stance phase). The first burst from Gastrocnemius occurs in the middle part of the stance phase when the heel is off the ground. The second burst occurs the late part of the pre swing phase (Whittle 1996).

It often proved impossible for volunteers to exactly reproduce identical averaged EMG activity in their two walks. This is easy to see in figure 5.7 of chapter 5. Some of this is due to minor variations in stride length. The volunteers maintained the same average walking speed but small short term changes allow them to move forward or backwards on the treadmill belt. Volunteers were familiarised

with the treadmill but it was not possible to eliminate this problem completely. Ultimately, it shows up as non-zero sections on the EMG difference signals of the figures in chapter 5.

There have been two previous reports of the successful use of the EMG difference methods. Duysens, Tax, Murrer and Dietz (1996) used it to investigate cutaneous reflexes in humans. The subtraction technique allowed them to measure both excitatory and inhibitory responses. In addition, Baken, Nieuwenhuijzen, Bastiaanse, Dietz and Duysens (2006) investigated cutaneous reflexes during human walking. In each case the technique has allowed reflexes to be investigated during on going movements. This may be more relevant than testing reflexes during periods of inactivity. The increased frequency of excitations during gait means that reflexes should in theory be detectable even when the muscles are inactive. Inhibitions would need a background EMG to be detected. The reflex excitations were never seen during periods of muscle inactivity and so the reflex effect is either too weak to raise motor neurones to their threshold or is modulated by some unknown mechanism.

Anatomical localisation of muscles and ligaments was done by physical examination of the limb with reference to anatomical landmarks. As a result, the author is confident that the recording and stimulating electrodes were positioned in correct places. Care was taken in the placement of electrodes to avoid restricting normal movement. As described above, reflexes were only ever elicited with the stimulating electrodes placed directly over the MCL or LCL.

The results in this study show that a wide range of muscles in the thigh and shank can be activated through short and medium latency reflexes elicited by stimulation of the LCL and MCL. It is not possible to predict the ultimate biomechanical actions of these reflexes but they may stabilize the knee during movement.

The reflexes in the current study were recorded in 34 volunteers during repeated experiments when they were standing, sitting and walking on the treadmill. The reflexes were elicited following stimulation of the medial and lateral collateral knee ligaments. MCL and LCL elicit very similar reflexes and there was no indication that MCL had stronger or faster actions on medial muscles such as Vastus Medialis than it had on Vastus Lateralis. Kim et al (1995) elicited reflexes with a protocol very similar to that used in this study. They observed reflexes in Sartorius, Gracilis, Vastus Medialis, Vastus Lateralis, Semitendinosus, Biceps Femoris Longus, and Tensor Fascia Lata following stimulation of the collateral ligaments of the human knee. However they reported that "medial muscles will be activated significantly more after MCL stimulation than LCL stimulation, and vice versa for lateral muscles". The data in this current study does not provide any evidence to support that view.

The most commonly observed pattern of the reflexes during sitting and standing in this study was a short latency inhibition but mixed inhibitory and excitatory responses were also observed. Dyhre-Poulsen et al (2000) also found a pattern of inhibitory reflexes following intra-capsular stimulation of the Anterior Cruciate Ligament. The shortest reflex latency which was elicited in this study was 46 msec after ligament stimulation. This is consistent with Golgi

tendon afferents and a short spinal pathway. The longest latency reflex was 150 msec after ligament stimulation.

The range of latencies observed in this study in stationary subjects is very similar to that reported by Kim et al (1995). This study found a range of 60 $\pm$ 7 msec for the fastest responses in Rectus Femoris to 125 $\pm$ 9 msec for the slowest responses in Lateral Gastrocnemius. Kim et al reported 88.4 $\pm$ 19.4 for the fastest responses in Sartorius to 115 $\pm$ 27.9 for the slower responses in Semitendinosus. In a study of reflexes elicited following stimulation of the Anterior Cruciate Ligament, Dyhre-Poulsen and Krogsgaard (2000) reported reflexes latencies ranging between 45 to 85 msec. Yang and Stein (1990) elicited cutaneous reflexes from Tibialis Anterior, Soleus and Rectus Femoris following tibial nerve stimulation during human walking on treadmill. They could elicit inhibition and excitation reflexes but the most reproducible reflexes that they elicited were inhibitory. The reflexes latencies were between 50 to 90 msec after the stimulation artefact. McIlroy and Brooke (1987) investigated Soleus, Lateral Gastrocnemius, Tibialis Anterior, Vastus Medialis and Rectus Femoris. They recorded reflexes with two different latencies. The earliest responses lay within a range between 85 to 132 msec after the onset of the movement interruption and the range of the later responses was between 121 to 195 msec.

Several investigations have reported detailed studies of muscle activity during joint and ligament stimulation. Most of the studies have been done on cats (Duysens and Stein (1978), (Grillner and Rossignol (1978), Forssberg (1979), Duysens and Loeb (1980), Baxendale and Ferrell (1981), Abraham et al (1985), Buford and Smith (1990), Perell, Gregor, Buford and Smith (1993)). Some studies have been

carried out in humans (Grillner and Rossignol (1978), Rossignol and Gauthier (1980), Crenna and Frigo (1987), Belanger and Patla (1987), Duysens, Tax, Trippel and Dietz (1993), Kim et al (1995), Duysens et al (1996), Dyhre-Poulsen and Krogsgaard (2000)). The ligament which has been investigated most frequently in humans is the ACL (Dyhre-Poulsen and Krogsgaard 2000). There are few studies that elicited responses after collateral knee ligament stimulation in human (Kim et al 1995) and cat (Andersson and Stener 1959).

Biomechanical studies in cadavers show that MCL and LCL work cooperatively to protect the knee against excessive valgus motion (Piziali, Seering, Nagel and Schurman (1980), Nielsen (1987), Kim et al (1995)). It may be physiologically significant that the vasti muscles are likely to support and stabilize the joint in the varus-valgus plane. In the experiments reported here it is obvious that the quadriceps were responsive to reflexes elicited by MCL and LCL stimulation. It also reinforces the need for rehabilitation and strengthening of quadriceps after knee ligament injury. The extent to which rehabilitation affects muscles and reflexes is an open question.

The results of this study support the suggestion by Kim et al (1995) that the knee ligaments not only have a role of passive joint stability but they have an important role in active stabilisation of the joint through ligamento-muscular reflexes.

The Nature of the Reflexes

Voluntary contractions might have played a role in the reflexes, since it was essential to have a background

contraction against which the reflex was identified. However, the onset latencies observed are typically 55-65 msec. This is much too rapid for voluntary modulation of ongoing contractions. Even the slower reflex responses seen during gait are still much too fast to have a voluntary component. A range of 215 to 220 msec is reported for voluntary contraction onset times of knee muscle (Pope, Johnson, Brown and Tighe (1979), Wojtys and Huston (1994)).

Distinguishing the source of responses to be either from receptors in capsules or ligaments is difficult. Observation of latencies shows that probably the main source of reflexes is from the stimulation of particular mechanoreceptors in the capsule and ligaments. It is unlikely that receptors with the very largest afferents, for example the Golgi tendon organs, are solely responsible, since relatively high intensity stimulation was needed to elicit reflexes. The minimum reflex latencies of 55-65 msec suggest afferent delays more consistent with slower group II or group III afferents. Thus a wide range of receptor types may be involved but probably not afferents from free nerve endings which would be very difficult to activate from electrodes on the skin.

These experiments have shown that ligamento-muscular reflexes are widespread after relatively strong electrical stimulation of medial and lateral collateral ligaments. They are present during maintained postural contractions and during walking. It is not possible to make any definitive statement of the biomechanical actions in protecting the joint. This is one obvious direction for future research. The experiments were all done in volunteers with healthy joints moving in a conservative way with low forces and low velocities. It would also be interesting to investigate if the reflexes are more powerful in high speed and high force

movements more closely resembling those used in sports where the joints are exposed to the risk of damage. It would also be useful to investigate the changes in reflexes during a period of recovery after joint injury.

There have been several recent studies in which the anterior and posterior cruciate ligaments have been stimulated directly during arthroscopic procedures Ochi, Iwasa, Uchio, Adachi and Sumen (1999)). The reflex effects described by (Kim et al (1995), Dyhre-Poulsen and Krogsgaard (2000)) were discussed earlier in section 1.6. The more recent studies have shown that ACL stimulation causes sensory evoked potentials in human volunteers (Pitman, Nainzadeh, Menche, Gasalberti and Song (1992) Ochi et al (2002)), so supporting the belief that a neurosensory function is important. In particular Ochi's paper reports that sensory evoked potentials were found in approximately half of their patients who had undergone ACL reconstruction and that those patients had better knee function that those whose ACL had not been re-innervated. Krogsgaard, Dyhre-Poulsen and Fischeer-Rasmussen (2002), reviewed the current literature on this field and concluded that the existence of long latency reflexes following ACL and PCL stimulation are unlikely to contribute directly to protecting the joint because of the delays involved. They believe that this system updates motor programmes which influence knee dynamics. This is consistent with Sjolander's summary diagram shown in figure 6.1. It is unfortunate that so far nobody has performed experiments with arthroscopic stimulation of the collateral ligaments.

Reflexes associated with ligament receptors contribute to proprioception, kinaesthesia, muscle co-ordination and joint stability. They act through projections in ascending

pathways, directly on alpha motor neurones or indirectly through reflex effects on the gamma motor neurone-muscle spindle system.

Information from joint afferents reaches supra-spinal structures via several ascending pathways. Signals from joint afferents travel through the dorsal columns, the spino-thalamic, the spino-reticular, the spino-cervical and the spino-cerebellar tracts to reach many different parts of the brain stem such as the cerebellum, the reticular formation, the thalamus and the somatosensory cortex. Neurones found in these supra-spinal structures are remarkably influenced by activity in low threshold joint afferents (Gardner and Noer (1952), Mountcastle, Poggio and Werner (1963), Sjolander et al (2002)).

Electrical stimulation of afferents found in the normal anterior cruciate ligament (ACL) during arthroscopic surgery elicits clear-cut somatosensory evoked potentials, which indicates that ACL afferents indeed activate pathways with cortical projections in man (Pitman et al (1992), Lavender, Laurence, Bangash and Smith (1999)). It is possible that ligament afferents may contribute to the control of muscle stiffness and co-ordination.

All of these neuro-anatomical and neuro-physiological observations combine with the results presented in this thesis to suggest strongly that ligament afferents may make important contributions to motor control. These ideas were recently reviewed by Sjolander et al (2002) and their summary is presented in the figure 6.1. The observation in this thesis of slower ligamento-muscular reflexes during gait and the change of balance from excitation to inhibitions

suggests that a locomotor controller might be added to this schema.

The results in this thesis support their idea of ligamento-muscular reflexes contributing to muscle co-ordination. The possible contribution made by ligament afferents to movement and position sense and to the process of learning new movements remains to be investigated. This will be particularly interesting for the skilled high velocity and high force movements required in sports. It will be doubly important if better understanding of these movements can reduce the frequency and severity of injuries caused by poor motor skills.

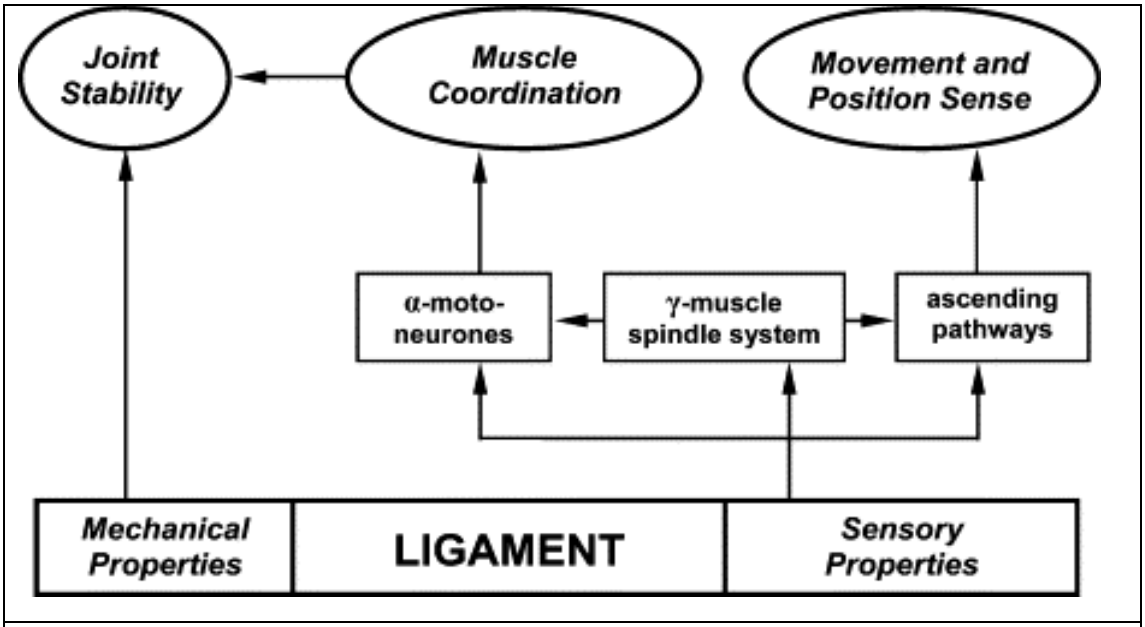


Figure 6.1.
The figure shows a schematic representation of the principal pathways by which mechanosensitive ligament receptors can contribute to joint stability, muscle co-ordination and proprioception.
Modified figure from Sjolander et al (2002).

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Appendix I

The following 2 figures illustrate the probability plots generated by Ryan Joiner tests performed in Minitab 15. Figure 1 shows the results of a normally distributed population of inhibition latencies after LCL stimulation of the quadriceps muscles from table 3.3 in this thesis. The data points lie close to the line and the p value shown in the inset ($p>0.01$) confirm the normality of the distribution.

Figure 2 shows the result of a Ryan Joiner test applied to a population which is not normally distributed. The data are from long latency inhibitions after MCL stimulation of the quadriceps muscles from table 3.3. The data points did not lie along the line but show systematic deviations and the p value was $P<0.01$.

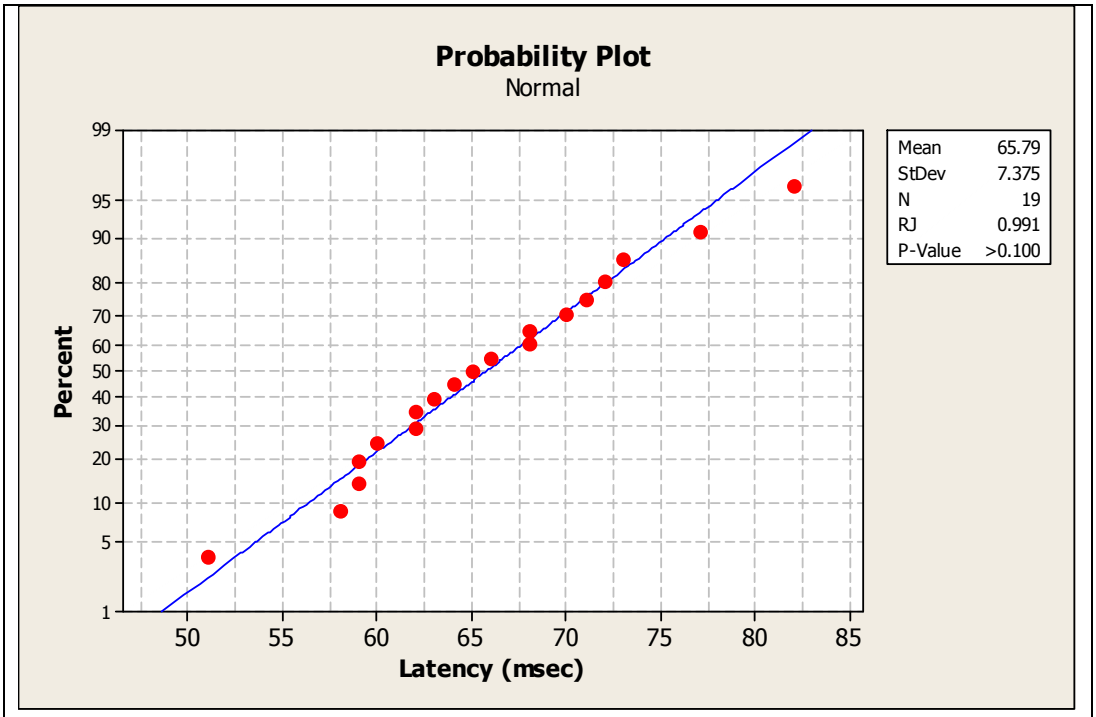

Figure 1. The figure shows a Ryan Joiner test applied to a population that shows the results of a normally distributed population of inhibition latencies after LCL stimulation of the quadriceps muscles (P> 0.1).

The values are from table 3.3.

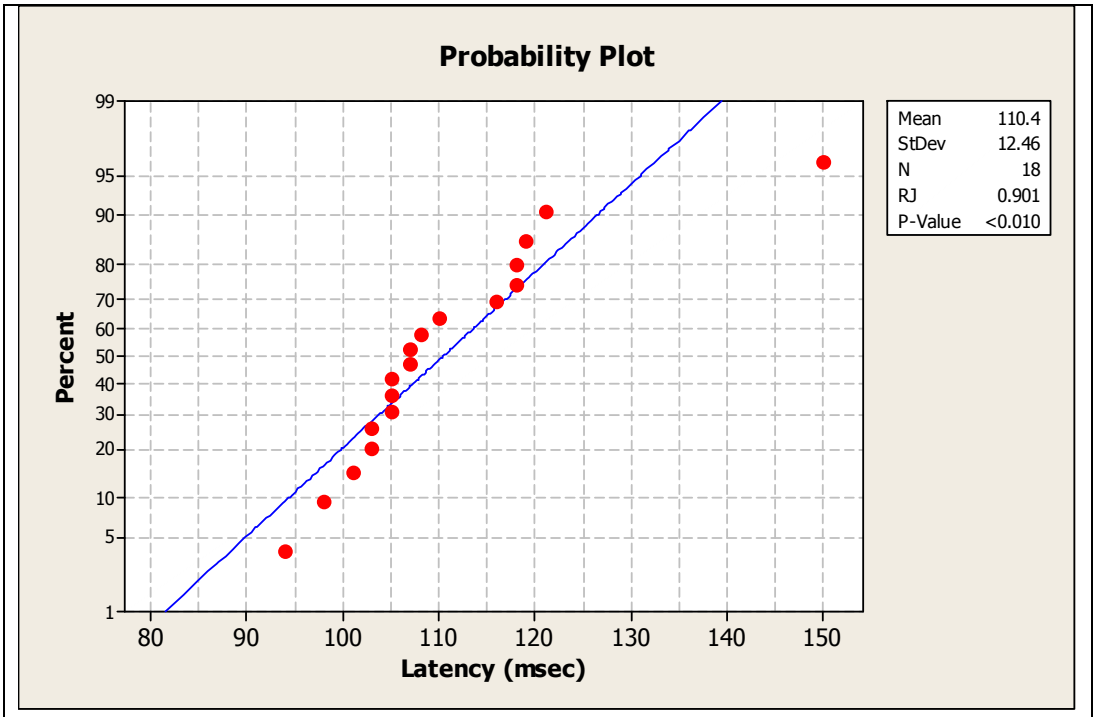

Figure 2. The figure shows a Ryan Joiner test applied to a population, which is not normally, distributed (P< 0.01). The data shows long inhibition latencies after MCL stimulation of the quadriceps muscles.

The values are from table 3.3.

Appendix II

An investigation of reflex elicited by percutaneous stimulation of the medial and lateral ligaments of the human knee

Abstract of the thesis presented on 13th of scientific conference of the union of Iranian student in Europe, Leeds University, UK. 2005.

The principal purpose of this study was to extend our knowledge of reflexes associated with joint disease and injury of joints, which are major causes of pain and disability in the population. Surprisingly, there have been very few neurophysiological studies of the reflex effects associated with stimulation of joints, ligaments and tendons. Similar experiments have been performed in humans and the existence of reflexes has been confirmed (1,2).
The immediate aim of this project was to investigate reflexes elicited following percutaneous stimulation of the medial and lateral ligaments (MCL, LCL) of the knee, during sitting position.
The longer-term aim is to provide a strong scientific foundation, which will guide the coaching of athletes to reduce the risk of joint injury and to enhance the rehabilitation of athletes after injury.
Seventeen subjects were seated on a testing bench and hip angle was 90 degrees. The age range of the subjects was from 21 to 49 years. The maximal voluntary contractions (MVC) of the quadriceps were measured at 180° of knee extension. This was then used to set the magnitude of subsequent sub-maximal contractions at 5, 10 and 20% of MVC. An identical stimulation sequence was delivered during sustained contractions electrodes were placed over the lateral and medial knee ligaments separately. The ligaments were stimulated by 3 pulses of 1 msec duration at 100 HZ. The stimulation intensity was the ranged of 0-45mamps. The EMG activity from three muscles was monitored simultaneously using surface electromyography by 1401 Electromyogram, and for data analysis used to Spike2 software system. Electrodes were placed over the muscles. The muscles selected were Rectus Femoris, Vastus Medialis and Vastus Lateralis. First of all no reflexes were elicited in very low current, effects have started in 20 mamps. The pattern of the muscle response and electromyography was relevant to the stimulation intensity. By increasing the stimulation intensity the EMG waves became bigger and also the magnitudes of the responses were increased. Inhibitions and excitations reflexes elicited and there is no significant difference between responses after MCL and LCL stimulation. Mostly effects were inhibition in both ligaments. The mean latency of early inhibition after LCL stimulation was 66 + 7 msec and After MCL stimulation was 68 + 12 msec. The difference was not significant (P=0.26). The mean latency of early excitation after LCL stimulation was 57 + 6 msec. And for the MCL stimulation was identical.

1.Dyhre-Poulsen, P. and Krogsgaard, M. R. (2000): Muscular reflexes elicited by electrical stimulation of the anterior cruciate ligament in humans. Journal Appl.Physiology. Vol.**89**, 2191-2195, 2249-2257.

2. Kim, A. W., Rosen, A. M., Brander, V. A. and Buchanan T. S. (1995). Selective muscle activation following electrical stimulation of the collateral knee ligaments of the human. knee.Arch.Phys.Med.Rehabil.Vol. 76:750-757.

UNIVERSITY OF GLASGOW

FACULTY OF BIOMEDICAL AND LIFE SCIENCES

ETHICS COMMITTEE FOR NON CLINICAL RESEARCH INVOLVING HUMAN SUBJECTS, MATERIAL OR DATA

APPLICATION FORM FOR ETHICAL APPROVAL

NOTES:

THIS APPLICATION FORM SHOULD BE TYPED, NOT HAND WRITTEN.

ALL QUESTIONS MUST BE ANSWERED. "NOT APPLICABLE" IS A SATISFACTORY ANSWER WHERE APPROPRIATE.

Project Title: An investigation of Reflex elicited by percutaneous stimulation of the medial and lateral ligaments of the knee.

Is this project from a commercial source? No

If yes, give details and ensure that this is stated on the Informed Consent form.

Date of submission to be entered: 13th May 2005_____

Name of all person(s) submitting research proposal
Dr Seyed Mohsen Rahimi, Dr Ronald Baxendale

_____

Position(s) held
PhD. student (IBLS), Senior Lecturer (IBLS)

_____

Division: Neuroscience and Biomedical Systems_

Address for correspondence relating to this submission: Dr Mohsen Rahimi, Lab 427 Kelvin Building, Glasgow University.

_____

Name of Principal Researcher (if different from above e.g., Student's Supervisor)
Dr Ronald Baxendale
Position held Senior Lecturer (IBLS)

2. Please give a summary of the design and methodology of the project. Please also include in these section details of the proposed sample size, giving indications of the calculations used to determine the required sample size, including any assumptions you may have made. (If in doubt, please obtain statistical advice).

We seek permission to invite up to 30 adults to participate in the experiment. Both male and female will be invited since there is no reason to suspect that the reflexes are different in men and women. The volunteers will be in good health and have no history of skeleto-muscular injury or diseases. Each volunteer will attend the lab for one visit lasting approximately on hour. During this period they will be familiarised with the experiment informed consent will be sought before the experiment starts.The volunteer will know they are free to stop the experiment at any time.

If they agree to participate, the surface electromyogram will be recorded from muscles of the lower limb using small skin mounted amplifiers. A pair of stimulating electrodes will be placed aver the collateral ligaments of the knee. Small switch is taped to the heel of the shoe to allow identification of the instant of ground contact during walking and running. The volunteer is then invited to stand for a few minutes. During this period electrical currents will be delivered once every two seconds to excite sensory receptors in the ligament. Reflex effects are identified by peri-stimulus averaging of the electromyogram. The averaged electromyogem before each stimulation is compared with that immediately afterwards when the reflex should occur. The technique is illustrated on a separate attached sheet with data from a polit experiment on one of the applicant.

As essentially similar process is repeated during walking and running on treadmill. The modification required is to time the application of the stimulus relative to a fixed point on the gait cycle (ground contact) rather than at a fixed time interval. Most adults walk at little slower than one step per second and so this effectively requires stimulation on every second or third step.

There is no data in the scientific literature to allow a formal power calculation to be employed. However, the results can be safely analysed since in each test the volunteer provides their own internal control period in the form of the averaged electromyogram for the priod before stimulation. This can be directly compared with the averaged electromyogram recorded at the same site immediately after stimulation. There is a widespread convention in reflex studies to consider the reflex to be significant it exceeds two standard deviations for the mean averaged electromyogram in the pre-stimulus control period. When a significant reflex is identified, its magnitude is measured as the area above or below the mean background electromyogram.

3. Describe the research procedures as they affect the research subject and any other parties involved.

The volunteer is invited to participate, given an information sheet, the nature of the experiment is explained and any questions answered. The volunteer is then invited to sign the consent form. The volunteer will know they are free to stop the experiment at any time.

The volunteer will have their skin cleaned with an alcohol wipe at the sites where electrodes will be applied. The electrodes will be fixed to skin using adhesive tape. They will be invited to stand and walk on a treadmill for periods of up to 5 minutes.

The volunteer will have electrical currents, each lasting 1 millisecond, applied to their leg over the medial or lateral collateral ligaments of their knee. The current intensity will be increased incrementally. The first series are always too weak to be preceived by the volunteer. With each increment the sensation becomes stronger until the volunteer indicates they wish to stop. It is hard to define an absolute current limit since this depends on the relative position of ligament and electrodes as well as the tolerance of the volunteer. The principal applicant has experienced stimulation up to 45 milliamps in polit experiments. This is uncomfortable but not damaging to tissues. It is important that the stimulation does not cause frank pain both to protect the volunteer and to avoid unwanted withdrawal reflexes.

The volunteer remains in control of the stimulation at all a times and is able to reduce or stop the stimulation wherever they wish.

4. What in your opinion are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

In our opinion the ethical considerations are minor.
Each volunteer is familiarised with the experiment and informed consent is sought before the experiment starts.
The experiment is short and pus the volunteer at no additional risk.
The volunteer is able to stop the experiment at any time.
The file naming strategy ensures the volunteer's anonymity.

5. **Outline the reasons which lead you to be satisfied that the possible benefits to be gained from the project justify any risks or discomforts involved.**

There is a reasonable balance between risk and benefit.
The "risk" or "discomfort" as explained above is very mild, if it exists at all. The gain is modest in terms of a better understanding of the behaviour of the central nervous system.

6. Who are the investigators (including assistants) who will conduct the research and what are their qualifications and experience?
Seyed Mohsen Rahimi, M.D., physician to the Iranian student National Team attending Student Olympic Games in China, 1998,Spain 2000,and Canada, 2001.
Dr Rahimi is a full time Ph.D. student in the University of Glasgow.

Ronald Baxendale BSc PhD is a senior lecturer in the Division of Neuroscience in Glasgow University.
Both have extensive experience of testing motor skills in humans.

7. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.
No. The applicants do not think such an emergency is likely.
There is a first aid box in the laboratory where the experiment will take place.
There is a telephone to call for assistance

8. In cases where subjects will be identified from information held by another party (for example, a doctor or hospital) describe the arrangements you intend to make to gain access to this information including, where appropriate, which Multi Centre Research Ethics Committee or Local Research Ethics Committee will be applied to.
No
9. Specify whether subjects will include students or others in a dependent relationship.
Students are not in a dependent relationship with the experimenter.

10. Specify whether the research will include children or people with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects.
The experiment will not test children or people with mental illness, disability or handicap

11. Will payment or any other incentive, such as a gift or free services, be made to any research subject? If so, please specify and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering payment or other incentive.
Yes, where appropriate.
A maximum payment of £10-15 will be made to cover expenses in travelling to the laboratory.
This will come from Dr. Rahimi, s bench fees.

12. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.
The volunteer will be given an information sheet.
They will be invited to discuss the experiment and any questions answered.
They will be invited to sign a consent form.

13. Comment on any cultural, social or gender-based characteristics of the subject which have affected the design of the project or which may affect its conduct.

In our opinion there are no cultural, social or gender-based issues in this project

14. Please state who will have access to the data and what measures which will be adopted to maintain the confidentiality of the research subject and to comply with data protection requirements e.g. will the data be anonymised?
The experimenter will have access to the data.
The data will be anonymised using a code known only to the experimenter.
The data files will be destroyed at the end of the experiment, to comply with the Data Protection Act.

15. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.
It is possible that the volunteers will participate in other experiments.
It is not the intention to recruit from other experiments.
The simple nature of this study will not place the volunteer at any additional risk

16.Date on which the project will begin
25th May 2005... and end ...30th June 2005

17. Please state location(s) where the project will be carried out.
Laboratory of Human Performance, Lab 427, Kelvin Building, University of Glasgow.

18. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects) e.g. where blood samples are being taken
The researchers are at no additional health and safety risk.

Signed _____

Date _____

(Proposer of research)

Where the proposal is from a student, the Supervisor is asked to certify the accuracy of the above account.

Signed _____

Date _____

Supervisor of student)

Email the completed form to: S.Morrison@bio.gla.ac.uk

And send the signed hard copy to:

Stuart Morrison
Faculty Research Office
Faculty of Biomedical & Life Sciences
West Medical Building
University of Glasgow
Gilmorehill
Glasgow
G12 8QQ

CONSENT FORM

Volunteer identification number

TITLE OF THE PROJECT: An investigation of reflexes elicited by percutaneous stimulation of the medial and lateral ligaments of the knee.

Name of the Researchers: Dr Seyed Mohsen Rahimi and Dr Ronald Baxendal

1). I confirm that I have read and understood the information sheet for the above mentioned study and I have had the opportunity to ask questions

2). I understand that my participation is voluntary and that I am free to withdraw at any time, without
giving any reason.

3). I agree to take part in this study.

Name date
signature

Researcher date
sign

VOLUNTEER INFORMATION SHEET

TITLE OF THE PROJECT: An investigation of Reflex elicited by percutaneous stimulation of the medial and lateral ligaments of the knee.

You are invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and discuss it with others if you wish. Ask us if there is anything that it is not clear or if you wish more information. Take time to decide whether or not you wish to take part.
Thank you for reading this.

FREQUENTLY ASKED QUESTIONS

WHAT IS THE PURPOSE OF THE STUDY?

The immediate purpose of this study is to investigate reflexes that follow stimulation of the ligaments in your knee.

The longer-term purpose is to understand how information from ligaments is used by the central nervous system. This should lead to a more scientific approach to coaching movement in sport and better rehabilitation of people with knee injuries.

WHY HAVE I BEEN CHOSEN?

You have been chosen because you are a healthy adult.

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part you will be given an information sheet and you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

WHAT WILL HAPPEN IF I TAKE PART?

You will have a series of wires taped on your skin to allow the activity of muscle in your leg to be studied. A second set of wires will deliver stimuli to the ligaments at the side of your knee.

You will be asked to stand for a few minutes and then to walk and then run at a moderate speed on a treadmill. Whilst you are standing or moving your ligament will be stimulated .You may feel this as a mechanical tap on your knee or as movement of the knee.

You will control the strength of the stimulation. You will be invited to increase it. The stimulation is not dangerous but it may feel uncomfortable at times. However, you will limit its strength and you can stop it at any time.

The muscle activity will be record on a computer for later analysis. Your anonymity is protected by using a serial number to name the file.

WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART?

The experiment is short and there is no significant additional risk associated with participation..
WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

The "risk" or "discomfort" to you is very small, if it exists at all. The gain is in terms of a better understanding of how movements are controlled.

WHAT IF SOMETHING GOES WRONG?

The chances of something going wrong are extremely small. All the procedure involved in this study are very low risk.
In the unlikely event that you are harmed due to someone's negligence, you may have grounds for a legal action, but you may have to pay for it.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

All the information collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the University will have your name and address removed so that you cannot be recognised from it.

WHO HAS REVIEWED THE STUDY?

This study has been reviewed and approved by the Research Ethics Committee.

CONTACT FOR FURTHER INFORMATION

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting either:

Dr Seyed Mohsen Rahimi

Tel: 0141 330 6197 E-Mail: 0223723r@student.gla.ac.uk

Dr Ron Baxendale

Tel: 0141 330 5344 E-Mail: R.Baxendale@bio.gla.ac.uk

You will be given a copy of this information sheet and a signed consent form to keep for your records.