Bi-articular muscles and their control of activity at the knee

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The candidate confirms that the work submitted is her own and that appropriate credit has been given within the thesis where reference has been made to the work of others.

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Abstract

This thesis addresses the role of biarticular muscles in the control of the knee joint during a static isometric and a dynamic balance task, and the question of whether these can be affected with training. Thirty-nine healthy participants participated in this study (F=17, M=25.46yrs±4.15): 17 in the static task (F= 8, M=24.29yrs±2.62), and 22 in the dynamic task (F = 9, M=26.23yrs± 4.69). Surface EMGs were recorded from multiple muscles at the knee and ankle of the right leg. The prevailing assumption during these tasks is that the anatomical position of the muscle underpins its activity, so its contribution to control is well-defined at all times. The agonist-antagonist interactions at the joint, aid maintenance of an upright posture. For example, at the knee joint it is the interaction between the flexors (semitendinosus (ST) and bicep femoris (BF)) and extensors (rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM) and vastus intermedius (VI)), together with the ankle flexors (tibialis anterior (TA)) and extensors (soleus (SL), lateral gastrocnemius (LG) and medial gastrocnemius (MG)). During a dynamic task, used to examine and restore balance, muscles at the ankle and trunk are assumed to be most involved in maintaining balance. My findings suggest that the biarticular muscles of the knee are involved significantly in both static and dynamic tasks, as well as when balanced, although there was an overall increase in activity in the ankle muscles. With training, these were found to be more involved, suggesting rehabilitation focussed on the knee muscles may speed recovery of balance. My data suggests further research is necessary to not only establish the role of the muscles acting at the knee during common tasks such as sit-to-stand, posture and walking but also in rehabilitation.

Contents

List of Abbreviations

sEMG	Surface Electromyography
RF / RFr	Rectus Femoris / Right rectus femoris
VL/ VLr,	Vastus Lateralis / Right vastus lateralis
VM/ VMr	Vastus Medialis / Right vastus medialis
TA/TAr	Tibialis Anterior / Right tibialis anterior
BF/BFr	Biceps femoris / Right biceps femoris
ST/STr	Semitendinosus / Right semitendinosus
LG/LGr	Lateral Gastrocnemius / Right lateral gastrocnemius
MG/MGr	Medial Gastrocnemius / Right medial gastrocnemius
SL/SLr	Soleus / Right soleus

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Chapter 1. Introduction

1.1 General introduction to motor control

Motor control is the study of the nature and cause of movement (Shumwaycook et al., 1987). Motor control is comprised of both biomechanical and neuromechanical aspects, and in this study, it was found that changes in muscle activity and associated movement are caused by neuromechanical rather than biomechanical events.

A significant proportion of our understanding of motor control is based on experimental evidence that is either purely kinematic or the recording of activity from a few select muscles that are anatomically defined as agonists and antagonists at a joint. In this thesis, we will explore how the muscles at a joint interact with other muscles during normal activity in healthy participants in a simple, currently-used, clinical tests.

The focus will be on the interactions between the muscles of the knee. This is the largest joint, which is essential for the performance of tasks in active daily living. The knee is classified as a modified hinge joint (Bohannon and Smith, 1987), with an ability to both flex and extend, thus acting in three main tasks: walking, stabilizing the posture and sit-to-stand. Without these normal functions of the knee, basic tasks become difficult. For example, amongst patients with post-stroke spasticity, the knee joint muscles are the most affected (37%) (Martin et al., 2014).

Normal functioning of the knee involves both the musculoskeletal and nervous systems, as observed in post-spasticity stroke patients with a significant neurological loss of the descending control pathways, who exhibit motor deficits such as knee hyperextension or knee stiffness.

Isometric knee extension is routinely used as a clinical test to assess the function of the lower limbs of patients and is graded by the examiner as poor, fair or good (Kendall and Kendall, 2005). However, there is no prescribed position for the knee to be placed in when these tests are carried out. This is likely to influence the local reflexes and interaction between the muscles.

Although the clinical test is an isometric task, the position of the knee during the extension is important in the Fugl Meyer test (Bohannon & Smith 1987), as muscles at the knee, are likely to be stretched in one position more than another, introducing sensory feedback which leads to altered muscle tone. This is because the movement is the product of coordinated and reflexive muscle activity, and is controlled by descending and sensory inputs (Sherrington, 1906). These local spinal reflexes are especially important to make a note of, as after injury, be it peripheral or central, these reflexes are severely modified.

1.2 Motor deficits and motor assessment

Knee extension is one of the crucial tasks such as sit-to-stand and standing balance. It can be performed by a knee extensor such as rectus femoris. Both flexor and extensor function as a muscle synergy controlled by supraspinal and proprioceptive afferent inputs. Normal movement is taken for granted by most until it is disrupted; we rarely think about how we walk or control the lower limbs. It is only when movement becomes difficult that we begin to think about how the joints are controlled, and how daily tasks can be performed smoothly. Spasticity is a major problem that affects the muscles at the knee, thereby disrupting walking and basic routines such as sit-to-stand and altering daily life. Consequently, it is a social and economic burden on both the subject and society (Payne et al., 2002a, Payne et al., 2002b). Over £100 billion is spent every year on the improvement of motor function in patients suffering from strokes and spinal cord injury. Direct costs for patients with post-stroke spasticity are approximately four times that of stroke patients without spasticity (Lundstrom et al., 2010). Spasticity is commonly evaluated by clinicians by assessing muscle tone in the quadriceps muscles, without any specification for the position of the knee.

Currently, spasticity measurement systems are subjective examinations, for example, the Modified Ashworth Scale, which rely on therapists scoring the spasticity. For reproducibility, a more quantitative measurement scale would be better. However, to introduce such a scale, knowledge of normal muscle interaction at the knee is necessary.

1.3 Benefits of a quantitative evaluation

1.3.1 Multiple treatments.

Spasticity is treated with multiple therapies such as Botox injection, passive stretching, and electrical stimulation. These are not always effective in improving motor outcome. In addition, it is expensive when multiple therapies are applied at the same time for the same patient.

1.3.2 Tailored treatment.

If we can classify the patients using more objective and quantitative measures, we will have a diagnosis that is more specific, allowing the therapy to be more specifically tailored to the individual.

1.3.3 Improving current practice.

An improved quantitative scale based on muscle activity will provide a uniform scale that can be used universally by clinicians without subjective bias. This will provide the basis for the stratification of therapies, which are then likely to be more effective, as the source of the problem should be identified more accurately. In this way, it will be possible to improve motor outcome with rehabilitation. Essentially, the more specific the diagnosis is, the more effective the recovery will be.

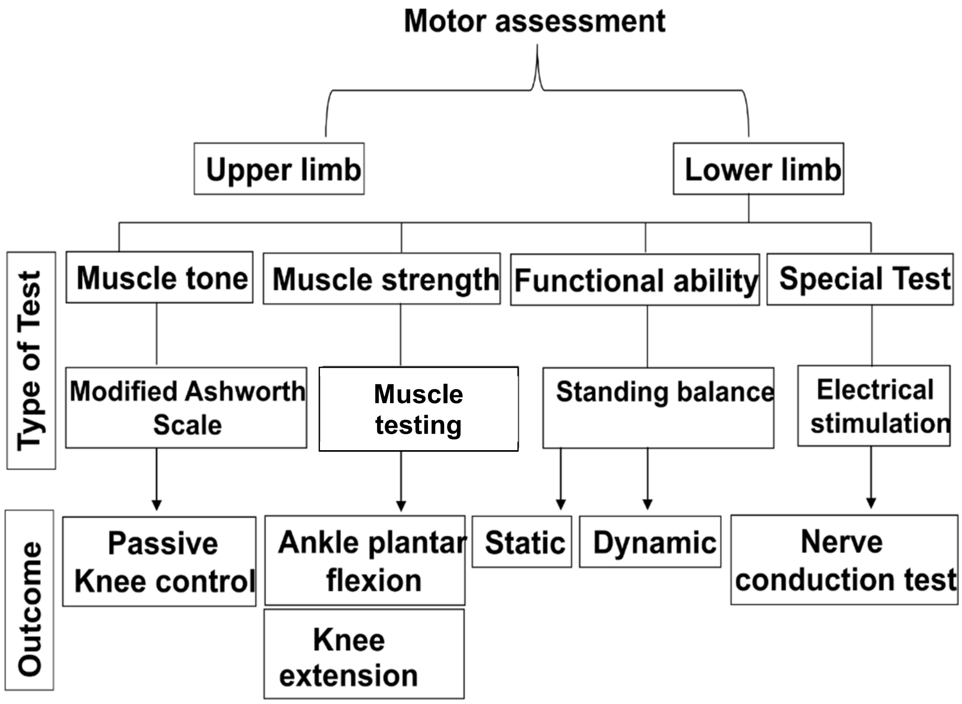

Figure 1-0-1 The assessment of lower limb function. Adapted from Bohannon and Smith (1987).

In order to observe the progression of motor deficit in patients such as stroke patients, clinicians utilize motor assessment guidelines to examine motor outcome, as shown in figure 1.1 (Bohannon, 2007, Bohannon and Smith, 1987). The motor outcome assessment (figure 1.1) can be divided into two aspects:

upper limb ability and lower limb ability. Lower limb ability is the focus of this thesis. There are four tests physiotherapists use in the clinic to assess the lower limb:

1) A muscle tone test, whereby lower limb muscle tone is scaled using the Modified Ashworth Scale, or MAS (grade 0 = no increase in muscle tone; grade 1= slight increase in muscle tone; grade 2 = more marked increase in muscle tone through most of the range of motion; grade 3 = considerable increase in muscle tone, with passive movement difficult; grade 4 = affected partial rigid in flexion or extension. An example of motor deficit affecting the knee is spasticity, the measure of a muscle's resistance towards the passive movement of the joints by the examiner (Duncan et al., 1985, Bohannon, 2007, Bohannon and Smith, 1987).

2) Muscle testing, where the Medical Research Council (MRC) scale is used to examine muscle strength (grade 0 = no muscle contraction, grade 1= trace of contraction, grade 2= active movement with gravity eliminated, grade 3= active movement against gravity, grade 4 = active movement against gravity, grade 5= normal strength).

3) A functional ability test, to test the activities used in daily life, using many tasks such as sit-to-stand, walking and dynamic balance. Functional ability is graded using the Fugl Mayer scoring system, which is sub-graded into three levels: poor, fair and good (poor = patients are not able to do the task, fair = patients are able to do the task with minor assistance, and good = patients are able to do the task completely without assistance.

4) in certain cases, a special test is required to assess nerve function, so electrical stimulation must be added to the lower limb ability test.

In terms of the outcomes of the four tests, five outcomes are relevant to this study. Firstly, for the muscle tone test, a passive knee control position is used to assess knee ability in study 1 (chapter 2). Secondly, in the muscle strength assessment, the knee extension outcome of the quadriceps muscle strength test is used in study 1 (chapter 2). Thirdly, ankle plantar flexion or ankle dorsiflexion is an example of lower limb muscle testing used to assess soleus muscle ability that I will refer to in study 2A in chapter 3. Fourthly, dynamic standing balance is used in study 2A to test the lower limb controlling balance. Before patients are discharged from the hospital, they must have good balance, both static and dynamic; therefore, dynamic balance is important in training patients to walk independently. This dynamic balance outcome is used in study 2A and will be referred to in detail in chapter 3. Finally, for the nerve conduction velocity outcome, the nerve conduction velocity test has been adapted for use in study 2B to assess the spinal reflex. In study 2B, electrical stimulation will be applied to assess the peripheral nerve function outcome, to assess the pathways involved in lower limb muscle activity. The lower limb assessment in this thesis mainly uses clinical tests. It is proposed that electrophysiological studies and clinical tests are integrated to improve lower limb assessment in rehabilitation in the future. For example, both the MAS scale and the MRC scale are qualitative scales that are normally used in the clinic. However, they should be revised according to suggestions for a more quantitative scale to improve reliability. Regarding the diagram in figure 1.1, clinical tests such as passive knee control, isometric knee extension, dynamic standing balance and electrical stimulation were applied as per the methods section. This is because the clinical tests and scales can be improved in the future when we know the basic neural pathways and muscle interactions better. The topics discussed will be divided according to the diagram (figure 1.1): neurophysiology and pathways control the lower limb, anatomy of the knee, knee range of motion, isometric assessment and dynamic standing balance.

1.4 Neurophysiology: modulation circuits and pathways

To smoothly coordinate lower limb muscle activity, the nervous system needs two types of information about the current state of a muscle. First, it needs to know the amount of tension in the muscle (Ia) and its associated tendons (Ib). This information is provided by the Golgi tendon organs. Second, it needs to know the length of the muscle. These two types (Ia and Ib) of proprioceptors play an important role in spinal reflexes, and also provide essential feedback to the cerebrum and cerebellum (Eccles and Lundberg, 1957). In this chapter, the diagram of the neurophysiology shows the muscle spindle and Golgi tendon and circuits (figure1.2). The nervous system enables the body to respond to continuous changes in its external and internal environment. It controls and integrates the functional activities of the organ systems. Anatomically, the nervous system is divided into the central nervous system (CNS) and peripheral nervous system (PNS). The CNS consists of the brain and spinal cord. The peripheral nervous system consists of the cranial nerve, spinal nerve and peripheral nerves that conduct impulses from (via efferent or motor nerves) and to (via afferent or sensory nerves) the CNS (Eccles, 1957, Davis, 1961)

The functional units of the nervous system are the neurone, which is divided into three categories: 1) sensory neurons, 2) motor neurons and 3) interneurons(Cleveland and Hoffman, 1991).

1) Sensory neurons convey impulses from the periphery to the CNS (Todd, 2010). This thesis focusses on the proprioceptive pathways from the muscles and tendon organs to provide the CNS with information related to the body and limbs.

2) Motor neurons convey impulses from the CNS or ganglia to effector cells. Somatic efferent neurons send voluntary impulses to skeletal muscles (Rothwell, 1994).

3) Interneurons form a communicating and integrating network between the sensory and motor neurons (Jankowska, 1992).

1.4.1 Motor innervation

Skeletal muscle fibres are richly innervated by motor neurones that originate in the spinal cord or brain stem. The axons of the neurons divide as they near the

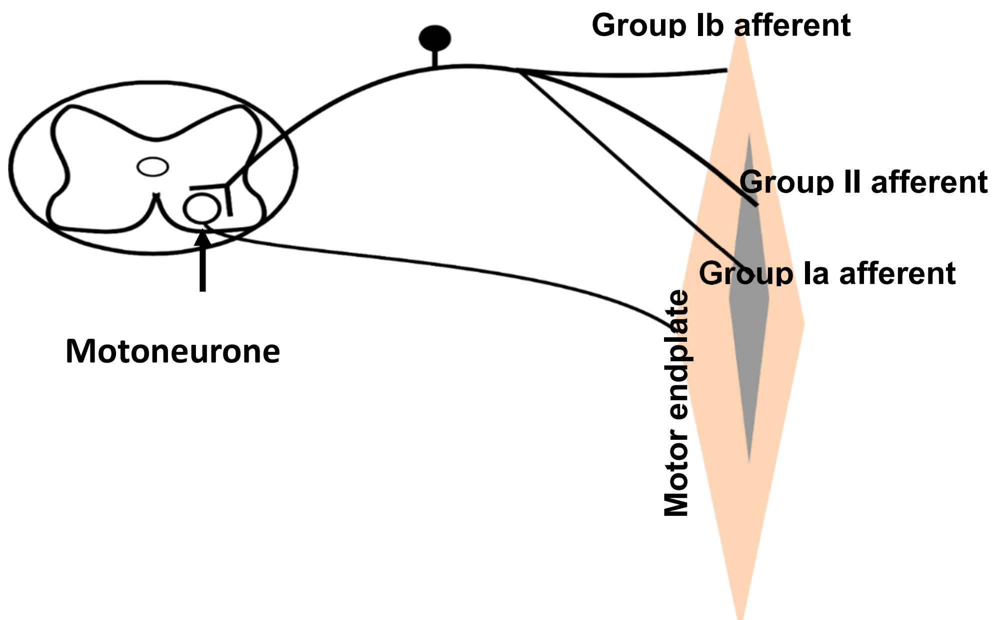

Figure 1-0-2 Diagram of proprioception showing motor innervation and sensory innervation. Muscle spindle is connected in parallel with extrafusal muscle. Group Ia (muscle spindle primary) conveys signals from the muscle spindle, Group Ib conveys proprioception from the tendon, and Group II conveys signals from the muscle spindle.

muscle, giving rise to many branches that end on individual muscle fibres. The neuromuscular junction (motor end plate) is the contact made by the terminal branches of the axon with the muscle. The end of the axon ramifies into several end branches, each of which lies in a shallow depression on the surface of the receptor region of the muscle fibre. The axon ending is a typical presynaptic structure with synaptic vesicles containing neurotransmitters (acetylcholine) (Eccles et al., 1954). The release of acetylcholine into the synaptic cleft initiates depolarization of the plasma membrane, leading to muscle contraction (Heuser et al., 1979).

1.4.2 Sensory innervation

Encapsulated sensory receptors in muscles and tendons provide information about the degree of tension in a muscle, and its position. The muscle spindle detects the degree of stretch in a muscle (Banks et al., 2009).

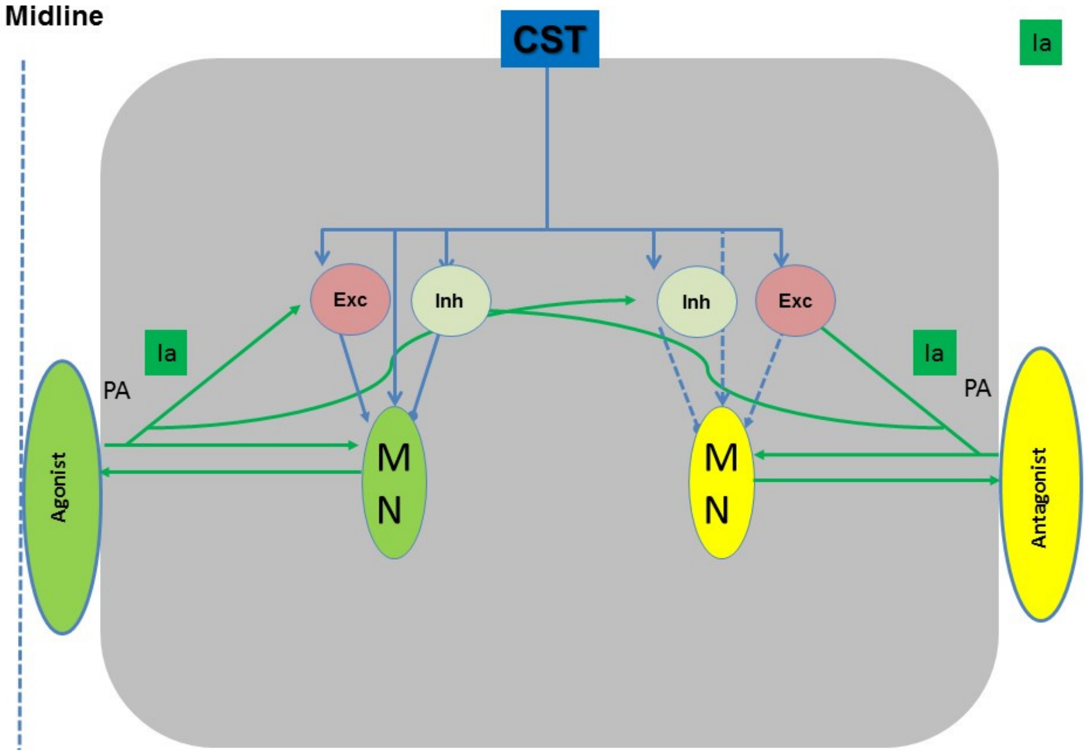

Figure 1-0-3 Primary muscle spindle afferent (Ia) schematic for the simplest healthy spinal circuit for control of agonist and antagonist muscles, under modulatory control of descending corticospinal tract (CST) and peripheral input (PA). This represent s specifically the role of Ia afferents as output modulators. Green represents all interactions associated with PA and blue represents all related to CST. Inh = inhibitory interneuron, EXC = excitatory interneurone, MN = motoneurone

The sensory (afferent) nerve fibres carrying information from the muscle spindle (Eccles and Lundberg, 1957). In addition, spindle cells receive motor (efferent) innervation from the spinal cord and brain via gamma (γ) efferent nerve fibres (Eccles, 1957, Granit et al., 1957), which are thought to regulate the sensitivity of the stretch receptor. When skeletal muscle is stretched, the nerve endings of sensory nerves become activated. They convey their impulses to the CNS, which in turn modulates the activity of motor neurons innervating that muscle (Burke et al., 1976). Tendon organs are found in the tendons of muscles and respond to stretch (PierrotDeseilligny and Burke, 2012) (figure 1.2).

1.4.3 Length feedback and muscle spindle apparatus system.

1.4.3.1 Muscle spinal primary afferent

Muscle spindle afferents (Ia) convey information about length changes from striated muscles. Ia fibres project to homonymous motoneurons directly (monosynaptic) and contact excitatory and inhibitory interneurones (polysynaptic) within the spinal cord. The inhibitory interneurones cause reciprocal inhibition of the antagonist muscles. Thus, during homonymous contraction, the collateral Ia branch inhibits heteronymous muscle (Baldissera et al., 1998) (figure 1.3).

1.4.3.2 Golgi tendon organ afferents

Disynaptic Golgi tendon organ afferent (Ib) reciprocal inhibition has been extensively studied in animals and humans (Sherrington, 1906, Lloyd and Chang, 1948, Eccles et al., 1956b, Eccles et al., 1956a, Eccles and Lundberg, 1957, Hultborn et al., 1971a, Hultborn et al., 1971b). There are a number of studies of the ankle but only a few of the knee (Bayoumi and Ashby, 1989), and due to lack of evidence some clinicians presume that reciprocal occurs between every agonist and antagonist muscle pair (Hamm and Alexander, 2010, Aslam et al., 2009, Kudina, 1980, PierrotDeseilligny and Burke, 2012) (figure 1.3).Tendon organ afferents (Ib). During isometric contraction, the corticospinal tract of the motor and the supplementary cortex excite motor pools in the spinal cord (Salenius et al.,1997), which later mediates signals via the femoral nerve to respective muscles as the final target outputs (Burke et al., 1979). In isometric knee extension, the rectus femoris length remains constant, but the muscle tone is changed. In this situation, we can assume that Golgi tendon (Ib) afferent may be involved. Ib afferent is active working when the tendon is stretched due to extreme movement of the joint. When you stretch the joint, Ib will directly affect the joint tendon. Ib’s activity seems to be the exact opposite of those of the Ia fibres. It may be that their activity is facilitating the Ib interneurons. If so, the consequence of this will be the decrease in the agonist muscle force (Rothwell, 1994). In addition, the excitatory effects of Ib interneurons on antagonist muscle may also reduce the speed of muscle contraction.

1.4.3.3 Muscle spindle secondary afferent

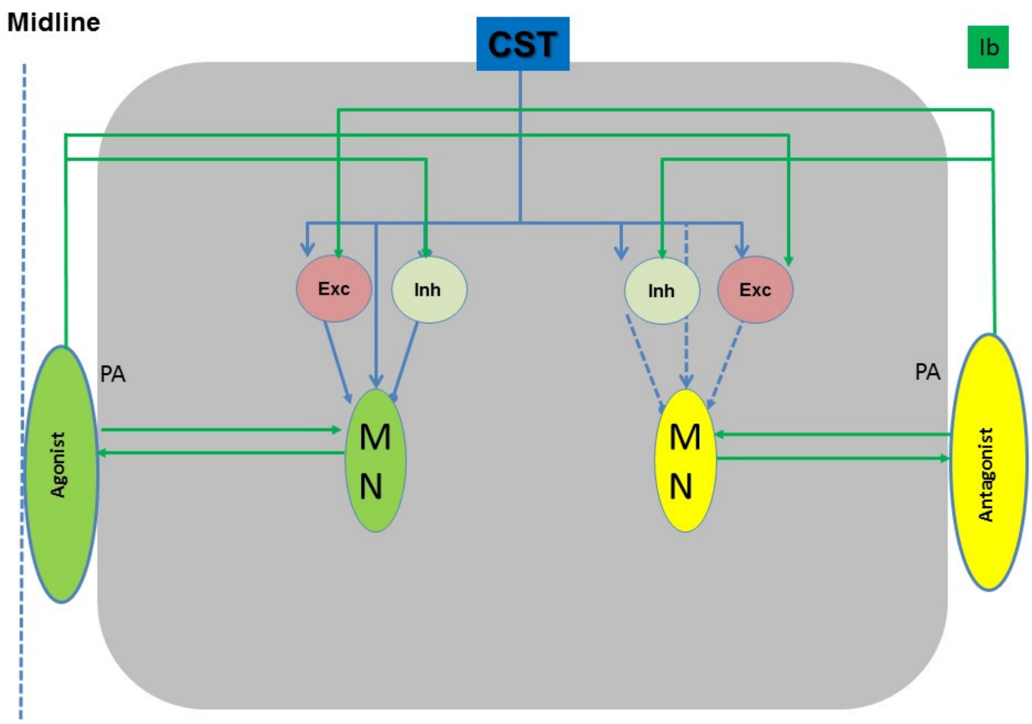

Figure 0-4 Golgi tendon organ afferent (Ib): schematic for the simplest healthy spinal circuit for control of agonist and antagonist muscles, under modulatory control of descending corticospinal tract (CST) and peripheral input (PA). This represent s specifically the role of Ib afferents as output modulators. Green represents all interactions associated with PA and blue represents all related to CST. Inh = inhibitory interneuron, EXC = excitatory interneurone, MN = motoneurone

Mathews suggested that secondary muscle spindle contributes to the inhibition of the extensor muscles (Matthews and Stein, 1969). In a study on humans, Roujeau and colleagues tested the heteronymous spindle afferent secondaries (group II) in the semitendinosus muscle by recording surface electromyography (sEMG) and placing electrical stimulation on the tibial nerve to examine the latency response (Roujeau et al., 2004). Their results suggested that the peak of the reflex can be found at 45 ms (Simonetta-Moreau et al., 1999).

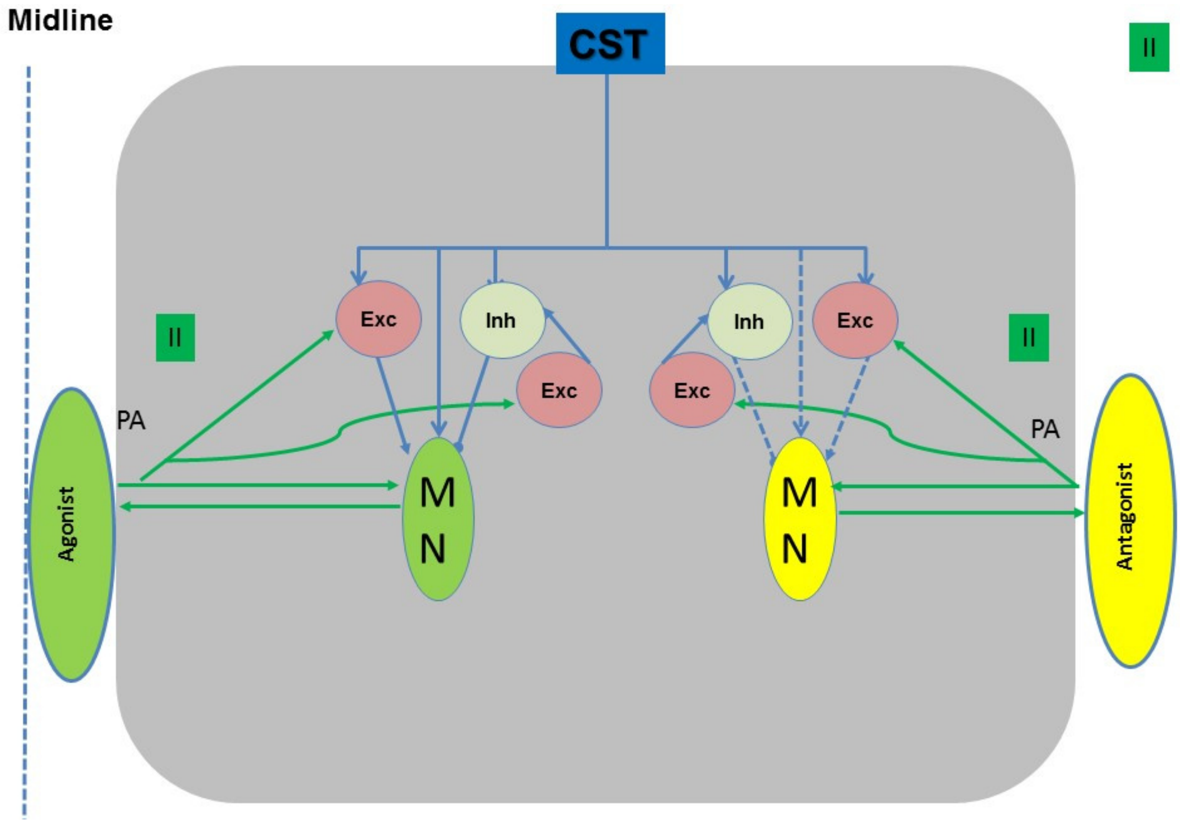

Figure 1-5 Group II secondary muscle spindle: Primary muscle spindle afferent (Ia) schematic for the simplest healthy spinal circuit for control of agonist and antagonist muscles, under modulatory control of descending corticospinal tract (CST) and peripheral input (PA). This represents specifically the role of Ia afferents as output modulators. Green represents all interactions associated with PA and blue represents all related to CST. Inh = inhibitory interneuron, EXC = excitatory interneurone, MN = motoneurone

1.4.3.4 Recording Muscle activity using electromyography

To record muscle activity via the skin surface, electromyography is widely used. The surface electromyography technique (EMG) is used to record muscle activity and detect short and long latency.

EMG is a neurophysiological technique used in both the clinic and the research environment. Currently, both invasive and non-invasive methods are used to record EMG to evaluate the neuromuscular function in both healthy individuals and those with motor deficits. In this study surface, EMG (sEMG) was used as it is convenient to use, especially when recording from multiple muscles simultaneously, and especially during dynamic tasks. This is in addition to the fact that subjects prefer this too invasive EMG recordings.

1.4.3.4.1 What is an EMG recording?

An EMG as presented by Basmajian and de Luca (Basmajian and De Luca, 1985) is shown in figure 1.7. sEMG is recorded from muscle fibres which are under the sensors, placed over the motor endplate. The recordings are a summary of the

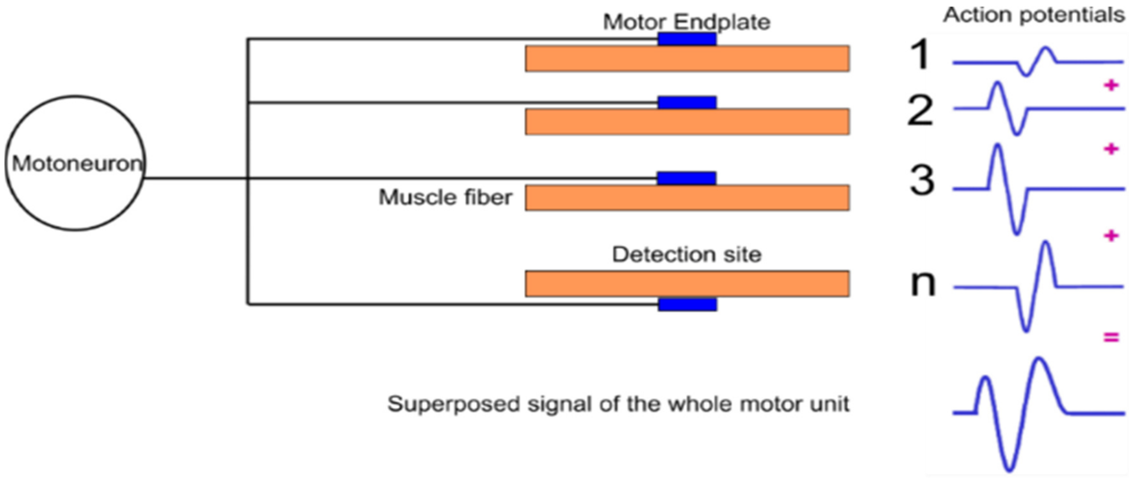

Figure 1-6 EMG signal recording from motor unit and accumulated signal from muscle fibres (adapted from Basmajian & Deluca, 1985)

effect of the activity of the fibres and not of a single motor unit. The myoelectric signals from several motor endplates are condensed into a triphasic motor unit action potential (MUAP). The depolarization-repolarization cycle produced at the neuromuscular junction endplate on the skeletal muscle fibre generates a depolarization wave which travels along the muscle fibre. There are two mechanisms that can modify the density of the signal: the recruitment of more MUAP, and their firing frequency.

1.4.4 Latency response (table 1.1)

1.4.5 Long latency

Hammond (Hammond, 1955) was the first to explain the long latency responses of about 100 ms or more. In the simple tendon jerk reflex, Rothwell suggested that under the proprioceptive feedback conditions, voluntary contraction can be as short as 90-100 ms. Two components can be involved in the long latency stretch reflexes: 1) Group II or secondary muscle spindle, and 2) the trans-corticospinal tract which travels up to the sensorimotor cortex and back to the spinal cord via the corticospinal tract (Rothwell, 1994). Feedback control, including long latency responses, also adapts to context demands as learning occurs (Pruszynski and Scott, 2012). In balance training, H reflex was suppressed by long latency, which could be via supraspinal control of presynaptic inhibition (Taube et al., 2006).

1.4.6 Short latency

To identify the origin of neural adaptation (spinal cord, brain stem or peripheral) latencies of the responses have been used to clarify the pathways.

The first peak is usually at 20-50 ms after the perturbation for a short latency (PierrotDeseilligny and Burke, 2012). Short latency response is mediated by peripheral and spinal pathways and has a voluntary response following the reflex in less than 100 ms. However, there is little evidence to confirm medium latency because it may be difficult to measure the brainstem and cerebellum.

Categories	Long latency	Short latency
Origin of reflex	Group II muscle spindle	Group Ia and Group II muscle spindle
Reflex post duration	>100 ms (Rothwell, 1994)	20-50 ms (Marchand-Pauvert et al., 1999)

Table 1-1 **Latencies to identify the origin of reflex evoked response divided into two categories: long and short latencies**

1.4.7 Anatomy of the knee

The knee is classified as a synovial joint, a weight-bearing articulation consisting of two mating members separated by articular cartilage, lubricated by synovial fluid enclosed in a fibrous capsule. Specifically, the knee consists of two articular joints: the tibiopatellar joint, and the tibiofemoral, which provides an articulation between the distal end of the femur and the proximal enlargement of the tibia. There are two cartilages, the medial meniscus and the lateral meniscus, providing shock absorption between the femur and tibia (Engin and Korde, 1974).

1.4.8 Anterior of the knee

The quadriceps consists of four parts: the rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM) and vastus intermedius (VI). The rectus femoris is in the middle of the thigh, is superficial, and takes a straight course down from the thigh to the knee. It is formed of two parts: 1) originating as a straight anterior tendon from the anterior inferior iliac spine, and 2) the reflected posterior tendon from the hip joint, which is close to the anterior capsule of the hip. The vastus lateralis is situated lateral to the rectus femoris, with its proximal origin in the greater trochanter of the femur bone. The vastus medialis is medial to the rectus femoris, with its proximal origin high up at the intertrochanteric line of the femur. The vastus intermediate is located beneath the rectus femoris. It is partially fused with the two other vastus muscles and its proximal origin is high up at the lesser trochanter of the femur. The distal insertion of these four parts of the quadriceps is fused as a common tendon of the quadriceps, or a patellar tendon, which is inserted into the patellar bone.

1.4.9 Posterior of the knee:

the hamstring has been found to be more important than the other muscles for knee function

This thesis focusses primarily on the leg and knee, discussing 1) the two muscles of the hamstrings, and 2) the gastrocnemius.

Hamstrings (semitendinosus, semimembranosus and biceps femoris)

The semitendinosus of the hamstring is a posterior muscle of the thigh, which is located medial to the thigh, originating from the ischial tuberosity of the hip bone. The semitendinosus can be easily palpated when the participant is facing down on the bed with resisted knee flexion. The muscle belly is to the medial posterior of the thigh, and the examiner can trace it by using the fold of the semitendinosus tendon.

The semimembranosus is another of the hamstrings, which is located beneath the semitendinosus. It is not easily palpable as an individual muscle because it is covered by the semitendinosus. The insertion of the hamstring is the medial condyle of the femur.

The biceps femoris. The long head and short heads of the biceps femoris are located at the lateral posterior of the thigh. They flex and laterally rotate the knee joint. In addition, the long head extends and assists in the lateral rotation of the hip joint.

Gastrocnemius. The two heads (medial and lateral) of the gastrocnemius begin above the femoral condyles and cover the knee joint on the posterior of the knee. It terminates at the Achilles tendon at the ankle joint. The function of both muscles is knee flexion.

1.4.10 Range of motion

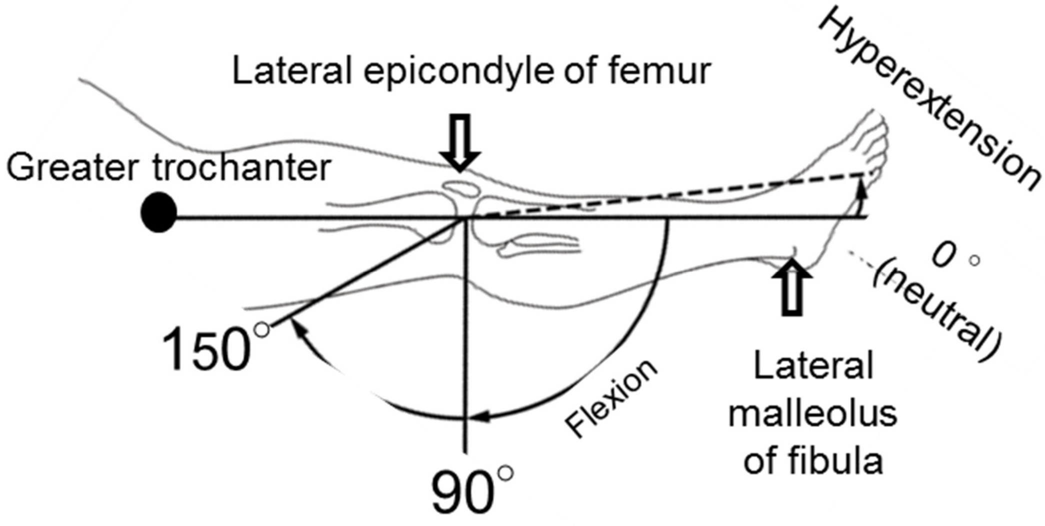

Figure 1-7 Range of motion and bony landmarks used to measure knee flexion/ extension

The knee movements can be flexion, extension, gliding or minor rotation. Both flexion and extension involve movement along the sagittal plane of movement. Extension of the knee is its movement in the anterior direction to a straight position, with the alignment of the thigh and lower leg such that the extended knee is in zero position, locked to prevent any rotation, and the thigh fixed, while flexion is a movement in the posterior direction. From the zero position of extension, the full range up to knee flexion is approximately 150º (Kendall and Kendall, 2005). In the clinic and my study 1, the motion of the knee joint is measured using a goniometer and a bony landmark is used to define the goniometer arm. As shown in figure 1.8, the proximal bony landmark is the greater trochanter, the centre of the goniometer is placed over the lateral

Knee position	Bony landmark to measure the knee angle			
Flexion/ Extension	Proximal reference	Centre		Distal reference
	Greater trochanter	Lateral epicondyle of the femur		Lateral malleolus
Range of motion and function				
Function	Allowable	Walking	Sitting	Climbing stairs
Range of motion	150 °/ -5°	70°/0°	100°-120°	70°- 90°

Table 1-2 Range of motion and knee function in activities in daily life

epicondyle, and the distal reference is placed over lateral malleolus. The hip is flexed when measuring full knee joint flexion to avoid restriction of motion by the rectus femoris, but the joint should not be fully flexed when measuring knee joint extension, to avoid restriction by the hamstring muscles. Lateral-rotation is a rotation away from the midsagittal plane. Medial-rotation is a rotation of the anterior surface of the leg toward the midsagittal plane. The table below (table 1.2) shows the range of motion (in degrees) at the knee during daily activities (Stewart and Hall, 2006). There are two main tasks to discuss here regarding their use as clinical tests and their application as a tool in this thesis: 1) isometric tasks 2) dynamic tasks.

1.5 Isometric tasks and maximal voluntary effort

In clinics, isometric procedures are used to test muscle strength and during rehabilitation. Knee isometric extension, as mentioned in the lower limb assessment (figure 1.1) is used as a task in study 1 to assess the activity of the quadriceps and hamstring. Isometric contraction can identify specific muscle weakness; therefore, developing muscle overload at the joint angle isometric knee extension is used to test quadriceps capability (Gandevia and McKenzie, 1988, Duchateau and Enoka, 2016). The isometric knee contraction defines muscle activation in which no observable change occurs in muscle fibre length. The firing rate of human muscle spindle afferents accelerates with pronounced irregularity during isometric contractions (Vallbo, 1970, Burke et al., 1976) in which the muscle gains tension but does not appreciatively modify its length. Furthermore, strength training can result in a greater activation of muscle, thus influencing strength production. Isometric exercise overloads the muscle and improves strength but offers only limited benefits compared to sports training. A manual activity test established by the Medical Research Council (MRC) of Great Britain is widely used for the prognosis of muscle strength (Paternostro-Sluga et al., 2008). However, little is known about the reliability and validity of the MRC scale in musculoskeletal and peripheral nerve lesions (Cuthbert and Goodheart, 2007). It is proposed in this study that maximal voluntary effort (MVE) be applied in all protocols because it can be used with neurological patients in the future. In addition, a maximal voluntary effort test (MVE), combined with the EMG technique is used in the experiments, because MVE can have a more quantitative scale. In isometric conditions, whether the neural activation generated by the CNS depends on muscle length or not remains an unsettled issue (Gandevia and McKenzie, 1988, Duchateau and Enoka, 2016). A static muscle contraction normally refers to a contraction where a person attempts to recruit as many muscle fibres in a muscle as possible to generate maximal force, often called maximal voluntary contraction (MVC), against a steady force. In the experiment, isometric or static exercises were used, and participants were asked to generate as much muscle activity as they could, as a voluntary muscle effort (MVE), without any resistance provided. Researchers generally use MVC, however, MVE was used in this study to ensure force feedback did not corrupt the muscle interactions. As Merton suggested, the term ‘effort’ is used to define an MVC, and removal of the force acting at the joint when they are making the effort should not eliminate any form of force feedback to the muscle that would modify the proprioceptive feedback (Merton, 1954). Participants were asked to perform isometric knee contractions while voluntarily contracting the rectus femoris during the task. This ensured consistency across subjects and angles, with similar recruitments in carrying out the task. In study 2A and 2B, MVC was used to examine the interaction between muscles during a soleus MVC.

Which are better: static or dynamic methods?

Isometric tests show the greatest strength improvement, while the dynamic task is a good way to apply resistance during a range of motion activities. A dynamic muscle action produces movement of the joint: for example, concentric and eccentric actions; for example, concentric (shortening) and eccentric (lengthening) muscle actions’ (Herzog, 2014, Edman et al., 1988, Morgan et al., 2000). The dynamic fusimotor neurons increase the response of the primary endings during the dynamic phase of stimuli, while the excitatory action of static neurons is dominant at a constant muscle length (Duchateau and Enoka, 2016, Enoka and Duchateau, 2016).

Review of the literature related to isometric and dynamic tasks

Antagonists of knee extensor muscles like the hamstring stabilise the agonists (quadriceps muscles). In 1996, Aagaard and colleagues published results regarding the capacity for joint stabilization by means of antagonist hamstring coactivation. They found that the knee seemed unstable if the hamstring muscles were not as strong as the quadriceps muscles. The hamstring/quadriceps strength ratio (H/Q) is typically 40-50% across velocity and contraction modes(Aagaard et al., 1996). Aagaard and Magnusson examined the H/Q strength ratio during knee isotonic contraction (eccentric) in a seated position (Aagaard et al., 1998). sEMG from the posterior of the thigh (biceps femoris, semitendinosus) and the vasti muscle at the front of the thigh were used for correlation analysis. They found that the activity of the biceps femoris was three times as great as that of the semitendinosus. These hamstring/quadriceps ratios are probably more indicative of the muscle grouping recruitment during a task.

1.6 Dynamic standing balance

In terms of rehabilitation weight-bearing exercise and performance, knee stability has to be tested before walking or stepping (Beutler et al., 2002). Neuromuscular control of the knee is important for lower limb balance control, especially dynamic strategies. Again, the response is focussed on the knee, while the control of posture is an adaptable feature of the motor system, depending on the sensory input and dynamic balance modulation output (Enoka and Duchateau, 2016, Sherrington, 1931). Sensorimotor control involves CNS control of movement, balance, posture and joint stability. Motor adaptation requires intact sensation: sensory system, visual, vestibular, somatosensory and proprioception (Franklin and Wolpert, 2011). Taube discussed balance training that can be used for rehabilitation, an ankle injury and postural deficits, and Taube believed that supraspinal and spinal pathways can be adapted to control balance (Taube et al., 2008a). Berg measured balance in the elderly, that is, the bipedal upright position controlled by the biomechanical (anatomy of whole body stability and segmental stability) and neurophysiological systems (Berg et al., 1992, Baudry et al., 2015). When standing upright, the body sways backwards and forward. Muscular activation prevents falls and represents automatic postural control activity (Dietz, 1992, Dietz et al., 1992).

The control of balance, along with proprioception reflexes, were described earlier; here the two pathways related to balance control are addressed: the vestibulospinal tract and the reticulospinal tract.

Evidence from human studies suggests that the vestibulospinal tract is for upright posture preservation, especially the extensor muscles for opposing gravity. However, the vestibulospinal tract does not facilitate voluntary movements dictated by the cerebral cortex: it is essential for such highly skilled accomplishments of motor coordination as the acts of an acrobat. The learning of these aspects of the skilled movement that involve posture and balance is affected by the vestibulospinal pathway. A caveat is that the vestibulospinal system excites extensor motoneurones and inhibits flexor motoneurones. The reticulospinal tract elicits both excitation and inhibition of flexor and extensor motoneurones (Lyalka et al., 2005). When we move, we are usually unaware of the complex neuromuscular process that controls our posture, but postural control is obvious enough when we accidentally fall or when disease damages parts of the postural system. The mechanical problem of maintaining posture is particularly challenging for upright bipeds (McCall et al., 2017). Balance training exercises are a challenge, and have been used to naturalise postural deficits, adapt motor skills and improve proprioceptive function (Taube et al., 2008a). Taube and colleagues studied the balance platform that could have central adaptions at multiple levels due to exercise. They found that increased co-activation of agonist and antagonist was related to improved joint stability. Studies have demonstrated the preventive effects of learning and training with respect to ankle and knee joint injuries. The main concern for a beginner in a motor task is to master the multiple and redundant degrees of freedom involved. Briefly, balance training is not only applied to rehabilitation and prevention, but also to improving motor performance, especially muscle power in the elderly (Taube et al., 2008a, Burdet et al., 2001). When participants perform balance control, Kiers and colleagues claimed that gastrocnemius-soleus may be important, while lumbar muscles receive more impact while standing on hard surfaces (Kiers et al., 2012). During the performance of tasks, the spinal cord can change the skills. After learning, muscle activity can be shifted, the H reflex may be greater or lesser, and training can affect the corticospinal tract (Wolpaw, 2007b).

1.7 The role of synergies

Synergy is where the whole is greater than the sum of its parts. Muscle synergists are muscles working together towards a single task. There are two views about synergy (figure1.7): fixed, and flexible (d'Avella et al., 2003). The synergy is fixed

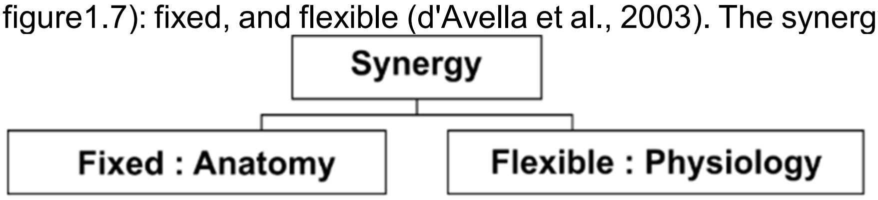

Figure 1-8 Diagram showing concept of fixed and flexible synergies, adapted from d’Avella 2003.

where a muscle group is predetermined, and each synergy is, therefore, simplifying the control of the process. In contrast, the flexible view is that there is no limit to the possible combinations of muscle activation patterns; each muscle is independently controlled (Cheung et al., 2012).

It has long been proposed that muscle co-activation during a task, commonly called muscle synergies are recruited by the cortex as motor primitives. This view of recruitment of primitives refers to the concept of a fixed synergy, proposed by neuroscientists to explain the control of muscles directly by the brain, often considering all things downstream like the central pattern generators in the spinal cord as primitives. Primitives were defined as the elements required for generating a stable mechanical response during motor behaviour. Muscle synergy has been studied in various vertebrates, for example, frogs, rats, cats and humans (Mussa-Ivaldi and Bizzi, 2000, Mussa-Ivaldi et al., 1994). Giszter and Cheung have proposed that the observed muscle synergies have a neuronal substrate and is hence related to neural coordination (Giszter et al., 1993, Cheung et al., 2012).They examined this in frogs, by stimulating the spinal grey matter and recording the evoked isometric forces acting at the ankle. Emergent patterns were considered to be the result of interaction between the mechanics of the limb and the contractions of muscle groups activated by microstimulation of the spinal cord. Using cluster analysis, they defined the patterns of synergy providing an insight into organsition of the frog's spinal cord for producing multi-jointed movement. Some of the frogs exhibited changes in the evoked force fields, which were likely due to modulation by either the descending or the afferent inputs. With respect to governance of the movement, the elastic properties of the muscles provided instantaneous correcting forces when a limb was moved away from the intended equilibrium. When the brain has accomplished the ability to control equilibrium, it can also master movements as temporal sequences for such postures. The use of an equilibrium-point strategy may be a characteristic of these spinally generated motor programmes within the frog's spinal cord. Some of these force fields clearly underlie phases of natural behaviours. These primitives are used in pattern generators and other spinal behaviours and are recruited by descending control systems to produce adjustable behaviours (Giszter et al., 1993).

An alternative approach to explain motor control proposes a combination of the theory of primitives and rather flexible control of muscle activity for gross motor skills. Bernstein (1967) proposed that the primary concern for a beginner in a motor task is to master the multiple and redundant degrees of freedom potentially involved (Caillou et al., 2002). Given an example from my study 2A dynamic balance board, participants tried to maintain balance using different groups of muscles. According to Bernstein's degrees of freedom problem (Bernshteĭn, 1967), or the motor equivalence problem in motor control, there are multiple ways for humans or animals to perform a movement in order to achieve the same goal. In other words, under normal circumstances, no simple one-to-one correspondence exists between a motor problem (or task) and a motor solution to the problem. For example, post-training there was a reduction in the number of observed muscle synergies recruited amongst the subjects to achieve balance in study 2A (chapter 3). However, it is still not established which variable, or a set of variables define the upright stance in these healthy participants. This is partly what Bernstein suggested as the broader issue that the DoF is introduced by the levels of variability within coordination, control and skill involved in performing a motor act. Degree of freedom (DoF) problem is simply put, related to the motion possibilities of rigid bodies. Kinematic definition for DoF for any system or its components can thus be "the number of independent variables or coordinates required to ascertain the position of the system or its component.

The flexibility to select different synergies to carry out the same task is possibly an evolutionarily conserved function, necessitated by the organisms necessity for survival (Liu et al., 2018). Principles of sensorimotor integration during postural control requires perception and corrections.(Liu et al., 2018, Horak et al., 1997, Shumwaycook et al., 1987). It has been suggested that the central nervous system, not only controls the output of the muscles, but also the degrees of freedom of the joints, often using the signal from the effector which converges within the spinal cord to produce movement (Todorov, 2004).

Given example my study 2A-standing balance , it has been suggested by Horak and Shumwaycook that the postural control used both sensory feedback and perception.

Therefore, it is not only nervous system control like as the fixed synergies view, but DoF become a key role. Why did we think about DoF? Because DoF gives us an idea about how to correct the posture by reducing the degree of freedom. For example, at the beginning, the participants used antero-posterior and medio-lateral direction to control balance, but after training, they used only antero-posterior. DoF suggested that muscle adaption and the sensory feedback improve the behavioral performance.

The sensorimotor system is a product of evolution, development, learning and adaptation—which work on different time scales to improve behavioural performance. Consequently, many theories of motor function are based on 'optimal performance': they quantify task goals as cost functions and apply the sophisticated tools of optimal control theory to obtain detailed behavioural predictions. The resulting models, although not without limitations, have explained more empirical phenomena than any other class. Traditional emphasis has been on optimizing desired movement trajectories while ignoring sensory feedback. Recent work has redefined optimality in terms of feedback control laws and focused on the mechanisms that generate behaviour online. This approach has allowed researchers to fit previously unrelated concepts and observations into what may become a unified theoretical framework for interpreting motor function. At the heart of the framework is the relationship between high-level goals, and the realtime sensorimotor control strategies most suitable for accomplishing those goals. However, the means by which these signals converge to produce an effective motor outcome is still not well understood. Muscle synergy can be a group of muscles that are active during the task. For example, the postural control study by Torres-Oviedo and Ting found that the set of multi-joint muscle synergies interactions between the hip, knee and ankle play an important role in postural control (Torres-Oviedo and Ting, 2007). Having examined activity at multiple joints and their associated muscles during different movements, they suggested that the synergy used is neuronally controlled and is based on the differing and often independent onset and duration of activity on these muscles (Ting, 2007).–Similar, fixed time associations amongst muscles within each muscle synergy has been reported too but using some very different mathematical tools (d'Avella et al., 2003), (Sartori et al., 2012).

Muscle synergies can be used in rehabilitation approaches, either to support or to inhibit the use of basic movement patterns. This may offer the clinician an ability to target the source of the drive and its modulation from within the neural structures underlying motor behaviours; thus a better handle for affecting the motor deficits and thus rehabilitation. Improved diagnosis would lead to more personalised treatment of a patient’s deficits (Safavynia et al., 2011).

1.8 Hypothesis

The clinical tests are used as the task in this thesis to better understand muscle interaction and to improve clinical scales, such as the modified Ashworth scale, (scoring system) in the future. In physiotherapy clinics, lower limb assessment makes use of both isometric and dynamic tasks, and clinicians normally select tests based on anatomical fundamentals, like muscle origin and insertion. However, both the position and the interaction of multiple muscles have been ignored. At the knee, the single joint at the anterior of the knee is comprised of the three vasti of the quadriceps, with proximal origin only at the femur bone, so they act only in knee extension. At the posterior of the knee, the short head of the biceps femoris (which is difficult to palpate) and the popliteus are one joint muscle, and these two can only perform knee flexion. In terms of multi-joints, the rectus femoris is a two-joint muscle functioning in both hip flexion and knee extension, and at the posterior of the knee, there are the biceps femoris long head semitendinosus and semimembranosus (knee flexion and hip extension) and the gastrocnemius (knee flexion and ankle plantar flexion). Day (1987) stated that if the lower limb position changes, the sensory input may be shifted. Therefore in this thesis, it was speculated whether the muscle activity at varied knee flexion angles might have different outcomes or not (Day et al., 1987). Priliutsky and Zatsiorsky (2002) stated that bi-articular muscles perform multiple functions during the same task. This study hypothesised that bi-articular muscles, especially at the knee joint, are important for both static and dynamic tasks (Prilutsky and Zatsiorsky, 2002). The knee is the largest joint in the body, and it is important to control multiple tasks in the activities of daily life, especially walking and standing balance. This has been addressed in this thesis by testing the role played by the bi-articular muscles that control the knee during defined passive and dynamic tasks. According to Wolpaw (2007), motor adaptation involves the mechanisms of the brain and spinal cord. Dynamic standing balance training was used in one of the dynamic tasks, as it is associated with changes in spinal cord reflexes (Wolpaw, 2007b). It was expected in this study that the peripheral pathways might be involved in balance training, and that muscle around the knee may be affected after training.

1.9 Aims

1.9.1 Study 1: Role of bi-articular muscles during a passive task.

The role of the bi-articular muscles and their relative contributions were examined under two controlled but variant conditions: 1) knee extension angles, and 2) limb position in relation to the hip. Using EMG recorded across multiple muscles activity in the muscles was compared at four knee angles for two different positions, which were adapted or modified: 1) Fugl-Meyer, 2) Thomas test.

Study 2: Aim1 (2A): the role of the muscles at the knee during a dynamic balance task was examined using EMG and balance training technique.

Aim 2 (2B): An exploration of the use of training to observe improvement in the ability to use these muscles, to be developed for use in clinical settings for improved balance and posture. EMG combined with electrical stimulation techniques were used to exam the reflex changes using balance training.

Chapter 2 Quadriceps muscle compartments are not agonistic during a static isometric knee task

2.1 Introduction

In the clinic, isometric contraction is commonly used to assess lower limb strength (Wilson and Murphy, 1996). Especially, in the stroke rehabilitation ward, the knee control test is used to assess the knee performance such as knee joint extension muscle strength (van Deursen et al., 2017). If patients can flex or extend the knee at good grade (full range of motion of knee extension and against gravity), then they can be discharged from the hospital (Perry et al., 1986). This is most commonly used to assess the power of quadriceps muscle. An isometric contraction is one where there is no movement at the joint with the muscle contraction. However, there is an obvious lack in the literature of a standard operating angle for these tests or corresponding details on what happens at the knee in normal subjects during these tests. To perform the muscle output, there are two main inputs integrated within the spinal cord 1) from the cortex called supraspinal control e.g. Corticospinal tract and 2) peripheral sensory input like from the muscle spindle (Lemon, 2008, Dum and Strick, 2005, Brouwer and Ashby, 1992b). In addition, in 1983, Day & Rothwell stated that lower limb positioning can lead to peripheral input alteration (Day et al., 1983). Therefore, if peripheral input changes, muscle output could be altered. One of muscle output problem is lower limb spasticity. Knee muscle spasticity affects about a third of the patients leading to lower limb mobility issues (Martin et al., 2014). The groups of muscles, which can contribute to the spasticity, have not clearly been understood yet (d'Avella et al., 2003). This may be best achieved by first exploring, the organisation and governing principles of interactions amongst these muscles of the upper leg, in the very positions where they are clinically assessed in patients. Common clinical tests used are the knee control test in stroke rehab ward and the hip flexor lengthening Thomas's tests (Bohannon and Smith, 1987, Kendall and Kendall, 2005).

Anatomically, as suggested by the literature, Rectus femoris, Vastus lateralis, Vastus medialis and vastus intermediate are defined as knee extensor (Kendall and Kendall, 2005). Knee extension is the orientation of the leg in an anterior direction with the alignment of the thigh and lower leg at 0º for a full extension or extended at an angle if the thigh and lower leg are at a given angle. The extended knee is in 0º position. Flexion, on the other hand, is a movement in the posterior direction, from the 0º position of extension, the range of knee flexion is approximately 140º. The hip should be flexed when measuring full knee joint flexion to avoid restriction of motion by the rectus femoris, but the joint should not be fully flexed when measuring knee joint extension to avoid restriction by the hamstring muscles. Assessment of their normal function might even lead to better stratification and thus improved therapy. Using a quantitative scoring based on surface electromyography (sEMG) might allow us to better apply the modified Ashworth scale (Bohannon and Smith, 1987). We may be able to better assess muscle activity and differentiate spasticity based on the activity of specific muscle groups and their likely neural source.

We have in this study, identified the recruitment of muscles that are acting as a group of muscles (synergy) across 2 distinct tasks that are based on the muscle strength and Thomas tests. We have examined the difference in the recruitment of the muscles, if there is between subjects and within muscles in the same subject, during a static task with identical isometric contractions of the major bifunctional muscle of the quadriceps, the Rectus femoris in healthy young participants. The 2 tests were used to crudely identify any difference that is introduced by the position of the tested leg and sensory feedback from the other leg in these static tasks.

Muscle synergies can be defined as muscles working together as a group (Turvey, 1990, Macpherson, 1988) or activation of a group of muscles to contribute to particular movement (Wojtara et al., 2014).

Taking this information together, I developed the hypothesis that the same set of muscle will interact differently when extending the knee under 2 conditions 1) angle 2) positioning. To assess the muscle activity, I utilize EMG from multiple muscles and then we compare it across 4 distinct angles which are further compared between the 2 positions adapted C1) Fugl-Meyer and C2) modified Thomas test.

2.2 Methods

[1]Study design: This cross-sectional, single-blinded control trial study is designed for comparing the criterion the amplitude of the muscle activity among four angles of knee isometric contraction. Participants were allocated to different groups randomly to reduce any bias during the tests (Chalmers et al., 1981). The measurements were taken in the motor control laboratories of the centre for sports sciences, in the School of Biomedical Sciences, University of Leeds, UK.

Participants: Healthy participants (n = 17, female =8) within the age of 18-30 (24.4± 2.57 years) without previous knee joint injury participated in the study. All participants were fully informed about the tests, and all gave their written, informed consent before participation. The study was conducted according to the declaration of Helsinki and was approved by the Local Ethics Committee of the University of Leeds (reference number BIOSCI 16-004). Participants were excluded if they reported 1) recently done exercise within 48 hours prior to testing 2) Knee stiffness, self-reported pain 3) Muscle or knee joint injury 4) Used recreational or performance-enhancing drugs 5) Ingested alcohol in the previous 24 hours 6) Unable to provide informed consent.

Task: Isometric knee extension (maximal voluntary effort) 6 x 5 sec per contraction with a 3min rest. EMGs were recorded from multiple knee muscles (Rectus Femoris, Vastus lateralis, Vastus medialis, Semitendinosus and Bicep femoris) at (0˚, 20˚,60˚, 90˚) multiple angles of Range of motion (ROM).

Equipment/Tools: EMGs were recorded using the wireless sEMG sensors (Delsys Trigno™ system; at 1.9 kHz, the bandwidth of 20 to 450 Hz). The data were recorded directly onto a PC running Spike 2.8. version 10 (Cambridge Electronic Designed limited, (CED)) software using the Delsys talker (CED) for later offline processing and analysis. A medical stretcher trolley (25”x74”x35”) for the subjects to lie on, during the tests. Physio clear plastic Goniometer angle ruler joint bend measure for measuring the knee flexion angles. Complete range of motion locking knee brace (Donjoy™) to support the knee at fixed knee positions.

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[1] Footnote: 1) In Fugl-Meyer (Fugl-Meyer et al., 1975), knee control test in stroke rehab ward: the knee control is tested for knee extensor strength which is graded into three grades (poor, fair and good). For example, if stroke patient can perform full range of motion of the extension task, then it will be good grade.
2) In Thomas test, the modified Thomas test is used for examining the flexibility of hip flexor such as iliopsoas and rectus femoris. If patients have normal length of the rectus femoris, the alignment of buttock, hip and knee will be straight on the bed. If they have shortening hip flexor, the hip flexor will raise up, not straight away Kendall and Kendall, 2005. Currently, the standard of the measurement was unclear yet because this test cannot tell us which muscle shows abnormal length (Kendall and Kendall, 2005, Harvey, 1998)

2.2.1 Electrode placements

The selected muscles and the recording sites were selected based on the SENIAM protocol (Pincivero et al., 2006b). Anatomical landmarks used to identify the muscles following the SENIAM guidelines (Pincivero et al., 2006b) and cadaveric observation as is summarised in table 1. For instance, the component of quadriceps, vastus lateralis (VL) - centred in the middle of the greater trochanter and the lateral epicondyle; vastus medialis (VM) - on the distal 1/5th of the muscle closed to the medial knee joint; rectus femoris (RF) - centred in the middle of the anterior superior iliac spine and the superior pole of the patella. The details for all muscles and their identification is presented in tables 2.1 and 2.2 (Pincivero et al., 2006a) and cadaveric observation as is summarised in table 1. For instance, the component of quadriceps, vastus lateralis (VL) - centred in the middle of the greater trochanter and the lateral epicondyle; vastus medialis (VM) - on the distal 1/5th of the muscle closed to the medial knee joint; rectus femoris (RF) - centred in the middle of the anterior superior iliac spine and the superior pole of the patella. The details for all muscles and their identification is presented in tables 1 and 2. (Rainoldi et al., 2004).

Prior to placement of sEMG electrodes, relevant skin areas were shaved, abraded with preparation gel (Nuprep, NRSign Inc., Canada), and the dead skin including oils and secretion of epidermis was cleaned with isopropyl alcohol, to reduce noise when recording EMG, (Merletti et al., 1998).

The electrode placement was verified in each subject by palpating the muscles and asking participants to perform a muscle contraction.

In addition, to minimise the crosstalk effect, EMG sensors were acquired with inter-electrode spacing ranging from 5 mm to 40 mm (Deluca et al, 2012). Crosstalk is the EMG signal detected from non-active muscle and generated by a nearby muscle. Therefore, crosstalk can be mistaken to originate from the muscle of interest. Several factors affect the level of crosstalk such as inter-electrode space, the thickness of subcutaneous tissue and EMG noise. Crosstalk is inherent in all surface EMG, and currently, there are no methods capable of eliminating EMG crosstalk. However, in my experiment, I used the inter-electrode space ranging from 10 mm to 40 mm to reduce the crosstalk effect.

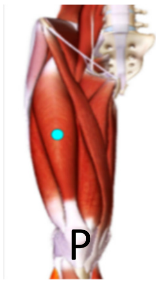
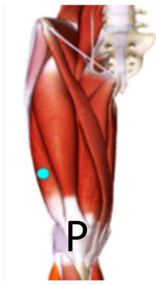
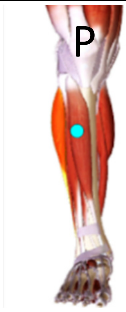
Muscle Anterior view	Electrode position	Palpation and inspection
Rectus femoris		When the hip is flexed, the tendon of origin may be observed and palpated in the V-shaped area between the Sartorius and the tensor fascia latae as seen.
Vastus lateralis		The muscle may be seen and palpated from just below the greater trochanter shown to the patella.
Vastus medialis		The distal portion of the muscle is bulky and is palpated in the lower third of the thigh medially.
Tibialis anterior		The muscle is superficial throughout its path, it may be observed and palpated all the way from its origin to its insertion. The muscular portion of the tibia when the foot is dorsiflexed. Its tendon is palpated as it passes over the ankle where it rises considerably when the foot is dorsiflexed.

Table 2-1 Shown above are electrode placement (blue - circular dot) on the anterior view lower limbs: the anterior upper leg muscles, which is composed of quadriceps muscles (rectus femoris, vastus lateralis, vastus medialis), and the anterior leg muscles perform ankle dorsiflexion (Tibialis anterior muscle) modified from -http://www.delsys.com/products/emg-auxiliary-sensors/trigno-im/). Abbreviations: P = Patella, T= Greater trochanter of femur, C= Calcaneus

Muscle Posterior view	Electrode position	Palpation and inspection
Biceps femoris		When knee flexion is resisted, the long head of biceps femoris may be observed and palpated from its origin all the way down to its insertion.
Semitendinosus		With the subject prone, the tendon may be observed and palpated posteriorly on the medial side of the knee. If the muscles of this medial side are then tightened without the joint movement, the semitendinosus rises markedly from the underlying tissue, the tendon in the back of the knee
Gastrocnemius (Medial side)		The muscular portion of the gastrocnemius may be seen contracting in resisted flexion of the knee. Medial gastrocnemius is seen medially contracting in rising on tiptoes.
Gastrocnemius (Lateral side)		The muscular portion of the gastrocnemius may be seen contracting in resisted flexion of the knee. Lateral gastrocnemius is seen laterally contracting in rising on tiptoes.
Soleus		Soleus is covered largely by the gastrocnemius, but in the lower portion of the calf, it bulges on both sides of the gastrocnemius. A comparatively isolated contraction of the soleus is obtained when plantar flexion is performed while the knee is flexed. The ankle against slight resistance.

Table 2-2 Shown are the electrode placements (blue dot) on the posterior view of the lower limb: the posterior upper leg muscles, components of Hamstring muscles (semitendinosus and bicep-femoris), and the ankle plantar flexor muscles (soleus, medial and lateral gastrocnemius modified from http://www.delsys.com/products/emg-auxiliary-sensors/trigno-im/);
Abbreviations: P = Patella, T= Greater trochanter of femur, C= Calcaneus

2.2.2 Procedures

For each session, the participants were introduced to electromyography (EMG) recording system and shown how to perform an isometric knee extension for the position presented (see below). The experiment was explained, and any questions or concerns answered. The electrodes were then, placed on various muscles to record from. Participants lay supine on the bed with the head, back and leg muscles being fully supported. To ensure the knee was supported we asked the participant to wear a knee brace.

Two Positions: Position (C 1) and Position 2 (C 2)

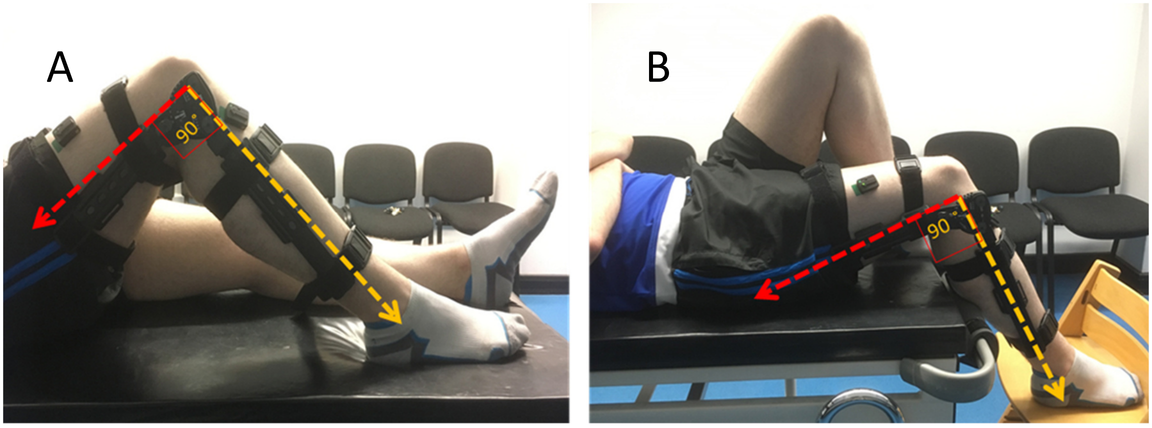


Figure 2-1 A representative angle of 90 ° is shown for either of the positions that the participants were tested. A position 1 (C1) - The participant is supine lying with both legs stretched forward with non-invasive sticky pads recording muscle activity from his dominant right leg. The right leg is supported at set angles (90 ° shown in the figure) using a knee brace. B. Position 2 (C2) - The participant is lying on the bed with the dominant right leg stretched forward, their foot resting on a platform such as the knee is bent through fixed angles (90 ° shown in the image), and while the non-dominant left leg is bent at knee to ensure that both hip and knee are flexed

C1 was adapted from the knee control test used in stroke rehabilitation ward and C2 adapted from the Thomas test for Hamstring lengthening test. The order in which the knee angles were presented for testing was randomised across participants. Overall, participants contracted their muscles at each angle for a total of 6 times, with each contraction lasting 5 seconds, with a 3-minute break between each contraction. Participants were asked to perform muscles contractions while lying in one of 2 positions in 2 independent sessions (the position not tested in session 1 would be tested in session 2).

For C1 as described in Figure 2.1A, the participants were supine lying on the bed with both legs stretched out in front, so at the start, the hip, knee and ankle are at 0 ° to each other.

For C2 as shown in Figure 2.2 B, participants were supine lying on the bed with the dominant leg stretched forward with the foot resting on a platform with the knee supported at different angles, while the non-dominant leg was kept bent at the knee (the ankle was close to the gluteal area). This meant that the non-dominant leg was fully flexed at both hip and knee. In figure 5.2 we show the experimental position where the knee was supported at 90 °. The angle at the knee was always measured against the hip joint and the bony prominence on the outside of the ankle.

In both C1 and C2, the participant contracted their muscles at the back of the thigh of the right leg while the knee is placed at 1 of 4 different angles. at 0 ° (foot extended, so straight at the knee), 20 ° (knee is slightly bent with foot pointing away), 60 ° (middle of the range of knee flexion), 90 ° (foot is at a perpendicular to the hip regarding the knee).

I used RF to normalise their activity against as during the task they were all asked to activate RF voluntarily, to ensure RF was the most active muscle based on current literature.

The root-mean-square (RMS) of the evoked muscle activity was then calculated using Spike2.8. The data were analysed without applying any additional filters. Activity across muscles was normalised to that in RF, which was voluntarily activated by the participants at each angle in both tasks.

2.2.3 Comparison of the EMGs across subjects

Activity in multiple muscles is related to one another in terms of timing and strength of their activity, which can be defined using different analytical tools that are widely accepted (Babault et al., 2002). According to Deluca, (1997) before comparing the EMG data of individuals from different days, the EMG should be normalized.

The recorded EMGs in our study was normalised to the activity in the RF muscle, considered the primary muscle in terms of activity and involvement in the task decided prior to assessment of the interactions between the muscles in the same subject and comparison across subjects to distinguish temporal and spatial detail about interactions (Knikou, 2008).

We compared three outcome measures of the recorded sEMGs—across participants and conditions.

a. Amplitude and latency

The activity in a muscle was examined based on the root mean square of the EMG used as a measure of its activity, illustrated in figure 2.2. The root-mean-square (RMS) envelope of the EMG signal was calculated using a moving window 5 second/contraction method in spike2.8.

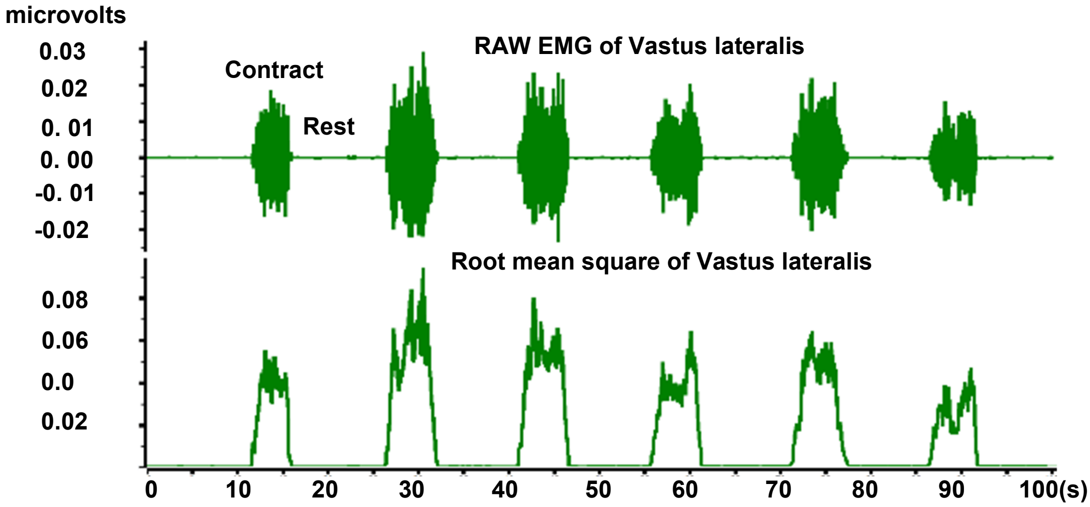


Figure 2-2 Demonstrate Root mean square (RMS) to assess muscle activity from raw EMG of muscle contraction, it was taken from isometric knee contraction. RMS was calculated area under the graph of six trials.

The RMS measures provide the best insight into the amplitude of the EMG signal since it gives a measure of the power of the signal too while producing a waveform that is easy to analyse (Farina et al., 2014).

To compare the similarity of muscle activity with respect to RF, cross-correlation function was used.

I assessed muscle synergy pattern across four different knee angles and positions by using cross-correlations. The similarity of muscle activity based on their RMS was examined to define interactions by using cross-correlations (De Luca and Merletti, 1988). One of analysis for cross-correlation widely used to investigate the similarity of muscle activity of the pairs is waveform correlation (Chang et al., 2012, Nielsen, 2003) as available as a function in Spike2.

2.2.4 Statistical tools.

Multiple statistical means were used to examine to examine the possible interactions and correlations

Pearson's correlation: To examine similarity of muscle activity (from RMS outcome) amongst the muscles a Pearson's Product correlation was used. This type of correlation is measured using a correlation coefficient (*r*) with the range of correlation being from -1 to 1 (Pearson, 1920), giving a measure of the strength and direction of association that exists between the two continuous variables; the muscle activity in the present study. Pearson's correlation attempts to draw a line of best fit through the data of two variables, and the Pearson correlation coefficient, *r*, indicates how far away all these data points are to this line of best fit (i.e., how well the data points fit this new model/line of best fit). Its value can range from -1 for a perfect negative linear relationship to +1 for a perfect positive linear relationship. A value of 0 (zero) indicates no relationship between the two variables (Zar, 1999).

Multivariate analysis

Multivariate analysis was used to identify relationships between activities across muscles. Cluster analysis - a multivariate technique which categorises a sample of subjects based on a set of measured variables into several different groups such that similar subjects are placed in the same group. In this thesis, Origin Pro 2016 8.0 version software is used to analyse the EMG data for cluster analysis. I have employed 2 forms of muscle activity classification tools similar to those previously used for gait and muscle synergy analysis (Krouchev et al., 2006)

To identify the lowest dimensional changes and similarities across these muscles during the different tasks, hierarchical clustering and Principle component analysis (PCA) is used.

Hierarchical clusters

Hierarchical clustering is commonly used in statistics for grouping data into 'clusters' that expose similarities or dissimilarities in the data. Hierarchical methods employ a precise definition of similarity, closeness and proximity of observations to group the data. Objects with the nearest values start in their own separate cluster and the two closest (most similar) clusters are then combined; this is done repeatedly until all participants are in clusters (Mardia et al., 1979). In the end, the best number of clusters is then chosen out of all cluster solutions. The steps for analysis are summarised below.

Step 1: Setting input

1) Select data for the Hierarchical Cluster Analysis.

Data in each column correspond to a variable. Five variables were chosen, as there were five muscles in this study (RF, VL, VM, ST, BF).

2) Cluster method setting: pairwise group averages was chosen for comparison and plotting.

Origin provides six methods (nearest neighbour, furthest, group average, centroid, median and ward) to calculate the distance between the new cluster and other clusters. In study 1, group average is selected, because it is commonly used to calculate the mean of two distances between two things: a single original cluster and 2) a new cluster which was made by merging two clusters.

For the group average version of hierarchical clustering, the proximity of two clusters is defined as pairs of the average pairwise proximity between all pairs of points in the different clusters.

Let clusters *j* and *k* be merged as cluster *jk*. Let n_i, n_j and n_k be the number of objects in Cluster *i*, Cluster *j* and Cluster *k* respectively, and let d_{ij}, d_{ik} and d_{jk} be the distance between two clusters. The distance between Cluster *jk* and Cluster *i* $d_{i,jk}$ can then be calculated in the following equation:

$$d_{i,jk} = \frac{n_j}{n_j + n_k} d_{ij} + \frac{n_k}{n_j + n_k} d_{ik}$$

(https://www.originlab.com/doc/Origin-Help/HCA-Algorithm#Distance_Matrix)

3) For variables that I am going to cluster, the correlation function is selected to calculate the difference between one variable and the correlation of two variables.

Step 2: Plotting group average dendrogram

The dendrogram plot is a hierarchical tree that shows the distance at which two clusters merge. Each stage is represented as a unit in the dendrogram. The top of the unit for each stage represents the new cluster created by the merging of two clusters. Its height corresponds to the distance between two merged clusters.

Principle component analysis (PCA)

The similarity of muscle synergies is commonly assessed by reducing the dimensionality of the data; PCA is used to identify the covariance amongst the multiple muscles, to establish the most common linear model that can explain the variance-covariance structure of a set of variables (Bolger et al., 2016). Determining this fact allows an experimenter to try and discriminate which combinations are important and which may be just redundant. We applied PCA to diminish the number of variables to assess the common muscle synergy used to control the knee. We identified the same muscles that were commonly used across participants. We used a custom script written in R to generate the PCAs for our dataset (Details of PCA analysis and R scripts (see Appendix)).

2.3 Results

2.3.1 Muscle activity (RMS)

NO.	subject	activity	Synergies' Position 1 (C1)				Synergies' Position 2 (C2)			
			0deg	20deg	60deg	90deg	0deg	20deg	60deg	90deg
1	AAD	cycling	VM	VM	VM	VM	VM	VM	VM	VM
2	AAG	cycling	BF	BF	BF	ST	VM	ST	ST	ST
3	AAF	cycling	VM	BF	ST	ST	VL	ST	ST	ST
4	AAK	cycling	VL	VL	RF	RF	VL	BF	BF	BF
5	AAL	cycling	VL	BF	ST	RF	VL	RF	RF	RF
6	AAN	boxing	VL	VL	RF	RF	VL	BF	ST	BF
7	AAM	boxing	VL	VL	VL	ST	VL	BF	VL	VL
8	AAO	cycling	VL	VL	VL	ST	VL	VL	ST	ST
9	AAC	rugby	VL	VM	VM	VM	VM	VM	VM	VM
10	AAI	walking	VL	BF	ST	ST	BF	ST	ST	ST
11	AAT	no activity	VL	BF	BF	VM	VM	BF	BF	BF
12	ABC	dancing	BF	VL	BF	BF	ST	VM	ST	ST
13	AAE	cycling	VL	BF	ST	ST	VL	BF	BF	ST
14	ABD	walking	VL	BF	ST	ST	VL	VL	ST	ST
15	ABE	squashing	VL	VL	VL	RF	VL	VL	VL	VL
16	ABF	football	VL	VL	BF	BF	BF	BF	BF	VL
17	AAX	walking	VL	VL	BF	ST	VM	ST	ST	ST

Table 2-3 characteristic of the 17 participants showing gender, physical activities, and maximal muscle activity (RMS) of isometric knee extension on five muscles recording (RF,VL,VM,BF,ST) for two position C1 and C2

There were 17 participants in the two EMG sessions (positions 1 and 2). Table 2.3 and figure 2.3 present the muscles that had the maximal activity during the task in each participant across the 2 tasks, compared to that in RF. We classified the data based on gender, and other details provided about their physical activities each undertook. In C1, at 0° and 20°, the activity in RF was not maximal in any of the participants, but it was in VL at 0° (13/17), and 20° (8/17). At 20°, BF had the second highest activity (7/17) and followed by VM (2/17). ST and BF were the most active the majority at 60° (5/17) and ST at 90° (8/17). In C2, at 0° the activity levels in RF were not maximal in any participants, but VL was at 0° (9/17) and VM (4/17). At 20°, BF was the maximal activity (6/17), followed by ST (4/17). At 60° and 90°, there was a similar pattern. At 60°, ST was the maximal activity (8/17) and BF (4/17). At 90°, ST was the maximal activity (8/17) and followed by BF (3/17).

Summary of the muscles with maximal activity for each position and angle amongst all the participants;

C1 0°: VL>VM>BF; **20°:** VL>BF>VM; **60°**: ST, BF>VL>VM, RF; **90°**: ST>RF>VM>BF

C2 0°: VL>VM>BF>ST; 20°: BF> ST>VM, VL> RF; 60°: BF> ST>VM, VL> RF; 90°: ST> BF, VL>VM>RF

2.3.2 Cluster and grouping

I provided the two positions to compare the possible role of proprioceptive feedback in shaping the output of the muscles at the knee. I hypothesised that the proprioceptive drive from the ankle muscles of the same leg and that from the muscles of the left leg would affect the output of the muscles in use. I assumed 4 clusters at each angle as I worked on the assumption that the muscles are likely to be grouped along anatomical rules of pairing for each angle.

2.3.3 Hierarchical cluster analysis was used for classification of the activity in these muscles in both C1 and C2 at different angles across all subjects.

In figure 2.4, C1 0°, RF and VL were closest together. ST and BF are being similar but farther from the rest. VM was separated from RF and VL.

C1 20°, RF and VL were closest together. ST and BF were being similar but farther from the rest. ST and BF are farther than 0°. VM was separated from RF and VL but it was not too far when compared to 0°.

C1 60°, RF and VL were closest together. ST and BF were closest together. However, VM was separated, farther than the rest.

C1 90°, VL and VM were closest together. ST and BF were closest together, but they both were further than VL, VM. RF was separated, farther than the rest.

C2 0°, RF and VL were closest together. VM, ST and BF were standing separately. ST and BF were farther than the rest of them. ST and BF in C2 0° were the farthest when comparing among positions and knee flexion

C2 20°, RF and VL were closest together. ST and BF were being similar but farther from the rest. ST and BF are farther than 0°. VM was separated from RF

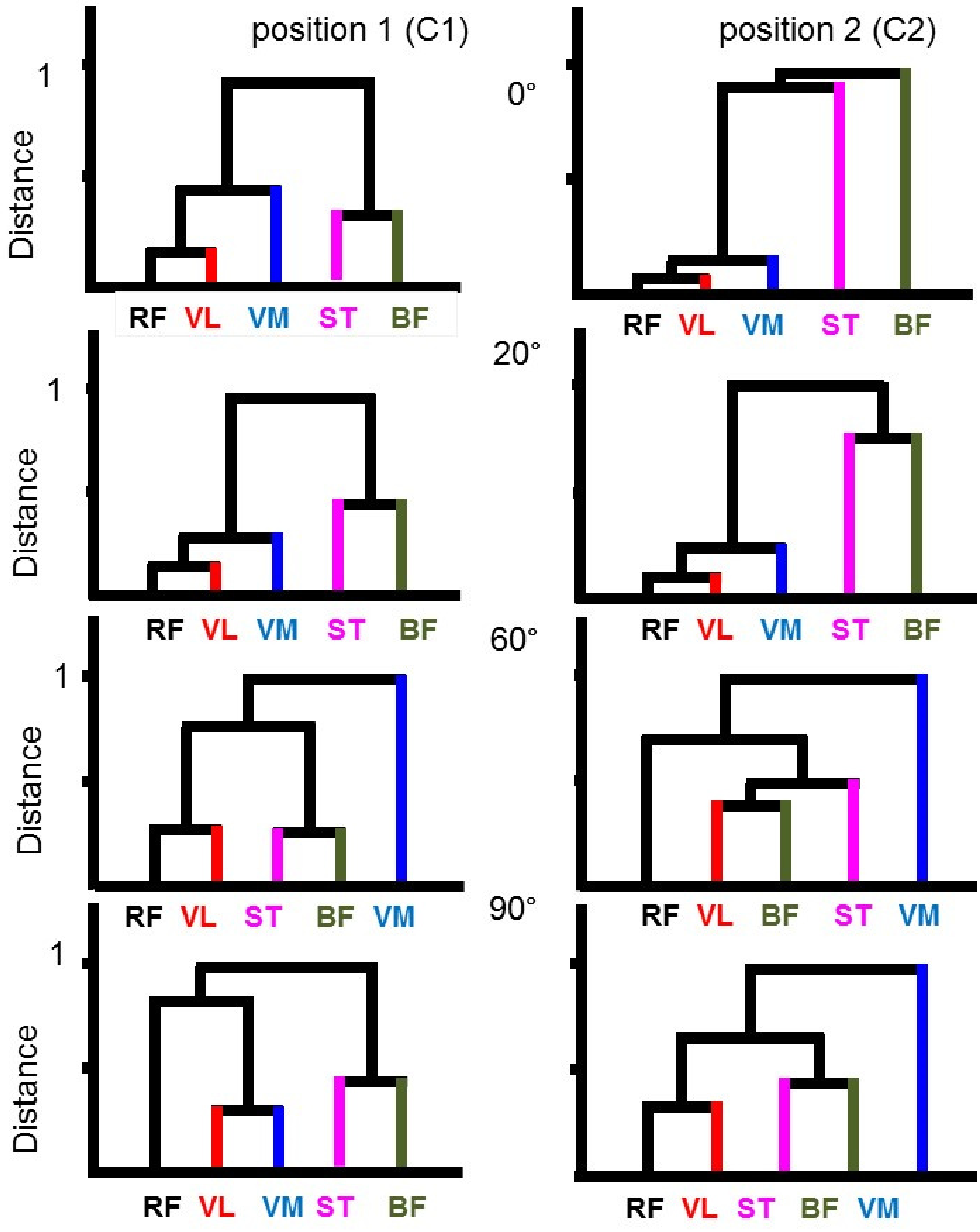


Figure 2-4 Hierarchical cluster of positions 1 (C1) and 2 (C2) across 4 angles (0 °, 20 °, 60 °, 90 °) of knee flexion. Overview of muscle synergies, 5 muscles recording (RF, VL, VM, ST, BF) from 17 participants

and VL but it was not too far when compared to 0°.

C2 60°, VL and BF were closest together and they were closed to ST. RF was separated. VM was the farthest and standing alone.

C2 90°, RF and VL were closest together. And ST and BF were closest. However, VM is alone and was the farthest than the rest. Between the two positions (C1 and C2), C1 differed at 0°, 60°, 90°, from C2. However, C1 at 20° was like C2, but not the same. At 20°, ST and BF were farthest in C2 but not in C1.

2.3.4 The similarity of muscle activity

2.3.4.1 Pearson correlation's test

The results of the Pearson's test (figure1.4) from 17 participants are summarised for the two positions across the angles tested (table 1.4), at 0° both positions have a similar pattern, though not the same. Most of the population in C1 and 2 showed a similar positive correlation between RF to VL and other three (VM, ST and BF) with the numbers 10/17 and 9/17, respectively. **At 20°, C**1 showed a different pattern from C2. The positive correlation between RF vs VL and RF vs VM at C1 was 5/17, but at C2 it was 12/17. It was noted that in C1, RF, VL and VM showed a negative correlation at 7/17. **At 60**°, C1 showed a different pattern from C2. C1 showed the highest number (9/17), with a negative correlation between the four paired muscles. In contrast, in C2, RF vs VL and RF vs VM had a positive correlation, though not for ST and BF (12/17). In addition, RF showed a negative correlation with BF and ST. **At 90°,** C1 showed a different pattern from C2. C1 showed a negative correlation for RF to each of the four muscles at 10/17. It was noted that in C2, RF correlated to VL and VM positively (9/17). In Pearson's correlation test (figure 4) described the results of the similar trend of the two positions.

At 0° (figure 2.5), both VL and VM, they were correlated positively in both positions with RF, but not correlated to ST and BF. At 20° (figure 2.6), C1 showed a different pattern to C2. In C1, there was a positive correlation between RF vs VL and RF vs VM, but not with ST and BF. In C2, RF had a strong positive correlation to VL- VM ($r > 0.5$), and RF was well correlated to ST, BF ($r = 0.2$, 0.3 respectively). At 60° (figure 2.7), C1 showed a different pattern from C2. C1 showed a positive correlation between RF and VL ($r = 0.53$). In contrast, in C2, RF vs VL were poorly correlated ($r = 0.009$).

At 90° (figure 2.8), C1 and 2 showed different patterns. In C1, VL was correlated to VM (r = 0.6). In C2, RF was not related to VL (r = 0.3), while VL was correlated to VM. Both positions had the same positive correlation of BF to ST (C1, r = 0.56 and C2, r = 0.57).

Knee angles	Participant numbers	Pearson's correlation between			
		RF vs VL	RF vs VM	RF vs ST	RF vs BF
0° C1	10/17	+	+	+	+
	4/17	+	+	-	-
	1/17	-	-	+	+
	1/17	-	-	+	-
	1/17	-	-	-	-
0° C2	9/17	+	+	+	+
	5/17	+	+	-	-
	3/17	-	-	-	-
20° C1	4/17	+	+	+	+
	5/17	+	+	-	-
	7/17	-	-	-	x
	1/17	-	-	-	-
20° C2	12/17	+	+	-	-
	5/17	-	-	-	-
60° C1	3/17	+	+	+	+
	5/17	+	+	-	-
	9/17	-	-	-	-
60° C2	3/17	+	+	+	+
	12/17	+	+	-	-
	2/17	-	-	-	-
90° C1	1/17	+	+	+	+
	1/17	+	+	+	-
	5/17	+	+	-	-
	10/17	-	-	-	-
90° C2	5/17	+	+	+	+
	9/17	+	+	-	-
	3/17	-	-	-	x

Table 2-4Summary of results from Pearson's correlation test. Overview of muscles synergies seen across the 17 participants for all angles in both positions (C1 and C2). The highest incidence amongst subjects at each angle and position is highlighted, + indicates positive correlation (r>0.05), - indicates negative correlation and x indicates no correlation

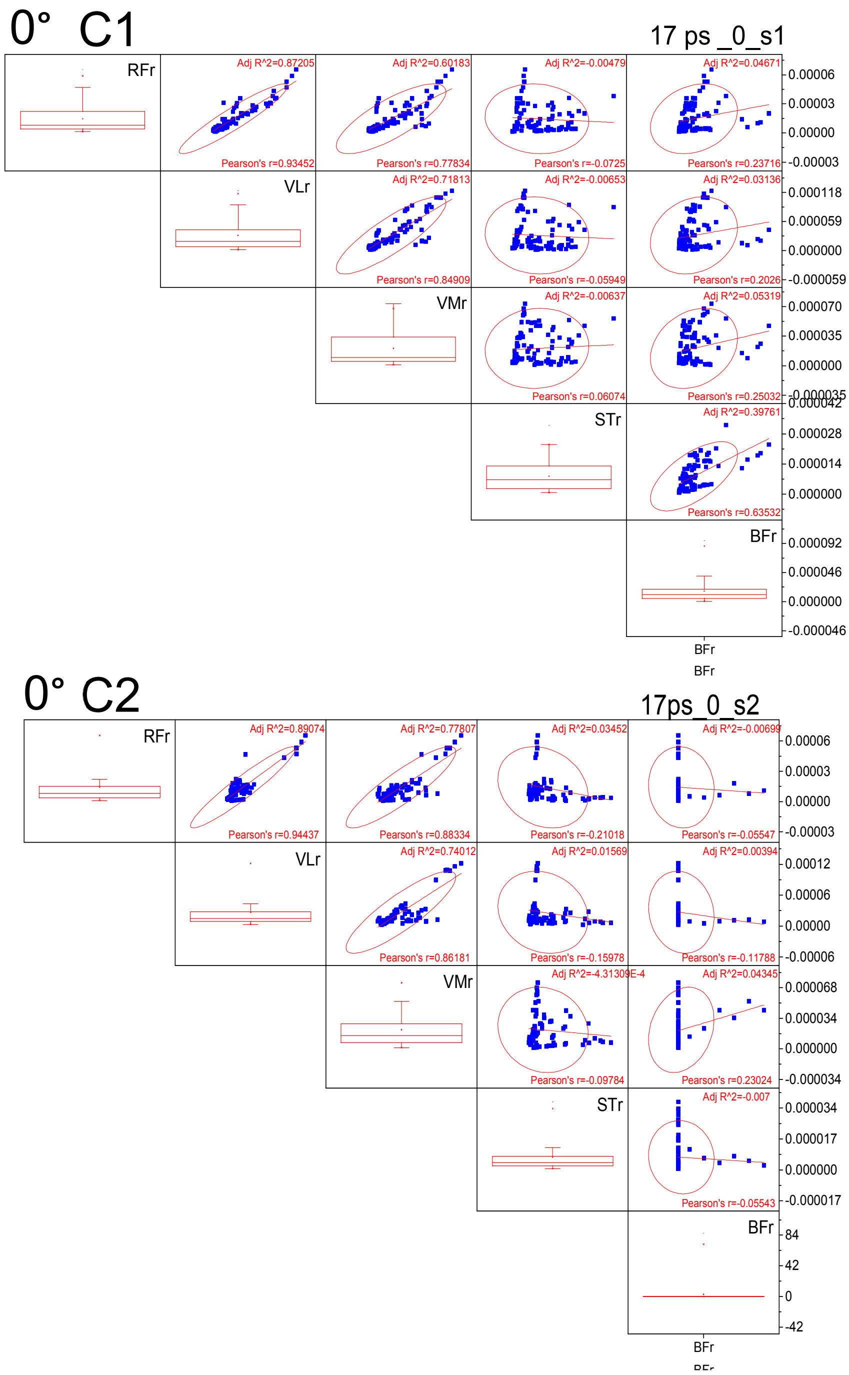
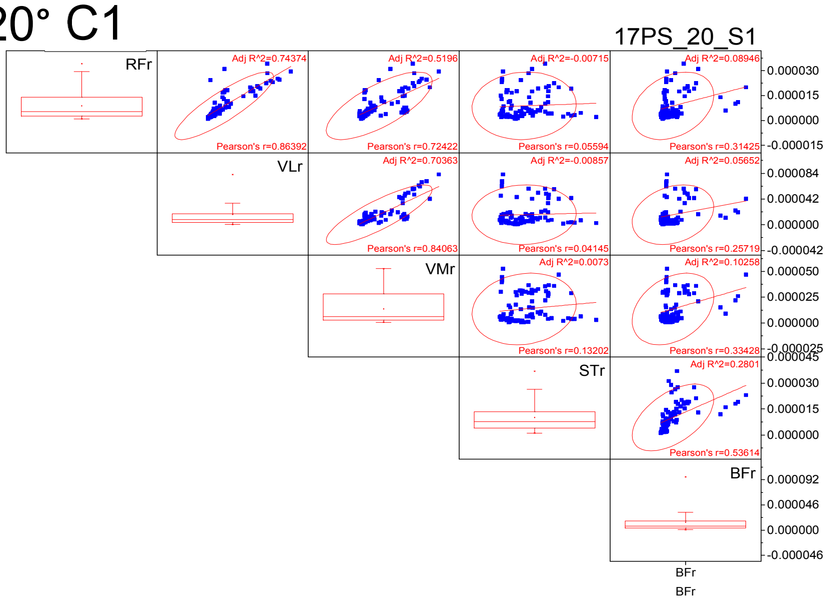


Figure 2-5 Pearson's correlation across 0° of knee flexion in two positions (C1 and C2). Overview of muscle synergies from 17 subjects recorded across five muscles: RF, VL, VM, BF, ST, note that r value presenting the positive and negative correlation between the two muscle, for example RF-VL, RF-VM, RF-BF, RF-ST.

20° C1



20° C2

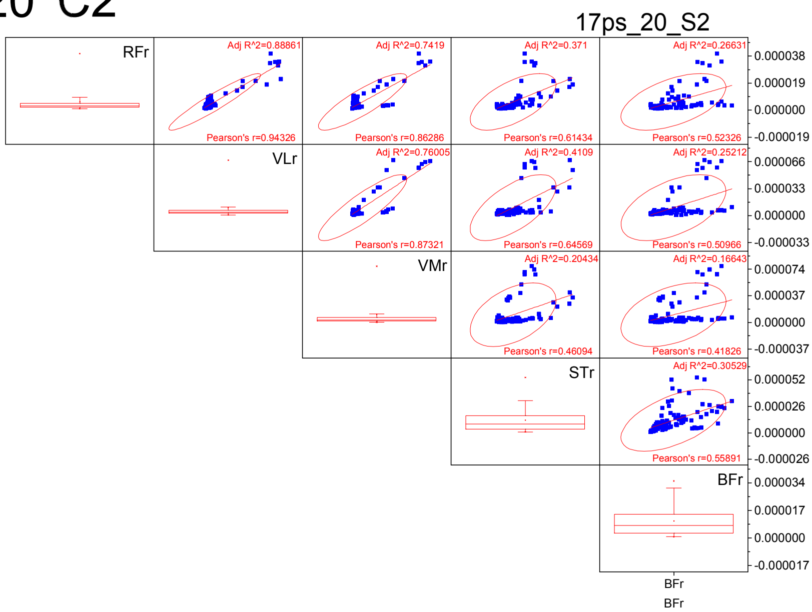


Figure 2-6 Pearson's correlation across 20° of knee flexion in two positions (C1 and C2). Overview of muscle synergies from 17 subjects recorded across five muscles: RF, VL, VM, BF, ST, note that r value presenting the positive and negative correlation between the two muscle, for example RF-VL, RF-VM, RF-BF, RF-ST.

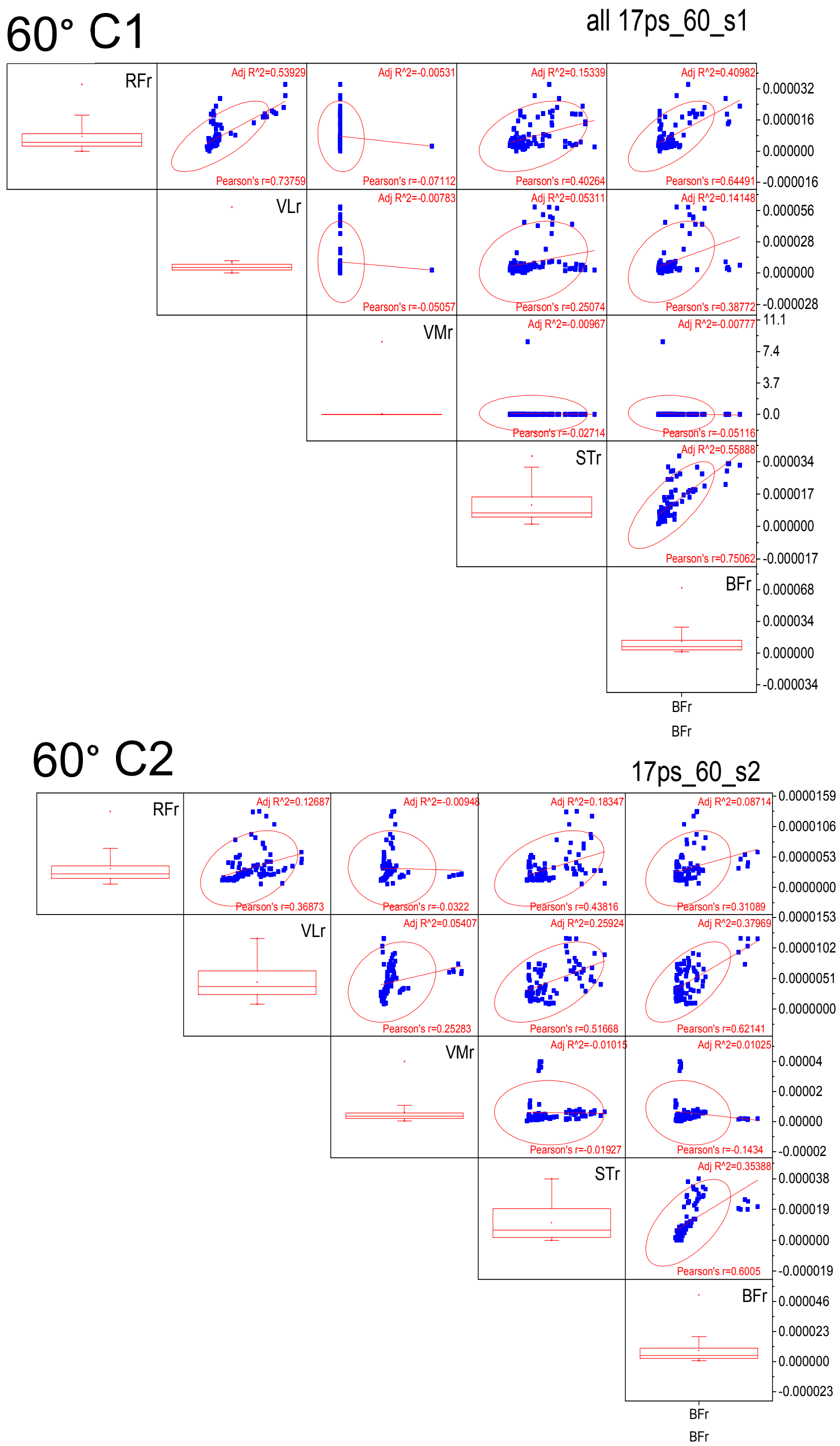


Figure 2-7 Pearson's correlation across 60° of knee flexion in two positions (C1 and C2). Overview of muscle synergies from 17 subjects recorded across five muscles: RF, VL, VM, BF, ST, note that r value presenting the positive and negative correlation between the two muscle, for example RF-VL, RF-VM, RF-BF, RF-ST

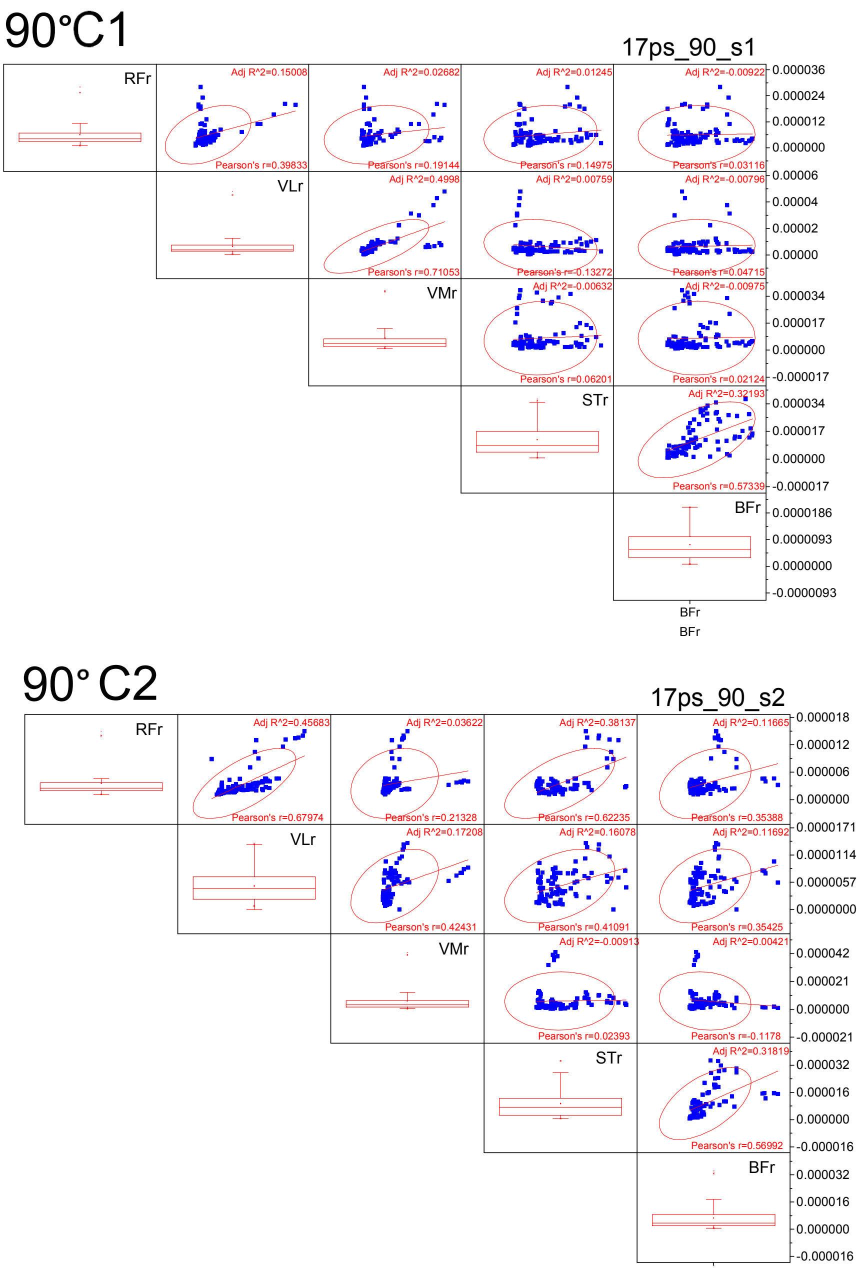


Figure 2-8 Pearson's correlation across 90° of knee flexion in two positions (C1 and C2). Overview of muscle synergies from 17 subjects recorded across five muscles: RF, VL, VM, BF, ST, note that r value presenting the positive and negative correlation between the two muscle, for example RF-VL, RF-VM, RF-BF, RF-ST

2.3.4.2 Principal component analysis (PCA)

As I have five synergist variables (RF, VL, VM, BF, ST), the principal component analysis was used to define the correlation among the five muscles activity (correlation above 0.5 is deemed important) (figure 2.9)

At 0°, C1, the first component was correlated with four variables (RF, VM, BF, ST). The second component (PC2) decreased with only one, decreasing VL. The third component (PC3) increased with only one, increasing ST. The PC3 decreased with VM. That was suggested that the more activity ST, the less activity of VM.

At 0°, C2, they were like PC1 of C1 by decreasing four variables (RF, VM, BF, ST). The second component (PC2), the variable direction differed from C1. PC2 increased with the only one variable, increasing VL activity. It was suggested that the more VL activity in C2. The third component (PC3 decreased with only one VM.

At 20°, C1, the first component PC1 was correlated with four variables (RF, VM, BF, ST) and they were like PC1 of C1 at 0°. The second component decreased with only one of five variables, increasing VL. The third component decreased with only ST.

At 20°, C2, the third component PC3 was the same as C1. The second component (PC2) increased with VL. The third component (PC3) decreased with only one of the five variables. decreasing ST. At 60° C1 and 90, ° C1 were presented in the same pattern across 3 components. The first component PC1 increased with all

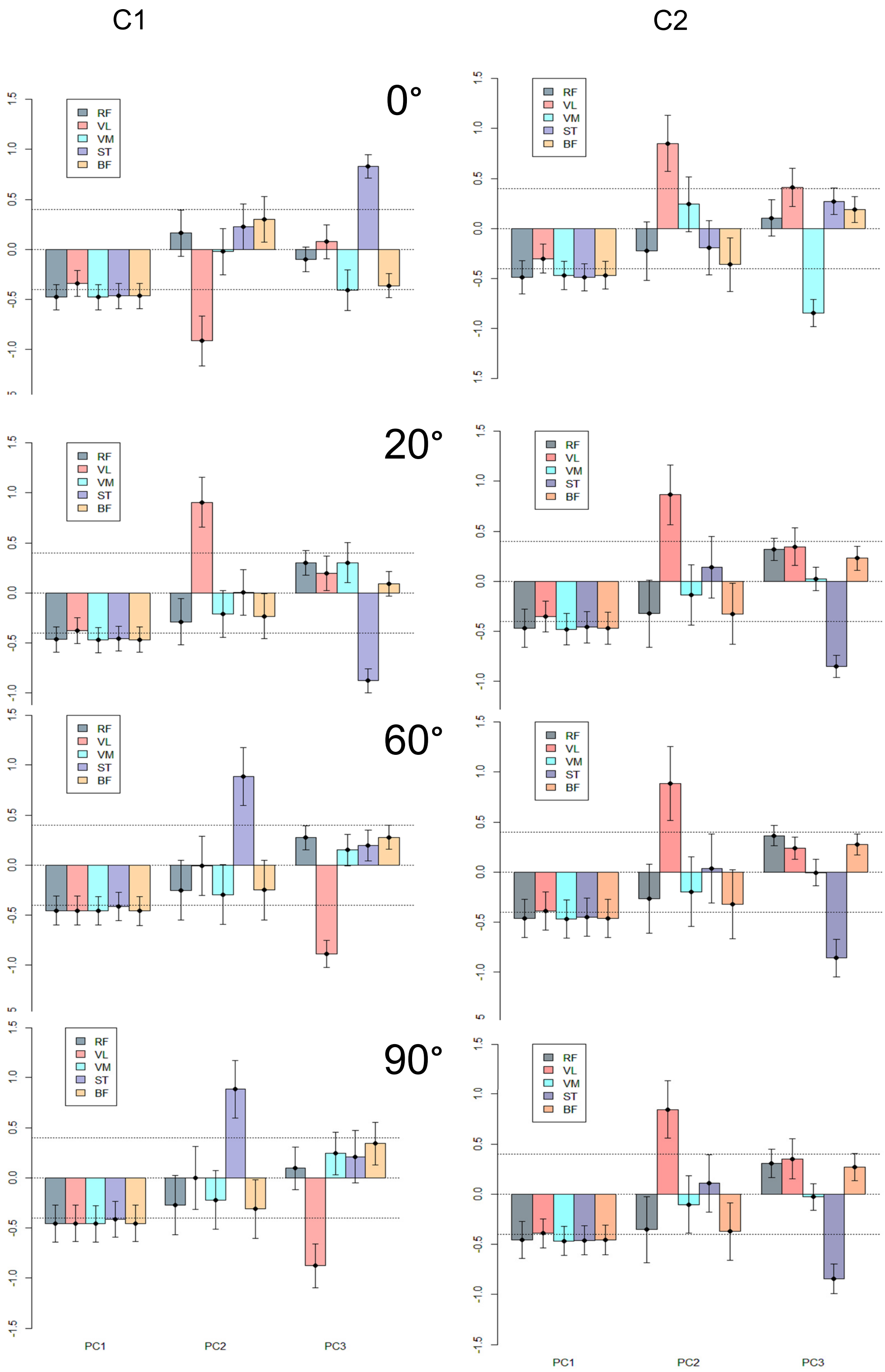


Figure 2-9 Principle component analysis (PCA) demonstrating three types of muscle synergistic patterns which vary across the four angles (0 º,20 º,60 º ,90º) of knee flexion in the two positions C1 and C2.

five variables (RF, VL, VM, BF, ST). The second component PC2 increased with only one variable, increasing ST. The third component PC3 decreased with only one, decreasing VL activity.

At 60° and 90° in C2 were the same pattern across 3 components. The first component was correlated with five variables (RF, VL, VM, BF, ST). The second component PC2 increased with only VL. The third component PC3 decreased with only ST.
In terms of comparison between the two conditions (C1 and C2), only 20° showed similar patterns across 3 components. At 60° and 90° showed the same pattern of muscle synergies. PC2 and PC3 were stated that VL and ST played a role of high correlation.

2.4 Discussion

2.4.1 Main finding

The output from result table 2.3 and figure 2.3: RF is not the primary muscle when performing knee isometric contraction.
The output from figure 2.4: Cluster analysis: Based on the literature, RF, VL and VM should act together as the knee extensor. However, having considered the results, in almost all cases, they are not working together, VL, VM is not the same group. Especially at 60 °, VM and VL play the role of knee flexors. At 90°, at C1 (10/17), RF is not related to VL, VM, ST or BF. In contrast, in C2, RF is related to VL, VM. In C2 (9/17), RF is related to VL, VM but not to ST, BF. Surprisingly, at 60°, the pattern is not as expected based on the gross anatomy of their origins and insertions. RF and VL are in the same cluster. ST and BF are in the same cluster. However, VM is not. VM is arranged separately and showed the most distance. Interestingly, at 60° in C1 and at 90° in C2 the patterns were similar: RF, VL, ST and BF were now in the same agonist cluster, but not VM. ST and BF are still in the same group. VM is not the knee extensor.
The output from table 2.4, comparing the 2 positions, they are almost the same at 0 °.
Output figure 2.5 to 2.8, the details from Pearson's correlation tell us two things: 1) at 60 º, looking at the variable patterns of the cluster's behaviour we see that muscle synergy is flexible. 2) RF and VL are not in the same group.
Output figure 2.9, PCA analysis, there are three components of the muscle synergies pattern. To define the muscle activity, PC2 and PC3 can be suggested

that various patterns of muscle activity in 17 participants. For example, at 20 º C1, PC2 increase only VL, but PC3 decrease only ST. That is suggested that the primary muscle in individual situations can have a different individual primary.

2.4.2 How this changes the original thinking

RF is not the primary muscle performing isometric knee contractions: bi-articular point of view.

Focusing on the highest muscle activity, we can see from the results of table 2.3 that Rectus femoris (RF) is not the primary muscle in knee extensions, while figure 2.3 shows that ST is the primary muscle. Therefore, the more knee flexion, the more ST activity. Table 2.3 and figure2.3, if RF is not primary, VL and VM should be the primary muscles. Based on the literature (Kendall and Kendall, 2005) RF is the primary muscle involved in performing knee extension; however, at greater knee angles, ST had the highest muscle activity (figure2.3) across all subjects. Using the RMS amplitude to observe each muscle' s activity in both positions at 90°, RF, VL and VM were showing the lowest activity, but ST the highest' the graph shows (figure 2.3), semitendinosus has the highest average for muscle activity among the five muscles (flexors and extensors), followed by the knee flexor.

RF, VL and VM is not the same group and they do not work together

Based on the literature (Kendall and Kendall, 2005), RF VL VM should act together as knee extensors; however, my results of the hierarchical cluster (figure 4), at 60 ° in C2 clearly shows that they work separately. At 60 ° position 1 & 90 ° position 2, it is similar: RF works with VL but not VM. At 90 ° in C1, VL correlates with VM but not RF (Nene et al., 2004).

Changing the understanding of anatomy: The quadriceps muscle is composed of RF, VL, VM (Sung et al., 2003, Kendall and Kendall, 2005), but my results suggest that this is not always the case. It depends on factors such as angles and positions. Nene and colleagues suggested the quadriceps femoris should have only VM, VI and VL, but not RF. From studies of both needle and surface EMGs, they stated that it is time to call it triceps femoris instead because RF does not work together with the other three (vastus lateralis, vastus medialis and vastus intermediate) (Nene et al., 2004).

Prilutsky and Zatsiorsky stated that there are three assumptions regarding the synergist pattern: the anatomy, the biomechanics and the task, (Prilutsky and

Zatsiorsky, 2002). In the 19th century, the two-joint muscle was difficult to assess, which is why people have taken the rectus femoris for granted as the anatomical agonist in knee extension. Because the rectus femoris is placed on the anterior of the femur bone, its function should be knee extension by anatomical placement. After the 19th century, biomechanical researchers suggested that the torque and moment of the joint draw the knee function into paradoxical action (Kamper et al., 2002). The moment of the knee has been divided into four quadrants: the upper two quadrants are to do with hip flexion, and the lower two to do with the momentum of knee extension. Therefore, the bicep femoris was situated in the upper left; however, the rectus femoris was in both the lower right and the upper right quadrants. Using studies of the torque moment, RF was defined as both knee extensors and hip flexors, (Dostal and Andrews, 1981). Later, Jacobs and Van Ingen Schenau suggested that the “knee extensor muscle depends on function or task”, after applying different forces to patella and distal quadriceps on 12 cadavers, measuring the pressure on the patellofemoral joint or Q angle in 4 different angles of knee flexion (20°, 30°, 60°, 90°),(Jacobs and van Ingen Schenau, 1992). The ratio of the pressure of the lateral side and the medial side of quadriceps are different at 30°The knee extensors and the flexors cannot be defined anatomically, but task dependent

VM and VL play the role of knee flexors, especially at 60 °. This changes the anatomy theory because the flexors are said to be semitendinosus and biceps femoris, (Kendall and Kendall, 2005), but my results show they are not always knee flexors.

Tasks demonstrate neuromuscular activation depending on the tasks (hip flexion, knee extension). Since the femoral nerve divided into two branches, proximal and distal, it could be that the proximal branch controls the hip, and the distal branch controls the knee, (Haffajee et al., 1972, Aagaard et al., 2000, Yang et al., 2013). Suzuki and colleagues studied EMG activity and the kinematics of human cycling movement at different constant velocities, and they concluded that the RF is not a quadriceps muscles,(Suzuki et al., 1982).

In VM RF VL isometric knee extension with seated position and using intramuscular needle EMG, VM comes to the final phase of knee extension (Miyamoto et al., 2012).

RF vs VL is not in the same group as the muscle synergy, especially at 60˚. It might have some collateral branch form RF afferent input drives to the interneuron of VL and VM as the same action to ST and BF (Bayoumi and Ashby, 1989).

ST is the primary muscle at 60˚, which is explained by neural mechanism support. It might be the Ib stretching, (PierrotDeseilligny and Burke, 2012).

2.4.3 Flexible synergies, not fixed synergies: Proprioceptive > biomechanics > anatomy

RF, VM and VL do not work together at some angles (figure 2.4). Tables 2.4 and table 2.5 show us why we must distinguish flexible and fixed synergies. Comparing the 2 positions, at 20˚, the positions are similar, while at 0 °, 60 ° and 90 ° the two positions are different. However, once we look at the detail of individual subjects, at 0˚, where we expect to see the same pattern, the two positions are different.

Knee angles (C1 and C2)	Comparison of the synergies using PCA
0 ◦	Different
20 ◦	Similar
60 ◦	Different
90 ◦	Different

Table 2-5 Summarise PCA results from comparison of the synergies classified using PCA between C1 and C2 at varying angles. At 20 **°** the synergies identified were similar for both c1 and c2.

At 20 °, both C1 and C2 had similar synergistic patterns of muscle activity, suggesting the sensory feedback from the non-dominant leg did not affect the dominant leg during the static isometric contraction of the knee flexed at 20°. This has significance in a clinical environment, suggests that ensuring the knee is flexed at 20° in either the modified Thomas test (c1) or the muscle strength test (c2) gives the most accurate measure of quadriceps muscle activity. In addition, the results suggest the qualitative score concerning their muscle strength like the Manual muscle testing (MMT), is largely defined by the task and is not uniform, thus requiring the protocol needs standardisation if it is used to make clinical and therapeutic decisions. Similarly, our findings suggest that if a clinician was interested in measuring the muscle strength of the semitendinosus muscle in an isometric contraction, having the knee bent at 60° or 90° would be appropriate in either of the 2 positions used for these tests. It is worth noting that in position C2 influence of sensory feedback from the other side is seen at 0°, 60° and 90°.

Many physiologists state that “isometric contraction should have all muscles co-act together” (Babault et al., 2003), but my results show clearly that this is not the case. It is good to use as an isometric model to convince biomechanics view. In my results for 0°, we expected to see the same pattern of muscle synergies, however, the PCA analysis shows that there are different patterns of muscle synergies across subjects in the two positions. Therefore, it is suspected that more of a neural mechanism is involved. I use this isometric task to answer the neural mechanism question because lower limb proprioceptive might be changed when the position of the lower limb is shifted (Marsden et al., 2000). Based on anatomical evidence and expected neural interactions, Kendall and Kendall predicted that at 0° in both positions, the same subject, if not all, should exhibit co-contraction of all muscles across every joint, (Kendall and Kendall, 2005). This ought to be improved further as the subject is asked to voluntarily contract the rectus femoris muscle alone. Instead, we find the same subject using different strategies to maintain the leg in position for both positions, the only distinguishing feature being the other leg folded back. This might suggest the proprioceptive feedback plays a role in defining the active synergy and not merely motor primitives that have been recruited centrally. Excitatory afferent inputs play a key role in muscle synergies (Burke et al., 1978). We need to further study the feedback from the other leg by recording from muscles of both legs.

The theory of fixed synergies should be changed, as individual subjects do not always have the same patterns of muscle synergies, and there can, therefore, be flexible synergies. Based on anatomy literature and the idea of fixed synergies (Macpherson, 1988), one assumes the flexors and extensors will always maintain their relationship. So, the hypothesis will instead be that “the muscles interact similarly across all angles”. This was assumed under passive examination (Avela et al., 1999). Flexible synergies suggest the best synergy required is chosen while fixed suggests at a given angle always the synergy of choice will be the same.

2.4.4 Possible mechanisms

In both conditions C1 and C2 in study 1, the primary input controlling the drive to the rectus femoris muscle was the same, as participants voluntarily contracted the muscle as best, they could. Therefore, the cortical control was defined for RF while the peripheral inputs to the spinal cord were varied, my results show that these inputs from the periphery were enough to modify the muscle interactions. Although, it was not examined explicitly if there was any change in length of the muscle in these tasks one can speculate based on previously published work, that there may be an increased sensory drive from the muscle tendons at the knee(Day et al., 1984, Day et al., 1983) at greater knee flexion of 60˚ and 90˚. This could be the cause of the alteration of the interactions leading to ST and BF being more active than the expected muscles of the Quadriceps, possibly via the reciprocal inhibitory networks. This study 1 also sought to investigate if sensory information from the non-dominant leg influences the dominant leg during an isometric contraction. To do this, we examined the effect of sensory input in 2 positions (C1 and C2). At 60˚, the muscles of the quadriceps, VM, VL and RF of the contracted leg acted independent of each other. It can be interpreted that VM, VL and RF were working separately. The more synergies active in knee flexion are therefore dependent on the degree of knee flexion and not the anatomical positions of the muscles.

The angles at which the knee was held, although similar between C1 and C2, had different interactions with the Hip, knee and ankle positions. The similarity and differences between them were examined using PCA as described in table 2.5.

2.4.5 Clinical implication

Recommendations for the clinical muscle test guidelines and improving gross motor function (Chang et al., 2017).

Clinically, this is critical, as the expected outcome of muscle testing is essentially task dependent. As an example, in clinical support, in a stroke rehabilitation ward knee control is used to test lower limb ability, with slight knee flexion and asking patients to move the quadriceps. We take the quadriceps for granted for knee extension, according to the anatomical view, but we do not know the interactions within the quadriceps itself. These findings are extremely important as they suggest that when clinicians conduct these basic tests, they ought to be aware of the angle used for these measurements, and not assume the outcomes based on anatomical knowledge. These synergies, once better defined, will provide comprehensive details about all muscles involved in knee function, thus ensuring more informed decision-making moving towards more personalised therapy. This, in turn, will lead to better functional outcomes and an improved quality of life.

2.4.6 Recommendations for MVC standards

The positioning in the lower limb is important to set up the MVE, but there are few defined standards. Now, we could suggest MVE testing for various purposes. If you wanted to measure the muscle synergy of the knee extensor, you should use 0° and 20° If you want to test the difference in muscle pattern, 60° is the best suggestion. One drawback of maximal voluntary effort and contraction is that it requires the full range of motion and muscle strength; however, some patients are not able to achieve the maximal force due to pain and swelling. Therefore, MVE can be used in both healthy adult participants and patients. Generally, researchers use MVC; however, MVE was used in this study to ensure force feedback did not corrupt the muscle interactions. As Merton suggested, the term 'effort' is used to define a maximal voluntary contraction (MVC), and removal of the force acting at the joint when they are making the effort should not eliminate any form of force feedback to the muscle that would modify the proprioceptive feedback (Merton, 1954).

In my study, I asked participants to do isometric contraction without resistance. Had I conducted the experiment with resistance, proprioceptive feedback might have had a stronger influence than without resistance. Bishop et al (2018) studied the index of gluteus medius and maximus and tensor fascia latae during a therapeutic exercise with and without elastic resistance. The isometric exercise with resistance increased the activation of the gluteus maximus, gluteus medius to greater than index during the isometric without resistance. It was possible that a heavier resistance may have generated a greater activation of these muscles compared to the activation observed when performing the exercises without resistance (Bishop et al., 2018).

How does this demonstrate the influence of proprioceptive feedback?

To assist in muscle contraction, maximize motor control and assist movement awareness, which leads to an increase in muscle response to the cortex. These stimulated to the cortical region will depend on the intensity of resistance, such as the greater the resistance, the greater the stimulus, noting that this resistance has to be enough movement occurs smoothly and coordinated, causing no pain or fatigue to patients. (Nelson et al., 1986). In terms of the more cortical input, the muscle activity output and proprioceptive feedback could be shifted.

Recommendations for prosthetics

After below-knee amputation, muscle synergy may be shifted. Therefore, the socket of prosthetic could not be fitted the amputee. To solve this problem, if we know the pattern muscle synergies at varied knee angles, we can modify the prosthetic by adapting to the synergies

Recommendations for the knee control position

We do not have a standard knee position for achieving isometric voluntary control. This study aimed to use applied knee control in stroke rehabilitation wards, measure the MVE at varied angles (0 °, 20 °, 60 ° and 90 °), and compare the knee extensor tasks. The idea behind this is to use the positions from the clinic, such as what stroke and orthopaedic wards use for testing: 0º = straight leg = control; 20º = slightly flex (used in the case of knee stiffness) 60º = normal angle for sports science set up for Biodex; 90º = function for walking upstairs (in the clinic, if patients can perform 90 º, they can be discharged).

C1	Muscle	Muscle	Muscle	Muscle	Muscle
0 °	VL	VM	RF	ST	BF
20 °	VL	VM	RF	BF	ST
60 °	ST	BF	VL	RF	VM
90 °	ST	BF	VM	VL	RF

C2	Muscle	Muscle	Muscle	Muscle	Muscle
0 °	VL	VM	RF	ST	BF
20 °	VL	VM	RF	BF	ST
60 °	ST	BF	RF	VL	VM
90 °	ST	BF	VL	RF	VM

Figure 2-10 Diagram of muscle synergies of the two positions (C1 and C2) in different four angles. Colour code – red – are muscle synergies with highest correlation to knee extension task, pink – are agonists, blue – Antagonists – antagonists to Quadriceps.

2.5 Conclusions

To conclude (figure 2.10), the main finding is that RF is not the primary muscle. At higher degrees, semitendinosus and biceps femoris were showed to be agonistic in the knee extensor. So, I suspect the Ib input from rectus femoris is related to semitendinosus via the excitatory interneuron in the spinal cord. In terms of future direction, we will apply electrical stimulation to see how muscle made for clinical guidelines that muscle testing cannot rely on the anatomical view alone, but tasks must also be considered.

Chapter 3 Identification of lower limb synergies during dynamic balance testIntroduction

Balance is important to stabilise the posture to prevent falling. We need adequate balance and postural control to perform any activities; for example, sit-to-stand, stand-to-walk and walking (Shumway-Cook et al., 2003). Postural control is a neurological loop consisting of the motor, the sensory and the integrative process used to maintain the body's position relative to gravity and of its body segment. And balance is a hallmark of postural control relies information from both motor and sensory (Alexandrov et al., 2001, Horak et al., 1997), Balance is related to the inertial force acting on the body and the inertial character of body segments (Winter, 1987, Winter et al., 1993) (Winter, 1995). Balance is divisible into two categories: static and dynamic balance. In this chapter, I will focus on dynamic balance which is useful for rehabilitation. Because dynamic balance will be trained before patients go walking confidently. To make them confident, we train patients with dynamic balance. During balance training, it is suggested that sensory integration occurs between the visual, vestibular and proprioceptive systems. Currently, dynamic balance called hypnotherapy or horses riding is widely used to improve proprioceptive function in cerebral palsy children (Moraes et al., 2016). The proprioceptive function can play a role to improve the balance function (Alonso et al., 2011).

The dynamic balance test is commonly used in the clinic to assess the ability of trunk, lower limbs and training is used for improving mobility. This information is subsequently used to make decisions about appropriate rehabilitation strategies (Horak et al., 1997, Gauchard et al., 2010). Therapists often use dynamic balance tests to assess muscle activity (Bohannon, 2007), or recruitment of muscle synergies (Cheng, 2016) in the lower limbs. During these dynamic balance tests, it is accepted that the anatomical location of the muscle underpins any muscle activity, or muscle synergy observed (Torres-Oviedo and Ting, 2007). For example, at the knee joint it is the agonist-antagonist interaction between the flexors (semitendinosus (ST) and bicep femoris (BF)) and extensors (rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM) and vastus intermedius (VI); together with the ankle dorsiflexor (tibialis anterior (TA)), and plantar flexor (soleus (SL), lateral gastrocnemius (LG) and medial gastrocnemius (MG), that aid the maintenance of an upright posture (Taube et al., 2008b). (Horak et al., 1997, Gauchard et al., 2010).

Rehabilitation is a complex interplay between muscles synergies, and that there is a dearth of literature about these particularly with respect to the hip and knee (Alexandrov et al., 2001). Therefore, this chapter will examine this and hopes to provide new data which might act as a platform for future research to investigate this further and/or in a clinical rehabilitation context.

3.1 Methods

3.1.1 Participants

Twenty-five participants volunteered to take part in the study. However, 3 participants' data were excluded from the analysis because EMG recording (Trigno wireless) system was error thus only data from 22 participants were analysed (26.23yrs± 4.69; F= 9). Exclusion criteria included (1) history of a neurological disorder that would affect motor coordination (2) use of prescription medication (3) recent illness or viral infection (within the last two weeks) (4) use of recreational or performance enhancing drugs (5) ingestion of alcohol in the previous 24 hours (6) history of anaemia, asthma, diabetes, epilepsy, family history of sudden death, fainting, heart disease, high blood pressure, respiratory disease, muscle or joint injury (7) Inability to provide informed consent or understand English (8) current pregnancy. (10) any current musculoskeletal injury. All experiments were carried out as per the guidance in BIOSCI 16-003, from the local human ethics committee at Leeds.

3.1.2 Task and Procedure

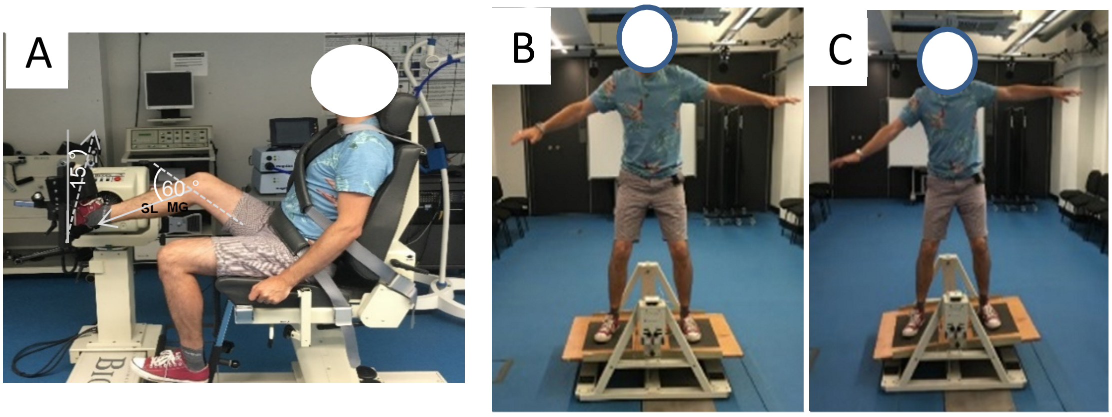


Figure 3-1 Procedure of the experiment A) MVC plantar flexion focusing on soleus on Biodex, B) participant was standing on balance board (stable phase) C) participant was on balance board (unstable phase)

3.2.2.1 MVC: Soleus maximal voluntary contraction

Since the soleus is the muscle that is presumed to be the primary muscle involved in the activity on the balance board (Di Giulio et al., 2009), we chose to establish its baseline maximal voluntary contraction (MVC) in each participant during an isometric task. The participant was seated, with the knee flexed at 60°) (figure3.1). Participants sat on the Biodex chair with body support with the right foot on the foot support, the MVC generated was measured and their EMG was recorded to provide visual feedback. Participants performed isometric plantar flexion for 5 seconds / trial for 5 trials with a second's rest between contractions.

I ensured that the position was suitable for obtaining MVC of soleus muscle alone and not the gastrocnemius. The MVC was also recorded before and after balance training to assess fatigue in line with Alonso and colleagues (Alonso et al., 2011). Given data from Bloem and colleagues stated that both legs produce the same output when working to control postural balance (Bloem et al., 2002), I focussed on muscles of the right leg alone.

3.1.2.2 Dynamic balance board task

During the dynamic task, the aim was to keep the platform about its a central pivot (stabilometer), level as close to horizontal as possible, while receiving no feedback about how to do so. A stable platform was defined stable phase by 30 % (Brouwer et al., 2019) of the total deflection either side of the pivot (figure 3.1 B and 3.3 A). An unstable platform was defined by over 30%. All participants completed 14 trials in all (2 x pre-training, 10 x training and 2 x post-training). Each trial lasted 30 seconds with a 60s rest between trials, and 5-minute rest between trial 2 and 3, and trial 12 and 13. sEMG activity was recorded from the leg muscles, the Rectus Femoris, Vastus lateralis, Semitendinosus, Biceps femoris, Tibialis anterior, Soleus, Medial gastrocnemius, and Lateral gastrocnemius (see table 1 in methods section chapter 2)

3.1.3 Analysis

All EMG activity was measured as an average of their RMS activity and duration (see methods section chapter2) across the 5 trials during soleus MVC and balance task.

3.1.3.1 Frequency analysis to define fatigue

In the dynamic balance study, participants were asked to complete a dynamic exercise using the soleus muscle frequency analysis of the sEMG was analysed to ensure the participants had not become fatigued. This is important given that fatigue can affect the performance of the individual leading to potentially confounding muscle activity with fatigue. Why be concerned about fatigue?
Fatigue is a natural physiologic response to exercise that describes the deadline

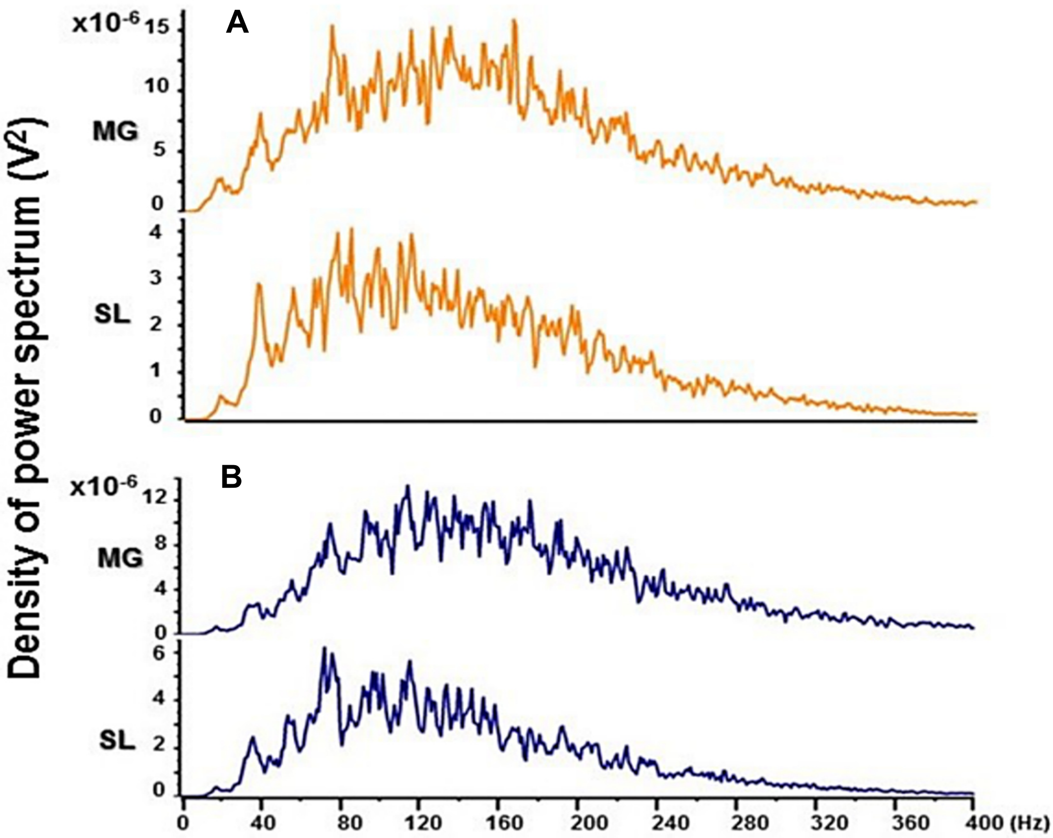


Figure 3-2 Frequency measures to assess fatigue An example of H7 frequency analysis (FFT2045) to assess central fatigue of SL and MG: A) pre-training MVC for SL and MG that continue to 400 Hz (represent non-fatigue) B) post-training MVC for SL and MG that being not changed from the pre-training, so they represented non-fatigue.

work output associated with the repetitive activity. Fatigue is typically attributed to neural or muscular origins (Gandevia, 2001; MacIntosh & Rassier, 2002). Neuromuscular fatigue has been defined as a progressive reduction in the ability of a muscle to produce power or force. Fatigue can reduce muscle contraction by inhibiting neural conduction from the central nervous system (CNS). It can also decrease proprioception in the musculature (Gandevia, 2001).

In these experiments, I avoid fatigue as I wanted participants to continue doing the exercise of dynamic standing for 60-90 minutes. In addition, I did not want fatigue resulting in decreased proprioception, as muscle fatigue has been shown to alter joint proprioception (Voight *et al* 1996). Voight and colleagues studied shoulder joint fatigue and related it to proprioception by using passive internal and external rotation of shoulder on the BIODEX machine. They found that the range of motion of the joint was significantly decreased with fatigue. In addition, Skinner et al (1986) reported a decrease in knee proprioception, with a 15% decrease of knee flexion and extension work output after general fatigue load. In our study, if the participant has fatigue conditions, their muscles responsible for balance might have a different output due to the shift of proprioceptive input.

We assessed fatigue in soleus (SL) as it is suggested in the literature that SL played an important role towards control of balance. If participants perform balance exercise for 90 minutes in the same session, they may be fatigued. There are a few shreds of evidence to support the standard position and duration of resting position between active isometric contractions. The standing position may result in muscle fatigue (central fatigue) due to actions of gravity on their own body mass. In this new protocol, I used a seated position using maximal voluntary effort and electromyography technique to examine the presence of fatigue during balance training. This procedure eliminates the likely role of central fatigue because no gravity was involved, and a minute rest was enough to achieve MVC without fatigue, as shown by the power spectra of the SL muscle, based on the tail-off frequency (Komi and Tesch 1985). Power spectral analysis has been used to ensure lack of central fatigue (neuromuscular fatigue). Fatigue, a decrease in the median frequency slope of EMG spectral power is associated with muscle fatigue. If the tail of the power spectra (frequency analysis) does not abruptly decline, it means that there is no evidence of fatigue. For instance, Fujisawa et al (2017) studied quadriceps muscle fatigue and change in surface EMG spectral power during dynamic knee extension contraction. They recorded EMG from Vastus lateralis (VL) and Vastus medialis (VM) and asked participants to perform knee dynamic contraction on the Biodex chair for 1s x 30 repetitions. They later monitored fatigue by using median frequency analysis. They reported a significant decrease in median frequency in VL of male volunteers, but not for the VM. Based on these, I applied power spectral analysis to assess fatigues in this study. If participants exhibited fatigue, I did not use their balance board data to further analysis. Frequency analysis of the EMG uses a Fourier transform function to transform the time domain of recorded muscle to the frequency domain. Non-Fatigue can be observed by tail off that can be seen until 400 Hz frequency or without shifting abruptly (Komi and Tesch, 1979). Figure 3.2 showed the frequency analysis to define fatigue as we found that there was non-fatigue in our procedure.

3.1.3.2 Waveform correlation

The activity of the muscles was calculated and compared with the position of the stabilometer that figure3.3 showed an example waveform of the balance board, SL and RF activity, measured in degrees of change from the baseline. The stabilometer was defined as stable if the balance board was held close to the baseline for at least 2 secs of the bout. The stable and unstable phases were subsequently classified into eight sub-phases 1) Stable peak minus 2) Stable peak positive 3) Stable trough minus 4) Stable trough positive 5) Unstable peak minus 6) Unstable trough minus 7) Unstable trough positive 8) Unstable peak positive.

Activity in the recorded muscles associated with these positions was then compared with the position of the stabilometer, using waveform correlation (100 bins, duration of the time domain = 50 ms) in spike 2.82. To examine the muscle activity correlative to the board on both stable and unstable phases, a waveform correlation was used to compare the activity in these muscles to that of the stabilometer and then applied to the co-efficiency correlation and onset to peak of the muscle activity. The balance board channel was used as the reference to compare the correlation to recording muscles. For further analysis, we processed the data, with the balance board channel interpolated to 2kHz to enable comparison with the muscle recordings using time window of -50ms to +50ms from the peak of activity of the balance board position. This was to examine the peripheral and short latency reflexes alone. Then, using R script performed by Gareth York to define the maximal correlation co-efficiency and maximal duration along onset (0 seconds) to peak (see figure 3.3).

To interpret the stable and unstable balance, it was suggested that 1) Stable balance: muscle active to maintain balance 2) Unstable balance: Muscles try to recover balance.

The details of the analysis were given in figure 3.3 to demonstrate how I capture the 3 seconds for stable and unstable phases.

In my study, balance board data (virtual channel V5 Stab) is calibrated to read the degree of deviation from 0 ° to 16° (maximum) and 0 ° to -16°. The data was visually split into three parts to help classify the data.

The protocol and associated figures are presented below.

Step 1: Choose the 30-second window and position the vertical cursor

1.1 Go to the stabilometer channel for balance board data (virtual channel V5 Stab degree) to examine the 30-second window and set the baseline at 0 ° (0%). The balance board is set to a degree of deviation from 0 ° to 16° (maximum) and 0 ° to -16°.

1.2 Position the horizontal cursor between -5 ° to +5 ° to capture the stable phase. Stable phase is defined as a participant maintaining the balance board at about 0 to 30% of baseline and 0 to – 30% of baseline. Again, set the horizontal cursors between 5° to 16 ° (above 30 %) and – 5 ° to – 16 ° (below -30%) to capture the

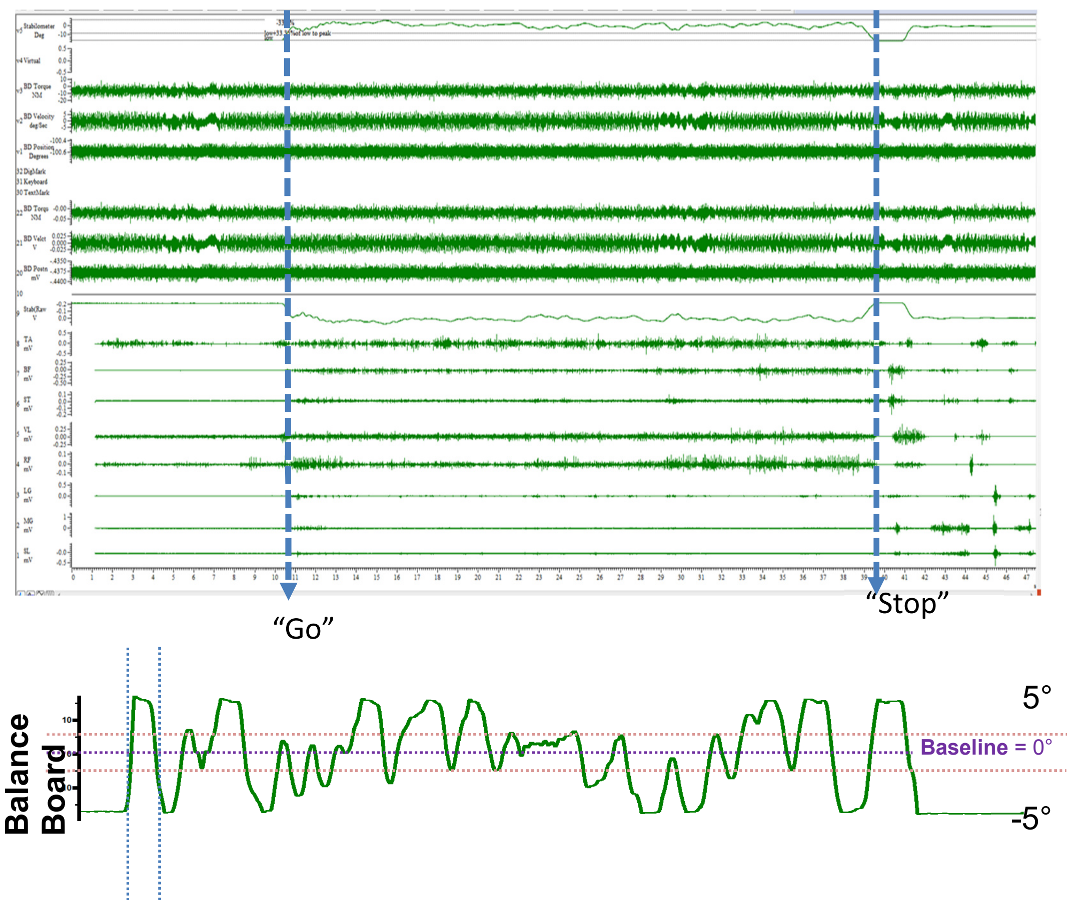


Figure 3-3 Raw image of the capture screen. Shown are all the recorded channels during the task, the very top trace was a virtual channel setup to record activity of the balance board. A portion of this has been presented in more detail under.

unstable phase.

1.3 Position the vertical cursors by using 3-second periods of time to define stable and unstable phases. For 3 seconds, onset is defined as cursor1, while offset is

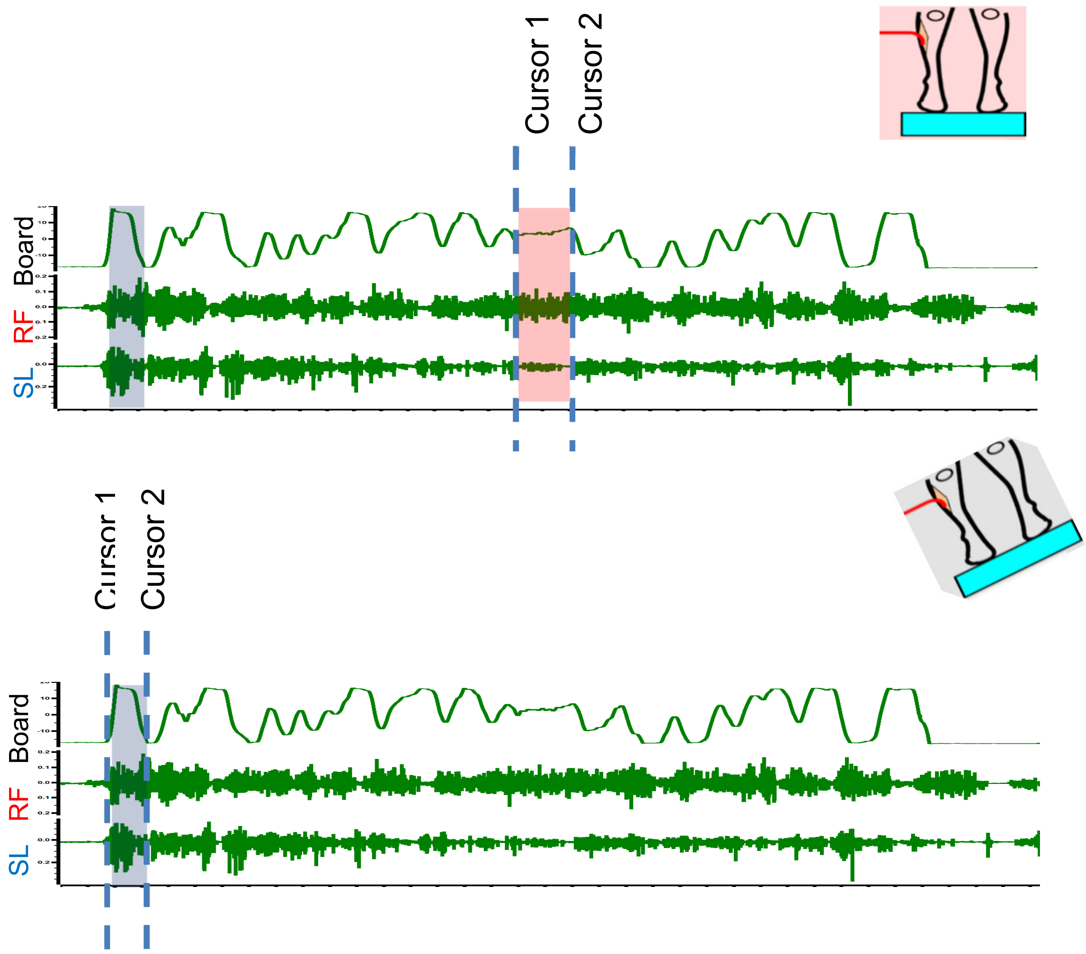


Figure 3.3 B Details of balance board analysis

defined as cursor 2.

Step 2. Comparing activity between the stable and unstable phases of balance board.

2.1 Position cursors 1 and 2 manually (stable phase: red)

2.2 Position cursors 1 and 2 manually on the raw signal of the balance board and demonstrate raw EMG SL and RF.

Step 3. The activity of the muscles was correlated with that of balance board over ± 50 ms from the peak position of balance board (0).3. Run spike 2.82 using the waveform correlation function and select balance board as a reference.

3.1. Select the period by 3 seconds as cursors 1 and 2 have been selected

3.2 Run the waveform correlation function using cursors 1 and 2 as the window of time and choose the balance board channel as the reference

3.3 activity across the eight muscles was correlated to that of the balance board, by using waveform correlations. If muscle activity was below baseline(negative), it meant the muscle was active prior to the activity of the balance board. If the muscle was active above baseline(positive), it meant that the muscle was active after the balance board.

Step 4. Find the peak of activity for the balance board position.

4.1 Position the horizontal cursor (blue) to define the highest peak of balance activity to examine the peripheral and short latency reflexes alone.

4.2 Position the vertical cursor (black) to define the onset to peak (ms) and the maximal correlation co-efficiency (mV).

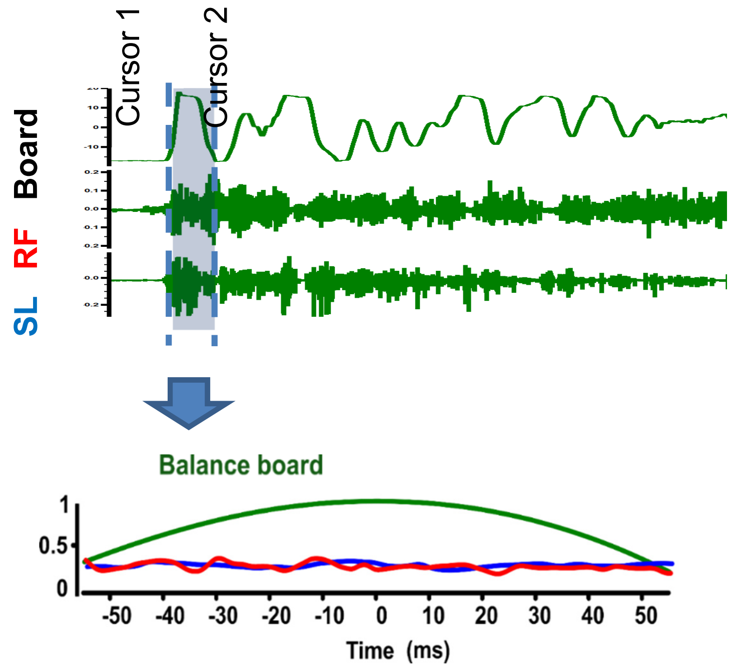


Figure 3.3C Details of analysis including Phases of balance board and activity in RF and SL demonstrating the stabilometer waveform and the procedure for cut-off of stable balance and unstable balance

4.3 Transport data to the spreadsheet to plot a graph for both pre-training and post-training.

Time duration: Onset to peak

Using waveform correlation, the peak of each muscle activity and stabilometer were calculated. Minus value (figure 3.3D) meant that the muscle time to peak occurred before the balance board and the positive peak value represented the muscle being active after the onset of the balance board. Time-lag such as the period between two closely related events (balance board event and response of the muscles, 50 to +50 ms were used to identify the time duration related to short-latency reflexes (see chapter 1 introduction). The activity of muscles was correlated with that of balance board over ± 50 ms from the peak position of balance board (0).

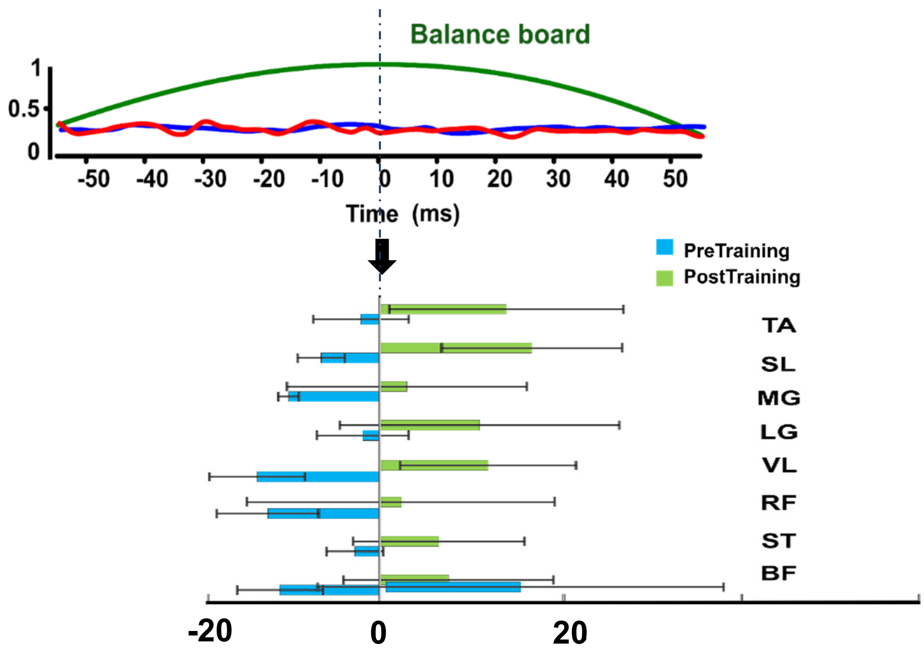


Figure 3.3D protocol for analysis of the data obtained using balance board along with distinction of activity phase. The figure shows the activity of the balance board and the muscles RF and SL. demonstrating the stabilometer waveform and the procedure for cut-off of stable balance and unstable balance.

Dependent variables and statistical analysis

Description of all analysis was calculated as the mean and standard error of the mean (±SEM). To investigate the difference between the group muscles and pre-post-training conditions, two-way ANOVAs were performed. When a significant difference was found ($p<0.05$), means were compared post hoc using Turkey's test.

3.2 Results

3.2.1 Maximal voluntary contractions: Normalisation MVC

Based on the pre-training soleus (SL) MVC, participants were categorized into two groups: Group 1: SL activity was greater than MG activity (n=14; see figure 3.4A), Group 2: MG activity was greater than SL activity (n=8, see figure 3.4B). In group1, participants had the highest activity level in the SL at 0.11mV (0.11

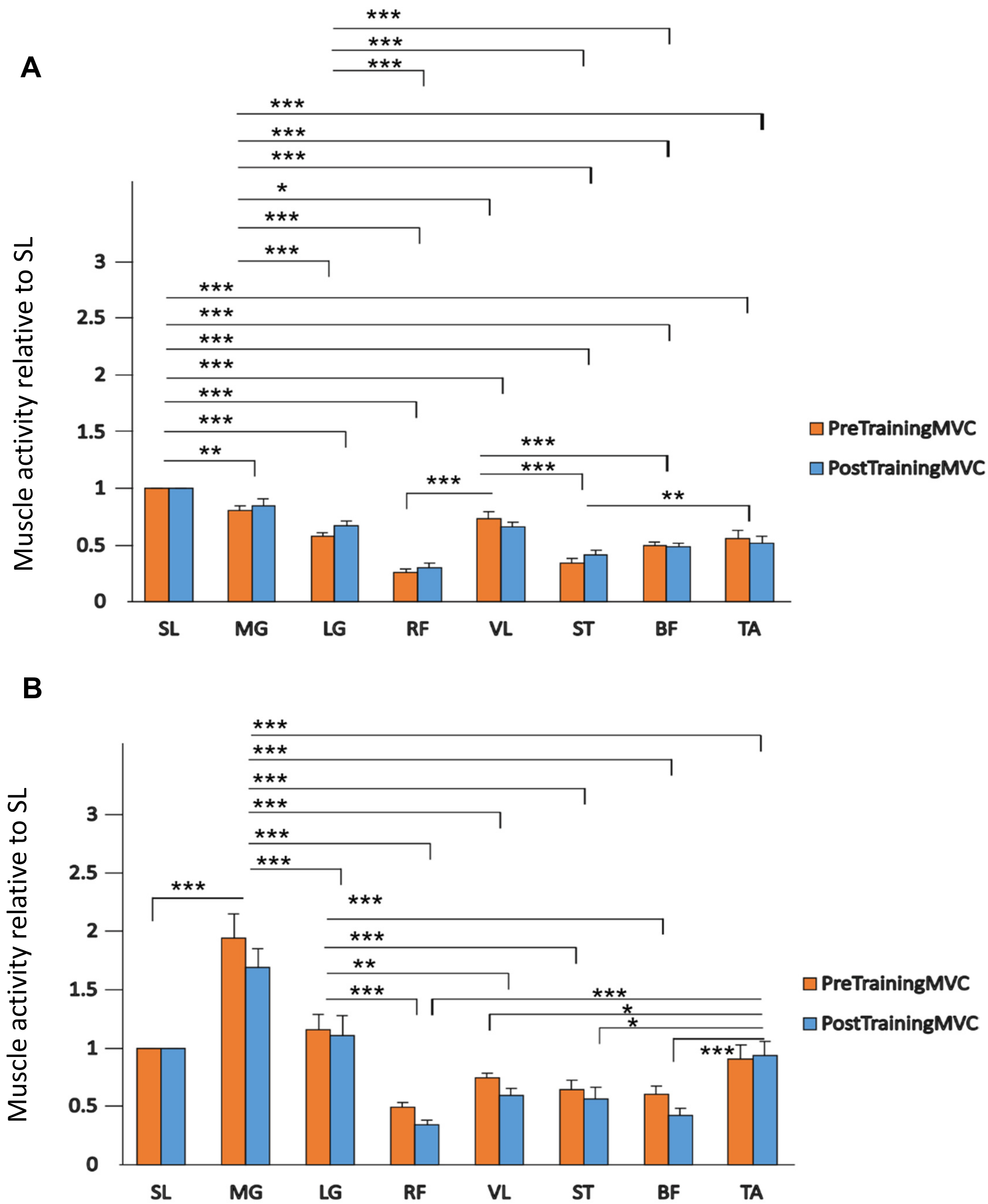


Figure 3-4 Normalisation of muscle activity to that of SL during MVC. Activity during plantar flexion MVC (mean± SEM) recording from eight muscles SL, MG, LG, RF, VL, ST, BF and TA. A) Represents group1 (n=14) and B) represents Group2 (n=8). * indicates p<0.05, ** indicates p<0.01 and *** p<0.001

mV±0.03), with MG activity about 0.07 mV±0.02 (pre-training) and 0.08 mV ±0.02 (post-training). In figure 3.4 A, in group 1, normalisation muscle activity relative to SL, SL activity had the highest ratio (1±0). MG relative to SL (ratio) was 0.8 ± 0.04 and VL ratio was 0.731 ± 0.061. In group 1, the overall data showed that there were no changes between pre and post-training ($F(1,1046) = 0.21$, $p=0.08$); however, there was a difference between muscle groups ($F(7,1046) = 0.54$, $p= 0.00001$). In pre and post-training condition, SL activity was higher than LG, RF, VL, ST, BF and TA significantly ($p<0.001$) and SL activity was higher than MG significantly ($p<0.001$). In pre and post-training condition, MG activity was higher than LG, RF, ST, BF, TA significantly ($p<0.001$) and MG activity was higher than VL ($p<0.01$). LG was significantly higher than RF, ST and BF ($p<0.001$) (See figure 3.4 A).

In Group 2 for pre-training and post-training, MG activity (0.105 mV±0.02) was higher than SL (0.057 mV±0.01). In terms of muscle activity relative to SL in Group 2, MG showed the highest activity (pre-training 1.942 ± 0.204, post-training 1.6906 ± 0.160) (Figure 3.4 B).There were changes with training ($F(1,615) = 3.961$, $p=0.047$) as well as a significant difference between means for muscle activity ($F(7, 615) = 37.474$, $p=0.00001$). MG activity was significantly higher than SL, RF, VL, ST and BF ($p< 0.001$). LG activity was significantly higher than RF ($p < 0.001$), VL ($p < 0.01$), ST ($p< 0.001$), and BF ($p< 0.001$). TA activity was higher than RF ($p<0.001$), BF ($p<0.001$), ST and VL ($p<0.01$) (see Figure 3.4B). To conclude, there was significance variance in Group 2 for all recorded muscles when comparing pre- and post-training.

3.2.2 Duration of MVC plantar flexion

Burst duration defined by the onset and offset of muscle activity of all muscles were measured for each participant when they performed the ankle plantar flexion MVC. We examined burst duration with respect to the MVC of the SL pre and post-training (figure 6). Based on the soleus (SL) MVC, participants were categorized into two groups: Group 1: SL activity was higher than MG activity (n=14; see figure 3.5A), Group 2: MG activity was greater than SL activity (n=8, see figure 3.5B). The trend for group 1 and 2 suggested differences in muscles burst duration.

In group 1, there was no significantly different with pre and post-training ($F(1,208) = 0.1$, $p=0.32$). There was significantly different between means of muscles (F

(7,208) =3.156, p=0.003). Participants had the longest burst duration in TA (5.77 ± 0.11 sec, pre-training) and 5.97 ± 0.18 sec, post-training. The participants had the shortest burst duration in BF with about 5.29 ± 0.14 sec (pre-training) and the shortest burst duration went for RF in post-training with 5.35seconds±0.2. TA post-training was significantly longer than RF pre-training (p=0.04) and TA post-training was significantly longer than BF pre-training (p=0.02). In post-training, ST, BF and TA had longer burst duration. There was suggested the trend that SL, MG, LG, RF, VL showed the unchanged pattern of data after training (see figure 3.5A).

In group 2, there was no significantly different with pre and post-training (F (1,112) =0.99, p=0.32). There was no significantly different between means of muscles burst duration (F (7,127) =0.28, p=0.96). Participants had the longest burst duration in VL with about 5.95 seconds ± 0.25(pre-training). The longest duration went for TA (5.82seconds ±0.34) and VL (5.80 seconds ±0.25) in post-training. SL, MG, LG, RF, VL and ST had a change in activity post-training (duration in post-training MVC was decreased by 0.2sec). BF and TA showed a similar data pattern, and do not change with training (see figure 3.5B). To conclude, in both groups, in comparison to pre and post-training, there was no difference in all recorded muscles.

3.2.3 Balance board

Using the activity of the stabilometer as a reference, we compared muscles activity of each of the stable and unstable phases outlined in section method. And

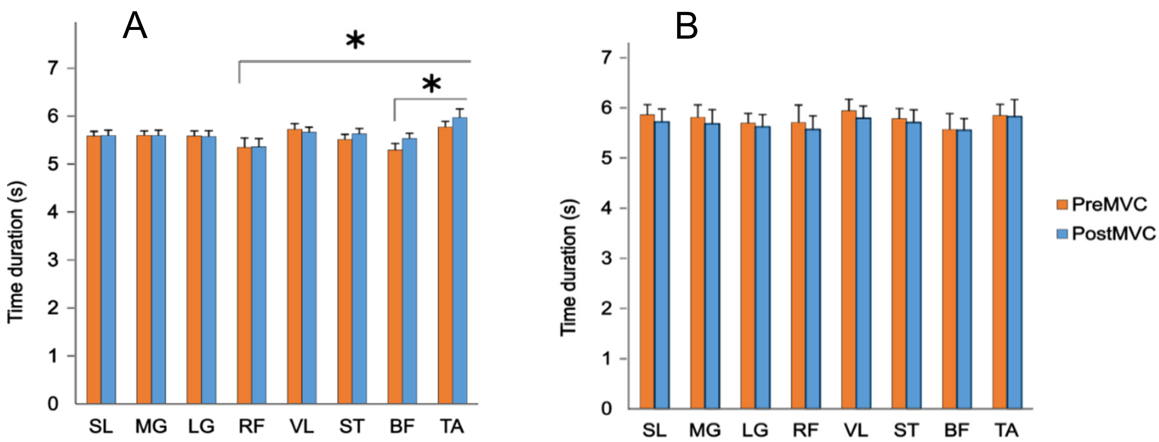


Figure 3-5 Average of burst duration of MVC plantar flexion (mean ± SEM) between pre and post-training recording from eight muscles SL, MG, LG, RF,VL,ST,BF,TA. A) Group1 n=14 B) Group2 (n=8). non-significant= p>0.05, * indicates p<0.05

then dataset of stable (figure 3.6 A) and unstable phases (figure 3.6 B) were taken to the ratio (figure 3.6 C).

In a stable phase, the average muscle activity from eight muscles was approximately 0.03 mV±0.04. The trend of muscle activity after training

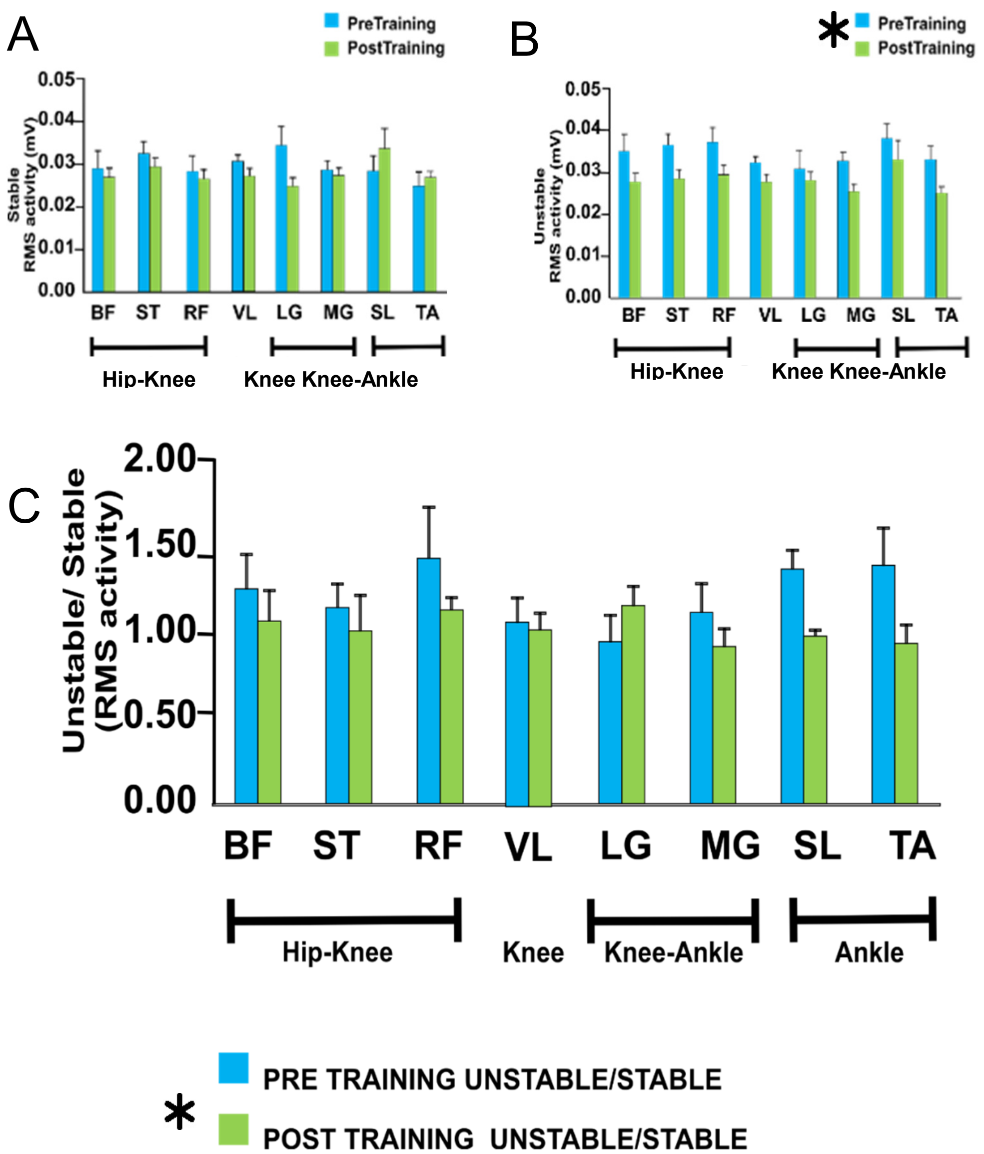


Figure 3-6 Muscle activity (mean ± SEM) during stable phase in relation to stabilometer recording from eight muscles BF, ST, RF, VL, LG, MG, SL, TA (in 22 participants) A) Muscle activity during stable phase B) Muscle activity during unstable phase C) The ratio of unstable/stable activity. Two-way ANOVAs and post-hoc (Tukey's tests) by indicating non-significant= $p>0.05$, * indicates $p<0.05$. There was significantly different between pre and post-training in figure A and C ($p<0.05$).

suggested decreasing pattern across six muscles (BF, ST, RF, VL, LG, MG) compared to pre-training, but there was no significantly different with training ($F_{(1,48)}=1.49$, $p=0.23$). There was no significantly different between muscles ($F_{(7, 48)} = 0.744$, $p=0.636$). There were two muscle activities suggested increasing the amplitude after training (SL and TA). In post-training, SL activity showed the highest amplitude (0.037±0.005 mV).SL activity suggests the trend improved stability by 1.5±0.1 mV.

In unstable phase (figure 3.6B), there was a significant difference between pre and post-training ($F(1, 48) = 9.913$, $p=0.003$), but the mean activity of muscles was the same ($F(7, 48) = 0.67$, $p=0.7$). The average muscle activity from the recorded muscles was approximately 0.035±0.03 mV. In pre-training, SL activity had the highest amplitude (0.038±0.004 mV) and followed by ST (0.037±0.004 mV), BF (0.035±0.006 mV) and RF (0.037±0.002 mV)). In post-training, SL activity had the highest amplitude (0.033±0.005 mV) and followed by RF (0.029±0.001 mV). After training, all recorded muscles activity suggested the decreasing trend.

To observe the ratio of unstable/stable phase, in figure 3.6 C, there was significantly different across pre and post-training ($F(1,48) =5.6$, $p=0.02$). There was no significantly different between means of muscles ($F(7,48) =0.62$, $p=0.73$). In pre-training, it was suggested the trend that RF (ratio=1.47±0.3) and SL (ratio=1.41±0.11) had the highest activity. After training, it was suggested LG (1.19±0.11) and RF (1.16±0.07) had the highest activity.

Balance board: Comparison of the time to peak for muscles and balance board during the same period

After calculating the onset to peak of onset along the peak time, unstable over stable phases were demonstrated in both pre and post-training. To examine the muscle activity, maintain balance both pre and post-training, the change in latency are depicted in figure 3.7. To assess the restore balance function, we observed by the unstable phase. Using waveform analysis (see method section), ± 5 ms was used to define the correlation between the muscle and balance board.

In the stable phase (figure 3.7A), there was significantly different between pre and post-training ($F(1,48) =12.342$, $p=0.001$). There was no significantly different between the means of muscles ($F(7,48) =0.4$, $p=0.9$). TA, LG and ST showed the onset to peak about 5 ms (TA= -2ms±5, LG=-2mec±5 and ST=-3ms±5), so they maintained balance at pre-training condition. It was suggested the trend after training, MG (3ms±13) and RF (2ms±1.7) was delayed at 5 ms from the peak of balance board activity. After training, both RF and MG were active.

In the unstable phase (figure3.7B), there was significantly different between pre and post-training ($F(1,48) =5.522$, $p=0.023$). There was no significantly different between the means of muscles ($F(7,48) =0.272$, $p=0.962$). In pre-training, using

the criteria of time lag ±5 ms, there was suggested that TA (4ms±3), SL (-2 ms±3) and MG (-1 ms±4). Therefore, TA, SL and MG were active to restore balance at pre-training condition by observing their time lags being approximately ±5 ms. Post-training, TA (1 ms±3) and VL (-2ms±3.4) were active to restore balance.
In figure 3.7 C, there was significantly different between pre and post-training (F (1,48) = 16.14, p=0.0002). There was no difference between means of muscles (F (7, 48) = 0.39, p=0.91). Observing the duration between 0 ± 5 ms of unstable -stable, the trend suggested that in pre-training condition, ankle muscle, MG (9±4 ms), SL (5±3 ms) and TA (6±4 ms) were active towards restoring balance. Post-training, knee muscles RF, VL and MG were active to recover balance instead of the ankle. In post-training, RF activity duration was about -9ms±13, VL was -10±9 ms and MG was -10±10 ms. RF activity was improved after training by four folds.

3.3 Discussion

3.3.1 Main findings

1. during the Plantar flexion MVC task, SL activity was greater than of the MG (14/22 participants) (figure 3.4). There was no evidence of fatigue, after training, when performing the MVC (figure 3.2).
2. During the balance task, MG, RF and ST helped maintain balance during stable phase (figure3.7A), while TA, SL, VL were active in improving recovery of balance (figure 3.7B).
4. There was a trend in the data to suggest a decrease in activity in most muscles post-training (figure 3.6) MVC

3.3.2 Possible mechanism: MVC

In group 1, 14 participants showed SL activity being higher than MG; however, the other group 8, MG was greater than SL (figure 3.4). In MVC group 1: soleus and medial gastrocnemius can be selectively activated for 11 participants (14/22 participants). MVC of soleus with the flexed knee at 60˚can be used for MVC test (14/22 participants achieved the MVC of soleus) and there were 8/22 participants demonstrated MG in Soleus MVC.

Anatomically, medial gastrocnemius (MG) acts not only as a plantar flexor but also as a knee flexor, meaning that it is an antagonist during knee extension. The soleus is a mono-articular plantar flexor (Suzuki et al., 2014). However, our initial data suggested that the activity of the MG is greater than that of the SL (figure 3.4B). This could be due to two reasons 1) MG and SL have the same insertion, which would allow them to work together during plantar flexion. During an MVC plantar flexion task, it can be challenging to achieve the output from soleus alone, because both MG and SL have the same insertion at the calcaneal tendon. 2)

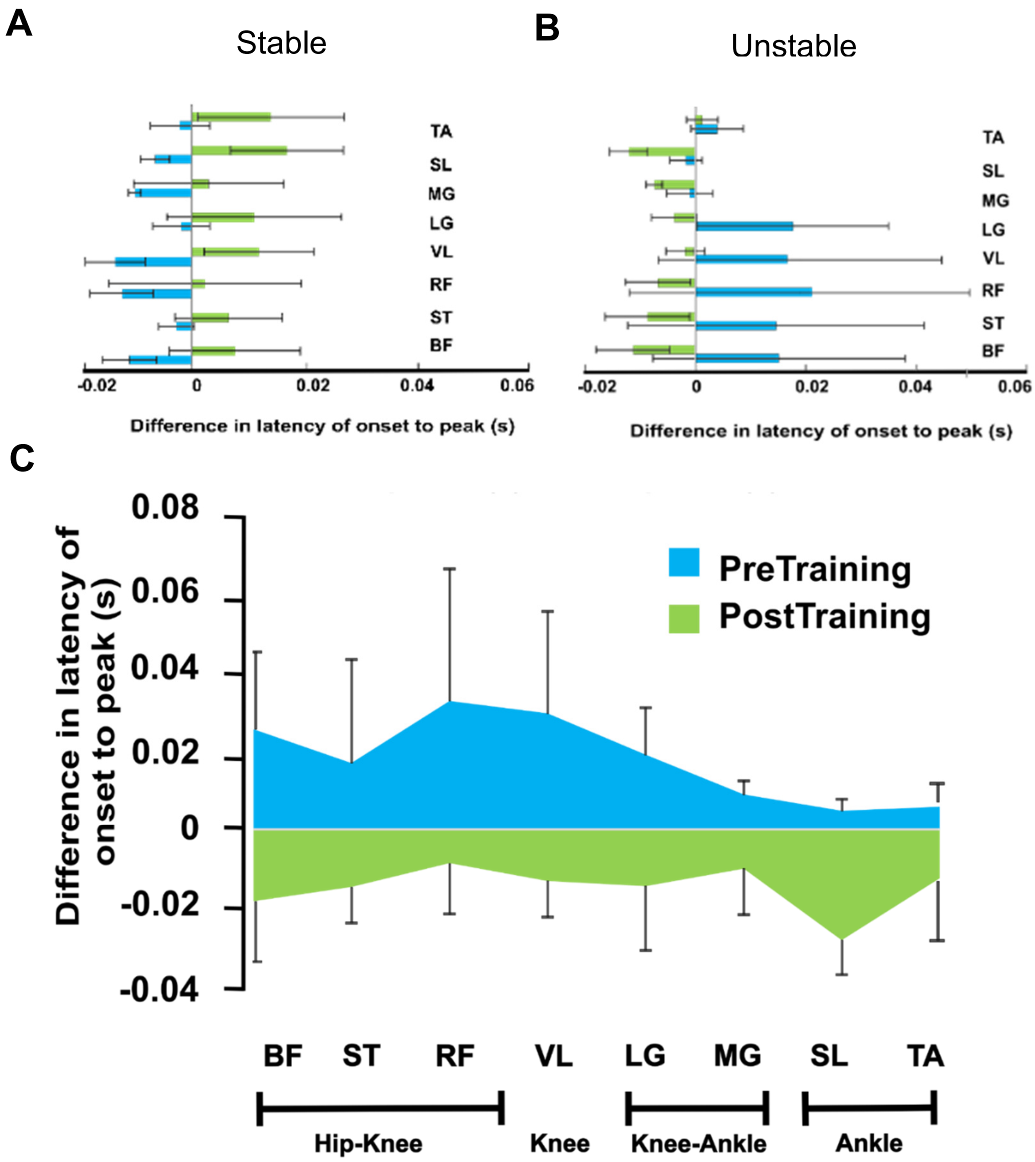


Figure 3-7 shows the change onset to peak in relation to stabilometer (mean ± SEM) in pre and post-training, recording from eight muscles of the lower limb (BF,ST,VL,RF,LG,MG, SL and TA). A) Change in latency in stable phase B) the change in latency in unstable phase C) shows the difference in latency onset to peak by Unstable (s) – Stable (s). Two-way ANOVAs was tested and post-hoc (Tukey's tests) by indicating non-significant= p>0.05. There was significantly different between pre and post-training in figure A, B and C (p<0.05), but there was no significantly different between the means of muscles *in figure A, B and C (p<0.05)*.

MG is bi-articular muscle, so it has a powerful action to achieve plantar flexion MVC (Suzuki et al., 1982).

Burst duration with respect to the MVC of the SL pre and post-training, burst duration was unchanged with training (figure 3.5). However, there was a dissimilar trend between group1 and group2 participants. For example, in group 1, 14 participants achieved SL muscle activity with remained the duration pattern SL, MG, LG, RF, VL after training. There were three muscles presenting increased duration (ST, BF and TA). In contrast, in group 2 (8/22 participants), burst duration after training of SL, MG, LG, RF, VL, ST was changed by 0.2 seconds (not significant $p > 0.05$) (figure 3.5). It was presumed that SL activity may lead in TA, BF and ST duration changes. MG may not influence TA and BF duration. However, it was shifted by 0.2 seconds but not significantly, so we might need further experiments to define the effect of TA and BF on MG.

3.3.3 Possible mechanism: Dynamic Balance

Balance is a complicated process involving modification of both axial and limb muscles function to compensate for the effects of gravity and alterations in body position and load to prevent a person from falling (Pratt et al., 1994). This procedure comprises input from a variety of sensory modalities (visual, vestibular and proprioceptive). During standing, the human sensory-motor system collects sensory input from the proprioceptive and supraspinal control to generate the appropriate movement output (Prieto et al., 1996). If the input has adaptation, the muscle activity output can have altered patterns. Because of inconsistency in signals due to various factors, such as non-constant biomechanics as the body is off- centre, relative to the base of support, non-constant sensory perception at the receptor and non-constant motor firing (Faisal et al., 2008). Because balance is complex, in order to maintain balance, bi-articular muscles, such as rectus femoris, acts to finely tune the movement with two-directional movement (hip flexor and knee extensor (Macpherson and Fung, 1999). The Rectus femoris may play a crucial role to maintain balance and may have deleterious effects when transferred to become a knee flexor. In terms of flexible synergises theory, bi-articular is different from the mono-articular muscle. For example, mono-articular has more rigidity (extensor acts extension, flexor acts flexion); however, bi-articular is more flexible by motion associated with peripheral feedback afferent (Macpherson, 1988).

To achieve stability, participants activated the VL, ST, RF, LG and TA earlier while delaying activity in SL when the activity of these muscles was compared to the unstable phase (see result figure 3.7 A, B). The shift in onset ranged from 5 to 20 ms. Overall, in a dynamic task commonly used to examine and restore balance (Spaan et al., 2017), it is assumed that muscles at the ankle and trunk are the most active when trying to maintain balance (Winter et al., 1993). However, my data show that while overall there was more activity of the muscles acting at the ankle, the activity of the muscles acting at the knee was greater when the participant was in balance (see figure 3.6,3.7). This suggests that training of the muscles active around knee may enhance or speed up the recovery of dynamic balance. Given their importance for balance (Winter et al., 1993), for the balance task used in the present thesis we expected to see activity in the muscles at the ankle (SL, MG, LG and TA) to be correlated with the position of the balance board, and a change in their activity would be reflected in the position of the stabilometer. Thus, activity in SL should be about the same in both stable and unstable states, but the other muscles at the ankle were reduced in a stable state. Instead, activity in the muscles at the hip and knee (especially VL, RF and ST) was more important in shaping the activity of the stabilometer, as a change in amplitude accompanied a change in onset and offset of activity of these appear to define stable over unstable states of the stabilometer (see figure3.7). Rectus femoris and knee muscle can be influenced by the positioning (squatting position). It has been previously reported that slightly flexed knee like a squat position can help control balance (Cheng et al., 2017) with suggestions that a slightly flexed knee at 30 ° can also be allowing auto balance correction or recovery (Runge et al., 1999). In this study, participants performed better at retaining balance when they stood with a slightly flexed knee like a squatting position. The squatting strategy can improve balance recovery (Hemami et al., 2006). Squatting position is a protective response and has been documented in backward falls (Runge et al., 1999).

To explain the paired muscle activity, in stable balance phase which was focused on ±5 ms, TA and LG showed balance maintenance (figure 3.7). TA is an ankle dorsiflexor muscle, but LG is an ankle plantar flexor. It was possible to work as an agonist-antagonist function. DiGiulio and colleagues studied gross anatomy using ultrasound on human SL and TA, they found that TA and SL are active together as agonist and antagonist to maintain balance (DiGiuilo et al, 2009). In post-training, VL and LG were active to maintain balance, again LG is a knee flexor, but VL is a knee extensor. These might be the counteraction of the knee to maintain an upright position.

To learn a new skill, the human nervous system is highly adaptable in response to different types of training (Adkins et al., 2006). These neural adaptations take place at both spinal and supraspinal levels and they are task and training dependent (Taube et al., 2007). In this regard, it is well known that resistant training promotes almost opposite adaptation (Aagaard, 2003). While balance training has been shown to decrease motor units firing rates, increased firing rates are observed after a period of resistance training. That is why RMS after training suggested the decreasing trend (see result figure3.6). In terms of balance control, the pathways suggesting supraspinal control (e.g. corticospinal tract, vestibulospinal tract) and proprioceptive afferent are modulated in the spinal cord to control the posture. However, there are a few studies in literature focusing on short periods of training. It is suspected that the spinal adaption may influence the muscle synergies and evoked response because of the latency period (10-20 ms) (see result figure 3.7). However, several studies have previously demonstrated that exercise produces similar, or even greater physiological adaptations (Aagaard, 2003). Neural circuitries in the spinal cord show adaptive alterations caused by changed afferent input to the spinal cord in relation to practice effects, immobilization, injury and neurorehabilitation (Christiansen et al., 2017). Participants practised the motor skill sequence and this training process was most likely affected by either experience or any balance- board skills. Because our participants have not had experience with balance training before participating in the study, so balance board practice was a new skill for them. In addition, they were not taught how to control the balance exactly, so it is simply that the response and muscle adaptation is caused by new skills training (Wolpaw, 2007a). New skills involve three programs 1) primary under new behaviour 2) compensate to maintain old behaviour 3) react plasticity (Wolpaw, 2007b). Chen and co-workers stated that the evoked response activity that conditioning can change motor function resulting from activity-dependent spinal cord plasticity (Chen and Zhou, 2011). Plastic changes in the central nervous system are well recognised in relation to the acquisition of new skills (Sanes and Donoghue, 2000), such neural alterations are generally reported mainly to take place in the initial stages of strength training (Tesch et al., 1983), and their significance for the increased strength compared with the well-documented muscular alterations is still discussed (Carroll et al., 2001, Gandevia, 2001). The changes in muscle activity after having the altered input can be monitored by muscle contraction. The contraction can be processed by motor axons; however, the sensory feedback performs as a timer to send the information about the error in movement (Franklin and Wolpert, 2011, Wolpert et al., 2011). Evoked responses transmission seems to be modified by any kind of physical motion (Perez et al., 2004). Balance training activity can cause spinal evoked response modulation. Evoked response changes seem important to explain how spinal cord cause circuitries (Papegaaij et al., 2016, Taube et al., 2008a, Horak et al., 1997, Wolpaw, 2001, Gauchard et al., 2010). This result can show you clearly that spinal circuit or local input plays a role in balance training and muscle adaptation (Christiansen et al., 2017).

3.3.4 Clinical implications

My findings provide two reasons to try and achieve better soleus MVC; 1) there was no compensation by hip-knee muscles because there was significantly different between SL and hip-knee muscles (figure 3.4) 2) There was no muscle fatigue. To achieve soleus MVC, with the seated position to achieve the MVC. Activity during MVC should reduce with fatigue but it was found to be higher even after training, possibly due to motor adaptation and recruitment of more motor units. Komi and Tesch used median frequency analysis to detect central fatigue, during voluntary contractions. If participants are exhausted, then the task will fail with a reduction in motor unit firing (Komi and Tesch, 1979, Chang et al., 2012). In my procedure, I asked participants to perform plantar flexion, and I asked them to rest between contractions for about a minute. It is thought to be challenging to avoid fatigue as many use rest of 3-5 minutes between bouts. There is no evidence of fatigue MVC (see methods section, MVC figure 3.2) present no fatigue using frequency analysis to see the frequency power spectrum. I wish to avoid fatigue confounding the MVC after an exercise or a rehab that is training dynamic balance, I use my methods. I examined this by examining the power distribution for the recorded EMGs of each subject. There are very good results of frequency analysis to show non-fatigue across all lower limb muscles (see figure 3.2). This can be the standard position for the clinical guide in the future. The likely reason for lack of fatigue is in this position, as it is a seated position, lack of gravity at the ankle joint is reduced along with the slightly relaxed position with support to the upper leg helps avoid the knee and hip influencing the ankle. During MVC's we minimised hip-knee function by supporting the participants with full support for buttocks, back and upper thigh (see figure3.1). This position, in which the activity of the knee extensor is reduced by bending the knee at 60˚, is similar to the position used by Suzuki in 1982, (Suzuki et al., 1982) during a cycling task. As participants we asked to maintain a 60 ° flexion at the knee, we also minimise quadriceps compensation (Suzuki et al., 2014), Given that MG has a bi-articular function, in that it acts in both knee flexion and plantar flexion (Kendall and Kendall, 2005), by flexing the knee at 60˚, we avoid activation of the MG muscles, in the upper portion of the leg/knee activating the lower muscle (SL) separately is single joint muscle that does not involve the knee or link with another muscle and is farther apart from MG. However, the bi-articular nature of MG means it is still active during plantar flexion at least in 22.5% of the participants (figure 3.4B). This could possibly be used as a standard position for a clinical guide to assess SL in the future; however, it should be called "gastro-soleus MVC"

Another important implication of my findings is that, given the trend of that SL MG and RF activity can improve balance after training, (figure 3.7) this initial dataset suggests that to improve dynamic balance, patients who have balance disturbance could be prescribed specific exercises to train the rectus femoris, soleus and medial gastrocnemius (RF) (Bloem et al., 2002). Besides, human balance and postural control involve both the sensory and motor systems. Dynamic

balance training as a closed chain exercise can improve proprioception, which can increase motor unit firing after training (Keshner et al., 1987).

3.4 Conclusions

For a dynamic balance task, commonly used to examine and aid recovery of dynamic balance (Spaan et al., 2017), it is assumed that muscles at the ankle and trunk are the most active when trying to maintain balance. However, my data shows that while overall there was more activity of the muscles acting at the ankle, the activity of the muscles acting at the knee was greater when the participant was in balance. This suggests that training of the muscles active around knee may enhance or speed up the recovery of dynamic balance. Taken all together, our data show that further research is required to not only establish the role of the muscles acting at the knee during common tasks (sit to stand, posture and walking) but also in rehabilitation. The role of the muscles acting at the knee is often overlooked by therapists and clinicians, although they are the commonly targeted muscles to improve stability in individuals with knee injuries, a very common occurrence.

Chapter 4 Pathways associated with altered lower limb muscle response to training during a dynamic task

4.1 Introduction

Acquisition of motor skill is correlated with changes in spinal cord reflexes (Wolpaw, 2001). Reflexes are modulated by prolonged practice and exercise. Balance training is an exercise that involves the plasticity of the spinal cord caused by modulating the motor unit activity (Taube et al., 2008). Standing balance is one of the lower limb ability assessment tests in stroke or motor disturbance patients in hospital (Bohannon, 2007). In addition, it is also used to train athletes to gain muscle co-contraction of the lower limb (McGuine et al., 2000). Muscle co-contraction may be associated with increased pre-synaptic inhibition and decreased reciprocal inhibition (Morita et al., 2001). Altogether, the spinal circuits themselves can be modulated by training. Schneider and Capaday reported reflexes to change over days and weeks into training in humans, manifested as a change in motor output. They studied the effect of backwards-walking over 10 days, with a recording of H-reflex in soleus muscle to assess the changes due to 10 days of training (Schneider and Capaday, 2003). They found that the reflexes were larger at the beginning and then it was not detected at 10th day. The change in reflex was attributed to the possible change in sensitivity of the proprioceptive or sensory input from the foot. The improved ability of the subjects to carry out the balance task, described in the previous chapter, was associated with an altered recruitment order of the muscles. The recruitment order can be altered due to multiple reasons, a) change in pathways defining muscle recruitment, b). Change in excitability of these pathways.

In this chapter, I describe interactions between leg muscles evoked by stimulation of the tibial nerve both before and after the subject was trained to perform the novel task of the balance board. This allowed us to assess any likely change in the spinal circuits associated with the muscles, induced by the training which improved the subject’s ability to balance.

Chen and Zhou (2011) reported that the soleus muscle training is used to improve balance in patients undergoing rehabilitation. Electrical stimulation of the tibial nerve is also commonly used clinically to stimulate the soleus muscle for both diagnosis and gain of function tests (Burke, 2016) testing spinal evoked response, assessment of nerve conduction and reciprocal inhibition (Abbruzzese et al., 1988, Pierrot-Deseilligny and Burke, 2012). My results presented in chapter 3 (balance board), indicated the same that the soleus is one of the muscles that help with maintenance of balance (see 3.7B and C).

4.2 Methods

4.2.1 Equipment/tools

Muscle activity was recorded as EMGs using the Delsys Trigno™ system (1.9 kHz, 20 - 450 Hz). Maximal voluntary contraction during plantar flexion was tested using a Dynamometer (Biodex system 3pro; Biodex medical, Shirley, NY, USA). For standing balance training a stability platform (42” x38”x 22”, Lafayette Ins. Co. USA) was used. Tibial nerve was stimulated with the single biphasic square pulse of 200 µs duration, stimulus strength ranging from 1-20 mA, at 2kHz, using a Digitimer DS7AH (Digitimer, Welwyn Garden City, Hertfordshire, UK). The data were recorded onto a PC running Spike 2.8.0 (CED) for later offline processing and analysis.

4.2.2 Participants

All experiments were carried out as per the guidance in BIOSCI 16-003, issued by the local human ethics committee at Leeds. Of the 25 healthy, participants in the previous study (Chapter 3), 15 were administered the Tibial nerve stimulation and recorded from for evoked responses. Physical attributes of the 15 participants (n=15, female=4); age of 18 – 30 (27.13yrs± 4.98), is detailed in table

SUBJECT	HEIGHT(m)	AGE	WEIGHT (kg)	ACTIVITY	SEX
H3	1.73	25	72	Gym	M
H7	1.61	29	65	NONE	F
H9	1.9	29	105	Karate	M
H10	1.79	25	75	Running	M
H12	1.8	24	70	Climbing	M
H13	1.85	39	81	Boxing	M
H14	1.75	23	63	Football	M
H15	1.55	36	45	Running	F
H16	1.78	28	63	Squash	M
H17	1.8	26	65	Jogging	M
H20	1.75	23	70	Cycling	F
H22	1.59	27	52	swimming	F
H23	1.9	27	82	Running	M
H24	1.75	19	72	Football	M
H25	1.78	27	82	Football	M

Table 4-1 Characteristic of the 15 participants showing height, age, weight, physical activities and gender who were administered tibial nerve stimulation

Participants were not permitted to participate if they had any history of 1) neurological disorders such as nerve injury 2) use of prescription medication 3) recent illness or viral infection within the past two weeks 4) use of recreational or performance-enhancing drugs 5) ingestion of alcohol within the previous 24 hours 6) history of anaemia, asthma, diabetes, epilepsy, family history of sudden death, fainting, heart disease, high blood pressure, respiratory disease, muscle or joint injury 7) Inability to provide informed consent or understand English 8) current pregnancy.

4.2.3 Procedure

In addition to the balance board training and the MVC described in the previous chapter, we used electrical stimulation of the tibial nerve to determine any change in the pathways involved in the control of the leg muscles active during balance

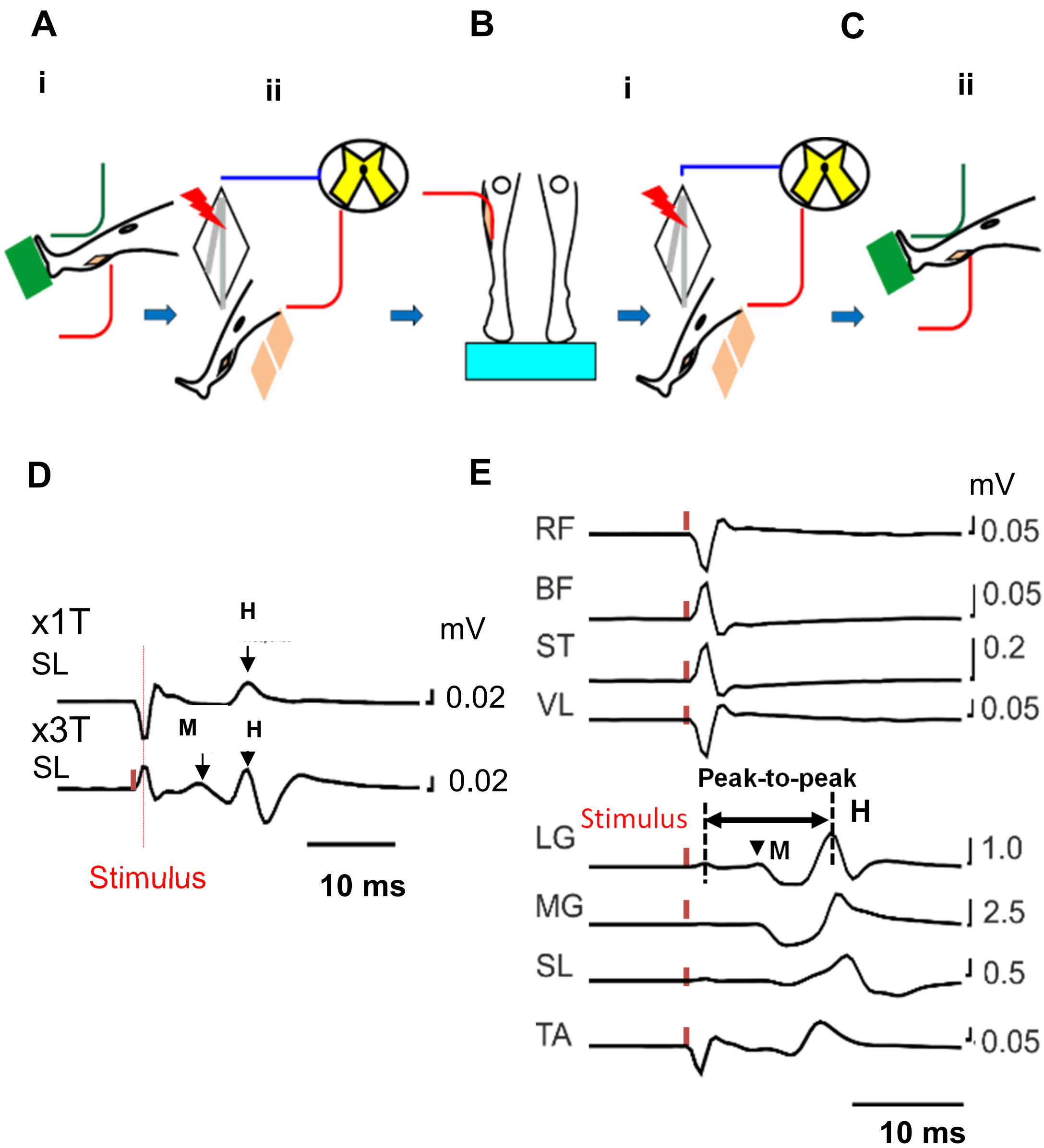


Figure 4-1 Standard testing and training protocol and example of evoked responses in muscles to tibial nerve stimulation. A) Pre-training session; i. Soleus MVC ii. Electrical stimulation of tibial nerve B) Training C) Post-training session; i Soleus MVC ii tibial nerve stimulation D) and E) Examples if evoked response in muscles to electrical stimulation of the tibial nerve, D) averages from H23 showing difference in response in SL muscles to stimulus strengths of 1x and 3x M threshold E) averaged recordings from all muscles (RF, VL, ST, BF, LG, MG,SL,TA) to the stimulation of the tibial nerve and the procedure to measure peak-to-peak. The response in RF, VL, ST and BF was negligible.

board tasks.

4.2.3.1 Peripheral stimulation using electrical stimulation

Stimulating electrode: The tibial nerve, innervating the plantar flexor muscles, identified by tracing the distal tendon of the semitendinosus muscle and moving laterally while palpating deep into the popliteal fossa. The optimal stimulation point was first located using a hand-held electrode. All peripheral nerve stimuli were delivered were a single square pulse using the constant current, high voltage stimulator (DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK) with the cathode being placed rostrally and anode towards the ankle to avoid an anodal block. The anodal block is a phenomenon where a local block in nerve conduction is caused by hyperpolarization of the neuronal membrane by an electrical stimulus. When steady direct current is passed through a nerve, excitability is raised near the cathode and lowered near the anode. When the polarization under cathode occurs, there is also hyperpolarization of the nerve beneath the anode. The threshold for the anodal block is always higher than for excitation that would be increasing the stimulation intensity when recording the muscle activity. To avoid anodal block, which causes the stimulus intensity to higher than required, the anodal end of the stimulator block should be placed carefully so that it is close to the muscle, and does not stimulate another nerve (Kirshblum et al., 1998). The electrical stimulation was repeated between 7-10 and the evoked responses, recorded from each subject at each stimulus strength tested. The stimulus current was increased incrementally until a plateau was reached for the peak-to-peak amplitude for the spinal reflex. Peak-to-peak measurement is widely used to assess the reflex changes. For example, Capaday and Stein (1996) used the peak-to-peak analysis to measure the amplitude modulation in the stepping procedure. Peak-to-peak is defined by measuring the difference between the maximum and minimum values in the time range (http://ced.co.uk/img/Spike8print.pdf). Using the peak-to-peak to define the amplitude and latency is convenient because the researcher could find the peak in activity and mark it properly and then ask the cursor to find the peak of reflex. The stimulus was increased in 5 mA steps after the motor threshold was established. The evoked responses were recorded from all 8 muscles, the thigh and leg muscles: soleus (SL), medial gastrocnemius (MG), rectus femoris (RF), vastus lateralis (VL), tibialis anterior (TA), biceps femoris (BF) and semitendinosus (ST). During the whole process, the subjects remained standing and relaxed, with the knee slightly bent at 20 º flexion. Peak-to-peak amplitudes and latencies were then computed offline from the average EMG waveform. Onset latencies of the evoked response in each muscle were measured as the

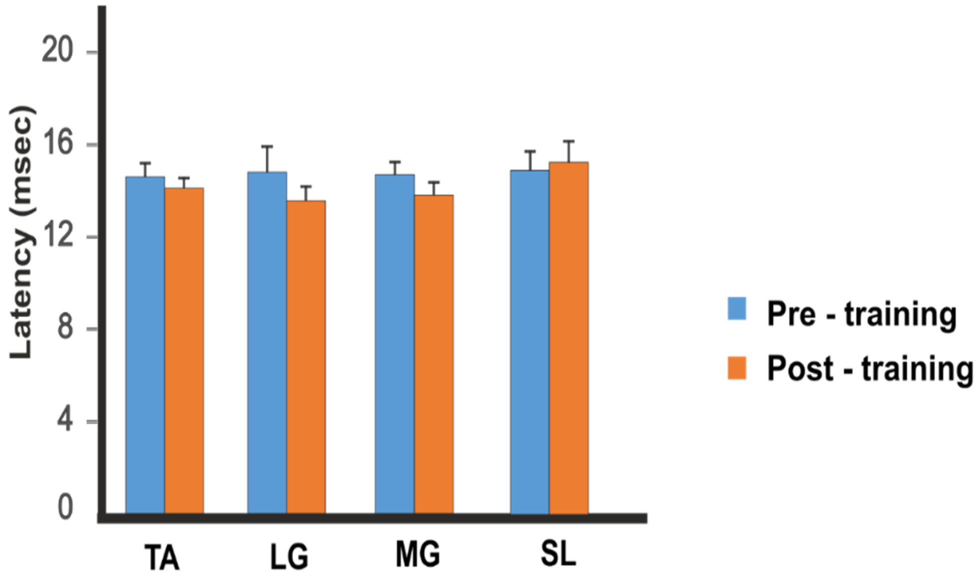


Figure 4-2 Change in latency to peak pre and post-training. Mean of the measured latency to peak for each muscle across all participants (n=15) is presented (mean ± SEM), pre-training in blue; post-training in orange.

time to peak from the peak amplitude of the evoked response was also measured. To avoid onset latency changes, the stimulating electrode was kept fixed throughout the experiment. The longest latency for an evoked response in the soleus to tibial nerve stimulation was 35 ms. The amplitude of evoked response in RF, VL, ST and BF was too low (<10% of baseline) to be used, hence only four muscles TA, MG, LG, SL were used for the analysis and further comparisons. All data were statistically compared using Two- way ANOVA to differentiate latency and amplitude changes under pre- and post-training conditions. When significant differences were found ($p<0.05$), means were compared post hoc using Tukey's test.

4.3 Results

4.4 4.Overall data

After recording the evoked responses to tibial nerve stimulation in 15 participants in both conditions (pre- and post-balance training), the amplitude and latencies were measured. The latency to peak in the muscles remained unaltered ($F_{(3,112)}=0.69$, $p=0.562$) as seen in figure 4.2; The latency to peak across four muscles (TA, LG, MG, TA) was approximately 15 ms and SL was slightly longer. There was no observed change after training across these four muscles (F (1,112) =1.24, p=0.27).

Change in evoked response amplitude is shown in figure 4.3. Between pre-and, post-training amplitudes of the muscles remained unaltered, F (1,112) =1.37, p=0.712). However, there was the statistically significant difference between muscles group means as determined by two-way ANOVA ((F (3,112) =18.18, p=0.0001)). Amongst the muscles, in both conditions, MG amplitude was significantly greater than that of TA, LG, SL. MG was greater than TA (p=0.0003) and MG (pre-training) was greater than TA post-training (p=0.0002). MG post-training was greater than TA post-training (p=0.0002). MG pre-training was greater than LG pre-training (p=0.02). MG pre-training was greater than LG post-training (p =0.003). MG post-training was greater than SL post-training (p=0.013). There was a trend in the data to suggest the pre-training amplitude was higher than post-training in three muscles (TA, SL), but for LG (figure 4.3), with an increase in MG activity. The reason the difference in their response is less obvious is possibly due to the high variability was seen especially in MG.

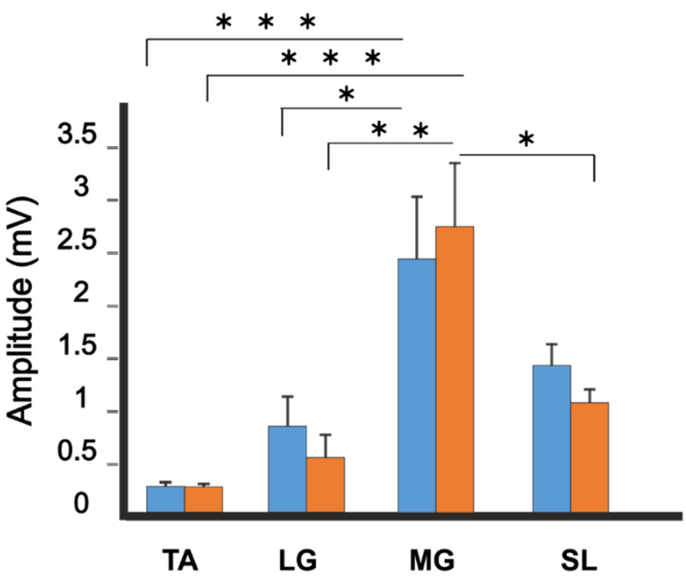


Figure 4-3 Change in amplitude of evoked response in TA, LG, MG and SL to Tibial nerve stimulation. Data from all participants (n=15) shown (mean ± SEM). MG activity is higher post-training while that in rest is lower although, not significant. post-hoc (Tukey's tests) to determine the significant between the muscles. Note that non-significant p> 0.05, * indicates p <0.05, ** p < 0.01, *** p<0.001

4.4.1 Grouped data

The latency and amplitudes when further examined seem to fall into 3 categories when compared to activity in SL. In group 1 (N=5), time to peak in muscles TA, LG, and MG both pre and post-training were compared to SL and they preceded by up to 3-5 ms (Figure 4.4A).In group 2 (n=2), the latency of TA, LG and MG was longer than SL approximately 5 ms (Figure 4.4B). In group3 (N=8, figure

4.4C), the latency by TA, LG and MG were significantly altered, such that all of them pre-training was delayed compared to SL, while after training, they preceded SL.

4.4.2 Comparison of latencies to that of SL

In group 1 (figure 4.5 A, B, C), the activity in MG, LG and TA peaked 18 ms ± 0.8before that in SL (n = 5; participants; H3, H9, H12, H13, H16). In group 2 (figure 4.5 D, E, F), (n = 2; participants; H10, H22) TA was most delayed by 15 ms. ± 1.2In group 3, activity in the four muscles were varied but had a similar trend approximately 14 ms ±0.9 (n =8; participants; H14, H15, H17, H20, H23, H24, H 25) (figure 4.5 G, H, I).

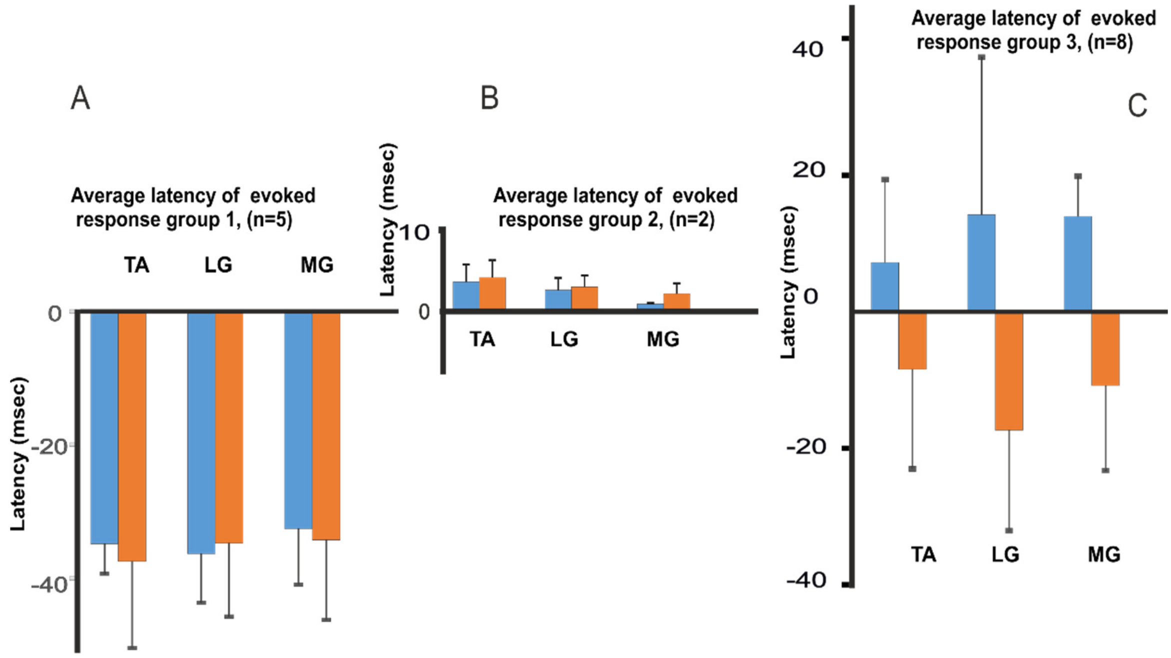


Figure 4-4 Latency changes classified based on the timing of the peak in SL. The latency of the evoked responses in the muscles was evaluated in comparison to that of SL and the averages (Mean $\pm$ SEM) of the 15 subjects is plotted. A) Showing group1 participants' average latency (n=5), SL latency was longer than TA, LG and MG. B) Showing group2 participants' average latency (n=2), SL latency was earlier than TA, LG and MG. C) Showing group3 participants' average latency (n=8).

In group1, comparison latency (figure4.5B), there was no changes with training between the groups means as determined by two-way ANOVA (F (1,32) =1.647, p=0.21). There was a significant difference between muscle groups (F (3,32) =6.95, p=0.001). The time to peak of SL was the longest (pre-training18 ms ±0.84). TA, MG, LG peaked at 15 ms $\pm$ 0.8, and SL was significantly longer than TA in latency of pre-training (p= 0.03). In terms of average response amplitude (figure4.5C), there were no changes with training between the groups means as determined by two-way ANOVA (F (1,32) =0.18, p=0.67). There was a significant

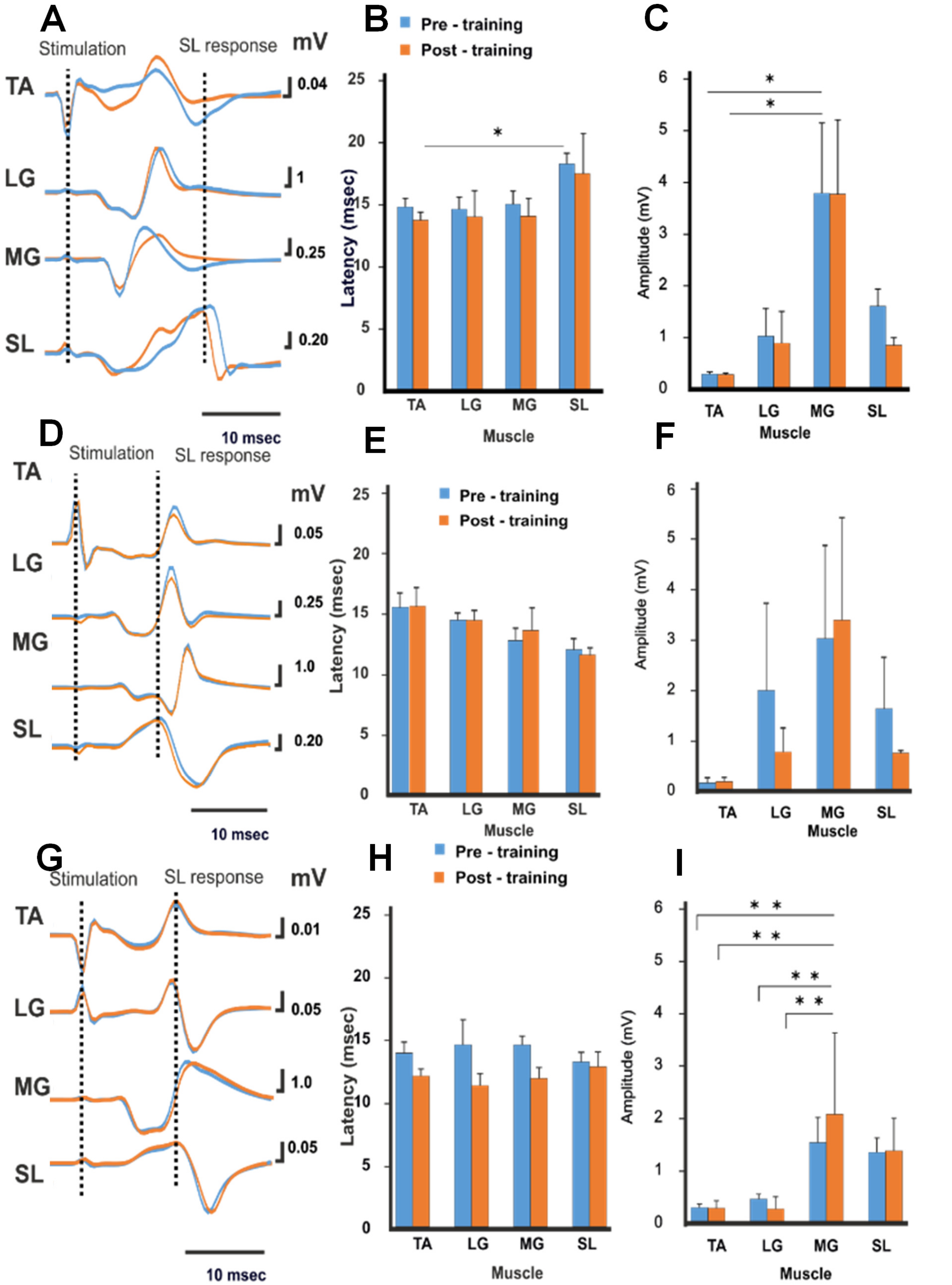


Figure 4-5 Comparison latency and amplitude of evoked response in three groups. Group1 n =5 (A-C), Group 2 n=2(D-F) and Group 3 n=8 (G-I). A, D, G) Raw EMG traces B, E, H) Average of onset to reflex (in ms) C,F,I) Amplitude response Two-way ANOVA applied to determine between pre and post-training conditions and across four muscles and using post-hoc (Tukey's tests) to determine the significant between

difference between means of muscle groups (F (3,32) =8.04, p=0.0004). MG had the highest amplitude (4mV±1.35), followed by SL, LG and TA respectively. LG and SL activity were reduced when compared to pre-training, (Pre-training LG (1±0.5 mV), Post-training LG (0.8±0.6 mV) and Pre-training SL (1.8±0.33 mV), Post-training SL (1±0.14 mV). TA amplitude was the lowest (0.2 ±0.04 mV) in both pre and post-training. MG activity was significantly greater (p=0.049) than that in TA, and LG.

In group2 shown in figure 4.5 D, E, F, comparison latency (figure 4.5D and E), there was no significant difference with training (F (1, 8) =0.02, p=0.89). There was no different across muscle groups (F (3, 8) =3.84, p=0.06). The onset of TA activity was delayed pre -training15.4 ms ±1.2 and post-training 16 ms ±0.8 compared to SL activity (pre-training 12 ms± 0.9 and post-training 11.5ms ± 0.6). The onset of LG was at 14.4 ms±0.6 (pre-training) and 14.3 ±0.8 (post-training) and MG at 12.7 ms ±1.0 (pre-training) and 13.5 ms±1.9 ms (post-training). In figure 4.5F, the response amplitude was presented, there were no changes with training (F (1,8) =0.25, p= 0.63). There was no different across muscle groups (F (3, 8) = 2.17, p=0.17). MG was the highest (pre-training 3.04 mV±1.84 and post-training 3.4mV± 2.06). The LG (pre-training 1.98mV±1.7 and post-training 0.76 mV±0.47), SL (pre-training 1.62 mV±1.0, post-training 0.74±0.05) and TA (pre-training 0.15 mV ± 0.01, post-training 0.17 mV± 0.8).

In group3 shown in figure 4.5 G, H and I, the onset of four muscles activity (figure4.5 G and H) were no changes with training (F (1, 56) =0.52, p=0.47) and there was no different across the means of muscle groups (F(3,56=0.03, p=0.99). In pre-training, the onset of TA was 14.2 ms±0.09, LG was 14.9 ms±0.2, MG was 14.8 ms±0.07 and SL was 13.5 ms±0.08). In post-training, the onset of TA was 12.3 ms±0.06, LG was 11.6 ms ± 0.09, MG was 12.1ms ±0.09 and SL was 13.1ms ± 0.1. It was suggested the trend that the onset of four muscles activity was reduced after training.

In figure 4.5 I presented the response amplitude, there was no significantly different with training (F (1, 56) = 0.21, p=0.65).

However, there was significantly different across muscle groups (F (3,56) =13.262, p=0.000001). The response amplitude of MG was the highest both pre and post-training (pre-1.38mV ± 0.44 and post 1.88 ± 1.43). LG and TA had the lowest amplitude. LG had the amplitude at pre-training 0.23mV±0.06 and post-training 0.21mV±0.13. SL had the amplitude at pre-training 1.21 mV ±0.25 and post-training 1.23 mV±0.57.MG post-training response was significantly greater than TA pre-training (p=0.002) and TA post-training (p=0.0013). MG post-training had greater than and LG pre-training (p=0.005) and LG post-training (p=0.0012). To summarise, in latency response, TA activity post-training was delayed in both group 1 and 3 by a millisecond. In amplitude response, pre-training TA, LG and MG were maximal after SL had reached its maximal amplitude in group 2 and 3.

4.5 **Discussion**

4.5.1 Main findings

1. TA muscle response was evoked with stimulation of the Tibial nerve (figures 4.1, 4.2).
2. There was a trend for the decreased amplitude of LG and SL after training (figure 4.2) along with a decreased latency to peak (figure 4.5 B, E, H).
3. MG activity was the highest compared to the rest of ankle muscles both pre and post-training (figure 4.3 and figure 4.5 C, F, I)

4.5.2 TA response after tibial nerve stimulation

It is known that the corticospinal, vestibulospinal and reticulospinal tract control balance tract (Woollacott et al., 1984, Woollacott and ShumwayCook, 1996, Newell and Vaillancourt, 2001). The corticospinal tract controls limb and trunk which can be studied in human easily. Originating from the primary motor cortex, supplementary motor cortex and cingulate gyrus, in a healthy subject, a small proportion of the contralateral corticospinal tract projects to motoneurones (monosynaptic), (Brouwer and Ashby, 1992a), while most, via collateral branches, project to inhibitory, excitatory and Renshaw interneurons (polysynaptic)(Baldissera et al., 1998).

Tibialis anterior (TA) muscle is innervated by common peroneal nerve (Kendall and Kendall, 2005); and the Tibial nerve activates MG, LG and SL. However, in this study, after tibial nerve stimulation, TA response was seen in both pre- and post-balance training (figure 4.1, figure 4.6 A, B). TA muscle response is possibly due to the facilitation of sensory or motor component of the circuit involved in the recruitment of the muscle. If the motor component alone was excited one assumes a motor collateral of the tibial nerve was more active in the conditions tested and would lead to an M wave or a direct motor response, which was not seen (figure 4.6), but if it was the reflexive path arriving back to the muscle via the spinal cord was more excitable it would produce a reflexive response as seen in this study. A possible mechanistic schema is presented in figure 4.6. Recently Bennett et al (2018) reported that heteronymous monosynaptic reflexes in rats were facilitated by the release of PAD from active muscle, which may be a mechanism similar to one responsible for the one observed by us here, and that which was suggested by Iles in 1996 (Iles, 1996). Therefore, TA response (figure 4.6 A, B) may be influenced by sensory and CST input as summarised in figure 4.6C.

4.5.3 Changes in spinal evoked response excitability after training

There was the trend suggested that decreasing of amplitude in LG and SL after

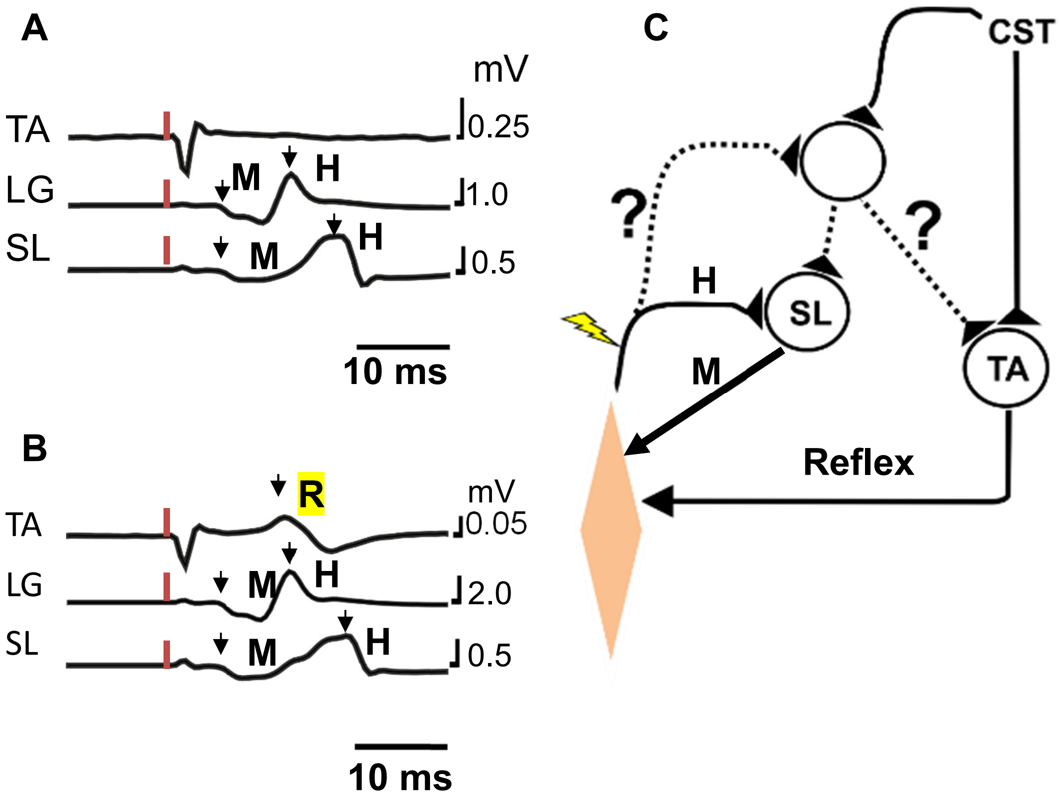


Figure 4-6 Tibial nerve stimulation evoked response in TA muscle. A) an example from subject H3 to 1.5xT stimulation showing an M and a H response on LG and SL but not in TA B) same subject H3 but after 3xT stimulation showing a reflex response (R) in TA while LG and SL have both M and H responses C) a possible of mechanism showing TA response modulated by several sources including Corticospinal drive (CST) and spinal reflex modulation (Adapted from Wolpaw,2007)

training (see results figure 4.2) and latency was decreased (figure 4.5 B, E, H). As you can see in this study, the balance training was set on the same day, but we enhanced training for 10 trials (see methods section). Therefore, the reduced amplitude may be due to training. The reduced amplitude of evoked response has been suggested after short-term balance board training (8 trial with 3 days follow-up) (Trimble and Koceja, 1994). A reduction in evoked response amplitude following a session of physical activity is a well-established phenomenon (Bulbulian and Darabos, 1986, Trimble and Koceja, 2001). In addition, Evoked response amplitude was more influenced by a sequential motor task training (Misiaszek et al., 1995, Mazzocchio et al., 2006). In this study, the trend of latency and amplitude was reduced about 4 ms and amplitude of SL reduced approximately 1-1.5 mV (50 %) when compared to pre-training.

In figure 3.5, the trend seems to be that the duration to peak between pre and post-training was 1-5 ms so it could be an oligosynaptic delay (Marchand-Pauvert and Nielsen, 2002). Therefore, latency changes in 15 participants suggested that changes in spinal evoked response excitability were due to changes taking place within the spinal cord. As the literature suggests the synaptic efficacy of afferent volleys can be modulated by an inhibitory process that depolarises the presynaptic terminals of the afferents. All afferents are subject to presynaptic inhibition, controlled by descending tracts (Rudomin and Schmidt, 1999). In future direction, to confirm latency change after training, I will conduct H reflex with paired-pulse and recruit more participants.

4.5.4 MG amplitude is greater than LG, TA by positioning

The classic position for researchers to achieve the spinal evoked response of soleus is a seated subject with the hip and knee flexed and the ankle fixed in 30° plantar flexion (Delwaide and Hugon, 1969). While applicable for the clinician, this situation is less convenient for diagnostic studies, and examining the patient lying on a bed is more convenient (prone for soleus). However, in my study, we would like to achieve the evoked response while participants are standing up to study the dynamic balance task properly. This takes stretch off the bi-articular gastrocnemius muscle. Medial gastrocnemius may be influenced by knee flexion and plantar flexion during standing. Therefore, the increasing of proprioception by sending proprioceptive signals to the knee joint which is the origin of the medial gastrocnemius. The increased proprioception in bi-articular muscles can result in both short-term and long-term alteration of spinal cord adaption(Gauchard et al., 2010). Increases in discharge probability at evoked latencies have been defined in post-stimulus time histograms of the discharge of low-threshold single motor units from these muscles. However, using the techniques described above, it has proved difficult to obtain convincing evoked response responses during voluntary contractions of some muscles in all healthy participants (Burke, 2016, Pierrot-Deseilligny and Burke, 2012).

My procedure used a standing position without support during evoked response stimulation because I wanted participants to maintain the position close to the balance board training. Position can alter the amplitude of the evoked response. Decreasing in evoked response excitability can be due to changes in presynaptic inhibition of group Ia afferent terminals or in the activity of homonymous motoneurone pools (Misiaszek, 2003). The amplitude of the soleus evoked response is smaller during active standing than when standing with back support (Katz et al., 1988). Therefore, spinal evoked response changes in the short term were most likely facilitated by presynaptic inhibition of Ia afferent.

4.6 Conclusions

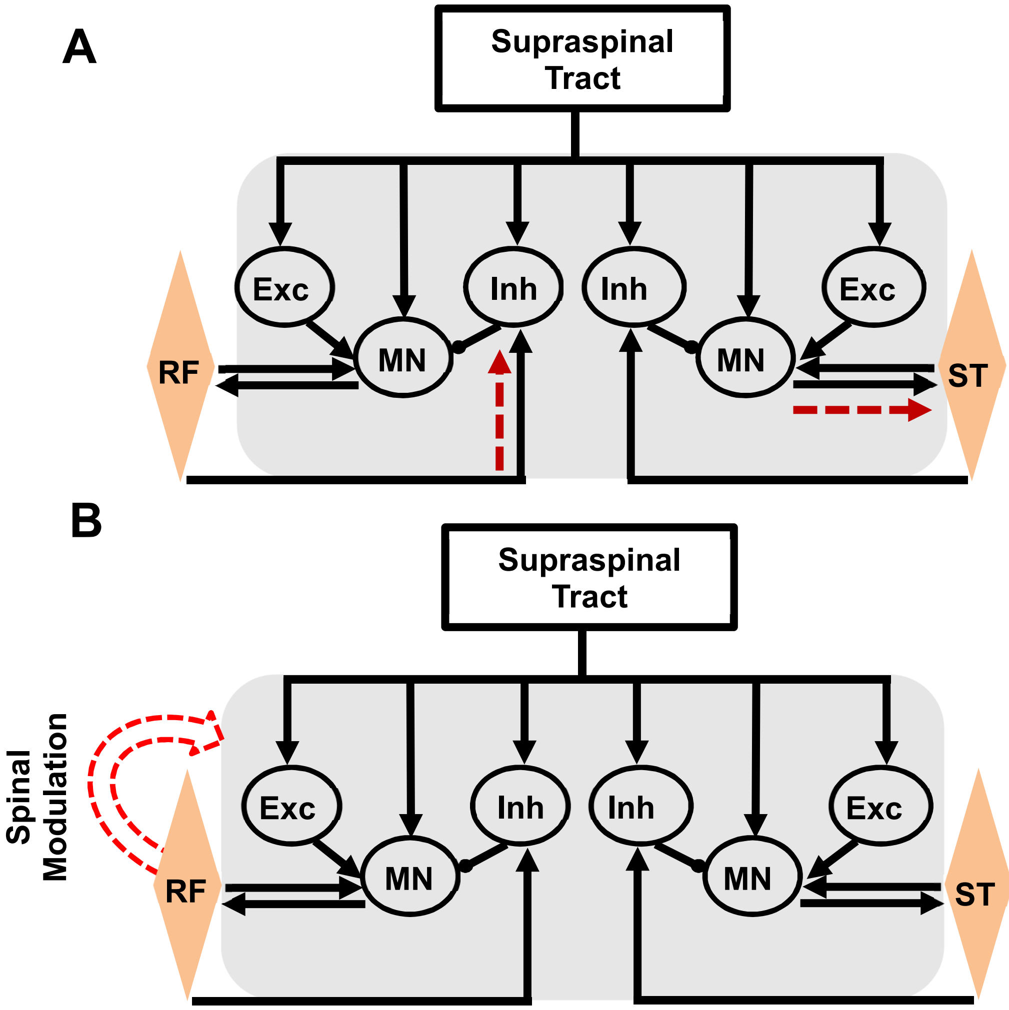
This study provides preliminary evidence that it is unlikely that pathways exhibited any organisational change, but modulation of spinal circuits after training influenced the subjects' ability to practice to balance after 14 trials.

Limitation and technically difficult.

An important limitation was of retaining participants, I began with 25 but 10 withdrew from the electrical stimulation session. They dropped out of the session owing to either fear of stimulation (n=2) and of difficulty standing during electrical stimulation (n=8).

Chapter 5 General discussion Possible mechanisms

My thesis comprised of three main studies based on the predicted mechanisms



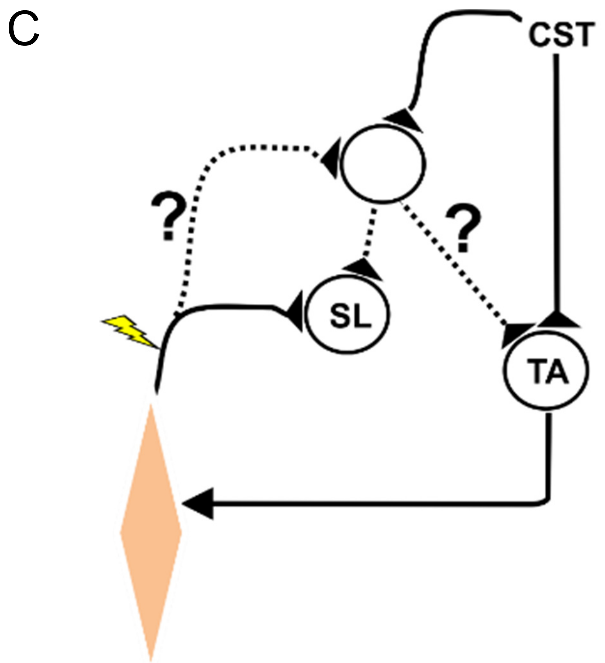


Figure 5-1 Summary of possible mechanisms from this thesis A) Study 1: The greater the knee flexion, greater is the ST activity. Ib afferents excites inhibitory interneurone onto RF (NOT illustrated here). **B)** Study 2A: Balance training might cause spinal modulation. Training leads to increased Ia led RF(Bi-articular muscles) activation, leading to more recruitment after balance training. **C)** Study 2B: Electrical stimulation of tibial nerve leading to increased TA recruitment. Multiple pathways may modulate the output. Exc- Excitatory interneurone, Inh-Inhibitory interneurone, MN-Motorneurone, RF-Rectus femoris, ST-semitendinosus, red arrow head –p increasing signal

described in figure 5.2; study 1 and study 2A demonstrated how muscle synergy switches from having one primary muscle to another like between RF and ST and finally study 2B explored how electrical stimulation is involved in TA muscle recruitment.

Study 1. There may be an increased sensory drive from the muscle tendons at the knee (Day et al., 1984, Day et al., 1983) at greater knee flexion of 60˚ and 90˚. This could be the cause for the alteration of the interactions leading to ST being more active than the expected muscles of the Quadriceps (RF), possibly via the reciprocal inhibitory networks (figure 5.2 A).

Study 2A. Balance training activity can cause spinal evoked response modulation. Additionally, propriospinal excitatory interneurons might play an important role to connect multiple spinal cord segments and participate in a complex of motor reflexes. Evoked response changes seem important to explain how spinal cord circuit excitability can produce different outputs (Papegaaij et al., 2016, Taube et al., 2008a, Horak et al., 1997, Wolpaw, 2001, Gauchard et al., 2010). This result shows clearly that either the afferent input plays a role in balance training and muscle adaptation like previously seen in other conditions involving the leg muscles (Christiansen et al., 2017).

Study 2B. After balance training and electrical stimulation on the tibial nerve, TA muscle had an evoked response, possibly due to the facilitation of the sensory or motor component of the circuit involved in the recruitment of the muscle. Therefore, TA response may be influenced by sensory and CST input (Iles, 1996) as summarised in figure 5.2

5.1 C. Clinical Correlation

In study 1, at 60 ° and 90 ° of knee flexion, I suspected the possible mechanism to be that the RF Ib inhibiting RF and increasing activity of ST. According to this

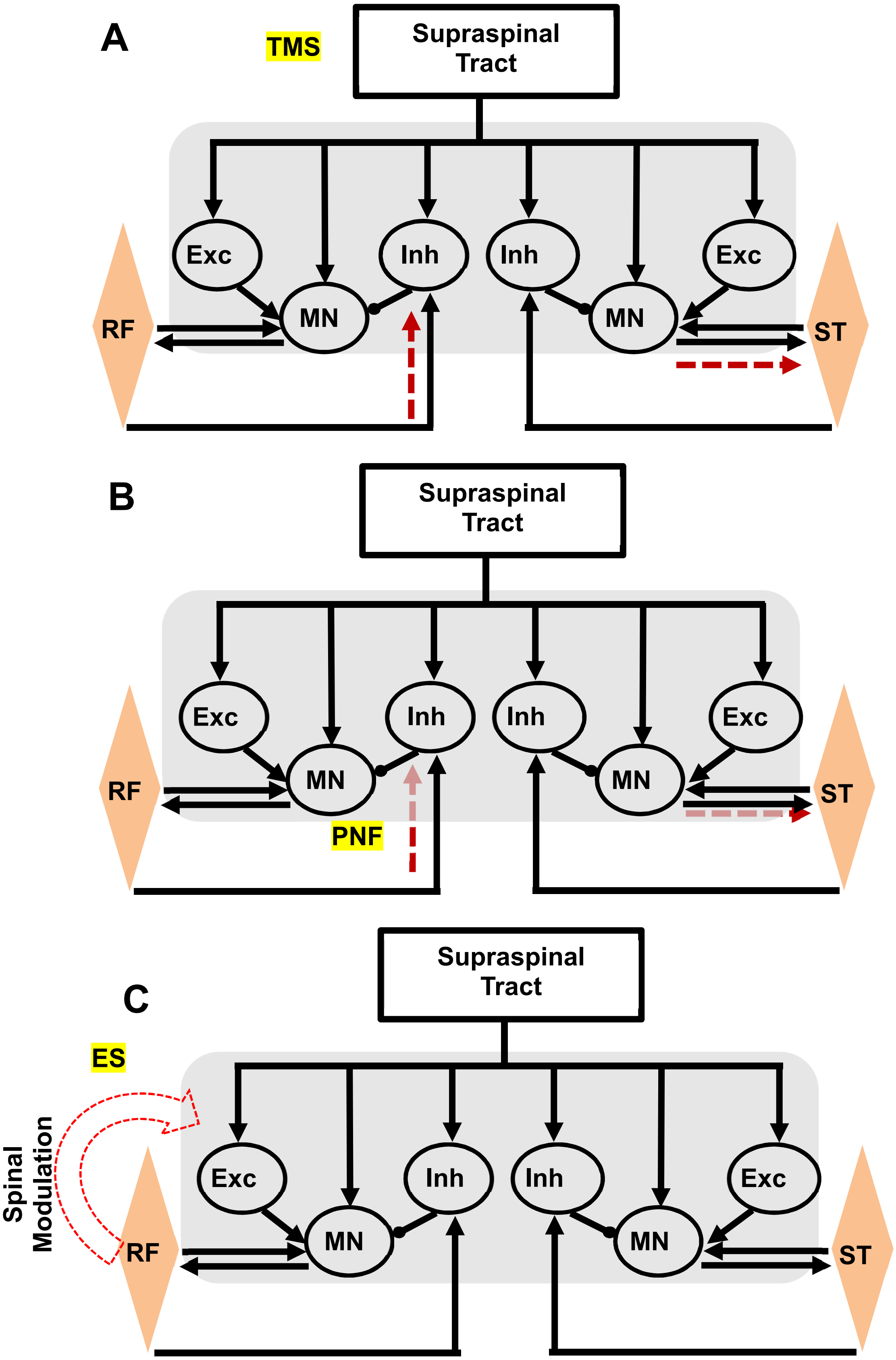


Figure 5-2 Clinical correlation relying on possible mechanisms from this thesis **A)** Transcranial magnetic stimulation (TMS) stimulation can activate excitatory interneurone to promote RF function. **B)** Proprioceptive Neuromuscular facilitation (PNF) exercise to increase range of motion and muscular function at a specific knee angle **C)** Electrical stimulation of the RF proprioceptive input combined with balance training to improve knee function after injury.

imbalanced muscle activity at the knee would be similar in producing knee stiffness seen in spasticity of stroke patients. Therefore, if we would like to increase RF activity at the knee flexion 60 °, two techniques in neurorehabilitation could be applied; first, to increase the drive from the descending tract using transcranial magnetic stimulation (TMS). Repetitive TMS can induce relatively longer lasting excitability changes, often used in the treatment of a neuromuscular disorder. To promote RF muscle function, TMS could be introduced in the cases which have a muscle imbalance between RF and ST. To promote an improved range of motion and RF function, proprioceptive neuromuscular facilitation exercise (PNF) is suggested. PNF is a common technique for increasing range of motion (Hindle *et al,* 2012). It is used to improve muscle elasticity by stretching; therapists can also passively work the knee to improve the range of motion (RF) of the patient while the patient contracts ST. It is so called contract-relax technique. As the results in study1 suggest Ib inhibits RF motoneurons, while ST has more activity at 60°, this angle will be used for the cases of knee stiffness or hemiplegic spasticity. To increase the range of motion, I would suggest that therapists apply PNF on RF muscle by tapping or stretching rectus femoris (RF) by asking patients to perform an isometric contraction of ST at 60°, followed by a therapist passively stretching RF to increase proprioceptive signal (Ib) to RF motoneuron. Using the PNF technique, one can then increase the range of motion of the knee (figure 5.3B).

In study 2, to improve balance stability, the results from chapter 3 suggests that rectus femoris (RF) is the key muscle to improve the ability to balance and the results from chapter 4 suggest that electrical stimulation can improve muscle recruitment. Imbalance in posture and stroke patients should be suggested to have both balance training combined with electrical stimulation to improve their knee function. For example, in figure 5.3C, spinal plasticity can be induced with balance training. I would suggest therapists apply both balance training and electrical stimulation to RF to increase muscle recruitment.

5.2 Future directions

1) EMG under condition1 (chapter 2, result section) can be used to improve the modified Ashworth Scale and MRC scale of muscle testing. In the future, I will combine the quantitative MAS and MRC scales with sEMG quantification to

improve the modified scale for lower limb muscle function.

2) It has been suggested that the increased proprioception in bi-articular muscles can result in long-term alteration of spinal cord adaption (Gauchard et al., 2010). I will conduct balance training and compare between trained and untrained participants to assess if force and muscle synergy patterns can be used to define better exercise regimes.

3) According to my data in chapter 3, I found that RF and SL are important to maintaining balance; however, due to kinematic redundancy, there would have been a variety of joint angles with which the task could be achieved. Therefore, I will measure the hip-knee-ankle angle during the stable phase and unstable phase by putting a marker on the bony landmark and visualise with 3D analysis further. It may be related to muscle activity and it might suggest clinical guidelines on how to fix the knee angle to stand on a balance board properly. Evidence suggested that ground force action from balance standing can influence bi-articular joint muscles and to prevent falling, this requires bi-articular joint muscle to undertake adaptive reflex quickly (10-20ms) (Cheng, 2016). Unfortunately, I did not test the reflex of RF, however, after training, RF (knee extensor) latency is faster, which is associated with recovery of balance (figure 3.7). If I were to test RF reflex, I expect the RF reflex to adapt after training. Because when participants stand on the balance board, the ground reaction force from the balance board can influence knee proprioceptive fibre (Ia). Muscle spindle (Ia) of the extensor motoneurone sends reciprocal inhibition to flexor motoneurone (Crone and Nielsen, 1989). Rectus femoris (bi-articular muscle) acts as the knee extensor, controlling the balance using local inhibitory interneurons.

4) In the future direction in chapter 4, I will do experiments with longer training and sessions to assess the training correlated to the pathways.

5) Electrical stimulation in chapter 4, was used to produce evoked response to establish the pathways associated leading to the suggestion for no change in pathways but a change in their excitability. However, the excitability was never explicitly tested in this study. This will be confirmed using the M to H ratio obtained by stimulating the tibial nerve to produce a recruitment curve for H-reflex to establish the role of increased excitability of these spinal circuits.

6) Further research is required to establish the role of the bi-articular muscles during common tasks like sit to stand, posture and walking but also in rehabilitation.

5.4 Conclusions

This thesis highlights the role of the bi-articular muscles and their role in shaping of muscle activity during these tasks, especially the knee muscles. The role of the muscles acting at the knee is often overlooked by therapists and clinicians, even though they are commonly targeted muscles to train to improve stability in individuals with knee injuries (Abbasi et al., 2017, Benson et al., 2017). Nevertheless, the knee quality score does not have high validity, changing from examiner to examiner who examines the subject. Clinical tools such as this do not rely on knowledge of the neural and muscular deficits which can manifest their effects in various unexpected ways, like spasticity(Dietz and Sinkjaer, 2007).To propose an appropriate therapy, this thesis shows the need to measure both muscle activity and explore the neural pathways which underpin this muscle activity to aid a better understanding of the knee movement.

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Appendix

Details of PCA analysis and R scripts

PCA is mathematically defined as an orthogonal transformation that transforms the data to a new coordinate system such that the greatest variance by some projection of the data comes to lie on the first coordinate (called the first principal component), the second greatest variance on the second coordinate, and so on.

To assess component analysis, the equation as W= T/X has been used.

Where W is weight of component analysis

Where T is defined by 5 Trials of muscle contraction

Where X is defined by using 17 subjects x 5 muscles column RF, VL , VM BF and ST of data sets

First component

To maximize variance, the first weight vector $w_{(1)}$ thus has to satisfy.

Where $x_{(i)} \cdot w_{(1)}$ is the first principal component of a data vector $x_{(i)}$ can then be given as a score $t_{1(i)} = x_{(i)} \cdot w_{(1)}$ in the transformed co-ordinates, or as the corresponding vector in the original variables, $\{x_{(i)} \cdot w_{(1)}\}\ w_{(1)}$.

Further components

The kth component can be found by subtracting the first $k - 1$ principal components from X: and then finding the weight vector which extracts the maximum variance from this new data matrix.

Dimensionality reduction

The transformation T = X W maps a data vector $x_{(i)}$ from an original space of p variables to a new space of p variables which are uncorrelated over the dataset. However, not all the principal components need to be kept.

To plot the PCA component, R scripts were used by online version as PTcomp in R script.

https://stat.ethz.ch/R-manual/R-devel/library/stats/html/prcomp.html

Description

Performs a principal components analysis on the given data matrix and returns the results as an object of class prcomp.

Usage

prcomp(x, ...)

S3 method for class 'formula'

prcomp(formula, data = NULL, subset, na.action, ...)

```
## Default S3 method:
prcomp(x, retx = TRUE, center = TRUE, scale. = FALSE,  tol = NULL, rank. = NULL, ...)
## S3 method for class 'prcomp'
predict(object, newdata, ...)
```

Arguments

formula	a formula with no response variable, referring only to numeric variables.
data	an optional data frame (or similar: see model.frame) containing the variables in the formula. By default, the variables are taken from the environment(formula).
subset	an optional vector used to select rows (observations) of the data matrix x.
na.action	a function which indicates what should happen when the data contain NAs. The default is set by the na.action setting of options and is na.fail if that is unset. The 'factory-fresh' default is na.omit.
...	arguments passed to or from other methods. If x is a formula one might specify scale. or tol.
x	a numeric or complex matrix (or data frame) which provides the data for the principal components analysis.
retx	a logical value indicating whether the rotated variables should be returned.
center	a logical value indicating whether the variables should be shifted to be zero centred. Alternately, a vector of length equal the number of columns of x can be supplied. The value is passed to scale.
scale.	a logical value indicating whether the variables should be scaled to have unit variance before the analysis takes place. The default is FALSE for consistency with S, but in general, scaling is advisable. Alternatively, a vector of length equal the number of columns of x can be supplied. The value is passed to scale.
tol	a value indicating the magnitude below which components should be omitted. (Components are omitted if their standard deviations are less than or equal to tol times the standard deviation of the first component.)

With the default null setting, no components are omitted (unless rank. is specified less than min(dim(x)).). Other settings for tol could be tol = 0 or tol = sqrt(.Machine$double.eps), which would omit essentially constant components.

rank. optionally, a number specifying the maximal rank, i.e., the maximal number of principal components to be used. Can be set as an alternative or in addition to tol, useful notably when the desired rank is considerably smaller than the dimensions of the matrix.

object object of class inheriting from "prcomp"

newdata An optional data frame or matrix in which to look for variables with which to predict. If omitted, the scores are used. If the original fit used a formula or a data frame or a matrix with column names, newdata must contain columns with the same names. Otherwise, it must contain the same number of columns, to be used in the same order.

Details

The calculation is done by a singular value decomposition of the (centred and possibly scaled) data matrix, not by using eigen on the covariance matrix. This is generally the preferred method for numerical accuracy.

Unlike princomp, variances are computed with the usual divisor *N - 1*.

Note that scale = TRUE cannot be used if there are zero or constant (for center = TRUE) variables.

Value

prcomp returns a list with class "prcomp" containing the following components:

Sdev the standard deviations of the principal components (i.e., the square roots of the eigenvalues of the covariance/correlation matrix, though the calculation is done with the singular values of the data matrix).

rotation the matrix of variable loadings (i.e., a matrix whose columns contain the eigenvectors). The function princomp returns this in the element loadings.

X if retx is true the value of the rotated data (the centred (and scaled if requested) data multiplied by the rotation matrix) is returned. Hence, cov(x) is the diagonal matrix diag(sdev^2). For the formula method, napredict() is applied to handle the treatment of values omitted by the na.action.

center, the centring and scaling used, or FALSE.

Note

The signs of the columns of the rotation matrix are arbitrary, and so may differ between different programs for PCA, and even between different builds of R.