The Relationship between Perception of Effort and Physiological Responses to an Acute Fatiguing Task of the Elbow Flexors. Evaluation of a New Rating Scale of Perception of Effort.

A thesis submitted for the degree of Doctor of Philosophy

by

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I. ABSTRACT

While fatigue is a common daily phenomenon, the exact relationship between perception of effort and fatigue is still unknown. Existing tools for assessing perception of effort are effectively limited to whole body exercise, while current methods for assessing voluntary activation are painful and not feasible for clinical application. The main aims of this thesis were to evaluate existing methodologies for their appropriateness in assessing perception of effort and voluntary activation following isolated muscle function testing, and to examine the relationship between subjective perception of effort and objective changes in the healthy motor control system. The implementation of reliable and valid assessment tools in clinical practice may enable clarification of the pathogenesis of many neurological conditions that have chronic fatigue as a key feature.

Four studies of within-subjects repeated measures design have been conducted. Sixty-nine healthy volunteers were recruited among staff and students of Brunel University. Magnetic stimulation was tested as a valid alternative to electrical stimulation in the conventional single-pulse Twitch Interpolation Technique. The 0–10 Numeric Rating Scale (NRS) was also tested for its reliability and validity in assessing the perception of effort during isometric exercise of elbow flexors. The changes of perception of effort following a submaximal elbow flexion fatiguing task, as well as following transcranial direct current stimulation (tDCS) over the motor cortex were also tested.

The main findings showed significant differences between peripheral and magnetic stimulation in conventional single-pulse Twitch Interpolation Technique. The 0–10 NRS demonstrated linear properties and reported excellent test-retest reliability and good concurrent criterion validity in recording perception of effort under repeated isometric contractions of elbow flexors. Ten minutes of a submaximal intermittent isometric fatiguing exercise produced a significant elevation in rating of perceived effort, which was associated with central and peripheral neurophysiological changes of the motor control system. In contrast, perception of effort did not change significantly following 10 minutes of tDCS. The major findings of this thesis suggest the 0–10 NRS is a valid and reliable scale for rating perception of effort in healthy individuals. Further testing of the scale on patients is needed to establish its validity in clinical settings. Additionally, the findings indicate a substantial role of perception of effort in the voluntary motor control system. However, further research towards revealing the underlying mechanisms of perceived effort regulation in both health and disease is required.

II. ACKNOWLEDGEMENTS

I still recall, when I decided to start this PhD, my supervisor told me to be aware that this would not be an easy process and that I should be patient and ready to face days and nights of frustration and many ups and downs in my mood. He was entirely right. Undertaking a PhD is neither a job nor a typical course of study. It is "a lonely journey", as my second supervisor described it, which leads you to many unknown and interesting paths that need to be discovered. I will never forget the days of extreme stress and loneliness I experienced during the three years of my doctoral studies. This laborious journey would never have been finished without the help of people who have supported me over these years.

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VI. LIST OF ABBREVIATIONS

ACh:	Acetylcholine
ANOVA:	Analysis of Variance
ATP:	Adenosine Triphosphate
ATPase:	Adenosine Triphosphatase
BB:	Biceps Brachii m.
Br:	Brachialis m.
BR:	Brachioradialis m.
Ca^{+2}:	Calcium ions
CFS:	Chronic Fatigue Syndrome
CI:	Confidence Interval
cm:	Centimetre
cMEP:	Compound Motor Evoked Potential
CNS:	Central Nervous System
CV:	Coefficient of Variation
EEG:	Electroencephalography
EMG:	Electromyography
FDI:	First Dorsal Interosseous m.
fMRI:	Functional Magnetic Resonance Imaging
GEE:	Generalized Estimating Equations analysis
H^{+}:	Hydrogen ions
LTD	Long-term depression
LTP	Long-term potentiation
ME:	Myalgic Encephalomyelitis (or Chronic Fatigue Syndrome)
MEP:	Motor Evoked Potential
mm:	Millimetre
MS:	Multiple Sclerosis
ms:	Millisecond
μs:	Microsecond
MSO:	Maximal Stimulator Output
mV:	Millivolt
μV:	Microvolt
MVC:	Maximal Voluntary Contraction
Pi:	Inorganic phosphate
PCr:	Phosphocreatine
Rms:	Root Mean Square
RMT:	Resting Motor Threshold
SEM:	Standard Error of Mean
sEMG:	Surface Electromyography
SR:	Sarcoplasmic Reticulum
T:	Tesla
TMS:	Transcranial Magnetic Stimulation
TES:	Transcranial Electrical Stimulation
V:	Volt

CHAPTER 1

BACKGROUND OF THE STUDY

1.1 Introduction

Fatigue is a common and daily phenomenon experienced by both healthy people and patients with neurological, neuromuscular and orthopaedic problems. It is a multidimensional phenomenon as it covers physiological and psychological aspects expressed as a sense of tiredness, exhaustion or reduced physical and mental activity (Zwarts *et al*., 2008). This thesis concentrates mainly on changes within the nervous system, specifically in the motor pathway, produced during muscle fatigue in healthy individuals. Muscle fatigue is defined as any exercise-induced reduction in voluntary ability to produce force (Taylor *et al*., 2006) which "begins almost at the onset of the exercise and develops progressively before the muscles fail to perform the required task" (Gandevia, 2001, p. 1732). Additionally, this thesis focuses on fatigue induced by isometric contractions of an isolated, single limb, rather than whole body exercise. Subsequently, the physiological dimension of fatigue is the main interest of this study which refers to a symptom reported by individuals who do not suffer from any obvious defect in muscle performance. The reduction in the production of voluntary force during exercise could be peripheral or central in origin (Gandevia, 2001). Recent neurophysiological research questions whether fatigue is caused only by factors affecting the contractile process of the skeletal muscle itself, as is traditionally believed to be the case by exercise physiologists. In contrast, a new component of muscle fatigue has been added, called central fatigue, which is defined as a reduction in the neural drive to the muscles resulting in a decrease in maximal voluntary force (Taylor *et al*., 2000a; Gandevia, 2001).

This central component of fatigue has attracted a lot of interest in neurophysiology research as it leads to new perspectives on the way that exercise is regulated. Ongoing research suggests that this central component of fatigue may be a protective mechanism of the Central Nervous System (CNS) to maintain peripheral homeostasis rather than a limiting factor that leads to exhaustion during voluntary exercise in humans (Noakes & St Clair Gibson, 2004). Indeed, observations in elite athletes as well as in untrained individuals give evidence of a central integrative system that leads to the termination of a motor task before real exhaustion (Noakes, 2007). Therefore, it is believed that the reduction in power output during a fatiguing exercise, leading to exercise termination, is not caused by a limiting physiological process in the peripheral skeletal muscles. Instead it may be caused by altered efferent motor commands from the brain. This altered brain function is a subconscious process, and it is believed to be consciously represented by a changed perception of effort (Noakes & St Clair Gibson, 2004). Thus, the increased effort that is perceived during a fatiguing exercise might be a central process that allows exercise performance to be precisely regulated.

People typically cease to maintain prolonged exercise when they believe that they have exceeded the limits of the effort that could be allocated for the task. However, paradoxically, strong external verbal encouragement at that stage typically can result in prolongation of the exercise for even longer, despite the feeling of exhaustion. This indicates that a reserve capacity is maintained which can be called up when needed. It is as if the brain would never allow the body and the peripheral organs to reach complete exhaustion. To what extent perception of effort interferes in that process is not clear yet. One theory speculates that the increased feeling of effort experienced during a fatiguing exercise may be the resulting conscious manifestation of subconscious calculations that the brain performs to complete a task by maintaining homeostasis (St Clair Gibson & Noakes, 2004). Additionally, indirect evidence proposes a close relationship between the altered brain commands, due to fatigue, and the perception of effort (McCloskey, 1981). This assumption drives the interest in exploring the perception of effort following alteration in corticospinal excitability induced by brain stimulation. Weak direct current electrical stimulation over the motor cortex has been reported for its potential benefits in neuromuscular fatigue (Cogiamanian *et al*., 2007). As such, its application in areas involved in the perception of effort may give further insights into the way it is involved in the human motor control system.

A better understanding of the way perception of effort is regulated during fatigue and its relation to central fatigue is crucial not only for understanding the way the healthy brain regulates everyday tasks, but also for explaining the pathophysiology of symptoms like chronic fatigue in neurological conditions. Observations in clinical environments suggest the relation between perception of effort and fatigue. Indeed many patients with neurological diseases as such as multiple sclerosis (MS), myasthenia gravis and stroke complain about continuous fatigue and exaggerated effort required even for easy tasks; while in many conditions, such as in chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME), fatigue is not alleviated by rest (Fukuda *et al*., 1994). Indeed, research has shown that these patients undertake greater effort during fatiguing exercise tasks than healthy, matched controls (Thickbroom *et al*., 2006; Wallman & Sacco, 2007). The origin of greater exercise-induced fatigue and indeed chronic fatigue in these conditions is not well understood. Moreover, the aetiology and pathophysiology of CFS/ME is a matter of debate that hinders the development of effective and well established treatment strategies.

The issue of fatigue, therefore, in relation to the perception of effort, requires a deeper understanding informed by neuroscience, and generates interest in research in this area. While it would be of greater clinical interest to evaluate the relationship between perception of effort and fatigue in patients with MS or CFS/ME, further work is needed to understand this relationship in the normal healthy individual, because perception of effort might be an important limit for exercise performance. An extensive review of the literature indicates that there is a need to try to establish main measurement outcomes for subjective perception of effort as an appropriate tool for further exploration in neurological conditions. Until now, research on perception of effort during fatigue has been based on the physical sensations a person experiences during whole body physical activity, where a subjective feeling of tiredness and exhaustion is usually connected to an increased heart rate, increased respiration or breathing rate, increased sweating and muscle fatigue (Borg, 1998). However, what is an easy estimate of perceived exertion during whole body physical activities, which result in increased heart and respiratory rate might be quite uncertain when isolated muscles are exercised, such as during fatiguing isometric contractions. Fatiguing tasks in isolated body joint movement serve the advantage of not straining the cardiopulmonary system to the same extent as whole

body exercise and therefore they are more suitable for assessing the perception of effort during fatigue especially in clinical settings.

The development of valid assessment tools that could be used repeatedly during and following fatiguing exercise without involvement of the whole body is therefore of great research and clinical value. The parallel assessment of objective voluntary activation is also necessary to investigate the relationship of the subjective feeling of effort with changes in muscle function due to fatigue. The development of a technique that could be used to assess voluntary activation without causing pain and discomfort would be very useful in clinical application settings when fatigue is a chronic complaint. The implementation of these tools in clinical practice may enable clearer diagnosis and clarification of the pathogenesis of many neurological conditions that have chronic fatigue as a key feature. Comprehension of the origin of fatigue might then lead to designing efficacious rehabilitation protocols for patients with low quality of life due to chronic fatigue. This is crucial not only for the care of those people but also for the cost effectiveness of the health care system.

Thus, the main aim of this thesis is to use existing methodologies as alternative methods for assessing perception of effort and voluntary activation following isolated muscle function testing, and to examine the relationship between subjective perception of effort and objective changes in the human motor control system.

This aim has been addressed through four different studies. The first two studies evaluate and develop some of the main measurement outcomes used in the main study, while the next two studies assess the alteration of the perception of effort as a result of stimulation of the motor cortex and as a result of fatigue, respectively. The thesis begins with a detailed review and critical analysis of the literature in which the main concepts and the controversies are presented. In this part, new directions in the research into fatigue have been analysed and gaps in the knowledge of the relation between the perception of effort and fatigue have been identified. Research findings in neurological patients are also included to point out the application of the concepts discussed in the clinical settings. The aims and objectives are presented at the end of the literature review. The development of the general methods follows with details about the main measurement outcomes used. Each study is presented in a separate chapter with the

tested hypothesis, the methods, results and discussion. A general discussion of the results with the limitations of the study, the implications for the clinical population and the directions for future research makes up the last chapter of the thesis.

1.2 Literature Review

1.2.1 Definition of Skeletal Muscle Fatigue

In neurophysiology, muscle fatigue is defined as a progressive reduction in the force generating capacity of an exercised muscle irrespective of whether the task can or cannot be continued. The decline in the force output starts once exercise begins so that fatigue really begins almost at the onset of the exercise and develops gradually to the point where the muscle is unsuccessful in performing the required task (Gandevia, 2001). Thus, physiological fatigue is not always accompanied by a feeling of fatigue (Zwarts *et al*., 2008). Indeed, muscle fatigue is a complex and multi-factorial phenomenon. The failure to maintain the initial maximal force during exercise depends on peripheral and central factors. Peripheral factors refer to factors intrinsic to the muscle which affect the capacity of the motor fibres to produce force, like metabolic factors, impaired excitation-contraction coupling and failure of neuromuscular transmission. Fatigue caused by these factors is called peripheral fatigue (Allen *et al*., 2008). Central factors refer to the extra-muscular factors that influence the central nervous system (CNS) control of the voluntary contraction and are associated with changes in sites higher up in the CNS, like motoneurons, and segmental and supraspinal circuits. Fatigue that might be caused by factors higher in the CNS is called central fatigue (Gandevia, 2001). Thus, changes in any level of the motor pathway from higher cortical and subcortical levels within the CNS and via descending paths to the motoneurons, neuromuscular junction, muscle fibre membrane and muscle fibre could affect the force production and cause fatigue (Taylor *et al*., 2006) (Fig. 1.1). This thesis refers to peripheral fatigue as a main contributor to force decline during fatiguing exercise and mainly concentrates on central fatigue. Central fatigue has a considerably broader definition as it encompassess the potential contribution of psychological factors such as motivation and perception of effort in the process of exercise regulation (Davis & Bailey, 1997).

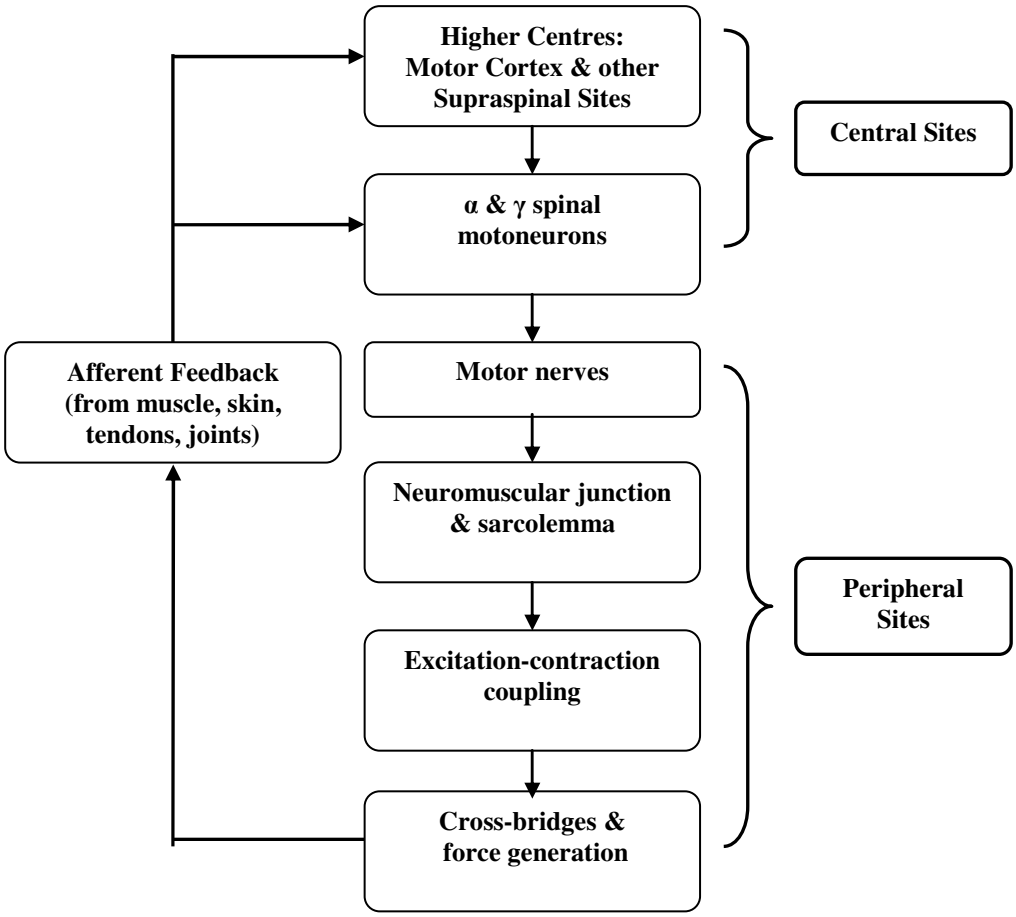


Figure 1.1: Diagram of potential sites at which fatigue may occur along the chain of motor command.

1.2.2 Peripheral Fatigue

A classical hypothesis about the mechanism of muscle fatigue suggests that metabolic and physiological changes in the muscle lead to system failure (Sahlin *et al*., 1998; Wilmore & Costill, 1999). Depletion of the energy supply systems, accumulation of metabolic by-products, failure in the nerve impulse transmission to the muscles and failure of the excitation-contraction coupling have been mentioned as the major peripheral causes of fatigue (Allen *et al*., 2008). Indeed, from research conducted within the last 20 years, it is now clear that reduced energy supply and metabolic changes are not the only factors to cause force reduction (Sahlin *et al*., 1998). In addition, time-dependent contribution from the various energy systems has been suggested (Gandevia, 1998). Thus, it seems that energy deficiency becomes a determinant of fatigue during high-intensity brief exercise, however, if voluntary contraction continues for more than

a few seconds discharge rates decline, suggesting that exercise performance is additionally based on central factors (Gandevia, 1998; Sahlin *et al*., 1998).

Thus, during high intensity of exercise when rapid production of ATP is needed, the phosphocreatine (PCr) is the first store of energy. However, the PCr energy system is limited and depletes very quickly, causing depletion to the ATP and failure to supply the energy for further contraction of the fibres (Sahlin *et al*., 1998). Glycogen is the subsequent source of energy. During prolonged moderate or high intensity exercise there is a reduction in the intramuscular glycogen pool. This occurs because the ATP dispersion in the cell is restricted and the large drop in glycogen stores leads to a local deficiency in ATP regeneration at sarcoplasmic reticulum membrane level. As such, any deficiency in ATP which in turn affects sarcoplasmic reticulum release or uptake of Ca^{+2} will interfere with the relaxation and the contractile process of the muscle (Giannesini *et al*., 2003). However, despite the reduction in the intracellular ATP during fatiguing exercise, ATP stores are highly protected and they do not fall below 60–70% of pre-fatigue levels even at exhaustion (Paul & Wood, 2002; Abbiss & Laursen, 2005). Thus, there is doubt about whether a reduction of ATP is a limited factor for the production of force.

The metabolic by-products of anaerobic glycolysis are another possible reason for exhaustion. Lactic acid increases at the end of glycolysis in the muscles and the body fluids. Additionally, as lactate and hydrogen ions (H^+) are released and accumulated in the muscle, acidosis occurs which leads to a reduction in force production (Wilmore & Costill, 1999). Although increased accumulation of H^+ and decreased pH is agreed to be a limiting factor for muscle performance, evidence that athletes can continue to exercise at relatively high intensities with low pH (Wilmore & Costill, 1999) calls into question the role of acidosis in the process of fatigue. Increased inorganic phosphate (Pi), through intense muscle contraction due to breakdown of PCr, is also regarded as a major cause of fatigue. However, findings that performance is improved after creatine supplementation (which is thought to be followed by increased PCr, with consequent increase in Pi during exercise) cannot be reconciled with the hypothesis that Pi increase is a major cause of fatigue (Sahlin *et al*., 1998).

In longer lasting activities, ATP is synthesized in the presence of oxygen through oxidation of the carbohydrate in mitochondria (Wilmore & Costill, 1999). It is suggested that during prolonged exercise muscle fatigue coincides with depleted glycogen stores in working muscles. After the depletion of glycogen, further energy is being supplied in the form of lipids through the circulation. The slower mobilization and delivery of lipid, as well as the relative inability of lipid metabolism to maintain power output, means that the muscles can no longer preserve their previous level of activity and fatigue occurs (Paul & Wood, 2002). However, the above model of fatigue is not so simple and includes contradictory findings. This is mainly because it has been found that the glycogen stores are not depleted during exhaustive exercise, and that carbohydrate supplementation has no effect on one-hour cycling performance, indicating that depletion of glycogen could have a larger effect after two or three hours of prolonged exercise (Abbiss & Laursen, 2005). In addition, muscle glycogen is heterogeneous between fibre-type and a considerable amount of glycogen may remain in fibres that are not recruited during fatigue (Sahlin *et al*., 1998).

Peripheral fatigue could be assessed by electrical stimulation over the motor nerve in conjunction with surface electromyography (sEMG) to measure the level of muscle electrical activity and hence voluntary drive to the muscle. A reduction in the twitch-evoked force at rest following a fatiguing exercise compared with pre-exercise twitch-evoked force indicates peripheral fatigue (Zwarts *et al*., 2008). The compound motor evoked potential produced by direct stimulation of the motor units is also seen in the sEMG signal and is called an M-wave. The size of the M-wave reflects the integrity of the neuromuscular transmission and muscle membrane, and it is usually included for the assessment of peripheral fatigue. Changes in the amplitude of the M-wave during or following fatigue indicate changes in the excitability of the neuromuscular junction and alterations in the neuromuscular transmission. It is also suggested that changes in the shape and size of the M-wave may also reflect changes in the fibre membrane excitability (Kent-Braun, 1997). These measurements are also included in studies that try to distinguish central from peripheral fatigue (Taylor & Gandevia, 2008).

1.2.3 Central Fatigue

Despite the evidence that limiting physiological and metabolic processes within the muscle cause reduction in force production and consequently fatigue, none of these changes have been shown directly to terminate exercise. In addition, a reported decline in the discharge rate of the motor units (Bigland-Ritchie *et al*., 1983) and an increase in the superimposed twitch evoked by electrical stimulation indicate central factors may be relevant to this reduction in force (Gandevia, 2001). It is as though the CNS acts as the "central governor" that regulates performance (Noakes, 2007). According to the "central governor" theory, the brain, after integrating all the afferent sensory information about any threat of hypoxia or ischaemia, reduces the efferent neural activation via the motor cortex and thereby limits the number of muscle fibres that can be recruited and hence reduces the exercise intensity that can be sustained (Hampson *et al*., 2001). This indicates that an exercise terminates before "real" exhaustion and that the feeling of exhaustion that develops during fatiguing exercise may be a protective mechanism to secure termination of the exercise before catastrophic failure of homeostasis (St Clair Gibson & Noakes, 2004). Thus, changes in the central nervous system should also be considered as a substantial component of fatigue although no obvious boundaries between peripheral and central changes exist.

1.2.3.1 Possible Mechanisms of Central Fatigue

To distinguish peripheral factors from central factors which contribute to fatigue is not always easy. One way to separate peripheral from central fatigue changes is by supramaximally stimulating the motor nerve of a muscle while the participant performs an isometric maximal voluntary contraction (MVC). The technique is called Twitch Interpolation Technique and it was first introduced by Merton (1954). Supramaximal electrical stimulation is used to ensure that all motor units are recruited in a maximal voluntary effort. There is evidence that all motor units in the exercising muscle may never be maximally recruited, even at the onset of a maximal isometric contraction (Gandevia, 2001). It has been reported that plantarflexors could be voluntary activated in a range of 90–99%. The same range of activation has been reported for elbow flexors,

while 85–95% of activation has been observed for quadriceps (Shield & Zhou, 2004). Additionally, intrinsic hand muscles (i.e., adductor pollicis) are activated less than elbow flexors during maximal contractions (Gandevia, 2001). A superimposed twitch force (interpolated twitch) evoked by the stimulation while the muscle is maximally contracted means that not all the motor units are recruited by the voluntary effort or that they are not firing fast enough to achieve a maximal force output. This indicates failure of the neural drive to the muscle and thus central fatigue (Taylor *et al*., 2006; Zwarts *et al*., 2008) (see Fig. 1.2). Merton (1954) in his study did not reveal any superimposed twitches evoked by electrical stimulation during fatigue, and he suggested that fatigue was caused by peripheral factors only. Later studies, however, using the same method showed that the twitches evoked by motor nerve electrical stimulation grow progressively with fatigue, suggesting a reduction in voluntary activation of the muscles (Taylor *et al*., 1999; Todd *et al*., 2003). Voluntary activation is an indicator of how well subjects can drive a muscle voluntarily to produce maximal force (Taylor *et al*., 2006). Impaired voluntary activation despite maximal effort suggests inadequate neural drive to the muscles and thus central fatigue (Todd *et al*., 2003; Søgaard *et al*., 2006) (Fig. 1.2). The suboptimal neural drive to the muscles, which is present when central fatigue develops, does not give any further information about the site in the CNS that caused the impaired voluntary activation. Recent studies using transcranial magnetic stimulation (TMS) give some insight to the origin of central fatigue.

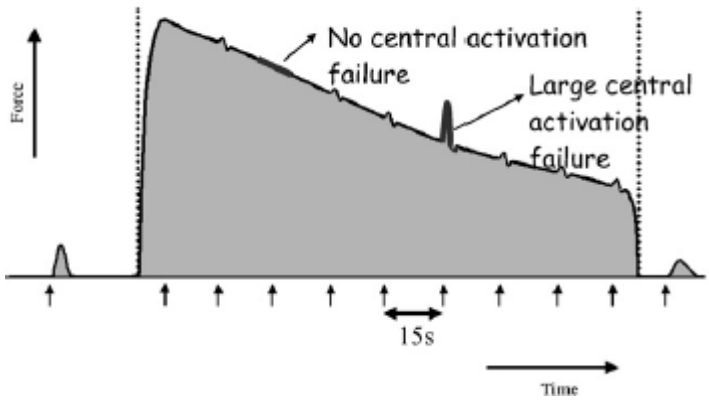


Figure 1.2: Decreased neural drive to the muscle as revealed by Twitch Interpolation Technique. Electrical stimulation was applied over the motor nerve during a 2 min maximum voluntary activation. The superimposed twitches evoked by electrical stimulation indicate central activation failure. The arrows indicate the moments of superimposed electrical endplate stimulation (Zwarts *et al*., 2008, p.4).

1.2.3.2 Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation (TMS) is a noninvasive technique for stimulation of neural tissue such as cerebral cortex, spinal roots, cranial and peripheral nerves. Unlike Transcranial Electrical Stimulation (TES), TMS is a painless method of activating the human cortex and providing information on the integrity of the central pathways (Kobayashi & Pascual-Leone, 2003). TMS has come into increasing use recently in normal subjects, as well as those with stroke and spinal cord injury, for a variety of clinical and scientific applications, including testing of motor function, vision, language and studying the pathophysiology of brain disorders (Nollet *et al*., 2003). TMS is based on the principle of electromagnetic induction (Faraday's law) which proposes that when the magnetic field is changed in one coil an electrical current of opposite direction is induced in a second coil (when the second coil is placed close to the first or it is wound with the first through an iron ring) (Barker, 2002). In magnetic stimulation of the body, the tissue serves the role of the second circuit while the stimulating coil represents the primary coil (Barker, 2002). Thus, if a pulse current of sufficient strength briefly passes through a coil placed over a person's head, rapidly changing magnetic pulses are generated (Kobayashi & Pascual-Leone, 2003).

This magnetic field passes unattenuated through the skull to the brain and induces an electric current in cortical tissue that is sufficient to discharge corticospinal neurons (Nollet *et al*., 2003). At a microscopic level, the induced electric field causes a change in transmembrane potential which can result in depolarization of the membrane and initiation of an action potential which then propagates along the neuron (Barker, 2002; Kobayashi & Pascual-Leone, 2003). The point of stimulation of a nerve fibre is the point along its length where the spatial derivate of induced current is maximum and sufficient to cause depolarization. In the case of a bent nerve, the current will continue in a straight line and pass out of the fibre across the membrane, although the fibre bends across the induced electric field (Kobayashi & Pascual-Leone, 2003) (Fig. 1.3). The TMS-induced current stimulates the superficial cortical layers and, by flowing parallel to the surface of the brain, preferentially excites horizontally oriented neurons. Indeed, depending on the orientation of the current induced in the brain, TMS preferentially activates pyramidal neurons via transynaptic inputs from excitatory interneurons,

probably through activation of cortico-cortical axons (Sandbrink, 2008) or their axon hillock or a nearby node of Ranvier directly (Taylor & Gandevia, 2000).

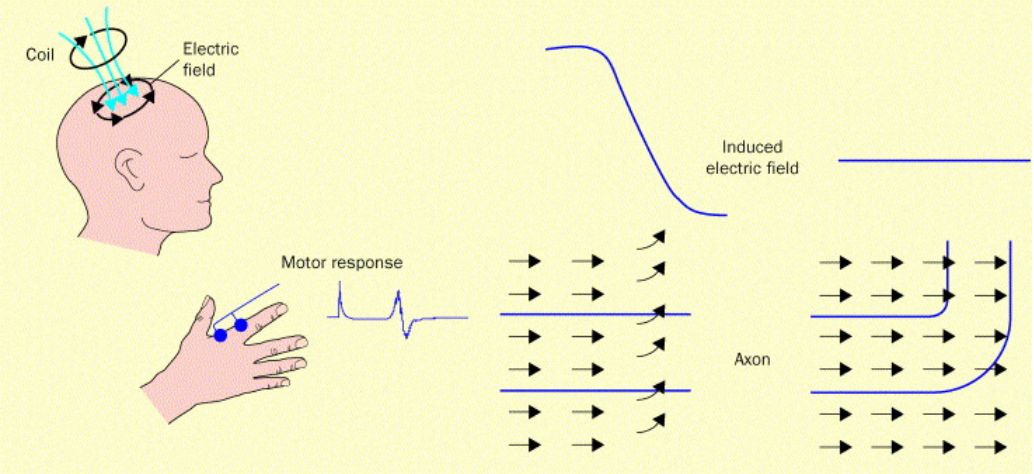


Figure 1.3: Principles of transcranial magnetic stimulation (TMS). The current flowing for short duration in the coil generates a changing magnetic field that induces an electric field in the brain, in the opposite direction. Illustration of the current flow due to induced electric field in a straight and a bend nerve resulting in a transmembrane current (Kobayashi & Pascual-Leone, 2003, p. 146.)

The technique has also gained increasing popularity in exploring the issue of central fatigue. The procedure painlessly activates cortical motoneurons and, in turn, spinal motoneurons by evoking a volley of descending excitatory waves in corticospinal pathways (Kobayashi & Pascual-Leone, 2003). Thus the behaviour of the cortical motoneurons can be assessed. When TMS is applied over the motor cortex during voluntary contractions both excitatory and inhibitory responses can be recorded in the sEMG. The excitatory response evoked by TMS, called motor evoked potential (MEP), and the silence in the sEMG activity of the muscle immediately after the TMS stimulation, called the silent period, are some of the measurements that are included in TMS studies exploring the brain motor function in health and in disease (Kobayashi & Pascual-Leone, 2003).

The amplitude of the MEP reflects the integrity of the corticospinal tract as well as the excitability of motor cortex and nerve roots and the conduction along the peripheral motor neuron to the muscle (Kobayashi & Pascual-Leone, 2003). Thus, patients with dysfunction at any level of the corticospinal tract may show abnormalities in MEP, such

as patients with stroke or multiple sclerosis (MS) who present small and dispersed MEPs (Kobayashi & Pascual-Leone, 2003). It is often assumed that the size of the MEP reflects the number of the activated motoneurons. However, the number of recruited motoneurons in the spinal cord, the number of motor units discharging more than once to the stimulus and the synchronization of the TMS-induced motoneuron discharges may influence the size of the MEP, making the interpretation of the MEP measurements difficult (Rösler & Magistris, 2008). Indeed, a characteristic of MEPs is their variability in size and shape from one stimulus to the next, even if the stimulus parameters are kept constant (Rösler & Magistris, 2008).

The silent period – the period from the end of the MEP response to the return of voluntary electromyographic activity – is believed to be due to long-lasting inhibition originating predominately within the motor cortex (Wolters *et al*., 2008). Only the early part of the silent period, up to its first 50 ms, seems to be generated by inhibitory mechanism in the spinal cord (Kobayashi & Pascual-Leone, 2003; Wolters *et al*., 2008). Thus, the duration of the silent period is another measurement that could be used to assess cortical inhibition in health and disease (Wolters *et al*., 2008). It is thought that the cortical silent period is mediated by gamma-aminobutyric acid $(GABA)_B$ receptors. Prolongation of the silent period suggests increased neural inhibition. Indeed, patients with movement disorders (i.e., Parkinson, stroke, dystonia, spinal cord injury) have silent periods of abnormally short or long duration (Kobayashi & Pascual-Leone, 2003).

TMS has also been used to stimulate directly the descending motor pathways at the level of the spine and supraspinally, over the back of the head at the level of the cervicomedullary junction. Stimulation at a spinal level is used to evaluate the behaviour of the motoneurons. The cervicomedullary MEPs are compared with the MEP evoked by motor cortex TMS to assess the drive to the motoneurons (Taylor *et al*., 2000b). Recent studies using TMS have demonstrated that some force loss in fatigue may be due to inadequate descending drive from the motor cortex, so-called supraspinal fatigue (Gandevia *et al*., 1996; Taylor *et al*., 1996). It is very important therefore to understand the supraspinal changes occurring with muscle activity in order to explain the overall neuromuscular performance during fatigue.

1.2.3.3 Evidence for Supraspinal Fatigue

Voluntary muscle activation requires sufficient activation of both the central as well as the peripheral nervous system. The motor cortex and the motoneuronal pool in the ventral gray matter of the spinal cord, as well as the peripheral motor nerve axon, the neuromuscular junction, the muscle fiber membrane and the cross bridge formation between the myosin heads and actin filaments could contribute to changes associated with fatigue. Thus, failure anywhere along the central or peripheral pathways can result in force reduction (Taylor & Gandevia, 2001). To further localize the site of failure of voluntary drive, magnetic stimulation over the motor cortex has been used (Todd *et al*., 2003; Todd *et al*., 2004). Increments in force elicited by TMS during maximal voluntary contractions indicate that some of the loss of force during fatigue occurs through suboptimal output from the motor cortex and that supraspinal fatigue has been developed (Taylor *et al*., 2006). Supraspinal fatigue is a component of central fatigue and it has been introduced to further localize the origin of fatigue at levels higher in the CNS.

Supraspinal fatigue has been well documented in the elbow flexors muscles during several fatiguing paradigms. Gandevia and his colleagues (1996) used the Twitch Interpolation Technique over the biceps brachii muscle (BB) while TMS was applied over the motor cortex during a 2 minutes sustained MVC. As the contraction proceeded, voluntary force declined while the additional force evoked by cortical stimulation increased from 1% at the beginning of the sustained MVC to 9.8% at the end of it. Additionally, the superimposed twitches evoked by electrical stimulation of the motor point of BB during the contraction increased from 0.7% of the control twitch force before the contraction to 9.3% at the final stimulus. The size of the force increments evoked by TMS cannot be compared directly with that obtained by motor point stimulation, as cortical stimulation contracts all the elbow flexors and not solely the BB. However, the force evoked by the cortical stimulation despite the maximal voluntary effort, indicates that the motor cortex was not optimally activated by volition at the moment of stimulation and thus some degree of supraspinal fatigue has been developed. Similarly, during intermittent sustained MVCs of elbow flexors the superimposed twitches evoked by motor cortical stimulation also increased (Taylor *et al*., 2000a; Todd *et al*., 2003). The increment in force twitches represents the extra force obtained from

the motor units that voluntary effort did not recruit or did not discharge at sufficiently fast rate. While the increase in TMS-evoked twitches indicates supraspinal fatigue, the increase in the superimposed twitches evoked by motor nerve stimulation indicates that although the axons of the motoneurons are capable of increased firing rates and the muscle fibres could produce more force, motoneurons firing has slowed, or some motor units have been de-recruited (Todd *et al*., 2003).

During sustained submaximal isometric contractions there is also evidence of central and supraspinal fatigue. In the study of Søgaard and his colleagues (2006) the subjects participated in a prolonged 43 minutes fatiguing contraction consisted of isometric elbow flexion at 15% of MVC. Throughout this sustained submaximal contraction, brief MVCs were performed during which cortical stimulation was undertaken followed by either brachial plexus or motor point stimulation. By the end of the submaximal contraction the force of the brief MVC decreased to 58% of the control MVC and was accompanied by increase in the superimposed twitches evoked by both motor nerve and motor cortical stimulation. The twitches evoked by motor nerve stimuli increased from 0.4% of control MVC to 2.3% in the final MVC while the twitches evoked by cortical stimuli increased from 1.2% of control MVC to 3.6% in the final MVC. An increase in twitches evoked by motor nerve stimulation indicates that voluntary drive to the muscle has decreased. Furthermore, the increase in twitches evoked by motor cortical stimulation indicates that some of this decrease occurred because of suboptimal output from the motor cortex and thus some of the impairment in motor unit discharge can be ascribed to supraspinal mechanisms (Søgaard *et al*., 2006).

Supraspinal fatigue is also evident during dynamic contractions. When TMS was applied to the motor cortex during both fatiguing elbow flexion and extension (cycles) a marked drop in voluntary torque and an increase in TMS-evoked torque was revealed. The increase in TMS-evoked torque in both types of contraction indicates that voluntary activation is not optimal during maximal dynamic actions, and the motor cortex output is not sufficient to drive the muscles maximally (Loscher & Nordlund, 2002). In another study (Prasartwuth *et al*., 2005) maximal voluntary activation, assessed with TMS, was reduced after eccentric exercise due to changes in motor pathways between the sites of stimulation at the motor cortex and motor axons.

Although a suboptimal output from the motor cortex may account for supraspinal fatigue, the mechanisms that underlie this failure are still unclear. It is postulated that either the motor cortex output is reduced during fatigue or the output is not sufficient to produce maximal force (Taylor *et al*., 2006). The former may include changes in the corticospinal neurons or input to corticospinal neurons, and the latter may include changes in the efficacy of the motoneurons to response to descending drive and changes in the muscle contractile properties (Taylor *et al*., 2006).

1.2.3.4 Behaviour of Cortical Neurons and Motoneurons during Fatigue

The behavior of the cortical neurons is assessed by changes in the sEMG recordings of excitatory and inhibitory responses evoked by TMS during voluntary contractions. These sEMG responses to TMS of the motor cortex are altered during fatiguing contractions (Taylor & Gandevia, 2001). Different types of fatiguing exercise have also been used to show how central fatigue develops during both maximal and submaximal voluntary contractions (Taylor & Gandevia, 2008). During a sustained isometric maximum voluntary contraction the MEP responses, evoked by TMS, increase in size and latency and the silent period increases in duration (McKay *et al*., 1996; Taylor *et al*., 1996; Taylor *et al*., 1999) (see Fig. 1.4). Similarly, an increase in MEP amplitude and in silent period duration is also remarkable during intermittent maximum voluntary contractions of the elbow flexors (Taylor *et al*., 2000a). The size of the evoked motor response could be influenced not only by the cortical excitability but also by changes in excitability of the spinal motorneuron pool. Therefore, non-invasive stimulation of the descending motor tracks in the spinal cord and supramaximal stimulation of the peripheral motor nerve of certain muscles have also been used to interpret the responses evoked by the cortex and to investigate the segmental behaviour of the motor pathway (Taylor & Gandevia, 2004). The findings showed that the growth of the MEP and the prolongation of the silent period during sustained maximal contractions were not followed by changes in the size of the cMEP evoked by stimulation of the cervicomedullary junction. This suggests that the increase in the MEP size is due to increased excitability of the motor cortex. The silent period following cervicomedullary stimulation lengthened but less than that following stimulation over the motor cortex suggesting that the motor cortex is primarily responsible for these changes (Taylor *et*

al., 1996). Although the initial part of the TMS silent period could reflect inhibition at a spinal level the later part is probably due to reduced cortical output. Thus the more prolonged silent period following TMS than following cervicomedullary stimulation indicates increased cortical inhibition (Taylor *et al*., 1996). In addition, the M-wave area evoked by supramaximal peripheral electrical stimulation grows during maximal contraction probably because of changes in the muscle action potential (Paul & Wood, 2002). However, because the M-wave increment is less than the increase in the area of MEP, the changes in the muscle action potential could not entirely account for the changes in the MEP. Hence, some of the increase in the MEP size should reflect changes in the motor cortex excitability (Taylor *et al*., 1999).

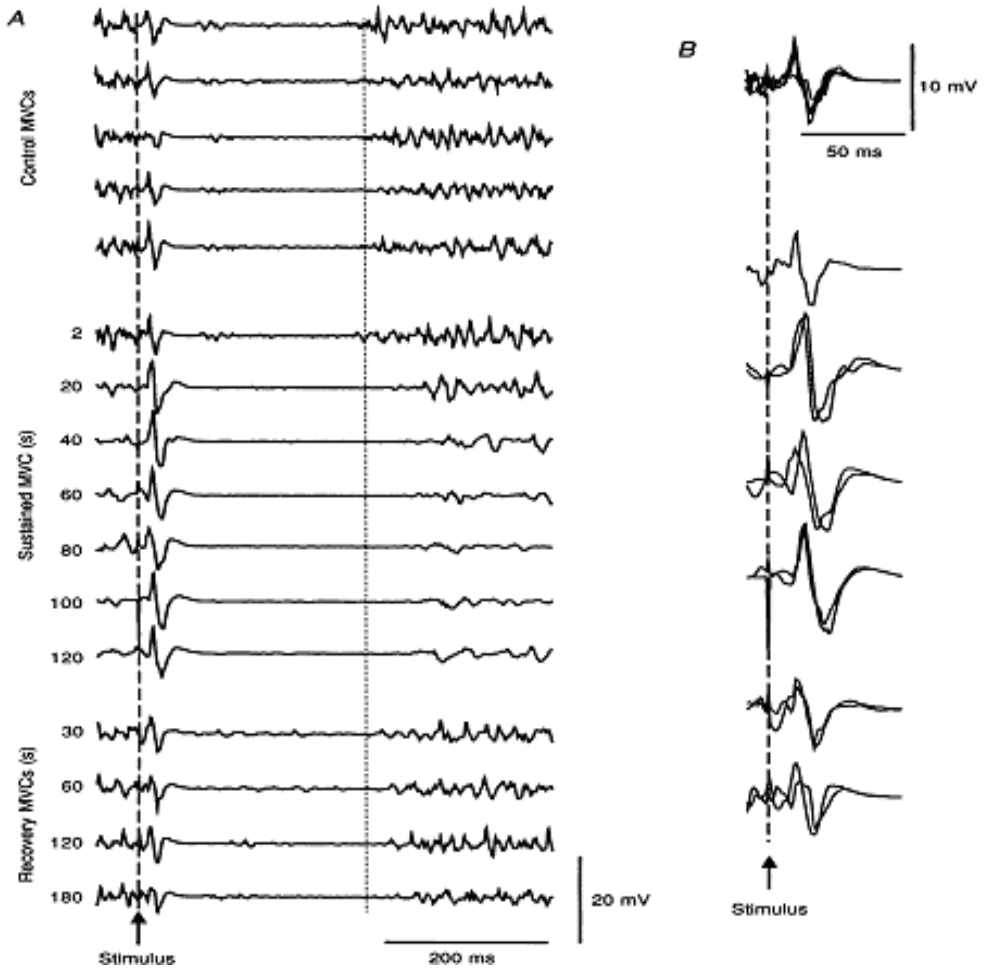


Figure 1.4: TMS changes during MVCs. Data from BB before, during and after the TMS. The dashed line represent the time of stimulation. The MEPs (just right to the dashed line) are shown in a larger scale in B. Dotted line marks the end of the control (before fatigue) silent period (Taylor *et al.*, 1996, p. 522).

Additional evidence that fatigue induced changes happen supraspinally, comes from TMS studies using ischemia or vibration as additional interventions (Taylor *et al*., 1996; Gandevia *et al*., 1996). When the blood supply to the arm is temporarily occluded by inflation of a blood pressure cuff around the upper arm, during sustained MVC, the MEPs evoked by TMS returned rapidly to control values, which suggest that changes during fatigue were not mediated by small-diameter muscle afferents (Gandevia *et al*., 1996). Additionally, vibration of the fatiguing muscle tendon, which has been used to augment muscle afferent input near the end of the sustained MVC, had no effect either on MEP area or on silent period duration (Taylor *et al*., 1996). These findings indicate that changes to MEPs were not affected by muscle spindle inputs and thus, it is impossible that changes in spinal excitability caused the increase in MEP size and silent period duration during maximal fatiguing contractions.

Similar MEP changes are reported during sustained submaximal muscle contractions (Ljubisavljevic *et al*., 1996; Sacco *et al*., 1997). The MEP increases in size and the silent period increases in duration as during maximal contractions. However, these changes are not as pronounced as during higher levels of activity and they may be more task specific (Taylor & Gandevia, 2001). Thus, during high levels of submaximal force (60% of MVC) the MEP and silent period show an initial decrease until the point when the voluntary force starts to decline (endurance point) and increase thereafter up to the end of the contraction irrespective its duration (Ljubisavljevic *et al*., 1996). During low intensity submaximal contractions (<30% MVC) of short duration (2 min) no increase in silent period was reported (Taylor *et al*., 1996). However, the silent period evoked by TMS increased in duration during longer lasting low submaximal contractions (Sacco *et al*., 1997; Søgaard *et al*., 2006). Although task specific, the changes in the silent period duration and MEP size during fatigue might be caused not only by changes in segmental spinal level but also by changes in the motor cortex output. During submaximal fatiguing contractions the silent period evoked by transcranial electrical stimulation (TES), which directly activates corticospinal neurons, did not change, indicating that the lengthening of the TMS silent period represents a cortical effect (Sacco *et al*., 1997). In addition, the increase in MEP size during submaximal contractions could not only represent an increase in motoneurons excitability. The MEP increment was followed by only a modest decline (10%) in M-wave amplitude, which implies that part of the MEP increment is due to increased motor cortex excitability (Sacco *et al*., 1997).

TMS changes are also present following a fatiguing task (Taylor & Gandevia, 2001). When the MEPs are evoked in relaxed muscle there is an increase in the MEP size compared to pre exercise baseline levels (Lentz & Nielsen, 2002). This post contraction facilitation of the MEP is short-lived (< 1 minute) and it is followed by a depression in magnitude that can last for more than 15 minutes (Brasil-Neto *et al*., 1993; McKay *et al*., 1995; Zanette *et al*., 1995; Sacco *et al*., 2000). This post exercise depression has been reported in various muscles, including: tibialis anterior (McKay *et al*., 1995), flexor carpi radialis (Brasil-Neto *et al*., 1993), and BB (Sacco *et al*., 2000) and is unrelated to the type of fatiguing contraction. It has been reported not only following dynamic, repetitive fatiguing contractions (Brasil-Neto *et al*., 1993; Zanette *et al*., 1995), but also following isometric contractions of maximal or submaximal intensity (Sacco *et al*., 2000; Lentz & Nielsen, 2002; Maruyama *et al*., 2006). This post exercise MEP depression is more likely to be confined to the contracting muscles as no MEP changes revealed in ipsilateral, non exercising muscles (McKay *et al*., 1995; Zanette *et al*., 1995). Both the post-exercise facilitation and depression probably reflect cortical changes (Taylor & Gandevia, 2001). When motoneurons were directly activated by TES, no significant effects of repetitive dynamic exercise on MEPs were reported (Zanette *et al*., 1995). However, McKay and colleagues (1995) reported a 50% decrease in motor responses of tibialis anterior to TES which lasted more than 5 minutes. This reduction might indicate reduction in excitability of the motoneurons but the subsequent absence of changes in H reflexes or M-waves following the fatiguing MVC does not support this hypothesis. Absence of changes in the M-waves, F-waves and H reflexes following the fatiguing exercise is also reported elsewhere, indicating that the post exercise depression in the MEP evoked by TMS while the muscle is relaxed represents a focal reduction of cortical excitability (Brasil-Neto *et al*., 1993; Zanette *et al*., 1995). This reduction in motor cortical excitability could be caused by intracortical or/and subcortical inhibitory mechanisms (Maruyama *et al*., 2006).

Motoneurons are also affected during fatiguing contractions. Their firing rate decreases during sustained maximal contraction (Bigland-Ritchie *et al*., 1983) (Fig. 1.5) and the sEMG responses to cervicomedullary stimulation remain unchanged (Taylor *et al*., 1996) or decrease (Taylor *et al*., 2000b). At the same time the voluntary force declines and extra descending drive is required to maintain the voluntary contraction (Taylor *et*

al., 2006). The reduction in the motoneurons firing rate which is called "muscle wisdom" seems to be a contributor to force decline and a promoter of fatigue (Gandevia, 2001). The muscle wisdom hypothesis proposes that the reduction in motoneurons firing rate happens to match the observed decline in motor unit firing rate and the contractile speed of the muscle during an MVC (Gandevia, 2001). Thus, the lowering of motor unit firing rates that accompanies the slowing of muscle relaxation is thought to ensure that the central drive to the fatiguing muscle would be just that necessary to produce the required force (Garland *et al*., 1997). This hypothesis was derived from experiments utilizing a sustained maximal voluntary contraction (MVC), typically held for 60–90 s.

The suboptimal firing of the motoneurons may be caused by the altered input from the muscles and the cutaneous afferents, by inhibitory input from interneurons in the spinal cord or by suboptimal drive from supraspinal sites (Taylor & Gandevia, 2001). During maximal voluntary contractions of a human muscle, the input from specialized muscle mechanoreceptors (innervated by large-diameter afferents: muscle spindle Ia and II, Golgi tendon organ Ib) and from chemosensors and nociceptors (innervated by small-diameter afferents: III, IV and non-spindle type II) will change (Gandevia, 1998). It has been reported that muscle spindle endings firing declines during sustained voluntary contractions while the sensitivity and the discharge of Golgi tendon organs also reduce (Gandevia, 1998; Taylor *et al*., 2000b; Gandevia, 2001). Additionally, the discharge of small-diameter muscle afferents (groups III and IV) increases during muscle fatigue as the chemical and noxious metabolites accumulate in the muscle (Gandevia, 1998). The kind of fibres that are innervated seem also to play a role in the change of firing rate of motoneurons as it has been found that the firing decline is more pronounced in the larger, faster and more fatigable motor units, like type II fibres (Taylor *et al*., 2000b). The intrinsic properties of the motoneurons however may not put the motoneurons in a state in which they fail to respond to excitatory drive. The plateau in the firing at the second part of the sustained MVC (Fig. 1.5) may be an indicator of the late adaptation of the motoneurons to the decreased firing rate (Gandevia, 2001). Thus the properties of the motoneurons play a crucial role in the modulation of fatigue. Supraspinal commands to interneurones and motoneurons, controlled by brainstem serotonergic pathways, classical reflex and recurrent inputs to α- and γ-motoneurons, and presynaptic modulation of reflex inputs to motoneurons could also affect the motoneuronal

behaviour during fatigue (Gandevia, 1998). However, the research in this field is still inconclusive. The combined monosynaptic and polysynaptic paths in addition to combined excitatory and inhibitory reflex input to the motoneuron pool make the assessment of motoneurons behaviour daunting.

In summary, both cortical neurons and motoneurons are affected during fatigue. Despite their robustness, changes in motor cortical excitability and changes in the motoneurons behaviour may not directly affect the central fatigue and cannot fully explain supraspinal fatigue during voluntary efforts. The question why the output from the motor cortex is insufficient to produce maximal force despite maximal effort remains (Taylor *et al*., 2006).

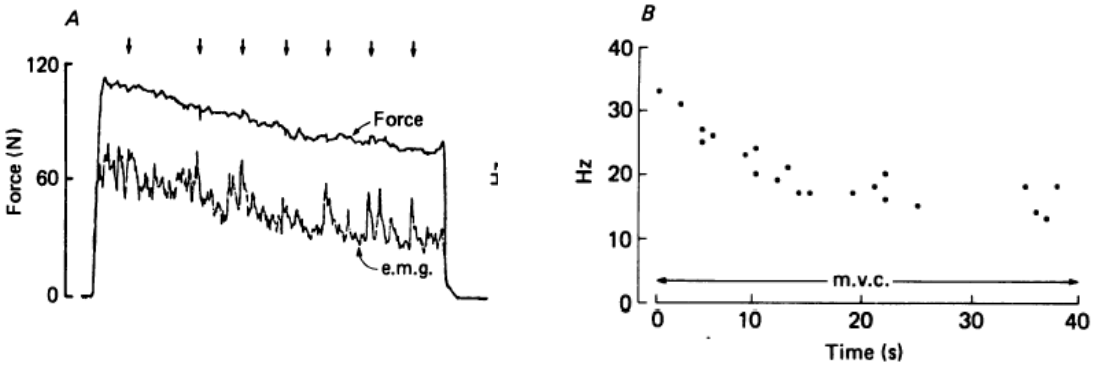


Figure 1.5: Effect of fatigue on voluntary force production and motoneurone firing rate during a sustained MVC of the adductor pollicis muscle. A) Voluntary force and surface SEMG during a sustained MVC of the adductor pollicis. Arrows show times when the M-waves evoked by electrical stimulation of ulnar nerve, were recorded. B) Discharged rates recorded from twenty one single motor units by insertion of tungsten micro-electrodes. Each point is the mean rate of a spike train recorded at the times shown (Bigland-Ritchie *et al*., 1983, p. 340).

1.2.3.5 Are the Changes in Cortical Excitability Related to the Changes in Voluntary Activation and Central Fatigue?

Although the output from the motor cortex is insufficient to drive the muscle maximally during fatigue its connection to central fatigue has been demonstrated by preventing the recovery of the fatigued muscle (Taylor *et al*., 2000c; Butler *et al*., 2003). This has been achieved by inflating a blood pressure cuff around the limb and holding the muscle ischaemic at the end of the fatiguing MVC. In that way the metabolites remain in the muscles but the activity dependent changes could recover (Taylor & Gandevia, 2004). When the elbow flexors were held ischaemic immediately after the end of the fatiguing maximal or submaximal voluntary contraction any changes in the motor cortex caused by the fatiguing contraction quickly returned to control levels despite the lack of blood flow to the muscles (Gandevia *et al*., 1996; Taylor *et al*., 1996) (Fig. 1.6). Similarly, the sEMG responses to transmastoid stimulation were not affected by the ischaemia and their recovery continued despite the blood flow occlusion (Taylor *et al*., 1996; Taylor *et al*., 2000b; Butler *et al*., 2003). However, when the motor pathways from the cortex to the muscle had recovered, the output from the motor cortex was still inadequate to fully activate the muscle. The voluntary force did not recover by rest during the period of ischaemia (Taylor *et al*., 1996) indicating that the muscle fatigue continued. At the same time the motor units firing rate, tested with motor nerve stimulation, also remained low (Butler *et al*., 2003). Additionally, the force evoked by cortical stimulation during a brief MVC remained large compared to that evoked before fatigue, keeping the voluntary activation low (Gandevia *et al*., 1996) (Fig. 1.6). These findings suggest that central fatigue continues while peripheral fatigue in the muscle is maintained and this comprises the first proof that changes in neural pathways are not directly associated with central fatigue.

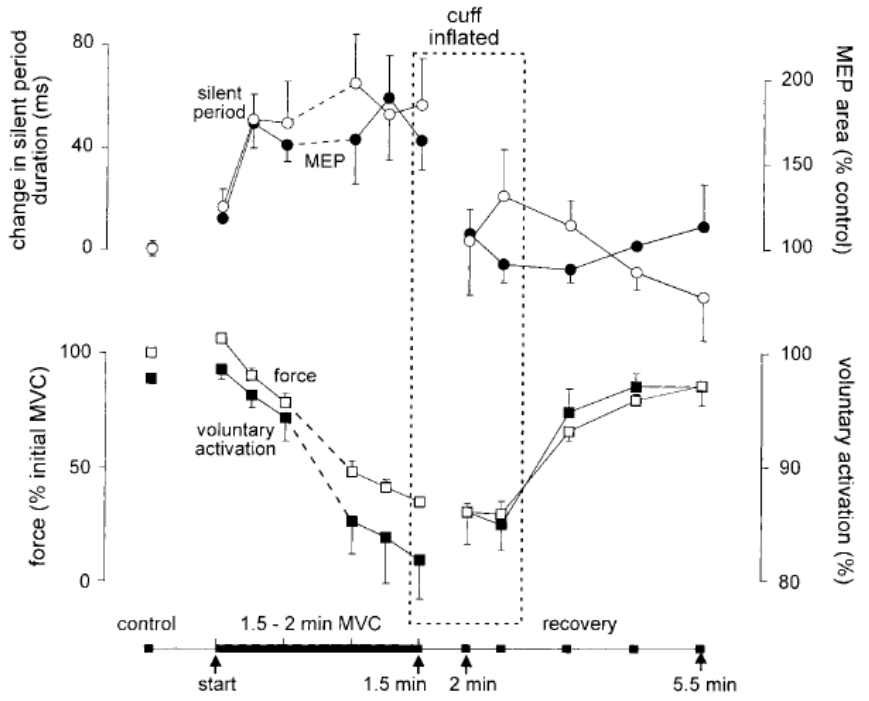


Figure 1.6: Effect of maintained ischaemia of elbow flexors muscles following sustained maximal voluntary contraction on recovery of MEPs (upper right axis, ●), silent period (upper left axis, ○), voluntary activation (low panel right axis, ■) and voluntary force (low panel, left axis, □). The shaded area (dotted box) indicates the period of ischaemia when the blood flow was occluded. Silent period duration and MEP recovered quickly after contraction while voluntary activation and force did not recover until blood flow had resumed (Gandevia *et al.*, 1996, p. 533).

A second source of evidence for this dissociation comes from studies evaluating the time course of recovery in voluntary activation and motor cortical responses to TMS following maximal or submaximal fatiguing contractions (Gandevia *et al.*, 1996; Taylor *et al.*, 2000a; Søgaard *et al.*, 2006). Following such contractions, the recovery of cortical excitability assessed by the sEMG responses to cortical stimulation during brief MVCs showed a different pattern of recovery from the recovery of the central fatigue assessed by voluntary activation. MEPs and the cortical silent period recovered almost immediately (in 15 seconds) after the end of the fatiguing contractions when the output of the motor cortex was still suboptimal and did not recover before 1–2 minutes had passed (Gandevia *et al.*, 1996; Taylor *et al.*, 1996; Taylor *et al.*, 2000a; Søgaard *et al.*, 2006). Indeed peripheral fatigue was maintained as it was assessed by the resting force twitches evoked by motor point stimulation (Søgaard *et al.*, 2006).

The above findings indicate that the changes in the silent period and the MEP are not directly associated with central fatigue. Although they represent cortical phenomena they are not affected by the afferent inputs from the muscle or intramuscular metabolite accumulation. Indeed, when the neural pathways from the cortex to the muscle had recovered from fatigue, the output of the motor cortex was still inadequate to fully activate the muscle. This indicates that central and supraspinal fatigue are related with the maintained fatigued state of the muscle, but this does not occur at the motoneurons or at the level of motor cortical output (Taylor *et al*., 2006). Instead, changes upstream of the motor cortex that may impair the voluntary descending drive have been suggested (Taylor *et al*., 2000a; Gandevia, 2001; Søgaard *et al*., 2006; Taylor *et al*., 2006).

Additionally, the MEP and silent period may occur as a consequence of changes in the excitability and inhibition in the input to the motor cortex and not in its output. Excitatory or inhibitory input from other cortical and subcortical areas may be a possibility (Sacco *et al*., 1997). Studies using TMS and magnetic imaging (fMRI) have explored changes in motor cortex excitability and function and its connections to other cortical and subcortical areas following fatigue (Benwell *et al*., 2005; Benwell *et al*., 2006b). These studies have examined the brain changes following fatigue to intrinsic hand muscles by comparing changes associated with fatigue of the first dorsal interosseous (FDI) and that of the un-fatigued abductor digiti minimi (ADM). MEP amplitude for both the fatigued and un-fatigued muscles increased above baseline during exercise and then decreased below baseline during the recovery period. This implies that the changes in motor cortical excitability due to fatigue are not restricted only to the brain areas involved in the motor control of the fatiguing muscles. Instead, fatigue caused more widespread interhemispheric changes (Benwell *et al*., 2006a; Benwell *et al*., 2007).

Similar results observed in the study of Humphry's and his colleagues (2004) where it was reported that corticospinal excitability was depressed following exhaustive exercise in both exercising and non-exercising muscles. Because there was not any measurable functional deficit in the non-exercising limb, as it was evaluated by force production in BB, hand-grip force, simple reaction times and movement times, it has been concluded that the reduced corticospinal excitability observed in this limb has little or no consequence on the aforementioned performance parameters measured (Humphry *et al*.,

2004). This study also suggests a generalizability of fatigue in other areas of the brain ostensibly unrelated to the fatiguing muscles. Additionally, the activated voxels (a relevant spatial unit for measuring local magnetic resonance reconstruction and therefore brain activation (Norman *et al*., 2006) of the fMRI signals, in the homologous areas of the contralateral hemisphere reduced following the fatiguing exercise (Benwell *et al*., 2005). This suggests more widespread changes through interconnections with brain areas other than those involved in the fatiguing task. The changes might be a central adaptive process to optimize motor output and motor control during and after fatiguing exercise (Benwell *et al*., 2005; Benwell *et al*., 2006b) rather than a failure in central motor drive during the development of fatigue (Gandevia, 2001; Taylor & Gandevia, 2001). Indeed, stimulation over the premotor area resulted in neural modulation in motor areas in the frontal cortex as well as in prefrontal and parietal cortices and provides an anatomical basis for the transformation of sensory information into motor actions (Chouinard *et al*., 2003). Furthermore, TMS over the dorsal premotor cortex suppressed the MEPs evoked from the controlateral primary motor cortex at interstimulus intervals of 8 to 10 msec (Mochizuki *et al*., 2004). These findings suggest interhemispheric cortico-cortical connections not only between premotor and primary motor cortices but also between different lobules. Based on these findings the suggestions for changes "upstream" of the motor cortex during fatigue (Gandevia, 2001) do not seem paradoxical.

Moreover, considering the behavioral changes that accompany muscle fatigue, such as changes in subjective effort (Søgaard *et al*., 2006), attention and pain (St Clair Gibson & Noakes, 2004), there may be many associated supraspinal changes measurable at an electrophysiological and biochemical level, but, as for events at a spinal level, it is difficult to determine which are secondary to peripheral fatigue and which contribute to central fatigue and eventual task failure (Gandevia, 1998).

Perception of voluntary effort has been reported to affect exercise performance (Hampson *et al*., 2001). Indeed, changes in perceived effort may allow exercise performance to be precisely regulated such that a task can be completed within the biomechanical and metabolic limits of the body (St Clair Gibson *et al*., 2006). Thus, perceived effort may serve an important role in an integrated system including the CNS and the peripheral organs. However, to date, a direct relation between any single

physiological variable and the perception of effort has not yet been established clearly. Research into this issue might give some insight to the ways the behavior is mediated during fatigue.

1.2.3.6 Assessment of Voluntary Activation

Electrical stimulation has been the preferred method of a non-volitional method of assessing muscle activation in human physiology since its early application (Cooper, 1930; Merton, 1954). When a single impulse is conducted by the nerve, a single twitch is generated, followed by a relaxation period. When further stimuli are delivered before relaxation is completed, greater tension is produced (Cooper, 1930; Man *et al*., 2004). This tension is the true tension of the muscle and it is increased further with the stimulation frequency until a plateau is reached (Fig. 1.7). Increasing the stimulus rate produces no extra tension and a complete fusion of force is developed (Cooper, 1930). However, use of tetanic stimulation is not a practical or tolerable method for assessing muscle capacity, especially in clinical environments.

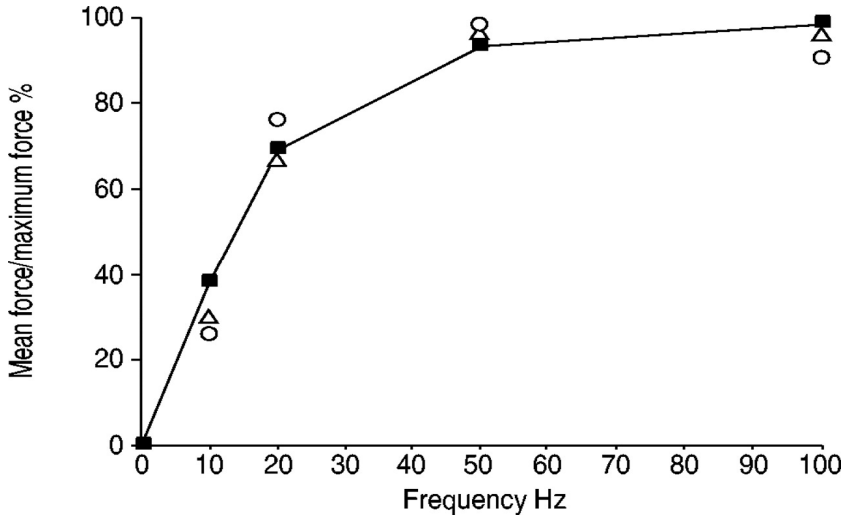


Figure 1.7: Force-frequency curve of electrical stimulation of the diaphragm (■), quadriceps (O) and adductor pollicis (Δ) from three healthy subjects (Man, 2004, p. 847).

Hence methods of single electrical stimulation have been introduced such as the Twitch Interpolation Technique, first established by Merton (1954). As mentioned earlier, the Twitch Interpolation Technique assesses the degree of voluntary activation by applying supramaximal electrical stimulation to the nerve or muscle during a voluntary contraction. If a twitch-like increment in force is evoked when the electrical stimulus is applied, then either the stimulated motor units were not all recruited voluntarily, as some of them were in a refractory state, or they were firing at sub-maximal rates. With increasing neural drive to the muscle, less motor units are available for recruitment and the superimposed twitches become smaller and finally undetectable if the muscle can be fully activated (Gandevia, 2001) (Fig. 1.8).

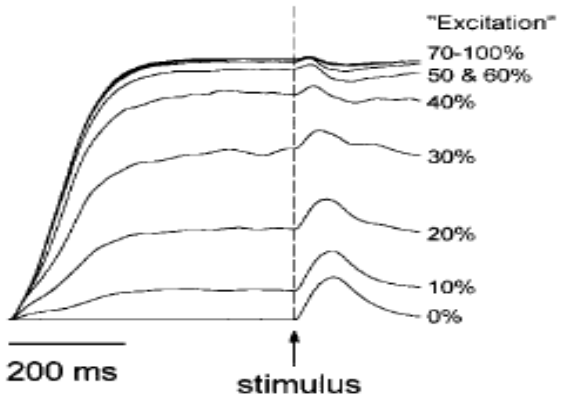


Figure 1.8: Superimposed twitch-like force responses of the adductor pollicis when excited from 0 to 100% of maximal excitation. The arrow indicates the timing of the electrical stimulus. The force increments progressively decrease as the muscle becomes fully activated (Gandevia, 2001, p. 1740).

Merton (1954) reported a negative linear relationship between adductor pollicis twitch force produced by a supramaximal stimulus to the ulnar nerve and voluntary isometric force measured during thumb adduction. He also reported no evidence of a superimposed twitch increment in force at maximal voluntary contractions and concluded that the muscle could be fully activated by voluntary command. However, later studies using more sensitive force transducers and electronic instrumentation for increased resolution of small twitch forces applied to this original technique have revealed that even at maximal voluntary force detectable small superimposed twitches (about 1% of original evoked twitch force in relaxed muscle) could still be apparent

(Herbert & Gandevia, 1999; Yue *et al*., 2000). Thus, the level of the motoneuron excitation is not sufficient to extract the maximal force that the muscle could have produced. The interpolated twitch is then used to quantify the level of motoneuronal excitation (Allen *et al*., 1998). The negative linear relationship between evoked and voluntary force found by Merton (1954) implies that the extent of inactivation can be quantified by expressing the interpolated twitch as a percentage of the twitch evoked in relaxed muscle. It also suggests that the muscle's true maximum force can be determined on the basis of a single interpolated twitch ratio. Accordingly, voluntary activation is usually derived by the formula: *voluntary activation=100×(1-superimposed twitch / control twitch)*, where the superimposed twitch is the force increment evoked during a maximal contraction at the time of the stimulation and the control twitch is that evoked by identical nerve stimulation in relaxed muscle (Shield & Zhou, 2004).

To provide a valid estimate of voluntary activation the formula above actually requires a linear relationship between evoked force and activation of the stimulated muscle (Gandevia, 2001). Some experimental factors that may distort the relationship between voluntary force and the size of the superimposed twitch include: failure to use a fully potentiated twitch and thus the initial twitch force will be lower than it should if the muscle twitch is not potentiated (Gandevia, 2001). However, even with potentiation, the superimposed twitch was found to be of similar magnitude with the un-potentiated twitch at high levels of force (>70%MVC) (Folland & Williams, 2007). Additionally, the force curve may not be linear because unstimulated synergists contribute disproportionately to increases in voluntary force in near maximal contractions, or because of inadvertent stimulation of antagonist muscles (Awiszus *et al*., 1997; Allen *et al*., 1998). Finally, the failure to prevent small changes in muscles length during testing can cause the otherwise near-linear evoked voluntary force relationship to become asymptotic at considerably submaximal forces (Herbert & Gandevia, 1999; Shield & Zhou, 2004). Mechanical factors are not the only ones that affect the relationship between the size of an interpolated muscle twitch to a single stimulus and the level of voluntary isometric force. Twitch amplitude is also influenced by collisions between orthodromic and antidromic actions potentials and axonal refractoriness (Yue *et al*., 2000).

For methodological accuracy there is a controversy about whether the single interpolated twitch ratio is a valid measure of the degree of failure of voluntary activation of motoneurons, as the non-linear relationship between evoked and voluntary force probably invalidates the equation (Gandevia, 2001). Thus, it is suggested that the Twitch Interpolation Technique cannot provide an accurate estimate of the true maximum activation. Instead, extrapolating the relationship to a predicted true maximum has been suggested as an appropriate alternative (Folland & Williams, 2007). The estimate of true maximum force may then offer a more precise and reliable measure of the maximum force capacity of the muscle than MVC, as it might avoid the influence of variables like motivation or attention (Folland & Williams, 2007). Voluntary activation is then determined by expressing the MVC force as a percentage of estimated true maximum (Shield & Zhou, 2004). Different ways have been proposed for estimating the true maximum force; by fitting a straight line to data points above 25% of MVC or by employing polynomial and exponential functions (Behm *et al*., 1996; Shield & Zhou, 2004; Folland & Williams, 2007). However, even these various methods do not overcome the problem of the non linearity. True maximum force exhibited similar variability to the MVC and in some cases it was unrealistically large (above 100% activation) (Herbert & Gandevia, 1999; Shield & Zhou, 2004; Folland & Williams, 2007).

Recent studies have introduced pairs of supramaximal stimuli in the Twitch Interpolation Technique rather than a single interpolated stimulus as initially described by Merton (1954) in order to obtain more accurate data for the assessment of voluntary activation. Paired (Behm *et al*., 1996; Folland & Williams, 2007) or trains of stimuli (De Serres & Enoka, 1998; Herbert & Gandevia, 1999; Yue *et al*., 2000) have been used at various frequencies and pulse durations. It has been suggested that more than one stimulus evokes force increments which are larger in amplitude and more readily detected. Additionally, the supramaximal multiple stimuli evoke less variable force increments than single stimuli at high levels of contractions (Shield & Zhou, 2004). The multiple stimuli, although widely used in assessing the voluntary activation, hide a practical problem; the increased discomfort involved. To overcome this problem, submaximal stimuli are often employed (Miller *et al*., 1999). However, longer trains of submaximal stimuli influence the estimates of voluntary activation. It has been reported that while the force increments evoked by both submaximal trains of 20 stimuli (100

Hz) and single supramaximal stimuli decline linearly with increasing voluntary force, the 95% confidence intervals for the relationship are considerably wider when trains of stimuli were employed (Newham & Hsiao, 1996 cited in Shield & Zhou, 2004). Furthermore, the use of submaximal stimulation, although minimizing the risk of activating antagonist muscles, may activate different portions of the muscle especially if small changes in voluntary drive occur immediately prior to stimulation (Behm *et al*., 1996; Awiszus *et al*., 1997; Yue *et al*., 2000). Accordingly, submaximal electrical trains may produce a less precise estimation of voluntary activation than single supramaximal stimuli.

1.2.3.7 Peripheral Magnetic Stimulation for Assessment of Voluntary Activation

Application of peripheral electrical stimulation of underlying nerves using surface electrodes requires currents flowing in a skin area and the electrical stimulus is often large enough to activate sensory fibre endings in the skin, thus causing pain (Man *et al*., 2004). This pain causes discomfort and even if tolerable in well motivated healthy subjects, it can hinder its successful application for voluntary activation assessment in a clinical setting with patients. While needle or implantable electrodes can be used clinically for certain neurophysiological assessments, they are not practical alternatives for assessment of muscle function, and have the added risk of infection, trauma or bleeding, and therefore make these invasive techniques unfeasible for such clinical use (Man *et al*., 2004). An alternative to peripheral electrical stimulation has been proposed which includes the use of peripheral magnetic stimulation of the nerve trunk for the assessment of muscle function (Polkey *et al*., 2000).

Peripheral magnetic stimulation, in contrast to peripheral electrical stimulation, can ensure nerve trunk stimulation without involving high currents in the skin, and does not cause painful sensations (Man *et al*., 2004). Based on the Faraday's electromagnetic induction, the magnetic field generated by the current flow in the coil induces an electrical current in the tissue proportional to the rate of change of the generated magnetic field (Barker, 2002). Thus magnetic stimulation causes an induced electric field without electrical contact with the tissue, which is of sufficient amplitude and

duration to depolarize nerve membranes and thus generate action potentials in a similar way to conventional electrical stimulation (Barker, 2002; Man *et al*., 2004). Additionally, the wider field of stimulation means that it is technically easier to perform and requires less trial stimulations to produce supramaximality. It has therefore been proposed to be more suitable for use in clinical environments (Polkey *et al*., 2000; Luo *et al*., 2002/6; Man *et al*., 2004). Previous studies have demonstrated that peripheral magnetic stimulation can be used for assessment of muscle strength in patients (Harris *et al*., 2001) and even for neonatal use (Rafferty *et al*., 2000). Furthermore, increased interest has developed recently for using the peripheral magnetic stimulation as an alternative to Twitch Interpolation Technique in assessing the level of activation of skeletal muscle during voluntary contractions (Harris *et al*., 2000; Hamnegård *et al*., 2004). In the study by Harris and his colleagues (2000), magnetic stimulation of the ulnar nerve was used for measurement of adductor pollicis twitch tension and compound action potential. The magnetic stimulation was applied to both healthy subjects and patients in the intensive care unit and operating theatre. To assess the level of voluntary activation of adductor pollicis, the healthy subjects were asked to perform a maximum voluntary contraction with the adductor pollicis during which a superimposed supramaximal electrical or magnetic stimulation (interpolated twitch) was delivered to the ulnar nerve when isometric force reached a plateau. A comparison was then made between electrical and magnetic stimulation. Close agreement was found between supramaximal twitch of adductor pollicis for electrical and magnetic stimulation, demonstrating that, with correct orientation of the magnetic coil, the magnetic field is likely to maximally excite the ulnar nerve without activation of the median nerve, or at least to no greater extent than electrical stimulation. No signs of discomfort were reported and the stimulation was well tolerated when the magnetic stimulation technique was used with patients (Harris *et al*., 2000). Similar results revealed in another study, when the same technique was used in a large sample size (n=45) (Hamnegård *et al*., 2004). Magnetic stimulation was applied at the femoral nerve for assessment of quadriceps voluntary activation. Supramaximal stimulation was delivered using a double circular coil with the appropriate orientation for better penetration and again the procedure was well tolerated in all 45 normal subjects participating in the study.

The results of the above studies point towards a promising new application of peripheral magnetic stimulation technique for the assessment of voluntary activation. Its painless applicability to patients makes motor nerve magnetic stimulation a valuable tool in research and clinical environments. It could be applied as a diagnostic tool for assessing any incomplete muscle activation, especially in cases where patients complain of exaggerated effort and chronic fatigue. Peripheral magnetic stimulation could be combined with use of TMS to evaluate further the properties of central fatigue as distinct from peripheral fatigue in the future.

To date, no study has compared the two techniques of peripheral magnetic and electrical stimulation in any detail. Given the limitations of the Twitch Interpolation Technique in the clinical setting, due to the pain and discomfort that electrical stimulation may cause, magnetic stimulation becomes an attractive alternative. However, before magnetic stimulation can be introduced for application in clinical settings, it is necessary to compare these two methods of peripheral stimulation – electric versus magnetic – to determine if magnetic stimulation is a reliable method for assessing the voluntary activation. This is therefore one of the main objectives of the present thesis which will be addressed in the first study of this research project, presented in chapter 3.

1.2.4 Perception of Effort

The concept of perception of effort is typically defined in a context-dependent manner. It is alternatively referred to as perceived exertion which is akin to Borg's concept of a "*gestalt*" or configuration of sensations from peripheral muscles and cardiopulmonary systems (Borg, 1998). Perceived exertion is referred to as the feeling of how hard and strenuous is the physical task. It is based on the physical sensations a person experiences during physical activity, including increased heart rate, increased respiration or breathing rate, increased sweating, and muscle fatigue (Borg, 1998). Thus, it is believed that changes in the perceived exertion during exercise result from multiple afferent signals from the periphery (Hampson *et al*., 2001).

Perceived exertion or effort is strongly related to the neuromuscular activation. If, for example, one carries a load for a prolonged period of time, that load is perceived to become heavier as the muscles supporting it become fatigued (McCloskey, 1981). This increased effort required to generate the same muscular force could be explained by the increased voluntary motor activity required to overcome the peripheral and central changes that accompany fatigue (Burgess & Jones, 1997; Presland *et al*., 2005). In many paretic limbs as well, weights are felt to be heavier than they actually are, because of the greater effort involved for this action (Henneman, 1974, cited in McCloskey, 1981). The importance of the perception of effort in judging heaviness is also clear when one tries to lift a very heavy object which cannot be moved in response to a great effort. In such a case, one cannot judge how heavy the object is; only that it is heavier than another object that has been lifted under the same effort. Nor is it possible to discriminate between the heaviness of two objects if neither of them can be lifted.

The above clearly indicates that some afferent signal of the success of a motor command is required before that command can be of use in judging heaviness (Gandevia & McCloskey, 1978). Thus, afferent signals are important not only for assessment of heaviness, but also in the interpretation of the perceived motor command by acting as indicators of the appropriate point at which the CNS changes the motor command (Gandevia & McCloskey, 1978). However, the way the various peripheral sources of information are processed in the brain and how the perception of effort is then regulated is not well understood. In an attempt to explain how effort is perceived during exercise and fatigue, the combined feedforward-feedback neurophysiological model has been developed (Noble & Robertson, 1996). Based on that model, feedback from mechano-(Ia, Ib, spindle II)- and chemo-sensitive receptors (non spindle type II, type III and IV) in muscular and tendon structures supply the CNS with afferent information (Pincivero & Gear, 2000). Thus, mechanoreceptors like Golgi tendon organs and skin receptors give signals about the force and the strain developed during a task. Consequently, a close and consistent relationship between the perceived effort and magnitude of force production has been reported (Somodi *et al*., 1995; Gearhart *et al*., 2002; Pincivero *et al*., 2003b; West *et al*., 2005). Other feedback, specific for whole body exercise, can generally be derived from cardiopulmonary activity and measured as respiratory rate, minute ventilation, heart rate and oxygen uptake (Hampson *et al*., 2001) (Fig. 1.9).

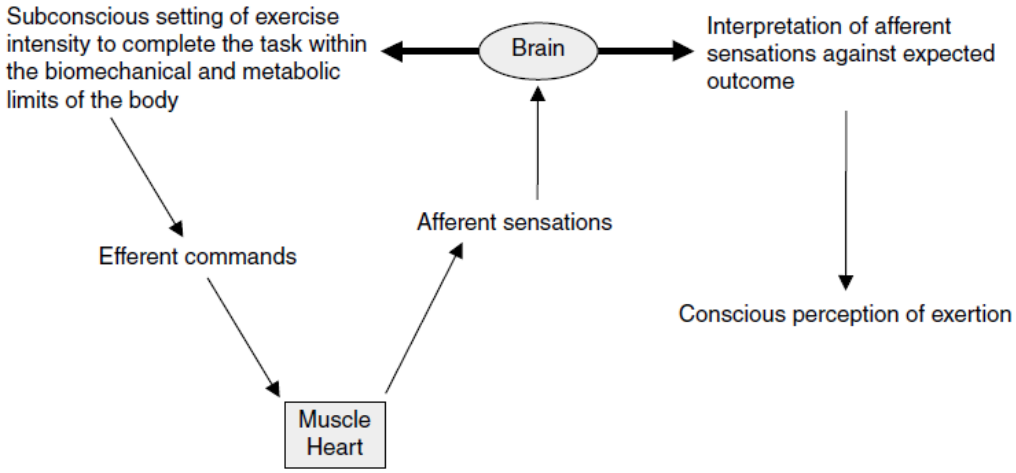


Figure 1.9: Schematic representation of perception of effort based on the feedforward-feedback neurophysiological model (Hampson *et al.*, 2001, p. 944).

At the same time, the feedforward model suggests that efferent commands from the motor cortex that are directed to the peripheral muscles are also transmitted to the somatosensory cortex for interpretation and conscious expression as perceived exertion (Noble & Robertson, 1996; Ulmer, 1996). Thus the input received from the sensory receptors is compared with the motor commands. That indicates how a movement might influence the input signal and allows the CNS to make adjustments for ongoing activities (McCloskey, 1981; Noble & Robertson, 1996). These internal signals that arise from the motor commands and radiate to the sensory centres have been called corollary discharges (Fig. 1.10) and it is suggested that they are the neural basis for the sensation of muscular force and perception of effort (McCloskey, 1981; Crapse & Somer, 2008). The exact mechanism for that is not clear yet. However, it has been suggested that the neurophysiological pathway of the perception of effort has its origin in efferent activity of motor commands and that the sEMG of the muscles could provide an indirect measure of the magnitude of this efferent motor command (Noble & Robertson, 1996).

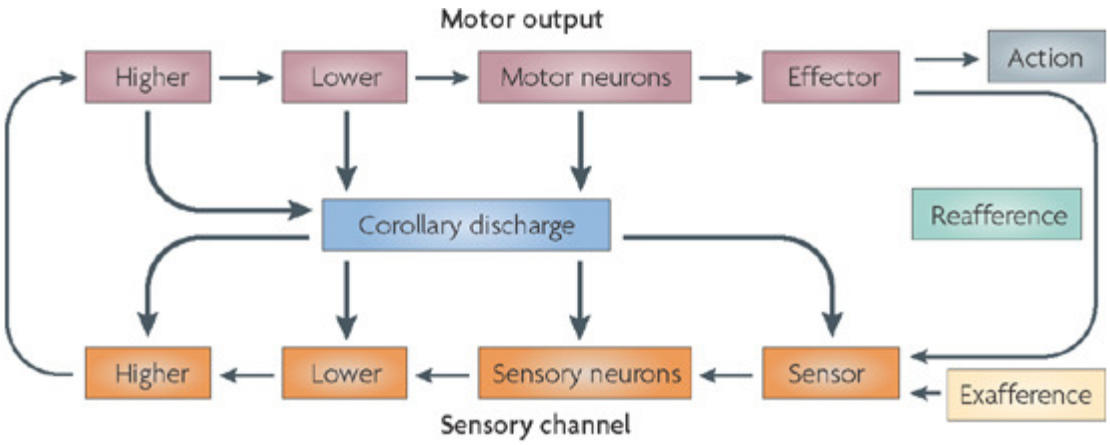


Figure 1.10: A schematic representation of the corollary discharge in the sensorimotor circuit composed of a sensory pathway (shown in orange) and a motor pathway (shown in mauve). Each pathway consists of a number of tiers that represent the complexity of the information processing in the motor control system. Motor-to-sensory signals (shown in thick blue arrows) occur at any number of levels of motor control (Crapse & Somer, 2008, p. 588).

The feedforward and feedback models find support in studies involving force judgments between contracting muscles of contralateral limbs. These studies give a neurophysiological link between exertional perceptions and central motor commands (Noble & Robertson, 1996). The study by Carson and his colleagues (2002), assessing the force accuracy using a contralateral limb-matching task has shown that, following eccentric fatiguing exercise, the level of force applied with the exercise limb working as indicator was lower than that required. The participants appeared to believe that they were generating more force than they actually produced. The peripheral pathways that give rise to force sensations may have been facilitated by the increased efferent activity due to fatigue. However, when the forces are expressed not in relation to pre-exercise forces but in terms of post-exercise peak voluntary forces, the matching errors were smaller (Carson *et al*., 2002). As the EMG recorded from the exercised arm increased in every instance and was always higher than that of the contralateral limb, it has been suggested that the participants relied on their sense of effort for the force matching.

Discrepancies in the force produced by contralateral limbs after fatigue are also reported by other studies as well (Proske *et al*., 2003; Proske *et al*., 2004; Weerakkody *et al*., 2003). In these studies the forces exerted by a muscle group in one limb (the reference limb) are matched in subjective magnitude by contractions of the corresponding muscle

group on the contralateral side (indicator limb). When the exercised arm was the indicator, the forces produced following fatiguing exercise were significantly lower than the reference level. Thus, even though participants believed that they have made an accurate match, they in fact made large matching errors in the direction where the exercised arm developed less force than the unexercised arm. The EMG also increased following the fatiguing exercise, indicating increase in the sense of effort that may have resulted in the matching errors.

1.2.4.1 Perception of Effort and Corollary Discharges

Indirect evidence that the perception of effort is attributable to the corollary discharges from the sensorimotor cortex comes from studies which correlate the perceived exertion with the sEMG activity and the level of force produced by the muscle during muscular contractions (Jackson & Dishman, 2000; Pincivero & Gear, 2000; Rosenbaum & Gregory, 2002; Pincivero *et al*., 2003a). All these studies have shown that the perceived exertion increased with the voluntary activity and was followed by an increase in the sEMG activity. The increase of sEMG parallels the level of produced force and therefore indicates that the central motor commands compensate for the changes in the peripheral system. A parallel increment in the perceived exertion suggests that it is attributable to these motor commands. Indeed, Pincivero's study (2000) showed a good correlation not only between force and sEMG activity but also between perceived exertion and sEMG activity.

The role of these motor commands in the perception of effort has also been objectively assessed experimentally through the use of contralateral-limb force matching tasks as has been already mentioned above (Gandevia & McCloskey, 1978; Cafarelli & Bigland-Ritchie, 1979; Carson *et al.,* 2002). These studies report that, without fatigue, the subjects accurately match the force between the fatigued-reference limbs and the contralateral-indicator limbs. However, as fatigue developed, the force produced by the indicator limb was greater than the force produced by the reference limb (Gandevia & McCloskey, 1978; Cafarelli & Bigland-Ritchie, 1979). Despite the differences in the force between the two limbs, the surface sEMG was the same for both reference and

indicator muscles (Cafarelli & Bigland-Ritchie, 1979). This indicates that for any constant degree of activation in the fatiguing muscle, the force declined but the sensation remained the same. It was therefore suggested that the overestimation in the force production is based on the central motor output commands, and not to peripheral proprioceptive feedback due to muscle tension (Cafarelli & Bigland-Ritchie, 1979). Similarly, when the muscles were partially paralysed with curare the lifted weight felt heavier; indicating that the perception of heaviness of achieved force in an isometric contraction does not rely on the afferent signalling from the muscular force achieved, but on sensing the effort, or the central input to motoneurons (Gandevia & McCloskey, 1977).

The above paradigms in perception of heaviness during isometric contractions indirectly indicate that the magnitude of the descending motor commands is the basis of the perception of effort. However, assumptions about the changes in perception of effort during fatigue based on assessments of the perception of heaviness might be misleading. Force and effort are potentially different sensory attributes that may represent different parts of the sensorimotor function (Carson *et al*., 2002). The issue that force and effort are different attributes of sensorimotor function finds support from the study of Burgess and Jones (1997), which assessed both the perception of force and effort during fatigue. Subjects were asked to judge either the heaviness of a test weight raised with the elbow flexors of the dominant arm, or the effort required to lift the test weight, while they became progressively more fatigued. The results showed that the effort increased more than the perceived heaviness during weight lifting in the presence of fatigue-impaired voluntary function. Thus it has been suggested that the perception of force represents an afferent copy coming from the periphery to aid in judging the heaviness of a lifted object or the force of an undertaken contraction; while the perception of effort is an efferent perception which involves higher centres in the CNS and is suggested to be proportional to the magnitude of voluntary motor command (Gandevia & McCloskey, 1977). In line with this suggestion, Slobounov and his colleagues (2004) found that the perceived effort during isometric force production tasks increased proportionally with the rate of force development but not with the actual amount of force produced. Indeed, the motor-related cortical potentials evoked by electrical stimulation, which reflect the corticospinal excitability, increased proportionally as a function of perceived effort but not as a function of force produced. Moreover, the motor-related cortical potentials at

frontal-central electrode sites, which represent pre-motor and primary motor cortical activity and are associated with planning and execution of movement (Siemionow *et al*., 2001, cited in Slobounov *et al*., 2004) were proportional to the perceived effort associated with achieving the required force level (Slobounov *et al*., 2004). The above robustly indicates that the perception of effort is associated with the frontal-central cortical areas of the brain and that it is probably related to dynamic aspects of motor activity more crucially than was believed until now.

However, the question that arises from these studies is what is the involvement of the peripheral feedback in regulation of the perception of effort? Studies that assess the perceived exertion at different levels of force production suggest that the CNS may not change its motor commands relative to the afferent feedback from the muscles (Pincivero *et al*., 2003a; West *et al*., 2005). Thus, the ability to match absolute force with target contraction intensities was more accurate for force at the level of 50% of MVC; while it was quite poor both at low and at high force levels (West *et al*., 2005). The tendency to overproduce low levels of forces and to underproduce higher level forces is reported elsewhere (Jackson & Dishman, 2000; Pincivero *et al*., 2003a). Indeed, in the studies of Pincivero and his colleagues (2003a, b) the torque exerted by the knee extensor muscles was significantly lower than the equivalent percentage values of perceived exertion, especially at the higher levels of force production. Thus, peak torque was less than 70%, 80%, and 90% of MVC at perceived exertion levels of 7, 8 and 9 on the Borg CR10 scale, respectively. Additionally, the sEMG was significantly less than the equivalent ratings of perceived exertion (Pincivero *et al*., 2003a, b).

The reason for this under- and over-production at the two limits of force production is not quite clear. One explanation for the overproduction of the low forces could be that the above studies tested lower limb muscles (e.g. knee extensors), which are commonly involved in gross movements related to power, and thus they show less consistency at low levels of contraction (West *et al*., 2005). The underproduction of the higher forces might be a protective mechanism. In an integrative motor system, there is evidence that the CNS fails to generate maximal force in maximal voluntary efforts and that all motor units in the exercising muscle may never be maximally recruited, even at the onset of a maximal isometric contraction (Gandevia, 2001).

More obvious examples of submaximal voluntary activation in maximal efforts come from studies showing that voluntary muscle strength increases with training (Enoka, 1997) and that during bouts of repeated voluntary maximal contractions the force output is higher when there is strong verbal encouragement compared with when there is no verbal encouragement throughout the exercise (St Clair Gibson *et al*., 2001a). Thus, it is suggested that a degree of muscle capacity reserve exists during maximal voluntary efforts that could theoretically be used in exceptional circumstances (Gandevia, 2001; St Clair Gibson *et al*., 2001a). This reserve capacity varies between muscles but has been reported to range between 10 and 15% of maximum. This reservation of about 10–15% capacity may exist to protect the muscle from metabolic or mechanical damage during maximum contractions and may be the reason for overestimation of the higher produced forces (West *et al*., 2005). The above findings further indicate that although feedback from the muscles exists to inform the brain of changes in the periphery, other sources of input may also exist and may be involved in regulation of task performance at ongoing activities (Taylor *et al*., 2006). Input from centres upstream from the motor cortex has also been suggested as a potential contributor to central fatigue (see section 1.2.3.5).

Indirect evidence that the sense of effort is associated with activity in neural centres upstream of the motor cortex comes from the study of Gelli and his colleagues (2005), which tested the possibility that changes into the input-output properties of the corticospinal pathway were associated with changes in the effort-force relationship. Specifically, the study assessed whether the force judgment is affected by changes in the force-generated capacity of abductor digiti minimi (ADM) muscle induced by changing shoulder position. The input-output changes of the corticospinal pathway were tested using TMS while the force estimation was based on a force matching task between the two arms without visual feedback. The participants had to perform a reference contraction with visual feedback and a test contraction without visual feedback, either with the two arms in the same position (the same input-output) or with the arms in opposite positions: the one anterior, 30° horizontal adduction, and the other posterior, 30° horizontal abduction (different input-output). When the reference and the test contractions were performed in the same position (anterior or posterior) the respective force levels were closely matched. In contrast, when the reference and test contractions were performed in opposite positions, the force applied was substantially different from that required. The above study was the first to connect the sense of effort directly with

the force output on the basis of the mechanisms taking place in the corticospinal pathway.

These results help us to pose some interesting suggestions. If the force estimation was based only upon the afferent input from the muscle, the reference and the test contractions should match in the two positions, as the target muscle was in the same biomechanical state in the two arm positions. On the other hand, if the force matching ability was based only upon the effort signals, then the produced force would be less in the position in which the muscle was weaker (posterior for the present experiment). The results however, showed that the test force levels were higher in posterior position than the reference forces (Gelli *et al*., 2005). This discrepancy could, therefore, be explained only if the participants estimated the levels of force based on the effort-force relationship as it was proposed by other studies as well (Carson *et al*., 2002). Because the changes in the corticospinal excitability, as recorded by the MEP, of the ADM muscle were observed to be significantly correlated with the degree of force mismatching, it is possible that the position-induced changes in the input-output relationship of the corticospinal pathway to ADM modified the relationship between the input command that forms the basis of the sense of effort and the motor output from which the sense of tension originates (Gelli *et al*., 2005).

These results, however, give rise to a new assumption. Probably the sense of effort is not simply a corollary of the central motor command (Carson *et al*., 2002). It seems that the relation between sense of effort and motor command is what it is altered following fatigue (Carson *et al*., 2002). The afferent signals may not impose directly upon the central mechanisms mediating the sense of effort but regulate the relationship between the neural commands that form the sense of effort and the output of the motor cortex (Carson *et al*., 2002). This could then lead to the possibility that the sense of effort is associated with activity in neural centres upstream of the motor cortex.

The aforementioned findings triggered the interest to explore further the role of the perception of voluntary effort in the motor control system. Whether perception of effort is changed primarily due to peripheral or central changes is not yet known. Indeed, it is still unknown whether perception of effort could be changed as result of alteration of the motor cortex excitability. Manipulation of perceived effort by artificially altering the

motor cortex excitability in the absence of changes in the peripheral feedback may help in understanding how perception of effort is mediated and how it is involved in motor performance with or without fatigue. Research in that direction may further help in finding ways of mediating perception of effort in conditions where perception of effort is disproportionally elevated, such as in CFS/ME and MS.

1.2.4.2 Perception of Effort during Exercise and Fatigue

Perception of effort is often used to assess fatigue during whole body exercise (e.g., treadmill running or cycling) where a subjective feeling of tiredness and exhaustion is usually connected with increased perceived exertion (Hampson *et al*., 2001; Presland *et al*., 2005). Presland's study (2005) showed that during prolonged cycling at 70% of peak oxygen consumption until exhaustion, the rating of perceived exertion measured on the popular Borg scale (Borg, 1998), increased progressively through the exercise and reached its highest level at exhaustion. The time to exhaustion was inversely related to the ratings of perceived exertion. Subjects who had the lowest rating at 15 minutes of exercise exercised the longest, while those who had the highest rating early in exercise became exhausted the most rapidly (Presland *et al*., 2005). Other studies also report an increase in perceived exertion as fatigue develops while the rating of perceived exertion increased with time and reached its highest rate at exhaustion (Hampson *et al*., 2001; Nybo & Nielsen, 2001; St Clair Gibson & Noakes, 2004; Noakes *et al*., 2005; Faulkner *et al*., 2008). Thus, perceived exertion has played a crucial role in understanding how the subjective sensation of strain alters with physical activity. In short duration exercise, local sensation from working muscles appears to be the primary stimulus for perceived exertion. As the level of work intensity increases, the strain from the cardiopulmonary system complements peripheral input from the neuromuscular mechanisms and the perceived exertion changes in relation to the heart rate and oxygen consumption (Borg, 1998).

1.2.4.3 Rating of Perception of Effort

Thus far, the method used most frequently to quantify perceived exertion has been the 15-point Rating of Perceived Exertion (RPE), or the modified Borg's category-ratio 10-item scale (Borg CR10 Scale) (Borg, 1998). The RPE scale ranges from 6 to 20, and has been reported to be highly correlated to a heart rate (HR) range of 60 to 200 beats per minute, while the Borg CR10 scale ranges from 0 to 10 (maximum) with an open end anchor for the absolute maximum (above 11 or even higher) (Borg, 1998). Although this is a subjective measure, a high correlation exists between a person's perceived exertion rating times 10 and the actual heart rate during physical activity; so a person's exertion rating may provide a fairly good estimate of the actual heart rate of healthy individuals during activity (Borg, 1998). For example, if a person's rating of perceived exertion (RPE) is 12, then 12 x 10 = 120; so the heart rate should be approximately 120 beats per minute. Based on this estimate, rating of perceived exertion is commonly used to assess exercise tolerance and to prescribe and regulate therapeutic training intensity (Noble & Robertson, 1996).

However, these scales are effectively limited to whole body exercise when a certain level of heart rate is achieved. As such they may be misleading in their recordings if they were applied following fatigue in the recovery period. Additionally, what is an easy estimate of perceived exertion during whole body physical activities, which result in increased heart and respiratory rate, might be quite uncertain when isolated muscles are exercised, as during fatiguing isometric contractions. Fatiguing tasks performed by isolated joints serve the advantage of not straining the cardiopulmonary system to the same extent as whole body exercise and therefore they are more suitable for research purposes. Assessing perception of effort during isolated joint exercise is also more feasible in clinical settings, when neurological patients may be unwilling or unable to undertake a strenuous exhaustive whole body exercise. Additionally, a whole body fatiguing exercise is very tough, even for athletes, who may not be willing to undertake this kind of testing. Therefore, the development, of a scale for effort assessment that can be used continuously during both fatigue and the recovery period in isometric fatiguing tasks is of great importance. Research is necessary in order to investigate further the way perception of effort alters during and following fatigue.

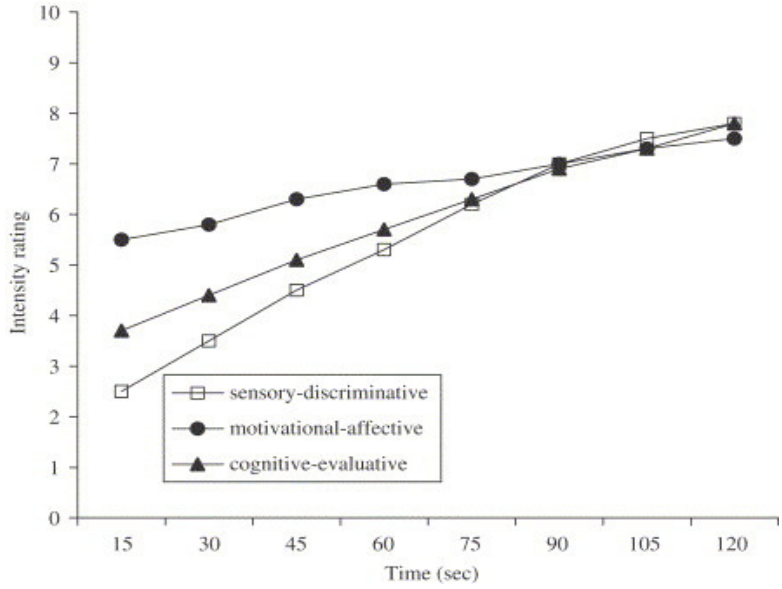
Moreover, the term exertion, although it has been broadly used in perception of effort research, has been criticized for its inappropriateness in describing an integral phenomenon like effort. Indeed, effort and exertion have been described as semantically different (Noble & Robertson, 1996). Perception of effort reflects the effort exerted by the person to complete a task, while perception of exertion mainly reflects the perception of strain or discomfort caused by the task (Noble & Robertson, 1996). The difference between the two is not always clear, however. Probably this is the reason that, during exercise and fatigue, these two terms are used interchangeably.

Hutchinson and Tenenbaum (2006) tried to distinguish between the two. They examined perception of effort as a gestalt. Within the gestalt conceptualization of perceived effort, the physical exertion, the motivation and the emotions were considered as physiological and psychological variants that contribute to the experience of effort. It was supported that participants might be able to distinguish between differentiated perceptual signals according to their specific physiological mediators. Thus, Hutchinson and Tenenbaum (2006) suggested that there are in fact three dimensions to perception of effort. The sensory dimension refers to the physical discomfort experienced during an exhaustive task and it is usually defined by muscle aches, pain and fatigue. These sensations are physical in nature. The motivational dimension is associated with the motivation and emotion during the discomfort stage. This dimension is defined by mental toughness, determination and concentration, referred to as the most silent first-order dimensions (Tenenbaum, 1999, cited in Hutchinson & Tenenbaum 2006). The third dimension is the cognitive, represented by the subjective interpretation of perceived physiological sensations. This dimension is defined through perceptual sensations of perceived effort and exertion and task aversion. Effort and exertion were distinguished according to Noble & Roberson's (1996) definitions, as they have already mentioned earlier. Task aversion reflected the motivation to avoid harm.

In their study (Hutchinson & Tenenbaum 2006), the perceptual sensations of the three suggested dimensions of perceived effort were examined independently during two exhaustive tasks; a sustained submaximal (25% of MVC) handgrip squeezing task, and a stationary cycling task of submaximal gradually increasing intensity (starting for 5 minutes at 50% of MVC, for further 5 minutes at 70% of MVC and then to volitional fatigue at 90% of MVC). The perceptual effort sensations were measured by verbal

report on a 0–10 rating scale with verbal anchors at 0 (*nothing*), 2 (*weak*), 5 (*moderate*), 8 (*strong*), and 10 (*extremely strong*). It was found that during both isometric and dynamic tasks perceptual sensations were perceived distinctly, and altered differently, with time and effort accumulation. Sensory and cognitive sensations followed the same pattern of changes. They were initially perceived to be slight but increased gradually with time. However, the cognitive sensations were rated higher than the sensory perceptions during both tasks. Motivational sensations remained relatively stable during the tasks (Fig. 1.11). These findings are not conclusive. However, they indicate that the perceived effort comprises various distinct inputs that are perceived differently during a task, and within different tasks, and that exertion is only one of many perceptual sensations that are felt during exercise (Hutchinson & Tenenbaum, 2006). As such, perception of effort may be very simply assessed in terms of perceived exertion, while effort and exertion could be dissociated under careful instructions to the participants. Additionally, the perception of effort should not only be assessed relative to the peripheral sensations of strain and heaviness, but also under continuous monitoring of the emotion state of the participants.

i)



ii)

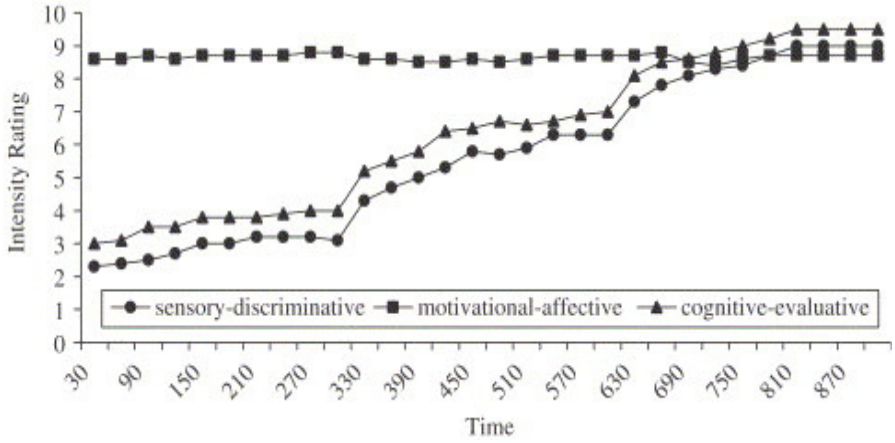


Figure 1.11: Mean ratings for each perceptual sensation within i) 120 s duration of the handgrip task ii) 900 s cycle task (30 s intervals) (Hutchinson & Tenenbaum 2006, p. 471 & 473).

1.2.4.3.1 Rating scales for effort assessment

Various scales have been proposed in the literature as valid and reliable methods of assessing clinical and subjective phenomena like pain and perception of exertion. These include: Visual Analogue Scale (VAS) (Crichton, 2001), Numeric Rating Scale (NRS) (Williamson & Hoggart, 2005), Borg scales (Gearhart *et al*., 2001; Dawes *et al*., 2005), Likert scales (Grant *et al*., 1999), Verbal Rating Scales (VRS) (Lund *et al*., 2005). VAS uses a horizontal line, 100 mm in length, anchored by word descriptors at each end ("no perception of feeling" and "maximum perceived feeling"). The subject indicates the

current state of their perception by placing a mark along the line. The VAS score is determined by measuring in millimetres from the left hand end of the line to the point that the subject marks (Crichton, 2001). The Likert scale is simply a statement about the level of agreement or disagreement which the subject is asked to evaluate according to any kind of subjective feeling (Grant *et al*., 1999). The Borg scales, as presented above, have various numeric points and are usually accompanied by verbal descriptors. The points are presented in an incremental order to indicate increase in the perceived feeling (Borg, 1998). The NRS is a scale of 11, 21 or 101 points where the end points are the extremes of the subjective or perceived feeling where one end denotes no feeling, and the other, the highest or worst, as appropriate (Williamson & Hoggart, 2005).

Comparative research has shown that VAS and NRS are much more sensitive to changes of the subjective feeling than are the VRS, for example (Williamson & Hoggart, 2005). VAS and Borg scales also appear to be better subjective scales than Likert scales during submaximal exercise (Grant *et al*., 1999). Both 15- and 10-point category-ratio Borg scales have been used extensively to assess perceived exertion during graded or progressive (aerobic) exercise. Borg scales have been validated and assessed on overall body perception when aerobic or dynamic exercise employs large muscle mass and the perceptual ratings were validated in relation to heart rate, blood pressure and lactate concentration (Suminski *et al*., 1997; Capodaglio, 2001). Therefore, the meaningful significant change in the Borg scale, following dynamic aerobic exercise where the whole body is exercised, could not be applied to changes when an isolated muscle group is contracted isometrically. Furthermore, although the Borg scale has been tested for its linearity when local exertion was monitored in active-only muscles undertaking dynamic, resistance exercise (Gearhart *et al*., 2001), no evidence exists about its reliability and validity for isolated muscle work. Additionally, the inter-individual variation in rating the verbal anchors of the 11-point category Borg scale (Dawes *et al*., 2005) suggests that participants might rate the perceived exertion differently when they are using the verbal anchors than when they are using the numeric value that corresponds to that anchor. Conceptual differences between exertion and effort mentioned earlier may also make the Borg exertion scale inappropriate for assessing effort.

VAS has been tested for its reliability and validity in recording the perceived effort during isolated hand muscle work and has also been tested with very positive results (Koppelaar & Wells, 2005). However, several limitations have been reported for this scale, such as; the subjective feeling can only be assessed in written form, and the tool itself may be rendered invalid when photocopying the scale, as this can lead to significant changes in its length (Williamson & Hoggart, 2005).

On the other hand, the NRS has been extensively used in pain assessment and it is considered valid, reliable and appropriate for use in clinical and research practice (Williamson & Hoggart, 2005). It has been recommended by the US National Institute of Health as a reliable indicator of the existence of pain (NIH Pain Consortium) and shares the advantages, when compared with the VAS, that: it is easy in administration and scoring, there are no age-related difficulties in using the scale, and it can be delivered either graphically or verbally (Williamson & Hoggart, 2005). When presented graphically the numbers are posed at equal intervals on a horizontal line indicating the interval level of the scale. Compared with the VAS, which obtains continuous ratio data, the NRS obtains discrete scores from 0 to 10 that may hinder subjects from getting continuous ratings and it may not permit the user to perform calculations such as percentage change in subjective feeling (Hartrick *et al*., 2003). It is also debated whether the NRS should be treated as a ratio scale (Hartrick *et al*., 2003). Indeed, it has been suggested that, in clinical research, the NRS should not be considered interchangeable with the VAS, and that a score of 40 mm on the VAS cannot be translated into a score of 4/10 in the NRS (Hartrick *et al*., 2003; Williamson & Hoggart, 2005). However, the NRS was sensitive enough in its ability to detect statistically significant differences between treatments, before and after treatment, when it was used for pain assessment (Breivik *et al*., 2000; Kendrick & Strout, 2005), and in that context, it does provide similar information to the VAS about subjective feelings. Indeed, the two scales showed similar sensitivity when tested in rating pain following extraction of impacted wisdom teeth (Breivik *et al*., 2000). Additionally, the NRS is an interval level scale and can provide data for parametric analysis (Williamson & Hoggart, 2005).

The reliability and validity of the 0–10 NRS in assessing pain (Kendrick & Strout, 2005; Williamson & Hoggart, 2005), as well as its ease of application, drives the interest in using the scale for effort assessment. However, although reliable and

sensitive in assessing pain, the 0–10 NRS has not been tested specifically for its reliability and validity in assessing perception of voluntary effort during isometric contractions. It is, therefore, necessary to review this scale to examine its reliability and validity in rating effort during and following isometric muscle exercise. The second study of this thesis is directed towards validating the 0–10 NRS as a suitable scale for rating effort for isolated exercise in healthy individuals which could be applied later, after validation, in clinical settings.

1.2.4.4 Perception of Effort and Central Fatigue

A relationship between perception of effort and changes in the motor cortex output has also been reported during fatigue, although this relationship is not well understood (Presland *et al*., 2005; Søgaard *et al*., 2006; Smith *et al*., 2007). During prolonged cycling at 70% of peak oxygen consumption until exhaustion, the increased perceived exertion was accompanied by reduction in the MVC of the quadriceps and development of central fatigue, as it was measured by use of tetanic (square wave stimulation pulses up to 400 V, 100 mA, and 0.5–1.0 ms) electrical stimulation for Twitch Interpolation Technique (Presland *et al*., 2005). This study also showed that the central component of fatigue persisted for 30 minutes post exercise and contributed to slow recovery of MVC, but the study did not monitor possible subsequent changes in the rating of exertion during the recovery phase. Thus, although the perceived exertion is presented as a main contributor for the termination of the exercise, it is not clear whether perceived exertion is directly related to the development and progress of central fatigue.

Additionally, during 45 minutes of a sustained, low-intensity (15% of MVC) isometric fatiguing task, the perceived effort increased by about 6 degrees in the Borg's rating scale (Søgaard *et al*., 2006). The increased effort was accompanied by a steady increase in voluntary sEMG of BB and brachioradialis (BR) muscle at 15% of MVC. The increase of the perceived effort was proportionally higher than the increase in sEMG. At the same time, the MVC torque decreased with fatigue and the twitch evoked by TMS over the motor cortex and motor point stimulation both increased, indicating development of central and supraspinal fatigue parallel to peripheral fatigue.

Additionally, changes in the excitability of the motor cortex have been shown (Søgaard *et al*., 2006). The MEP area increased and the silent period lengthened, which is consistent with the increased sEMG indicating an increased descending drive to maintain the target force. The increased perceived effort may represent this increased motor command as it is observed by the increased sEMG. However, following fatigue, the changes in MEP size and silent period recovered more quickly than the voluntary activation, indicating that these changes are not directly related to central fatigue. The sEMG at submaximal contractions and the superimposed twitches decreased after the end of the fatiguing task and returned to control values in about 8 minutes (Søgaard *et al*., 2006). This reduction indicates that the descending drive was reduced as the muscle was recovering from fatigue. However, the perception of effort was not assessed during the recovery period, so no conclusions could be made whether it follows the same trend of change with the motor commands.

Similarly, during a low (5% MVC) submaximal contraction sustained for 70 minutes, perceived effort also increased in parallel with peripheral and central fatigue (Smith *et al*., 2007). The increased perceived effort was accompanied by an increase in the sEMG. However, similarly to the study of Søgaard and colleagues (2006), the perception of effort was disproportionally higher than the sEMG. Thus, when the subject was fatigued, the mean rating of perceived effort increased to about 40% compared with the prefatiguing baseline testing; while the increase in the sEMG was 11%. This disproportionate increase in effort compared with the level of sEMG may be related to central fatigue (Smith *et al*., 2007). It may be caused by changes in the properties of the motor neurons due to repetitive contractions or by changes in their afferent input (Smith *et al*., 2007). However, it could also be related to changes in the input to the motor cortex and not to its output. It may indicate that the perceived effort is not attributable to actual motor commands but is related to activity in areas upstream of the motor cortex (Smith *et al*., 2007).

The findings from the studies described above, although not conclusive, do point towards a close relationship between the perception of effort and the development of peripheral as well as central fatigue. The use of the Borg scale, which is mainly applied to whole body exercise and is closely related to changes in the heart rate, may give misleading results when it is applied to exercise that fatigues isolated muscles.

Additionally, the lack of monitoring of the ratings of perceived effort during recovery from fatigue does not allow for conclusions about the extent that perception of effort is related to central fatigue. However, studies that assess perception of effort in neurological patients who complain of fatigue indicate that perception of effort may be significantly related to the development of fatigue in these patients (Sacco *et al*., 1999; Ng *et al*., 2004; Wallman & Sacco, 2007).

1.2.4.4.1 Transcranial Direct Current Stimulation

Various noninvasive transcranial stimulation techniques have raised considerable interest due to their potential therapeutic application by altering motor cortex excitability. These techniques include the use of repetitive TMS (rTMS) and Transcranial Direct Current Stimulation (tDCS) to modulate excitability in the cerebral cortex (Fitzgerald *et al*., 2007; Nitsche *et al*., 2008). Repetitive TMS has been shown to increase or decrease the excitability of corticocortical pathways depending on the intensity or frequency of stimulation, while producing effects that outlast the period of stimulation (Fitzgerald *et al*., 2007). The mechanism of these effects is not clear, however, it is widely believed to reflect changes in synaptic efficacy compatible with the changes involved with the long-term potentiation (LTP) and long-term depression (LTD) processes (Classen & Stefan, 2008).

Compared with rTMS, which may cause discomfort, pain or even seizures especially in patients on medication and patients with epilepsy (Wassermann, 1998), tDCS is considered safe for both healthy people and patients (Nitsche *et al*., 2003b). Direct current brain polarization is not “stimulation” in the same sense as transcranial magnetic stimulation or the stimulation of the brain and nerves with conventional electrical techniques. It does not appear to cause nerve cell firing on its own and does not produce discrete effects such as the muscle twitches associated with classical stimulation (Nitsche *et al*., 2008). Hence, tDCS could be considered as a neuro-modulatory intervention which modulates the neuronal excitability by a de- or hyperpolarization of the resting membrane potential (Nitsche & Paulus, 2000). Indeed, anodal polarization of the motor cortex increases the motor evoked potentials evoked by single transcranial

magnetic stimulation of the same area, while a reduction of these responses is observed with cathodal polarization (Nitsche & Paulus, 2000). Moreover, it is reported that these effects last for about 10–20 minutes after exposure. The same results have been confirmed by later studies as well (Liebetanz *et al*., 2002; Nitsche *et al*., 2003c; Furubayashi *et al*., 2008). The underlying mechanisms of these observed changes are not yet known. It is believed that the initial, short-lasting effects of tDCS may be explained by membrane changes in cortical neurones (Nitsche & Paulus, 2000; Liebetanz *et al*., 2002; Nitsche *et al*., 2008). On the other hand, two possible mechanisms have been proposed for the longer-lasting effects, but are still under continued investigation. One mechanism may include changes in neural membrane function such as alterations in trans-membrane proteins and changes in intracellular pH (Ardolino *et al*., 2005). The other may be related to changes in the strength of synaptic transmission, probably because of changes in the efficacy of the NMDA receptors (Liebetanz *et al*., 2002; Nitsche *et al*., 2003a).

Despite the debate about the exact mechanisms of the after-effects of the tDCS, this noninvasive stimulation is now undergoing a renewed interest because of its potential for modulating neuroplasticity and hence for clinical neuroplasticity research, as well as use in the potential treatment of neurological and psychiatric disorders (Wassermann, 2008). Indeed, it has been reported that tDCS could modulate neuromuscular fatigue (Cogiamanian *et al*., 2007). In Cogiamanian's study, a submaximal (35% of MVC) fatiguing isometric contraction was undertaken before and one hour after 10 minutes of tDCS over the motor cortex or no stimulation (control group). The endurance time decreased at the post-tDCS evaluation. However, following anodal tDCS, the endurance time was decreased significantly less than following cathodal or no stimulation. This reduction in the expected shortening of the endurance time owing to fatigue, observed post anodal tDCS, implies that anodal tDCS could improve endurance time and therefore modulate muscle performance by decreasing muscle fatigue. Parallel with the changes in the endurance time, the MEPs evoked by TMS increased by nearly 30% at the end of the anodal stimulation, indicating that the prolongation of the endurance time might be due to an increase in excitability of the motor cortex. However, because there were no significant effects of tDCS on sEMG, the prolongation of the endurance time following anodal tDCS might also have arisen from changes in premotor areas, or be due to reduced pain sensation arising from the muscles (Cogiamanian *et al*., 2007).

Indeed, the effects of tDCS have been reported not only for the motor cortex but also for the somatosensory cortex (Matsunaga *et al*., 2004). Cognitive effects in terms of learning and memory, by application of anodal tDCS of the prefrontal cortex, and effects on mood, by application of tDCS over the orbits, have also been observed (Wassermann, 2008).

The reversible modifications of perceptual, cognitive, motor and behavioural functions as a result of tDCS application merits further research of the possible effects of tDCS on perception of effort. The tDCS-induced modulation of the human sensorimotor cortical excitability at low current intensities, as well as its painless and safe application, make tDCS a potentially valuable tool for studying the sensorimotor system. Perceptual changes of effort could then be assessed further as a result of tDCS-induced changes in the corticospinal excitability.

1.2.4.5 Perception of Effort in Neurological Conditions

Findings in patients with neurological diseases also support the assumption that the perception of voluntary effort may be related to central fatigue. Patients with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) experience continuous or relapsing fatigue for more than six months which is unrelated to tasks performed and is not alleviated with rest (Fukuda *et al*., 1994). Studies have shown that these patients experience higher levels of effort in response to fatigue than healthy controls for both low and high levels of force (Sacco *et al*., 1999; Wallman & Sacco, 2007). Interestingly enough, the capacity for producing maximum voluntary contractions did not differ between these patients and healthy people (Wallman & Sacco, 2007), although the time that patients could sustain a submaximal (20% MVC) elbow flexor contraction until exhaustion was significantly shorter than the endurance time in healthy controls (Sacco *et al*., 1999). Additionally, the surface sEMG activity during the fatiguing task and the recovery did not differ between healthy people and patients (Wallman & Sacco, 2007). Thus, deconditioning of the muscular system could not be the reason for the higher ratings of effort in these patients. However, the patients revealed greater TMS evoked twitches at the second half of the endurance fatiguing task than healthy controls, which

may be related to the increased rating of effort they reported (Sacco *et al*., 1999). The studies of Sacco (1999) and Wallman (2007) did not include assessment of voluntary activation so it is not clear if the increased perceived effort is accompanied by the development of central fatigue. However, an impaired central activation in patients with CFS/ME has been reported elsewhere (Schillings *et al*., 2004). Indeed, these patients have shown less peripheral fatigue but greater central activation failure than healthy controls (Schillings *et al*., 2004).

Similar results are shown in patients with multiple sclerosis (MS) (Thickbroom *et al*., 2006). When patients were compared with healthy controls, they experienced higher levels of rating effort in the Borg scale during 20 minutes of fatiguing intermittent isometric contractions of first dorsal interosseous (FDI) at 40% of MVC. This higher level of perceived effort in patients was accompanied with a larger increase in both MEP and duration of the silent period (Thickbroom *et al*., 2006). This increased central drive as fatigue develops may again be related to the increased perceived effort in patients with MS. An electroencephalographic (EEG) study conducted in healthy participants has shown that the amplitude of the initial part of the motor-related cortical potential increased as a function of anticipated effort, indicating that the motor cortical drive may be considered as a relevant measure of the perception of effort associated with force production (Slobounov *et al*., 2004). However, the study of Thickbroom and his colleagues (2006) did not present data following fatigue during the recovery period. The changes in the central drive during the fatiguing task may be not directly related to central fatigue and thus the increased perception of effort may just reflect task related alterations. Other studies have shown that fatigue in MS is associated with abnormal activation of the motor cortex (Leocani *et al*., 2001; Ng *et al*., 2004) and with impaired interactions between functionally related cortical and subcortical areas, as revealed by fMRI (Colombo *et al*., 2000, Filippi *et al*., 2002). This does not exclude the possibility that perception of effort is linked with this abnormal cortical activity during fatigue. Assessment of perception of effort in these studies might have given better insight to the causes of this abnormal activity. Further research using these methodologies could be essential for elaborating changes in perception of effort in recovery from fatiguing exercise.

It is therefore important to assess how the perception of effort accompanies the changes in the voluntary activation following fatigue. If perception of effort increases with the central activation failure following fatigue, then the subjective rating of perceived effort and subsequent changes in this rating may reflect an underlying process involved in central fatigue. Until now, the presence of fatigue in these patients is of unexplained origin (Fukuda *et al*., 1994) and the pathophysiological features are hindered by the debate between psychiatric approaches (mental, non-physical symptoms) and physical approaches (illness) (Lawrie *et al*., 1997). The perception of effort seems to be a main contributor to the development of fatigue in CFS/ME patients (Lawrie *et al*., 1997). It has been suggested that a reduced effort tolerance results in the decreased motor performance (Van Houdenhove *et al*., 2007). In addition, the complaint of fatigue in patients with MS is not well understood. It is suggested that it also originates centrally, and may be related to depression and impaired connectivity between motor and somatosensory cortex (Romani *et al*., 2004). Additionally, central fatigue is also a significant part of the changes following fatiguing motor tasks (Schubert *et al*., 1998; Petajan & White, 2000). More extensive research is needed to assess the relationship between perception of effort and the feeling of fatigue in these neurological patients. Based on the "central governor" theory (Noakes, 2007), the increased perception of effort may be a centrally mediated construct, caused by the imbalance between afferent and efferent signals (Van Houdenhove *et al*., 2007) resulting partially in the presence of symptoms in these patients.

1.2.5 Aim and Objectives.

The literature review has revealed that muscle fatigue is a complex and multi-factorial phenomenon, and that the failure to maintain the initial maximal force during exercise depends not only on peripheral factors intrinsic to the muscle, but also on factors higher in the CNS that cause what it is called central fatigue. However, the changes that occur in the motor cortex during fatigue cannot fully explain the reduction in voluntary activity of the muscles. Indeed, it has been found that when the neural pathways from the motor cortex to the muscles recover, following the fatiguing exercise, the output from the motor cortex is still suboptimal to that required to maximally activate the muscles. Thus, it has been suggested that changes upstream of the motor cortex, possibly in the input to the motor cortex from other cortical or subcortical areas, may contribute to central fatigue. A concurrent increase in the subjective rating of perceived exertion during whole body exercise suggested that other supraspinal psychological factors, such as perception of effort, may have an important role in central motivation, and may influence the central components of fatigue.

However, the exact relation between the subjective feeling of effort and the progress of fatigue has not been adequately explored. Central changes in motor cortex may be the primary regulator of perceived effort during fatigue, but research findings are not conclusive in that area. Additionally, the extent to which peripheral feedback contributes to the changes in the perception of effort following fatigue is not well established. This is mainly due to the methodologies used for assessing perception of exertion, which are limited to whole body exercise, where exertion is triggered by changes in the peripheral motor and cardiopulmonary systems, and therefore are used during the fatiguing task but not before or after. Therefore, there is a need to develop a method which would be valid and reliable in rating the perceived effort during isolated joint exercise, and as such, could be used repeatedly before and following a fatiguing task. An additional method for distinguishing between central and peripheral fatigue that is less painful and more feasible for clinical application than the Twitch Interpolation Technique needs to be established. Peripheral magnetic stimulation has been proposed as a feasible alternative to the Twitch Interpolation Technique using electrical stimulation, but a detailed comparison between the two methods has not been conducted. The use of appropriate and valid methodologies could help in identifying

how the central and peripheral components of fatigue are related to the increased feeling of effort, which seems to be one of the main characteristics that is reported in chronic fatigue neurologic conditions. The way perceived effort is changed following fatigue may further help in understanding how perception of effort is regulated.

The literature review has also revealed that the neurophysiological pathway of the perception of effort has its origin in efferent activity of the motor commands that arise in the motor cortex and radiate to the sensory centres, but the exact mechanism for the formation and regulation of the perceived effort is not yet clear. Peripheral feedback from the muscles has been suggested to be a primary contributor to the changes in the motor commands and subsequently to the perception of effort. However, the way perception of effort changes, relative to the motor commands, and to what extent afferent feedback interferes with that process, has never been assessed directly. Perhaps a more direct way to evaluate this is by changing the efferent drive to descending neural pathways artificially by altering the corticospinal excitability, using transcranial stimulation techniques now currently being investigated, where a condition can be created in which there is no change in the peripheral motor control system.

The use of such methods might give further insight into the role of perceived effort as a central mediator in regulation of exercise and to the exact relationship between perception of effort and fatigue in both health and disease. The implementation of reliable and valid tools in clinical practice may enable clearer diagnosis and clarification of the pathogenesis of many neurological conditions that have chronic fatigue as a key feature. Comprehension of the origin of fatigue might then lead to the design of efficacious rehabilitation protocols for patients with low quality of life due to chronic fatigue. This is crucial not only for the care of those people, but also for the cost effectiveness of the health care system.

Based on findings in the literature, the main aim of this thesis, therefore, is to evaluate existing methodologies for their appropriateness in assessing perception of effort and voluntary activation following isolated joint function testing, and to examine the relationship between subjective perception of effort and objective changes in the motor control system following fatigue.

Furthermore, the objectives of the thesis are:

1) To assess whether magnetic stimulation could be used as an alternative to standard electrical stimulation in the Twitch Interpolation Technique for evaluating the voluntary activation of BB muscle.
2) To adopt a rating scale and to test its reliability and validity for assessing perception of effort during isometric elbow flexion exercise.
3) To assess the changes in the rating of perception of effort following a submaximal isometric fatiguing exercise of elbow flexors and to see how these changes are associated with neurophysiological changes accompanying fatigue.
4) To test whether localized alteration of motor cortical excitability affects perception of effort.

Each of the four objectives is addressed separately in chapters 3 to 6, respectively. The general methodology of the studies is presented in chapter 2. Specific research hypotheses, materials and protocols for each study are presented in the relevant chapters.

CHAPTER 2

GENERAL METHODS

2.1 Introduction

In order to address the objectives of the thesis, four studies have been designed. Every study addresses one of the research objectives as they are presented in the introductory chapter. The general methods applied to all studies of the thesis are presented in the following chapter. Methods that are specific to individual studies are presented in the relevant chapters.

2.2 Recruitment and Ethics Consideration

In total, 69 healthy volunteers (23 males, 46 females) were recruited for the studies of the present research (mean age: 33.5 ± 8.9(SD) years). They were recruited from staff and students at Brunel University by an e-mail advertisement. Participants who expressed an interest in the research received an information sheet, previously approved by the University Ethics Committee. Details of the experiment were provided in the information sheet along with the statements that participation was completely voluntary as well as indicating the right to free withdrawal at any time (see the information sheet for every study in Appendices: A, B, C, D). Participants were asked to sign the consent form (find the consent form for every study in Appendices: E, F, G) on the day of the experiment. All the experiments had the ethical approval of the Research Ethics Committee of Brunel University (see Appendix H).

2.3 Design of the Studies

All studies used a within-subjects design where participants served as their own control. The within-subjects design was selected to overcome the problem of increased error and variability between groups due to individual differences inherent in between-subjects design (Hicks, 2004). Thus, variability in the data is reduced and the power to detect an effect is increased (Field, 2005). Additionally, fewer subjects are required compared to the between-subjects designs as the same group of subjects participates in all conditions. Repeated measures before and after the interventions were conducted in most of the studies to assess the effect of the intervention on the dependent variables. A group of 10 to 15 individuals participated at each study. TMS studies, with similar sample size (n=8 to 10), assessing central fatigue and perceived exertion have shown significant central and peripheral changes due to fatigue (Todd *et al*., 2003; Søgaard *et al*., 2006; Smith *et al*., 2007).

2.4 Apparatus

2.4.1 Measurement of Isometric Elbow Flexion Force

Force measurements were obtained from the isometric right elbow flexion by using a purpose built static rig containing a force transducer (Model 615, S-Type Load Cell, Tedea-Huntleigh Electronics, UK, force range 50–10 00kg, with tension or compression applications, calibrated for tension throughout the studies). The analogue force signals were amplified 300 or 1000 times and filtered (high pass DC-offset, low pass 2 KHz (Quad 1902, 4 channels, Cambridge Electronic Design (CED), Cambridge, UK)). Additionally, the force signal was simultaneously sampled and digitized (4 KHz) using a personal computer based data acquisition system (micro 1401, 12 channels, Cambridge Electronic Design, Cambridge, UK). The force transducer signals were simultaneously recorded with all surface EMG (sEMG) signals. All digitized data was obtained, stored and processed using a computer and CED software Spike 2 (version 6) and Signal software (version 4) for Windows.

During the initial experiment (voluntary activation assessment via peripheral electrical and magnetic stimulation) the participants were seated with their arm relaxed and restrained in a rig (see Figure 2.1). The arm was positioned at 90° flexion of the elbow and 90° flexion of the shoulder and secured on the device with straps around the wrist. The forearm was in supination (Fig. 2.1). This position had been used previously by Todd et al. (2003, 2004) and has been validated for the study of voluntary activation with peripheral electrical stimulation and transcranial magnetic stimulation to the motor cortex, using the standard Twitch Interpolation Technique. The arm position has been shown to minimize the coactivation of other neighbor muscles so that a more isolated peripheral activation of the biceps brachii muscle (BB) is the major contributor to the isometric elbow force flexion. The straps around the wrist restricted any movement of the wrist, while the shoulder immobilized in 90° constrained most of the undesired movements of other muscles of the shoulder or trunk area.

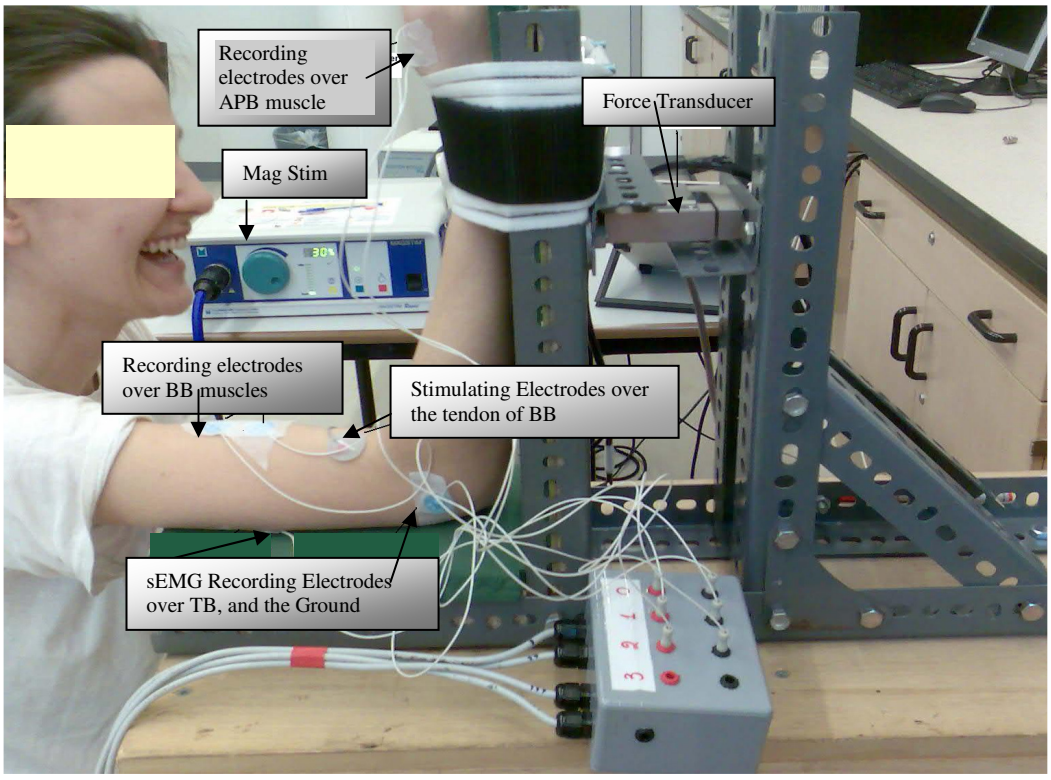


Figure 2.1: The position of the participant during the 1st experiment. The arm is flexed at the shoulder and elbow at 90° and strapped in a home-made device connected with the force transducer.

However, since the majority of participants complained about the ongoing discomfort and pain resulting from prolonged arm positioning, re-design of the rig and positioning was considered and implemented for all additional studies. This was particularly important for subsequent studies involving perception of effort which may be negatively affected by both pain and discomfort in maintaining arm position for extended times during the experiment.

Therefore, a new rig design was tested to improve prolonged positioning and increased comfort which was implemented for subsequent experiments. While using the same force transducer, subsequent modifications were made to the positioning platform and wrist attachment to ensure improved comfort while retaining sensitivity to measurement of elbow flexor forces during isometric activity. The modified rig had participants seated with their arm relaxed on a fixed inclined forearm platform which was padded (Fig. 2.2). The positioning allowed for 90° elbow flexion while the shoulder remained in a slightly flexed and abducted position which maintained a more comfortable anatomical position. Subsequent instructions and attention to seating position ensured that the right shoulder in each participant was not more elevated than his/her left shoulder. The forearm, nearly supinated, was positioned in a resting splint (Fig. 2.2). An adjustable plastic block lined with foam, secured the wrist in position. Elbow flexion force was measured by the positioning of the force transducer rigidly mounted to the adjustable frame and wrist restraint. A small downward force was applied but kept constant to ensure good contact between wrist and restraining block. Upward pressure on the wrist block could then be monitored by the load cell and was recorded as force. Forearm supination and 90° elbow flexion were selected to secure that the BB is working at its greatest effectiveness during the contractions. Indeed, more isolated activation of BB in isometric elbow flexion is achieved in that position (Neumann, 2002).

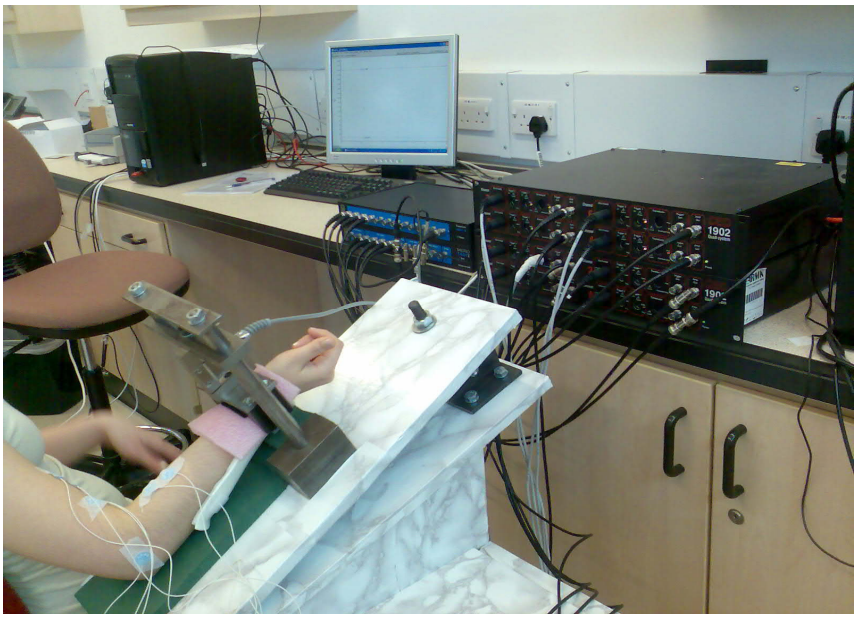


Figure 2.2: Posture set up. Participants were seated with their arm resting on a purpose built force-rig device with their shoulder slightly (15^0) flexed and abducted and their elbow flexed at 90^0. The wrist was secured under an adjustable blocker connected with the force transducer. The forearm was rested in a comfortable splint in semi-full supination. sEMG was recorded from Biceps, Brachialis and Brachioradialis.

Participants undertook isometric elbow flexion at different levels of voluntary force (defined appropriately for each study) against the wrist block (see Fig. 2.2). Because the moment arm of the muscle and the centre of rotation of the joint remain constant throughout the experiments the net torque could be reasonably and directly related to the net force acting in the joint (De Luca, 1997). Thus, force and not torque level is being reported in this thesis. The force levels were pre-determined as a percentage of everyone's MVC ranging from 10 to 100% of MVC. The MVC was measured by asking the participant to perform three 5-seconds maximum contractions with the elbow flexors with a 30-seconds rest in between each to eliminate fatigue. The MVC was defined as the average of the three maximum contractions at the beginning of each experiment. MVCs were delivered under verbal encouragement to ensure maximum effort undertaken by the participant (Shield & Zhou, 2004). Reinforcing the "true" maximum would also allow normalization procedures to maximum force and EMG during later data analysis while reliable and valid comparisons could be made among subjects and conditions. An internal program script within Spike computed the target levels of force based on the MVC. Each subject was provided with a visual cue for each required target force level. The cue was a horizontal line produced on the PC monitor.

This line might or might not be accompanied by a visible vertical force scale (according to the specific protocol requirements). The force trace produced was seen and the subject quickly adjusted this to the target level as shown by the horizontal line (see Fig. 2.12). The isometric contraction was maintained for about 6 seconds while participants were asked to maintain the isometric contraction as stable as possible. Stability of the force trace during the contraction was of great importance as force twitches evoked by peripheral stimulation needed to be detectable during the experiments. Participants, therefore, were trained to undertake stable isometric contractions during the familiarization session.

2.4.2 Surface Electromyography (sEMG)

Muscle electromyography of various arm muscles were recorded simultaneously in the individual experiments. However, the main muscle groups which were the focus of all of the experiments in this study were the elbow flexors of the right arm. Due to limitations in the design of the force rig, and because of the majority of right hand dominance in humans, all experiments utilized right side recordings. BB as one of the major muscles of elbow flexion and most superficial was generally the main one used in sEMG recordings. All sEMG signals were simultaneously digitized and recorded with the subsequent isometric elbow flexion force signal. Other muscles from which sEMG recordings were made included m. triceps brachii (TB), m. brachioradialis (BR), m. brachialis (Br), and the m. abductor pollicis brevis (APB). TB was selected as the main antagonist muscle of the elbow flexors, and used to monitor the degree of spread of stimulation from the chosen stimulation site over BB which is likely to occur at supramaximal intensities. The monitoring of antagonist muscles is necessary in twitch interpolation experiments examining voluntary activation, as force signals represent only the net force from the intact elbow. Similarly, the APB was used to monitor the possible effect of stimulation through underlying nerves in the upper arm (e.g., median nerve) whose trajectory is parallel and superficial to BB muscle as it innervates forearm and hand. The APB is innervated by the median nerve (Neumann, 2002) and is therefore an appropriate one to monitor to determine the spread of superficially applied stimulation to upper arm regions. In addition, where appropriate, sEMG from both BR

and Br were also recorded as they act as the main synergists for elbow flexion (Neumann, 2002).

Standard recording sites for arm and hand muscles were used (Cram *et al*., 1998). For the sEMG recordings, pairs of silver/silver chloride (Ag/AgCl) disposable gel recording electrodes (Arbo infant electrodes, circular, 22mm, Henleys Medical supplies, Herts, UK) or woven, cloth monitoring electrodes, with full-surface solid, adhesive hydrogel (KENDAL, SOFT-E, H59P, Tyco Healthcare, by Henleys Medical, Brownfields, Welwyn Garden City, UK) were used. These surface electrodes were placed parallel to the muscle fibres over the muscle belly of each muscle respectively where the highest density of motor end plates can be found and the inter-electrode distance for each pair was approximately 2 cm, according to surface electrode placement recommendations (De Luca, 1997; Cram *et al*., 1998; SENIAM, nd.). In respect to this transverse location, the recording electrodes were placed in the midline of the muscle belly, away from the border with the other muscles, to reduce the likelihood of detecting a crosstalk signal (De Luca, 1997). A ground electrode was placed over the medial epicondyle of the humerus bone. The recording sites were cleaned with alcohol to reduce impedance caused by poor skin contact. The electrodes were then fixed in place with micropore. To permit analysis of the quantitative relationship between force and EMG signal, fidelity of the EMG signal was required (De Luca, 1997). The stability of the EMG signal was ensured by introducing isometric contractions as the main muscle contraction in all the experimental trials. The isometric contraction, although less physiologically interesting than the dynamic contraction, ensures that the distance between the electrode and the active motor units remain fixed during the contraction and therefore the same active motor units are detected and assessed (De Luca, 1997). For comparisons to be made among subjects all EMG data were normalized to the EMG signal during MVC. Special consideration was given to assure that the participants generated the maximum force when asked to do so. This was secured by giving verbal encouragement during every MVC (see above: section 2.4.1).

The recording electrode pairs for each muscle and the ground electrode were connected in a bipolar, differential configuration to a programmable signal conditioner using common mode rejection, and the EMG signals were amplified 1000 or 3000 times, filtered (1 Hz high pass, 2KHz low pass). Filters were used to pass frequencies related

to muscle activity (1Hz – 1KHz) and to reject frequencies that are associated with electromagnetic noise. All EMG signals were simultaneously sampled and digitized (sampling rate of 4 KHz) using an analogue to digital converter (ADC) as described above. The hardware and software equipment used for signal detection, processing and digitization are commercially available and purpose built for research use with human subjects (Cambridge Electronic Design). All digitized data were obtained and stored on a PC. Typical recordings were data files continuously sampling EMG and force responses over time (seconds to minutes) and simultaneous recording of event trigger TTL pulse for time locked analysis of evoked responses to stimulation. The CED software package, Spike v. 6 for windows enabled continuous high fidelity recording of several EMG signals, force recordings and event trigger recording and subsequent data analysis typical of EMG studies (Fig.2.3).

The amplitude of the surface EMG activity during generation of voluntary force levels, produced typically in these experiments, was determined by the root mean square (rms) method of analysis. The European Project "Surface EMG for Non Invasive Assessment of Muscles" (SENIAM, 1997–2000) provided various techniques for EMG signal processing and analysis such as average rectified value, mean amplitude value, root mean square, spectra analysis. Rms amplitude has been recommended for measuring the level of activation of a muscle under contractions of constant force and constant angle as the most appropriate method of analysis for EMG signals detected during voluntary contraction, because it represents the power of the signal and thus has a clear physical meaning (De Luca, 1997). Rms amplitude is calculated by squaring each data point, summing the squares, dividing the sum by the number of observations (data points) and taking the square root. The CED software permitted the online calculation of the rms amplitude. The use of stimulation techniques to evoke a myoelectrical and muscle twitch responses required the additional use of software designed to capture the subsequent evoked responses over time (from millseconds to seconds) which are time locked to presentation of the stimuli. CED Signal software contains a number of purpose built analysis tools for reliable quantification of event related signals including: digital signal processing, averaging and graphical presentation.

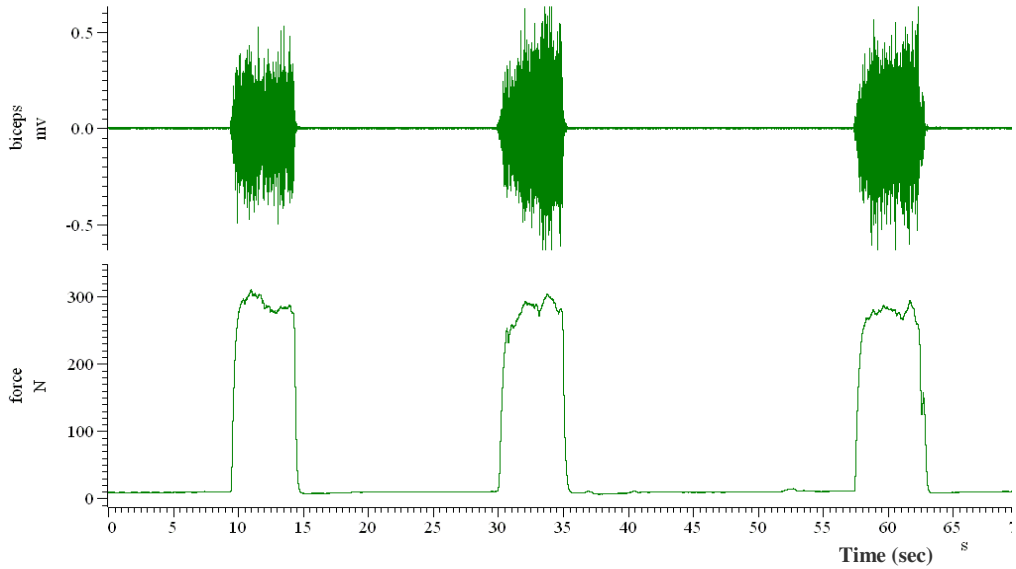


Figure 2.3: Continuous and simultaneous recording of surface EMG with force records. The raw suface EMG activity of biceps during isometric maximum contractions of elbow are shown.

2.4.3 Electrical Stimulation of Musculocutaneous Nerve

Peripheral electrical stimulation was used for the assessment of voluntary activation of BB by evoking force twitches during different levels of voluntary contractions. Single constant-current electrical stimuli (pulse width of 1 ms) were delivered to the musculocutaneous nerve innervating BB over the motor point of BB (point that the musculocutaneous nerve inserts into BB) using a commercially available isolated constant current electrical stimulator certified as safe for human use (Digitimer, DS7A, UK range from 1–100mA, current pulse width 0.05–2 ms). The pulse width used for electrical stimulation is standard for electrical stimulators and based on the strength-pulse duration electrophysiological principles of the nerve stimulation (Low & A. Reed, 2000b). A pair of self-adhesive, circular (2.5 cm), reusable, woven, gel electrodes (PALS Platinum, neurostimulation electrodes, model J10R00, Axelgaard manufacturing, Denmark) were used for the electrical stimulation. A surface cathode (–) was placed over the motor point located between the anterior edge of deltoid and the elbow crease, with the elbow flexed at 90°, and a surface anode (+) was placed over the bicipital tendon. This electrode arrangement with the cathode set over the motor point has been suggested for uniphasic electrical stimulation of a nerve because the positive charged membrane would more easily driven away of threshold by increasing negativity

and a action potential would more readily occur (Low & A. Reed, 2000a). Prior to electrode placement, the skin was first cleaned and mildly abraded with alcohol swabs to reduce electrical contact impedance. While the anode was fixed, a small saline soaked, movable probe (0.5 cm) was first used to identify the motor point in each subject. Once the motor point was identified, the cathode electrode was then affixed to this site.

Full activation of the muscle during the electrical stimulation utilizing supramaximal intensity was first determined by monitoring the twitch responses to increasing levels of current intensity (range typically 3 to 100mA, 1ms). The use of supramaximal stimulus intensity is recommended in twitch interpolation studies to ensure that any associated small movement of the electrodes on the surface of the skin, relative to the underlying muscle or nerve has a minimal effect on the proportion of the muscle that is activated (Allen *et al*., 1998; Shield & Zhou, 2004). The supramaximal stimulus intensity used was set at 20% above the current intensity which produced a resting twitch of maximum amplitude in a relaxed muscle for each participant. Muscle fibre potentiation due to repeated stimuli (Binder-Macleod *et al*., 2002) and to voluntary contractions (Baudry & Duchateau, 2004) was avoided by stimulating with a 15s interval between each stimulus applied. From pilot trials this interval was considered as sufficient enough to eliminate muscle fibre potentiation. In order to determine the stimulus intensity to be used, a stimulus response curve of single twitch force versus stimulus intensity was first determined over a range of intensities. The stimulus intensity which produced a plateau in the evoked twitch force enabled the determination of the required maximal intensity (Fig. 2.4). The amplitude of the twitch was measured from baseline to peak in each twitch response.

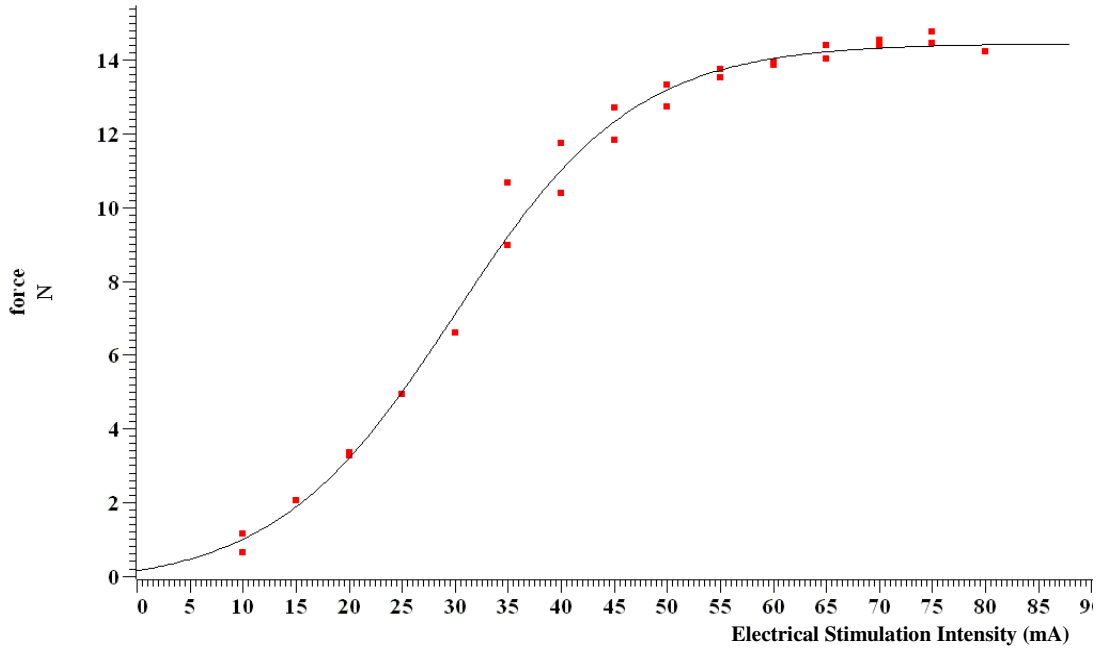


Figure 2.4: Intensity response curve for musculocutaneous nerve electrical stimulation. Y axis represents the peak force twitches evoked by single pulse electrical stimulation (pulse width 1ms) of gradually increased intensity with the arm completely relaxed (two single responses at every intensity level). Intensity started from sub threshold (5mA) and increased gradually until the evoked twitch force plateau (max intensity). (Example from one participant).

2.4.4 Magnetic Stimulation

2.4.4.1 Magnetic Stimulation of Musculocutaneous Nerve

Peripheral magnetic stimulation was used for comparison with the standard peripheral electrical stimulation in assessment of voluntary activation using the Twitch Interpolation Technique (see Chapter 3). In order to determine supramaximal magnetic stimulation intensity, a stimulus-response curve stimulator output intensity was applied from sub threshold to maximal stimulator output (MSO=100%) to obtain the full range of evoked twitch forces (Fig. 2.5). The supramaximal intensity was set at 20% above maximum and remained constant throughout the experiment.

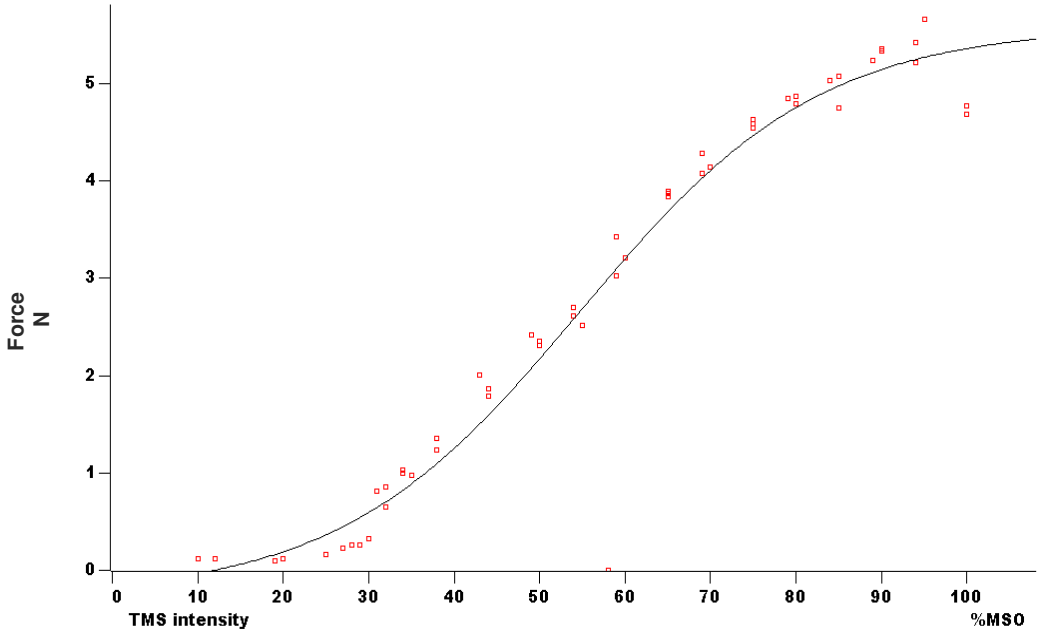


Figure 2.5: Intensity response curve for musculocutaneous nerve magnetic stimulation. The trend plot represents the force twitches evoked by peripheral magnetic stimulation as they gradually increase with the magnetic stimulator output (two single responses at every intensity level). Intensity started from sub threshold and increased gradually until the evoked twitch force plateau (max intensity). Example from one participant.

Single pulse magnetic stimulation was performed using a 70mm figure of eight coil powered by a Magstim Rapid (pulse duration 250 μs) biphasic stimulator, (Magstim Company Ltd, Spring Gardens, Whitland, Wales, UK). The coil was positioned firmly against the skin with the crossover positioned on the motor point. The optimal stimulation site was detected before the experiment by moving the coil over BB near the cathodal electrical stimulation site, and it was defined as the stimulation site that yielded the largest force twitch elicited from the muscle. The optimum position was marked with a pen to assure consistent placement of the coil during the experiment. Induced current flow is directional when using magnetic stimulation (Sun *et al.*, 1998), and therefore, optimal orientation of the coil was tested before the experiment by comparing the twitch size evoked when the magnetic stimulator double coil was parallel or perpendicular to the axis of the upper arm. It was found that the most effective orientation of the induced current flow, produced by the coil, was perpendicular to the trajectory of the musculocutaneous nerve as previously reported (Sun *et al.*, 1998) (Fig. 2.6).

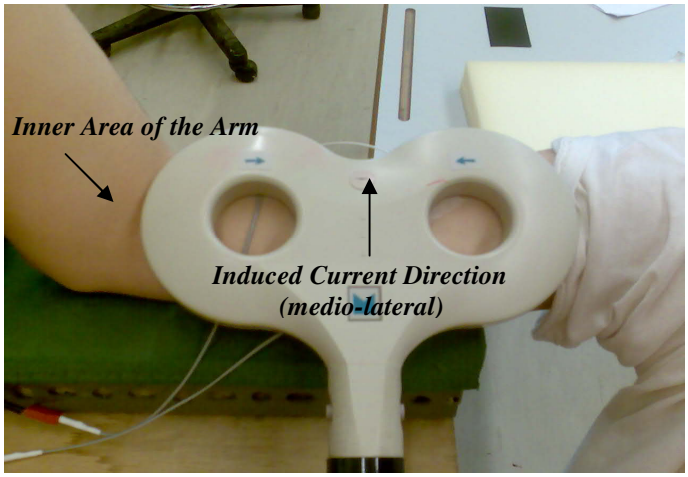


Figure 2.6: Position of the magnetic coil over the motor point of the biceps brachii muscle. The orientation of the coil was parallel to musculocutaneous nerve with the induced current vertical to the nerve in a medio-lateral direction. This position was decided after practice at different angles as the most appropriate to produce the biggest relaxed force twitch when the muscle was rested at 90° elbow and shoulder flexion (old rig/posture).

During each recording session, both the magnetically evoked and electrically evoked force twitches were obtained from each participant in order to compare these directly. Both electrical and magnetic stimulation of the musculocutaneous nerve were delivered while elbow flexors were at rest and during isometric elbow contractions of various levels of voluntary force (10, 25, 50, 75 and 90% of every subject's MVC). Target force levels were displayed using horizontal cursor lines on the computer screen showing the force signal over time (see section 2.4.1). The vertical force scale was visible to provide visual cues of the actual amount of force produced. A set of three contractions was performed at every target level of force in random order, with 30 sec rest to avoid fatigue. During each contraction and when force reached a plateau, a supramaximal stimulus was delivered to the musculocutaneous nerve over the motor point. Two single pulses of magnetic or electrical stimulation were delivered to the biceps muscle while relaxed (resting twitches) at the beginning and at the end of every set of contractions (Fig. 2.7). The twitches evoked by the stimulation (interpolated twitch) where displayed on the PC screen while an automated twitch peak analysis was used by first setting cursors for the peak search within user specified regions of the force record (Fig. 2.8).

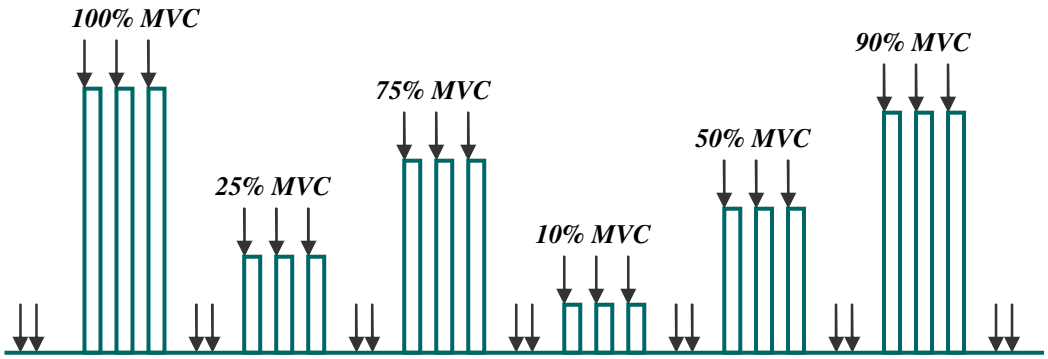


Figure 2.7: Schematic representation of experimental set up in peripheral stimulation for assessing voluntary activation. The arrows indicate the time of stimulation (magnetic or electrical) over the BB motor point while BB was at rest or during voluntary elbow contractions of various levels of force.

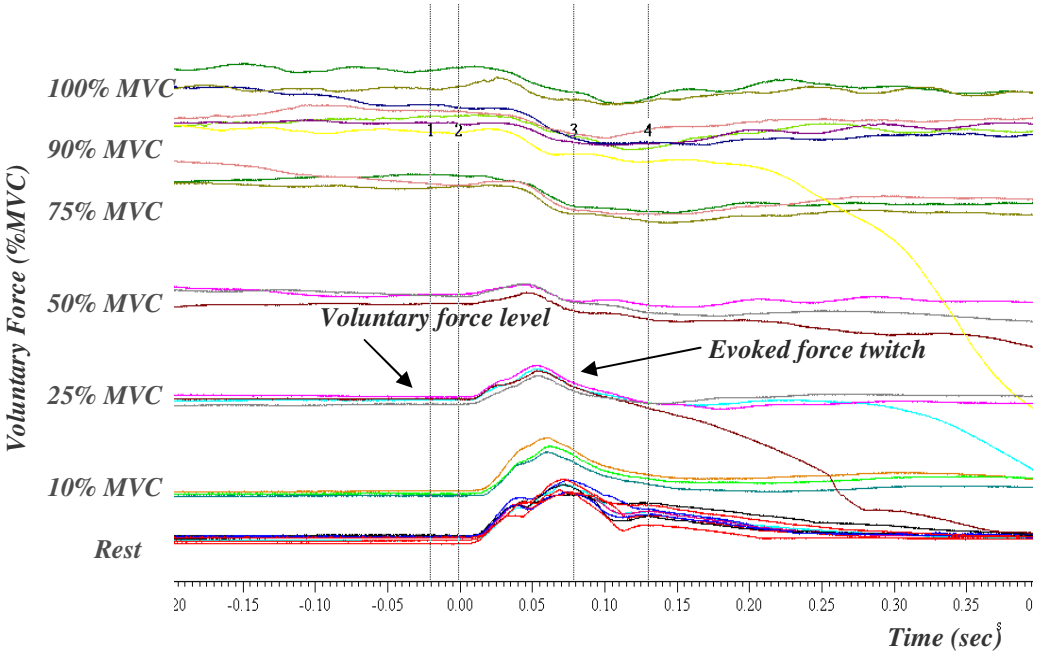


Figure 2.8: Force twitches evoked by supra-maximal magnetic stimulation over musculocutaneous nerve at various levels of voluntary force defined as percentages of an individual's MVC. Data from one participant.

2.4.4.2 Transcranial Magnetic Stimulation (TMS)

Single-pulse TMS over the motor cortex was used to assess changes in the motor cortex excitability of elbow flexors in experiments where fatigue and transcranial direct current stimulation (tDCS) were used as interventions (see Chapters 5 & 6). Painless and safe in its application (Wassermann, 1998), single-pulse TMS has been extensively used in central fatigue research (Todd *et al*., 2004) as well as in assessment of changes of the global cortical excitability due to tDCS (Nitsche & Paulus, 2001). After pilot experiments, BR was used instead of BB as the site for monitoring the motor evoked potentials in response to TMS. In this study, BR motor evoked potentials (MEPs) were significantly larger than BB MEPs and therefore localization of the BR motor "hot spot" (the optimal point on the cortex for eliciting a MEP in the muscle of interest) in the left motor cortex was more easily found. As it was not possible to conduct subsequent experiments with two different stimulation sites, the use of the BR as the larger of the two stimulation sites ensured monitoring of changes in excitability over the time course of these complex experiments. As both muscles were voluntarily activated, only BR MEPs were analyzed as part of an evaluation of changes in motor cortical excitability.

Single-pulse TMS was applied over the scalp using biphasic magstim rapid (30 Hz, 2 boosters Magstim Company Ltd, Whitland, Wales, UK), through a 70mm figure of eight coil of maximum magnetic field strength of 2T. This double coil has greater focality compared to a circular coil where the greatest induced current is located in circumference of the coil (Epstein, 2008). This type of focal stimulation with the double coil makes it crucial to place the coil over the "hot spot" when maximal responses are desired (Rösler & Magistris, 2008). This optimal position was defined as the stimulation site that yielded the largest MEP in the resting BR at suprathreshold stimulus intensities (Tergau *et al*., 2000) and it was found on average in the participants of this study to be about 4 cm left and 0.5 cm posterior of vertex. The "hot spot" of BR was determined by moving the coil in small steps over left motor cortex, from the vertex towards the posterior anterior direction and monitoring the MEP from elbow flexors. The vertex (Cz) was designated by three main measurements: nasion–inion, preauricular points and circumference of the head in accordance with the American Electroencephalographic Society guidelines for standard electrode position nomenclature (10-20 System of electrode placement; American Electroencephalographic Society, 1991). The "hot spot"

was marked with a pen to assure constant placement of the coil during the experiment. The center of the double coil, which is the point where the maximum field strength is produced (Rösler & Magistris, 2008), was held tangentially to the scalp with the handle of the coil pointing backwards and slightly lateral to the interhemispheric line so that the induced current flowed from a posterior to an anterior direction. This orientation was chosen as it was found to be the most effective for eliciting MEPs in the particular target muscle (Tergau *et al*., 2000). The coil was maintained on the head by the experimenter and its position and orientation were constantly checked to ensure that no slippage occurred during the experiment.

The resting motor threshold (RMT) was defined as the lowest stimulus intensity to elicit a reliable MEP (at amplitude more than 50 μV) in more than half of 10 consecutive stimuli with the muscle relaxed (Reid *et al*., 2002). Once the threshold was determined the stimulus intensity of the experiment was set at 1.2 × RMT. The standardization of the stimulus intensity with respect to an individual's RMT is adopted in both clinical (Rösler & Magistris, 2008) and research settings using TMS (Furubayashi *et al*., 2008). The difficulty lies in the use of magnetic stimulation with regards to the actual induced current whereby the stimulus intensity uses an arbitrary linear scale (0–10 0%) of magnetic stimulus current to discharge the coil, and standardize this to something comparable (i.e. electrical current). The magnetic stimulus strength is determined by a number of parameters as the duration of the magnetic pulse, the induction coil size and shape while the effective stimulus strength may also be affected by many variables that are still unknown (Rossini *et al*., 1994). Thus, it can only be measured in relative terms (% of the maximal stimulator output (MSO)) by the voltage stored in the stimulator storage capacitor compared to that required to give a threshold response (Rossini *et al*., 1994). Consequently, to provide a measure of stimulation intensity that can be generalized across different TMS-configurations, stimulation output is expressed as a percentage of the motor response threshold (Stokes *et al*., 2007).

After the hot spot, the RMT and the stimulus intensity (% MSO- defined earlier and explained) had been defined, the experimental protocol started with the baseline measurement. MEPs were recorded before and after the intervention (fatigue or tDCS) at the same stimulus intensity and with the arm resting in the rig. The MEP response is a summation of individually activated motor units discharge in response to the applied

stimulation to the motor cortex, and represents activation via corticospinal tract and spinal motor neurons. Therefore, the wave shape and amplitude is complex. The quantification of the MEP response is the main measure of changes in excitability of this descending voluntary motor system, and in order to improve signal to noise, averaging techniques of multiple MEP responses were used (Rösler & Magistris, 2008), Typically 10-15 consecutive MEPs were collected, evoked every 5 to 10 sec over a particular time point of interest. These were then averaged to produce a mean MEP response at each time point before and after any intervention. Further off line quantification of the MEPs was by means of analysis using Signal software. Fixed position cursors were set from the onset to the offset of the mean MEP response and the computed area was by means of the modulus function. This adds both the negative and positive components of the multiphasic waveform to compute the MEP area (Fig. 2.9). The MEP area is less dependent on single point amplitude measures which are also typically used for assessing changes in excitability through MEP measurement.

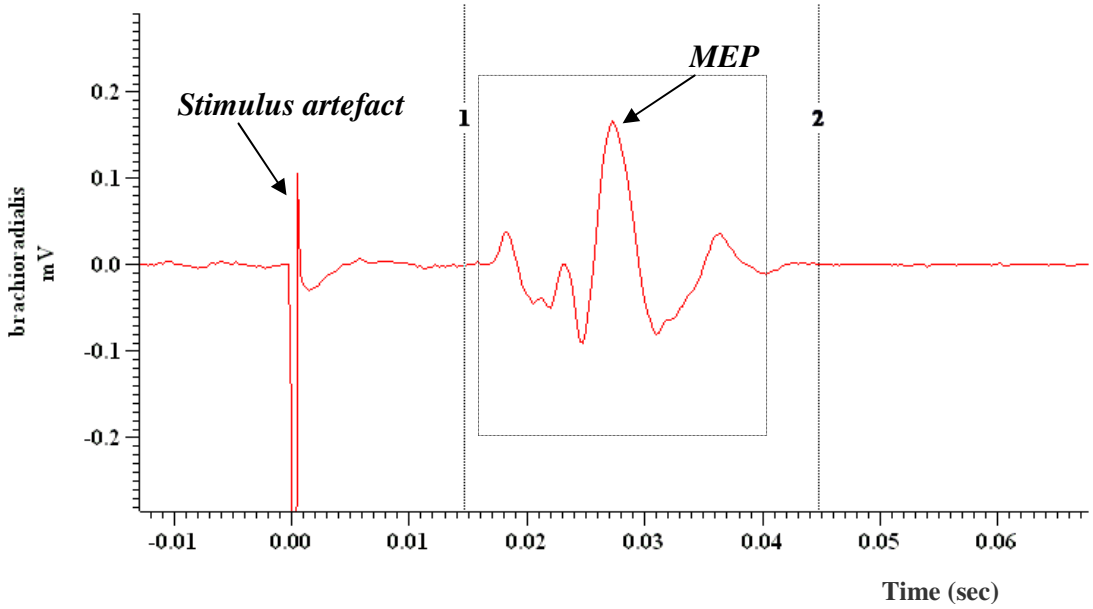


Figure 2.9: MEP of brachioradialis. The MEP area defined between cursors 1 and 2.

Data collection of MEP responses by TMS were taken before assessment of rating of perceived effort at different levels of voluntary force to eliminate potential MEP facilitation due to the voluntary contractions over time. TMS studies show that excitability of circuits involved in MEPs changed during or following voluntary activation of the target muscle and that even a brief activation could lead to post-exercise facilitation (Rothwell, 2008). The collection of responses over time was therefore standardized to ensure that changes in post-pre comparison were specific to the phase of the experiment or intervention.

2.4.5 0–10 NRS for Rating Perception of Effort

Although sensitive and reliable enough, in pilot work undertaken before the experiments, practical difficulties emerged with the utility of the VAS. In the current experiments, participants could not use their right hand to mark the line (the right arm was restricted for the purposes of the experiment). Therefore the researcher had to mark on subject's behalf as precisely as possible wherever the participant pointed with his left index finger. However, this method of marking gave an error in effort rating of about 5 to 10 mm on the VAS. Additionally, the importance of keeping the direction of the line and the angle at which the subject views the VAS consistent throughout the study has been noted in the literature (Wewers & Lowe, 1990). Any changes either in the direction of the line or the distance between the VAS and the subject could alter the placement of the mark on the line. Unfortunately, in the present experiments such requirements could not be maintained. It was decided therefore, that the VAS was inappropriate for use in the current experimental setup. For reasons extensively presented in the Literature Review (see section 1.2.4.2.1) the Borg scale has also been excluded for effort assessment. In contrast, the 0–10 NRS was decided as an appropriate effort rating given that it would show good reliability and validity.

Thus, the 0–10 NRS was validated for rating perception of effort. The end points are the extremes of no effort at all and the maximum effort that could be experienced during production of a certain level of voluntary force with the elbow flexors. The 0–10 NRS was presented graphically during the familiarization session when instructions were

given to participants in regards the utility of the scale (Fig. 2.10). In subsequent experiments the 0–10 NRS was delivered verbally and each response was recorded with the associated force data. (See following sections 2.5 & 2.6)

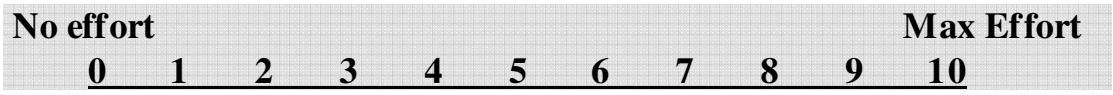


Figure 2.10: 0–10 Numeric Rating Scale that has been presented to subjects graphically at the beginning of the familiarization session.

2.4.6 Mood Rating Scale

The Positive and Negative Affect Schedule (PANAS) was used as a secondary measurement outcome for assessing the general state of mood of the participants at the beginning of each session and following the intervention. (Appendix I). The scope of this measurement was twofold: firstly to secure that no changes in the mood occurred from session to session, and secondly to assess whether the intervention (tDCS or fatigue) had any effect on the mood that might indirectly affect perception of effort. Psychological factors such as attention, mood, and motivation have been suggested to affect exercise performance (Zwarts *et al*., 2008). The PANAS is a brief, 20-item self-report adjective list containing 10 positive mood adjectives and 10 negative mood adjectives developed by Watson, Clark, and Tellegen (Watson *et al*., 1988 cited in Crawford & Henry, 2004). Sample positive mood adjectives include interested, excited, enthusiastic, and inspired. Sample negative mood adjectives include distressed, upset, hostile, and ashamed. Each item is rated on a 5-point scale ranging from 1 = "very slightly" or "not at all" to 5 = "extremely" to indicate the extent to which the rater has felt this way in the indicated time frame (Crawford & Henry, 2004). The PANAS scale has been tested for its reliability and validity in the healthy population (Crawford & Henry, 2004) and has also been used to examine mood state changes in interactive video game exercise contexts (Russell & Newton, 2008).

2.5 Experimental Procedure

All studies were undertaken in the Motor Control Laboratory (located in Mary Seacole Building) on the Uxbridge campus of Brunel University. When participants came to the laboratory a familiarization session was first undertaken. The experimental procedure was explained, and the participants received a test trial of the forms of stimulation used during the experiments. They were also asked to undertake test isometric contractions of elbow flexion to become familiar and comfortable with using the force rig, positioning the arm in the rig and with producing visually guided stable isometric contractions. Stable and accurate isometric contractions were of great importance for the reliability of the ratings of the perceived effort (see below: section 2.5.1). When a perception task was to be performed (Chapters 4, 5, 6) the task was also explained to participants and they were asked to use the rating scale in a test trial before the experiment, to ensure its correct utility.

Following the brief (20 minutes) familiarization, the participant was positioned correctly in the force rig, and all EMG recording electrodes were positioned on the muscles of interest. The optimal peripheral stimulation sites for electrical and magnetic stimulation were also determined. When applicable (see Chapters 5 & 6) the motor "hot spot" for the BR in the left motor cortex was determined using TMS; while the RMT and the stimulus intensity for magnetic and electrical stimulation were identified. The maximal (isometric) voluntary contraction (MVC) in elbow flexion was also defined for every participant as described above (see section 2.4.1).

Both magnetic and electrical stimulation were well tolerated. Magnetic stimulation was painless and only some discomfort was caused by the electrical stimulation due to repeated stimuli applied for identifying the optimum intensity during the familiarization session. Despite the discomfort that the electrical stimulation did cause, the unpleasant sensation produced by stimulation through the skin was well tolerated and attenuated with time. However, to avoid any potential interference with the rating of the perceptual effort whenever applicable (see Chapter 5) the use of the electrical stimulation followed the perception of effort assessment during these experiments. The actual experimental session began shortly after the familiarization and preparation session. Each study followed a specific procedure which is identified in the following chapters.

2.5.1 Perceived Effort Task

The perception of effort task consisted of ratings of perceived effort monitored at the end of short elbow flexion isometric contractions at different levels of voluntary force. The target levels of force were predetermined based on the subject's MVC. The MVC was defined as the mean of 3 maximum contractions (as previously described) undertaken every time the perception task was performed. Participants were prompted to think about the perceived effort during these contractions and to rate that feeling of effort as 10 on the NRS. A point 0 on the NRS, that was explained as remained still and relaxed, corresponded to no effort at all. After they had been given the lower and higher anchors of the scale as a perceptual guide for their rating and the MVC was established, intermediate levels of force were set (as already described in section 2.4.1) in a random order to avoid bias in the results. In pilot work it was found that the way the target levels of force were selected had an effect on the ratings of perceived effort. When the levels of force were selected in incremental order (from 10 to 100% of MVC), higher levels of ratings were indicated than when the levels were randomly selected. Additionally, random selection of the target levels of force resulted in more accurate results for the perceived effort (see Fig. 2.11) and therefore randomized presentation was chosen as the most appropriate way of using the selected levels.

The target force levels were presented by a horizontal line displayed on the PC monitor (Fig. 2.12). The horizontal line lacked a visible vertical force scale to ensure that participants would not use any visual cues of the actual force produced that could help in rating the effort. This horizontal line always appeared in the middle of the screen regardless of the actual level of force that it represented. After each target level was set, participants were asked to make an isometric elbow contraction and to adjust the produced force trace to the target horizontal line, and to maintain that level as steadily as possible for 6 seconds. At the end of the contraction they were asked to think about the effort they undertook to make the contraction and to translate it into a number between 0 and 10 on the NRS. Every time the participant had finished with one contraction the screen was turned out of view, and then a new target level was set. During each experiment, all target force set levels and force trials produced were recorded, along with each perception of effort rating by means of keyboard entry to recording data file.

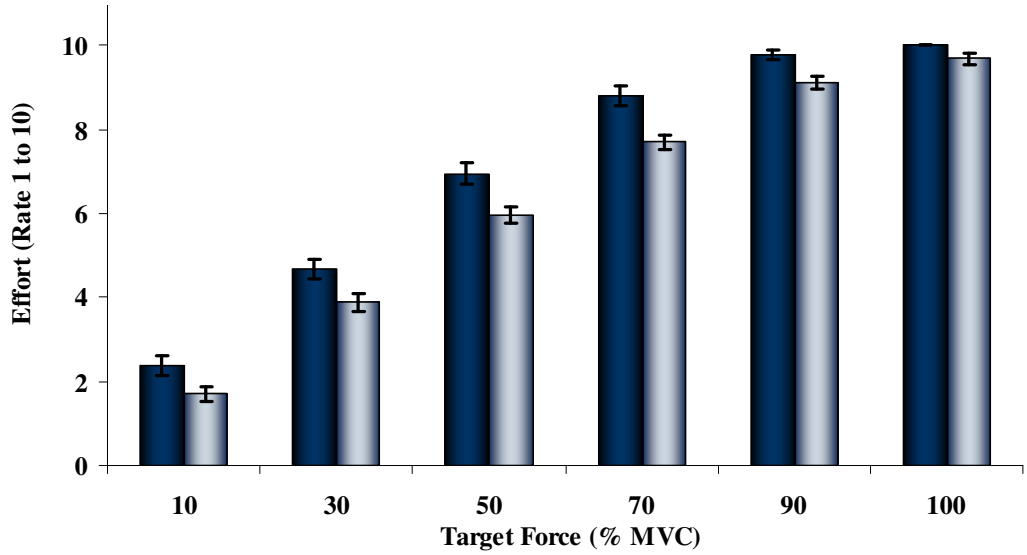


Figure 2.11: The way of selection of the target levels of force affected the rating of the perceived effort. The columns represent the mean values of 3 ratings of effort at every force level when this was chosen in an increased order (dark blue ■) or randomly (light blue ■). Group mean ± SEM of n=21 participants.

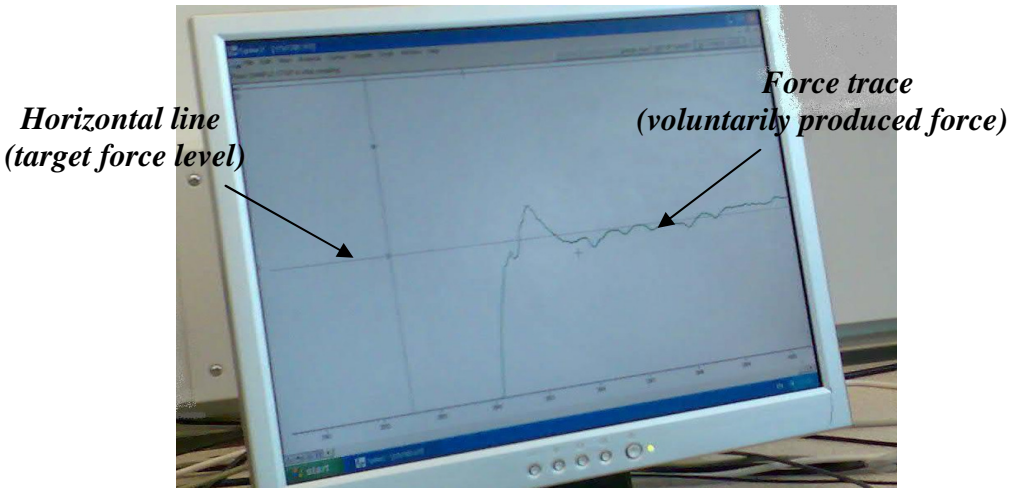


Figure 2.12: The target level of force was shown by a horizontal cursor set in the middle of the PC screen. The horizontal line had no vertical force scale visible. During each trial, visual feedback of the produced force ensured adequate matching to the required target force shown.

2.5.2 Fatiguing Exercise

The fatiguing exercise comprised the intervention for the second and the third study of this thesis (look Chapters 4 and 5). Fatigue was induced by repeated intermittent isometric elbow flexions at 50% MVC for 10 minutes. Each contraction lasted 15 seconds, followed by 2 seconds rest. In pilot work these intervals produced rapid fatigue in a fairly standard and reliable manner. At the beginning and at regular (2 min) intervals during the fatiguing session the subjects performed an MVC which was used to determine the degree of fatigue. When the MVC was reduced to 40% of initial MVC, the exercise ceased. This drop in the amount of MVC has been reported previously as a satisfactory drop that accompanies fatigue-induced peripheral and central motor changes, and it has been used for assessing central fatigue (Taylor *et al*., 2000). The arm flexors were chosen because they have the advantage of being extensively used in central fatigue research (Taylor *et al*., 2000a; Todd *et al*., 2003, Søgaard *et al*., 2006; Smith *et al*., 2007). As such, elbow flexors research does provide a robust background of validated techniques for assessing central fatigue on top of which new methodologies could be built in assessing perception of effort. Various intermittent fatiguing exercises at submaximal levels of voluntary force (5-15%MVC) (Søgaard *et al*., 2006; Smith *et al*., 2007) have been used in fatiguing exercises and have been reported to cause central fatigue. Here, the middle range of voluntary force (50% of MVC) was chosen as the most appropriate to test perceived effort following fatigue, as it is a level that is commonly used in everyday activities. The isometric contractions have been employed to ensure EMG stability (see section 2.4.2) and to allow interpretation of the EMG-force and EMG-effort relationship. Given that the EMG signal has been considered as a measure of the efferent motor command to the periphery, as discussed in the literature review (see section 1.2.4) and therefore a consequent objective indicator of the perception of voluntary effort, fidelity in sEMG signal detection and processing is required. This is applicable only when careful application of the sEMG is considered. Isotonic (e.g., concentric and eccentric) contractions, although physiologically interesting, cause various mechanical (e.g., change in muscle fibres length), anatomical (e.g., change in the shape of the motor unit that construct the EMG signal due to altered position of the electrode relatively to the contracting fibres), physiological and electrical (e.g., change in the signal due to changes in the number of the motor units recruitment

during different phases of dynamic contraction) modifications that affect the consistency of the sEMG signal over time (De Luca, 1997).

2.5.3 Transcranial Direct Current Stimulation

Transcranial Direct Current Electrical Stimulation (tDCS) was used as an intervention to assess its impact on perception of effort by artificially changing the corticospinal excitability (see Chapter 6). The use of standard low intensity (1.5 mA) direct current was applied for 10 minutes as it has been found to cause excitability changes (Cogiamanian *et al*., 2007), lasting for several minutes (up to about 10-15 min) and has been considered safe according to Nitsche and Paul (2000). The electrical current, delivered by a battery driven constant current stimulator (DC-Stimulator: CX-6650, model TRCU-04A, Rolf Schneider Electronics, Germany) (Fig. 2.13) was applied over the motor cortex at the area that represents the BB muscle via moistened sponge electrodes (3×3.5cm with 1,2m cables red & blue and 2mm-plugs). The sponge covers (viscose pockets 5×4cm) were dampened with 1% of NaCl. Use of near isotonic saline solution was found to be associated with decreased voltage at the skin, that is required to drive the current and consequently decreased sensation and discomfort. Additionally, it does allow good conduction of current (Dundas *et al*., 2007). The motor cortex electrode (active) was placed over the hot spot of the right BB and the other electrode (reference) was fixed over the left shoulder. This extracephalic positioning of the reference electrode has been used elsewhere (Cogiamanian *et al*., 2007) and was chosen against the conventional motor cortex-contralateral forehead, to limit the source of the tDCS after effects in one hemisphere. Additionally, this arrangement of the electrodes was chosen to prevent the reference electrode of producing its own effects over the brain (Wassermann, 2008).

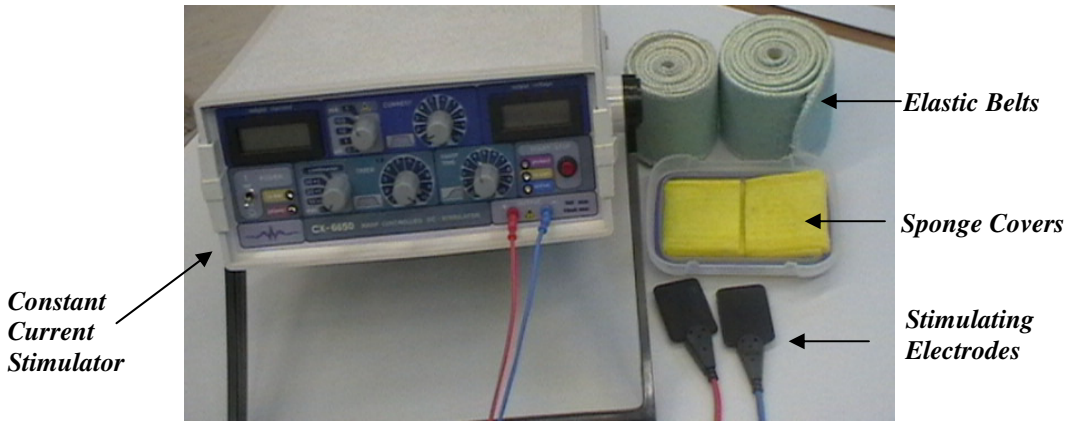


Figure 2.13: Constant-current stimulator for tDCS. The current was induced through sponge stimulating electrodes (3×3.5cm with 1,2m cables red & blue and 2mm-plugs). The sponge covers (viscose pockets 5×4cm) were dampened in a 1% saline and secured in place with elastic belts.

2.6 Analysis of Data

To minimize variability in the measurements, due to inter-individual biological differences and to variability in the brain signal (Wassermann, 2002; McIntosh *et al*., 2008) analysis was undertaken on normalized data. Specifically, the sEMG signals and the voluntary force were always normalized to the maximal values taken from every subject for comparisons between conditions. Repeated measurements were also conducted before and after the interventions to allow monitoring of the behavioural and physiological changes for a sufficient period of time.

Whenever applicable, voluntary activation was calculated by the formula: *voluntary activation=100×(1- superimposed twitch / control twitch)*, where the superimposed twitch is the force increment evoked during a voluntray contraction at the time of the stimulation and the control twitch is that evoked by identical nerve stimulation in relaxed muscle (Shield & Zhou, 2004).

Repeated measures analysis of variance (ANOVA) and paired-samples *t*-test were used to measure the effects of an intervention in this within subjects design scenario. The F-value was always reported and Partial Eta squared (η_p^2) calculation indicated the effect size of the variables. The significant level was set at a p value equal or less than 0.05. During the repeated measure ANOVA, the homogeneity of the variances of the

differences was tested with the Mauchly's sphericity test which is produced automatically in SPSS. If homogeneity is violated the type I error is inflated and therefore the probability to reject the null hypothesis when it is true is increased (Kinnear & Gray, 2009). Whenever sphericity was violated the Greenhouse-Geisser correction was used to produce a valid F-ratio (Field, 2005). Greenhouse-Geisser calculates the degree to which the sphericity assumption is violated and then adjusts the degrees of freedom accordingly to produce a more accurate significance (p) value (Field, 2005). The Huynh-Feldt correction is another way to correct the F-ratio when the assumption of sphericity is violated (Field, 2005) but because it tends to overestimate sphericity, the more conservative Greenhouse-Geisser correction has been used instead. Planned posthoc comparisons of significant factor effects were undertaken using Bonferroni corrections, which is robust for controlling the type I error especially when the assumption of sphericity is violated (Field, 2005).

As in all parametric statistics normally distributed data are required. Normality was tested by the Kolmogorov-Smirnov test (Kinnear & Gray, 2009). Whenever the data were not normally distributed, a Wilcoxon signed-rank test, which is the non-parametric equivalent to the paired samples t-test, was used (Field, 2005).

To estimate the relationship of the evoked twitches with the level of voluntary force a Generalized Estimating Equations (GEE) analysis was employed, using an exchangeable correlation structure for non-independent variables (Hanley *et al*., 2003). GEE analysis is based on a generalized linear model to estimate more efficient and unbiased regression parameters relative to ordinary least squares regression, where an unknown correlation is present (Ballinger, 2004). This method was chosen for its applicability to within subjects repeated measures research designs in which data are clustered within subgroups (Hanley *et al*., 2003; Ballinger, 2004).

In all the experiments using NRS for perception of effort, assessment of the stability of the scale over time was conducted via a test-retest reliability method of analysis among the baseline measurements (Yen & Lo, 2002). The Intra Class Correlation (ICC) was used to assess the agreement between test and re-test recordings of the NRS for the perception of effort ratings given by the same raters (participants) among repeated measures (undertaken in the same day or within 1-week interval (see Chapter 4). ICC is

computed as the relationship among multiple observations of the same variable to distinguish it from the Pearson's Correlation which is usually between different variables (Streiner & Norman, 2003). The ICC is a coefficient that expresses the total variance in the measurements (ratings) which is due to true differences between subjects. Thus, the ICC (varies from 0 to 1) yields a value of 1 only if the measurements on each participant are identical at test and re-test recordings (Streiner & Norman, 2003; Weir, 2005). In that way, the ICC is a measure of homogeneity, and it is 0 when the within subjects variance equals the between groups (between repeated measurements) variance indicating that the grouping variable has no effect, or alternatively that the instrument does not give reliable records (Streiner & Norman, 2003). The ICC categories of reliability are as follows: 0.0-0.4: poor, 0.4-0.75: fair to good, and 0.75-1.00: good to excellent (Fleiss, 1986). Given that the magnitude of the coefficient is affected by the time interval between the administrations of the measurement, in a way that short intervals may yield estimates of reliability which are too high (Streiner & Norman, 2003), the reliability outcome of this assessment could not be generalized to other experimental conditions with longer time intervals between test and retest measurements. Specifically, the model that was used was the two-way fixed effects model: ICC2(A,2 or 3) where the "class 2" indicates that all participants took part at all time points, and "2" or "3" indicates the number of the time points that the subjects were assessed (the number of baseline measurements; specific to experimental protocol) and A indicates the absolute agreement among the measurements. The method of absolute agreement was used for comparison among the measurements instead of consistency because the scale was tested for its reliability and therefore under the same conditions it should give the same results when it is operated by the same person (researcher) and undertaken on the same participant (rater).

ICC is population based, meaning that the ICC measures how distinguishable the participants are and consequently it can be changed by choosing a heterogeneous or a homogeneous population (Quan & Shih, 1996; Shoukri *et al*., 2008). The within subjects Coefficient of Variation (CV) therefore, was also used as a measure of reproducibility. Within subjects CV was also undertaken to test the closeness of repeated measurements taken on the same participant by the same instrument and it is distinguished by the CV which is similar more population based such as ICC (Shoukri *et al*., 2008). The within subjects CV was calculated with the root mean square

approach, by taking the coefficient variation for each subject separately, square these, find their mean, and take the square root of this mean (Bland's home page, 2006).

To assess the criterion validity of the scale, the ratings of the perceived effort were correlated with the objective measurements of the voluntary force produced at every effort rating and with the EMG activity of the muscles participating in the voluntary contractions. Spearman's correlation coefficient was used for the correlations of non-normally distributed data.

Due to its ability to fit GEE analysis, StataCorp STATA statistical software (release 9.0, College Station, Texas: Stata Corporation 2005) was employed for the GEE analysis. All the other statistical tests were performed using SPSS (version 13 and 15; SPSS for Windows, Rel. 15.0.1. 2007. Chicago: SPSS Inc).

CHAPTER 3

MAGNETIC VERSUS ELECTRICAL STIMULATION IN THE TWITCH INTERPOLATION TECHNIQUE

3.1 Introduction

The muscle force-generating capacity during a motor task is frequently measured to assess human muscle function in clinical and research settings (Man *et al*., 2004; Shield & Zhou, 2004). Muscle activation is a core measurement in assessment of motor function in cases of disruption of force-generating muscle capacity such as during and following fatigue, or due to muscle disorders, or due to neurological diseases (Sacco *et al*., 1999; Prasartwuth *et al*., 2005). The force generated during the MVC is one way to assess the motor capacity however, the ability to perform true maximum contractions relies on an individual's cooperation and motivation (Gandevia, 2001; Man *et al*., 2004), and therefore cannot be a reliable objective way of assessment. The need to develop non-volitional methods of assessing muscle activation has then been developed (Merton, 1954; Allen *et al*., 1998; Man *et al*., 2004).

Electrical stimulation activates skeletal muscles by inducing a twitch contraction. Hence, methods based on single electrical supramaximal stimulation of the nerve have become standard for assessment of human muscle function (Merton, 1954). However, use of electrical stimulation as a means of assessment is confounded by the practical problem of the pain and discomfort caused when it is applied at supramaximal intensities (Delitto *et al*., 1992). On the other hand, use of magnetic stimulation offers a pain-free and more comfortable alternative (Man *et al*., 2004). Peripheral magnetic stimulation for assessing voluntary activation in clinical settings has been investigated with promising results (see section 1.2.4.5) (Harris *et al*., 2000; Polkey *et al*., 2000).

However, to date no studies have compared the two techniques of peripheral stimulation directly in the assessment of voluntary activation using twitch interpolation. Before magnetic stimulation is used in patients, a study was conducted as part of this thesis, to evaluate the characteristics and methodological variables of magnetic and electrical stimulation. Peripheral stimulation of the musculocutaneous nerve innervating BB was undertaken using single pulse magnetic and electrical stimulation. The orientation of the coil, the placement of the stimulating electrodes, the intensity and the duration of the electrical pulse were considered and tested in supplementary experiments.

The objective of this study was to evaluate both the similarities and differences between the two techniques with the further aim to test whether magnetic stimulation of the motor nerve innervating biceps is similar to electrical stimulation and therefore, could be used interchangeably in the single pulse Twitch Interpolation Technique.

The specific hypotheses of this study have been set as:

H_0 (null): ***There are significant differences between electrical and magnetic stimulation when used in conventional single pulse Twitch Interpolation Technique for biceps motor nerve.***

H_1: ***There are no significant differences between electrical and magnetic stimulation when used in conventional single pulse Twitch Interpolation Technique for biceps motor nerve.***

3.2 Methods

3.2.1 Sample

This study comprised 4 experimental sessions. A total of 24 healthy participants (8 males, 16 females) took part in all experiments with an average age of 34.99 ± 9.72 (SD) years and range between 23 and 63 years. During the first main experiment, a group of 13 participants (3 males, 10 females; mean age 32.83 ± 8.22 (SD) years), took part in both conditions of peripheral electrical and magnetic stimulation in a within-subjects crossover design. The subsequent three supplementary experiments tested the effects of the coil orientation, the position of the stimulating electrodes and the electrical stimulation pulse duration respectively. A group of 6 participants (3 males, 3 females; mean age 32.50 ± 8.55(SD) years) participated in the coil orientation and the electrodes positioning experiments, while a group of 5 subjects (2 males, 3 females; 37.4 ±6.27(SD) years) participated in the last experiment. All participants except two were right handed.

3.2.2 Apparatus

The details of the measurement of the isometric force of elbow flexors (see section 2.4.1), surface electromyographic techniques (see section 2.4.2), the electrical (see section 2.4.3) and magnetic stimulation (see section 2.4.4.1) of the musculocutaneous nerve have already been described in general methods chapter (see section 2.4).

3.2.3 Experimental Procedure

While participants were seated with their arm positioned in the force measuring rig the MVC for every subject, the supramaximal intensity for both electrical and magnetic stimulation and the motor point for stimulation were defined as described in sections 2.4.1 to 2.4.4. Both electrical and magnetic stimulation of the musculocutaneous nerve

were delivered while elbow flexors were at rest and during isometric elbow contractions of various levels of voluntary force (10, 25, 50, 75 and 90% of every subject's MVC). The main experimental session started with the electrical stimulation and was followed by the magnetic stimulation without randomizing the pattern of delivery. The design of the protocol of the main experiment is depicted in figure 2.7. To test further conditions under which the magnetic stimulation could replace electrical stimulation in the Twitch Interpolation Technique, methodological issues were evaluated in supplementary experiments.

- **Coil Orientation**.

During the main experiment the coil was positioned perpendicularly to the trajectory of the musculocutaneous nerve (induced current vertical to the nerve in the medio-lateral direction) (see Fig. 2.6). In a supplementary experiment while participants were seated in the new force-rig (see Fig. 2.2), three different coil orientations were used to test differences in the evoked resting twitches. The coil was placed with its handle in three different directions, i) perpendicular to the nerve, towards the outer part of the arm (induced current in latero-medial direction (L-M)), ii) towards the shoulder (induced current parallel to the musculocutaneous nerve in a proximal to distal direction (P-D)) and iii) towards the body (induced current perpendicular to the nerve in a medio-lateral direction (M-L) (Fig. 3.1). After the supramaximal intensity was defined for the magnetic stimulation as described in section 2.4.4.1 and while participants were seated with their arm rested in the new rig with elbow in 90^{o} flexion, 5 resting force twitches were evoked with magnetic stimulation at each of the coil orientations.

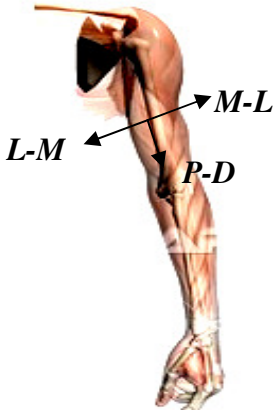


Figure 3.1: Direction of the induced current at three different coil orientations. The arrows indicate the direction of the induced current: i) latero-medial (L-M), ii) proximal to distal (P-D), and iii) medio-lateral (M-L). The photo has been downloaded by www.photosearch.com, k0094599).

- **Matched Amplitude of Electrically and Magnetically Evoked Resting Twitches**

To test whether the change of the superimposed twitches with the level of voluntary force followed the same trend between the two methods of stimulation, the intensity of the electrical stimulation was adjusted to match the resting twitches evoked by the magnetic stimulation intensity used. The same protocol was followed as in the main experiment (see section 2.4.4.1 and Fig. 2.7). The orientation of the coil was perpendicular to the musculocutaneous nerve positioned so that the induced current was in L-M direction.

- **Position of the Stimulating Electrodes**

An alternative electrodes position was also tested in that experiment. Parallel with the motor point-tendon placement (see section 2.4.3) both electrodes were also placed over the BB muscle belly with the cathode specifically positioned over the motor point. This placement aimed to test whether by reducing the distance of the electrodes there was more selective stimulation of the BB through minimizing current spread and reducing coactivation of synergistic and antagonist muscles. After the supramaximal intensity had been defined and while participants rested their arm in the new force rig (see Fig. 2.2), 5 resting twitches were evoked by electrical stimulation in each of the two electrode pair configurations.

- **Matched Electrical and Magnetic Pulse Duration**

The electric current used in the Twitch Interpolation Technique in the main experiment shared the characteristics of a constant current with 1000 μs pulse duration and delivered via single stimuli in supramaximal intensity (see also section 2.4.3). During a supplementary experiment, the duration of the single pulse was reduced to 200 μs to more closely match the characteristics of the magnetic stimuli which have a reported induced current duration of 250 μs (Magstim rapid operating manual, 1355/1450-23-11). This electrical current pulse duration was the best that could be achieved from the range of durations available (e.g., 50, 100, 200, 500, 1000 and 2000 μs) of the Digitimer stimulator available. After the supramaximal intensity was determined using the shorter electrical stimulation pulse width, the same protocol was followed as in the first study (see section 2.4.4.1 & Fig. 2.7).

3.2.4 Data Analysis

The twitch forces evoked at different levels of voluntary contraction were normalized as a percentage of each participant's MVC. EMG signals and force twitches evoked by electrical and magnetic stimulation were analyzed as outlined in sections 2.4.2 and 2.4.4.1. Whenever applicable, voluntary activation was calculated by the formula: *voluntary activation=100×(1- superimposed twitch / control twitch)*, where the superimposed twitch is the force increment evoked during a voluntray contraction at the time of the stimulation and the control twitch is that evoked by identical nerve stimulation in relaxed muscle (Shield & Zhou, 2004). Additional statistical analysis was performed as described in section 2.6. The results are presented in tables and graphs as mean values ± standard deviation (SD) or ± standard error of mean (SEM).

3.3 Results

3.3.1 Main Experiment

A two-way repeated measures ANOVA revealed that there was a significant main effect of the mode of stimulation on the twitch force evoked and that the twitches evoked by electrical stimulation were significantly larger than those evoked by magnetic stimulation ($F_{(1, 10)}$=4.972, p=0.05, Partial Eta Squared=0.332) (Fig. 3.2). Furthermore, there was a significant interaction effect between the type of stimulation used and the level of voluntary force ($F_{(2.27, 22.69)}$=5.54, p=0.009, Partial Eta Squared=0.357). This indicates that the twitch forces changed differently as the level of voluntary contraction increased depending on the type of stimulation used. Further statistical analysis via Generalized Estimating Equations (GEE) analysis for modelling the relation between the twitches evoked by each kind of stimulation and the level of voluntary contraction showed that the trend of changes of the twitches evoked by electrical stimulation followed a cubic curve as the level of voluntary contraction increased: $Twitch\%MVC = 12.54 - 0.14EForce - 0.0009EForce^2 + 0.00001EForce^3$ (95%CI=1.73×10^{-06}–2.1×10^{-05}), p<0.021, where twitch%MVC are the interpolated twitches normalized to every subject's MVC and EForce is the Voluntary Force produced by the participant when electrical stimulation used. In contrast, for magnetic stimulation the relationship fitted a quadratic trend:

$Twitch\%MVC = 9.8 - 0.18\ MForce + 0.0009MForce^2$

(95%CI= 6×10^{-04} – 1×10^{-03}), p<0.001, where MForce is the voluntary force produced by the participant when magnetic stimulation used.

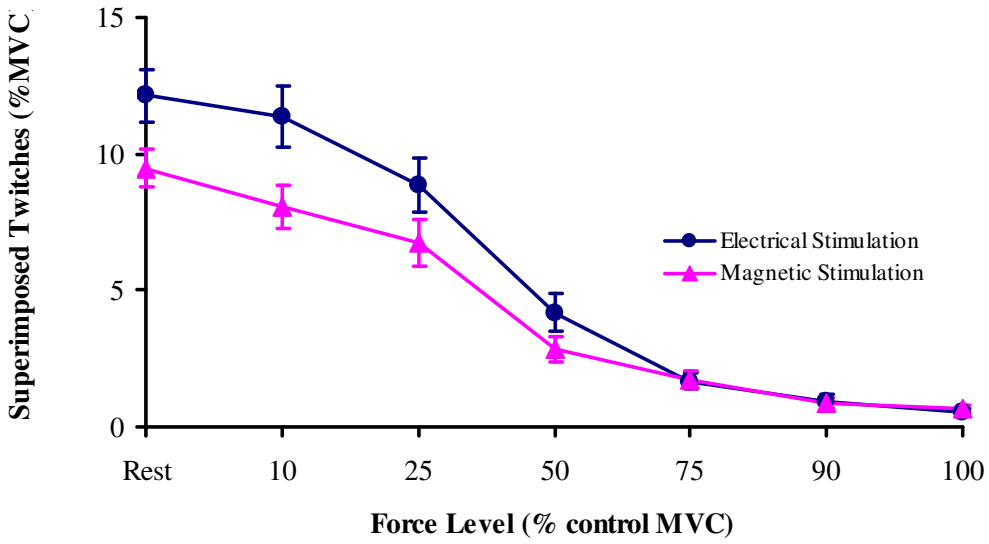


Figure 3.2: The changes of the amplitude of the twitch force (%MVC) (mean ± SEM) evoked by Electrical and Magnetic Stimulation at different levels of voluntary contraction (0, 10, 25, 50, 75, 90, 100%MVC) (Summarized data from n=12).

3.3.1.1 Similarities between the Two Methods of Stimulation

- **Evoked Twitch Forces as the Level of Voluntary Contraction Increases:**

As the level of voluntary contraction increased the superimposed twitches evoked by both kinds of stimulation decreased (Fig. 3.3). The mean resting evoked twitch was about 10% of MVC and significantly reduced to 0.6% of MVC (almost negligible) during MVCs. The amplitude of the twitches evoked at MVCs did not differ significantly between electrical and magnetic stimulation (mean difference = 0.1 ± 0.62% MVC, $t_{(11)} = -0.7$, p=0.5, paired samples t-test).

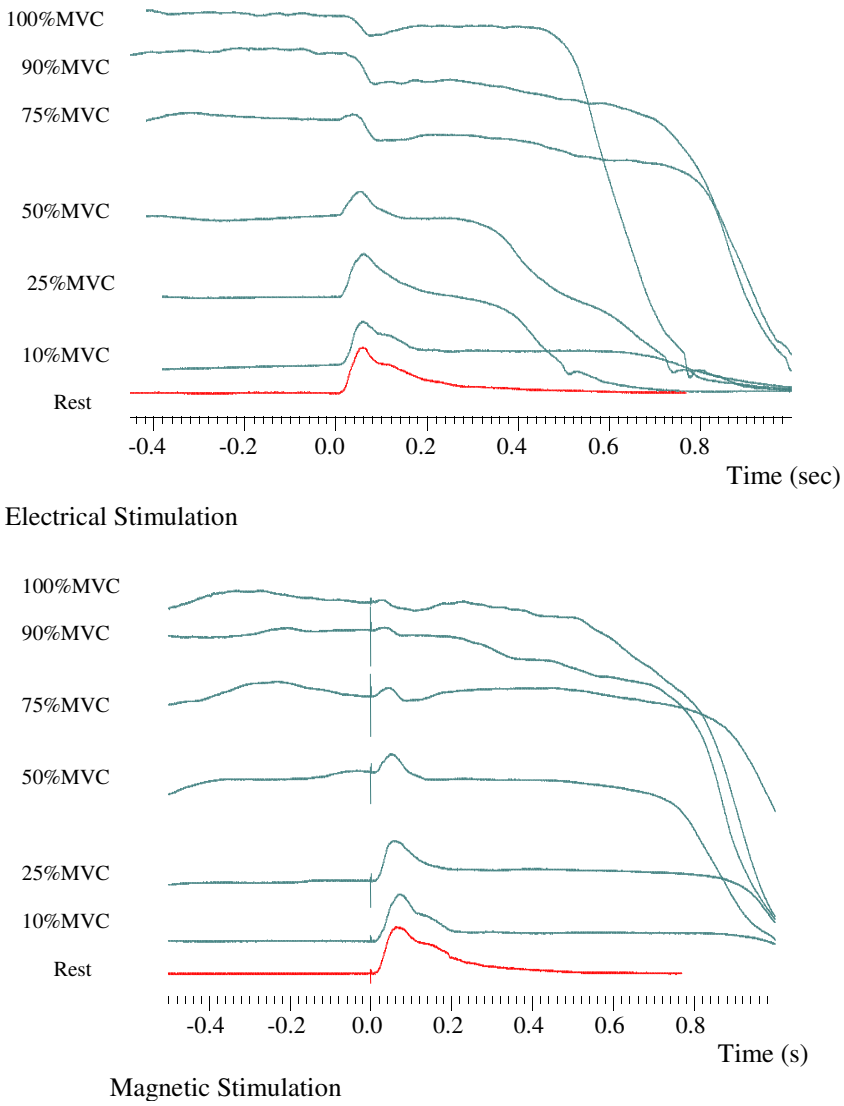


Figure 3.3: Superimposed Twitches evoked by Electrical and Magnetic Stimulation at different levels of voluntary contraction (0, 10, 25, 50, 75, 90, 100%MVC). The red lines represent resting twitches. At time point zero is the point of motor nerve stimulation. The background voluntary contraction is present for 50msec before the stimuli. Representative single trials of each force from one participant.

- **Background EMG of Agonist & Antagonist Muscles:**

The level of maximum voluntary contraction (MVC) was constant during the two sessions of different mode of stimulation ($t_{(11)} = -0.019$, p=0.985, paired samples t-test), while the EMG amplitude of BB, determined for a time period 100ms before the stimuli, was the same during the two series of stimulation as revealed by Repeated Measures ANOVA ($F_{(1, 10)} = 0.05$, p=0.830, Partial Eta Squared=0.005). Additionally, the background EMG activity of BB was significantly increased as the level of

voluntary contraction increased, ($F_{(6, 60)}$ =29.58, p<0.001, Partial Eta Squared=0.747) but the way of increment did not differ between the two modes of stimulation. ($F_{(6, 60)}$ =0.62, p=0.715, Partial Eta Squared=0.058) (Fig. 3.4). The EMG activity of the antagonist TR remained low compared to BB EMG activity even at maximum contractions (mean rmsEMG: 0.044 ±0.03(SD) mV during electrical stimulation (30% of BB EMG) and 0.042 ±0.02(SD) mV during magnetic stimulation (28% of BB EMG). Furthermore, this activity was not different between the two types of stimulation ($t_{(11)}$=0.686, p=0.507, paired-samples t-test).

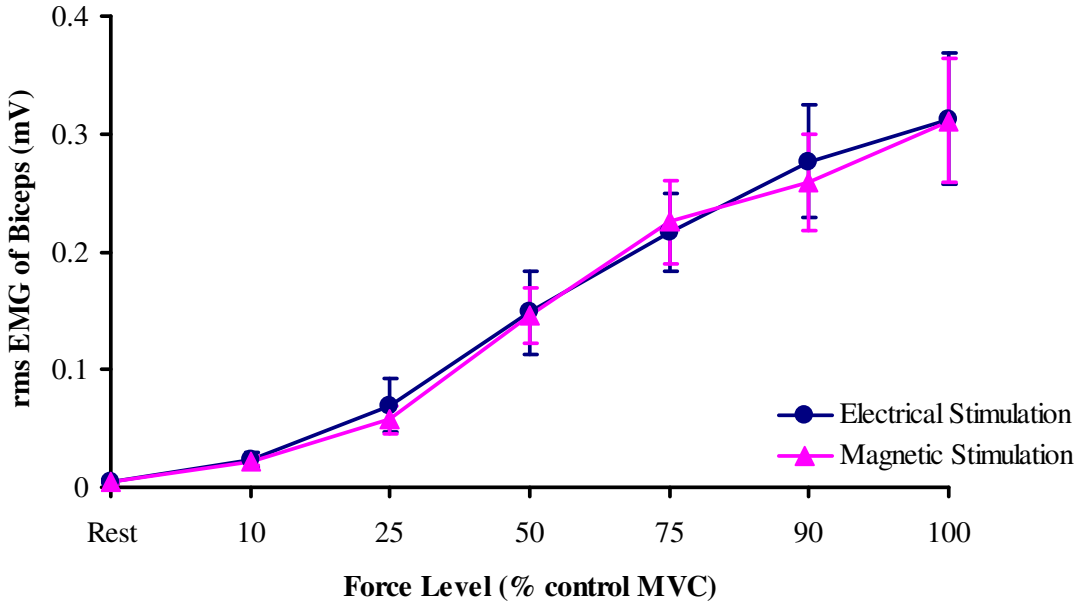


Figure 3.4: The background EMG activity of BB with the level of voluntary contraction. The graph presents mean amplitude at every force level ± SEM (n=12).

- **MVC during main experiment**

The MVC did not change significantly due to repeated voluntary contractions as compared in before and after the electrical stimulation session ($t_{(6)}$ =0.79, p=0.461, paired samples t-test) and the magnetic stimulation session ($t_{(6)}$ =0.07, p=0.947, paired samples t-test). Thus, no pronounced effect of fatigue was present.

- **M-waves of BB and APB at rest:**

The mean evoked M-waves of BB were not significantly different between the two modes of stimulation (mean difference=0.01 ± 2.8(SD) mV.ms, $t_{(8)}$ =0.11, p=0.991, paired-samples t-test) (Fig.3.5). Likewise, the mean evoked M-waves of APB did not differ significantly between electrical and magnetic stimulation (mean difference=3.85 ±9.42(SD) mV.ms, $t_{(10)}$=1.35, p=0.205, paired-samples t-test) (Fig. 3.5).

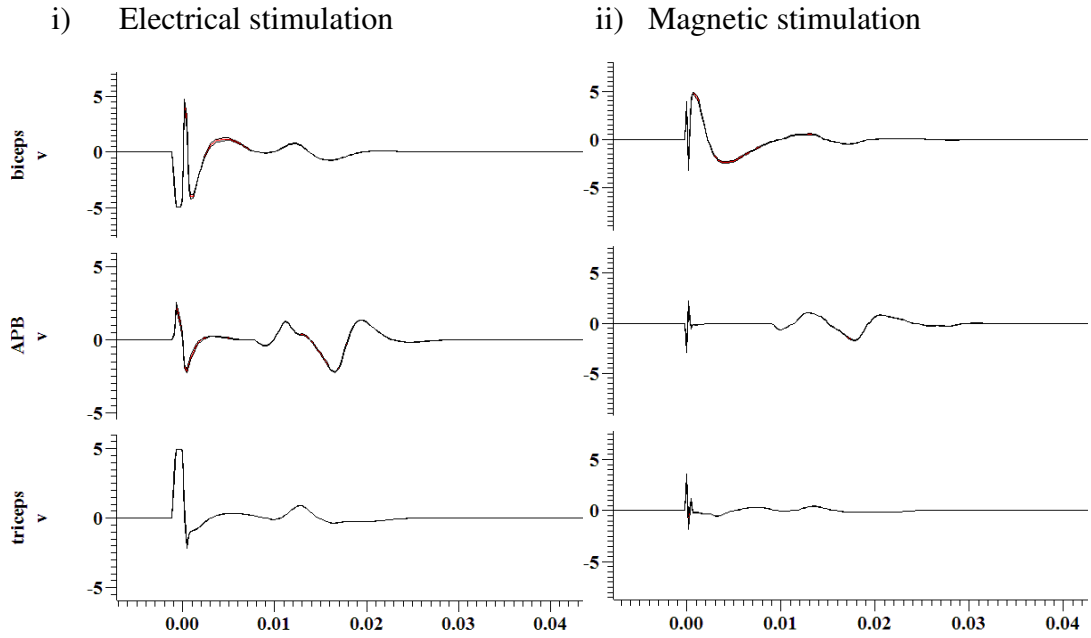


Figure 3.5: M-waves of BB, APB & TB at rest i) during electrical stimulation of biceps motor nerve (average (±SD) from 17 resting twitches) ii) during magnetic stimulation of biceps motor nerve (average (±SD) from 21 resting twitches). Data from one participant.

- **M-Waves of BB during voluntary contraction**:

Due to the influence of the stimulus artefact, the M-waves of BB were analyzed only in seven out of the 12 subjects, as it was not possible to remove the stimulus artefact from the data in these experiments. The Two-Way Repeated Measures ANOVA revealed no significant differences between the two types of stimulation for the BB M-waves ($F_{(1, 6)}$=0.604, p=0.466, Partial Eta Squared=0.092). Additionally, the M-waves did not change significantly as the level of voluntary contraction increased, ($F_{(1.5, 9)}$=1.23, p=0.323, Partial Eta Squared=0.170) and there was no significant interaction effect between the level of voluntary contraction and mode of stimulation ($F_{(2.2, 13.13)}$=1.898, p=0.187, Partial Eta Squared=0.240) (Fig. 3.6).

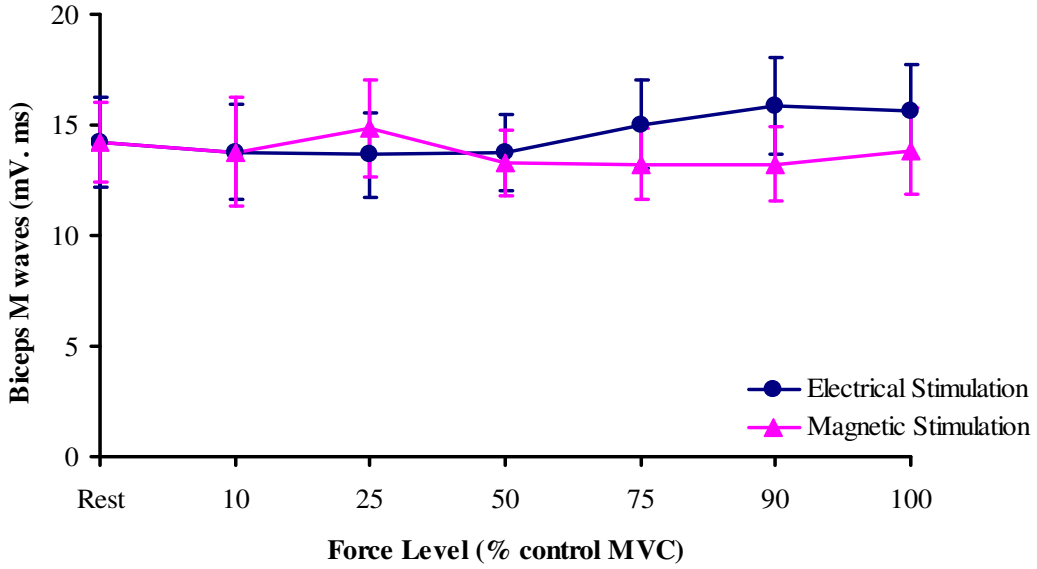


Figure 3.6: Changes of the BBs M-Wave area (mV. Ms ± SEM) at different levels of voluntary contraction (0, 10, 25, 50, 75, 90, 100%MVC) during electrical and magnetic stimulation (n=7).

- **Voluntary Activation of BB**

Two-Way Repeated Measures ANOVA revealed that voluntary activation of BB significantly increased with the level of voluntary contraction ($F_{(6, 60)}$=307.407, p<0.001, Partial Eta Squared=0.968). Additionally, the type of stimulation did not have any significant effect on voluntary activation of BB ($F_{(1, 10)}$=0.245, p=0.632, Partial Eta Squared=0.024) and the interaction effect between voluntary force level and mode of stimulation was not statistically significant ($F_{(6, 60)}$=1.269, p=0.285, Partial Eta Squared=0.113). During maximum contractions the mean voluntary activation of biceps determined using electrical stimulation was 95% (range 90%–99.8%) while that determined using magnetic stimulation was 92% (range 90%–98.9%). Maximum voluntary activation of BB was not significantly different between electrical and magnetic stimulation ($t_{(11)}$=1.954, p=0.077, paired-samples t-test). Figure 3.7 shows the voluntary activation of biceps with the evoked twitches for both methods of stimulation. This figure shows the nonlinear relationship of voluntary activation for the evoked twitches and the level of voluntary contraction. This is in agreement with the non-linearity of the evoked twitch force/voluntary force relationship (see above: section 3.3.1.1).

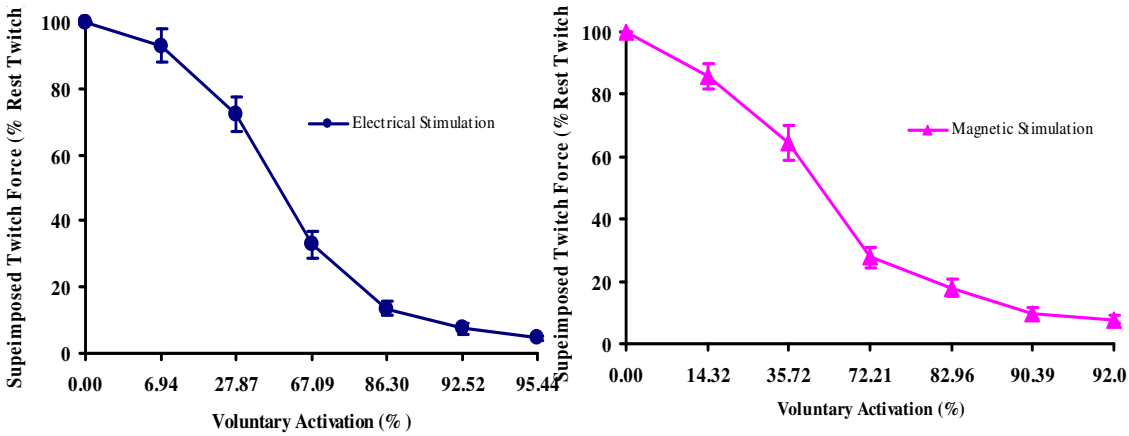


Figure 3.7: Changes of the evoked twitches with the level of voluntary activation of BB (mean±SEM), during electrical and magnetic stimulation (n=12).

3.3.1.2 Differences between the Two Methods of Stimulation

- **Supra-maximality using Magnetic Stimulation**

For 7 of the 13 participants the determined supramaximal stimulus intensity (20% above maximum from stimulus response data) was greater than the maximum magnetic stimulator output. Because it is not possible to exceed this using the magnetic stimulation equipment, 100% of stimulation output was used here, and therefore this limitation may confound comparison between the two methods of stimulation used here. The mean supramaximal intensity used here was 93 ±11%(SD) (n=13).

- **Resting Superimposed Twitch Force**

The mean resting twitch force amplitude (Table 3.1) evoked by the electrical stimulation (16.87 ± 1.22(SD) N) was significantly larger than that evoked by the magnetic stimulation (12.8 ± 0.94(SD) N), (z= –7.709, p<0.001, Wilcoxon test). Indeed, the twitch force evoked by electrical stimulation was 12.15 ± 3.6(SD) %MVC (120% of maximal evoked twitch) and decreased to 0.53 ±0.4(SD) %MVC when participants maximally contracted. The magnetically evoked resting twitches were 9.27 ± 2.5(SD) %MVC (90% of maximal evoked twitch) and reduced to 0.65 ±0.5(SD) %MVC during MVC. The time to peak amplitude (TTP) of the resting twitches also differ between electrical and magnetic stimulation (Table 3.1). The resting twitches evoked by magnetic stimulation reach their peak force later than the twitches evoked by electrical

stimulation (mean difference = 12.55 ± 10.58(SD) ms, $t_{(11)} = -4.107$, p=0.002, paired-samples *t*-test).

Table 3.1: Mean (± SD) amplitude of Resting Twitch Forces (N) and time to peak (TTP) (ms) from every subject during Electrical and Magnetic Stimulation.

	Electrical Stimulation		*Magnetic Stimulation*	
Subjects	***Twitch Force (N)***	***TTP (ms)***	***Twitch Force (N)***	***TTP (ms)***
1	7.22 ± 1.09	55.90 ± 8.02	5.73 ± 0.65	90.54 ± 10.76
2	13.24 ± 1.71	73.36 ± 3.07	11.56 ± 1.47	83.97 ± 4.26
3	14.38 ± 1.92	69.95 ± 5.73	11.58 ± 1.31	73.83 ± 4.17
4	17.69 ± 5.01	51.93 ± 2.73	20.66 ± 2.12	79.96 ± 3.20
5	12.65 ± 2.24	79.20 ± 13.95	11.47 ± 1.25	82.69 ± 17.09
6	11.43 ± 1.27	67.41 ± 11.96	6.69 ± 1.62	69.56 ± 12.86
7	40.74 ± 4.52	77.31 ± 8.18	21.71 ± 4.30	81.23 ± 10.65
8	20.43 ± 3.01	51.63 ± 2.01	10.35 ± 1.67	66.52 ± 3.42
9	10.75 ± 1.84	55.69 ± 11.67	10.90 ± 1.30	75.04 ± 5.27
10	14.72 ± 1.89	49.95 ± 3.43	11.96 ± 2.18	64.60 ± 19.59
11	21.11 ± 2.26	49.60 ± 1.40	14.94 ± 2.64	60.41 ± 3.29
12	18.09 ± 2.78	58.22 ± 3.96	16.09 ± 1.29	66.36 ± 2.19
Avg	**16.87 ± 1.21**	**61.68 ± 11.07**	**12.80 ± 0.94**	**74.23 ± 9.06**

- **Resting Triceps M-waves**

The mean M-wave of triceps, evoked by electrical stimulation was significantly greater than that evoked by magnetic stimulation (mean difference=1.00 ± 0.9(SD) mV.ms, $t_{(10)}$ =3.56, p=0.005, paired-samples *t*-test) (See also Fig. 3.5).

- **APB M-waves during Contractions**

Two-Way Repeated Measures ANOVA revealed that the M-waves of APB during electrical stimulation (15.99mV.ms ± 2.36(SEM)) were significantly bigger than those evoked by magnetic stimulation (8.97mV.ms ±1.66(SEM)), ($F_{(1, 10)}$ =17.515, p=0.002, Partial Eta Squared=0.661) (Fig. 3.8). No significant effect of the level of voluntary contraction on the APB M-waves was revealed ($F_{(1.8, 16.32)}$ =2.210, p=0.145, Partial Eta

Squared=0.197), although the way the M-waves changed over the different levels of force was statistically different between the two modes of stimulation as revealed by the significant interaction effect between force level and mode of stimulation ($F_{(2.20,\ 19.79)}$ =7.58, p=0.003, Partial Eta Squared=0.46) (Fig. 3.8). Specifically, the M-waves evoked by magnetic stimulation reduced with the level of contraction from 13.16 ±6.5(SD) mV.ms when the participants were completely relaxed to 7.4 ± 3.74(SD) mV.ms when they performed a maximum contraction (analysis in 11 subjects). In contrast, the APB M-waves were quite stable through the different levels of voluntary contraction during electrical stimulation.

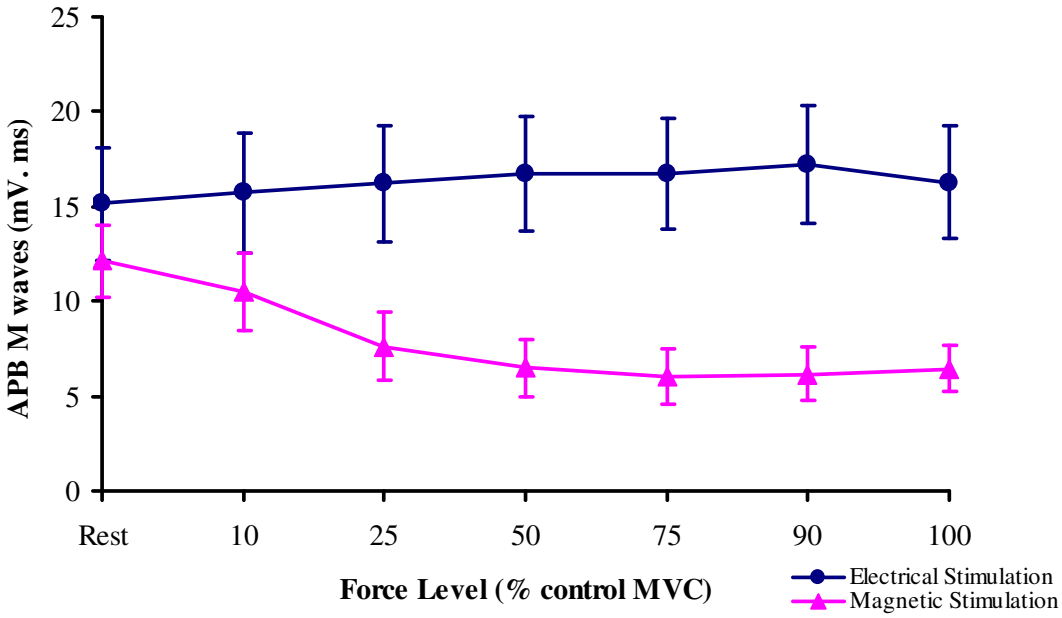


Figure 3.8: The area (mV. ms ± SEM) of the APB M-Waves during electrical and magnetic stimulation (n=11).

3.3.2 Supplementary Experiments

3.3.2.1 Coil Orientation

The coil orientation had a significant effect on the twitch force evoked by magnetic stimulation (supramaximal intensity) at rest ($F_{(2,\ 10)}$=16.02, p=0.001, Partial Eta Squared=0.76, One-Way Repeated Measures ANOVA). Figure 3.9 shows the resting evoked twitches from one participant from the three coil orientations. The greatest force of the resting twitches was evoked when the coil was orientated to induce current in L-M direction (12.7 ± 5.74(SD) N) and it was statistically higher than the force at P-D current direction (mean difference=8.14 ± 1.75(SEM) N, p=0.017). Additionally, the twitch force evoked while the induced current was in M-L direction (9.27 ± 3.33(SD) N) was significantly bigger than the twitch force evoked by magnetic stimulation of induced current at P-D direction (4.56 ± 1.90(SD) N), (mean difference=4.70 ±0.99(SEM) N, p=0.015). Pairwise comparisons of Repeated Measures One-Way ANOVA revealed that the difference of the twitch force evoked by the magnetic stimulation when the direction of the induced current changed from L-M to M-L did not reach statistical significance (mean difference=3.44 ±1.49(SEM), p=0.207).

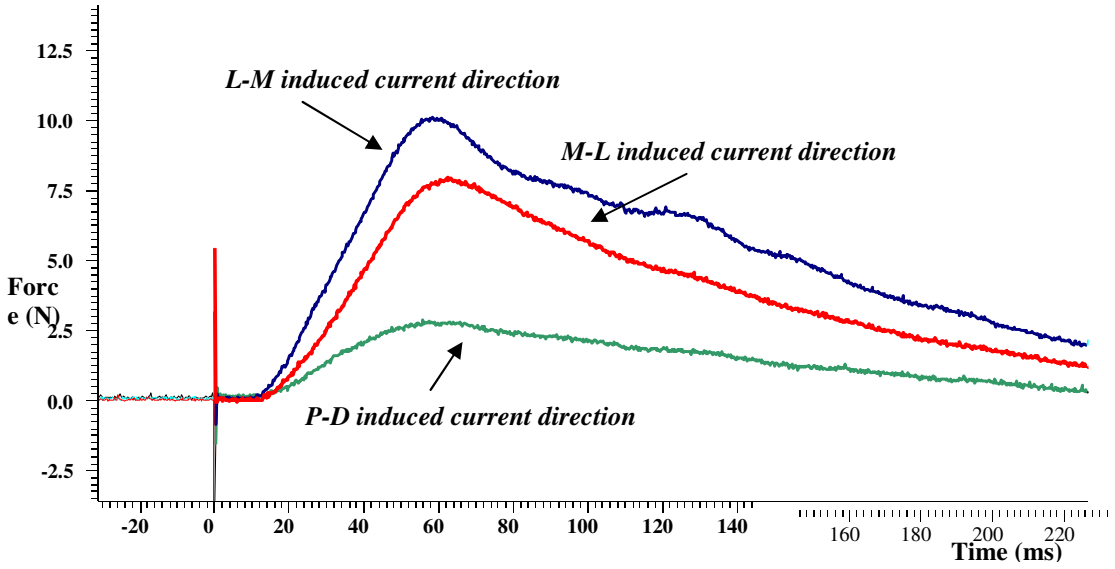


Figure 3.9: Force twitches (N) evoked by magnetic stimulation (100% of stimulator output) at 3 coil orientations so as the induced current was directed towards the shoulder (P-D), towards the outer part of the arm, perpendicular to musculocutaneous nerve (M-L) and towards the inner part of the arm, perpendicular to the trajectory of musculocutaneous nerve (L-M). Every trace is the average of 5 twitches at every coil orientation. Data from one participant.

3.3.2.2 Placement of Stimulating Electrodes

The distance of the stimulating electrodes also had a significant effect on the resting twitch force evoked by electrical stimulation. Paired-Samples *t*-test revealed that the mean resting twitch force evoked by electrical stimulation with the standard-wide placement of electrodes (25.8 ± 9.4(SD) N) was significantly greater than the twitch force evoked by stimulation of closed spaced electrodes (19.3 ± 9.3(SD) N) (mean difference=6.5 ± 6.2(SD) N, $t_{(5)}$=2.59, *p*=0.049, paired samples *t*-test).

3.3.2.3 Effect of Intensity of Electrical Stimulation

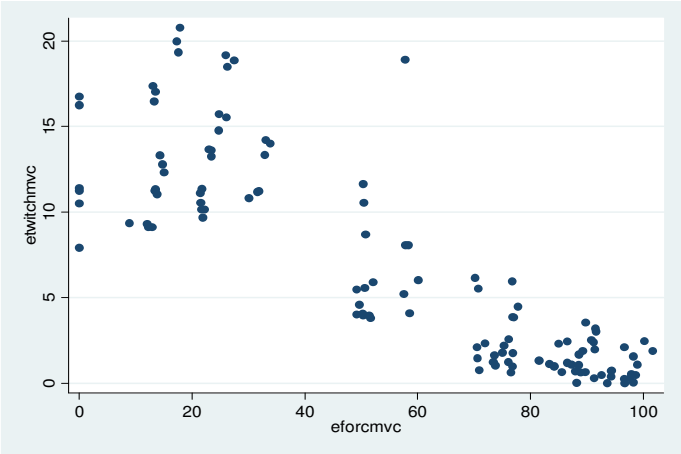
When the intensity of the electrical current was reduced (submaximal) to evoke resting twitches of the same amplitude of those evoked by magnetic stimulation of supramaximal intensity, differences were revealed again between magnetic and electrically evoked twitches with the level of voluntary contraction (Fig. 3.10). The twitches evoked by electrical stimulation were significantly bigger than those evoked by magnetic stimulation (mean difference =2.67 ± 0.7(SEM) %MVC, *p*=0.013, pairwise comparisons of Two-Way Repeated Measures ANOVA). Additionally, the amplitude of the superimposed twitch force evoked at MVCs was statistically different between electrical and magnetic stimulation (mean difference=0.13 ± 0.84(SD) %MVC, $t_{(5)}$=0.38, *p*=0.72, paired-samples *t*-test). Despite the absence of differences in the resting twitches (mean difference=2.15 ± 2.34(SD) %MVC, $t_{(5)}$=2.26, *p*=0.073, paired-samples *t*-test) a significant interaction effect was revealed between the type of stimulation and the level of voluntary contraction ($F_{(2.17, 10.84)}$= 8.54, *p*=0.005, Partial Eta Squared=0.631, Two-Way Repeated Measures ANOVA). When the superimposed twitches evoked by electrical stimulation the relation between force peak amplitude and voluntary force followed a linear curve, as found by GEE analysis:

Twitch %MVC=15.95 – 0.165 EForce (95%CI= – 0.20 – 0.13), *p*<0.001,

where Twitch%MVC is the amplitude force of the superimposed twitches normalized to every subject's MVC and EForce is the level of voluntary force (% MVC) (Fig. 3.10i). In contrast, when magnetic stimulation was used, the relationship between force amplitude and voluntary force fitted a quadratic curve:

$Twitch\%MVC=11.07–0.16MForce+0.00048MForce^2$, (95%CI=$2.2\times10^{-4}$ 7.3×10^{-4}), *p*<0.001, where MForce is the level of voluntary force (% MVC) when magnetic stimulation was delivered to BB (Fig. 3.10ii).

i) Electrical Stimulation



ii) Magnetic Stimulation

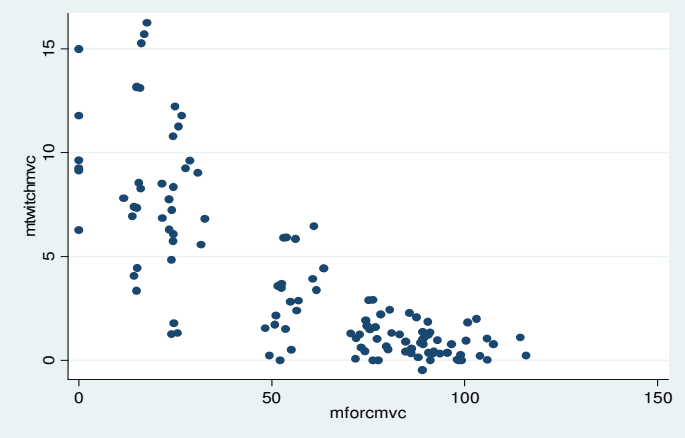


Figure 3.10: Relationship of the evoked twitches (%MVC: y-axis) to voluntary force (%MVC: x-axis) with submaximal intensity of electrical stimulation. i) during electrical stimulation (submaximal intensity) the force amplitude of the twitch superimposed twitches reduced linearly with the level of voluntary force, ii) during magnetic stimulation (supramaximal intensity) the force amplitude reduced in a quadratic curve as the level of voluntary force increased (n=6).

3.3.2.4 Effect of Reduced Electrical Stimulation Duration

When the duration of the electrical current was reduced from 1000 μs to 200 μs, the resting twitches evoked by electrical stimulation were not significantly different in amplitude than those evoked by magnetic stimulation (mean difference 1.6 ± 2.33(SD) %MVC, $t_{(4)}$ =1.5, p=0.20, paired samples t-test, two tailed). The time to peak for the resting twitches force also did not differ significantly between the two modes of stimulation (mean difference = 2.6 ± 4.45(SD) ms, $t_{(4)}$ = –1.30, p=0.26, paired samples t-test). Mean values of evoked twitches (%MVC) at rest from every subject between electrical and magnetic stimulation and the time to peak are presented in Table 3.2.

Table 3.2: Mean amplitude of Resting Twitch Forces (%MVC) and the time to peak (TTP) amplitude (ms) during Electrical and Magnetic Stimulation with matched pulse duration. The values presented as mean ±SD (n=5). The averages mean amplitude and TTP of the resting twitches from all subjects is also included.

	Electrical Stimulation		Magnetic Stimulation	
Subj	***Twitch Force (%MVC)***	***TTP (ms)***	***Twitch Force (%MVC)***	***TTP (ms)***
1	11.16 ± 1.13	62.46 ± 2.13	10.56 ± 0.88	62.50 ± 2.54
2	14.73 ± 1.43	59.00 ± 1.44	10.59 ± 1.35	66.40 ± 3.97
3	14.03 ± 0.94	58.02 ± 1.84	13.91 ± 1.28	58.32 ± 2.05
4	6.75 ± 0.60	61.52 ± 1.80	7.67 ± 0.89	68.90 ± 5.90
5	11.14 ± 1.07	64.70 ± 1.45	7.15 ± 0.74	62.60 ± 1.59
Avg	**11.56 ± 3.14**	**61.14 ± 1.73**	**9.98 ± 2.71**	**63.74 ± 3.21**

Additionally, the mode of stimulation used did not have any significant effect on the force twitches evoked when participants produced different levels of force ($F_{(1.00, 4.00)}$= 3.73, p=0.126, Partial Eta Squared=0.483). In contrast, the level of voluntary force had a significant effect on the superimposed twitch force ($F_{(1.48, 5.95)}$= 74.83, p<0.001, Partial Eta Squared=0.949). As the level of voluntary force increased the superimposed twitch force evoked by the stimulation decreased significantly (Fig. 3.11). Furthermore, there was a non significant interaction effect between type of stimulation and level of voluntary force ($F_{(1.30, 5.19)}$= 2.56, p=0.17, Partial Eta Squared=0.39), indicating that the superimposed twitch force changed in the same way as the level of voluntary force increased regardless of the type of stimulation used (Fig.3.11). Indeed, GEE analysis showed that the relationship between superimposed twitch force and level of voluntary contraction followed a cubic curve during both electrical and magnetic stimulation. For electrical stimulation this relationship is represented by the formula:

$Twitch\%MVC = 3.27 - 0.03EForce + 0.11EForce^2 - 0.004EForce^3$

(95%CI= 4.7×10^{-3}, 3×10^{-3}), p<0.001, where twitch%MVC are the superimposed twitches normalized to every subject's MVC and EForce is the Voluntary Force produced by the participant when Electrical stimulation was used. Similarly, for magnetic stimulation the relationship fitted a cubic trend:

$Twitch\%MVC = 10.57 – 0.09\ MForce – 0.002MForce^2 + 0.00001EForce^3$

(95%CI= 1.43×10^{-6}, 2.6×10^{-5}), p=0.029, where MForce is the voluntary force produced by the participant when magnetic stimulation was used.

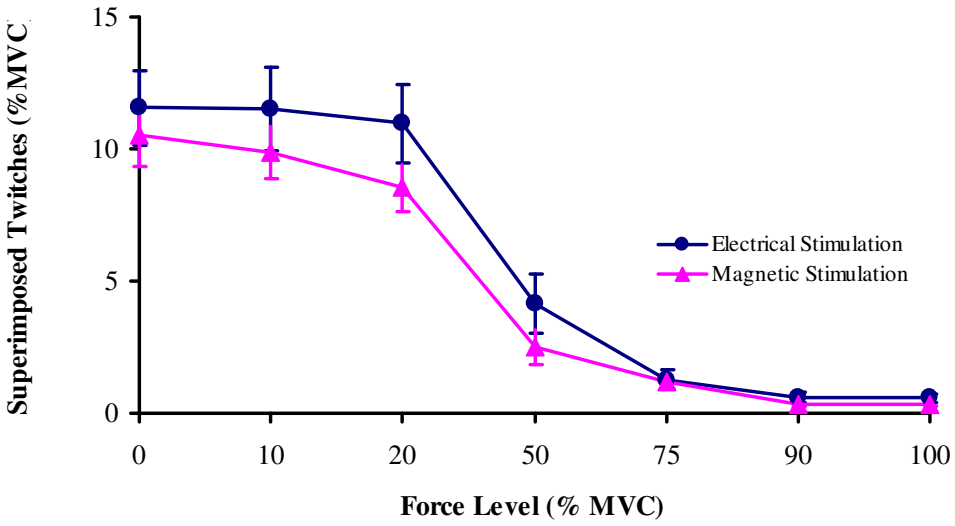


Figure 3.11: Change of the force amplitude of the superimposed twitches with the level of voluntary contraction during electrical and magnetic stimulation. Summarized data from 5 participants (mean ± SEM). The electrical current's pulse duration has been reduced to 200µs to match with the duration of magnetic current.

3.3.3 Summary of Results

- The amplitude of the evoked twitches decreased as the level of voluntary contraction increased in a non-linear trend during both electrical and magnetic stimulation. At maximum contractions the twitches were almost negligible (0.6%MVC). Additionally, the M-waves of BB evoked by electrical and magnetic stimulation did not differ significantly.
- The twitches evoked by magnetic stimulation were consistently smaller in amplitude and reached their peak 13 ms later than those evoked by electrical stimulation.
- The amplitude of the evoked resting twitches was affected by placement of the stimulating electrode pairs (closely spaced versus widely spaced), and for magnetic stimulation, by the orientation of the induced current produced when the magnetic coil was perpendicular or parallel to the general orientation of the trajectory of the underlying nerve.
- The curve fitting of twitch versus voluntary force relationship remained different between magnetic and electrical stimulation even when electrical stimulation was reduced to submaximal intensities to closely match the amplitude of the resting twitch evoked by the supramaximal (120%max) magnetic stimulation.
- Reduction of the electrical stimulus pulse duration from standard 1000 μs to 200 μs resulted in reduced twitch amplitude which more closely matched the evoked twitch produced by magnetic stimulation (120%max intensity, 250 μs pulse duration). Both twitch/voluntary force profiles were best described by a cubic function, and the resting twitches did not differ significantly between these two methods of stimulation.

3.4 Discussion

The main findings of this study point towards significant differences between use of peripheral magnetic and electrical stimulation in conventional single pulse Twitch Interpolation Technique for biceps as the main elbow flexor. Although both techniques evoked twitches which reduced with the level of voluntary force in a way that very small, almost negligible twitch forces were evoked when the muscle was maximally contracted, the resting twitch forces evoked by electrical stimulation (120% of max) were significantly bigger than those evoked by magnetic stimulation (90% of max). Comparison of measures of M-waves evoked by both methods of stimulation for the biceps were not significantly different, however the M-waves evoked by electrical stimulation recorded from the triceps, the elbow antagonist, were bigger than those evoked by magnetic stimulation. Additional key experiments examined the stimulus characteristics of the two methods of stimulation.

Reduction in current duration of electrical stimulation to more closely match the duration of magnetic stimulation carried out by standard methods, did produce both amplitudes which were smaller, resting twitches of similar amplitude and curve fitting of twitch versus voluntary force relationship which was similar between the two methods of stimulation. Manipulation of these parameters for the electrical stimulation, including electrode positioning are all easily carried out with this method of stimulation for twitch interpolation. However, these cannot be modified with magnetic stimulation where the induced current duration is fixed by the method of electromagnetic induction characteristics of the equipment used. Alternative approaches of evoking twitches using magnetic stimulation may be possible. For example pairs of magnetic stimulation pulses could be used with short interpulse delays (10-30ms) in a manner similar to dual paired electrical stimulation. However this was not investigated in this study. The parameters which can be changed for magnetic stimulation under the present experimental conditions, including the coil orientation with respect to the nerve trajectory, were also explored here. The findings are consistent with previous studies which have shown that orientation of the induced current is directional (Sommer & Paulus, 2008). Coil placement is also critical (Sun *et al*., 1998). The differences in the M-wave evoked from distal APB with magnetic but not electrical stimulation strongly suggests that an

increase in the distance between the surface coil position and the underlying median nerve when the muscle is contracted, could account for these findings. This is a likely explanation given that the known relationship between the magnitude of the induced current is proportional to the distance from the source of the magnetic pulse produced by the coil (Epstein, 2008), which is positioned on the surface of the upper arm.

3.4.1 Explaining the Differences between Magnetic and Electrical Stimulation

3.4.1.1 Resting Evoked Twitches

This study is the first to report differences in the electrically and magnetically evoked resting twitches and to examine some of the parameters which may lead to those differences. Other studies which compared the two techniques suggest magnetic stimulation as a suitable alternative to electrical stimulation because of the similar evoked twitches at maximum contractions (Man *et al*., 2004; Harris *et al*., 2000; Hamnegård *et al*., 2004; Cros *et al*., 1992). However, these were used for different muscles which may share different inherent physiological properties and therefore, their responses to electrical or magnetic stimulation may be markedly different (Clamann, 1993 cited in Miller *et al*., 1999). Some of the differences in the resting evoked twitches using electrical stimulation could be a function of the stimulus intensity-duration characteristics as well as a result of the wide spread of the electrical current. Differences in the type of neural fibres activated and consequently in the type of motor units recruitment by each type of stimulation (Lotz *et al*., 1989) may also account for the differences between electrical and magnetic stimulation. The resting twitches evoked by electrical stimulation reached their peak 13 ms earlier than magnetically evoked twitches supporting the above assumption. The differences found in the time to peak cannot be fully explained, however they may indicate that magnetic stimulation activates preferably the slower small-diameter alpha motor neurons innervating slow fatiguing muscle fibres, while electrical stimulation activates the faster-conducting motor neurons. Temporal differences between magnetic and electrical stimulation have also been revealed in respiratory studies, when the phrenic nerve was tested (Similowski *et al*., 1997).

3.4.1.2 Supramaximality of Magnetic Current

A possible explanation of the difference could be in that it is not possible to produce a similarly matched, supramaximal magnetic stimulation intensity under the particular experimental conditions. Seven of the thirteen participants required a stimulus-intensity for 120% of maximum which exceeds the stimulator intensity maximum (an arbitrarily linear scale from 0 to 100% of the 2.2T max magnetic field intensity produced by conventional stimulator). Therefore 100% MSO was used which may not have been truly supramaximal intensity in 50% of the sample of participants in this study. This is not in agreement with the study of Hamnegard and his colleagues (2004) who reported that only two of the 45 participants were not demonstrably supramaximally stimulated when femoral nerve stimulation was performed with a 70mm double circular coil. Our results are also different from those of Harris and co-workers (2000) who reported supramaximality in all the healthy participants when magnetic stimulation was tested over adductor pollicis using a 45mm magnetic coil. Supramaximality was also reported when magnetic stimulation with a 45mm figure of eight coil used, for assessing quadriceps strength (Polkey *et al*., 1996). The contradictory results may be due to different muscles tested and the different size and geometry of coil used indicating that supramaximality of magnetic stimulation for peripheral twitch interpolation may be highly dependent on the size of the coil and the type of nerves and muscles examined in human research and clinical monitoring.

3.4.1.3 Orientation of Magnetic Coil

Although the duration-intensity characteristics of the conventional magnetic stimulators cannot be altered to match those of electrical current, positional alterations which affect the direction of the induced current with respect to peripheral nerve and anatomical fibre trajectory should be considered when magnetic stimulation is to be used in the Twitch Interpolation Technique. The orientation of the magnetic coil plays a significant role in the stimulation effect to the nerve (Maccabee & Amassian, 2008). The present study revealed that the perpendicular direction of the induced current in relation to the musculocutaneous nerve trajectory, regardless of the specific current flow (clockwise or counter clockwise), was the most efficient for evoking the biggest twitch forces when the muscle was completely relaxed. These results are in agreement with those of Sun

and his colleagues (1998) who reported that the stimulation effect on the median nerve at the elbow was the highest when the current, induced by a small (32 mm outer diameter) figure of eight-shaped magnetic coil, flowed perpendicularly to the nerve. Results from other studies however disagree with the present findings and indicate that a single nerve is more excited when the current flow is in a longitudinal direction along the nerve (Maccabee *et al*., 1990 cited in Sun *et al*., 1998; Harris *et al*., 2000). These contradictory results were probably due to the different dimensions of the coil used in every study, the different muscles examined and the differences in the position of the limb (Calder *et al*., 2005). The reasons for the difference in the twitches evoked by various coil orientations are not completely understood. They could be explained by differences in the distribution of the current within the nerve or that at various directions different subsets of the neural tissue are preferentially activated (Orth & Rothwell, 2004).

The absence of significant differences between M-L and L-M direction of the induced current while perpendicular to the nerve trajectory may be due to the biphasic stimulator used. For a biphasic wave, the induced current in the neural tissue, flows in both clock- and anti-clockwise directions (Orth & Rothwell, 2004; Sommer & Paulus, 2008) and therefore, may cause the same results in both directions. Furthermore, with the double round coil the field is symmetrical around the centre of the coil regardless the direction of the induced current (Sun *et al*., 1998) which may also explain why the twitches did not differ significantly when the axis of the coil remained vertical to the nerve, but the direction changed from medio-lateral to latero-medial.

3.4.1.4 Spread of Electric Current

The motor point-tendon configuration for placement of the stimulating electrodes is the most commonly used, as it provides the most selective way of stimulation of the muscle of interest (Low & A. Reed, 2000a; Shield & Zhou, 2004). However, widely spaced electrodes (more than 5 cm in the case of biceps) increase the degree of current spread to antagonists and may activate both superficial as well as underlying agonists (e.g. brachialis), whereas with magnetic stimulation a much smaller volume of muscle fibre may be activated (Allen *et al*., 1998). This may have led to larger twitches evoked by

electrical stimulation. The M-waves of triceps evoked by electrical stimulation were consistently larger in area in all the subjects in the present study which does suggest greater current spread of electrical stimulation compared to magnetic stimulation. Additionally, the larger M-waves of the APB evoked by electrical compared to magnetic stimulation, at all -apart from resting- levels of voluntary force, points towards a more wide spread application of electrical stimulation than magnetic stimulation. This assumption is supported by the findings of a supplementary experiment, when the resting twitches evoked by electrical stimulation of the close spaced configuration (stimulating electrodes placed closer over the muscle belly) reduced in amplitude to 73% of those evoked with the wide spaced configuration. Additionally, although the possible coactivation of synergists was not recorded in this study, stimulation of brachialis and brachioradialis -as the main synergists in elbow flexion- during the Twitch Interpolation Technique has been reported elsewhere (Allen *et al*., 1998).

3.4.1.5 Intensity of the Electrical Stimulation

Although the intensity of the electrical current reduced to submaximal levels, the twitches evoked by electrical stimulation were again greater in amplitude than those evoked by magnetic stimulation at low levels of voluntary force. Only at levels above 75% of MVC were these differences attenuated. Consequently, the relationship between evoked twitch and voluntary force was also different between the two types of stimulation. These differences could be explained by differences in the effusion of the electric field between the two modes of stimulation. The distribution of the electric field induced by magnetic stimulation is more homogenous and is parallel to the surface of the coil while the electric field of the electrical stimulation flows beneath the electrodes in all directions away from the cathode (Nollet *et al*., 2003). That could result in a wider distribution of the current of electrical stimulation and consequently greater twitches. The linear twitch-voluntary force relationship revealed by electrical stimulation could be explained by less coactivation of the antagonist and synergist due to submaximal intensity. These results however, should be examined cautiously as intensity less than supramaximal could alter the threshold of the motor axons and their terminals, resulting in fewer axons being recruited during contractions. As a result, changes in the axonal excitability could lead to smaller induced twitches (Burke & Gandevia, 1998).

3.4.1.6 Pulse Duration of Electrical Stimulation

The similarities in resting twitches, in M-waves of the biceps and triceps and in curve fitting of twitch-voluntary force relationship, revealed when the duration of the electrical pulse was reduced to match that of magnetic stimulation look very promising. They indicate that, when the current parameters of the two modes of stimulation are manipulated to closely match, the two techniques could be comparable and thus, magnetic stimulation could replace electrical stimulation in the Twitch Interpolation Technique. This is very important for the application of a painless method of assessment of muscle voluntary activation in clinical settings.

3.4.2 Nonlinearity of Twitch-Voluntary Force Relationship

The present study revealed that although the evoked twitch force reduced as the level of voluntary contraction increased, both electrical and magnetic peripheral stimulation failed to show a negative linear relationship as was first proposed by Merton (1954). As has now been suggested by other research since Merton, this relationship is nonlinear (Behm *et al*., 1996; Allen *et al*., 1998; Herbert & Gandevia, 1999; Folland & Williams, 2007), and in this study, can be described by a cubic function for electrical stimulation and a quadratic for magnetic stimulation respectively.

Mechanical and physiological parameters could influence the relationship between evoked and voluntary force. The method of single interpolation twitch has been criticized because of its difficulty in detecting small increments in force at near-maximal contractions (Behm *et al*., 1996; Kent-Braun & Le Blanc, 1996). As an alternative, both doublets or brief trains of stimuli have been used in order to improve the sensitivity of the Twitch Interpolation Technique to study voluntary activation (Kent-Braun & Le Blanc, 1996; Miller *et al*., 1999). The use of a train of stimuli generates larger force increments at MVCs than single impulse stimulation and may be the preferred method for detection of central activation failure during isometric contractions (Miller *et al*., 1999). In the present study trains or doublets of stimuli were not examined as it was the aim of this study to utilize the most commonly used protocol for electrical stimulation which could be then compared to single pulse magnetic

stimulation. While pairs and brief trains of magnetic stimulation are possible, these require more technically advanced equipment which may not be so readily available in a clinical setting for neurophysiological testing.

Additionally, co-activation of other synergistic muscles and their disproportionate contribution in near maximal contractions has been suggested as a contributory factor to nonlinearity (Allen *et al*., 1998; Herbert & Gandevia, 1999; Shield & Zhou, 2004). The contribution of antagonists could also be a significant parameter although the way antagonists affect the decay of the biceps twitch force as the level of voluntary force increases is not yet well understood (Awiszus *et al*., 1997). Additionally, the antidromically propagating action potentials elicited in the motor and sensory axons (Tucker *et al*., 2005) could influence the evoked twitch by colliding with the voluntary produced action potentials and reducing the motoneurons discharge immediately after the stimulus (Herbert & Gandevia, 1999; Shield & Zhou, 2004; Tucker *et al*., 2005). This effect although having its greatest effect at voluntary forces between 40 to 80% of MVC may also cause diminished evoked twitches at voluntary contractions higher than 80% of maximum (Herbert & Gandevia, 1999) and therefore, result in a nonlinear twitch-voluntary force relationship.

3.4.3 Implications for Evaluating Voluntary Activation

For both electric and magnetic stimulation the evoked twitches decayed with increased biceps voluntary activation in a non-linear fashion (see Fig. 3.7). This is in agreement with the nonlinearity of the evoked twitch-voluntary force relationship. Due to this non-linearity, the results about voluntary activation should be considered with caution. They may be misleading as they are based on the use of a formula (see section 3.2.4) that requires the evoked twitches to decrease in a linear way as the level of voluntary force increases. Indeed, studies reported that at low levels of voluntary contraction the twitches evoked by stimulation are disproportionately large compared to control ones (resting twitches) (Behm *et al*., 1996). The same phenomenon has been noticed in this study in some of the participants. The bigger twitches evoked at low levels of contraction, compared to control ones, resulted in negative values for the voluntary

activation –that is paradoxical– and may have caused the convex relationship of twitch-voluntary activation. These negative values of voluntary activation, therefore, questions the validity of the equation used for the assessment of voluntary activation.

During maximum contractions the very small twitches evoked resulted in biceps voluntary activation of 92 and 95% for electrical and magnetic stimulation respectively. This difference was not significant indicating that maximum voluntary activation can be estimated similarly by both electrical and magnetic stimulation. The same curve fitting of voluntary activation-voluntary force also suggests similar sensitivity of the Twitch Interpolation Technique in the assessment of voluntary activation between magnetic and electrical stimulation. The above findings suggest that magnetic stimulation could be used interchangeable to electrical stimulation whenever muscle activation is required. This could be more applicable in clinical settings when the evaluation of the muscle inactivity of patients is the main aim. It might be wiser however for research purposes and whenever the twitches-voluntary force relationship is required that electrical stimulation is used due to its reproducibility at least in normal subjects.

3.4.4 Conclusions

In this study, the twitches evoked by electrical stimulation were greater than those evoked by magnetic stimulation. The more widespread effect of electrical stimulation, perhaps on synergists, may explain part of the differences in the evoked twitches observed here. Due consideration of the anatomical and biomechanical aspects of the joint movement should be paramount. Choice of appropriate limb position for isolating the muscle of interest, choice of muscle groups to study, use of the appropriate duration and intensity of the current could minimize current spread. The inability of the magnetic stimulation to elicit supramaximal intensities in all cases due to manufacturer restrictions may also account for the smaller twitches evoked by magnetic stimulation. This is a limitation that should be taken into consideration when magnetic stimulation is used for assessment of peripheral muscle activity. Although supramaximality is a key limitation for the magnetic stimulation, manipulation of the intensity, the geometry, and the orientation of the coil is essential if maximal responses are desired. The

aforementioned differences provide limitations in the substitution of electrical stimulation with magnetic stimulation for peripheral monitoring of twitch interpolation.

Although magnetic stimulation is unlikely to offer an important advantage over electrical stimulation for subjects who are able to properly perform an MVC, its great advantage of not producing pain and discomfort would allow measurement of muscle strength and voluntary activation in the clinical environment. Most importantly, the similar sensitivity of magnetic stimulation to electrical stimulation in assessing voluntary activation makes this stimulation method a very valuable technique for the detection of central activation failure in disorders of the central nervous system which prevent full muscle activation. The technique could also be of particular value in studies involving rehabilitation or training, as a painless method to monitor possible improvements in voluntary muscle activation. Further research is required to examine the suitability of using peripheral magnetic stimulation for twitch interpolation in studies examining both peripheral and central contributions to fatigue.

CHAPTER 4

RELIABILITY AND VALIDITY OF THE 0–10 NRS IN RATING EFFORT PERCEPTION FOLLOWING ISOMETRIC ELBOW FLEXION: A FEASIBILITY STUDY

4.1 Introduction

Perceived effort is strongly related to neuromuscular activation (McCloskey, 1981) and it is often used to assess fatigue because a subjective feeling of exhaustion is usually connected with increased perceived effort (St Clair Gibson & Noakes, 2004). Hitherto, the 15-point Ratings of Perceived Exertion (RPE) and the modified Borg category-ratio 10-item scale (Borg, 1998) have been the methods most frequently used to quantify perceived exertion (for details, see section 1.2.4.3). Perceived exertion is usually referred to as perceived effort, however, the two terms might not refer to the same concept (see Literature Review: section 1.2.4.3) and therefore, a scale of perceived exertion might be misleading in recording ratings of effort. Additionally, the Borg scales are effectively limited to whole body exercise where a certain (sustained) level of heart rate is achieved (Borg, 1998). Very often, however, it is preferable that less strenuous exercises be used in research and clinical settings for assessing central fatigue and perception of effort (Thickbroom *et al*., 2006; Smith *et al*., 2007). These exercises which include isolated muscles contractions serve the advantage of not straining the cardiopulmonary system to the same extent as whole body exercise and as such are more applicable in cases when patients are unwilling or unable to undertake an exhaustive whole body task. The use of an appropriate scale of rating effort for isolated isometric muscle exercise is therefore of great clinical and research value. Establishment of such a tool is necessary to investigate further the way perception of

effort alters during and following fatigue in healthy people and in patients. Various scales have been used in assessing subjective feelings such as perception of pain. Likert scales (Grant *et al*., 1999), the Visual Analogue Scale (VAS) (Crichton, 2001), and the 0–10 Numeric Rating Scale (NRS) (Williamson & Hoggart, 2005) are some of these scales which are extensively used in rating pain, but none of them have been validated for perception of effort assessment.

Among the others, 0–10 NRS seems to be the most appropriate for assessing the perception of effort (see sections 1.2.4.3 & 1.2.4.3.1). Its practicability, ease of verbal utility and acquisition of interval data made the 0–10 NRS a potential candidate for assessing changes in the perception of voluntary effort during these studies where the subject was required to briefly maintain isometric, isolated elbow force. Although reliable and sensitive in assessing pain (Kendrick & Strout, 2005; Williamson & Hoggart, 2005), the 0–10 NRS has not been tested specifically for its reliability and validity in assessing perception of voluntary effort during isometric contractions.

Thus, the main aim of this study was to assess the 0–10 NRS for its appropriateness in recording ratings of perceived effort under isometric submaximal contractions of elbow flexors. Consequently, the hypotheses of this study are:

H_{01} (null 1): ***0–10 NRS is not a valid and reliable measurement tool to assess perception of effort during isometric elbow flexion.***

H_1: ***0–10 NRS is a valid and reliable measurement tool to assess perception of effort during elbow flexion.***

Additionally, it has been hypothesized that:

H_{02} (null 2): ***0–10 NRS is not sensitive enough to monitor changes in the perceived effort of healthy individuals with the level of exercise intensity.***

H_2: ***0–10 NRS is sensitive enough to monitor changes in the perceived effort of healthy individuals with the level of exercise intensity.***

4.2 Methods

4.2.1 Sample

The 0–10 NRS was tested for its repeatability and validity under two experimental conditions: i) between measurements repeatedly taken over the same session (1 hr) and ii) between measurements taken over time in three weekly sessions. Every condition was tested in a separate experimental setup. Given that the magnitude of the ICC coefficient is affected by the time interval between the administrations of the measurement, in a way that short intervals may yield estimates of reliability which are too high (Streiner & Norman, 2003), the reliability and validity of the scale was tested in both short and longer time intervals between test and retest measurements. This study was one which used a within-subjects repeated measures design. Twenty one healthy participants took part in the first (short test-retest interval) experiment (14 women and 7 men), with an average age of 32.62 ±10.93(SD) years and range between 18 and 63 years. This experiment gave data for the reliability of the NRS in a within-session repeated baseline measures, when perception of effort was not expected to have changed, and tested the sensitivity of the scale in measuring changes in effort as the level of the voluntary contraction of the elbow flexors gradually increased from 10 to 100% of MVC. Twelve healthy participants took part in the second (longer test-retest interval) experiment (8 women and 4 men), with an average age of 32 ± 6(SD) yrs and range between 24 and 42 years. All participants except one were right handed. The second experiment gave data for the repeatability and validity of the NRS among sessions separated by a week. The accuracy of voluntary contractions at target levels of force was also tested in both experiments.

4.2.2 Apparatus

The details of the apparatus in regards to the measurement of the isometric force of elbow flexors (see section 2.4.1), the surface electromyography (see section 2.4.2), and the ratings of the perceived effort (see section 2.4.5) were outlined in section 2.4 of the General Methods chapter. Electromyography was recorded from Br, BB, BR in the first experiment and from BB and BR at the second experiment.

4.2.3 Experimental Procedure

After signing a consent form, participants were seated with their arm fitted in the rig (see section 2.4.1 and Fig. 2.2). The MVC for every subject was defined as described in sections 2.4.1, 2.4.2, and 2.5.1. In both experimental sessions the participants started the experiment with a familiarization session (see section 2.5). Following the familiarization session the experiment started with the baseline measures taken every 20min. Three baseline measures were taken in the first experiment. Ratings of effort on the NRS were taken for six levels of voluntary contraction intensity: 10, 30, 50, 70, 90, and 100% MVC. During the second experiment the perception of effort task was repeated twice at every session. Three sessions were undertaken with a week interval, to test the repeatability of the NRS in longer time intervals. Effort was recorded at 30, 50, 70 and 100% of MVC. In both experiments the levels of voluntary contraction were randomly selected to avoid bias in the effort ratings because of learning or habituation effects. Three contractions at every level were undertaken to further enhance unbiased results.

4.2.4 Data Analysis

All force data were normalized to the maximum MVC, while all EMG data were normalized to the EMG during the MVCs. To assess the stability of the NRS over time, the test retest reliability used the Intra Class Correlation (ICC) analysis between measurements as described in section 2.6 of the General Methods chapter. The Coefficient of Variation (CV) was also calculated to test reproducibility of the NRS

records (see section 2.6) and variation of the measurements between subjects. The criterion validity of the scale was tested by correlating the ratings of the perceived effort with the objective measurements of the voluntary force produced at every effort rating and with the EMG activity of the muscles participating in the voluntary contractions. Because the data were not normally distributed, the Spearman correlation coefficient was used for the correlations. Changes in the perception of voluntary effort and the EMG activity of all muscles due to increased level of voluntary contraction were analyzed through repeated measures statistics as described in section 2.6. This study is part of a bigger study undertaken to evaluate the validity and reliability of the NRS in rating effort and to assess perception of effort following fatigue at relative levels of MVC. This chapter presents only the data which are related to the reliability and validity analysis of the effort NRS. Data related to the effort and EMG changes following fatigue at relative levels of MVC are presented in the next chapter as part of the subsequent evaluation (see section 5.2) of the impact of fatigue on perceived effort.

4.3 Results

4.3.1 Repeatability of the 0–10 NRS

4.3.1.1 Accuracy in Voluntary Contractions

All subjects were accurate in producing voluntary force equal to the target level during the perception of effort task. The ICC for the absolute agreement between target level of force and voluntary force was excellent at every baseline (ICC=0.99 (95%Confidence Interval (CI): 0.98, 0.99) for baseline 1, ICC=0.98 (95%CI: 0.97, 0.99) for baseline 2, ICC=0.99 (95%CI: 0.98, 0.99) for baseline 3). The subjects were consistent in producing a force level equal to the target level of force that was set in advance and only at the very highest level of the maximum contractions some variability between the subjects was revealed (Fig. 4.1). The correlation coefficient, measured by Spearman's Correlation, between target level of force and the produced voluntary force was also very high at every baseline measurement (ρ=0.981, $p<0.001$, ρ=0.975, $p<0.001$, ρ=0.980, $p<0.001$, for the baseline 1, 2, 3, respectively).

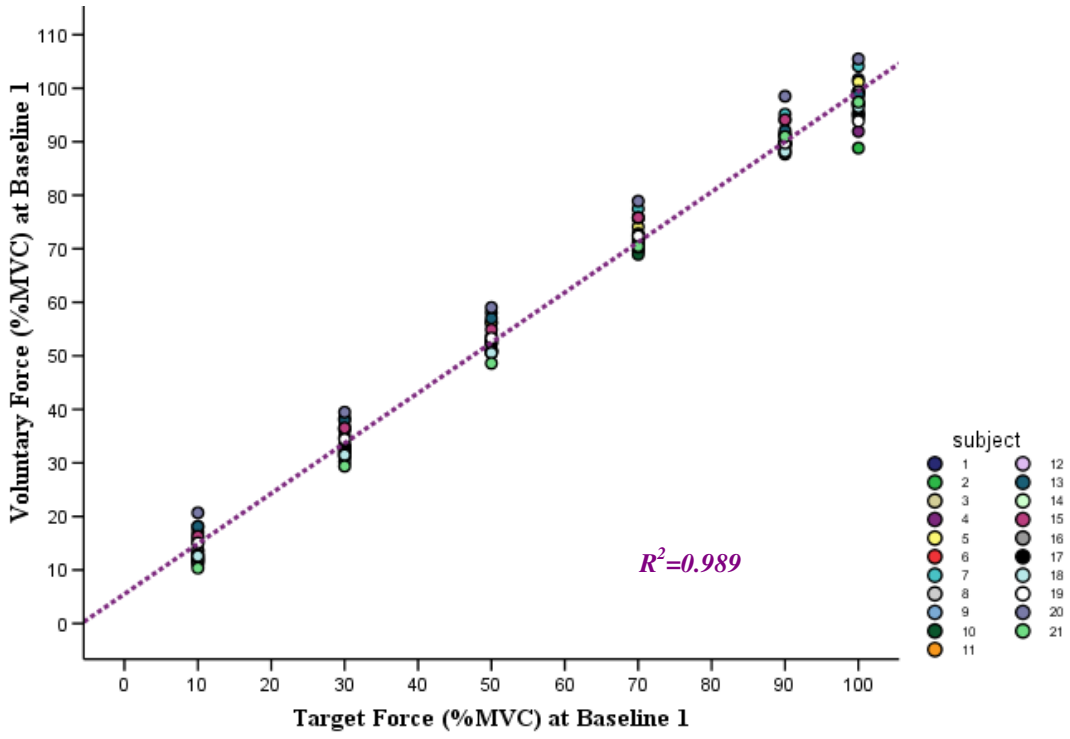


Figure 4.1: Scatter plot between the target level of force and the voluntary force produced at each level of voluntary contraction (mean of 3 attempts) at baseline 1. Data are clustered by participant (n=21).

4.3.1.2 Test-Retest Reliability and Reproducibility of the 0–10 NRS

The ICC among the three baseline measurements separated by a 20 min time interval for the rating of the perceived effort was 0.99 (95% CI: 0.98, 0.99; excellent reliability) indicating that the participants were constant in their rating for the same level of force over a short time (Fig. 4.2). When the interval between test and retest measures was increased to one week, the NRS again revealed excellent reliability (Fig. 4.3). The ICC for the ratings among the 3 sessions was 0.96 (95% CI: 0.96, 0.97). Repeated measures ANOVA revealed no significant differences in the ratings of perception of effort from session to session ($F_{(2, 20)}$=0.31, p=0.74, Partial Eta Squared=0.03). Additionally, the within subjects CV for the three measurements taken within one session, separated by a 20 min interval was 6%, while the CV for the 3 measurements taken with a week interval was 9.1% (Table 4.1). The between subjects CV was greater for the measurements taken with a week interval than those taken within a session but it did not exceed the 17% (Table 4.1).

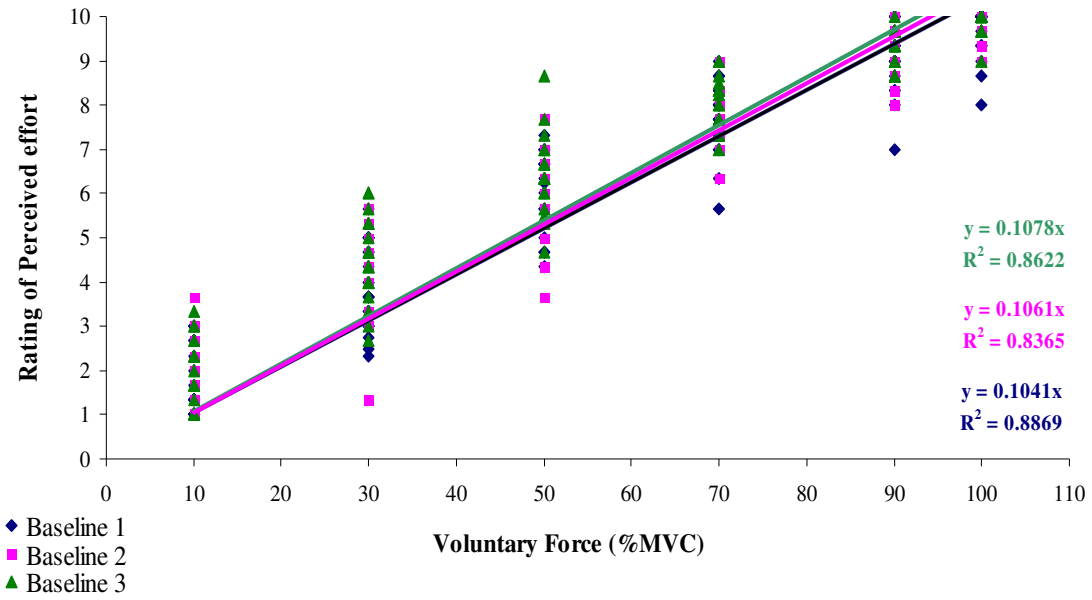


Figure 4.2: Correlation between perceived effort and level of voluntary contraction (mean of 3 contractions at every level) at every baseline separately (n=21). The linear equation and the coefficient of determination (R^2) are presented for every baseline.

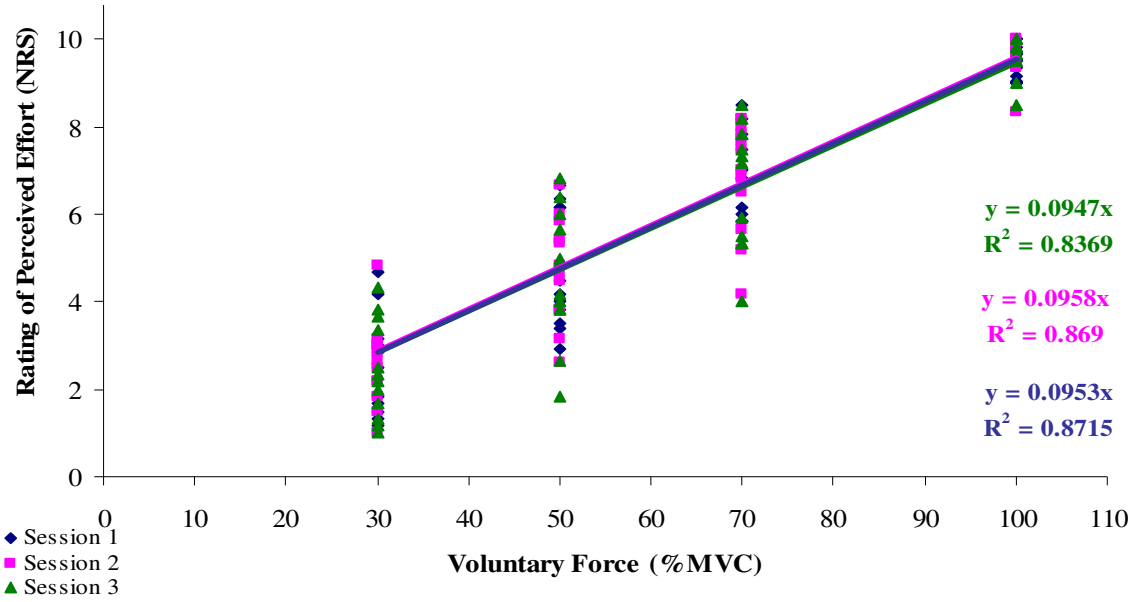


Figure 4.3: Correlation between perceived effort and level of voluntary contraction (mean of 3 contractions at every level) between test and retest measures separated by a week (n=12). The linear equation and the coefficient of determination (R^2) are present for every session.

Table 4.1: Within and Between Subjects CV ($SQRCV^2$) from measuremenst taken with a 20min interval (within a session, n=21) and with a week interval (n=12).

	Between Subjects CV Measurement 1	*Between Subjects CV Measurement 2*	*Between Subjects CV Measurement 3*	*Within Subjects CV*
Within a session	9.3%	8.6%	6.7%	6%
With a week interval	16.3%	12.3%	17.4%	9.1%

4.3.1.3 Validity of the 0–10 NRS

The rating of the perception of voluntary effort was highly correlated with the produced voluntary force (Fig. 4.3). The ratings of the perceived effort on the NRS increased in a proportional way with the level of produced force (Table 4.2). Additionally, the perceived effort was strongly correlated with the EMG recorded by all flexor muscles (Fig. 4.4). Specifically, as the level of voluntary contraction increased, the ratings of the perceived effort increased in parallel with the group mean normalized EMG of the flexor muscles (Fig. 4.4). BB EMG increased from 8 ± 3.4(SD) %Max at level 1 (10%MVC) of the voluntary contraction to 104 ± 16(SD) %Max at level 10 (100%MVC) (Table 4.2). All correlation coefficients measured by Spearman's Correlation between effort and EMG or voluntary force were above 0.89 (range 0.89 to 0.95) when measures were taken 20min apart (Table 4.3). Correlations were above 0.86 (range 0.86 to 0.93) when measures repeated with a week interval (Table 4.4). Least squares of best-fit trend lines, forced through zero, demonstrate the linear relationship between the NRS values and the level of voluntary contraction (Fig. 4.2 & Fig. 4.3).

Table 4.2: Ratings of perceived effort, rmsEMG of Br, BB, BR (mean ± SD) at voluntary produced levels of force (mean ± SD) (n=21).

Voluntary Force (%MVC)	*Effort (NRS rating)*	*Br rmsEMG (%Max)*	*BB rmsEMG (%Max)*	*BR rmsEMG (%Max)*
14.51 ± 3.02	1.84 ± 0.73	10.60 ± 4.32	8.10 ± 3.42	6.25 ± 2.90
34.18 ± 2.97	4.28 ± 0.82	24.00 ± 5.78	21.16 ± 4.89	19.97 ± 5.63
53.57 ± 2.83	6.17 ± 0.81	44.66 ± 7.06	41.49 ± 8.15	42.86 ± 9.50
72.70 ± 2.82	7.81 ± 0.52	72.44 ± 8.08	69.48 ±10.38	73.60 ± 11.23
90.63 ± 2.79	9.16 ± 0.47	95.38 ± 9.62	94.77 ± 13.34	94.85 ± 10.70
97.09 ± 3.95	9.83 ± 0.25	105.73 ± 12.00	104.10 ± 16.01	100.86 ± 10.51

Table 4.3: Spearman's rho (ρ) Correlations between perceived effort and i) voluntary force (%MVC), ii) rmsEMG (%Max) of biceps, iii) rmsEMG (%Max) of brachialis and iv) rmsEMG (%Max) of brachioradialis at measures taken 20min apart within a session (n=21).

Spearman's rho Correlation	*Baseline 1*	*Baseline 2*	*Baseline 3*
Perceived effort-Produced Force	ρ=0.941*	ρ=0.938*	ρ=0.954*
Perceived effort-Br rmsEMG	ρ=0.930*	ρ=0.923*	ρ=0.937*
Perceived effort-BB rmsEMG	ρ=0.924*	ρ=0.911*	ρ=0.924*
Perceived effort-BR rmsEMG	ρ=0.913*	ρ=0.891*	ρ=0.931*

* $p<0.001$

Table 4.4: Spearman's rho (ρ) Correlations between perceived effort and i) voluntary force (%MVC), ii) rmsEMG (%Max) of biceps, iii) rmsEMG (%Max) of brachioradialis at measures taken one week apart (n=12).

Spearman's rho Correlation	*Session 1*	*Session 2*	*Session 3*
Perceived effort-Produced Force	ρ=0.897*	ρ=0.883*	ρ=0.891*
Perceived effort-Br rmsEMG	ρ=0.897*	ρ=0.901*	ρ=0.863*
Perceived effort-BR rmsEMG	ρ=0.902*	ρ=0.934*	ρ=0.861*

* $p<0.001$

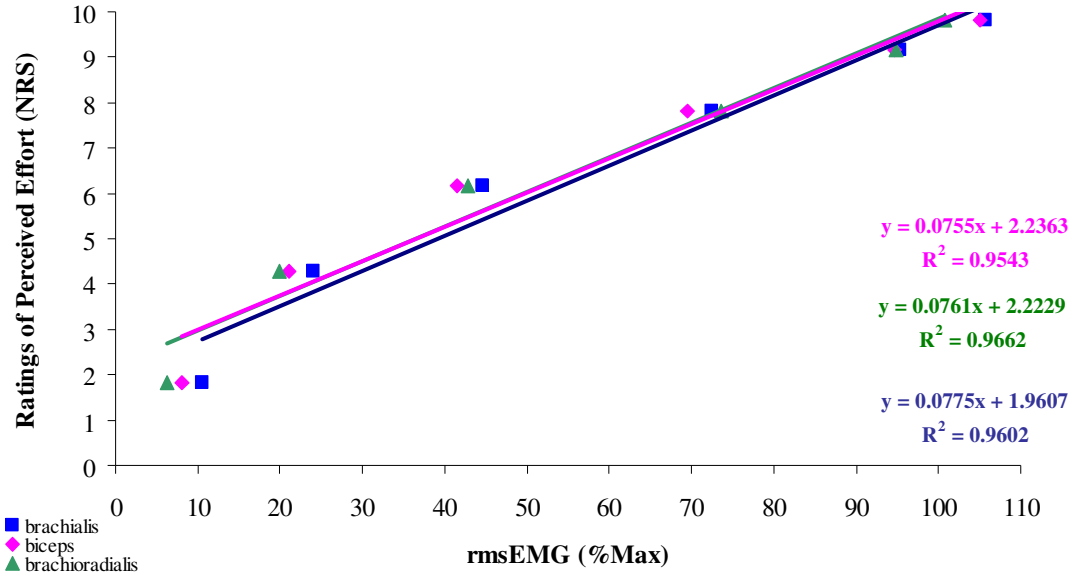


Figure 4.4: Strong correlation between perceived effort and EMG of BB, Br & BR. Mean data of 21 participants from average baseline measurements.

4.3.2 Sensitivity of the 0–10 NRS

Two-Way Repeated measures ANOVA revealed a statistically significant effect of the intensity of the contraction (level of voluntary force) on perception of effort ($F_{(2.23, 44.66)}$=805.70, p<0.001, Partial Eta Squared=0.98) (Fig. 4.2). Participants reported an increase in the perceived effort with parallel increases in the contraction intensity. Pairwise comparisons revealed that the effort ratings at every intensity level were significantly higher than the ratings at the preceding level (p<0.001) (Table 4.5). Table 4.5 presents the differences in the NRS ratings between levels of voluntary contraction. The smallest difference presented in the table to be statistically significant was a difference of 0.67 points on the NRS. The main effect of intensity of voluntary contraction on EMG of BB, Br, BR was also significant (for BB: $F_{(1.82, 36.46)}$=493.77, p<0.001, Partial Eta Squared=0.96, for Br: $F_{(1.76, 35.17)}$=625.20, p<0.001, Partial Eta Squared=0.97, for BR: $F_{(2.51, 50.19)}$=795.85, p<0.001, Partial Eta Squared=0.98).

Table 4.5: Difference in ratings of perceived effort (points on NRS) among levels of force production (n=21).

Level of Force (%MVC)		*Mean Difference (2-1)*	*Sig.*	*95% Confidence Interval*	
Level 1	*Level 2*	*(Mean ± SEM)*		*Lower Bound*	*Upper Bound*
10	30	2.44 ± 0.12	0.001*	2.83	2.04
30	50	1.89 ± 0.10	0.001*	2.23	1.55
50	70	1.64 ± 0.13	0.001*	2.08	1.20
70	90	1.35 ± 0.09	0.001*	1.65	1.05
90	100	0.67 ± 0.08	0.001*	0.92	0.41

4.3.3 Summary of Results

- The test-retest reliability analysis showed an excellent ICC between 3 measurements of the 0–10 NRS taken within one session (20min apart) and between 3 consecutive weekly sessions.
- A strong linear association was revealed between NRS ratings and levels of voluntary contraction. Indeed, the perceived effort, as it was reported on the NRS, significantly increased with the intensity of the voluntary contraction. Additionally, the levels of perceived effort matched %MVC target force intensities.
- Significant correlations were revealed between ratings of perceived effort on the 0–10 NRS and EMG recordings of all flexor muscles.
- The 0–10 NRS was sensitive and showed significant changes of the perceived effort as the intensity of the voluntary contraction increased. A change of 0.7 points on the NRS was the smallest to cause statistically significant changes in the perception of effort.

4.4 Discussion

The 0–10 NRS has been assessed for its reliability and validity in recording ratings of the perceived effort under various intensity levels of isometric elbow flexion. The main findings of this study showed that participants were accurate in reproducing voluntary forces equal to the target forces and consistent in reporting their perception of effort for the same levels of voluntary force. The test-retest reliability of the 0–10 NRS was very high among measurements within a single session and between three consecutive weekly sessions. The excellent association of the NRS to the voluntary force and the EMG activity of the muscles of interest support the concurrent criterion validity of the measure and present the linear properties of the scale. Additionally, the sensitivity of the scale to record changes in the perception of effort as the intensity of the work load increased points towards a scale that reflects well the changing physiological demands of the exercise tasks presented. These findings confirm the research hypothesis that the 0–10 NRS is a reliable and valid method of recording perception of effort when isolated muscles are contracted.

The excellent test-retest reliability (0.99) of the 0–10 NRS indicates that 99% of the variance in the scores results from "true" variance among subjects and not from measurement error (Streiner & Norman, 2003). Indeed, the between subjects variance was greater than the within subjects variance indicating that the ratings were reproducible from session to session. The very small variance, as well as the consistency of the ratings not only when measurements were taken within the same session, but also when a week separated the test from the retest measurements, indicate that the 0–10 NRS is both easy to use and reliable. One could argue that the excellent repeatability of the NRS is due to the short interval between test and retest measurements especially when the measurements are taken within the same session. The short interval might lead to a learning effect in a way that participants could easily recognize stimulus (level of voluntary contraction) of the same intensity when the stimulus is repeated a number of times (Williamson & Hoggart, 2005). However, this could happen only if limited levels of intensity were provided. In the present study, six different levels of voluntary contractions were applied, taken from the full available range of contractions, and they were repeated randomly three times. In that way bias in

the responses was eliminated. Furthermore the excellent repeatability of the scale between sessions separated by a week, and the absence of significant changes in the perception of effort among these measurements, further supports the conclusion that the excellent repeatability of the NRS is not due to habituation or any learning effect. The participation of the same subjects every time and the administration of the scale by the same person may have enhanced these results.

Additionally, the strong linear association of the NRS with the level of voluntary contraction makes the 0–10 NRS an appropriate measurement tool in clinical practice for the assessment of voluntary effort. It has been suggested that, when assessing pain, if intense stimuli are applied after a level of moderate pain is reached, relatively small increases in stimulation may result in exponential (nonlinear) enhancement of pain report (Janal, 1995, cited in Hartrick *et al*., 2003). The same could be applied to the effort ratings, although pain and effort are different subjective feelings that are perceived under different processes. However, in the present study even small perceptual changes of 0.7 points on the NRS, when the intensity of the voluntary contraction increased from 90 to 100% of MVC, did not affect the linearity of the scale.

Indeed, each level of perceived effort on the 0–10 NRS matched equivalent percentile levels of voluntary contraction. This finding is not in accordance with the studies of Pincivero (2003a, b) and West (2005) which reported a perceptual overestimation at moderate to high levels of voluntary contraction and an underestimation of effort at nearly maximum levels of voluntary contraction. This disagreement may be due to differences in the experimental procedures. In the studies above, healthy participants had to perform sub-maximal contractions at prescribed levels of perceived voluntary effort which could involve a greater cognitive process, resulting in inaccurate force estimation or a subconscious underproduction of force at higher intensities as a protective mechanism. Additionally, the use of a different rating scale might also have led to these differences. The CR10 Borg scale has been used in these previously published studies (Pincivero *et al*., 2003a; Pincivero *et al*., 2003b; West *et al*., 2005). One advantage of the NRS over the Borg scale is that, apart from the anchors presented at the limits of the scale, it lacks other intermediate anchors that may confuse the subjects and increase the inter-individual variation in rating, due to differences in rating the perceived exertion when they are using the verbal anchors or the numeric value that

corresponds to that anchor (Dawes *et al*., 2005). It would be interesting therefore if the studies above used the 0–10 NRS scale for setting prescribed levels of perceived effort. The 0–10 NRS requires less mental and cognitive work in its utility than the Borg scales, and lacks intermediate anchors, and consequently it could be used in studies that test the neuromuscular activation during perceptually guided levels of voluntary contraction.

Additionally, although the establishment of the validity of the 0–10 NRS has not been based on the relationships of the NRS ratings to other tools that measure the same construct, the constant associations of the NRS ratings with the voluntary force and the EMG recordings provide further evidence to support the concurrent criterion validity of the 0–10 NRS. It is difficult to measure perception of effort objectively because invariably it is a complex process involving many areas in the central nervous system in addition to primary sensori-motor activity (McCloskey, 1981). The strong linear association of the NRS with the EMG when the intensity of the voluntary contraction increases supports the assumption that perception of effort is an efferent perception, which involves higher centres in the CNS, and it is proportional to the magnitude of voluntary motor command which changes relative to afferent inputs from the periphery (Gandevia, 2001). When the demands in the periphery increase due to enhanced workload, the increased EMG activity of the muscles, which indicates increased central drive to the muscles for recruitment of more motor units, is followed by an increase in the perceived effort (Liu *et al*., 2003). This may be explained by the feedforward-feedback system where perception of effort does change whenever there is a mismatch between the corollary discharges that radiate to the somatosensory cortex and the afferent impulses evoked in the periphery as result of the motor command (Wallman & Sacco, 2007).

The parallel between actual EMG and the changes of the perception of effort with the level of exercise intensity, as they have been detected by the NRS, point towards a measurement tool that is able to follow neurophysiological alterations due to exercise. The close correlation of the perceived effort with the EMG which is a physical measurement suggests that the tested scale is a tool that provides measurements similar to physical ones and gives ratings that are sufficiently valid not only for variables that present a growing trend but also for detecting changes of intensity level. Consequently,

the NRS could be used not only as a recording measurement, but also as diagnostic tool for healthy people and patients who may report disproportionate levels of effort to the actual workload magnitude. Its ability to detect changes in the effort when isolated muscles are exercised supports its potential applicability in assessing perception of effort in patients who present restricted whole body activation. This would have an additional research interest as it could lead to designing research protocols with a scope for evaluating the mechanisms of central fatigue and its relationship with the underlying perception of effort changes in these patients.

A significant change of 0.7 points on the scale indicates a sensitive tool that could detect changes in perception of effort of even a small magnitude. Because a statistically significant change is not as reliable as the clinically important change, validation of the scale in patient populations could be of great importance in order to determine the minimal clinically important change.

In conclusion, the 0–10 NRS demonstrated linear properties and reported excellent test-retest reliability and good concurrent criterion validity in recording perception of effort under repeated isometric contractions of elbow flexors. As the validation of the NRS for the perception ratings has been confined to a healthy population, the effectiveness and applicability of the effort NRS within the clinical field has yet to be explored. Future research should seek to evaluate the construct validity and the minimal clinically important change in the 0–10 NRS measure of perception of effort. Its ease of use and repeatability, as demonstrated by the data, makes the 0–10 NRS a promising candidate for widespread clinical and research use for evaluating changes in the perception of effort, and useful in prescribing resistance exercise in clinical settings. Combined with the Twitch Interpolation Technique, the 0–10 NRS could be used to evaluate the central mechanism of fatigue and its relationship to perceived effort in healthy people and patients who may be extremely reluctant to participate in strenuous whole body exercise protocols.

CHAPTER 5

CHANGES IN PERCEPTION OF EFFORT FOLLOWING SUBMAXIMAL ISOMETRIC FATIGUING EXERCISE

5.1 Introduction

Previous studies have demonstrated that during a fatiguing task there is an increase in the rating of the perception of voluntary effort and an increased sense of fatigue which limits the duration of exercise (St Clair Gibson *et al*., 2001b; see also reviews Hampson *et al*., 2001; St Clair Gibson *et al*., 2006). Most of these studies however, are limited to whole body exercise where fatigue is mainly assessed by changes in the peripheral cardiopulmonary systems. Indeed, laboratory research has suggested a central component of fatigue which is associated with changes in various sites of the CNS and is potentially affected by behavioral changes in perception of effort (Søgaard *et al*., 2006). Whether alteration in perception of effort is secondary to peripheral fatigue or primary to development of central fatigue and eventually task failure is yet to be determined. Indirect findings suggest that the motor commands play a crucial role in mediation of perceived effort (Gandevia & McCloskey, 1978; Noakes, 2007). Indeed, changes in perceived effort may allow exercise performance to be precisely regulated such that a task can be completed within the biomechanical and metabolic limits of the body (St Clair Gibson *et al*., 2006). Thus, during a fatiguing task of maximal or submaximal intensity the potential force output is reduced and the perception of exertion is increased over time and at the end of the task the person feels exhausted (Taylor & Gandevia, 2001; Søgaard *et al*., 2006). However, verbal encouragement results in increasing not only the force output but also the duration for which the task can be sustained (St Clair Gibson *et al*., 2006). Similarly, marathon runners despite their

feeling of exhaustion can still walk at the end of the race (Noakes, 2007). The above indicate that individuals are not really exhausted even if they feel so and there is a reserve energy capacity that can be used in exceptional conditions (St Clair Gibson *et al*., 2006). The "central governor' theory (Noakes, 2007) suggests that the brain regulates our performances in such a way that the exercise is always terminated before real exhaustion in order to prevent biological harm. Thus, the feeling of exhaustion and the perception of effort and fatigue might react as protective mechanisms (St Clair Gibson *et al*., 2006).

The above findings suggest that perception of voluntary effort is involved in the motor control system. However, to date, a direct relationship between central fatigue and perception of effort has not yet been clearly established. Research into this issue might give some insight into the ways that behaviour is mediated during fatigue. Additionally, there is limited research which examines the time course of the perception changes following the fatiguing exercise or the underlying neurophysiological mechanisms involved. Post fatigue changes in perception of effort and corticospinal excitability would give the advantage of relating psychometric with neurophysiological measurements while monitoring their progress due to fatigue. Furthermore, by introducing a fatiguing task which simulates the intensity and type of exercise used for everyday activities, a better understanding would be gained about underlying mechanisms involved in our everyday experiences. Daily load bearing tasks, such as carrying shopping bags, or holding a baby, or cooking, or gardening involve a submaximal muscle activity of the upper limbs. Frequently, this activity involves isometric contraction of the elbow flexors when the load should remain stable against gravity during the task. These are some of the tasks that people with neurological problems, like chronic fatigue syndrome or multiple sclerosis, find quite demanding and fatiguing (Zwarts *et al*., 2008). The 0–10 NRS for perception of effort during isolated isometric muscle activity, which was shown to be a readily applicable and reliable method of assessment in the previous chapter, is now used here to assess the possible changes in perception of voluntary effort directly following fatiguing exercise. In addition, monitoring electromyographic activity during the effort rating task allows for an assessment of the changes in effort rating with possible underlying neurophyisological measures of fatigue. A better understanding of this relationship in normal healthy people may then be applied to examine further the changes in effort

associated with chronic neurological conditions where fatigue is a significant symptom of the condition.

Thus, the aim of this study is to examine the changes in the perception of effort during isometric elbow flexion following fatiguing exercise using the effort 0–10 NRS. A further aim is to evaluate the neurophysiological changes accompanying fatigue. The main specific hypotheses for this study have been set as:

H_{01} (null 1): ***Perception of voluntary effort will not change following a submaximal intermittent isometric elbow flexion at 50% of MVC.***

H_1: ***Perception of voluntary effort will change following a submaximal isometric intermittent isometric elbow flexion at 50% of MVC.***

Additionally, it has been hypothesized that:

H_{02} (null 2) : ***Central fatigue changes will not be revealed by peripheral nerve electrical stimulation following 10 min fatiguing isometric elbow flexion at 50% of MVC.***

H_2: ***Central fatigue changes will be revealed by peripheral nerve electrical stimulation following 10 min fatiguing isometric elbow flexion at 50% of MVC.***

Additionally it has been hypothesized that:

H_{03} (null 3): ***There will be no changes in the indices of the motor cortex excitability following 10 min fatiguing isometric elbow flexion at 50% of MVC.***

H_3: ***There will be changes in the indices of the motor cortex excitability following 10 min fatiguing isometric elbow flexion at 50% of MVC.***

5.2 Methods

5.2.1 Sample

This study consisted of two experimental sessions. During the main experiment, the participants undertook a 10 minute session of fatiguing exercise and the post-fatigue changes were compared with pre-fatigue baseline measurements. Twelve healthy participants took part in this experiment (4 men and 8 women), with an average age of 34.5 ± 8.7(SD) years and range of age between 24 and 55 years. The supplementary experiment assessed fatigue-induced changes in perception of effort at relative levels of force (see next section 5.2.2) using the same exercise protocol. Twenty-one subjects participated in the supplementary experiment (sample characteristics as described in the previous chapter for validation of 0–10 NRS study (short interval experiment): see section 4.2).

5.2.2 Apparatus

The details of the measurement of the isometric force of elbow flexors (see section 2.4.1), the surface electromyographic techniques (see section 2.4.2), the electrical stimulation of the musculocutaneous nerve (see section 2.4.3), the Transcranial Magnetic Stimulation (see section 2.4.4.2), the mood (see section 2.4.6) and perception of effort (see section 2.4.5) measurements have been described in the General Methods chapter. Target levels of 30 and 50% MVC for the perceptual ratings were the main levels of interest for the perception of effort task in the main experiment and the ones that have been analysed. Pilot work on perceptual levels showed that immediately following fatigue, levels above 70% of pre-fatigue MVC were almost impossible to be performed and they were perceived as maximal. Therefore, to avoid ceiling effects in the post-fatigue perceptual ratings, no levels above 50% of absolute MVC were included for assessing post-fatigue perceptual changes. To compare with the baseline measurements, the same force levels were chosen for effort assessment at baselines. Additionally, very low levels were avoided to minimize any potential floor effect in the post-fatigue effort ratings. Other randomly chosen levels were also included in the task

to eliminate bias in the rating responses. For the post-fatigue ratings, percentage levels of before fatigue (absolute) MVC (30 and 50%) were used. For convenience they will be called "absolute" levels of MVC.

Additionally, perception ratings at relative levels of MVC (post-fatigue MVC recorded at the beginning of the perception task at the particular time point of interest) were also recorded in the supplementary experiment. For convenience they will be called "relative" levels of MVC. The idea of assessing effort during relative levels of MVC derives from Carson's study (2002), where the force accuracy was assessed by using a contralateral limb-matching task while force was expressed relative to either pre- or post-fatigue MVC. The study showed that the errors in force matching decreased when the force was expressed in relative terms (Carson *et al*., 2002). Based on that study, the present study intended to assess whether perception of effort changed differently when force was expressed relative to post-fatigue MVC from that recorded at actual levels of voluntary contraction. This would give further information on the way perception of effort changed following fatigue and would provide better data to test the first hypothesis of this study. Perception ratings at relative force levels of 10, 30, 50, 70, 90, and 100% of post-fatigue MVC were recorded at 1, 20, and 40 minutes following fatigue (see also next section 5.2.3).

5.2.3 Experimental Procedure

While participants were seated with their arm positioned in the force-measuring rig, the MVC for every subject, the supramaximal intensity for peripheral electrical stimulation, the hot spot, RMT and stimulus intensity for TMS stimulation were defined as described in sections 2.4.1 to 2.4.5. The protocol of the main experiment consisted of two baseline measurements (30 min and 15 min before the fatiguing exercise), the fatiguing exercise and the 20 min recovery phase. The 15 resting MEP responses were followed by the perception task which involved rating of perceived effort during contractions of percentile levels of individual's MVC randomly selected. Electrical stimulation of musculocutaneous nerve followed the perception task and was delivered

while the elbow flexors were at rest and during maximal isometric elbow contractions. The design of the protocol of the main experiment is depicted in Figure 5.1.

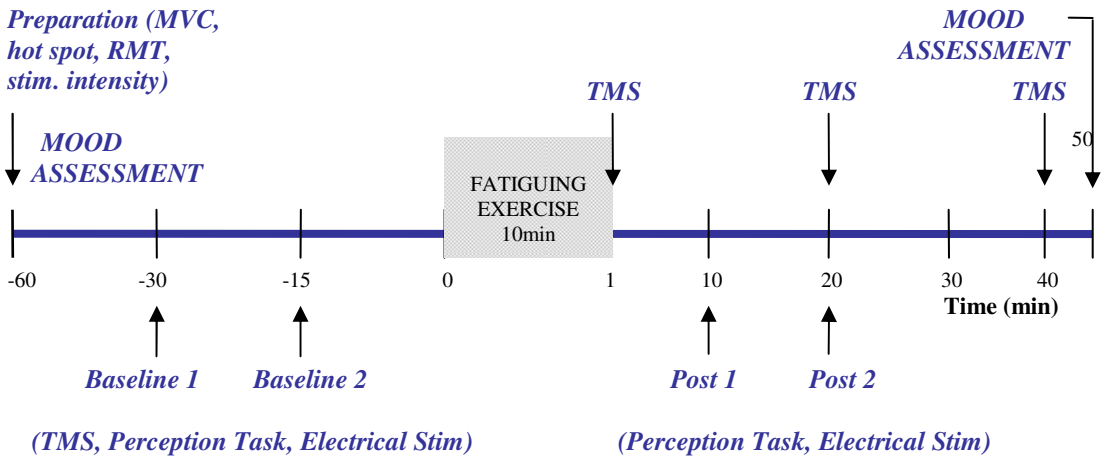


Figure 5.1: Schematic representation of the experimental procedure of the main experiment.

The supplementary experiment consisted of three baseline measurements, the same fatiguing excersise and three post fatigue measurements. Schematic representation of the supplementary experiment is depicted in Figure 5.2. The experimental process before the fatiguing exercise is as described in the previous chapter (see section 4.2.3). The post-fatigue measurements were taken to see the changes of perception of effort at relative levels of force during recovery from fatigue over a period of one hour (Fig. 5.2). The fatiguing exercise was the same as in the main experiment and as described in section 2.5.2 of the General Methods chapter.

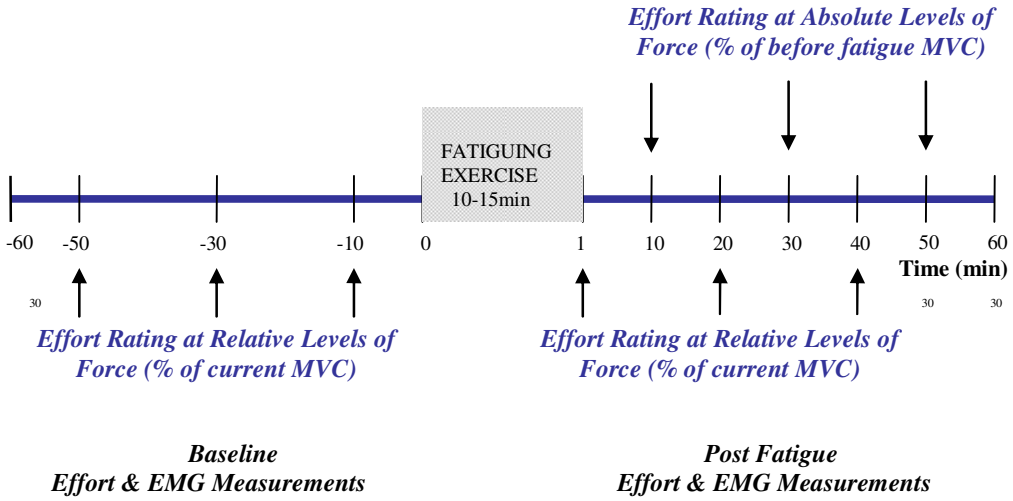


Figure 5.2: Schematic representation of the experimental protocol of the supplementary experiment.

5.2.4 Data Analysis

EMG and force data analysis were performed, as outlined in section 2.6. All force data were normalized to the maximum MVC, while all EMG data for baseline contractions and the relative post-fatigue contractions were normalized to the EMG during the MVCs. Averaged data of volition EMG activity during the five MVCs undertaken at every time point, as well as averaged data of five M-waves caused by motor point electrical stimulation during MVCs (EMG_{max} & $M\text{-}wave_{max}$), were used for the statistical analysis. M-waves evoked by motor point stimulation at rest were also included. All data on M-waves were normalized as percentiles of baseline values. The post-/pre-fatigue ratio was used in the analysis for the effort and EMG at absolute levels of force. Averaged data of 15 consecutive MEP responses at every particular time point were used in the analysis, as described in section 2.4.4.2.

Two-Way Repeated Measures Analysis of Variance (ANOVA) was used to assess changes in the perception of effort and EMG following fatigue with the time from the fatiguing exercise and the level of voluntary contraction being the two factors. For the purposes of the statistical analysis, the effort ratings of the same level of contraction (three contractions were taken at every contraction level) were averaged within the perception tasks in the entire experiment. One-Way Repeated Measures ANOVA was used for the impact of fatigue on the MEP and M-wave responses.

5.3 Results

5.3.1 MVC, EMG_{max} and M-waves

There was a significant main effect of the fatiguing exercise on the MVC as revealed by one way Repeated Measures ANOVA ($F_{(1.79,\ 19.66)}$=29.66, p<0.001, Partial Eta Squared=0.73) (Fig. 5.3). Ten minutes of intermittent isometric elbow flexion at 50% of MVC caused a 44% drop of the MVC of the initial value, (from 174.2 ± 69.7(SD) (at the beginning of the fatiguing exercise) to 97.3 ± 28.9(SD) N at the end of the exercise, range of decline from 27 to 140 N). The maximum voluntary force remained constant at baselines (mean baselines average=178.95 ± 69.41(SD) N). After the end of the exercise there was some recovery in the MVC (the drop reduced from 44% to 20% of the initial value, 20min post exercise, range of recovery from –13N (meaning no recovery but continuing 13N decline from the initial MVC value) to 108 N) but remained significantly lower to baseline MVC even 20min post exercise (mean difference between pre20 to post20 min MVC = 35.74 ± 7.36 (SEM) N, p=0.013, n=12, One-Way Repeated Measures ANOVA).

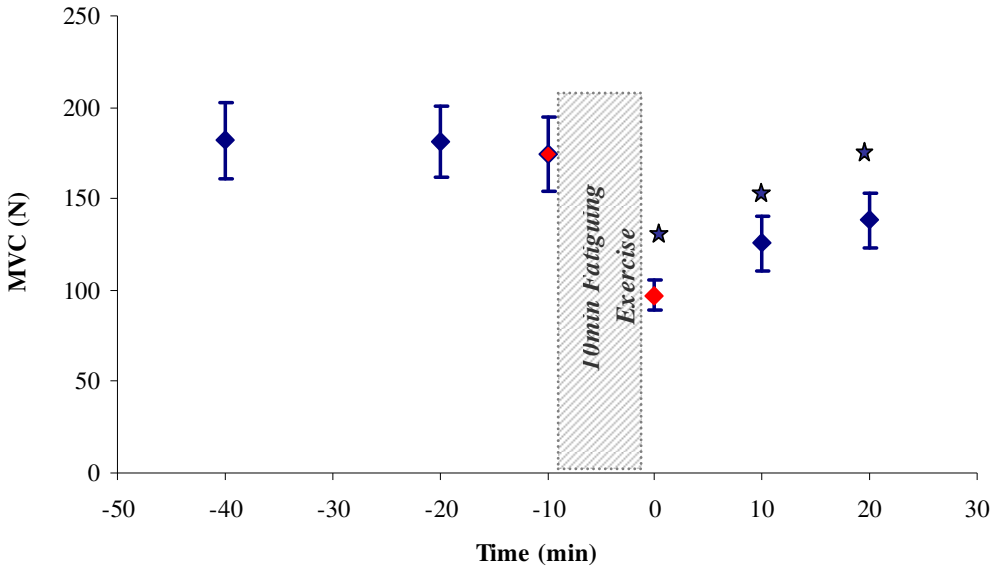


Figure 5.3: MVC (mean ±SEM) changes over course of experiment (n=12). The dotted box corresponds to the fatiguing exercise. Asterisks indicate significant differences (p≤0.05) compared to baselines.

The reduced MVC was accompanied by a 15% reduction in the EMGmax of BB 10min post exercise compared to baseline measurements, which however did not reach significance ($F_{(3,\ 33)}$=2.18, p=0.108, Partial Eta Squared=0.17, One-Way Repeated Measures ANOVA). There was a significant reduction of the EMGmax of BR due to fatigue ($F_{(3,\ 33)}$=6.46, p<0.001, Partial Eta Squared=0.37, One-Way Repeated Measures ANOVA). A 25% drop was revealed in the BR EMG_{max} 10min post exercise which reduced to about 15% 20min post exercise.

One-Way Repeated Measures ANOVA was used for assessing the M-waves of BB and BR caused by motor point electrical stimulation during MVC. The analysis revealed that ten minutes post exercise the M-$wave_{max}$ of BB (revealed by data of 12 subjects) did not differ significantly to baseline measurements ($F_{(1.32,\ 14.54)}$=2.26, p=0.15, Partial Eta Squared=0.17). M-$wave_{max}$ of BR was significantly decreased 10 and 20min post exercise ($F_{(3,\ 33)}$=7.85, p<0.001, Partial Eta Squared=0.42) (Fig. 5.4). There was a 20% drop in the BR M-wave area compared to baselines which remained at 20min post the fatiguing exercise (78.32 ± 17.89% (mean±SD) of the baseline Mw_{max} 10min post exercise and 79.74 ± 19.81% (mean±SD) of baseline M-$wave_{max}$ 20min post exercise).

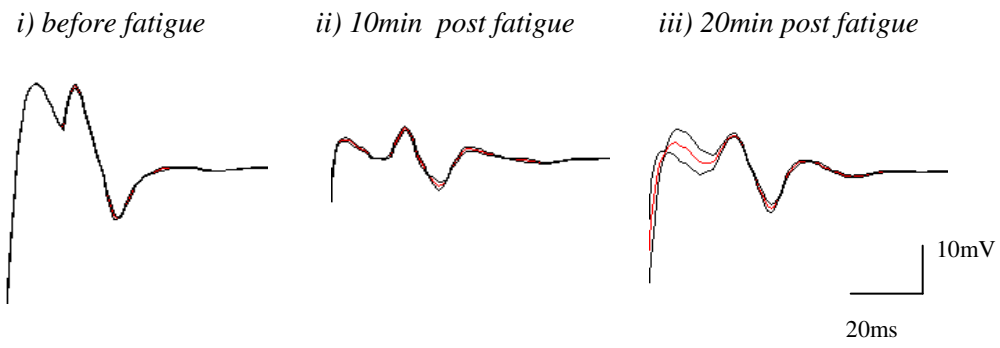


Figure 5.4: M-$wave_{max}$ (average ± SD during 4 MVCs) of BR i) before fatigue ii) 10min post fatigue iii) 20min post fatigue. Data from one participant.

Post fatigue M-waves of BB evoked by electrical stimulation of biceps motor nerve while subjects were at rest were not significantly different from baselines ($F_{(2.06, 22.61)}$=0.95, p=0.41, Partial Eta Squared=0.08, One-Way Repeated Measures ANOVA. BB M-wave area of averaged twitches at rest was 17 ± 8.95(SD) mVms before the fatiguing exercise. 10min post fatigue the M-wave were 17.48 ± 6.44 8.95(SD) mVms and 20min post exercise it was 16.16 ± 6.79(SD) mVms. In contrast, BR M-waves evoked at rest significantly decreased following the fatiguing exercise ($F_{(3, 33)}$=12.34, p<0.001, Partial Eta Squared=0.53, One-Way Repeated Measures ANOVA (Fig. 5.5). 20% drop of BR M-wave 10min post fatigue (79 ± 16(SD) % of baseline) and it remained almost unchanged 20min post fatigue (80 ± 14(SD) % of baseline).

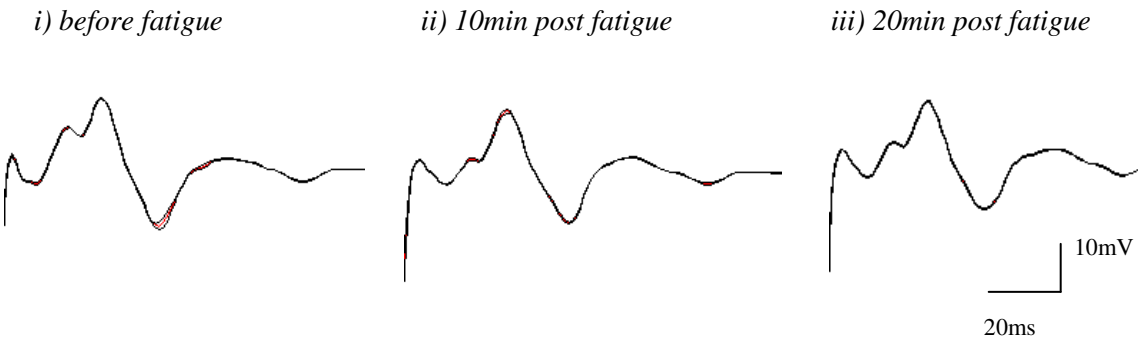


Figure 5.5: BR M-wave at rest (average ±SD of 8 resting twitches) i) before fatigue ii) 10min post fatigue iii) 20min post fatigue. Data from one participant.

5.3.2 Brachioradialis MEP Responses

Brachioradialis MEP responses evoked by TMS did not change significantly after the fatiguing exercise ($F_{(3, 30)}$=0.85, p=0.48, Partial Eta Squared=0.08, One-Way Repeated Measures ANOVA (Fig. 5.6). Immediately after the end of the exercise there was a small, non-significant reduction of about 13% compared to baseline measurements (Fig. 5.7). 20min and 40min post exercise the MEP increased to about 15% and 11% respectively compared to baselines.

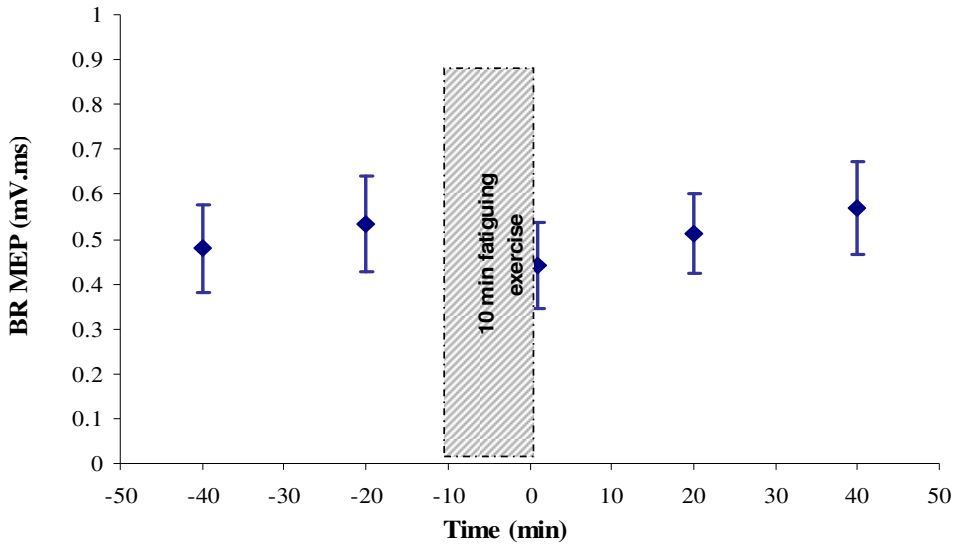


Figure 5.6: Brachioradialis resting MEPs (mean ± SEM) before and after fatigue (n=12). The baselines have been averaged (Blavg) for the purposes of the statistical analysis.

i) before fatigue ii)1min post fatigue iii) 10min post fatigue iv) 20min post fatigue

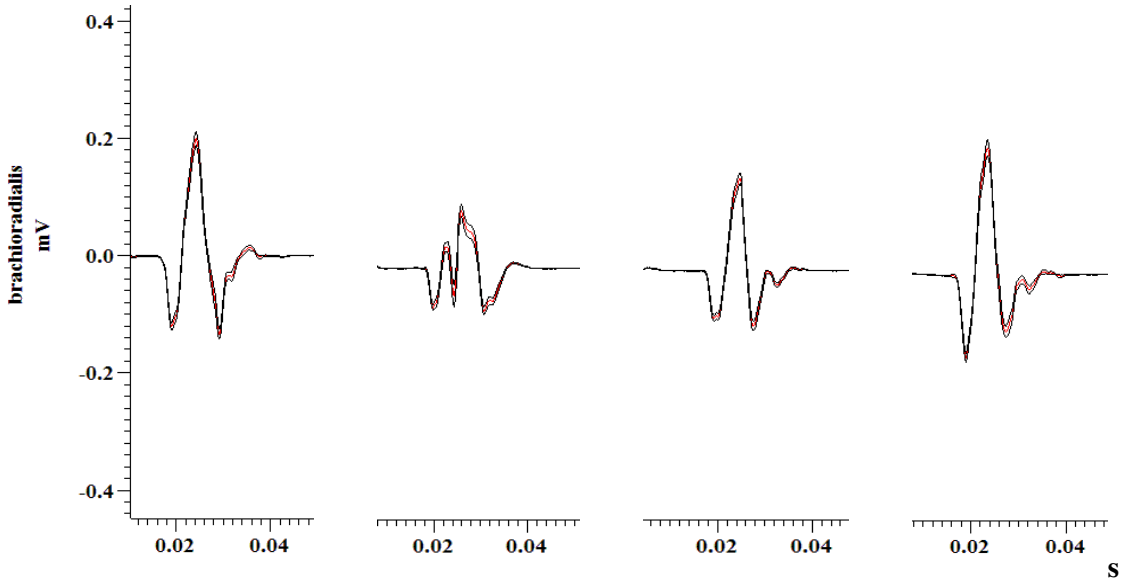


Figure 5.7: Sample of mean (±SEM) BR resting MEP (n= 15) i) 10min before and ii) immediately after the fatiguing exercise iii) 20min post exercise iv) 40min post exercise (data from one participant).

5.3.3 Amplitude of Twitch Force Evoked by Nerve Electrical Stimulation

The resting twitch force significantly decreased following the exercise ($F_{(1.55, 17.07)}$=23.97, p<0.001, Partial Eta Squared=0.69, One-Way Repeated Measures ANOVA) (Fig. 5.8). Ten minutes after the exercise, the resting twitch was 63% of the baseline value (37% drop) and 20 minutes post exercise it remained significantly less by 35% of the baseline value (65% of baseline amplitude).

The effect of the fatiguing exercise was significant on the amplitude of superimposed twitches evoked at MVCs ($F_{(2, 20)}$=32.83, p<0.001, Partial Eta Squared=0.75, One-Way Repeated Measures ANOVA) (Fig. 5.8). The superimposed twitches significantly increased (more than double in size) following the fatiguing exercise (from 2.24 ± 1.27(SD) N before fatigue (1.3% of baseline MVC) to 4.71 ± 2.36(SD) N (2.9% of baseline MVC) 10min post exercise and 3.35 ± 1.71(SD) N (2% of baseline MVC) 20min post exercise).

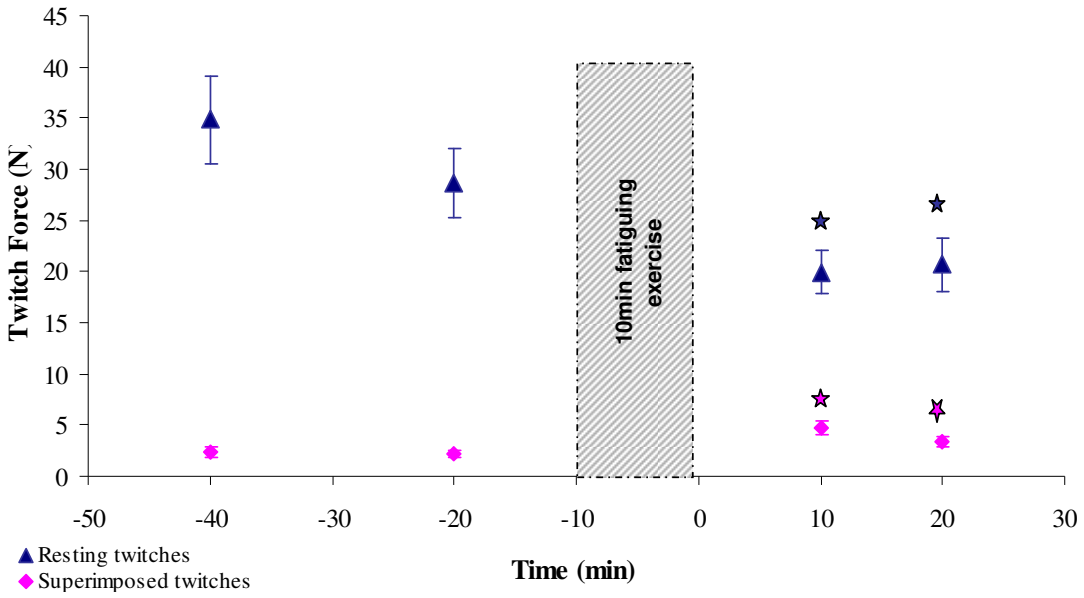


Figure 5.8: Resting and superimposed twitch force amplitude (mean ± SEM) before and after fatigue (n=12). Asterisks indicate significant differences (p≤0.05) compared to averaged baselines.

5.3.4 Voluntary Activation of BB

The fatiguing exercise did cause significant change to the voluntary activation of BB ($F_{(1.52,\ 16.73)}$=46.40, p<0.001, Partial Eta Squared=0.81, One-Way Repeated Measures ANOVA) (Fig. 5.9). The voluntary activation declined significantly following the fatiguing exercise from 92.81 ± 4.20% (mean ±SD) before fatigue to 74.52 ± 9.80% (mean ± SD) 10min post exercise (20% drop). There was some recovery as the time passed from the fatiguing exercise (from 20% to 10% drop 20min post exercise) but it remained significantly reduced 20 min post exercise (82.81 ± 8.49(SD)%).

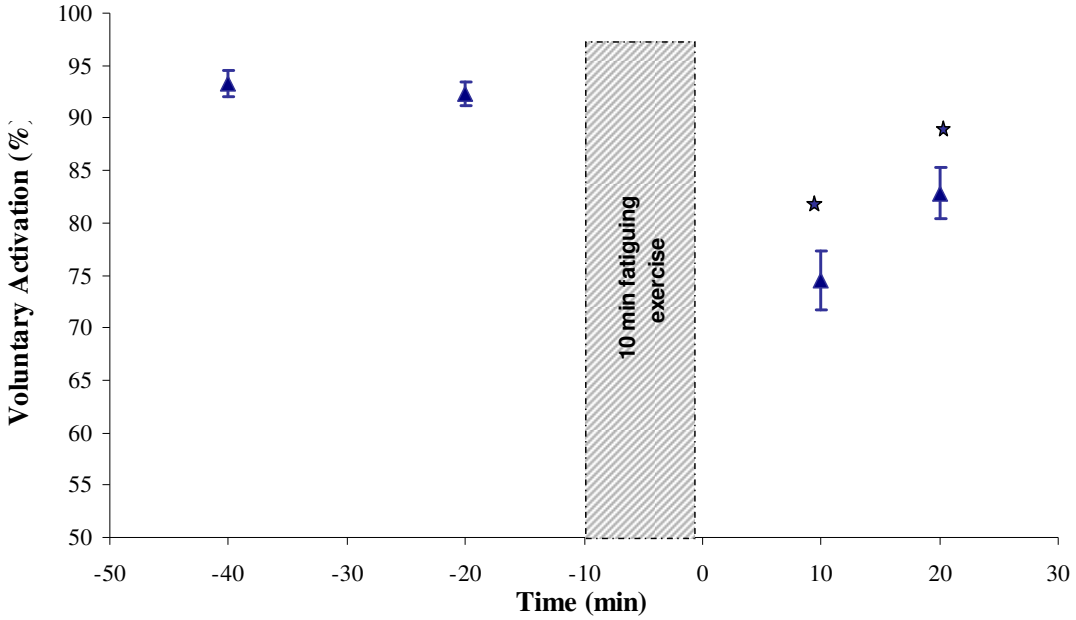


Figure 5.9: Voluntary Activation of BB (mean ±SEM) before and after fatigue (n=12). Asterisks indicate significant differences (p≤0.05) compared to averaged baselines (Blavg).

5.3.5 Ratings of Perceived Effort at Absolute Levels of MVC

The perception of voluntary effort did change significantly following the fatiguing exercise ($F_{(2,\ 22)}$=29.17, p<0.001, Partial Eta Squared=0.73, Two-Way Repeated Measures ANOVA) (Fig. 5.10). For the same (before the exercise) levels of voluntary force the ratings of perceived effort increased about 1 to 2 points on the 0–10 NRS. Thus, the rating effort for 30%MVC increased from a mean of 3.1 ± 0.93(SD) points on the NRS before exercise, to 4.2 ± 1.25 (mean ± SD) rating points 10 min after the fatiguing exercise and to a mean of 4.05 ± 1.06(SD) rating points 20 min after the

exercise. Accordingly, the rating effort for 50%MVC which before fatigue was a mean of 5.22 ± 0.61(SD) rating points on the NRS, increased to a mean of 7.70 ± 1.16(SD) rating points 10min after the exercise and a mean of 7.31 ± 1.02(SD) rating points 20min later.

Two-Way Repeated measures ANOVA revealed that the perception of effort also increased linearly with the level of voluntary force ($F_{(1.00, 11.00)}$=198.59, p<0.001, Partial Eta Squared=0.95) (Fig. 5.10, see also Fig. 5.14i). Additionally, the interaction effect between time and force level was also significant ($F_{(2, 22)}$=5.96, p=0.009, Partial Eta Squared=0.35, Two-Way Repeated Measures ANOVA). Specifically, the increment in the perception of effort 10min post exercise was greater for the 50%MVC (mean rating of effort difference post 10min to baselines 2,5 ± 043(SEM) points on NRS) than the 30%MVC (mean rating of effort difference post10min to baselines 1.2 ±0.34(SEM) points on NRS; $F_{(1, 11)}$=11.67, p=0.006, Partial Eta Squared=0.52, Two-Way Repeated Measures ANOVA). Similarly, the difference between baselines rating and post exercise effort rating was greater for the 50%MVC than the 30%MVC 20min post exercise ($F_{(1, 11)}$=16.18, p=0.002, Partial Eta Squared=0.59, Two-Way Repeated Measures ANOVA).

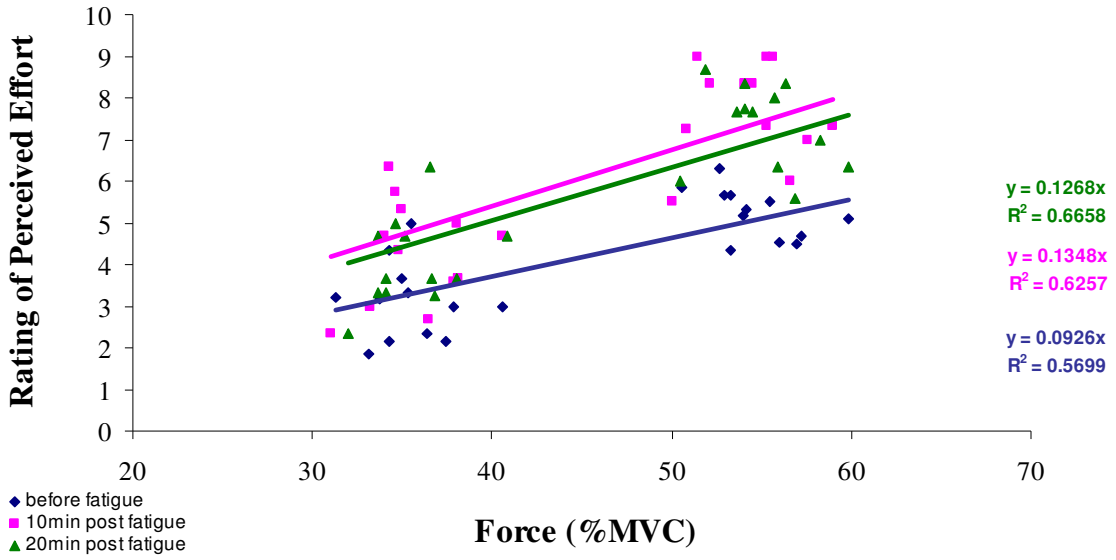


Figure 5.10: Ratings of perceived effort on the 0–10 NRS (n= 12) for 30 and 50% of absolute MVC.

Perception of effort was strongly correlated with the EMG of BB (ρ=0.65, *p*=0.001) and BR (ρ=0.49, *p*=0.015) as it was revealed by Spearman Correlation analysis 10min post exercise (Fig. 5.11). Following fatigue, perception of effort at 30% of absolute MVC increased as the size of the resting twitches evoked by electrical stimulation at biceps motor point were increased (ρ=–0.6, *p*=0.040 Spearman Correlation) (Fig.5.12). In addition, the perceived effort at 30% of absolute MVC increased the superimposed twitches (expressed % of the resting twitches at baselines) increased (ρ= 0.58, *p*=0.049, Spearman Correlation). No correlation was found between the perceived effort and the MEPs of Brachioradialis (Fig. 5.13). Perception of effort did not correlate with the MEP responses.

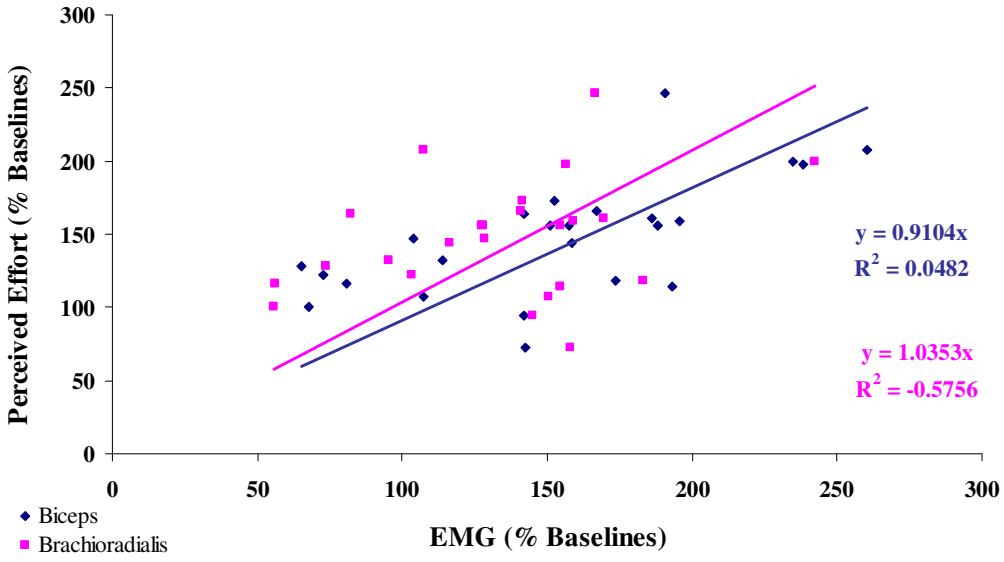


Figure 5.11: Correlation of the ratings of perceived effort (% of baselines) with EMG of BB and BR (% of baselines) for 30 and 50% of absolute MVC (n= 12).

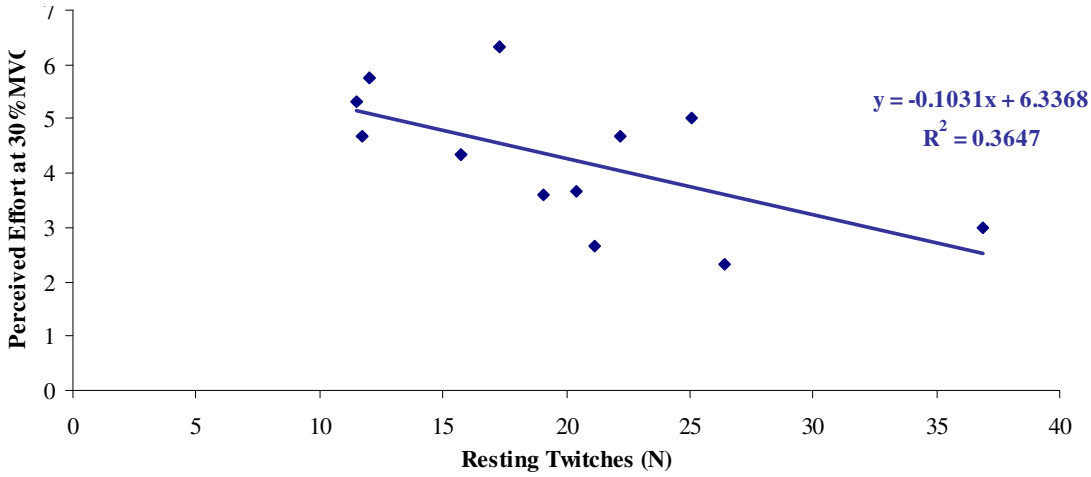


Figure 5.12: Correlation of the ratings of perceived effort at 30% of absolute MVC and resting twitches (n= 12).

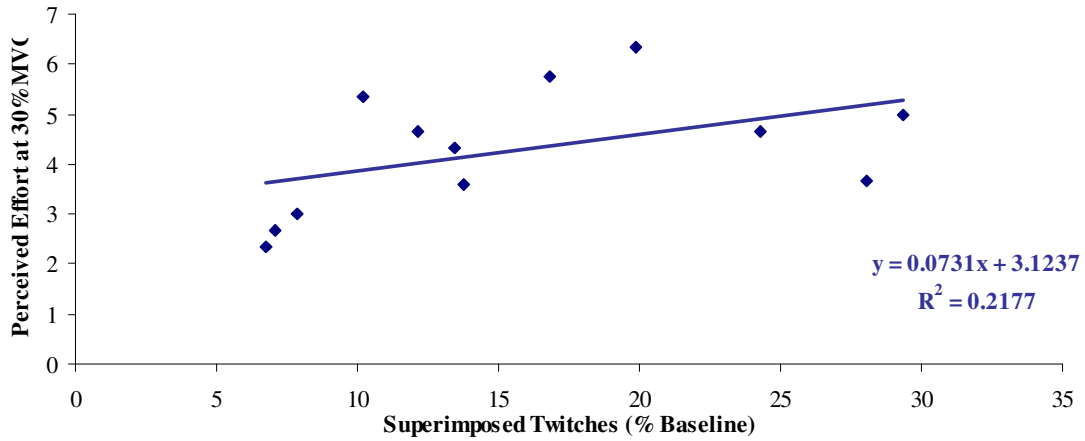


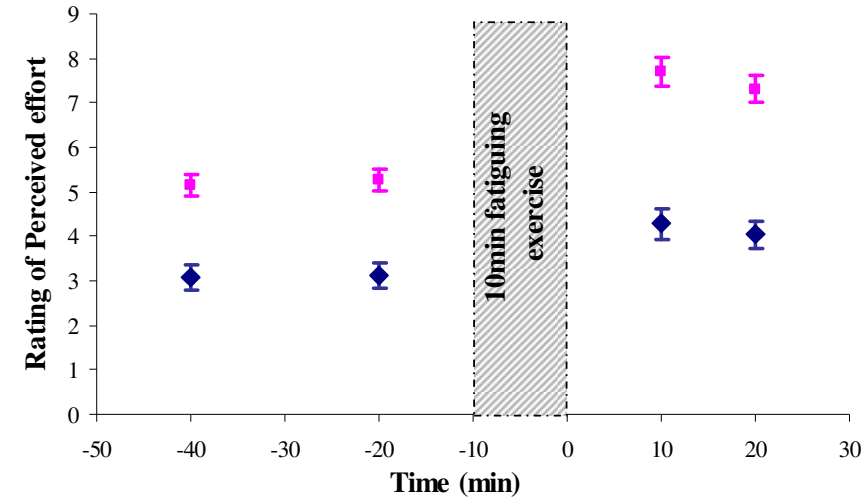
Figure 5.13: Correlation of the ratings of perceived effort at 30% of absolute MVC and superimposed twitches (n= 12).

5.3.6 EMG at Absolute Levels of MVC

BB EMG increased significantly following fatigue for 30 and 50% of absolute MVC ($F_{(1.74,\ 19.16)}$=13.72, p<0.001, Partial Eta Squared=0.55, Two-Way Repeated Measures ANOVA). 10min post the fatiguing exercise the biceps EMG activity was about 55% above the baseline activity (Fig. 5.14ii). The EMG activity remained significantly increased (51% above baseline) 20min post the fatiguing exercise.

BR EMG also increased (35%) significantly following the fatiguing exercise ($F_{(1.72,\ 18.99)}$=9.02, p=0.002, Partial Eta Squared=0.45, Two-Way Repeated Measures ANOVA) (Fig. 5.14iii).

i)



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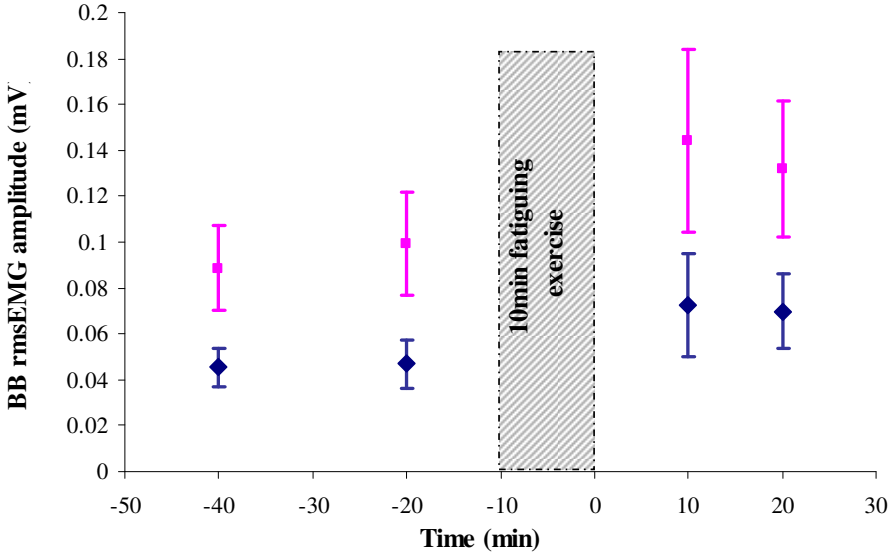


iii)

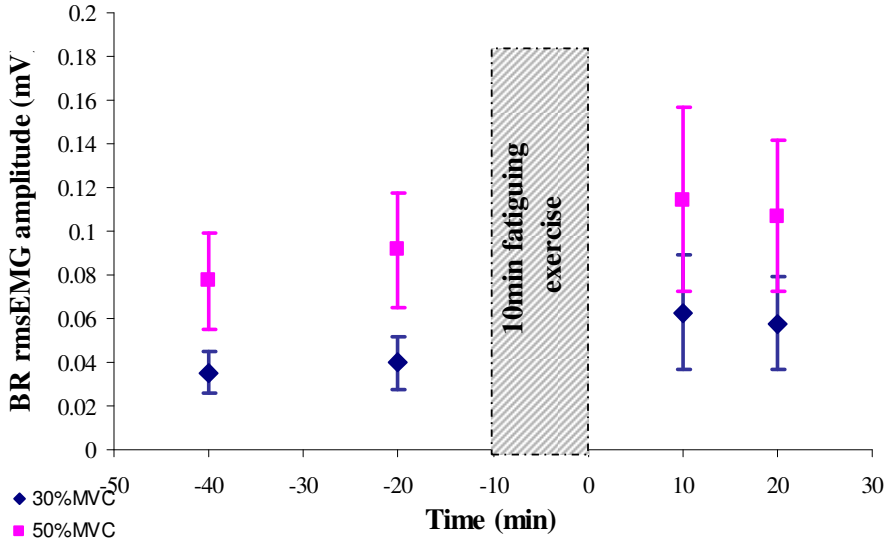


Figure 5.14: Effort and sEMG of BB and BR (mean ±SEM) before and following the fatiguing exercise at 30 and 50% of absolute MVC (n=12).

5.3.7 Perception of Effort at Relative Levels of MVC

Data from the supplementary experiment revealed that the perceived effort significantly increased in a linear manner with the level of contraction ($F_{2.22,\ 44.46}$=984.55, p<0.001, Partial Eta Squared=0.98, Two-Way Repeated Measures ANOVA) (Fig. 5.15). The post fatigue linear increase of perceived effort with the level of force remained despite the increased correlation coefficient (b_1 slope) (see figure 5.16). A significant effect of fatigue over time on the perceived effort was also revealed ($F_{1.84,\ 36.88}$=8.00, p=0.002, Partial Eta Squared=0.28, Two-Way Repeated Measures ANOVA). Further analysis however revealed that it was only at a target force level of 90 and 100% of the relative MVC that the perceived effort decreased significantly, immediately following the fatiguing exercise (Fig. 5.15). No significant differences to pre fatiguing exercise ratings existed 20min later. Additionally, there was a significant interaction effect between force level and time ($F_{7.39,\ 147.83}$ =3.58, p=0.001, Partial Eta Squared=0.15, Two-Way Repeated Measures ANOVA).

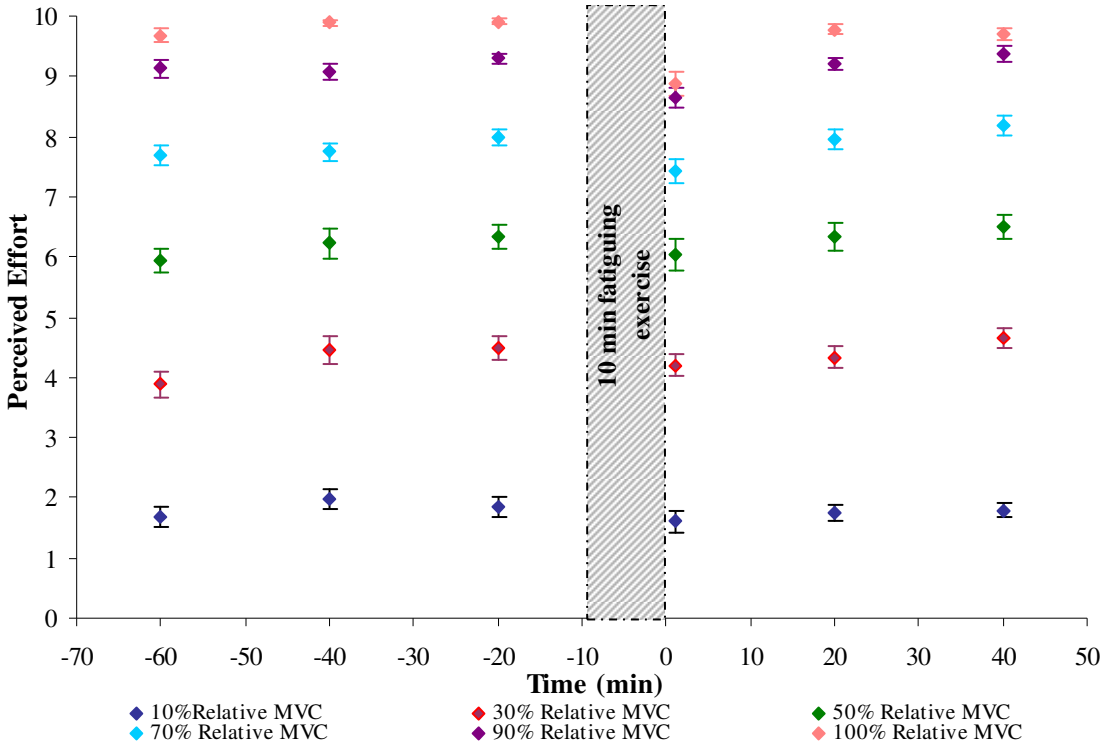


Figure 5.15: Perceived effort rating on the NRS (mean ±SEM) at 10, 30, 50, 70, 90, and 100% of relative MVC (n=21).

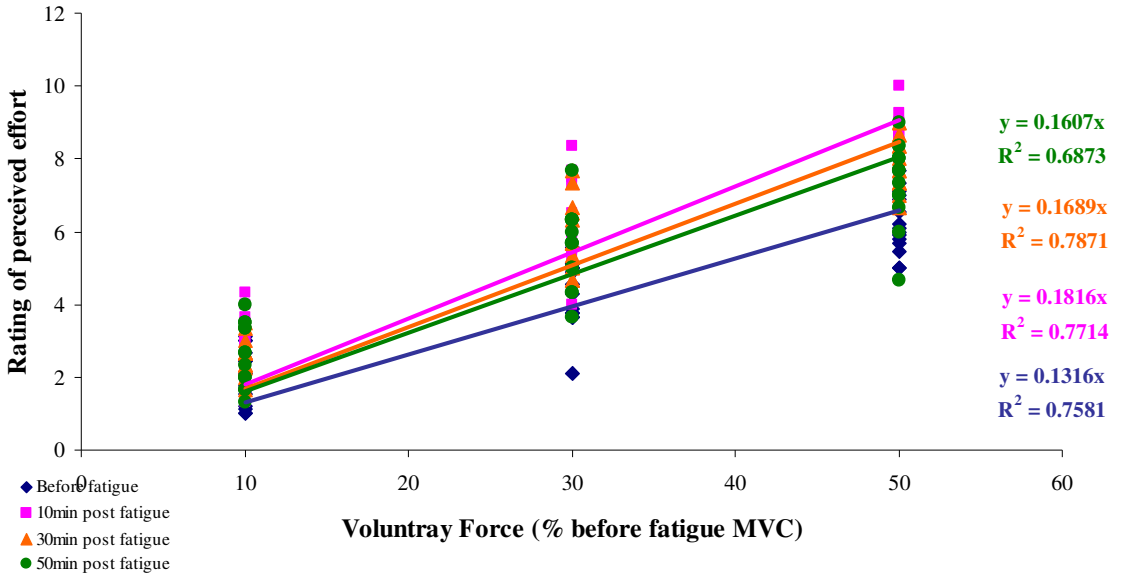


Figure 5.16: Rating of perception of effort (data from 13 subjects at 10, 30, 50% of pre-fatigue MVC) performed following the fatiguing exercise at every time point separately.

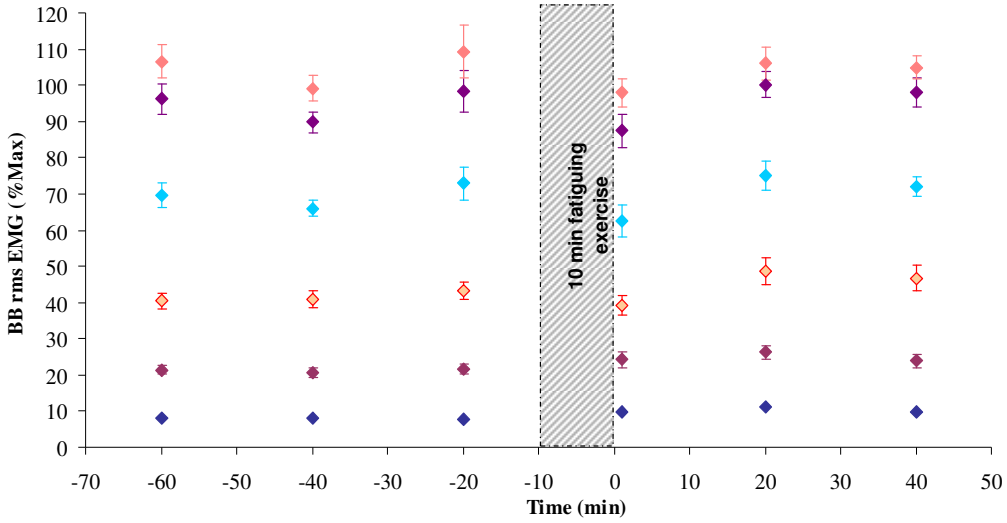
5.3.8 EMG at Relative Levels of MVC

Similarly to the effort ratings, BB EMG increased with the level of voluntary contraction ($F_{(2.59, 51.73)}$=768.14, p<0.001, Partial Eta Squared=0.98, Two-Way Repeated Measures ANOVA) (Fig. 5.17i). There was also a significant effect of fatigue ($F_{(3, 60)}$=2.93, p=0.041, Partial Eta Squared=0.13, Two-Way Repeated Measures ANOVA). Specifically, immediately following the fatiguing exercise no significant changes existed to the BB EMG at any relative level of force. Only 20min post exercise the BB EMG increased significantly compared to pre fatigue values at low levels of relative force but 40min later no significant changes existed. Additionally, no significant interaction effect was revealed between the level of force and the time ($F_{(4.37, 87.40)}$=1.75, p=0.142, Partial Eta Squared=0.08, Two-Way Repeated Measures ANOVA).

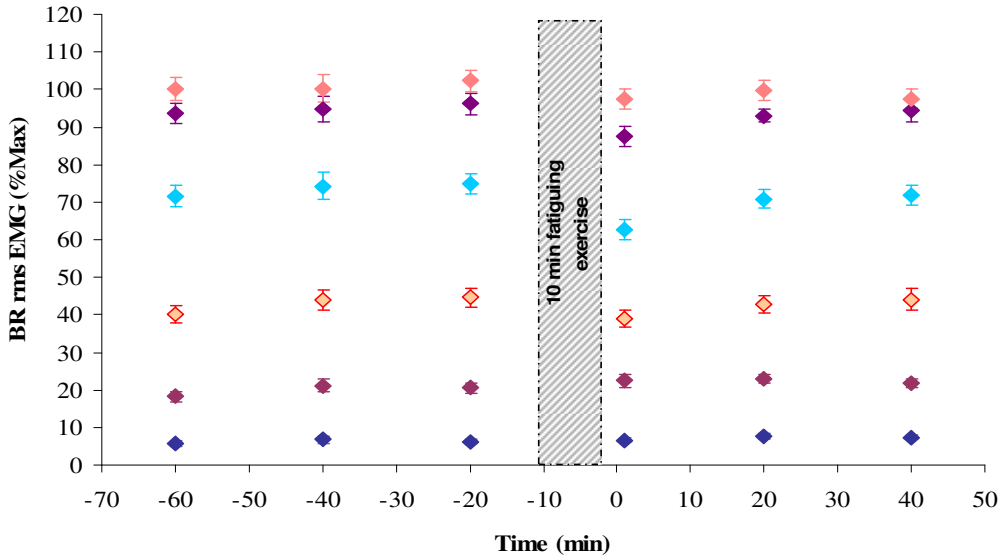
Br EMG also increased significantly with the force level ($F_{(2.37, 47.31)}$ = 791.45, p<0.001, Partial Eta Squared=0.98, Two-Way Repeated Measures ANOVA) (Fig. 5.17ii) but no significant effect of fatigue was revealed ($F_{(3, 60)}$=1.01, p=0.40, Partial Eta Squared=0.05, Two-Way Repeated Measures ANOVA).

BR EMG was increased significantly with the level of voluntary force produced ($F_{(2.50, 49.96)}$ = 1105.32, p<0.001, Partial Eta Squared=0.98, Two-Way Repeated Measures ANOVA) (Fig. 5.17iii). There was no significant effect of time on the rmsEMG ($F_{(2.14, 42.83)}$ = 1.94, p=0.15, Partial Eta Squared=0.09, Two-Way Repeated Measures ANOVA).

i)



ii)



iii)

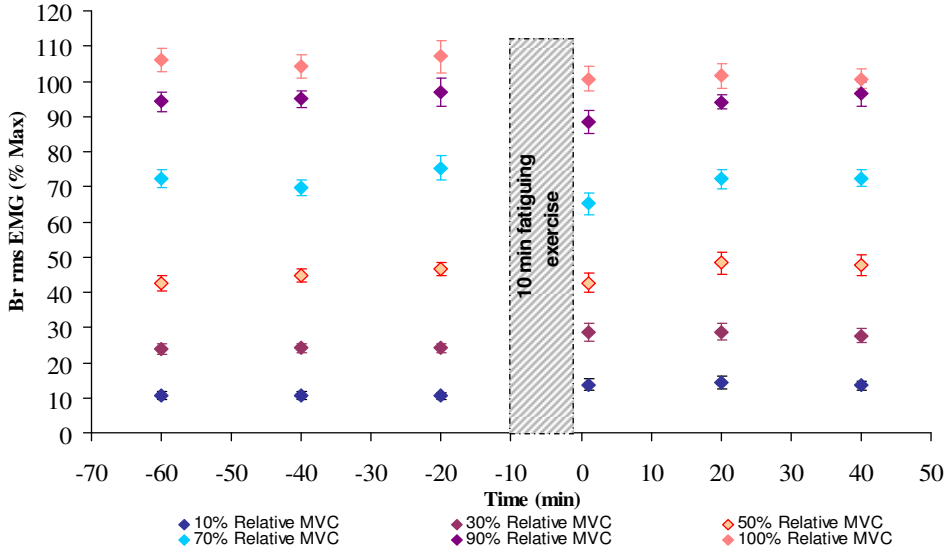


Figure 5.17: rmsEMG (mean ±SEM) of i) BB, ii) BR and iii) Br at 10, 30, 50, 70, 90, and 100% of relative MVC (n=21).

5.3.9 Mood

The general mood of participants did not change significantly between the beginning and the end of the session either with respect to Positive Affect (mean difference 0.25 ±4.9(SD), t_{11}=0.176, p=0.86, paired-samples t-test) or to Negative Affect (mean difference 1.08 ±3.4(SD), t_{11}=1.11, p=0.29, paired samples t-test), according to the responses given to the PANAS questionnaire.

5.3.10 Summary of Results

- 10min of intermittent, isometric elbow flexion did cause a 44% reduction in the MVC which, despite some recovery, remained significantly decreased (20%) compared to baseline at 20 min post exercise. It was also accompanied by a significant reduction in the EMGmax (25% drop) and M-wave (20% drop) of BR. BR M-wave evoked at rest reduced by 20% post fatigue. A small but non-significant reduction in the EMGmax and M-wave of BB was also observed.
- The group mean 13% reduction of the amplitude of the BR MEPs revealed 1min post exercise did not reach significance, and by 20 min post exercise, the mean responses had returned to pre-exercise levels.
- A statistically significant 37% drop of the mean relaxed twitch amplitude was observed at 10min post exercise. At 20 min post exercise relaxed twitch amplitudes were significantly reduced at 65% of their mean baseline values.
- In contrast, the superimposed twitches evoked by motor nerve stimulation during MVCs increased in amplitude from 1.3% of baseline MVC to 2.9% of baseline MVC 10min post exercise. 20 min post exercise they were still significantly increased (2% of baseline MVC). Consequently, maximum voluntary activation of BB was significantly reduced from 93% at baseline to 75% 10min post exercise and 83% 20min post exercise respectively.
- The mean effort ratings increased significantly by 2 points on the NRS for the 50% of absolute MVC and 1 point for the 30% of absolute MVC post exercise. The ratings remained significantly increased at 20 min post exercise. For relative levels of MVC, a short lasting decrease in the perceived effort at 90 and

100%MVC was revealed. No significant differences were detected in the effort ratings at all the other levels of relative MVC.

- The mean EMG levels of both BB and BR at submaximal contractions increased post exercise and consistently correspond to significant increases of the perceived effort for absolute levels of MVC throughout the 20 min post-exercise monitoring period. For the relative levels of MVC no significant changes were revealed immediately after the exercise at EMG of BB, Br and BR.
- Perception of effort significantly correlated with the EMG of BB and BR while the perceived effort at 30% of absolute MVC was correlated with the resting and superimposed twitches evoked by peripheral electrical stimulation.

5.4 Discussion

The aim of this study was to examine the possible changes in the perception of effort during isometric elbow flexion following a fatiguing exercise using the effort 0–10 NRS and to evaluate the neurophysiological mechanisms underlying fatigue. The main findings of this study show that 10 minutes of isometric elbow flexion at 50% MVC was associated with central and peripheral fatigue. This study is in agreement with published data that provide evidence for the presence of central changes in addition to already known peripheral ones following submaximal intermittent isometric fatiguing exercise (Allman & Roci, 2001; Søgaard *et al*., 2006; Smith *et al*., 2007). A reduction in twitch force evoked by local electrical stimulation demonstrates a peripheral component of fatigue that was elicited by the exercise protocol. A central contributor to fatigue was also revealed as it was demonstrated by the increase in the superimposed twitch force evoked during MVCs. An important role of the perception of effort in the motor control system is also indicated by the marked increase of the perception of effort following fatigue for the equivalent to pre-exercise levels of voluntary contraction as well as by the correlation of the perception of effort with the neurophysiological changes following fatigue. As such, the data confirm the fist hypothesis of this study. The absence of significant change in MEP response evoked by TMS to the contralateral motor cortex in BR, despite a small reduction immediately following the fatiguing exercise, is contrary to the second hypothesis of this study.

5.4.1 Peripheral and Central Components to Fatigue

The above findings point towards peripheral and central changes following 10 minutes of a submaximal intermittent isometric exercise of elbow flexors. The decreased resting twitch force and the accompanied reduction in the M-wave of BR during MVCs indicates impairments within the muscle as the main changes due to fatigue, for which the likely candidates responsible include impaired neuromuscular transmission or impaired excitation-contraction coupling (Zwarts *et al*., 2008). The increased superimposed twitch force at maximal contractions indicates that not all muscle units are recruited, or they are not firing fast enough to produce a maximal force, and thus

central fatigue has been developed at the same time as peripheral fatigue (Taylor *et al*., 2006). The steady decrease of voluntary activation of BB further points towards a central component of the fatigue (Gandevia, 2001). The post-fatigue decrease in the EMGmax of BB (although not statistically significant) and BR indicates reduced neural drive to the muscles and, consequently, central fatigue (Gandevia, 2001; Gibson & Noakes, 2004). The absence of significant changes 10 minutes after exercise at EMGmax and M-wave of BB might be explained by a quicker recovery of EMG response in BB than in BR. Søgaard *et al*. (2006) reported a recovery of the BB EMGmax within 8 minutes after exercise. However, recovery of the EMG responses, while the MVC and resting twitch force were still reduced, again indicate that additional changes, other than those localized in the muscle, had occurred. Although voluntary activation by peripheral nerve stimulation does not identify the origin of central fatigue, studies using voluntary activation of the motor cortex during elbow flexion isometric contractions reported reduced activation which is attributable to supraspinal changes (Todd *et al*., 2003; Søgaard *et al*., 2006; Smith *et al*., 2007).

The central changes as they were revealed by motor point stimulation were not accompanied by corticospinal excitability changes for BR as it was assessed by resting MEPs evoked by TMS. This is in disagreement with other studies which report a short lived (<1 min) post-exercise MEP facilitation followed by a depression that lasts for more than 15 minutes (Brasil-Neto *et al*., 1993; McKay *et al*., 1995; Sacco *et al*., 2000). The absence of depression in the MEPs after exercise may be due to the frequent contractions subjects performed during the recovery period as part of the perception of effort task. Continuous voluntary muscle activation could require an increased neural drive for meeting the requirements of the effort task, which might have masked any reduction in the corticospinal excitability during recovery from fatigue. Additionally, the conveniently recorded MEPs from BR may have given different results to those obtained if the MEPs from BB had been recorded. Compared with BB, which is the major elbow flexor when the forearm is supinated, as in the present experimental apparatus, BR is a minor elbow flexor. Consequently changes in corticospinal excitability following a fatiguing task that activated BR to a lesser extent than BB might be insufficient to reveal statistically significant alterations in MEPs. Alternatively, the absence of changes in corticospinal excitability, while peripheral fatigue persists, as it was revealed by the parallel reduction of the BR M-waves, might suggest that changes

in neural pathways from motor cortex to motoneurons are not directly associated with central fatigue (Taylor *et al*., 2006). A quicker recovery of corticospinal excitability following maximal or submaximal fatiguing contractions, assessed by the sEMG responses to cortical stimulation during brief MVCs, while the voluntary descending drive (assessed by motor cortex voluntary activation) was still suboptimal, has also been reported elsewhere (Gandevia *et al*., 1996). This indicates that central and supraspinal fatigue are related to the maintenance of the fatigued state of the muscle. This may not occur at the spinal motoneurons or at the level of motor cortical output, but at levels upstream of the motor cortex that may impair the voluntary descending drive (Gandevia, 2001; Taylor *et al*., 2000a; Taylor *et al*., 2006).

The time course of central and peripheral changes following the fatiguing exercise in this study show dissimilar findings to previously reported studies (Søgaard *et al*., 2006; Smith *et al*., 2007). While these studies report quicker recovery of the central fatigue (within 10 min post exercise) than peripheral fatigue changes, our results show significantly longer lasting changes in both central and peripheral measures following this fatiguing exercise protocol. Both resting and superimposed twitch forces remained significantly changed, compared with pre-fatigue levels, for 20 minutes after exercise. Similarly voluntary activation, despite some recovery to pre-exercise levels, was still reduced at 20 minutes after exercise. One likely explanation for this is the cumulative effect of the voluntary contractions, which were repeated during the recovery period as necessary part of the perception of effort task, and which may have delayed the reported rapid recovery from fatigue. These continuing contractions could also account for the delay in recovery of the MVC. Incomplete recovery of voluntary force following intermittent (3 s on, 2 s off) contractions of the elbow flexors at 60% of MVC within a 60-minute recovery profile has been reported elsewhere (Allman & Rice, 2001). This incomplete recovery might be due to peripheral limitations in the muscle such as muscle fibre damage, or a failure in the excitation-contraction coupling (Allman & Rice, 2001). The increased discomfort due to position restrictions and also due to fatigue might have enhanced the accumulation of noxious and chemical metabolites in the muscle. Prolonged discharge of the small diameter muscle afferents (groups III and IV) therefore, might have altered the motoneuronal excitability (Gandevia, 1998) resulting in prolongation of fatigue. Lack of motivation in the subject, due to the long duration of the experimental session, might be another reason for delayed recovery of the MVC

despite the strong verbal encouragement given during the contractions. The longer lasting central changes revealed by the superimposed twitches may explain why it is more difficult to exercise continually in the fatigued state and in fact could be a consequence of normal voluntarily imposed limitations to continued exercise in the post-fatigue period.

5.4.2 Perception of Effort Following Fatigue

Increase in the perception of effort with fatigue has been indirectly demonstrated in other studies by means of increased perceived exertion (Søgaard *et al*., 2006; Smith *et al*., 2007). The present study, however, is the first to record perceived effort changes directly during recovery from fatigue by using a suitable measurement tool for its assessment which has been previously tested for its reliability and validity in measuring effort during isolated repetitive exercising activity. The 0–10 NRS was sensitive enough to detect post-fatigue changes of 1 to 2 points on the scale and its linearity remained unaffected even following fatigue. The changes in the perception of effort that were detected by the 0–10 NRS following fatigue further support the potential applicability of the scale for assessing improvement in perception of effort following an intervention.

The time course of perceived effort changes following fatigue, with the accompanied changes in the twitch force and voluntary activation of BB, show that increased perceived effort correlates with well characterized neurophysiological changes of fatigue in the human voluntary motor system. Indeed, perception of effort was found to correlate significantly with the volitional EMG activity of the flexor muscles as well as the resting and superimposed twitches evoked by electrical stimulation after fatigue.

The proportional increase in the flexor EMG activity with that of an increased rating of perceived effort during submaximal voluntary contraction clearly confirms the correspondence between subjective effort rating and objective measurement of muscle activity (Pincivero *et al*., 2003b). Indeed, perceived effort followed the increased voluntary drive required to match target force level in the presence of motor fatigue, indicating the correlation of the perceived effort with the motor command (Gandevia,

2001). The increase in the EMG at submaximal levels of voluntary contraction has also been reported in other fatigue studies and may be explained best by the recruitment of extra motor units via increased descending drive or increase in the firing frequencies of motor units to compensate for the reduced number of active motor units due to fatigue (Søgaard *et al*., 2006; Smith *et al*., 2007). As the muscles recover from fatigue and more motor units are available for recruitment, the voluntary neural drive gradually returns to pre-fatigue levels and the effort consequently starts to decline.

The absence of post-fatigue changes in the ratings of perceived effort for relative levels of MVC suggests that individuals correctly rate their effort relative to their maximal effort despite fatigue. The linear correlation of the perceived effort post-fatigue with the level of voluntary contraction further confirms that fatigue does not disturb the normal ability of healthy individuals to rate the effort relative to their maximal ability. Indeed, they are still able to differentiate various levels, set to relative MVC, consistently in this new, albeit fatigued, state.

The findings about the changes of perceived effort post fatigue further indicate that the perceived effort follows the changes in the motor neural drive when the afferent signals give feedback for changes in the contractile apparatus due to fatigue. The disproportionate increase in the afferent signalling due to fatigue-induced sensations of pain and discomfort, or the presence of infection caused by muscle fibre damage (Sacco *et al*., 1999) could have led to a mismatch between the afferent feedback and the feedforward signals, resulting in greater amplitude of the corollary discharge and consequently in a gain in effort perception. The increase in the corollary discharge which is indirectly connected with an increase in the neural drive can be further supported by the parallel post-fatigue increase of the EMG at absolute levels of contraction. Thus, perception of effort is attributable to the magnitude of the voluntary motor command (Gandevia & McCloskey, 1977). The increase in the perceived effort with the central activation reduction following fatigue may reflect an underlying mechanism involved in central fatigue that could explain why patients with ME and MS experienced higher levels of effort during fatiguing exercise than healthy controls (Thickbroom *et al*., 2006; Sacco *et al*., 1997). As central fatigue is a significant part of the changes following fatiguing motor tasks in these patients (Schubert *et al*., 1998; Petajan & White, 2000), the increased perception of effort may be a centrally mediated

construct, caused by the imbalance between afferent and efferent signals resulting in the presence of symptoms in these patients (Van Houdenhove *et al*., 2007). Whether perception of effort is regulated primarily by changes in the motor commands as a result of altered afferent input, or subsequently by altered input from other areas upstream from the motor cortex (Gandevia, 2001), is yet to be determined. Further studies changing the excitability of the motor cortex by artificial or non-exercise protocols without an alteration in the afferent signal may help to explore the way perception of effort is regulated in the human motor system.

5.4.3 Summary

Ten minutes of a submaximal intermittent isometric exercise protocol not only reliably produced fatigue but also produced a significant elevation in the subject's rating of perception of voluntary effort. This increase in the perception of effort was associated with changes in neurophysiological measurements previously identified as indicators of both peripheral and central fatigue mechanisms. The fatigue-induced increase in perception of effort lasted throughout the post-exercise monitoring period. The close relationship of the elevation of the subjective ratings of perceived effort with the objective neurophysiological measures of fatigue could explain the disproportional increase in the perceived effort in conditions where chronic fatigue is the main symptom. The use of a measurement tool extensively tested in this thesis does appear to be able to detect significant changes in the effort ratings following a fatiguing exercise. The effort 0–10 NRS has the advantage, compared with the Borg scales (15 points RPE, CR10), that it can be used repeatably without requiring the prolonged, strenuous, fatiguing exercise necessary to achieve a certain level of cardiovascular activity. This has some benefit for clinical application too. The adoption of the 0–10 NRS for assessing perceived effort in research involving neurological patients might give further insight into the possible interaction between central fatigue and perception of effort in these patients. Ideally neurophysiological measurements of central fatigue via voluntary activation, measured by motor cortex and peripheral nerve stimulation, should be included in studies examining altered perceived effort. The good correlation revealed between the subjective rating scale and motor performance further points towards a

useful research tool that could be used in other types of studies in clinical conditions. Whether perception of effort is altered primarily by central changes in the motor cortex could further be tested by changing the excitability of the motor cortex in the absence of changes in the peripheral afferent signalling.

CHAPTER 6

CHANGES IN PERCEPTION OF EFFORT FOLLOWING TRANSCRANIAL DIRECT CURRENT STIMULATION

6.1 Introduction

The changes in the perception of effort with the level of voluntary activity and following fatigue, and the proportional changes in the sEMG, revealed in the previous chapters, give indirect evidence that the perception of effort is attributable to the motor commands as it has been suggested by the literature (Jackson & Dishman, 2000; Pincivero & Gear, 2000; Rosenbaum & Gregory, 2002; Pincivero *et al*., 2003a). The increase of sEMG parallel with the level of produced force indicates that the central motor commands compensate for the changes in the peripheral system while the parallel increment in the perceived effort suggests that these motor commands may be subjectively represented in terms of perception of effort (Pincivero & Gear, 2000). The findings above further suggest that the peripheral afferent signals are of great importance to the CNS to adjust its motor commands whenever it is necessary as in the case of fatigue. However, it is not clear what the involvement of the peripheral feedback is in regulation of the perception of effort. Specifically, it is not clear whether perception of effort could be altered primarily as a result of changed motor cortex excitability or alternatively whether perceived effort could be changed without changes in the afferent input from the periphery. Given that perception of effort may be primarily altered due to changes in the motor commands it would be of research interest to see whether by artificially altering the motor commands there would be any change in the perception of effort. The main aim of this study therefore is to assess potential

alterations in the perception of voluntary effort following changes in the motor cortex corticospinal excitability.

Repetitive TMS (rTMS) is the most commonly used technique to modify corticospinal excitability (Fitzgerald *et al*., 2007). However, recently another non-invasive technique has gained great interest in the neurophysiological research. Compared to rTMS which may cause side effects (Wasserman *et al*., 1998) and changes not only at the site of rTMS but also at distant connected sites, transcranial Direct Current Stimulation (tDCS) has been reported to cause a painless, selective, focal and reversible excitability modulation of the cortex (Nitsche & Paulus, 2000; Lang, 2005). These potentially beneficial applications of tDCS on the motor control system suggested that this technique could be applied in evaluating perception of effort. The involvement of the subjective feeling of effort in the neuromuscular fatigue will be better understood if perception of effort is altered by brain polarization using tDCS. Modulation of perceived effort due to tDCS may open the way for improving the complaint of fatigue in neurological patients by reducing the disproportional increment in effort.

The main aim of this study therefore, is to test whether 10 min of tDCS over the motor cortex will alter the corticomotor excitability and if this could then lead to changes in perception of effort during isometric elbow flexors contractions. Thus, a two fold hypothesis has been set for this study:

H_{01} (null 1): ***10min tDCS over the motor cortex will not alter the corticospinal excitability assessed by single pulse TMS over the motor cortex.***

H_1: ***10min tDCS over the motor cortex will alter the corticospinal excitability assessed by single pulse TMS over the motor cortex.***

Additionally, it has been hypothesized that:

H_{02} (null 2): ***Changes in the corticomotor excitability, caused by 10 min tDCS over the motor cortex, will not be followed by alteration in the perception of effort as it will be tested by effort ratings on the 0–10 NRS during isometric elbow flexors contractions***.

H_2: ***Changes in the corticomotor excitability, caused by 10 min tDCS over the motor cortex, will be followed by alteration in the perception of effort as it will be tested by effort ratings on the 0–10 NRS during isometric elbow flexors contractions***.

6.2 Methods

6.2.1 Sample

This within subjects repeated measures study comprised 3 experimental sessions. Anodal, cathodal or sham condition of tDCS was applied during one of each session randomly, in a double-blind sham-controlled experimental trial. Twelve healthy participants took part in all sessions (8 women and 4 men), with an average age of 32 ±6(SD) yrs and range between 24 and 42 years. All participants except one were right handed.

6.2.2 Apparatus

The detailed apparatus in regards to the measurement of the isometric force of elbow flexors (see section 2.4.1), the surface electromyography from BB and BR (see section 2.4.2), the ratings of the perceived effort (see section 2.4.5) and mood (see section 2.4.6) as well as the TMS (see section 2.4.4.2) is outlined in section 2.4 of the general methods chapter. The tDCS is as described in section 2.5.3.

6.2.3 Experimental procedure

After having signed a consent form, participants were seated with their arm secured in the rig (see section 2.4.1 and Fig. 2.2). The MVC for every subject was defined as described in sections 2.4.1, 2.4.2, and 2.5.1. In every experimental session the participants were familiarized with the equipment used and the utility of the NRS (see section 2.5). Following the familiarization session, the experiment started with the baseline measures taken every 20 minutes. Two baseline measures were taken in every session before the tDCS. Three more measurements were taken at 40 minutes after the stimulation to monitor the duration of potential after-effects of tDCS on perception of effort (Fig. 6.1). The perception of voluntary effort, motor cortex excitability and mood

assessment were measured before and after the stimulation at every session. One of each of the three types of tDCS, anodal, cathodal and sham, was applied in one of the subsequent sessions separated by a week to eliminate possible cumulative effects of the stimulation. In all sessions both subjects and raters were blinded to the intervention type. The experimenter who applied the tDCS was different from the experimenter who collected the data. The tDCS devise was also covered to eliminate any possibility of subjects or the investigator of the study to identify the intervention type (Gandiga *et al*., 2006). Anodal and cathodal direct current was maintained for 10 min. In sham tDCS the current was turned off 30 sec after its application, while the stimulating electrodes remained over the scalp for 10 min. This duration has been suggested by other studies for use as the sham/control condition, where electrical current application to the scalp causes habituation to any sensation, and shown not to be detectable thereafter (Gandiga *et al*., 2006). Ratings of effort on the NRS were taken during isometric contractions of elbow flexors at 30, 50, 70, 100% MVC. The schematic representation of the experimental procedure at every session could be seen in Fig. 6.1.

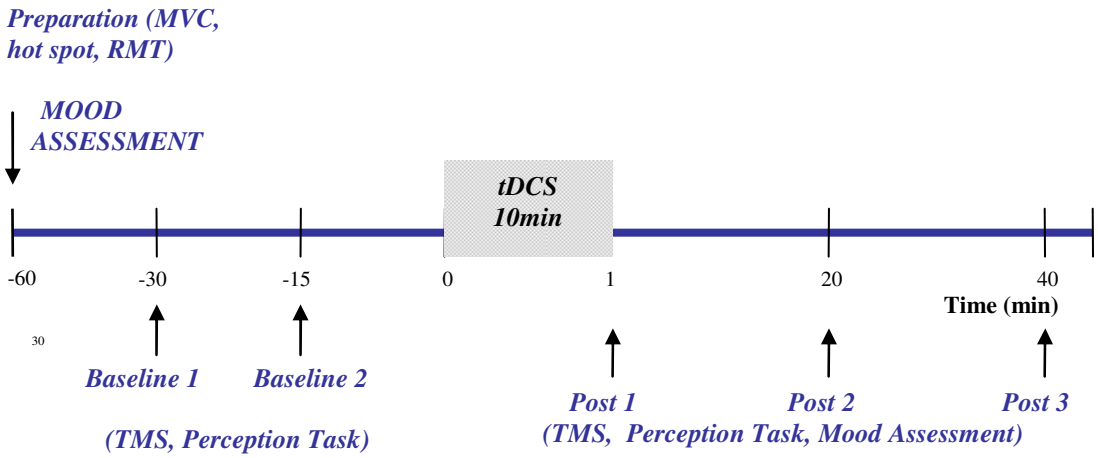


Figure 6.1: Schematic representation of every experimental session.

6.2.4 Data Analysis

All the force and EMG data were normalized as described in section 2.6. The mean of 15 consecutive MEP responses at every time point before and after the intervention was used in the analysis for the excitability assessment as described in section 2.4.4.2. The sum of the scorings on the PANAS questionnaire separately for the positive and negative affect questions was entered in the analysis for the mood assessment.

All dependent variables (MEP, mood, perceived effort, sEMG) were tested not only for their consistency among baselines for every session separately, but also for their consistency among baselines between the three sessions, separated by a week, to secure that behavioral stage of participants was stable before intervention. Changes in the MEP area, mood, perception of voluntary effort and the EMG activity of all muscles due to tDCS were analyzed through 2 and 3-factors repeated measures ANOVA with the type of treatment, the time post stimulation and the level of voluntary contraction as the factors. For the purposes of the statistical analysis the ratings of the three contractions at every force level were averaged during all the perception tasks in the entire experiment. Additionally, the two baseline measurements were also averaged for the EMG and perception measurements. Repeated t-tests have been conducted with Bonferoni adjustments and the level of significance has been set at ≤ 0.05 as described in section 2.6.

6.3 Results

6.3.1 Consistency among Baselines Within and Between Sessions

- **Accuracy in Voluntary Contractions**

Participants were accurate and consistent in producing voluntary force equal to the target level during the perceptual effort task. The Spearman's rho Correlation (ρ) between target level of force and voluntary produced force was significant at every session and at every baseline with high correlation coefficients (ρ =0.98, p<0.001).

- **MEPs**

The baseline motor cortex excitability as it was assessed from the brachioradialis MEP responses did not change significantly between sessions ($F_{(2, 16)}$=0.13, p=0.88, Partial Eta Squared=0.02, One-Way Repeated Measures ANOVA) indicating that any changes after the stimulation have not been caused merely because of possible changes in the baseline measurements. Additionally, the resting motor threshold did not change from session to session ($F_{(2, 16)}$=0.96, p=0.41, Partial Eta Squared=0.11, One-Way Repeated Measures ANOVA).

- **MVCs**

During a total period of 3 weeks the participants retained the same ability to maximally contract their elbow flexors. The MVC (before the tDC stimulation) did not change significantly from session to session as revealed by the repeated measures ANOVA for the baseline 1 between sessions ($F_{(2, 22)}$=0.19, p=0.83, Partial Eta Squared=0.02).

- **MOOD**

Additionally, the general mood of the participants remained stable from session to session without any significant changes either to Positive Affect ($F_{(2, 22)}$=0.81, p=0.50, Partial Eta Squared=0.07) or to Negative Affect ($F_{(2, 22)}$=0.34, p=0.72, Partial Eta Squared=0.03), implying that any changes found in perception did not occur due to changes in mood or motivation, factors that interfere with cognitive tasks like perception.

- **Ratings of perceived effort on 0–10 NRS**

Participants were consistent in their effort ratings of the same levels of contraction between the three sessions before the stimulation. The ICC for the effort ratings at baseline 1 for the three sessions was 0.96 (95% Confidence Interval 0.96, 0.97; excellent reliability).

6.3.2 tDCS Effects

6.3.2.1 Effect of tDCS on Corticospinal Excitability

Nine out of 12 participants (5 women, 4 men) gave complete TMS data for all sessions and were included in the analysis of MEP and RMT. The type of tDCS used (anodal, cathodal, sham) did not have any significant effect on the way the MEPs changed over time and there was no significant interaction effect between time and intervention (Table 6.1). There was however a significant effect of time on the MEPs (Table 6.1). Specifically, One-Way Repeated Measures ANOVA showed that the MEP area increased significantly following the sham ($F_{(4,\ 36)}$=8.75, p<0.001, Partial Eta Squared=0.49) and following the cathodal stimulation ($F_{(2.11,\ 18.99)}$=4.25, p=0.03, Partial Eta Squared=0.32,) compared to baseline measurements (Fig. 6.2), (Table 6.2). Anodal stimulation did not cause any significant change to the MEP responses ($F_{(4,\ 32)}$=1.03, p=0.41, Partial Eta Squared=0.11). Mean MEP area before and after the intervention at every session is presented in Table 6.3.

Additionally, the RMT did not change significantly due to stimulation or due to time, and no significant interaction effect was revealed for time and intervention factors (Table 6.1) & (Table 6.4).

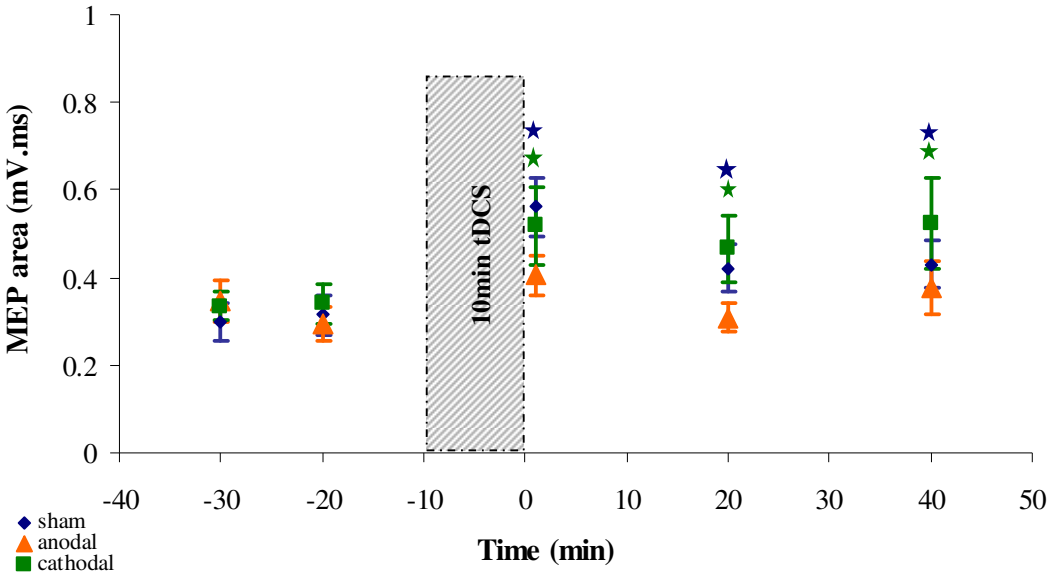


Figure 6.2: MEPs (mean±SEM) during every treatment (anodal, cathodal & sham) before and after the tDCS (n=9). The asterisks indicate significant differences (p<0.05) compared to baselines at every session separately (indicated by the colour of the asterisk: ★ sham session, ★ cathodal stimulation).

Table 6.1: Repeated measures ANOVA for MEPs, RMT, Positive & Negative affect of MOOD questionnaire and MVC. Within measures factors were the type of intervention (Sham, Anodal, Cathodal) and Time (min from stimulation:-20, -10, 1, 20, 40). The asterisks indicate significant effect (p<0.05) of the factors on the dependent variables, (n=9).

Variables	*d.f.*	*F ratio*	*P value*	*Partial Eta Squared*
MEPs				
Intervention	2	1.67	0.22	0.17
Time	4	8.47	<0.001*	0.51
Intervention × time	8	1.49	0.18	0.16
RMT				
Intervention	2	0.63	0.55	0.07
Time	1.00	1.62	0.24	0.17
Intervention × time	2	1.13	0.35	0.12
MOOD: PA				
Intervention	2	0.38	0.69	0.03
Time	1.75	2.73	0.10	0.20
Intervention × time	3.21	2.14	0.11	0.16
MOOD: NA				
Intervention	2	0.97	0.40	0.08
Time	1.22	0.48	0.54	0.04
Intervention × time	2.07	0.24	0.80	0.02
MVC				
Intervention	2.22	0.14	0.87	0.01
Time	1.43	0.31	0.66	0.03
Intervention × time	2.88	0.50	0.68	0.04

Table 6.2: MEP area (mean ±SEM) over time during the Sham and Cathodal Sessions (n=9).

Time from stimulation (min)		*SHAM*		*CATHODAL*	
Time (1)	*Time (2)*	*Mean Difference(1-2)*		*Mean Difference(1-2)*	
Pre 20	Pre 10	-0.014 ± 0.04,	p=0.74	-0.006 ± 0.02,	p=0.76
	Post 1	-0.260 ± 0.06(*),	p=0.002	-0.185 ± 0.08(*),	p =0.04
	Post 20	-0.122 ± 0.03(*),	p=0.004	-0.131 ± 0.05(*),	p =0.04
	Post 40	-0.130 ± 0.06(*),	p=0.04	-0.190 ± 0.08(*),	p =0.05
Pre 10	Post 1	-0.246 ± 0.07(*),	p=0.004	-0.179 ± 0.07(*),	p =0.02
	Post 20	-0.108 ± 0.04(*),	p=0.02	-0.125 ± 0.05(*),	p =0.03
	Post 40	-0.116 ± 0.05(*),	p=0.06	-0.184 ± 0.07(*),	p =0.03
Post 1	Post 20	0.139 ± 0.06(*),	p=0.04	-0.054 ± 0.08,	p =0.50
	Post 40	0.131 ± 0.03(*),	p=0.003	-0.005 ± 0.05,	p =0.92
Post 20	Post 40	-0.008 ± 0.05,	p=0.88	-0.059 ± 0.06,	p =0.38

Table 6.3: MEPs area (mean ±SD) mVms and range at every session (n=9).

Time	*Sham*	*Anodal*	*Cathodal*
20min Before Stimulation	0.30 ± 0.14 (range 0.10 – 0.57)	0.34 ± 0.15 (range 0.32 – 0.59)	0.33 ± 0.11 (range 0.16 – 0.51)
10min Before Stimulation	0.31 ± 0.15 (range 0.07 – 0.56)	0.10 ± 0.39 (range 60 - 73)	0.34 ± 0.15 (range 0.13 – 0.61)
1min After Stimulation	0.56 ± 0.22 (range 0.14 – 0.91)	0.40 ± 0.14 (range 0.20 – 0.62)	0.52 ± 0.29 (range 0.23 – 1.24)
20min After Stimulation	0.42 ± 0.18 (range 0.16 – 0.75)	0.15 ± 0.37 (range 60 - 73)	0.46 ± 0.25 (range 0.20 – 1.0)
40min After Stimulation	0.43 ± 0.18 (range 0.12 – 0.77)	0.13 ± 0.39 (range 60 - 73)	0.52 ± 0.35 (range 0.19 – 1.4)

Table 6.4: RMT (mean ±SD) before and after every session (n=9).

Time	*Sham*	*Anodal*	*Cathodal*
30min Before Stimulation	67.4 ± 5.91 (range 60 - 70)	65 ± 7.31 (range 60 - 80)	67.9 ± 8.97 (range 60 - 87)
60min After Stimulation	68.6 ± 4.92 (range 60 - 75)	68 ± 6.10 (range 60 - 73)	67.1 ± 7.69 (range 60 - 83)

6.3.2.2 Effect of tDCS on Mood

The positive and negative affect components of the PANAS general mood questionnaire did not change significantly due to the type of stimulation or to time. Additionally, no significant interaction effect revealed between intervention and time (Table 6.1).

6.3.2.3 Effect of tDCS on MVC

No significant changes of the MVC were revealed due to the type of tDCS used or due to time passed from stimulation (Table 6.1). The mean MVC of all participants (n=12) was 182± 19(SEM) N (range 69.35 - 294.32 N), 184± 20(SEM) N (range 86.34 - 324.99 N) and 183± 20(SEM) N (range 90.03 – 315.59 N) during sham, anodal and cathodal sessions respectively. Additionally, the MVC remained stable for about an hour following the stimulation (Table, 6.5) (Fig. 6.3), and no interaction effect was revealed between time and treatment (Table 6.1).

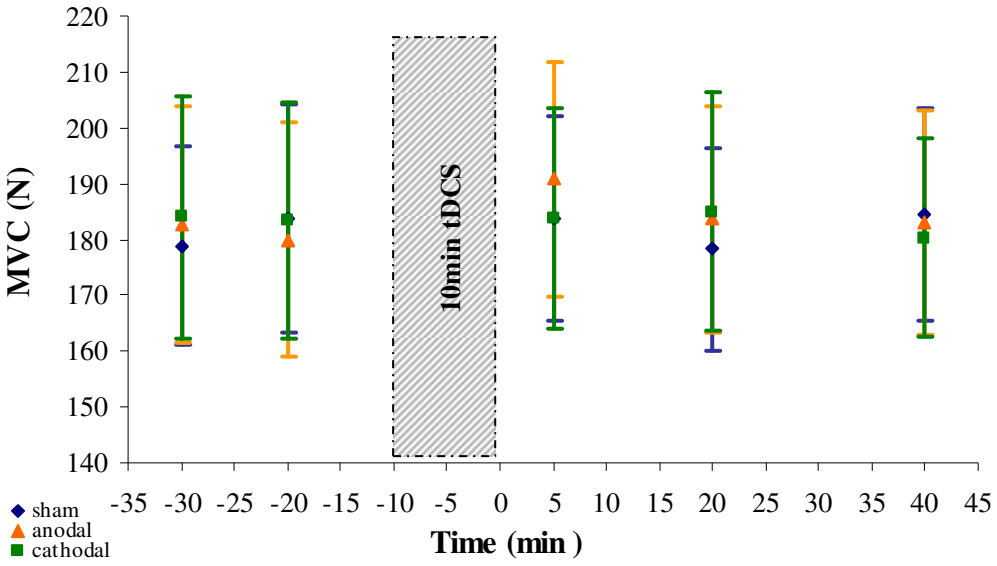


Figure 6.3: MVCs (mean ±SEM) performed during every experimental session before and after the tDCS (n=12).

Table 6.5: MVC (mean ±SEM) over time (n=12).

Time (min from tDCS)	MVC (N) (mean ±SEM)	95% Confidence Interval	
		Lower Bound	Upper Bound
20 pre	181.81 ± 19.65	138.560	225.058
10 pre	182.36 ± 20.30	137.671	227.045
5 post	186.12 ± 19.42	143.380	228.860
20 post	182.29 ± 19.61	139.126	225.451
40 post	182.63 ± 18.67	141.538	223.713

6.3.2.4 Effect of tDCS on Perceived Effort

The absence of changes in the MVC following the stimulation compared to baseline values, made unnecessary the discrimination of the ratings of perceived effort at absolute and relative levels of voluntary force. Both relative and absolute levels of target force were equal and therefore, the effort and the rmsEMG of biceps and brachioradialis will be presented only at relative levels of force (% of post stimulation MVC).

The three-way repeated measures ANOVA revealed that the type of tDCS used did not cause any significant change in the perception of voluntary effort (Table 6.6) (Fig. 6.4). The mean perceived effort was 6.13±0.23(SEM), 6.16±0.25(SEM), 6.07±0.28(SEM) at sham, anodal and cathodal stimulation respectively (range 1 to 10). The perceived effort significantly increased with the level of voluntary contraction (Table 6.6). In addition, a significant effect of time on the perceived effort was revealed (Table 6.6). In subsequent analysis with One-Way Repeated Measures ANOVA this effect of time was found only following cathodal stimulation ($F_{3,\ 33}$=4.36, p=0.01, Partial Eta Squared=0.29). Specifically, 40min after the cathodal stimulation the perception of effort significantly increased compared to baseline measurements. No significance was found in any of the interaction effects (Table 6.6). The mean and range of effort ratings at every level of contraction before and after each intervention are presented in Table 6.7.

When correlations were performed between the changes post tDCS of perception of effort and MEP at almost every treatment for most post tDCS time points no significant correlations were revealed. Only during the sham session the post stimulation changes

in the perception of effort immediately after the intervention correlated with the MEP changes (ρ=0.71, *p*=0.022, Spearman Correlation).

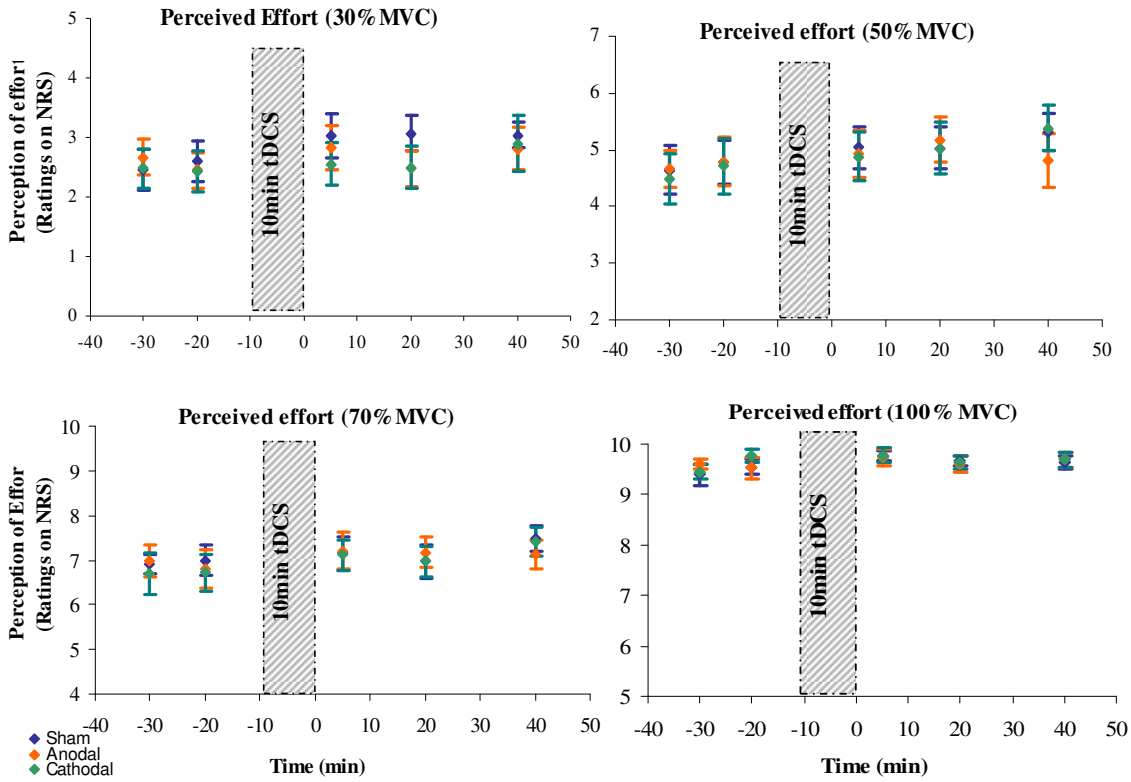


Figure 6.4: Ratings of perceived effort (mean ±SEM) over time and type of stimulation at every level of voluntary force separately (n=12).

Table 6.6: Repeated measures ANOVA for the effect of type of Intervention (Sham, Anodal, Cathodal), time (min from stimulation) and level of contraction on perceived effort and EMG of BB and BR. The asterisks indicate significant effect (p≤0.05), (n=12).

Variables	*d.f.*	*F ratio*	*P value*	*Partial Eta Squared*
Effort				
Intervention	2	0.08	0.93	0.007
Time	3	3.20	0.04*	0.24
Force Level	1.56	268.80	<0.001*	0.96
Intervention × time	6	1.78	0.12	0.15
Intervention × force level	6	0.42	0.87	0.04
Force level × time	9	1.03	0.42	0.09
Intervention × time × force level	5.8	0.99	0.44	0.09
Biceps EMG				
Intervention	2	0.99	0.39	0.09
Time	2.04	1.60	0.23	0.14
Force Level	1.56	421.74	<0.001*	0.98
Intervention × time	6	1.34	0.25	0.12
Intervention × force level	2.37	0.99	0.40	0.09
Force level × time	3.81	2.33	0.08	0.19
Intervention × time × force level	5.28	0.77	0.58	0.07
Brachioradialis EMG				
Intervention	2	0.85	0.44	0.08
Time	3	2.77	0.06	0.22
Force Level	1.52	530.74	<0.001*	0.98
Intervention × time	6	1.77	0.12	0.15
Intervention × force level	6	0.42	0.86	0.04
Force level × time	9	3.31	0.002*	0.25
Intervention × time × force level	6.11	1.18	0.28	0.11

Table 6.7: Effort ratings (average of three trials ± SD) and range of values for every level of contraction at every session (n=12).

Time to stimulation	*Levels of MVC*	*Sham*	*Anodal*	*Cathodal*
20min pre	30	2.45 ± 1.18 (range 1 – 4.3)	2.66 ± 1.01 (range 1 – 5)	2.47 ± 1.11 (range 1 – 4.33)
	50	4.64 ± 1.47 (range 3 – 6.6)	4.66 ± 1.11 (range 3.33 – 6.66)	4.58 ± 1.56 (range 2.33 – 6.66)
	70	6.91 ± 0.80 (range 6.3 – 8.3)	6.98 ± 1.23 (range 4.33 – 8.33)	6.68 ± 1.62 (range 4 – 8.33)
	100	9.39 ± 0.72 (range 8 – 10)	9.61 ± 0.34 (range 9 – 10)	9.44 ± 0.51 (range 8.33 – 10)
10min pre	30	2.60 ± 1.2 (range 1 – 5)	2.44 ± 1.02 (range 1 – 4.66)	2.42 ± 1.19 (range 1 – 4.33)
	50	4.77 ± 1.31 (range 2.5 – 6.6)	4.79 ± 1.48 (range 2 – 6.66)	4.71 ± 1.64 (range 2 – 6.33)
	70	7.15 ± 1.33 (range 6 – 8.6)	6.80 ± 1.53 (range 4 – 8.33)	6.72 ± 1.44 (range 4 – 8.66)
	100	9.55 ± 0.51 (range 9.3 – 10)	9.53 ± 0.77 (range 7.33 – 1.0)	9.77 ± 0.48 (range 8.33 – 10)
5min post	30	3.04 ± 1.29 (range 1 – 5)	2.83 ± 1.31 (range 1 – 5.66)	2.55 ± 1.20 (range 1 – 4.66)
	50	5.03 ± 1.32 (range 2 – 6.6)	4.92 ± 1.42 (range 2.75 – 7.33)	4.88 ± 1.45 (range 2.33 – 7.33)
	70	7.14 ± 1.33 (range 4.6 – 8.6)	7.21 ± 1.39 (range 5.33 – 8.66)	7.11 ± 1.14 (range 5 – 8.66)
	100	9.76 ± 0.34 (range 9 – 10)	9.75 ± 0.57 (range 8 – 10)	9.78 ± 0.52 (range 8.66 – 10)
20min post	30	3.07 ± 1.07 (range 1 – 4.5)	2.47 ± 1.00 (range 1 – 4.66)	2.5 ± 1.26 (range 1 – 4.66)
	50	5.03 ± 1.31 (range 2 – 6.6)	5.17 ± 1.39 (range 2 – 7.53)	5.02 ± 1.58 (range 4 – 6.33)
	70	6.97 ± 1.29 (range 6 – 8.6)	7.16 ± 1.18 (range 5.33 – 8.66)	6.97 ± 1.20 (range 6 – 9)
	100	9.66 ± 0.37 (range 9 - 10)	9.61 ± 0.60 (range 8.66 – 1.0)	9.43 ± 0.46 (range 8.66 – 10)
40min post	30	3.04 ± 0.70 (range 1.33 – 3.66)	2.80 ± 1.23 (range 1 – 5.66)	2.89 ± 1.63 (range 1 – 5.33)
	50	5.33 ± 1.12 (range 2.66 – 7.33)	4.80 ± 1.62 (range 3.66 – 7.66)	5.38 ± 1.38 (range 2.33 – 7.33)
	70	7.47 ± 0.97 (range 5.25 – 9)	7.13 ± 1.12 (range 4.33 – 8.66)	7.40 ± 1.13 (range 4.66 – 8.66)
	100	9.64 ± 0.41 (range 9 – 10)	9.69 ± 0.48 (range 8.33 – 10)	9.69 ± 0.50 (range 8.33 – 10)

6.3.2.5 Effect of tDCS on rmsEMG

- **BB rmsEMG**

BB rmsEMG was not affected by the type of tDCS or the time passed from stimulation (Fig. 6.5) but was significantly increased with the level of voluntary contraction (Table 6.5). No significant interaction effect was revealed between force level and time, time and type of intervention or type of intervention, time and force level (Table 6.5).

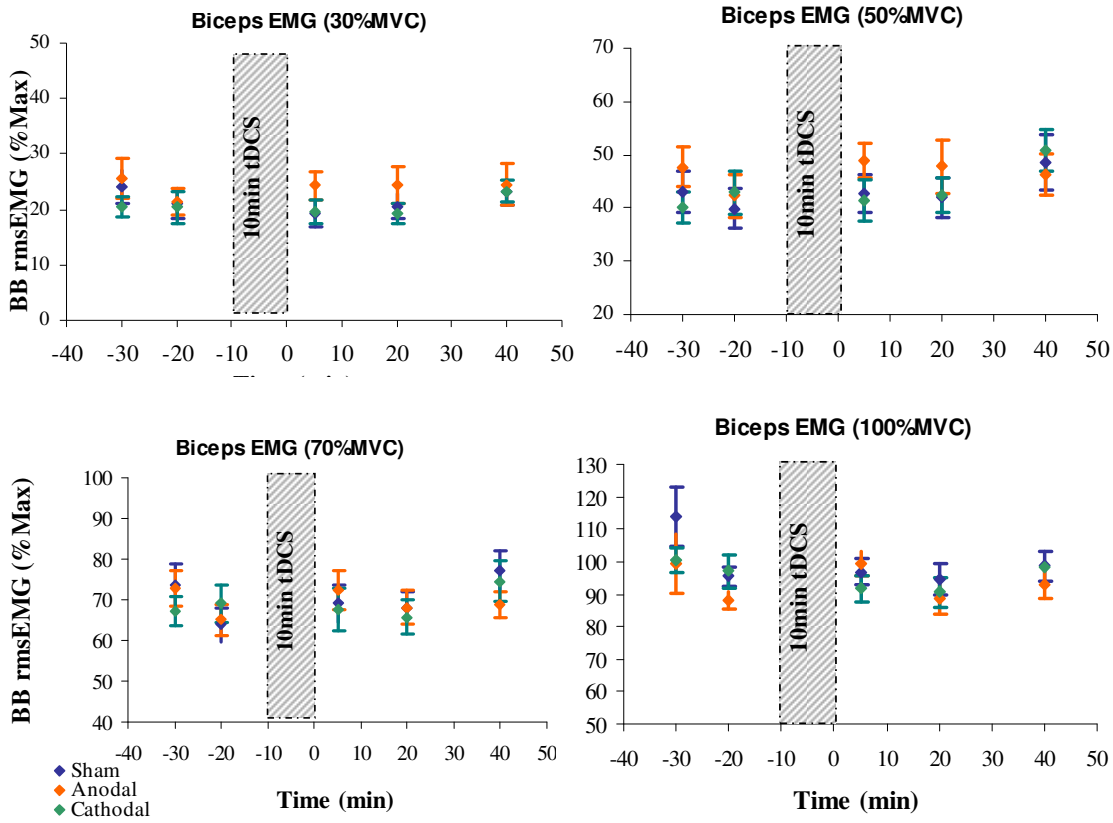


Figure 6.5: BB rmsEMG (mean ± SEM) %EMGmax over time and type of stimulation at every level of voluntary force separately (n=12).

- **BR rmsEMG**

BR rmsEMG also increased significantly with the force level but not with time or the type of intervention (Fig. 6.6). All the interactions of Three-Way Repeated Measures ANOVA were not significant apart from the one between level of voluntary force and time (Table 6.5). As revealed from further analysis, in contrast this interaction effect was significant only after cathodal stimulation ($F_{(4.04,\ 44.41)}$=2.76, p=0.04, Partial Eta Squared=0.20, Two-Way Repeated Measures ANOVA). Specifically the within subjects contrasts revealed that the increment in the EMG from the 50% to 70% of voluntary contraction was significantly less 40min post stimulation compared to the same increment before the intervention ($F_{(1,\ 11)}$=9.80, p=0.01, Partial Eta Squared=0.47, Two-Way Repeated Measures ANOVA).

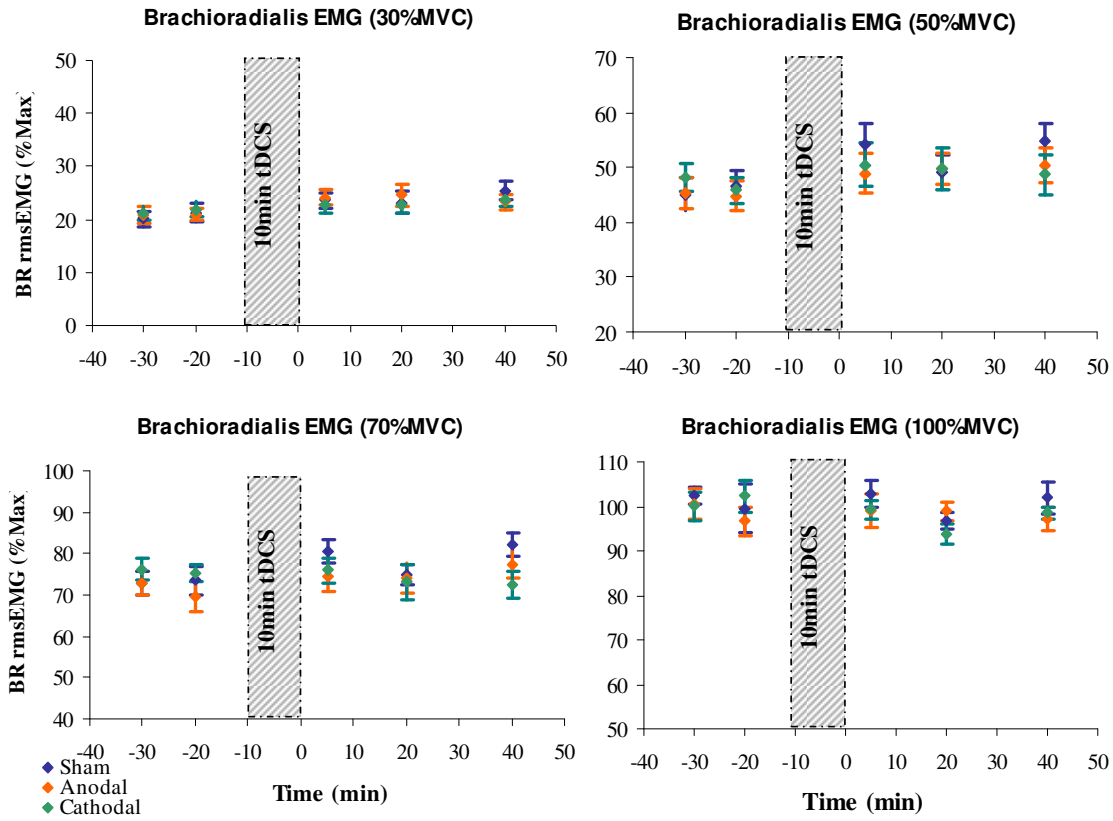


Figure 6.6: BR rmsEMG (mean ±SEM) %EMGmax over time and type of stimulation at every level of voluntary force separately (n=12).

6.3.3 Summary of Results

- 10 minutes of 1.5mA cathodal and sham tDCS did cause a significant increase in the MEP area of BR, while anodal stimulation did not cause any significant effect. The way the MEP responses changed over time was not affected by the type of stimulation used.
- Additionally, the tDCS did not cause any change in the perceived effort immediately after the stimulation. Only 40min after the cathodal stimulation the perception of effort significantly increased compared to baseline measurements.
- The perceived effort increased with the level of voluntary contraction but the way it changed from one level to the next was the same over time, and it was not affected by the type of stimulation used.
- Similarly, the tDSC did not cause any significant change in the rmsEMG of BB and BR and it did not affect the way the EMG changed over time.

6.4 Discussion

The main aim of this study was to assess potential alterations in the perception of voluntary effort resulting from changes in the motor cortex corticospinal excitability by passing non-invasively low direct current through the scalp applied over the motor cortex. The main findings point towards a different pattern of excitability changes following tDCS to those already reported in the literature (Nitsche & Paulus, 2000; Nitsche & Paulus, 2001). Thus, 10 minutes of 1.5 mA cathodal but not anodal tDCS over the motor cortex increased the corticospinal excitability as measured by the MEPs evoked by TMS from the motor cortex. The concomitant changes in the corticospinal excitability following sham stimulation indicate that additional factors other than the tDCS might account for the excitability changes. The absence of changes in the maximum voluntary force produced over time and in the general mood, as it was reported by the participants in the PANAS questionnaire, indicates that any changes in the excitability are not due to fatigue or to changes in the motivation and mood of the participants. Furthermore, the absence of changes in the perception of voluntary effort, following tDCS, further suggests that any potential effects of tDCS might have been hindered by the extensive voluntary activity required for the perceptual tasks after stimulation. The EMG activity of BB and BR followed the same post-stimulation pattern with the perception of effort without any significant change regardless of the mode of tDCS used.

A plausible explanation for the absence of perceptual changes post tDCS could be that the rating scale is not sensitive enough to pick up such changes. However, the scale has been tested in advance for its sensitivity and proved sensitive enough to pick up changes of 0.7 points of the rating scale. The fact that the EMG activity followed the same pattern of changes as the perception of effort indicates that the aforementioned explanation is not supported. Alternatively, the absence of changes in the perception of effort might indicate that the tDCS application, although effective in causing alterations at the level of neurons, may not be so efficient in causing behavioural changes at least in the doses used in the present experimental apparatus. Absence of behavioural changes post tDCS has also been reported previously. In the study of Koenigs and colleagues (2009), tDCS application failed to cause any systematic effect on emotional and

psychometric functions when it was applied over the anterior frontal lobe with an extra-cephalic reference for 35 minutes with a current density of 0.05mA/cm^2. The absence of such changes calls into question whether tDCS at moderate doses applied to healthy subjects is effective in modulating perception of effort and other psychometric functions. Stimulation of longer duration, more than 10 minutes, may yield more reliable results in future studies.

The absence of changes in the perception of voluntary effort in cases when the motor cortex excitability has been increased, as it was assessed by single TMS, calls into question whether the perceived effort is proportional to the magnitude of voluntary motor command (Gandevia & McCloskey, 1977). It has been suggested that the efferent copy of the motor command is projected from the motor cortex to the primary somatosensory cortex where it is compared with afferent inflow coming from the periphery after a motor action (Jones, 1995, cited in Wallman & Sacco, 2007). Every time there is an alteration in the relationship between the afferent inflow and the efferent copy, the motor signal is forced to readjust, with a consequent alteration in the perception of effort (Gandevia & McCloskey, 1978). In the present study, instead of changing the afferent inflow, which most studies did through fatiguing exercise (Gandevia & McCloskey, 1978; Wallman & Sacco, 2007) or peripheral anaesthesia (Gandevia & McCloskey, 1977), a change to the efferent motor command by changing the corticospinal excitability of the motor cortex was investigated. However, the perception of effort failed to change parallel to the changes in the motor cortex output, suggesting that perception of effort is closely related to the afferent inflow in such a way that perceived effort will change only when internal alteration in the afferent inflow has occurred, such as following fatigue. This is not paradoxical, given that the perception of effort reflects the effort exerted by the person to complete a task (Noble & Robertson, 1996) and it may not be expected to change if this effort does not change.

However, the afferent inflow might not only mean inflow from the peripheral muscles. It might also mean inflow to the motor cortex by other areas upstream to it (Gandevia, 2001). It is possible that the perception of effort is influenced by this inflow and that the corollary discharges involved may have to arise in the pathway before, or upstream of ,the corticospinal neurons (McCloskey, 1981). If that is the case, then the absence of changes in the perceived effort might be explained by an absence of change in the input

to the motor cortex by these areas above the motor cortex following the tDCS. Other studies using PET (Lang *et al*., 2005) have shown widespread changes in cortical and subcortical neuronal activity during tDCS, when both tDCS stimulating electrodes were placed in contralateral hemispheres. The cerebral blood flow was increased in regions underlying both electrodes, such as the contralateral primary motor cortex, the anterior cingulate cortex, the contralateral parieto-occipital junction, the temporal sulcus and the cerebellum (Lang *et al*., 2005). In the present study, however, only the active electrode was positioned over the cerebral cortex; the reference one was placed outside of the brain for more focal application of the direct current. No studies to date have reported the regional activity of the brain while using this focal tDC application method. Therefore, no conclusions could be made as to the possible alteration of the motor cortex inflow from other cortical or subcortical areas following tDCS. This focal tDCS application might have restricted the widespread changes in the association areas which are major inputs to the motor cortex. Our results could only suggest that either the perception of voluntary effort is not altered by isolated changes of the motor cortex output without any alteration in the afferent inflow, or that the effects of the tDCS, in the way that it was applied, are not localized in the areas where the perception of effort is regulated.

The absence of changes in the EMG of BB and BR means that the neural drive to muscles during the perception task did not change following the tDCS for the same levels of voluntary contraction. This indicates that, whatever the changes in the corticospinal excitability following the stimulation, these did not cause a detectable alteration in the motor neuronal activation in this study. This further implies that the long-lasting changes of tDCS might have an intracortical origin. The intracortical origin of the tDCS-induced changes in the MEP amplitude has also been mentioned elsewhere (Ardolino *et al*., 2005; Nitsche *et al*., 2005). To what extent these long-lasting changes involve synaptic efficacy is not yet determined. There are contradictory findings in the literature regarding the underlying mechanisms of brain stimulation using tDCS. Some studies support the view that the long-lasting after-effects of tDCS involve enhanced synaptic activity (Nitsche *et al*., 2008); while others favour changes in neural membrane function (Ardolino *et al*., 2005). The absence of changes in the RMT revealed in the present study, which has been suggested to reflect neuronal membrane excitability

(Nitsche *et al*., 2005) implies that the changes in the corticospinal excitability following tDCS are not due to changed membrane function.

Nevertheless, whatever is the mechanism of action of tDCS, if these changes did occur in the corticospinal tract, then this change in the descending input from supraspinal centres would have caused subsequent changes in the excitability of the motor neurons. During voluntary contractions it has been suggested that motoneuronal excitability is influenced by; the descending input to the motor neurons, the input from muscle afferents, and recurrent inhibition from reflex input, all interacting at a segmental level to influence spinal interneurone excitability (Taylor *et al*., 2000b). In the absence of changes in muscle afferents and reflex input, changes in the descending drive may be the only cause of changes in the motor neurone excitability, which could subsequently change the neural drive to the muscles and hence be recorded as changes in the EMG activity. The absence of EMG changes regardless of the kind of tDCS applied, therefore, points towards an intracortical origin of the post-tDCS MEP changes. Interestingly, in our experiment, the motor cortex excitability significantly increased following cathodal and sham tDCS conditioning, but not following anodal stimulation as it is reported in the literature (Nitsche *et al*., 2008). However, the study presented here is the only one whereby there was continued voluntary activation in the post-application time, as required for the continuous assessment of force and perception of effort, and this may explain the results from this study.

The extracephalic positioning of the reference electrode could be a reason for the unexpected results found in regards to the motor cortex excitability changes following tDCS. The more focal current application, with the reference electrode placed far from the brain, might have enervated the effectiveness of the tDCS in causing the polarization changes reported in the literature. The current orientation with respect to the target cells is a crucial parameter for the effects of tDCS and it has been pointed out in the literature that any different current orientation to the cortex-contralateral orbit one might cause different results (Nitsche *et al*., 2008). According to the literature, anodal and cathodal stimulation cause MEP changes of opposite directions (Nitsche & Paulus, 2001). Increase in the motor cortex excitability is reported following anodal tDCS and decrease in excitability following cathodal stimulation (Nitsche *et al*., 2005; Nitsche *et al*., 2008). All the above studies, however, use the conventional motor cortex-contralateral

forehead positioning of the stimulating electrodes (Nitsche & Paulus, 2000; Nitsche & Paulus, 2001), which enables the reference electrode to cause its own effects. This is supported by the study of Lang and his colleagues (2005), who reported increased brain activity under both electrodes.

One study that has used extracephalic positioning for the reference electrode reports less decrement in endurance time during a fatiguing task when it was repeated following anodal stimulation than following cathodal or sham stimulation, while increased MEP responses occurred following anodal stimulation compared with the baseline (Cogiamanian *et al*., 2007). This behavioural improvement following anodal stimulation, although sounding promising for mediating fatigue, should be compared cautiously with our results. The relative prolongation in endurance time following anodal tDCS found by Cogiamanian and colleagues (2007) might be due to changes in motor cortex excitability induced by anodal stimulation, which might have occurred secondarily due to motor fatigue. The absence of EMG changes despite the prolongation of the endurance time might further point towards an effect of tDCS not on the corticospinal neuronal projections but intracortically, which, however, might not have happened without fatigue. The increased corticospinal excitability revealed by the increased MEPs following anodal stimulation cannot be considered as proof of the efficacy of the anodal stimulation because it was tested in a separate experiment in the absence of a fatiguing motor task. Additionally, it was not compared with cathodal and sham conditions which made the statistical analysis simpler and consequently increased the probability of revealing significant changes.

The discrepancy between the results here and previous studies using tDCS for cortical excitability changes might be explained by other methodological differences including the size of the stimulating electrode and/or the intensity of current. Both of these parameters affect the density of the electrical current delivered to the motor cortex. However, compared with previous studies which used about 0.03–0.04 mA/cm^2 current density (Nitsche & Paulus, 2000; Cogiamanian *et al*., 2007), the present study used a density three times greater than that commonly used (0.14 mA/cm^2). It is unlikely, therefore, that the density could be the reason for the absence of changes in the corticospinal excitability.

Another possible explanation for the absence of significant changes in motor cortex excitability due to anodal stimulation could be the involvement of extensive voluntary activation of the muscles of interest and cognitive effort for the perception task. It is suggested that massive activation of the motor cortex by prolonged muscle contraction, as well as extensive cognitive effort, abolishes the results of anodal tDCS (Antal *et al*., 2007). Furthermore, it has been found that the tDCS-induced changes are highly dependent on the state of the participant during stimulation (Antal *et al*., 2007). Indeed, when subjects participated in a cognitive task during stimulation, the motor cortex excitability was lower after anodal stimulation and higher after cathodal stimulation (Antal *et al*., 2007). The absence of significant changes following anodal stimulation as regards the brain activity, as it was assessed by the blood oxygenation level dependent (BOLD) MRI responses over the motor cortex during a sequential finger opposition task, has also been reported (Baudewig *et al*., 2001). The authors explain the results as a "ceiling effect" due to maximum blood supply of the central sulcus required for the finger task, so that any further increase of neuronal excitability could not lead to further upregulation of blood flow (Baudewig *et al*., 2001).

The increased MEP found in our experiment after sham and cathodal stimulation might therefore reveal a significant interaction between tDCS, the cognitive state of the participants and motor exercise. That could give rise to new assumptions regarding the efficacy of the tDCS, but also to the way perception of voluntary effort could be regulated. If the participant's state during and following the stimulation does affect the brain excitability, then, by altering the cognitive or emotional state of the participants during stimulation, neuroplastic changes and subsequent perception changes might be revealed. Indeed, studies have reported a positive effect of music in motor performance, especially during submaximal exercise, through dissociation, a diversionary technique that promotes a positive mood state, turning the attention away from thoughts of physiological fatigue and lowering the perception of effort (Bishop *et al*., 2007). This gives rise to the need to investigate further alternative, more natural, methods of modifying perception of effort.

In conclusion, the absence of changes in perception of voluntary effort following 10 minutes of 1.5mA tDCS suggests that either the perceived effort is not altered by isolated changes of the motor cortex output without any alteration in the afferent inflow

from the muscles, or the effects of the tDCS are not localized in the areas where the perception of effort is regulated. The most likely explanation is that the perception of voluntary effort is not regulated by the output of the motor cortex but by the input from other centres upstream of the motor cortex. The overall state of the participant and the kind of task performed before and after the stimulation could have played a significant role in the tDCS effects on brain polarization. Further research on the regional neural activity in the human brain following focal tDCS might give insight into the issue of perception of effort. Widespread changes in cortical activity following brain polarization could be assessed by fMRI scanning while individuals undertake a motor task. Whether perception of effort is associated with activity in areas associated with the motor cortex may be determined. Changes in the cognitive and emotional state of participants while performing a motor task could also be followed by tDCS to see whether such changes interfere with the polarization effects of the direct current. The findings might help to identify a research tool that would mediate perception of effort in cases where it is reported to be disproportionally elevated, such as in chronic fatigue conditions.

CHAPTER 7

OVERALL DISCUSSION, SUGGESTIONS FOR FURTHER RESEARCH AND CONCLUSIONS

7.1 Overall Discussion

7.1.1 Overview of Aim and Objectives Achieved of the Thesis

Fatigue is a multidimensional phenomenon including both physiological and psychological aspects (Gandevia, 2001). Perception of effort increases with fatigue (Søgaard *et al*., 2006) but the way it is related to the recovery from fatigue is still inconclusive. Additionally, the existing methodologies in monitoring perception of exertion during whole body exercise may be misleading when assessing perception of effort during isometric exercise. The overall aim of the thesis therefore, was to evaluate existing methodologies for their appropriateness in assessing perception of effort and voluntary activation following isolated muscle function testing. The appropriate tools were then used to examine the relationship between subjective perception of effort and objective changes in the motor control system in cases where the motor cortical excitability was altered due to fatigue or motor cortex stimulation. Four studies were conducted to serve the main aim of the thesis and each of them has addressed one of the following objectives:

1) To assess whether magnetic stimulation could be used as an alternative to the standard electrical stimulation in the Twitch Interpolation Technique for evaluating the voluntary activation of biceps brachii muscle.

2) To adopt a rating scale and to test its reliability and validity for assessing perception of effort during isometric elbow flexion exercise.
3) To assess the changes in the rating of perceived effort following a submaximal isometric fatiguing exercise of elbow flexors and to see how these changes are associated with neurophysiological changes accompanied with fatigue.
4) To test whether localized alteration of motor cortical excitability affects perception of effort.

7.1.2 Main Overall Findings

The main findings of this research revealed both similarities and significant differences between the use of peripheral magnetic stimulation and electrical stimulation in conventional single-pulse Twitch Interpolation Technique for biceps brachii as the main elbow flexor. Thus, both techniques were sensitive enough to detect decrements in the evoked twitches as the voluntary contraction increased and voluntary activation did not differ significantly, as assessed by the two techniques. However, the twitches evoked by the former were greater than those evoked by the latter. The more widespread effect of electrical stimulation by activating other synergists, and the inability of the magnetic stimulation to always elicit supramaximal intensities may be the major contributors to these differences.

The findings of this thesis also provide evidence that the 0–10 NRS perception of effort rating scale is a reliable and valid tool for monitoring the perception of voluntary effort during isometric elbow flexion at various levels of contraction. Specifically, the 0–10 NRS demonstrated linear properties and reported excellent test-retest reliability and good concurrent criterion validity in recording perception of effort under repeated isometric contractions of elbow flexors in healthy volunteers. It also showed consistency of the ratings not only when measurements were taken within the same session, but also when a week separated the test from the re-test measurements.

Additional findings of the thesis revealed that the fatigue-induced increase in perception of effort lasted throughout the post-exercise monitoring period, and was significantly

correlated with changes in neurophysiological measurements identified as indicators of both peripheral and central fatigue mechanisms. Specifically, 10 minutes of intermittent isometric elbow flexion at 50% of MVC caused an increase in perceived effort of about 2 points on the 0–10 NRS for similar pre-exercise levels of contraction which may be explained by a gain in the feedforward-feedback information. A proportional increase in the sEMG activity of the elbow flexor muscles at submaximal levels of contraction indicates a correlation over this range of effort with voluntary command output in order to achieve the pre-set load levels required. The absence of changes in corticospinal excitability, as assessed by monitoring changes in the resting MEPs of brachioradialis (BR) evoked by TMS, while central and peripheral fatigue persist, may suggest that central fatigue occurred because of changes outside of the corticospinal path and it may well fit with the "upstream" of motor cortex changes (Gandevia, 2001). Alternatively, the continuous voluntary muscle activation and the use of a minor elbow flexor such as BR for assessing corticospinal excitability may have masked any expected results.

To further test whether perception of effort changes as a result of alterations in corticospinal excitability, direct current stimulation was delivered to the motor cortex. The results from this study showed that immediately following 10 minutes of 1.5mA tDCS over the motor cortex there was no significant effect on the perception of effort or sEMG. Indeed, no differences were revealed between ratings of effort following anodal, cathodal or sham tDCS. Paradoxically, both cathodal and sham stimulation, but not anodal stimulation, caused the observed increase in the measures of motor cortical excitability.

7.1.3 Contribution to Knowledge

The main findings of this thesis not only support the findings of other studies reporting the presence of central fatigue during and following a variety of fatiguing tasks (Sacco *et al*., 2000; Taylor *et al*., 2000a; Loscher & Nordlund, 2002), but also give a better insight into the way perception of effort changes during recovery from fatigue. Thus, it has now been proved that when fatigue is caused by isometric, intermittent, submaximal elbow flexor contractions, perception of effort is elevated with fatigue and remains

increased for at least 40 minutes after the end of the fatiguing task while central and peripheral fatigue persist. This information supplements what was already known about increase in perception of effort during a fatiguing task (Søgaard *et al*., 2006; Smith *et al*., 2007). Whether perception of effort after exercise follows the temporal changes of peripheral or central fatigue requires further investigation beyond the scope of this thesis. By prolonging the recovery period, we could investigate whether the elevation of perceived effort recovers with the complete recovery of central or peripheral fatigue. While these studies have explored the research aim in healthy individuals with a younger age profile than most clinical populations, nonetheless the above findings have important implications for clinical practice and research. Knowing the duration of elevated perception of effort post-fatigue is important. It will help clinicians and exercise trainers to advise patients or athletes on the time frame in which they could work to minimize fatigue. Thus, the performance at a new task/exercise may be reduced if the new task is undertaken while the individuals has not yet recovered from fatigue caused by a previous task. As such, individuals who undertake a fatiguing task could be advised not to perform another task before the complete recovery from the first task.

It is not yet clear how perception of effort regulates exercise performance, however, the present study showed that it is clearly related to central and peripheral components of fatigue. This finding may lead patients who complain about chronic fatigue to monitor their effort during everyday tasks and to avoid a task when it requires more effort than usual. In that way, individuals may be protected from enhanced fatigue. This is because the current study, and other studies also, suggest that increased perception of effort is a sign that fatigue is present (Presland *et al*., 2005; Søgaard *et al*., 2006), even when – as the present findings show – the cause of fatigue has been removed. The presence of central fatigue is a signal for reduced performance even if the individuals do not feel exhausted (Gandevia, 2001). Thus, if a usual task now requires more effort than usual, that may mean that the individual is already fatigued, even if he or she does not feel so.

The increased EMG activity of elbow flexors at submaximal levels of contraction, which was closely associated with increased perception of effort, indicates an underlying responsive compensation through an increase in motor unit recruitment (St Clair Gibson & Noakes, 2004). These neuromuscular changes, which are measured with surface electromyography, are objective measures of this increased voluntary output.

Taken together with the increased effort, this may support the suggestion in the literature that the perception of effort is attributable to the magnitude of the voluntary motor command (Gandevia & McCloskey, 1977).

The reduction of recruitment of muscle units revealed by the reduced rms EMG of the muscles at maximal levels of contraction may fit well with the suggested failure of the CNS to drive the muscles maximally due to central fatigue or a drop in the firing frequencies (Gandevia, 2001). The reduction of the resting twitch force and the increase in the superimposed twitch during contractions indicate that central and peripheral changes are induced not only by sustained maximal contraction (Gandevia *et al*., 1996) or by intermittent maximal (Todd *et al*., 2003) and prolonged submaximal contractions (Søgaard *et al*., 2006), but also by a short bout of an intermittent, isometric fatiguing task in the middle range of MVC.

The close association of the perceived effort with the central and peripheral fatigue changes could also have relevance to the increased perceived effort reported by patients with CFS/ME and MS during fatiguing exercise, which is significantly higher thanthat reported by suitably matched healthy controls (Sacco *et al*., 1997; Thickbroom *et al*., 2006). As central fatigue is a significant part of the changes following fatiguing motor tasks in these patients (Schubert *et al*., 1998; Petajan & White, 2000), the elevated perception of effort may reflect a protective mechanism of the CNS to regulate motor output when both an exaggerated feeling of effort and fatigue are present. Alternatively, it may be a centrally mediated mechanism, caused by the imbalance between afferent and efferent signals resulting in the presence of symptoms in these patients (Van Houdenhove *et al*., 2007).

In addition, it has also been shown that subjects, even when they are fatigued, retain their ability to rate their effort relative to their maximal ability accurately, a finding that is important for the safety of the individuals. It indicates that individuals are always aware of their limits even if these limits have changed due to fatigue. Although the present study was based on isometric and not dynamic muscle work, the findings may explain why, during prolonged exercise, elite athletes are typically guided by their perceived effort and they terminate the exercise when they believe that they have exceeded the limits of the effort that could be allocated for the task (Noakes, 2007).

The important contribution of the present thesis in neurophysiology and psychology research constitutes the establishment of the 0–10 NRS – usually used and validated for assessing pain – for rating the perceived effort during isolated isometric exercise. In order to establish proof of the principle for the subsequent use of effort-rating scales in clinical settings, it was important first to establish the validity of a measurement tool for assessing perception of effort and muscle voluntary activation. This has been demonstrated now by the findings of this thesis, and their application will be useful for further research and clinical application in cases when fatigue is the dominant and persistent complaint in chronic conditions. Now effort can be assessed directly in terms of perception of effort rather than indirectly using the more widespread rating of perceived exertion that is used particularly in exercise physiology research (Borg, 1998; Pincivero *et al*., 2003a; West *et al*., 2005). While using the NRS, participants were instructed to rate the effort needed to complete the isometric contraction, which included the effort to achieve the target force level and the effort to maintain the contraction for six seconds. These instructions ensure that the ratings reflected the effort required to complete the task, and not the strain or discomfort caused by the task. As such, the effort NRS has an advantage over the Borg scales, which rate the perceived exertion (Borg, 1998). Additionally, the use of the 0–10 NRS scale for repeated assessment of the perceived effort before and following fatigue has now provided data not only to assess directly the relationship between perceived effort and fatigue, but also to evaluate the duration of the fatigue effect on the perception of effort. The post-fatigue monitoring of the perception of effort was restricted in studies when the Borg scales were used, mainly because perception of exertion – not effort – was assessed, which was more reliably tested by correlating these with increased steady state of heart rate during graded exercise (Presland *et al*., 2005).

The reproducible ratings, its test re-test reliability, and the criterion validity in assessing perception of effort during isometric elbow exercise, suggest that more widespread use of the NRS could lead to significant contributions in the clinical study of chronic conditions. The detection of even small perceptual alterations due to changes in workload or to fatigue make the 0–10 NRS a reliable tool that could be used for evaluating the improvement or deterioration of neurological patients who report exaggerated effort as a continuous limiting factor for their everyday activities. Indeed,

the sensitivity of the 0–10 NRS to neurophysiological changes with exercise points towards a valuable recording measurement outcome for rating effort in cases when healthy people and patients report ratings of effort that are disproportional to the magnitude of the workload. The adoption of this method of assessment could lead to implementation of research protocols to study central fatigue in patients such as those with paraplegia, traumatic brain injury, MS or CFS/ME, and who are unable to undertake strenuous whole body exercises as part of rehabilitation or future interventions. Further research to test whether this scale would give valid and reproducible results when used in patients should be undertaken first. A potential obstacle could be the absence of anchors in the NRS. While research has shown that the presence of anchors on a rating scale leads to increased variation in rating (Dawes *et al*., 2005) patients may prefer the guidance provided by the anchors.

This research is the first to present a detailed comparison between electrical and magnetic stimulation of a peripheral nerve for the assessment of the voluntary activation of elbow flexors, commonly used for distinguishing between central and peripheral fatigue. Previous studies have been based on comparisons of the twitches evoked at maximum contractions between the two techniques and suggested that magnetic stimulation is a suitable alternative to electrical stimulation, but this was not tested in any great detail (Harris *et al*., 2000; Hamnegård *et al*., 2004). The relevant study of this thesis has tested the characteristics and methodological constraints of both magnetic and electrical stimulation, such as the coil orientation, the placement of the stimulating electrodes, and the intensity and duration of the electrical current, and has presented a detailed comparison between these two techniques. The data presented here do not support the superiority of one techniques of stimulation over the other. However, these findings should be considered for future research and possible clinical evaluation. Thus, reduction of nondiscrete effects of electrical stimulation to other muscles can be minimized both by reducing the inter-electrode distance of electrode stimulation pairs, and by reducing the pulse duration of the electric current. In contrast, orientation of the magnetic coil and the induced current with respect to the nerve trajectory should be tested in advance to ensure maximal evoked twitch forces for use in twitch interpolation methodologies.

The differences revealed between the two techniques indicate that the substitution of electrical stimulation with magnetic stimulation for peripheral monitoring of twitch interpolation should be done cautiously. One needs to weigh up, with due consideration of the limitations, the practicality in use with a healthy population versus an unwell patient population. The bigger twitches evoked by electrical stimulation may be an advantage for research purposes, and therefore electrical stimulation may be preferable when research is being conducted. However, the discomfort caused by electrical stimulation may be an obstacle for using the technique with patients. On the other hand, the present study revealed some advantages of the magnetic stimulation which make it a desirable technique for the peripheral assessment of muscle strength in cases when electrical stimulation is not readily tolerable. The results of the present study suggest that magnetic biceps stimulation is safe and efficient in detecting incomplete maximal contractions while the supramaximal intensities used are tolerated well by healthy participants. The minimal activation that it causes to antagonist and distal muscles, compared with electrical stimulation, provides evidence for the focality of the magnetic stimulation. Its pain-free application during muscle function assessments could also be beneficial for patients who are prevented by pain, including those in the intensive care unit. Most importantly, magnetic stimulation could be a useful technique for detecting central failure in disorders of the central nervous system which cause incomplete muscle activation. The technique could also be a valuable assessment tool in studies involving rehabilitation or training as painless evaluation of muscle activation gains could be assessed routinely. This non-volitional and painless assessment of muscle activation is particularly important given that not only physiological but also psychological parameters such as motivation, attention and mood could interfere with the ability of a person to perform maximal muscle activation (Zwarts *et al*., 2008).

Finally, the study in the use of transcranial direct current stimulation (tDCS) over the motor cortex for assessing the impact of possible changes in excitability on perceived effort was the first of its kind to evaluate this possible effect on effort. tDCS has been used before now; to alter cortical excitability artificially, and to modulate fatigue (Cogiamanian *et al*., 2007), pain, visual and cognitive functions (for review, see Nitsche *et al*., 2008). However, the technique has never been used in assessing perception of effort. The findings revealed by this study were unexpected and suggest that (i) the perceived effort is not altered by isolated changes of the motor cortex output without

any alteration in the afferent inflow from the muscles, or (ii) the effects of the tDCS are not localized in areas that the perception of effort is regulated, or (iii) the changes that the tDCS cause at neuronal level are not sufficient to alter a behavioural function. The overall cognitive and emotional state of the participants and the kind of task performed before and after the stimulation may also play a significant role in the tDCS effects. Despite the failure of this study to modulate perception of effort with DC polarization of the motor cortex, the findings of the study indicate that an acute, 10 minutes of tDCS over the motor cortex at 0.14 mA/cm^2 current density, and extra-cephalic reference, is safe and does not cause any side effects at least in healthy subjects.

In addition, the unexpected increase in motor cortical excitability found after both sham and cathodal stimulation is in contrast to the previously reported effect of tDCS applied to the motor cortex (Nitsche & Paulus, 2000; Nitsche *et al*., 2005). The more widely reported increased excitability has been attributed to anodal tDCS stimulation in a number of TMS-based studies (Nitsche & Paulus, 2000). The extensive voluntary activity and the cognitive effort required for the perception of effort task might have obscured any weaker effects of tDCS alone. Alternatively, these findings may further indicate that when two tasks are involved in the post-stimulation period it reverses the effects of tDCS. More research is needed, as to the efficacy of tDCS stimulation in fatiguing protocols should be undertaken.

7.1.4 Critical Overview of the Thesis

Strengths

The studies of this thesis were designed to address the gaps in the literature on the perception of effort, through further exploration using fatiguing exercise. To date there have been many neurophysiological studies that have explored the question of the contribution of central fatigue in voluntary movement in health and disease, but have not linked this to a detailed exploration of perception of effort. The main aim and objectives were selected to address some of the unanswered questions, not only because they were crucial in the ongoing research but also because the answers could have clinical application. The findings may then help to optimize assessment and

rehabilitation strategies designed to improve function in patients who report impaired motor performance.

The studies presented in chapters 3 to 6 were designed to answer the identified research questions. The study designs adopted here have the advantage of providing measurements over time and, therefore, in contrast to other studies, the intervention effects could be monitored and analysed in a more reliable way.

The force rig was designed to offer isolated limb positioning by minimizing the coactivation of other body parts, as required for testing the objectives of the studies. A redesign of the original force rig was seen to improve prolonged positioning. It increased comfort, ensuring sensitivity of the force measurements, as well as minimizing the negative effects of discomfort-induced pain on perception of effort when there was prolonged and maintained arm position. One limitation of the force rig was its restricted design to accommodate right-hand positioning only. However, the majority of the human population is right-handed, making it unnecessary to design a more complex bilateral force rig. In these studies, only one participant from the present sample was left-handed.

The simultaneous recording of the sEMG was essential for the studies of the present research. Continual monitoring of antagonist and synergist muscles was readily achieved, and quantification of EMG activity with force measurements is an indispensable method of monitoring voluntary activity objectively. The well known limitations of the use of sEMG regarding the stability of the signal, and the quantity of the motor units detected and assessed, were obviated by using standard recording sites and complying with guidelines for sEMG signal processing and analysis.

The perception of effort task was also designed in such a way that various levels of voluntary contraction were rated for the effort required to be achieved and maintained for six seconds. The random method of selection of force levels minimized bias in the results due to a habituation or learning effect. Additionally, effort ratings at various levels of force production, although time consuming and difficult in more widespread practical application, did give required depth of data for the adequate validation of the scale.

Single-pulse TMS, which can be used to monitor the motor cortex output, is limited in its ability to detect underlying intracortical changes (Kobayashi & Pascual-Leone, 2003), but does offer a rapid, painless and safe assessment tool for monitoring corticospinal excitability. Additionally, the short time required to record the TMS-evoked responses during the extended duration of these long-lasting experiments allowed the combination of a number of measures required to be obtained repeatedly.

The exercise interventions chosen can be replicated in clinical practice. Ten minutes of isolated fatiguing exercise of the elbow flexors, and ten minutes of tDCS could be considered a feasibly easy method of application to patients, who are unable or unwilling to undertake strenuous interventions.

Limitations

The present research has concentrated on the physiological aspect of fatigue while trying to bridge the gap between objective neurophysiological assessments and subjective psychological assessment of possible underlying factors. Changes in neural output from the motor cortex were examined in parallel with changes in perception of effort and mood, to give a better understanding of the epiphenomena of fatigue in the human voluntary motor control system. However, the use of fMRI and EEG may have helped further in testing whether perception of effort is affected by the inflow to the motor cortex from upstream areas. Additionally, it may have shown the active areas post-tDCS and may have explained the absence of post-stimulation changes of the perceived effort. The already complicated and long-lasting experiments involved in this research made it somewhat difficult to make extra measurements; however, novel approaches and techniques may be useful in future studies of perception of effort.

The length of time, which participants were immobilized in the force rig for the purposes of the experiments, might have been another limiting factor for assessing a psychological component, such as perceived effort. External factors that may have affected the perception of effort due to an extended experimental duration, such as fatigue (when it was not part of the intervention) and mood were continuously

monitored and the results showed that the possible effects of this type of restraint were minimal. However, other psychological factors, which had not been monitored, such as concentration, attention and cognitive fatigue, which are more difficult to control for in these types of experiments, may have inevitably had an effect on our results. Monitoring with the use of additional psychological questionnaires may also be required when studies test psychological phenomena.

The validation of the 0–10 NRS for rating perception of effort during isometric elbow flexion has been limited in testing only the intra-rater reliability and criterion validity. For a measurement in psychometrics, such as perception of effort, testing criterion validity is of great value because it measures the agreement between the results obtained by the given subjective instrument and more "objective" results for the same population (Streiner & Norman, 2003). However, the scale had not been tested for its construct validity and therefore it is not known how it correlates with or is at variance with other measurements of the same construct. Correlation of the NRS with the Borg CR10 scale would be another key area of future research for the validation of the 0–10 NRS for effort rating.

Additionally, the population used for the present experiments was younger than most clinical populations and not equally gender-balanced. That should be kept in mind before any implications of the results for patients are made. The age and gender profile of the subjects, although important for the generalization of the results in a clinical population, may be irrelevant at least to fatigue-induced changes. It is reported that the time to produce fatigue or the rate of voluntary force loss is not age related, and that the delayed recovery of the voluntary force was present in both young and older participants (Allman & Rice, 2001). The specific fatiguing task may be of greater importance in the development and recovery from fatigue than proposed alterations in the aged neuromuscular system (Allman & Rice, 2001). Similarly, gender differences may be applicable to the absolute levels of MVC produced, but when relevant pre-fatigue data are analysed, such differences become muscle specific and absent in elbow flexors (Albert *et al*., 2006). However, because it is also reported that females are more resistant to fatigue and maintain the contractions longer than males (Albert *et al*., 2006), matched population characteristics is always essential for valid results.

Additionally, the use of paired-pulse magnetic stimulation to compare with electrical stimulation in the Twitch Interpolation Technique may have overcome the problem of systematically evoked twitches smaller than those evoked by peripheral electrical stimulation.

7.2 Recommendations for Further Research

The present thesis has contributed to a better understanding of how perceived effort changes with fatigue. It has also provided useful methodologies for clinical and research applications. However, many questions remain unanswered about the underlying mechanisms of fatigue and the way perception of effort is involved in the human voluntary motor control system.

The brain areas that are both directly and indirectly involved in formation and regulation of the perception of effort are still unknown. There are widespread changes in the brain during fatigue (Mochizuki *et al*., 2004; Benwell *et al*., 2005) while perceived effort as a conscious process was found to be closely related to anterior cingulate cortex activity, which plays a role in functions such as error detection, motivation and anticipation (Mulert *et al*., 2005). The absence of changes in perceived effort following changes in corticospinal excitability, as shown in the study of this thesis, and the aforementioned suggestions in the literature spur further research towards revealing the precise role of the various brain areas involved in the regulation of perceived effort. The use of fMRI scanning while the participants rate their effort may give further insight in the way perceived effort is involved in the processes underlying motor performance.

The validation of the 0–10 NRS for rating effort in healthy people opens the way for its use in clinical practice and research. However, validation of the scale in patient populations is now necessary also. Estimating the minimal clinically important change on the scale that patients perceive as beneficial would be useful for implementation of progressive changes designed for rehabilitation of patients as well as in the further management of their conditions. Testing the construct validity of the scale, as well as

determining the inter-rater reliability, would help to establish the applicability of the effort NRS in the clinical field.

The limitations revealed for electrical peripheral stimulation in assessing voluntary activation, and the advantages of magnetic versus electrical stimulation, also spur further research interest for more widespread testing of the magnetic stimulation technique in a clinical environment. Testing the reproducibility of the technique in patients whose ability to produce a real MVC is impaired is fundamental if the technique is to be useful for clinical practice. Well designed, controlled experimental trials could give further evidence of whether the technique is sensitive enough to detect changes in both healthy people and patients following an intervention. A rehabilitation programme could also be given to these patients to test whether the technique is feasible in recording improvement in muscle strength and voluntary activation.

Both the NRS and the peripheral magnetic stimulation could now be applied to monitor the tendency to fatigue in people with MS and CFS/ME, while the effectiveness of appropriate rehabilitation programmes and/or medical therapies could be tested. The parallel use of EEG and fMRI could also help to correlate the subjective feeling of fatigue in terms of increased perceived effort with objective measurements of brain activity. It would be interesting, therefore, to test cognitive activity for its relationship with the increased perceived effort following fatigue. Additionally, the emotional state of individuals could be correlated with the ratings of perceived effort in studies where different populations with established emotional impairments are tested while performing exercise tasks. Furthermore, it would be interesting to test whether natural sources, such as music, could affect perception of effort by indirectly altering the emotional state of individuals and consequently alleviating the feeling of fatigue.

7.3 Concluding Remarks

The studies presented here have established appropriate methodologies for measuring the objective voluntary activation and subjective ratings of perception of effort in order to examine further the role of the perception of voluntary effort in the motor control system. For the 0–10 NRS, the excellent test-retest reliability, the very high criterion validity and the sensitivity to pick up changes of the perceived effort with the intensity of the voluntary contraction as well as subsequent to fatigue indicate that it is a valid and reliable rating of effort scale. Comparisons between electrical and magnetic stimulation of the musculocutaneous nerve in assessing the voluntary activation of biceps indicated significant differences between magnetic and electrical stimulation which indicate that substitution of electrical stimulation with magnetic stimulation in conventional single-pulse twitch interpolation technique for biceps as the main elbow flexor may have limitations for research use. However, the painless and well tolerated application of magnetic stimulation on naïve healthy individuals suggests the technique is a valuable method of muscle strength assessment in clinical practice.

In addition, this thesis documents the relationship between the psychological variable of perceived effort and the physiological variable of fatigue. The main findings of the present study confirm the presence of peripheral and central fatigue following a short bout of isometric intermittent submaximal exercise undertaken by the elbow flexors at 50% of MVC. The fatigue-induced increase in perception of effort for the same levels of voluntary contraction lasted throughout the 40-minute post-exercise monitoring period. The increase in perception of effort was followed by a proportional increase in the sEMG activity of the elbow flexor muscles, indicating a correlation over this range of effort with voluntary command output. Interestingly enough, the findings revealed that the ability of individuals to rate their effort relative to their maximal effort has not been affected by fatigue.

Whether perception of effort is regulated primarily by changes in the corticospinal excitability has not been answered by the present study. This study was unsuccessful in modulating perception of effort by applying 10 minutes of tDCS with current density 0.14 mA/cm^2 over the motor cortex. These findings raise the question whether

perception of effort is regulated by centres that were activated by tDCS or whether tDCS is inefficient in altering such behavioural functions. The increase of corticospinal excitability after cathodal and sham tDCS, but not after anodal stimulation, as revealed by the MEP responses of BR evoked by single TMS over the motor cortex, were not predicted and in contrast to the previously reported findings of post-tDCS changes in excitability. The prolonged muscle activation as well as the extensive cognitive effort required for the effort tasks may account for this finding.

The establishment of useful methodologies for assessing voluntary motor activity and perception of effort now offers the opportunity to design new research projects for the clinical environment. Such methodologies may help towards providing an effective implementation of adequate outcome evaluations of rehabilitation strategies and may enable further research into neurological conditions where chronic fatigue is a key debilitating symptom.

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APPENDIX A

Participant Information Sheet: Study I

(Magnetic vs. Electrical Peripheral Stimulation)

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RESEARCH PARTICIPANT INFORMATION SHEET (experiment I)

Study Title

The impact of fatigue on perceived voluntary effort.

Invitation Paragraph

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

What is the purpose of the study?

This study is being undertaken as part of a PhD project. We plan to conduct two experiments, which will help us understand how fatigue in the central nervous system affects muscle performance. During the first experiment we plan to develop a new technique to measure voluntary activation of the arm muscles. We hope that this technique will be easier to perform than current methods and more transferable to environments outside the laboratory, for example, hospitals.

Why have I been chosen?

You have been chosen because you are found to fit the initial inclusion criteria of this study.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your status as a student or member of staff at the University.

What will happen to me if I take part?

The study will take place at the laboratory of Brunel University. In the first session (experiment I), magnetic and electrical stimulation will be delivered to biceps brachii muscle's motor nerve or the neck (at the spinal nerve root). You will be seated comfortably in a chair with your arm resting on a table. You will be asked to perform sets of arm bends of different levels of force. At rest and during each bend single magnetic or electrical stimuli of the biceps nerve or at the neck will be delivered.

Expenses and payments

No payments or expenses will be given for this study.

What do I have to do?

You should visit the laboratory of Brunel University for two hours. Additionally, you should be healthy. Pregnant women and children will be excluded from the study.

Are there any possible risks of taking part?

The use of electrical stimulation for peripheral nerve stimulation, which will be used during experiment I, is a safe procedure but may produce some discomfort in the arm muscle and may be moderately painful at certain stimulus intensities. In the range of electrical stimulation used in the present study, the unpleasant sensation produced by stimulation through the skin is usually well tolerated. However, in any case of intolerable discomfort the session will be stopped and you have the right to withdraw from the study.

Are there any possible benefits of taking part?

We cannot promise that the study will help you but the information we get will be of great importance as might help us to understand how Central Nervous System contributes to fatigue changes of motor system. This knowledge will be helpful in designing effective treatment protocols for patients with neurological and orthopaedic disorders in the future.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible discomfort you might feel will be addressed. The research team will discuss your issue before taking any decision.

What if I want to complain about anything?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do by contacting the supervisors of this study. The contact numbers are at the end of this information sheet.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation will be kept confidential. As the experiment will take place in the University laboratory all the data will be stored in computer and discs in the laboratory where no unauthorised persons have access. Moreover, the files with the data will be protected by security codes and any data saved in papers will be kept in locked cabinets and only the researchers will have access to them. Once the research has been completed the confidential data will be stored for about 5 years for further study or educational use. In such case you will be aware of the reasons of retention and you will be asked for permission.

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the intervention that is being studied. If this happens, the researchers will tell you about it and discuss whether or not you want to continue in the study. If you decide not to carry on, your status as a student or staff will not been affected in the University. If you decide to continue in the study you will be asked to sign an updated consent form.

What will happen if I don't want to carry on with the study?

You have the right to withdraw from the study at any time without giving a reason. If you withdraw from the study we will destroy all data related to you.

What will happen to the results of the research study?

The results of this study will be included in the PhD thesis. Additionally, it is intended to publish the results. If you wish, a report of the results could be sent to you. You will not be identified in any report/publication.

Who has reviewed the study?

The study has received ethical approval from Research Ethics Committee of Brunel University.

Contact details

For further information please do not hesitate to contact with:

Sofia Lampropoulou,
Enhanced PhD Student, Brunel University
Email: sofia.lampropoulou@brunel.ac.uk
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Or with the supervisors of this experiment:

Dr. Alexander Nowicky
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Head of the School of Health Science & Social Care, Brunel University
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Thank you very much for taking the time to read this information sheet!!!

APPENDIX B

Participant Information Sheet: Study II

(Validation of NRS for Perception of Effort)

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RESEARCH PARTICIPANT INFORMATION SHEET

The Study Title

The impact of fatigue on perceived voluntary effort.

Invitation Paragraph

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

What is the purpose of the study?

This study is being undertaken as part of a PhD.

It is believed that muscle performance following fatigue is influenced by the subject's perceived effort. However, the relation of perception of effort during exercise and fatigue is still not well understood. The aim of the experiment therefore, is to investigate how the perception of voluntary effort affects the muscle performance especially under fatiguing conditions in healthy volunteers. We hope that the experiment will help us understand how the sense of effort accompanies with motor actions and how central nervous system then compensate for reduced muscle performance induced by fatigue. This knowledge is of great interest as it may help the treatment of people with neurological diseases suffering from fatigue.

Why have I been chosen?

You have been chosen because you are found to fit the initial inclusion criteria of this study. One group of healthy people will participate in this study. The group will include 15 participants.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your status as a student or member of staff at the University.

What will happen to me if I take part?

The experiment will take place at the Motor Control Lab of the Mary Seacole Building of Brunel University.

During the experiment you will be required to produce and briefly maintain arm bends at different levels of force and report your perceived effort. Force will be monitored with a device called a force transducer and it will be displayed on a computer screen. Muscle activity during the task will be recorded through small electrodes placed on the skin of your arm. Your perception of voluntary effort will be reported by using a numeric rating scale (ranging from 0 to 10, where 0 indicates no effort while 10 indicates maximum effort).

This procedure will be repeated before and after a fatiguing task. The fatiguing exercise will consist of repetitive sustained (10sec) arm bends set at a submaximal level of force.

Expenses and payments

No payments or expenses will be given for that study.

What do I have to do?

You should attend the laboratory of Mary Seacole Building for about two hours. You should be dressed appropriately (short-sleeves shirt) so as the surface of the right upper limb is exposed during the experiment. Additionally, you should not be suffering from any kind of pain on the right upper limb. It should also be preferable if you avoided any kind of fatiguing exercise that involves the right upper limb for the last three days before the experiment.
Pregnant women will be excluded from the study.

Are there any possible risks of taking part?

Muscle soreness in the arm following the fatiguing exercise may occur, but normally it disappears within a week.

Are there any possible benefits of taking part?

We cannot promise that the study will help you but the information we get will be of great importance as will help us to understand how Central Nervous System and perception of effort contributes to fatigue changes of motor system. This knowledge will be helpful in designing effective treatment protocols for patients with neurological and orthopaedic disorders in the future.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible discomfort you might feel will be addressed. The research team will discuss your issue before taking any decision.

What if I want to complain about anything?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this by contacting the supervisors of this study. The contact numbers are at the end of this information sheet.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation will be kept confidential. As the experiment will take place in the University laboratory all the data will be stored in computer and discs in the laboratory where no unauthorised persons have access. Moreover, the files with the data will be protected by security codes and any data saved in papers will be kept in locked cabinets and only the researchers will have access to them. Once the research has been completed the confidential data will be stored for about 5 years for further study or educational use. In such case you will be aware of the reasons of retention and you will be asked for permission.

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the intervention that is being studied. If this happens, the researchers will tell you about it and discuss whether or not you want to continue in the study. If you decide not to carry on, your status as a student or staff will not been affected in the University. If you decide to continue in the study you will be asked to sign an updated consent form.

What will happen if I don't want to carry on with the study?

You have the right to withdraw from the study at any time without giving a reason. If you withdraw from the study we will destroy all data related to you.

What will happen to the results of the research study?

The results of this study will be included in the PhD thesis. Additionally, it is intended to publish the results. If you wish, a report of the results could be sent to you. You will not be identified in any report/publication.

Who has reviewed the study?

The study has received ethical approval from the Research Ethics Committee of Brunel University.

Contact details

For further information, please do not hesitate to contact with:

Sofia Lampropoulou,
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Or with the supervisors of this experiment:

Dr. Alexander Nowicky
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Thank you very much for taking the time to read this information sheet!!!

APPENDIX C

Participant Information Sheet: Study III

(Perception of Effort & Fatigue)

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RESEARCH PARTICIPANT INFORMATION SHEET

Study Title

The impact of fatigue on perceived voluntary effort.

Invitation Paragraph

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

What is the purpose of the study?

This study is being undertaken as part of a PhD project.

It is believed that muscle performance following fatigue is influenced by the subject's perceived effort. However, the relation of perception of effort during exercise and fatigue is still not well understood. Additionally, it has been found that perception of effort changes as a consequence of alteration in brain activity. The aim of this experiment therefore, is to investigate how the perception of voluntary effort affects the muscle performance especially under fatiguing conditions in healthy volunteers while the brain activity will be monitored before and following a fatiguing exercise. We hope that this experiment will help us understand the changes in the central nervous system following fatigue, and it may help the treatment of people suffering from different types of fatigue in the future.

Why have I been chosen?

You have been chosen because you are found to fit the initial inclusion criteria of this study. One group of 10 healthy volunteers will participate in this study. People with neurological diseases, orthopaedic problems in upper limbs, or those who are suffering from migraines will not be included in this experiment. Additionally, pregnant women and children are excluded.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your status as a student or member of staff at the University.

What will happen to me if I take part?

The study will take place at the laboratory of Mary Seacole Building at Brunel University and it will include one session of about 2.30 hours duration.

During the experiment you will be required to produce and briefly maintain arm bends at different levels of force and report your perceived effort. Force will be monitored with a device called a force transducer and it will be displayed on a computer screen. Muscle activity during the task will be recorded through small electrodes placed on the skin of your arm. Your perception of voluntary effort will be reported by using a numeric rating scale (ranging from 0 to 10, where 0 indicates no effort while 10 indicates maximum effort). This procedure will be repeated before and after a fatiguing task. The fatiguing exercise will consist of repetitive sustained (10sec) arm bends set at a submaximal level of force.

Additionally, Magnetic Stimulation will be applied to the motor cortex (at the optimal position of the scalp to evoke motor response of the arm muscles), to see the changes in brain activity before during and following the fatiguing exercise. Electrical stimulation will be delivered to peripheral motor nerve, to see if changes in the nerve muscle responses have peripheral origin.

Expenses and payments

No payments or expenses will be given for this study.

What do I have to do?

You should visit the laboratory of Brunel University once for two and a half hours. Additionally, you should wear comfortable clothes and preferably a short-sleeves t-shirt so as the upper arm is easily exposed during the experiment. You should also not

experience any kind of pain in your right upper arm. It is also suggested you do not participate in any kind of fatiguing exercise that involves the upper arms at least three days before the experiment as fatigue will interferes with the perception of voluntary effort that we are measuring during the experiment. Finally, the day of the experiment you should feel well and in a good mood.

Are there any possible risks of taking part?

The use of electrical stimulation for peripheral nerve stimulation, which will be used during experiment, is a safe procedure but may produce some discomfort in the arm muscle and may be moderately painful at certain stimulus intensities. In the range of electrical stimulation used in the present study, the unpleasant sensation produced by stimulation through the skin is usually well tolerated. However, in any case of intolerable discomfort the session will be stopped and you have the right to withdraw from the study.

Single & paired pulse Transcranial Magnetic Stimulation is safe and without any known long-term risk. It is a non-invasive method of stimulating the brain through the scalp. Although it is a painless procedure, the activation of muscles using this technique may cause brief discomfort. However, once over the novelty of the sensation of this type of stimulation, it is well tolerated. In a small number of cases, a mild headache may ensue for about 24 hours. To minimize such issue you should feel well at the day of the experiment and not have received medication on that day or have headache for at least two days before the experiment. Nevertheless, in any case of pain or discomfort the procedure will be stopped and you have the right to withdraw from the study.

Muscle soreness in the arm following the fatiguing exercise may occur but normally, it disappears within a week.

Are there any possible benefits of taking part?

We cannot promise that the study will help you but the information we get will be of great importance as might help us to understand how Central Nervous System contributes to fatigue changes of motor system. This knowledge will be helpful in designing effective treatment protocols for patients with neurological and orthopaedic disorders in the future.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible discomfort you might feel will be addressed. The research team will discuss your issue before taking any decision.

What if I want to complain about anything?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do by contacting the supervisors of this study. The contact numbers are at the end of this information sheet.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation will be kept confidential. As the experiment will take place in the University laboratory all the data will be stored in computer and discs in the laboratory where no unauthorised persons have access. Moreover, the files with the data will be protected by security codes and any data saved in papers will be kept in locked cabinets and only the researchers will have access to them. Once the research has been completed the confidential data will be stored for about 5 years for further study or educational use. In such case you will be aware of the reasons of retention and you will be asked for permission.

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the intervention that is being studied. If this happens, the researchers will tell you about it and discuss whether or not you want to continue in the study. If you decide not to carry on, your status as a student or staff will not been affected in the University. If you decide to continue in the study you will be asked to sign an updated consent form.

What will happen if I don't want to carry on with the study?

You have the right to withdraw from the study at any time without giving a reason. If you withdraw from the study we will destroy all data related to you.

What will happen to the results of the research study?

The results of this study will be included in the PhD thesis. Additionally, it is intended to publish the results. If you wish, a report of the results could be sent to you. You will not be identified in any report/publication.

Who has reviewed the study?

The study has received ethical approval from Research Ethics Committee of Brunel University.

Contact details

For further information please do not hesitate to contact with:

Sofia Lampropoulou,
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Dr. Alexander Nowicky
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Thank you very much for taking the time to read this information sheet!!!

APPENDIX D

Participant Information Sheet: Study IV

(Perception of Effort & tDCS)

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RESEARCH PARTICIPANT INFORMATION SHEET

The Study Title

The impact of fatigue on perceived voluntary effort.

Invitation Paragraph

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

What is the purpose of the study?

This study is being undertaken as part of a PhD.

It is believed that muscle performance following fatigue is influenced by the subject's perceived effort. However, the relation of perception of effort during exercise and fatigue is still not well understood. Perception of effort alters as result of changes in the brain and these changes might explain some of the findings following fatigue. The aim of this experiment therefore, is to assess the changes in the perception of effort following brain alterations caused by weak transcranial direct current stimulation.

This technique of transcranial direct current stimulation has been recently developed and has been effectively used in neuroscience and neurorehabilitation research as it has shown that by changing the level of activity in the brain it can modulate the pain, the working memory, the depression and the endurance time of a motor task. We are particularly interested in testing the changes that transcranial direct current stimulation might cause to the perception of voluntary effort. The information gained from that experiment might help us understand how the perception of effort is regulated in the central nervous system with the intention to further examine how it is modulated by fatigue. This knowledge is of great interest as it may help the treatment of people with neurological diseases suffering from fatigue.

Why have I been chosen?

You have been chosen because you are found to fit the initial inclusion criteria of this study. One group of 10 healthy people will participate in this study. People with neurological diseases, migraines, scalp operations, or with orthopaedic problems in the right upper arm will not be included in the study. Additionally children and pregnant women will also be excluded.

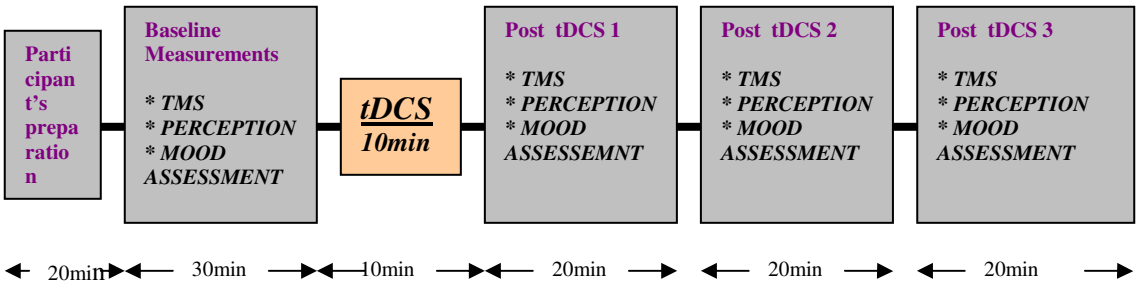
Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your status as a student or member of staff at the University.

What will happen to me if I take part?

The experiment will take place at the motor control laboratory of Mary Seacole Building of Brunel University and it requires three visits of about 2 hours every visit. Every session will consist of measurements of the brain activity and the perception of effort before and after the intervention. Additionally, your mood state will be assessed before and twice after the intervention at each session to see whether possible interactions with the time and the intervention might affect the perception of effort (flow chart). The intervention will be the application of low intensity transcranial Direct Current stimulation (tDCS) for 10 minutes through surface electrodes over the motor area of the brain responsible for the motor control. The visits will differ in the polarity of transcranial direct current stimulation applied (anodal, cathodal or sham).

For the brain activity measurements Transcranial Magnetic Stimulation (TMS) will be delivered over the motor area of the brain at the optimal site of the scalp to evoke motor response of the arm muscles. The perception of voluntary effort will be recorded while you produce forearm bends at various levels of force. At the end of every bend you will have to report your effort in an 11-point Numeric Rating Scale. Finally, your mood state will be assessed by filling in a self –report 20-items mood questionnaire.



Expenses and payments

No payments or expenses will be given for that study.

What do I have to do?

You should attend the laboratory of Mary Seacole Building three times, two hours every time. Additionally, you should feel well on the day of the experiment. People who feel unwell or suffer by headaches in a regular basis will not be included in the study. Pregnant women and people with neurological conditions will be also excluded. Additionally you should not experience any kind of pain in your right upper limb. Because fatigue might cause disturbance in the perception of effort it is recommended that you will not participate in any kind of fatiguing exercise that involves the upper limbs for at least three days before the experiment. Furthermore, you should be dressed appropriately (short-sleeves shirt) so as the surface of the right upper limb is exposed during the experiment.

Are there any possible risks of taking part?

Single pulse Transcranial Magnetic Stimulation (experiment II) is safe and without any known long-term risk. It is a non-invasive method of stimulating the brain through the scalp. Although it is a painless procedure, the activation of muscles using this technique may cause brief discomfort. However, once over the novelty of the sensation of this type of stimulation, it is well tolerated. In a small number of cases, a mild headache may ensue for about 24 hours. To minimize such issue you should feel well at the day of the experiment and not have received medication on that day or have headache for at least two days before the experiment. Nevertheless, in any case of pain or discomfort the procedure will be stopped and you have the right to withdraw from the study.

Transcranial Direct Current Stimulation is also a non-invasive technique of stimulating the brain through electrodes placed over the scalp. It has been increasingly used the last 10 years in Neuroscience Research as it is easy to administer, safe and painless. The parameters of the tDCS we use in our study are the common ones used in neuroscience research and have been suggested for their safeness. No side effects have been reported previously other than a sense of itching under the electrode during the first seconds of stimulation which however, fade very quickly and a short light flash as the current turned on and off.

Are there any possible benefits of taking part?

We cannot promise that the study will help you but the information we get will be of great importance as will help us to understand how Central Nervous System and perception of effort contributes to fatigue changes of motor system. This knowledge will be helpful in designing effective treatment protocols for patients with neurological and orthopaedic disorders in the future.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible discomfort you might feel will be addressed. The research team will discuss your issue before taking any decision.

What if I want to complain about anything?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this by contacting the supervisors of this study. The contact numbers are at the end of this information sheet.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation will be kept confidential. As the experiment will take place in the University laboratory all the data will be stored in computer and discs in the laboratory where no unauthorised persons have access. Moreover, the files with the data will be protected by security codes and any data saved in papers will be kept in locked cabinets and only the researchers will have access to them. Once the research has been completed the confidential data will be stored for about 5 years for further study or educational use. In such case you will be aware of the reasons of retention and you will be asked for permission.

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the intervention that is being studied. If this happens, the researchers will tell you about it and discuss whether or not you want to continue in the study. If you decide not to carry on, your status as a student or staff will not been affected in the University. If you decide to continue in the study you will be asked to sign an updated consent form.

What will happen if I don't want to carry on with the study?

You have the right to withdraw from the study at any time without giving a reason. If you withdraw from the study we will destroy all data related to you.

What will happen to the results of the research study?

The results of this study will be included in the PhD thesis. Additionally, it is intended to publish the results. If you wish, a report of the results could be sent to you. You will not be identified in any report/publication.

Who has reviewed the study?

The study has received ethical approval from Research Ethics Committee of Brunel University.

Contact details

For further information, please do not hesitate to contact with:

Sofia Lampropoulou,
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Thank you very much for taking the time to read this information sheet!!!

APPENDIX E

Participant Consent Form: Study I & III

(Magnetic vs. Electrical Peripheral Stimulation & Perception of Effort following Fatigue)

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CONSENT FORM

The impact of fatigue on perceived voluntary effort

The study has received ethical approval from Research Ethics Committee of Brunel University

General Health Screening for Participation in Research using Transcranial Magnetic Stimulation.

Confidential

Please answer the following health related questions. You should only complete this screen if you know that you are fit and healthy. If you answer yes to any of these questions then you should not participate in the study.

Please circle your responses

Question	
I feel unwell today.	Yes No
I suffer from dizziness.	Yes No
I suffer from balance disturbances.	Yes No
I am on prescribed medication.	Yes No
I have an orthopaedic condition (injury to my joints).	Yes No

I have a medical condition.	Yes No
I have a heart condition and /or have a cardiac pacemaker.	Yes No
I have a respiratory problem other than asthma.	Yes No
I have a dermatological condition.	Yes No
I have a (metal) prosthesis or implant in my body.	Yes No
I have had a neurosurgical procedure (operation to the skull).	Yes No
I have an aneurysm clip in my head.	Yes No
I have a neurological condition (including epilepsy).	Yes No
I suffer regularly by headaches (migraine, or other).	Yes No
I am pregnant.	Yes No

If you have answered "no" to all of the above questions then you may participate in the research using these techniques. Your participation is entirely voluntary. You may withdraw at any time from any session for any or no reason. Should you choose not to participate or to withdraw from a session, your status as a student or staff of Brunel University will in no way be affected.

If you have any concerns please feel free to ask for further information.

Risk and Discomfort

The use of electrical stimulation for peripheral nerve activation, during experiment I, is a safe procedure, but in some cases it may cause a mild discomfort and may be moderately painful at certain stimulus intensities. In the range of electrical stimulation used in the present study, the unpleasant sensation produced by stimulation through the skin is usually well tolerated. However, in any case of intolerable discomfort the session will be stopped and you have the right to withdraw from the study.

The use of magnetic stimulation either for single pulse transcranial activation of the brain's voluntary motor control area or peripheral nerve stimulation is safe and without any known long term risk. This technique has been used throughout the world for over 20 years in both research and clinical screening. Although it is a painless

procedure, the activation of muscles using this technique may cause brief discomfort. However, once over the novelty of the sensation of this type of stimulation, it is well-tolerated. In a small number of cases, a mild headache may ensue for about 24 hours. Nevertheless, in any case of discomfort or mild headache the procedure will stop and you can withdraw from the study.

Muscle soreness in the arm following the fatiguing exercise may occur but normally, it disappears within a week.

Participant's Statement

Please, read this form carefully. If you have any further questions, please do ask. You have the right to change your mind at any time, including after you have signed this form.

Please tick the appropriate box

	YES	NO
Have you read the research participant information sheet?	☐	☐
Do you understand that your name will not be referred to in any report regarding the study?	☐	☐
Do you understand that your are free to withdraw from the study:		
▪ at any time	☐	☐
▪ without affecting your status as student or member of staff at this University	☐	☐
Do you understand that your participation is entirely voluntary?	☐	☐
Do you understand that your are free to refuse to participate?	☐	☐
Do you agree your anonymized data, recorded during the study, to be stored beyond the completion date, for future study or educational use?	☐	☐
Do you agree for to take part in the study?	☐	☐

I understand the information provided for me and agree to participate in the research project and give my consent.

Name: _____ Signature: _____

Age: _____ Date: _____/_____/_____

Witness Details

Witness: _____ Signature: _____

APPENDIX F

Participant Consent Form: Study II

(Validation of 0–10 NRS)

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CONSENT FORM

The impact of fatigue on perceived voluntary effort

The study has received ethical approval from Research Ethics Committee of Brunel University

General Health Screening for Participation in Research.

Confidential

Please answer the following health related questions. You should only complete this screen if you know that you are fit and healthy. If you answer yes to any of these questions then you should not participate in the study.

Please circle your responses

Question	
I feel unwell today.	Yes No
I suffer from dizziness.	Yes No
I suffer from balance disturbances.	Yes No
I am on prescribed medication.	Yes No
I have an orthopaedic condition (injury to my joints).	Yes No

I have a medical condition.	Yes No
I have a heart condition and /or have a cardiac pacemaker.	Yes No
I have a respiratory problem other than asthma.	Yes No
I have a dermatological condition.	Yes No
I have a (metal) prosthesis or implant in my body.	Yes No
I have had a neurosurgical procedure (operation to the skull).	Yes No
I have an aneurysm clip in my head.	Yes No
I have a neurological condition (including epilepsy).	Yes No
I suffer regularly by headaches (migraine, or other).	Yes No
I am pregnant.	Yes No

If you have answered "no" to all of the above questions then you may participate in the research using these techniques. Your participation is entirely voluntary. You may withdraw at any time from any session for any or no reason. Should you choose not to participate or to withdraw from a session, your status as a student or staff of Brunel University will in no way be affected.

If you have any concerns please feel free to ask for further information.

Risk and Discomfort

Muscle soreness in the arm following the fatiguing exercise may occur but normally, it disappears within a week.

Participant's Statement

Please, read this form carefully. If you have any further questions, please do ask. You have the right to change your mind at any time, including after you have signed this form.

Please tick the appropriate box

	YES	NO
Have you read the research participant information sheet?	☐	☐
Do you understand that your name will not be referred to in any report regarding the study?	☐	☐
Do you understand that your are free to withdraw from the study:		
▪ at any time	☐	☐
▪ without affecting your status as student or member of staff at this University	☐	☐
Do you understand that your participation is entirely voluntary?	☐	☐
Do you understand that your are free to refuse to participate?	☐	☐
Do you agree your anonymized data, recorded during the study, to be stored beyond the completion date, for future study or educational use?	☐	☐
Do you agree for to take part in the study?	☐	☐

I understand the information provided for me and agree to participate in the research project and give my consent.

Name: _____ Signature: _____

Age: _____ Date: _____/_____/_____

Witness Details

Witness: _____ Signature: _____

APPENDIX G

Participant Consent Form: Study IV

(Perception of Effort & tDCS)

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CONSENT FORM

The impact of fatigue on perceived voluntary effort

The study has received ethical approval from Research Ethics Committee of Brunel University

General Health Screening for Participation in Research using Transcranial Magnetic Stimulation.

Confidential

Please answer the following health related questions. You should only complete this screen if you know that you are fit and healthy. If you answer yes to any of these questions then you should not participate in the study.

Please circle your responses

Question	
I feel unwell today.	Yes No
I suffer from dizziness.	Yes No
I suffer from balance disturbances.	Yes No
I am on prescribed medication.	Yes No

I have an orthopaedic condition (injury to my joints).	Yes	No
I have a medical condition.	Yes	No
I have a heart condition and /or have a cardiac pacemaker.	Yes	No
I have a respiratory problem other than asthma.	Yes	No
I have a dermatological condition.	Yes	No
I have a (metal) prosthesis or implant in my body.	Yes	No
I have had a neurosurgical procedure (operation to the skull).	Yes	No
I have an aneurysm clip in my head.	Yes	No
I have a neurological condition (including epilepsy).	Yes	No
I suffer regularly by headaches (migraine, or other).	Yes	No
I am pregnant.	Yes	No

If you have answered "no" to all of the above questions then you may participate in the research using these techniques. Your participation is entirely voluntary. You may withdraw at any time from any session for any or no reason. Should you choose not to participate or to withdraw from a session, your status as a student or staff of Brunel University will in no way be affected.

If you have any concerns please feel free to ask for further information.

Risk and Discomfort

The use of magnetic stimulation for single pulse transcranial activation of the brain's voluntary motor control area or peripheral nerve stimulation is safe and without any known long term risk. This technique has been used throughout the world for over 20 years in both research and clinical screening. Although it is a painless procedure, the activation of muscles using this technique may cause brief discomfort. However, once over the novelty of the sensation of this type of stimulation, it is well-tolerated. In a small number of cases, a mild headache may ensue for about 24 hours.

Additionally, the use of transcranial direct current stimulation is considered to be safe at the parameters used in this experiment. Only a slight tingling sensation may be caused under the electrodes during

the first seconds of stimulation which however fade quickly in a few seconds. A sensation of a short light flash might also be occurred when the stimulation is switched on and off. Nevertheless, in any case of discomfort or mild headache the procedure will stop and you can withdraw from the study.

Participant's Statement

Please, read this form carefully. If you have any further questions, please do ask. You have the right to change your mind at any time, including after you have signed this form.

Please tick the appropriate box

	YES	NO
Have you read the research participant information sheet?	☐	☐
Do you understand that your name will not be referred to in any report regarding the study?	☐	☐
Do you understand that your are free to withdraw from the study:		
▪ at any time	☐	☐
▪ without affecting your status as student or member of staff at this University	☐	☐
Do you understand that your participation is entirely voluntary?	☐	☐
Do you understand that your are free to refuse to participate?	☐	☐
Do you agree your anonymized data, recorded during the study, to be stored beyond the completion date, for future study or educational use?	☐	☐
Do you agree to take part in the study?	☐	☐

I understand the information provided for me and agree to participate in the research project and give my consent.

Name: _____ Signature: _____

Age: _____ Date: _____/_____/_____

Witness Details

Witness: _____ Signature: _____

APPENDIX H

Brunel University Ethics Committee:

Approval of Final Research Protocol with Ammendments

Brunel
UNIVERSITY
WEST LONDON

School of Health Sciences and Social Care

Research Ethics Committee

School of Health Sciences and
Social Care
Brunel University,
Uxbridge
Middlesex UB8 3PH
Telephone: +44 (0)1895 274000
Web www.brunel.ac.uk

22 September 2008

Approval of Amendment to Protocol

Proposer: **Sofia Lampropoulou – PhD Student**

Title: **The impact of fatigue on perceived voluntary effort (Request for amendment to protocol)**

Reference: **08/08/PHD/08**

The School Research Ethics Committee has considered the amendment to protocol recently submitted by you in relation to the above study. Acting under delegated authority, the Chair is satisfied that there is no objection on ethical grounds to the amendment. Approval is given on the understanding that the conditions of approval set out below are followed:

- *The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee.*

NB:

- **Research participant information sheets and (where relevant) flyers, posters and consent forms, should include a clear statement that research ethics approval has been obtained from the School of Health Sciences and Social Care Research Ethics Committee.**
- **Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.**

Elizabeth Cassidy

Elizabeth Cassidy
Chair, Research Ethics Committee
School of Health Sciences and Social Care

APPENDIX I

PANAS scale

PANAS

Directions

This scale consists of a number of words that describe different feelings and emotions. Read each item and then circle the appropriate answer next to that word. Indicate to what extent you have felt this way during the past week.

Use the following scale to record your answers.

(1) = Very slightly or not at all (2) = A little (3) = Moderately (4) = Quite a bit (5) = Extremely

	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
1. Interested	1	2	3	4	5
2. Distressed	1	2	3	4	5
3. Excited	1	2	3	4	5
4. Upset	1	2	3	4	5
5. Strong	1	2	3	4	5
6. Guilty	1	2	3	4	5
7. Scared	1	2	3	4	5
8. Hostile	1	2	3	4	5
9. Enthusiastic	1	2	3	4	5
10. Proud	1	2	3	4	5
11. Irritable	1	2	3	4	5
12. Alert	1	2	3	4	5
13. Ashamed	1	2	3	4	5
14. Inspired	1	2	3	4	5
15. Nervous	1	2	3	4	5
16. Determined	1	2	3	4	5
17. Attentive	1	2	3	4	5
18. Jittery	1	2	3	4	5
19. Active	1	2	3	4	5
20. Afraid	1	2	3	4	5