THE ASSOCIATION BETWEEN EXERCISE-INDUCED MUSCLE DAMAGE AND CORTICAL ACTIVITY IN THE ALPHA AND BETA FREQUENCY RANGE

BY

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DECLARATION

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THESIS ABSTRACT

This thesis examines the regulation of muscle function following exercise-induced muscle damage (EIMD), in an attempt to determine whether regulation occurs primarily in the muscle (neuromuscular) or further upstream. Upstream regulation has been hypothesized to occur in the lower brain structures, but one may assume that the efferent output to the muscle should be guided by the motor and pre-motor cortex alongside other associated cortical areas.

There are five chapters comprising of theoretical and experimental sections. Chapter one is a review of the current understanding, knowledge and controversies around EIMD, pain, electromyography (EMG) and electroencephalography (EEG). More specifically this chapter focuses on exercise-induced muscle damage (EIMD) and the associated clinical symptoms and neuromuscular changes. A further focus is on acute pain, mainly muscular pain and pain associated with movement and EIMD. Central nervous system pathways connecting the periphery with the brain are also discussed. This chapter further explores the use of EMG to measure the neuromuscular changes associated with EIMD. In the third section the effect of pain and movement on the activity of cortical alpha (α) and beta (β) frequency bands is discussed. The use and applications of EEG during movement and whilst experiencing acute pain, are also discussed. It was apparent after this review that there is a need to investigate the effects of the symptoms and neuromuscular changes caused by EIMD on the EMG activity as well as the cortical α- and β- frequency band activity.

The second chapter is the first experimental chapter which investigates the effect of the symptoms of EIMD on the neuromuscular function and EMG activity during maximal isometric contractions (MVC) and submaximal flexion-extension movements. Twenty-five right-handed males were recruited for this study. They were allocated to either a control (n = 13) or experimental group (n = 12). In contrast to the control group, the experimental group participated in an EIMD induction protocol. Subsequently, both groups and their symptoms were closely monitored for 132 hours. After the induction of EIMD, the experimental group displayed symptoms of muscle pain, swelling, muscle shortening, increased serum creatine kinase activity, decreased force output and altered neuromuscular function. Muscle pain scores in the experimental group peaked after 36 hours with creatine kinase activity peaking after 108 hours. Twelve hours after EIMD was induced, EMG activity had decreased by 20% during a MVC in the

experimental group and decreased by a further 10% until the end of the study was reached 132 hours after the EIMD inducing protocol. In contrast EMG during a submaximal flexion-extension movement increased significantly until the end of the study (132 hours). In conclusion this study showed that EIMD disturbs neuromuscular function during maximal isometric and submaximal shortening-lengthening exercises but these changes are not directly related to the clinical symptoms. The dissociated EMG activity at submaximal and maximal movement intensity also suggests that central regulation influences the neural firing patterns and motor unit activity.

The aim of the study in the third chapter was to investigate the effects of EIMD to determine the relationship between the peripheral symptoms, neuromuscular changes and delayed pain sensation during a submaximal movement of the biceps brachii on cortical alpha (α) activity. In contrast to the control (n = 12) group, the experimental (n = 16) group participated in an EIMD inducing protocol and both groups were monitored for 132 hours post-EIMD induction. At twelve hours neuromuscular functioning was already disturbed while the sensation of pain was perceived but not fully developed. Muscle pain scores in the experimental group peaked after 36 hours with the lowest torque reported at twelve hours. Alpha-1 activity increased significantly in the motor and somatosensory area twelve hours post-EIMD while α-2 activity increased in the contralateral fronto-central area. At 36 hours pain had further increased and neuromuscular function improved whilst α-1 and α-2 activities had decreased. We hypothesise that α-1 activity over the motor and somatosensory cortex of the experimental group displays a compensatory increase in response to the changes in neuromuscular function during movement, whilst an increase in α-2 activity is related to the suppression of pain experienced within the first twelve hours.

The fourth chapter describes an experiment in which the response of cortical β activity to pain and neuromuscular changes caused by EIMD was studied. This study explored the manner in which cortical β activity changed during a testing protocol lasting for 144 (pre to 132 hours post) hours. In more detail this study investigated the effect of neuromuscular changes and pain induced by EIMD and the recovery of the symptoms of EIMD on the global cortical β range activity in the experimental group (n = 16) and the effect of repeated submaximal movements on cortical β activity in the control group (n = 12).

Muscle pain scores in the EIMD group peaked after 36 hours with the lowest muscle torque reported at 12 hours. Beta -1 activity was up-regulated in the frontal and parietal

area in the experimental group at 12 and 36 hours. This suggests an impact of EIMD induced neuromuscular changes on the cortical proprioceptive and motor perceptive networks. Beta-2 activity decreased in the control group over time suggesting a loss in focused attention and greater familiarization with the protocol as the study progressed, while it increased in the EIMD group. These data suggest a change in activity in the upstream regulator integrating movement perception and proprioception post-EIMD. Furthermore our data indicates that β-2 activity is associated with the anticipation of pain induced by movement.

Finally chapter five synthesises the data from the previous studies to create a hypothetical model to explain how EIMD symptoms, neuromuscular function and cortical activity interact with each other in a feed-forward and feedback regulation mechanism. This chapter also includes suggestions for future directions of research in EIMD and muscular pain.

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elbow angle, creatine kinase activity

Figure 2.2

(a) The change in current pain measured with the NPS scale over seven days is shown the control (●) and experimental (○) group.

p < 0.01 at 84 hours post versus pre in the experimental group
p < 0.001 at 36 and 60 hours post versus pre in the experimental group

(b) The change in the difference in relaxed elbow girth (cm) between the left and right arm of the control (●) and experimental (○) group over seven days.

 # p < 0.05 at 108 hours post versus pre in the experimental group
p < 0.01 at 36 and 60 hours post versus pre in the experimental group
p < 0.005 at 84 hours post versus pre in the experimental group

(c) The change in the difference in elbow angle (degrees) between the left and right arm of the control (●) and experimental (○) group over seven days.

p < 0.01 at 12 hours post versus pre in the experimental group
p < 0.001 at 36, 60 and 84 hours post versus pre in the experimental group

(d) The change in creatine kinase activity ($U.l^{-1}$) over seven days is shown the control (●) and experimental (○) group.

 # p < 0.05 at 132 hours post versus pre in the experimental group
p < 0.01 at 108 hours post versus pre in the experimental group

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(indicates results of the Kruskal-Wallis non-parametric test, # indicates results of the Friedman's non parametric test)*
** p < 0.05 control versus experimental group.*
*** p < 0.01 control versus experimental group*
**** p < 0.001 control versus experimental group*

Figure 2.3

(a) The maximal force output produced on seven consecutive days is shown in the control (●) and experimental (○) group.

 # p < 0.05 at 60 hours post versus pre in the experimental group
p < 0.01 at 84 hours post versus pre in the experimental group
p < 0.005 at 12 and 36 hours post versus pre in the experimental group

(b) Change of maximal EMG amplitude on four different occasions

** p < 0.05 control group versus experimental group.*
 # p < 0.05 at 132 hours post versus pre in the experimental group

(c) Change in neuromuscular efficiency measured as a ratio of torque/EMG during the MVC in the control (●) and the experimental (○) group.

 # p < 0.05 at 12 hours post versus pre in the experimental group
† p < 0.001 at 132 hours post versus 12 hours post in the experimental group
‡ p < 0.01 at 132 hours post versus 36 hours post in the experimental group

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(indicates results of the Kruskal-Wallis non-parametric test, # indicates results of the Friedman's non parametric test)*
** p < 0.05 control group versus experimental group.*
*** p < 0.01 control versus experimental group*
**** p < 0.001 control versus experimental group*

f: Percentage change of EMG amplitude produced during a low force isometric contraction with a 1 kg wrist weight normalized to the maximal EMG amplitude measured on four different occasions in the control (●) and the experimental (○) group.
(indicates results of the Kruskal-Wallis non-parametric test, # indicates results of the Friedman's non parametric test)*
* $p < 0.05$ control group versus experimental group, ** $p < 0.01$ control versus experimental group, *** $p < 0.001$ control versus experimental group, # $p < 0.05$ post versus pre in the experimental group, ## $p < 0.01$ post versus pre in the experimental group, ### $p < 0.005$ post versus pre in the experimental group,#### $p < 0.001$ post versus pre in the experimental group

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Figure 4.5 Twelve different electrodes representative of the change (%) of β-1 activity in the frontal, central and parietal areas of the brain in the control (●) and experimental (○) group. Each graph is labeled with the corresponding electrode number. The horizontal dotted line represents the time of the EIMD- inducing protocol in the experimental group. The vertical grey line marks the time of the EIMD inducing protocol in the experimental subjects.
(indicates results of the Kruskal-Wallis nonparametric test, # indicates results of the Friedman's non parametric test)*
** p < 0.05 control group versus experimental group, ** p < 0.01 control versus experimental group*
 # p < 0.05 at 12 and 36 hours post versus pre in the control group, ## p < 0.01 at 12 hours post versus pre in the control group
p < 0.001 at 36 hours post versus pre in the control group

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Figure 4.7 Twelve different electrodes representative of the change (%) of β-2 activity in the frontal, central and parietal areas of the brain in the control (●) and experimental (○) group. Each graph is labeled with the corresponding electrode number. The horizontal dotted line represents the time of the EIMD- inducing protocol in the experimental group. The vertical grey line marks the time of the EIMD inducing protocol in the experimental subjects.
(indicates results of the Kruskal-Wallis nonparametric test, #, †, ‡ indicate results of the Friedman's non parametric test)*
** p < 0.05 control group versus experimental group, ** p < 0.01 control versus experimental group, *** p < 0.001 control versus experimental group*
 # p < 0.0512 and 36 hours post versus pre in the control group, ## p < 0.01 at 12 and 36 hours post versus pre in the control group.
† p < 0.05 at 36 versus 12 hours post in the experimental group, ‡ p < 0.05 at 132 versus 12 hours post in the experimental group.

CHAPTER 5

Figure 5.1 Schematic overview of the main findings of chapter 2

Figure 5.2 Schematic overview of the main findings of chapter 3

Figure 5.3 Schematic overview of the main findings of chapter 4

Figure 5.4 Schematic overview of the hypothesized model.

Figure 5.5 An abstract model of the communication pathways between the peripheral muscle and the cortical areas. Each colour represents a frequency. Blue = β-1, green = α-2, red = α-1 and yellow = β-2.

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LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
AMY	Amygdala
ANOVA	Analysis of Variance
Ca^{2+}	Calcium
CK	Creatine Kinase
CNS	Central nervous system
ECG	Electrocardiogram
EEG	Electroencephalography
EIMD	Exercise-induced muscle damage
EMG	Electromyography
ERP	Event related potential
FFT	Fast Fourier Transform
HC	Hippocampus
HT	Hypothalamus
MEG	Magneto-encephalography
MI	Primary motor area
MRI	Magnetic resonance imaging
MVC	Maximal voluntary contraction
PAG	Periaqueductal grey matter
PAR-Q	Physical activity readiness questionnaire
PCC	Posterior cingulate cortex
PET	Positron Emission Tomography
PF	Prefrontal area
PMA	Supplementary motor area
PMC	Pre-motor cortex
RF	Reticular formation
RMS	Root Mean Square
SI	Primary somatosensory area
SII	Secondary somatosensory area
THAL	Thalamus
VAS	Visual Analogue Scale
α	Alpha
β	Beta
δ	Delta

θ	Theta
γ	Gamma
μ	Mu

Measuring units

Cm	Centimetre
dB	Decibel
h	Hour(s)
Hz	Hertz (frequency)
Kg	Kilogram
kΩ	Kilo Ohm
m	Metre
min	Minute(s)
ml	Millilitre
mm	Millimetre
ms	Millisecond
$m \cdot s^{-1}$	metres per second
Nm	Newton meters (force)
S	Second(s)
$U \cdot l^{-1}$	Units per litre
μV	Micro-Volt
$\mu V \cdot ms^{-1}$	Micro-Volt/millisecond
°	Degree
°C	Degrees Celsius
$° \cdot s^{-1}$	Degrees per second

CHAPTER 1

REVIEW OF THE LITERATURE

1.1 INTRODUCTION TO THE REVIEW OF THE LITERATURE

Humans, unlike other mammals have developed a muscular skeletal system specially adapted for bipedal upright locomotion [46] with front limbs free to be used for fine controlled grasping and holding tasks [307]. In response to the demands of searching for food or fighting for survival, their musculo-skeletal systems, especially the skeletal muscles have adapted to accommodate a multitude of requirements. However even this well adapted system experiences severe stress and consequently is subjected to structural damage and associated acute pain.

Today the skeletal system has to adapt to the demands of voluntary exercise, such as marathon running, endurance cycling, downhill running or weight training sessions at the gymnasium. These types of exercise can cause damage to the muscle, particularly if the person is not accustomed to the exercise mode and when the exercise is predominated by muscle lengthening under tension [16, 17, 113, 121, 251].

This unaccustomed exercise immediately leads to structural and metabolic changes such as, pH changes in the tissue, intramuscular enzyme and metabolite leakage into the circulation system, muscle fibre tears and the initiation of inflammatory processes [16-18]. Furthermore there is an immediate loss in force output and changes in neuromuscular functioning [296]. These structural and metabolic changes lead to a disturbance of the system homeostasis and need to be counteracted to reinstate homeostasis. Recent research has shown that pain, just like hunger, thirst and itch, is an emotional response to reinstate the system homeostasis [86, 241]. Pain is also an expected response associated with exercise-induced muscle damage (EIMD). Most interestingly the sensation of pain associated with EIMD is delayed and only appears twelve hours after the exercise, with severity intensifying 24 hours after the protocol. Pain peaks at 48 hours and subsides within seven days after the exercise which caused EIMD. Clinical symptoms also decline within seven days. However, structural changes to the muscle fibres have been documented to last six weeks after prolonged exercise in events such as a marathon [169, 395].

The pain that is associated with EIMD is not only delayed in its onset but is also not tightly associated with the other symptoms of EIMD. Interesting questions arise from this phenomenon. For example, how is this noxious stimulus received by the muscle tissue, and how is it perceived and integrated in the brain? Of special interest is to

determine if there is an involvement of cortical structures during the subsequent recovery period from EIMD and if cortical activity influences the neuromuscular activation patterns as well as the delayed onset of pain.

To understand the underlying factors in greater detail a review of the literature was prepared and divided into three parts. The first part introduces EIMD, its symptoms, peripheral changes and how it might disturb the homeostasis of the system. In addition the sensation of pain and the pathways leading from the painful stimulus to the emotional and physiological integration of the sensation of pain is introduced.

The second part describes the methodology of electromyography (EMG), including what it measures and how the data are recorded and analysed. This is of importance as EMG reflects the changes in activation of the muscle (peripheral changes) in response to the EIMD before measuring the EIMD associated changes in the cortical areas. The third and last part of this review illustrates the involvement of the cortical areas in movement execution and pain perception. This is followed by a discussion of electroencephalography (EEG) as a mode to measure the electrical activity and by implication the changes in the activity of the cortex.

The goal with this review of the literature is to convince the reader that to further the knowledge on EIMD and its implications on the system, the field of research needs to be widened to include not only peripheral (clinical signs and EMG) but also central measures (EEG) to understand EIMD and its effects, particularly those of pain and neuromuscular changes better.

1.2 EXERCISE-INDUCED MUSCLE DAMAGE AS AN EXAMPLE OF DISTURBING SYSTEM HOMEOSTASIS

1.2.1 Introduction to muscle damage as a result of unaccustomed exercise

Unaccustomed exercise, especially exercise during which the muscle is lengthened under tension, can lead to structural, metabolic and biochemical changes at a cellular level in the involved muscle tissue. Although this type of exercise is often referred to as eccentric exercise [76, 85, 124, 133, 142], the technically appropriate term is 'lengthening under

tension' [130], which will be used subsequently.

Clinical symptoms of EIMD include swelling of the tissue, loss of strength and muscle function and a decrease in the resting length of the muscle. There is also increased activity of intramuscular enzymes, such as creatine kinase in the blood [56, 264, 267]. The circulating enzyme activity is possibly a result of the disruption of the sarcolemma or change in membrane permeability caused by the EIMD inducing protocol. This would lead to the leaking of intracellular enzymes and proteins into the serum [80, 264]. The activity of serum creatine kinase increases rapidly 48 hours after an EIMD protocol [153] and usually remains elevated for five days or longer after the intervention [210, 215, 264, 266].

The muscle stiffness associated with EIMD is usually felt within the first twelve hours and develops into soreness, which peaks between 24-72 hours after the muscle damage was induced [142, 267]. The pain usually subsides within seven days after the damage inducing exercise [173, 210]. Importantly it is still not known which biochemical and mechanical processes are associated with the delayed perception of pain following EIMD. For example, the symptoms associated with EIMD[233], particularly the decrease in neuromuscular functionality measured as force output [79], usually occur within the first 0-12 hours after the injury [80] and peak at different time points thereafter [80, 265]. The increase in arm circumference is caused by oedema as a result of tissue fluids leaking from the injured muscle tissue and accumulating in the connective tissue [173]. The oedema [172] and changes in the connective tissue [81] have been proposed as causes for the shortening of the muscle leading to a change in the elbow angle. Oedema in the damaged muscle, for example, leads to a peak in limb circumference 72 hours after the EIMD inducing protocol, as does the shortened muscle length associated with the damage [79, 265], while creatine kinase activity in the blood only peaks 72 or 96 hours after the EIMD inducing protocol . The exact timing seems to be dependent on the exercise protocol used [79, 80, 329].

The symptoms of oedema and muscle shortening disappear after 5-7 days [81, 113], while the peak soreness usually occurs at 24-48 hours after the EIMD inducing exercise [403]. Therefore, the asynchronous appearance and disappearance of the clinical symptoms and pain makes it difficult to establish a cause and effect relationship between these variables.

1.2.2 Processes during EIMD

Armstrong [17] has proposed that EIMD is an inflammatory process. He used a 4-stage model to describe this process. The four stages are as follows; an initial stage, an autogenic stage, the inflammatory stage followed by the regenerative stage. Not much is known about the initial stage besides that it triggers the following degenerative, inflammatory and regenerative processes in the muscle tissue.

The autogenic stage which follows can last up to several hours and includes an increased concentration of calcium (Ca^{2+}) in the damage tissue due to the disturbance in the release and reuptake of calcium by the sarcoplasmic reticulum. The free calcium leads to the activation of calpain and phospholipase leading to the breakdown of cell structures [27, 269]. This stage merges into the inflammatory stage when phagocytic and other inflammatory cells enter the damaged tissue [232, 375]. The regenerative phase is the last stage of the process and describes the phase when the first regenerative processes take place. The regeneration includes the revascularization of the damaged area, the activation of satellite cells resulting in proliferation and fusion of myoblasts and the maturation of the myotubes [65, 142, 159, 365]. This occurs within 4-6 days after the initial event and after 10-14 days the tissue appears normal again [142]. In cases of severe muscle damage, for example after an ultra marathon race, the regeneration process can take several weeks (10—12 weeks) [395]. This type of exercise leads to severe muscle fibre tearing, intra and extra cellular oedema, myofibrillar lysis and focal mitochondrial degeneration within the tissue, even without displaying inflammatory processes [156, 395].

Most of the above mentioned processes (break down of cell structures, oedema etc) are similar in EIMD and inflammatory processes [233, 264, 354, 381]. But inflammatory processes do not entirely explain the events in the muscle tissue after the unaccustomed exercise, because the inflammatory response to the EIMD induced tissue damage does not provide sufficient strength to elicit the pain response as seen in EIMD [264].

Besides the inflammatory response, mechanical damage to the contractile apparatus is often observed. This includes damage to the myofibrils and the cytoskeleton which is regularly analysed in biopsies taken from muscle tissue exposed to excessive exercise [140, 142]. It has been shown that the first structural changes of the cytoskeleton, including

rupturing of sarcomeres [140-142], damage to the cytoskeleton (desmin) and the cell membrane [140-142, 251] occur within the first 5-15 minutes after the onset of repetitive lengthening muscle actions [218]. Other signs of damage include z-band streaming and dissolution, A-band disruption, disintegration of the intermediate filament system and misalignment of the myofibrils [2, 140-142]. As a consequence of the damage to the myofibrillar structure and change in membrane permeability, the internal chemical balance is disturbed. This results in enzyme leakage, cellular degradation and failure of the excitation-contraction coupling in the muscle [17, 53, 80, 141]. Intermediate filament damage is caused by direct tension on the tissue during exercise or indirect tension due to the pressure of oedema. The apparent damage to the intermediate filaments could be a secondary response to muscle damage and may be the initial step of sarcomereogenesis [140].

The major symptoms of EIMD include soreness and tenderness. It has been suggested that these pain symptoms are triggered by intracellular fluids leaking out of the damaged muscle fibres and activating nociceptors. As the pain during EIMD is dull and not well localized, it is assumed that the nociceptive signal is mainly conducted by the type C unmyelinated pain fibres [268, 370].

1.2.3 Measurement of muscular pain

To be able to compare the pain caused by a variety of stimuli such as capsaicin [69, 70], hypertonic saline injection [75, 214], laser stimulation [185, 188] or muscle damage a reliable system to measure pain is needed. Several systems have been developed including subjective (pain scales) and objective (pressure probe) [210] pain measures. Over the years the visual analogue scale (VAS), a subjective pain measure, has proven to be valid [146, 277], reliable and repeatable [40, 122, 277], even when compared to more objective pain measurements

The VAS, which is a widely used scale to measure pain, is a type of a ratio-interval scale [297]. It consists of a line or scale, with each end of the line/scale labelled with statements appropriate for describing the extreme values of the sensation: one end is often labelled “no pain” and the other end is often labelled “worst possible pain.” The subjects rate the perceived pain by placing a marker or simply tick the line in relation to the two extremes. The VAS has been shown to be useful for separating measures of pain intensity and unpleasantness [40, 108].

In conclusion, EIMD with its symptoms of tissue damage, enzyme leakage, tissue healing and pain has been shown to be a good model for acute pain and its effects on the peripheral as well as central structures. Of particular interest in the context of this thesis are the delayed pain response and the association between neuromuscular changes and electrical activity in the muscle (EMG) and brain (EEG).

Therefore the next two parts of this review will elucidate the use and background of EMG and EEG in measuring the effects of EIMD. In particular the review will focus on pain associated with EIMD and the electrical activity in the peripheral nerves and muscles as well as affected brain structures.

1.3 PAIN: THE PERCEPTUAL RESPONSE TO DISRUPTION OF HOMEOSTASIS

1.3.1 Introduction to pain

Pain is a sensation experienced by all mammals and consists of a nociceptive signal accompanied by an emotion or feeling. The nociceptive signal is a physiological activation of niceptive receptors and their afferent nerves (physiological sensation of pain is discussed in greater detail from page eight onwards). The emotional component of pain exists largely to produce a behavioural response associated with the avoidance of the pain inflicting stimulus [244, 392, 402]. To fully understand pain one cannot separate the nociceptive signal from the emotional response [86]. Similar to thirst, hunger, temperature and itch, pain is an emotional motivation to restore homeostasis which has been disturbed by the sensation causing the stimulus [86, 241].

1.3.2 Definition and teleology of pain

The International Association for the Study of Pain defines pain as: ”P*ain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*” [1].

The sensation of pain heightens awareness of a problem such as an inflammatory process or injury. The sensation of pain may also protect the involved area from further injury and aid in the process of healing by eliciting an emotional response and causing

an avoidance reaction [56, 86].

1.3.3 Classification of pain

According to the time frame of inflammation, tissue damage and healing process, the sensation of pain can be divided into three subdivisions; transient, acute and chronic pain [61, 242]. Transient pain is of very short duration such as a needle prick or stubbing a toe and usually does not require medical attention. Acute pain, which is elicited by damage to tissue and can last anything from a few hours to a few weeks or even months but usually subsides before the healing of the damaged tissue is completed. Examples for acute pain are the pain associated with burning with hot water, exercise-induced muscle fibre tears or a broken bone. Acute pain requires medical treatment in some cases and can also be related to trauma or surgical interventions [61, 242]. Chronic pain lasts from a few months to several years and is often difficult to treat, as the tissue damage is either so severe that healing is impaired, or the pain is due to peripheral or central neuropathic pain. Examples of conditions associated with chronic pain included multiple sclerosis and osteoarthritis [1, 242]. However in this review the focus will mainly be on the effects of acute muscular pain and in particular the pain associated with EIMD. This review as well as the following chapters will mainly focus on the effects of pain rather than nociception as, the experimental studies focused on the emotional/cognitive response to nociception which is included in the broader term pain. Therefore the spinal pathways will be called nociceptive pathways but as soon as the noxious stimulus reaches the brain I will be talking about pain as one cannot discriminate anymore between the noxious stimulus, the sensation and the emotional /cognitive response.

1.3.4 Physiology of pain

1.3.4.1 Noxious stimulus

There are several muscular [66, 68, 69] or cutaneous [70] stimuli which are used in research to induce a noxious stimulus. Most studies of evoked pain use heat, laser, hypertonic saline or capsaicin injection to induce pain in the arm or foot area [66, 70, 103, 175].

1.3.4.2 Nociceptors

The painful stimulus caused by, for example, a muscle fibre tear due to exercise is first received in the nociceptors. Nociceptors are high threshold, free nerve endings which act as receptors for noxious stimuli (i.e.. needle prick, boiling water, muscle fibre tear,

skin cut) and are found in most organs of the body. They are stimulated either by a mechanical, chemical, thermal, electrical or other stimuli, but mostly are activated by chemical mediators before they can react to the noxious stimulus [182]. These chemical mediators can be prostaglandins, proteolytic enzymes, potassium, bradykinin, histamine and other chemical or metabolic substances released by injured tissue [406]. Local tissue acidity (pH change) can also lead to a depolarisation of the receptors, which in return leads to a hyper sensitivity to noxious stimuli. By activating the pain receptors these substances can enhance the transmission of the nociceptive signal to the spinal cord. This process is known as peripheral sensitization [409].

1.3.4.3 Nociceptive fibres

The nociceptive component of pain is transferred by two different types of nociceptive fibres; the fast conducting Aδ fibres and the slower conducting unmyelinated C fibres. Aδ fibres are thinly myelinated neurons conducting information at about 20 $m \cdot s^{-1}$, and are believed to be responsible for the acute and sharp pain felt immediately after an injury or tissue damage occurred [182]. The Aδ fibres terminate in the dorsal horn, from where the second order neurons directly transmit the nociceptive signal through the spinothalamic tract (Figure 1.1) to the thalamus and sensory cortex [182].

However in this review the pathway of only the slower type C fibres will be discussed as it has been hypothesised that they are more relevant to the pain felt while experiencing the symptoms associated with EIMD. This is due to the observation of EIMD causing a slow, longer lasting dull pain, which is associated with C fibre conduction, rather than the short sharp pain associated with Aδ fibre conduction [370]. However it has been proposed that the pain felt during movement after an EIMD protocol is often sharper and more localised and could therefore be transmitted by the Aδ fibres [268].

1.3.4.4 Nociceptive signal entry into the spinal cord

Nociceptive inputs are mediated through a complex system of receptors and pathways. From the affected organ, the nociceptive signal is transmitted through the first order neuron to the unmyelinated dorsal root C fibre at a speed of 0.5-2.0 $m \cdot s^{-1}$. The first order neurons enter the dorsal horn and synapse in lamina I [86]. The neurons usually terminate shortly after their point of entry into the dorsal horn and fan out over several segments [406] and therefore lose some of their localization specificity. The synapses in the dorsal horn show great malleability and therefore pain signals can be intensified or inhibited in this region [241, 243, 244].

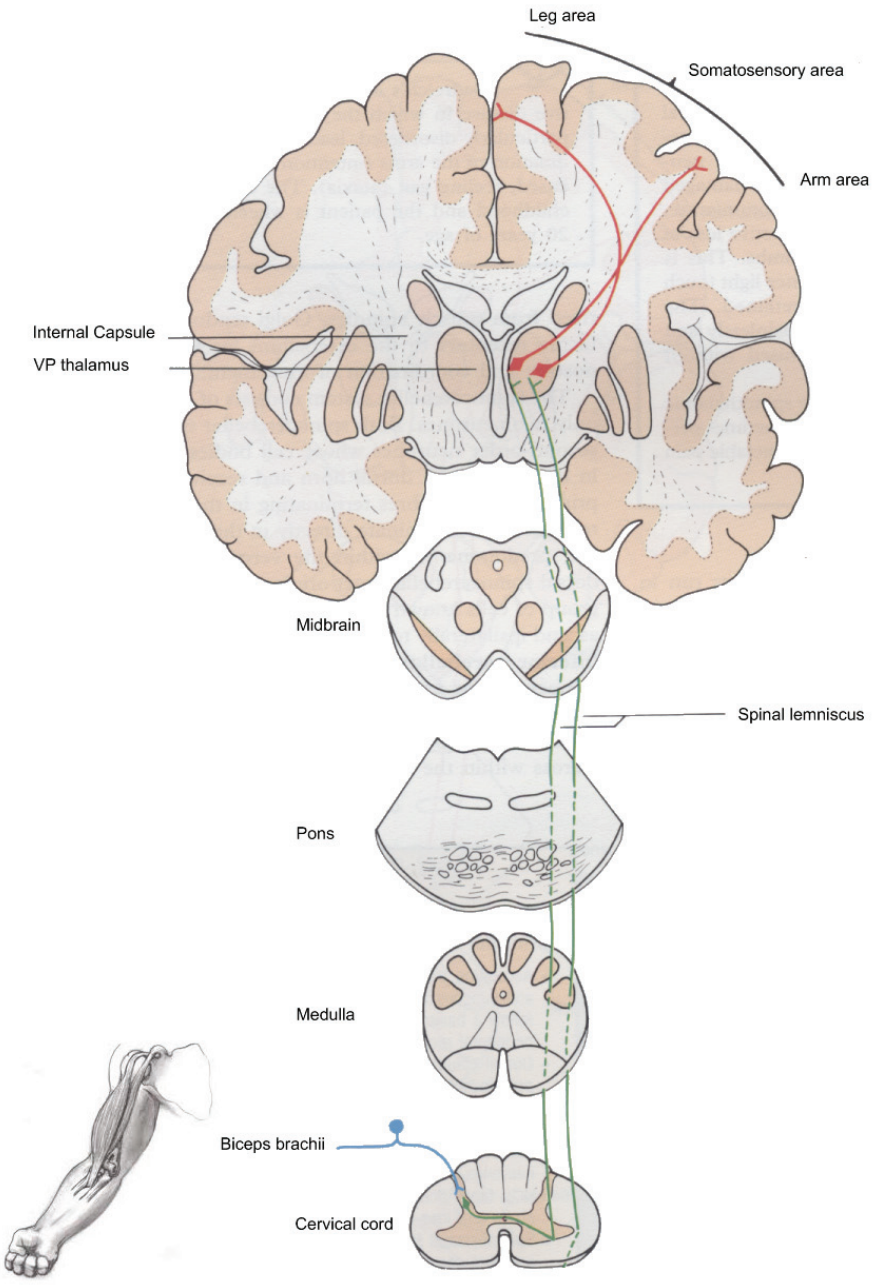

Figure 1.1 A graphical representation of the central pain pathways from the biceps brachii via the spinothalamic tract to the somatosensory area.
** Figure modified from Crossman AR and Neary D. Spinal cord In: Neuroanatomy, An illustrated colour text Churchill Livingstone, Pearson Professional Limited 1996:49* [88]

1.3.4.5 Gate control theory of pain

In particular impulses from collateral and interneurons can inhibit the transmission of pain signals from the dorsal root fibres to the spinothalamic neurons by interfering at the first point of synapsing between the first and second order neurons in the dorsal horn [185, 192, 406, 407].

Figure 1.2 shows the entry point to the nociceptive C fibre and the Aβ mechanoreceptor into the dorsal horn. It shows how interneurons can relay information from the mechanoreceptor to the pain fibre to inhibit pain transmission. This explains why, for example, rubbing the skin decreases a painful sensation. The rubbing stimulates the mechanoreceptors which then interact with the nociceptors at the dorsal horn level and decrease (or increase) the feeling of pain [298, 399]. Furthermore, efferent neuronal signalling from the brain and the subsequent release of neurotransmitters can interact with, and impede flow of, information at this level and hence inhibit the further transmission of the pain signal [99, 164, 181].

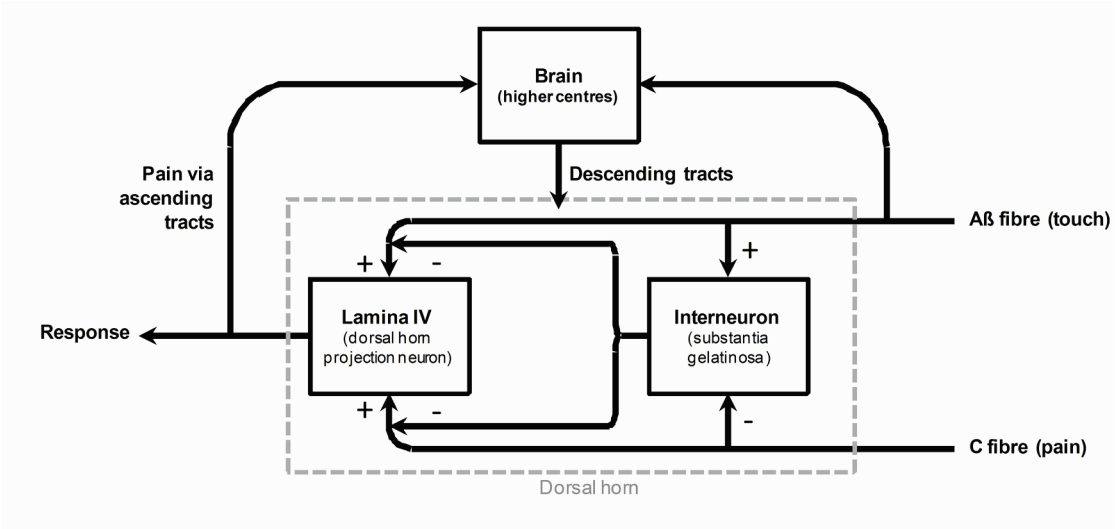

Figure 1.2 The basis arrangement of the sensory GATE mechanism in the dorsal laminae of the grey matter of the spinal cord.
**Adapted with permission from Crossman A R. Spinal Cord. In: Standring S, ed. Gray's Anatomy Elsevier; 2005:307-326.)*[87]

Information from the frontal somatosensory area, the cingulated cortex, hypothalamus and amygdala descends via the reticular formation and the spinal cord to the first order synapses in the dorsal horn (the areas of the brain will be discussed on page twelve). This efferent information is used by relay cells and interneurons to either inhibit or enhance signal transmission at the synapses in the dorsal horn [241, 406], hence the term GATE for these synapses as suggested by Melzack and Wall [244] in their GATE theory of pain. This GATE is the first point at which the transmission of a painful stimulus can be inhibited by signals transmitted from the brain and is therefore of interest when discussing the interaction of a painful stimulus and the brain later. Therefore the dorsal horn GATE is the first central point at which an efferent signal from the brain can inhibit or enhance afferent pain transmission.

1.3.4.6 Spinal pathway

From the point of synapsing in the dorsal horn, the second order neurons ascend towards the brain [406]. A number of neuronal axons terminate in the spinal cord (information used for reflex arc) while other axons enter the anterolateral system including the lateral spinothalamic tract [87] (Figure 1.1). The main pathway ascends through the dorsal horn of the spinal cord, crosses the midline to the opposite side of the spinal cord and often synapses several times before entering the brain stem at the reticular formation [87, 405]. Some of the neurons end in the reticular formation and near the aqueduct in the midbrain. Further neurons project from the reticular formation, which mediates the central inhibition and arousal effect of pain, to the midline and intralaminar non-specific projection nuclei of the thalamus. From the ventral nuclei of the thalamus the signal is transmitted through the third order neuron to the somatosensory area [44, 171, 302] in the cortex.

1.3.5 Perception of pain

1.3.5.1 Brain areas associated with pain

As discussed in the previous paragraph the painful stimulus reaches several brain areas. It has been found that pain is not evaluated and integrated in a single area of the brain but rather that the activation of the small unmyelinated type C pain fibres lead to the activation of several cortical and subcortical areas. A pain stimulus activates parts of the primary somatosensory area (SI), secondary somatosensory area (SII), anterior insular cortex and cingulate gyrus (both part of the limbic system), all on the side opposite to the stimulus (Figure 1.3) [14, 182, 392]. The noxious stimulus is also received by the periaqueductal grey matter in the midbrain, inferior parietal area, rostral insula, the hypothalamus and thalamus as well as the ipsilateral (to the painful stimulus) cerebellum [14, 262, 293, 294, 362, 367, 367].

Studies using magnetic resonance imaging (MRI) could show that brain areas associated with pain have an increase in blood flow with an increase in pain intensity [294, 367]. There are also negative correlations (i.e.. decreased blood flow) in the medial and parietal cingulate cortex, and medial prefrontal regions. These changes in blood flow were only correlated with the actual intensity of pain and not the anticipated intensity of pain [294].

Further studies have shown that various pain activated areas are also activated in the

anticipation of pain [14, 136, 293]. These areas include the anterior cingulate gyrus, insular cortex, pre frontal area, primary somatosensory area and the cerebellum [14]. The same studies have shown that if a painful stimulus is expected in the foot, the correlated area on the homunculus of the primary sensory cortex will show increased activity, but at the same time the activity in the areas related to hand and face decreases [293].

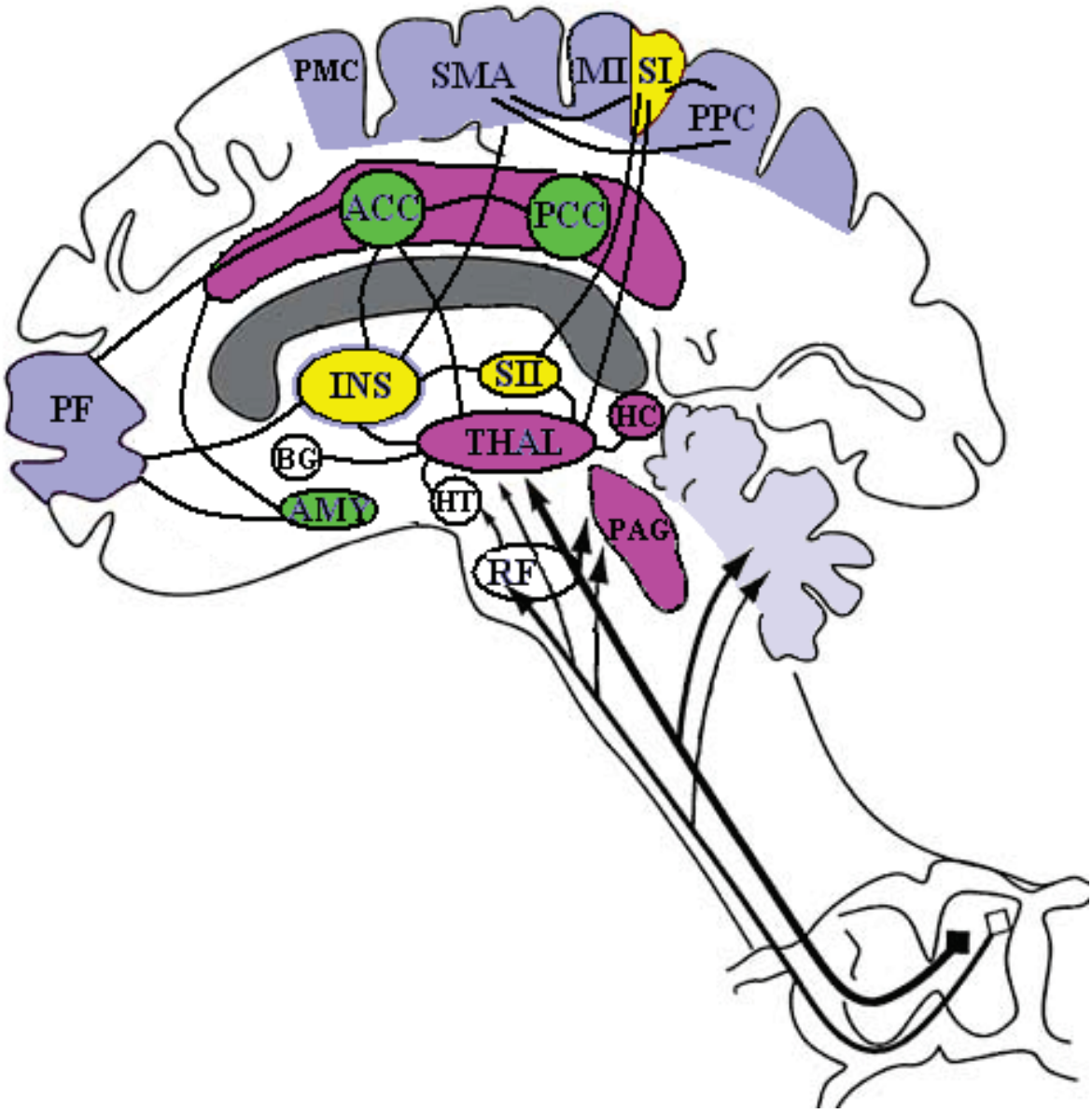

Figure 1.3 Pain pathways in the brain, modified from Apkarian *et al.* [14]. The areas labelled from the cortex to the brain stem are; pre-motor cortex (PMC), supplementary motor area (PMA), primary motor area (MI), primary somatosensory area (SI), posterior parietal area (PPC), anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), prefrontal area (PF), insula (INS), secondary somatosensory area (SII), hippocampus (HC), basal ganglia (BG), thalamus (THAL), amygdala (AMY), hypothalamus (HT), periaqueductal grey (PAG), reticular formation (RF). The black lines indicate connections between areas which interact during the perception of pain.

1.3.5.2 Pain neuromatrix in the brain

1.3.5.2.1 *Medial pain system = perception and affectionate component*

The prefrontal cortices, amygdala, hypothalamus, posterior insula, motor area, hippocampus, cingulated cortex, thalamus and periaqueductal grey are collectively referred to as the medial pain system, because of their medial location and their activation whenever a noxious stimulus occurs [205, 392]. In the medial pain system the neural and endocrine information related to pain are integrated [14, 15, 82, 205]. Together these areas are associated with the affectionate component and perception of pain [205, 290, 392]. The periaqueductal grey area is known to not only be associated with the emotional component of pain but is also able to inhibit the sensation of pain in the conscious animal [14, 14, 185, 406].

1.3.5.2.2 *Lateral pain system = localization*

The primary and secondary somatosensory area as well as the inferior parietal areas are part of the lateral pain system which is associated with the localization of the painful stimulus [205 341, 383].

However, recent research by Ohsiro et al [272] has shown that the medial and lateral pain system are not as discriminated from each other as previously thought . Functionality rather overlaps between the two systems and no complete discrimination is possible between the sensory-discriminative (previously thought to be lateral pain system) and emotional-affectionate (previously thought to be medial pain system) component of pain in the cortical areas. Of special importance is that the pain sensation is represented on a fronto-parietal network as are perception and attention processes (page 18) [272].

1.3.5.2.3 *Memory of pain*

Further parts of the limbic system, especially the insular cortex and anterior cingulate cortex, as well as the pre frontal cortex are not only associated with the cognitive, emotional and affectionate processing of pain, but also form memories, including previous responses to pain [14, 26, 185, 258, 361, 362, 392, 402]. The posterior part of the anterior cingulate cortex, also associated with the feeling of unpleasantness and suffering, has a delayed response of 0.5 to 1.5 s [205, 290] which might indicate that it reacts to the pain transmitting C fibres which are activated during EIMD[370], rather than the faster transmitting Aδ fibres.

Collectively these pain related systems and networks are called the pain neuromatrix of

the brain [243], a highly complex network integrating the sensation of pain.

1.3.6 Further pathways activating the pain response to reinstate homeostasis: Inflammatory processes and their association with the perception of pain

Injured sites or inflammatory processes trigger the damaged tissue to release cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor (TNF) into the blood within one to four minutes after tissue trauma occurred [239, 396]. This trauma, can be an injury or an infection but also tissue damage caused by over training [355] or EIMD. The cytokines (IL-1, IL-6 and TNF) cross the blood-brain barrier and attach to receptors found on glial cells. Interestingly glial cells are the main producers of cytokines within the central nervous system [235]. Cytokines also bind to receptors in the hypothalamus and hippocampus [89, 90, 224, 235, 355, 373, 384] and subsequently act as mediators to activate the pain modulating processes in the brain [58, 239, 241, 309, 371, 404].

The activation of the pituitary dependent adrenal response provides evidence that the inflammatory stimuli can activate anti-inflammatory signals from the central nervous system [36, 384], as the peripheral inflammatory process may alter the neuronal signalling of the hypothalamus [384]. The cytokines released from the glial cells activate the hypothalamic-pituitary axis which increases circulating glucocorticoids leading to the suppression of cytokine release in the periphery [33, 36, 224, 384].

Therefore there seems to be a bi-directional communication between the immune system and the brain during an inflammatory process which functions without the involvement of the peripheral nervous system [235], but is dependent on the glial cells rather than the neurons situated in the central nervous system. The glial cells in the central nervous system possess a membrane potential slightly larger than that of central nervous system neurons. When localised changes in the extra cellular potassium concentration of the central nervous system occur the glial cells depolarize. Thus it appears that the glial cells have an amplifying effect on extra cellular field potentials [282]. This possible amplification of the electrical field potential may be recorded in EEG.

Due to the extensiveness of this topic and the emphasis on acute pain in this review, the immune system and glial cells will not be discussed in further detail. They are only

mentioned above to acknowledge the fact that there are other systems of information transfer besides the neuronal pathways which might be important in the information transmission process during peripheral inflammatory processes.

1.3.7 Interaction between movement and pain

Small, soft and brief movement is known to lead to a reduction in C fibre transferred pain [390]. Therefore the next section will focus on movement and its control by the brain. It is of special interest where the information about pain overlaps with motor control. Le Pera [213] showed that motor cortex activation due to movement preparation could inhibit cortical pain processing as well as increase the pain threshold and decrease the pain rating.

Since the general pathways of pain conduction have been outlined, the following section will discuss EIMD, a model for muscular pain. This section will emphasize that the symptoms consequent to EIMD will lead to a pain response.

However, the experience of EIMD does not only lead to an increased sensation of pain but also influences proprioception [299, 300, 380] and motor control [43, 206, 300] in the affected limb. Proprioception is the afferent information from the periphery to the brain which is altered due to the altered state and disturbed homeostasis of the muscle tissue. Motor recruitment and motor activation on the other hand change due to the altered efferent response by the motor control centres in the brain, especially the cortex.

1.4 VOLUNTARY MOTOR CONTROL: AFFERENT AND EFERENT FEEDBACK

1.4.1 Afferent sensory pathways associated with changed proprioception

Information about proprioception received by the muscle spindles and Golgi tendon organs ascend to the brain via the anterolateral system (which includes the spinothalamic tract involved in the transmission of pain) to the somatosensory are of the brain and to the cerebellum via the spinocerebellar tracts (Figure 1.4) [87, 123].

While the cerebellum is known to integrate proprioceptive feedback [87, 123], recent research suggests that the cerebral cortex is also involved in the integration of proprioception, motor perception, posture control and the determination of force output [315, 320, 350-353, 387]. Electroencephalographic research has shown that the frontal and parietal-occipital areas are activated when receiving and integrating proprioceptive information [320, 351, 387].

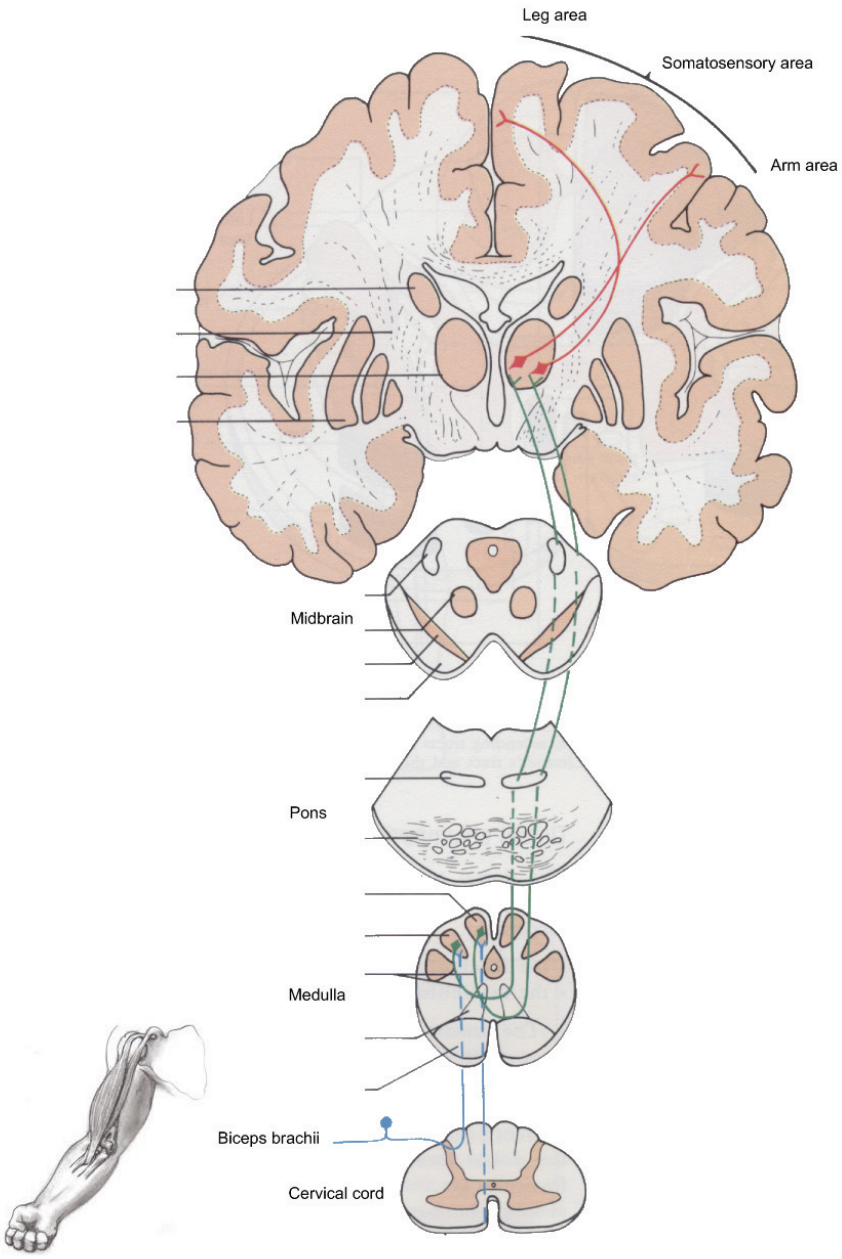

Figure 1.4 A graphical representation of the dorsal columns system carrying information about proprioception.
** Figure modified from Crossman AR and Neary D. Spinal cord In: Neuroanatomy, An illustrated colour text, Churchill Livingstone,* Pearson Professional Limited *1996:48* [88]

Proprioceptive information is needed to guide motor output to adjust force output and neuromuscular recruitment to altered circumstances (i.e.. during EIMD). Recent research shows that this integration takes place in the cerebral cortex [147, 206, 249].

1.4.2 Brain areas involved in voluntary motor control

Figure 1.5 depicts the areas of the brain which are directly involved in the control of voluntary movement. This has not only been detected indirectly (by MRI) but also directly by the stimulation of cortical regions with transcranial magnetic stimulation (TMS) or stimulating needle electrodes [77, 149, 216, 338, 343, 372, 376, 377].

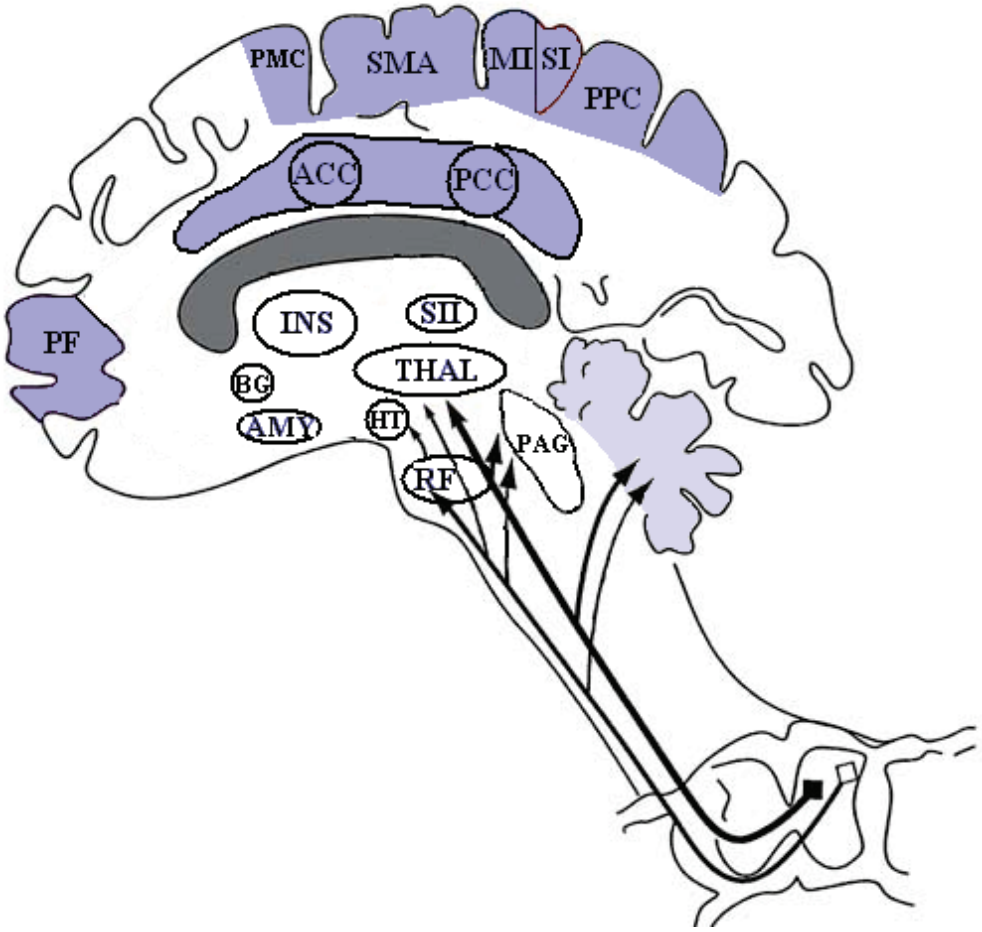

Figure 1.5 Brain areas activated during voluntary limb movement, adapted from Apkarian *et al.* [14]. Highlighted in blue are the brain areas activated during voluntary movement which can be measured with EEG. Highlighted in light blue is the cerebellum which also plays a great role in movement regulation but cannot be measured with EEG. The white areas are also activated during movement but measuring the activity by means of EEG is difficult.

The most prominent areas of the brain which control voluntary movement include the pre-motor, motor and supplementary motor areas [194, 304, 336, 342]. Also activated are the

sensory motor area ipsilateral to the movement (during limb movement), the somatosensory area contralateral to the movement, the parietal area and the posterior insula [92, 136, 137, 151, 270].

1.4.3 Function of the motor control areas

While the efferent control of the movement is guided by the primary motor cortex, the secondary and associated areas are not only activated by voluntary movements but are also interacting with each other to learn and integrate movement as well as form memories of motor sequences [137, 151]. The supplementary motor area is assumed to be important for the preparation and organisation of voluntary movement [92] while the parietal lobe is involved in the procedural organisation of movement as well as proprioception and movement perception [134, 135, 137].

1.4.4 Efferent motor pathways

The motor pathway for voluntary movement consists of the pyramidal tract also called the corticospinal tract (Figure 1.6). The motor impulses originate in the giant pyramidal cells, the so called primary motor neurons, of the motor area in the precentral gyrus. The axons of the pyramidal neurons pass from the cerebral cortex to the corona radiata and then descend to the midbrain and the medulla oblongata[117 87]. In the lower part of medulla oblongata neuronal fibres sending information to the extremities (limbs) pass to the opposite side. The fibres of the corticospinal tract terminate in the anterior horn of the grey matter of the spinal cord and synapse with the secondary motor neurons. These peripheral motor nerves transmit the motor impulses from the anterior horn to the voluntary muscles, where the nerve innervates the muscle fibres forming the motor units [117 87].

1.4.4.1 Efferent communication between the central nervous system and the periphery

As described before efferent output from the brain to the muscle is guided by the precentral motor area during voluntary movement [22, 49, 83, 148, 157, 300]. This notion is supported by the discovery of a coherent 15-30 Hz activity in the muscle, peripheral and central nervous system during movement [12, 13, 64, 83], measured by EEG and EMG [13, 64]. This motor-cortical coherence is assumed to be due to the firing behaviour of the corticospinal tract (Figure 1.6) or other spinal motor neurons [83]. The time delay

between the first occurring EEG rhythm and the following EMG rhythm can be explained by the conduction velocity of the nerves [157] and is 15 ms longer for EMG recorded in the leg than in the arm [50], due to the longer pathway.

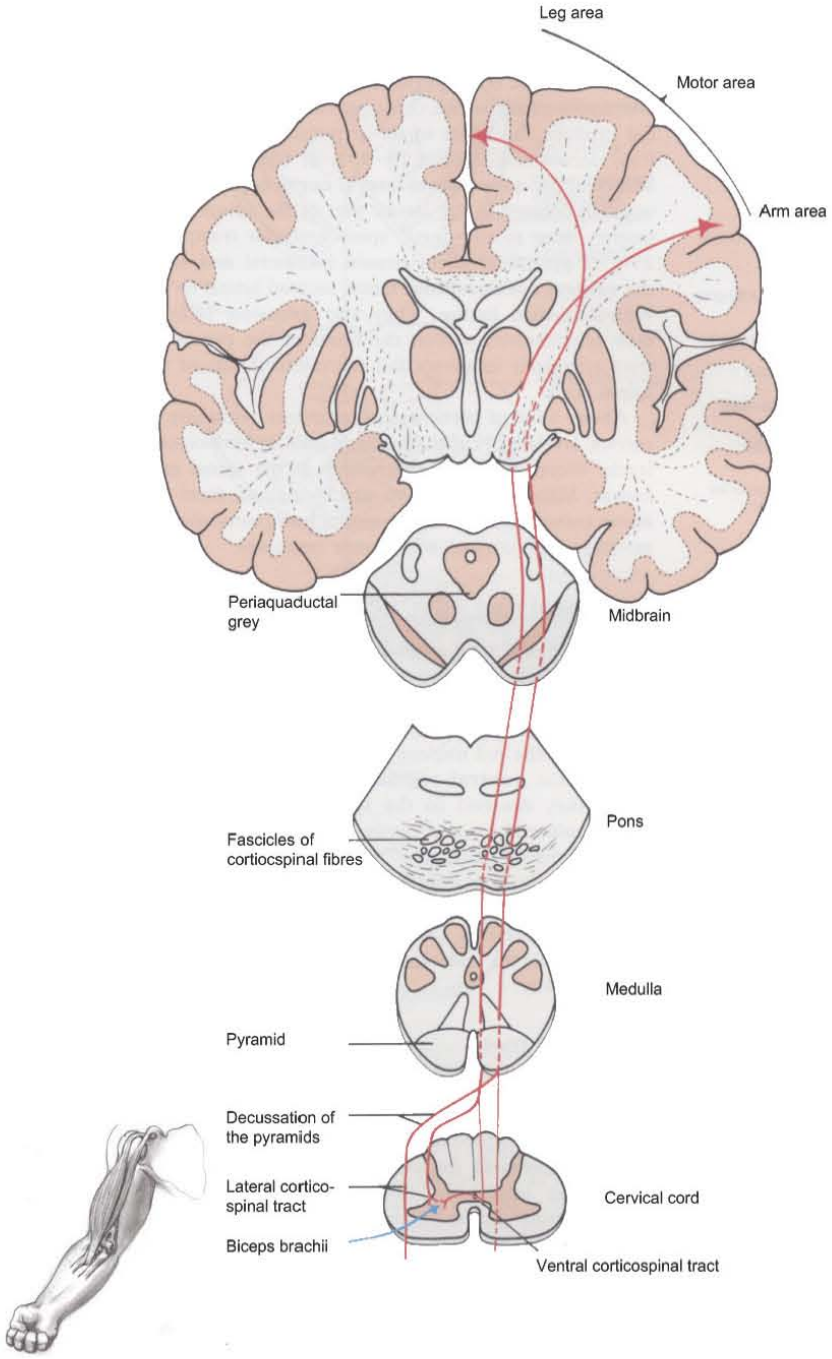

Figure 1.6 A graphical representation of the corticospinal (pyramidal) tracts.
** Figure modified from Crossman AR and Neary D. Spinal cord In: Neuroanatomy, An illustrated colour text Churchill Livingstone 1996:51*[88]

Further cortico-muscular synchronization during movement has been shown, especially between the contralateral primary motor area and the activated muscle in the 15-35 Hz activity [83, 129, 157, 193, 317]. A 40 Hz oscillation in the motor area contralateral to the moving side precedes a 40 Hz rhythm in the EMG during strong voluntary isometric contractions and phasic wrist movements [50]. The coherence between EEG and EMG is strong in the 15-35 Hz [64] frequency range and is recorded during dynamic and isometric tasks[50, 157, 193]. The coherence is also positively correlated to the force output by the activated muscle [64, 193, 194].

Therefore there seems to be a link between the 15-35 Hz frequency activity and motor output, however it has not been determined if this is a bottom-up afferent connection or a top- down efferent connection. Furthermore, it is not known whether this could be interfered with by neuromuscular functioning, pain or other peripheral or central changes.

1.5 ELECTROMYOGRAPHY: MEASURING ELECTRICAL ACTIVITY IN THE MUSCLE

1.5.1 Introduction to Electromyography

Surface electromyography (EMG) is the electrical activity which is measured in a muscle by electrodes placed on the skin overlaying the muscle of interest [37]. An EMG signal represents a global measure of the active motor units within the muscle, and does not have the precision to measure the signal of individual motor units [37]. As the recorded signal is comprised of the neural recruitment of the motor units as well as the firing rate of the active motor unit, it can be stated that the EMG reflects on the properties of the peripheral as well as the central components of the neuromuscular system and that the two cannot be separated from each other [127]. EMG can also measure the amplitude of the signal [9, 223] as well as its power frequency spectrum [223, 229]. The power spectrum is the frequency distribution of the signal which is influenced by the muscle fibre type which is recruited [223]. The EMG signal can be disturbed by several factors, since the electrical activity is created within the muscle, but the EMG signal is measured on the surface (skin). For example, the subcutaneous fat layer [263], the electrode position [125, 128, 165, 187, 189, 246] and muscle fibre characteristics and length [118, 127, 127, 187, 252, 253] have a large effect on the accuracy of surface EMG readings [125]

and therefore need to be considered in an experimental design. The next section will discuss the methodology of EMG measurement, followed by applied aspects of EMG measurements, particularly when applied to EIMD.

1.5.2 Methods of recording and electrode placement

Studies on maximal and submaximal, as well as raw and normalised data have not shown conclusively which method is the most appropriate for EMG data analysis after an EIMD protocol [95, 133, 339, 385, 413]. EMG activity in the elbow flexors (biceps brachii and brachialis), for example, may be influenced by the type of contraction and the required force level and both of these factors are also important for the normalisation of the data. However, the most common way of displaying the EMG data is to normalise the submaximal recordings to the EMG amplitude during a maximal isometric recording [95, 339, 385, 398]. But with careful electrode placement [187, 200], well controlled contractions and normalisation of the data, interpretable measurements of surface EMG amplitude can be obtained from the biceps brachii. It has also been shown that correct and repeatable electrode placement is very important for EIMD studies on the biceps brachii. Different locations of electrode placement can lead to varying results as a consequence of uneven muscle fibre damage, reduced neural drive due to pain or central fatigue and changes in muscle geometry like fibre length and orientation. All these factors can influence the EMG recording at different recording sites on the biceps brachii [287].

1.5.3 Methods of analysing the EMG signal

The first step when analysing EMG data is to filter the raw data (Figure 1.7a) using high and low pass filters. Filtering the signal is necessary to remove artefacts, especially muscular artefacts and non–physiological artefacts such as interference from electromagnetic sources. The data are then rectified using root mean squared analysis, which calculates the squares of the raw signal followed by 'weighted' averaging over a suitable time window (Figure 1.7a,b) [245]. The aim of this process is twofold; (i) to ensure that opposing amplitudes do not cancel each other out and (ii to smooth the data (Figure 1.7c). The surface EMG signal represents the summation of all motor unit action potentials active at that instant.

(a)

(b)

(c)

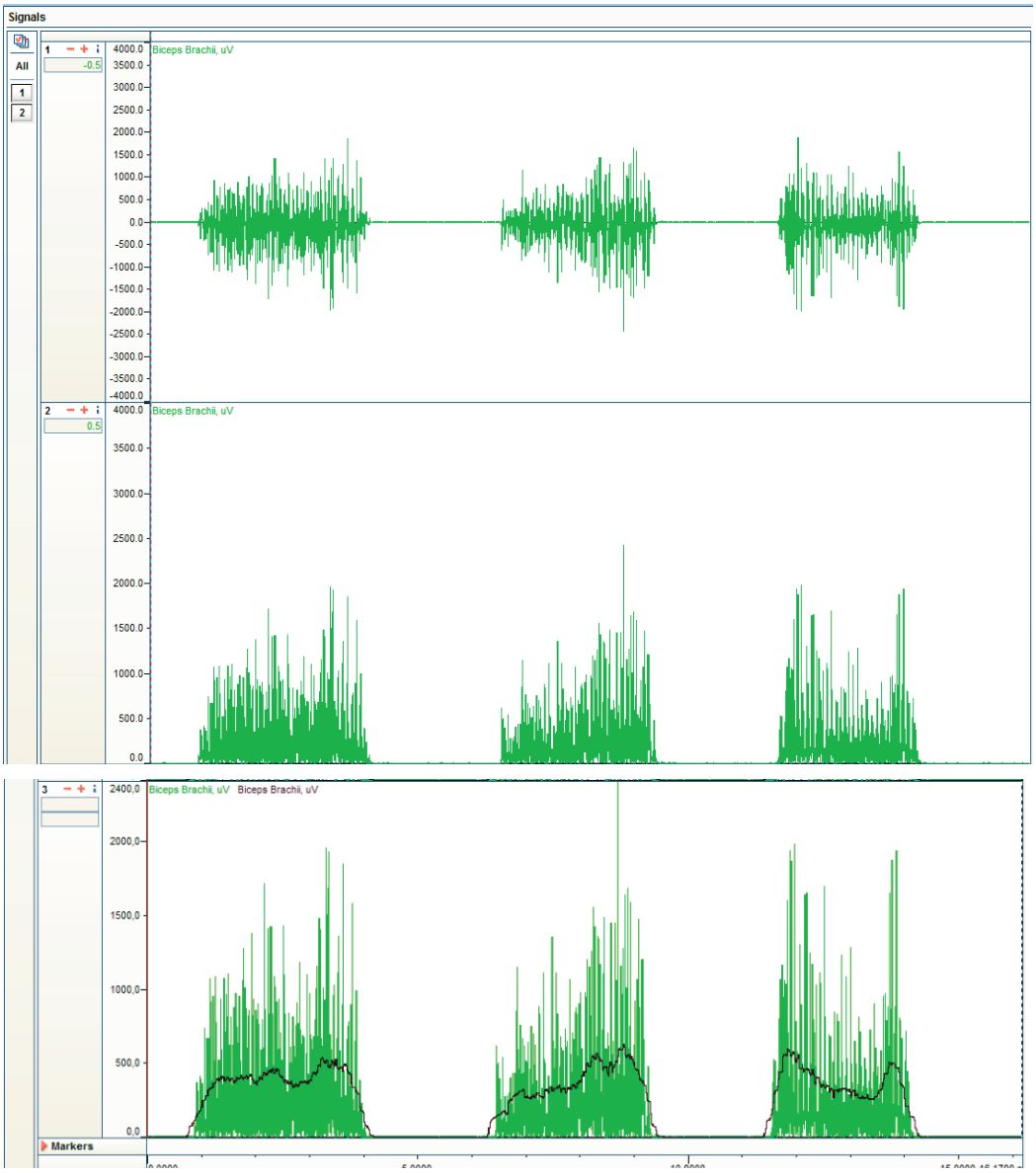

Figure 1.7 Processes of EMG signal analysis. (a) raw EMG data, (b) full wave rectified data, (c) smoothed data.
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1.5.4 EMG and force relationship

Many researchers during the 1950 to 1970's have shown that the relationship between EMG and force produced by the muscle is linear [38, 39, 364]. Since 1970 it has been shown that this linear relationship only exists in muscles such as the soleus or adductor pollicis and not for muscles such as the biceps brachii and brachialis [78]. The biceps brachii and brachialis muscles follow two linear responses with two different slopes intercepting when the force output reaches about 35% of the maximal voluntary contraction (MVC) [39]. At this point the slopes (EMG versus force) increase. This could

be due to the mixed muscle fibre composition of the biceps brachii and brachialis muscle [179]. It was suggested that at the change over point (approximately 35% of MVC), recruitment changes from a higher number of slow twitch type I fibres to more fast twitch type II fibres [39, 222]. A type II fibre can generate more force than a type I fibre [222]. Therefore when more type II fibres are recruited the resultant force increases.

1.5.5 Pain as an inhibitory factor for EMG

Certain research designs mimic the effects of acute pain by injecting hypertonic saline or capsaicin into the muscle [120, 154]. These methods have shown that under resting conditions, the acute pain caused by hypertonic saline does not cause any EMG hyperactivity in the tibialis anterior and gastrocnemius muscles[119]. However the torque output and EMG amplitude during a maximal voluntary contraction is reduced [154]. Similar results were found when hypertonic saline was injected into the biceps brachii muscle followed by elbow extension and flexion exercises [120]. The pain induced by the hypertonic saline decreased EMG activity in the biceps brachii during the movement ~23%. This inhibitory effect of muscular pain on EMG activity is greatest during maximal contraction.

1.5.6 EMG responses to EIMD

Exercise protocols which require that the muscle is repeatedly lengthened under tension have been used to induce muscle damage and associated pain and neuromuscular changes [21, 287]. In EIMD muscle tenderness occurs 1-2 days after the exercise protocol, pain occurs during muscle contraction 1-4 days after the induction of EIMD and the force output during a MVC decreases by about 38% directly after the EIMD protocol and remains low for several days [296]. The EMG amplitude of the affected muscles also decreases during the maximal isometric contraction after EIMD [95, 201, 332]. Although Day *et al.* [96] did not show a decrease in EMG amplitude during a MVC after an EIMD protocol, mean frequency power and MVC decreased on day two and three. The unchanged EMG amplitude may have been due to a small subject numbers (n = 8) coupled with large standard deviations in the EMG data and their data should be interpreted with caution.

The decrease in average EMG amplitude during the MVC in the study by Day *et al.* may have been caused by the damage inflicted on the muscle by the EIMD protocol

leading to a decrease in functional capacity. This skeletal muscle damage could have influenced the motor unit activation [220, 296].

As previously discussed, muscle activation rates can only be changed by either recruiting more motor units or by increasing their firing rates. Decreased muscle activity after EIMD has been attributed to several factors. These include damage of the myofibrillar structure such as disruption of the contractile machinery, inexcitability of the muscle or changes in the release and reuptake of calcium and the ability of troponin C to bind to the calcium within the cells to activate the actin-myosin binding [18, 251].

The muscle damage as a consequence of the exercise could also lead to changes in the excitation-contraction coupling rates and this in return would lead to a disturbance in the contractile properties of the individual muscle fibres [54]. Furthermore, it has been proposed that most of the damage is selective to the type II muscle fibres [142]. The changes in EMG amplitude and frequency over time show that the functional capacity of the muscle decreases after an exercise protocol dominated by muscle lengthening under tension [95, 385]. At low levels of force, without isometric preactivation, mean spike amplitude is higher when the muscle lengthens under tension, than when the muscle shortens. This might indicate a selective activation of type II motor units [220]. Type II fibres are more prone to fatigue and therefore also to damage by the lengthening action [142]. EMG studies have also shown that the median frequency of muscle activity decreases after EIMD exercise protocols [133]. This is a sign that more damage occurs in the type II than in the type I fibres [133, 219]. The shift to lower median frequencies can be detected for up to seven days after an EIMD protocol [133].This suggests that during a maximal voluntary contraction, after an exercise protocol designed to induce muscle damage, the entire muscle fibre population would not be available for activation and that some fibres which are activated might not be capable of full activation. Inhibition of muscle activity could be situated in another part of the activation pathway. This could be upstream, at spinal or cortical level, where, for example, there would be a decrease in overall motor unit recruitment or neural firing rates [39, 132]. A change in firing rates would be due to a change in either central activation or at the level of the motor nerve or the neuromuscular junction [39, 95, 132, 240] and EIMD could have an influence at any of these levels.

It has been shown that the EMG amplitude during submaximal static and dynamic contractions increases in participants who have been exposed to an exercise protocol

designed to induce muscle damage [21, 43, 95, 339, 385, 398]. Exercise involving lengthening muscle actions resulted in a larger than proportional increase in elbow flexor EMG for a given level of force at low levels of activation [339]. Studies also found that the increases in EMG amplitude are more pronounced in lower force ranges (submaximal contractions at 0-40% of MVC) in most muscle groups including the biceps brachii [21, 95]. The increases in EMG amplitude are also more pronounced in isometric and shortening (90-60°) contractions compared to lengthening (90-120°) actions [21, 385].

It has been shown that EMG can be decreased during the maximal contraction and during controlled lengthening actions for up to a week after EIMD [43]. However, most EMG studies following an EIMD protocol have only been conducted up to 24 [339, 385] or 48 hours [21, 398] after the EIMD inducing exercise protocol. A study by Prasartwuth *et al.* [296] showed that the decrease in maximal voluntary force and the associated EMG were dissociated from the changes in elbow angle, soreness levels and force production. They reported that EMG activity during the submaximal contraction of the elbow flexors increased significantly compared to control values at two and 24 hours after the EIMD protocol, but did not show any further differences 48 hours later. They suggest that motor units might have become damaged during the EIMD protocol and therefore produced less force even with sarcolemmal activation being preserved. Cytochemical studies have shown myofibrillar damage occurs following EIMD therefore the contractile (myofibrillar damage) but not the excitatory properties (sarcolemmal activation) of the muscle could be disturbed [140].

Muscle fibre repair starts within the first 72 hours after the EIMD protocol however some damage is still seen 132 hours later [140, 142]. This could explain both the increase in EMG activity during submaximal exercise, as the neural activation rates as well as the firing rates of the non-damaged muscles have to be higher to produce the same force output, compared to before the EIMD protocol. During the maximal voluntary contraction after an EIMD protocol the EMG activation remains lower, because some fibres are damaged and therefore fewer intact muscle fibres are available for activation [296].

As previously discussed the damage caused by lengthening under tension movements mainly affects the type II fibres, with the greatest effect shown during the first three days after the EIMD protocol [140-142]. Therefore the increased EMG activity during the submaximal dynamic contraction could be caused by increased firing patterns of the type I fibres, as they are trying to generate more force to sustain the dynamic

contraction. A higher firing rate of type I fibres would lead to an increase in submaximal EMG amplitude [414]. While type I fibres fatigue at a slower rate than type II fibres, they are also not able to produce as much force as the type II fibres do at the same firing rates [222]. Therefore they need to fire more rapidly or more fibres need to be recruited to attain the same force output leading to an increase in EMG amplitude during a submaximal contraction. But as the overall fibre population is still damaged and unable to fire to full capacity, EMG amplitude during a maximal contraction would therefore decrease.

Another explanation for the increase in submaximal EMG is that after the damage inducing exercise protocol the firing of the motor units becomes more synchronised [414] as has been shown in the biceps brachii after an EIMD protocol [95]. This leads to an increase in EMG amplitude and an increase in power in the lower frequency domain. EMG amplitude increases during submaximal exercise while experiencing the symptoms of EIMD [110, 133, 385]. The increased EMG during low force contractions (< 40% of MVC) could be explained by motor unit synchronisation and also by motor unit coherence, as motor unit coherence was 34% greater in the biceps brachii, 24 hours after EIMD was induced [95]. This led the author to conclude "*that the series of events leading to muscle damage after an EIMD* p*rotocol alter the correlated behaviour of the motor units in the bice*p*s brachii* "[95].

Therefore one could assume that there is an upstream control mechanism which regulates the motor unit recruitment and firing rates following EIMD.

1.5.7 Upstream drive of EMG

Upstream drive is defined as the central activation of the muscular system. It can be measured at the motor nerve level by nerve activation [295, 296, 377] or can be directly induced on the motor cortex by transcranial magnetic stimulation (TMS) [147, 170, 216, 296, 372, 377, 378]. Fatigue leads to a decrease in voluntary activation of the biceps brachii during MVC, as measured by nerve stimulation and transcranial magnetic stimulation of the motor cortex [372, 377]. Todd *et al.* [377] have shown that when the MVC torque decreased by 40% about a quarter of this reduction in force can be attributed to the failure of optimal activation. Prasartwuth *et al.* [296] performed a similar study but measured voluntary activation, rather than nerve stimulation [377] of the biceps brachii after an EIMD protocol. They concluded that the decrease in torque immediately after

the EIMD was caused by a reduced voluntary activation either at the spinal or motor cortex level. The authors also suggested that the decrease in force output was due to decreased voluntary activation but was not caused by the soreness felt due to the EIMD protocol [296]. Another study on EIMD and voluntary activation found that elbow angle recovered eight days after an EIMD protocol in contrast to MVC and voluntary activation [295] which were still reduced at this stage. They also concluded that EIMD did not only lead to peripheral factors limiting activation but also lead to central changes, from the spinal cord up to the brainstem and the motor cortex. It has also been shown that for eight days following an EIMD protocol, the median frequency power of the surface EMG signal during 50% and 80% MVC contraction is of lower spectral power than before the EIMD protocol [133]. This phenomenon as mentioned before, suggests either a recruitment shift towards type I muscle fibres, or a lower drive by the upstream regulator.

Spectral analysis, also called time-frequency analysis (used to calculate frequency power values) was done on EMG data recorded during finger contractions at 50% MVC. In the spectral analysis, three frequency peaks were discovered; 10, 20 and 40 Hz [240]. The authors suggest that the frequency peaks are too large to be created by single motor units and must therefore be due to synchronised firing of several motor units and could therefore be created by central oscillators (to be discussed in more detail in the next section). These central oscillators are neuronal bundles firing in a synchronised pattern, and are most likely needed for inter-neuronal communication, between different areas in the brain as well as for communication between the brain and the periphery [22, 29, 162, 240]. McAuley *et al.* [240] also mention that the three frequency peaks are also represented in the EEG frequency spectrum as α, β and gamma (γ) activity bands [240]. This will also be explained in more detail in the next section.

In summary this section has shown that there are peripheral changes including changes in muscle activation and muscle fibre recruitment patterns following EIMD. It has also been suggested that these changes could be guided by an upstream regulator which is most likely situated in the brain.

1.6 ELECTROENCEPHALOGRAPHY:

MEASURING ELECTRICAL ACTIVITY IN THE BRAIN

1.6.1 Introduction to electroencephalography

Electroencephalography is the measure of spontaneous electrical activity of the brain, similar to electromyography in the muscle. EEG measures brain activity as neural oscillation or as wavelike sinusoidal changes in voltage amplitude and can detect changes which appear within seconds to milliseconds.

There are o methods of analysing brain activity like functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) scans [92, 150, 275, 346, 367, 400, 415]. MRI, as well as PET scans, are more accurate in localising an active brain area, especially in deeper lying structures but are not as useful in movement trials as the subjects need to remain motionless over the recording period. While fMRI is an indirect measure of brain activity, as it only measures blood flow, EEG is a direct measurement of the electrophysiological signalling at anyone point in time. Limited limb movement is possible during EEG recording which makes it the better option for movement and exercise studies and it also has a greater time resolution than fMRI or PET scans. Therefore fMRI and EEG are regularly used in combination to study changes in the activity of the brain [42, 91, 92, 306, 346, 415]. However to obtain data during movement with a greater time resolution and direct association with cortical functionality; EEG is the more preferred measure.

1.6.2 Neural Oscillations: EEG signal generators

Neural oscillations are rhythmic or repetitive neural activities in the central nervous system. Neural tissue can generate oscillatory activity in many ways, driven either by mechanisms localized within individual neurons or by feedback interactions among populations of neurons [51, 97, 335]. As mentioned previously the oscillations are similar to the electrical activity in the muscle measured by EMG. The oscillations can appear either as:

(i) sub-threshold rhythms of membrane potentials rising and falling, which cannot be measured by EEG, or

(ii) increases and decreases in action potential activity, reflective of changes in the postsynaptic (i.e.. dendritic) membrane potentials [3, 211, 260]. As a result they produce a rhythmic activation of synapses in the target neurons leading to the propagation of action potentials along the target neurons [211].

When action potentials occur consecutively within the same neuron they are called spikes or bursts [97, 98, 211]. Neurons can generate multiple action potentials in sequence forming so-called spike trains [202]. These spike trains are the basis for neural coding and information transfer in the brain [51, 97, 98, 177, 368]. Spike trains can form all kind of patterns, such as rhythmic spiking and bursting as well as irregular spike patterns. Different types of neuronal coding schemes have been proposed, such as rate coding (frequency of action potentials), temporal coding (dependent on interval length between action potentials), independent- spike coding and correlation coding [163, 177]. All the coding schemes contribute to the oscillatory activity. Apart for single neurons that can generate oscillatory spike trains, oscillations can be created by the combined electrical activity of multiple individuals or groups of neurons [51, 97, 335]. These large-scale oscillations arise to synchronize the activity of multiple neurons or neuronal assemblies in different brain areas [357]. Apart from the intrinsic properties of neurons, network properties are also important for oscillatory activity [227, 273]. Neurons are connected locally, forming small clusters that are called neural assemblies or neural populations [60, 163]. Some of these network structures promote oscillatory activity at specific frequencies. This is determined by the type of neurons, i.e.. *excitatory* or *inhibitory* neurons, as well as time delays and neuronal coupling. Neural synchronization is the process by which the activities of two or more neurons or neural assemblies tend to oscillate with a repeating sequence of relative phase angles [273, 345].

Experimentally these oscillations can be measured by fluctuation in the local field potential or by means of EEG and magneto-encephalography (MEG) [97, 129, 163], as synchronized oscillations of large numbers of neurons can give rise to macroscopic oscillatory electric fields, which can be observed in the EEG. This synchronized firing of large neuronal assemblies is believed to be driven by a pacemaker most probably situated in the thalamus [51, 374]. It has been proposed that these coherent oscillations are important for interactions and communication between close and distant neuronal populations [8, 178, 225, 335, 345].

This synchronized activity of large numbers of neurons and neural assemblies results in electromagnetic fields that can be measured on the surface of the scalp with EEG.

Using these techniques, synchronized neural activities have been observed throughout the central nervous system and during various tasks [98, 363]. Neural synchronization can be modulated by different events, tasks, states and behaviours such as attention, movement and cognition [98, 314, 363], and is thought to play a role in neuronal communication [144, 211, 319, 357] and motor coordination [12]. A change in neural synchronization can be observed by measuring the increases and decreases in activity (amplitude^2 or power of the signal) in the different oscillatory frequencies. Increased amplitude^2 means greater synchronised firing of the neurons at any one time. Lower amplitude^2 implies that neuronal firing is desynchronized. "*Event-related desynchronization (ERD) and event-related synchronization (ERS) is the change of signal's* p*ower (amplitude^2) occurring in a given band, relative to a reference interval"* [111, 282].

Oscillatory EEG is a result of firing of localized neuronal population and coherent global activation of neuronal population is seen as wave-like EEG signals. These wavelike signals originate not only in the cortex but also areas deeper in the brain [8]. The difference between oscillations and waves can be seen in the EEG as follows: In oscillations the alternation between two states is important, while the regularity and the extreme values are less important, while a wave is a single variation of a parameter between two extremes [8].

Different brain structures, for example, the thalamus and the cortex, can form connections or feedback loops which support synchronized oscillatory activity [51, 196]. Oscillations recorded from multiple cortical areas become synchronized and form a large-scale network, where dynamics and functional connectivity can be studied by spectral analysis. This coherent behaviour of brain areas can be responsible for dynamic links of large-scale brain activity which is required for the integration of distributed information [357, 368].

1.6.3 Interpretation and understanding EEG

Due to different firing rates of the neurons the EEG signal is comprised of a range of different frequencies. The frequencies have been segmented into groups with different wavelength properties. As a result there are different frequency peaks in the EEG spectrum (e.g. delta (δ), theta (θ), α, β and γ [260]. This will be discussed in more detail in the next section (see Table 1.1, page 39).

Usually the frequencies of brain activity, measured by time-frequency (spectral) analysis on the EEG, are negatively correlated to their amplitudes (i.e.. the lower the frequency the higher its amplitude). This is due to the amplitude being proportional to the number of synchronously active neural elements [115]. Slowly oscillating cell assemblies comprise a larger number of neurons than fast oscillating cell assemblies [347, 348]. Therefore with an increasing number of interconnecting neurons and an increasing number of synchronously active neurons the amplitude of a recorded signal increases and the frequency decreases [226].

1.6.3.1 How to measure EEG

Electroencephalography was recorded for the first time in the late 1920's by Berger [30] with a simple galvanometer and a one channel recording. Since then the equipment has been developed extensively and now the most common used EEG system is the 10:20 system [24, 260]. Twenty-three electrodes are set on a cap which covers the scalp and measures the EEG amplitude at pre determined locations (Figure 1.8).

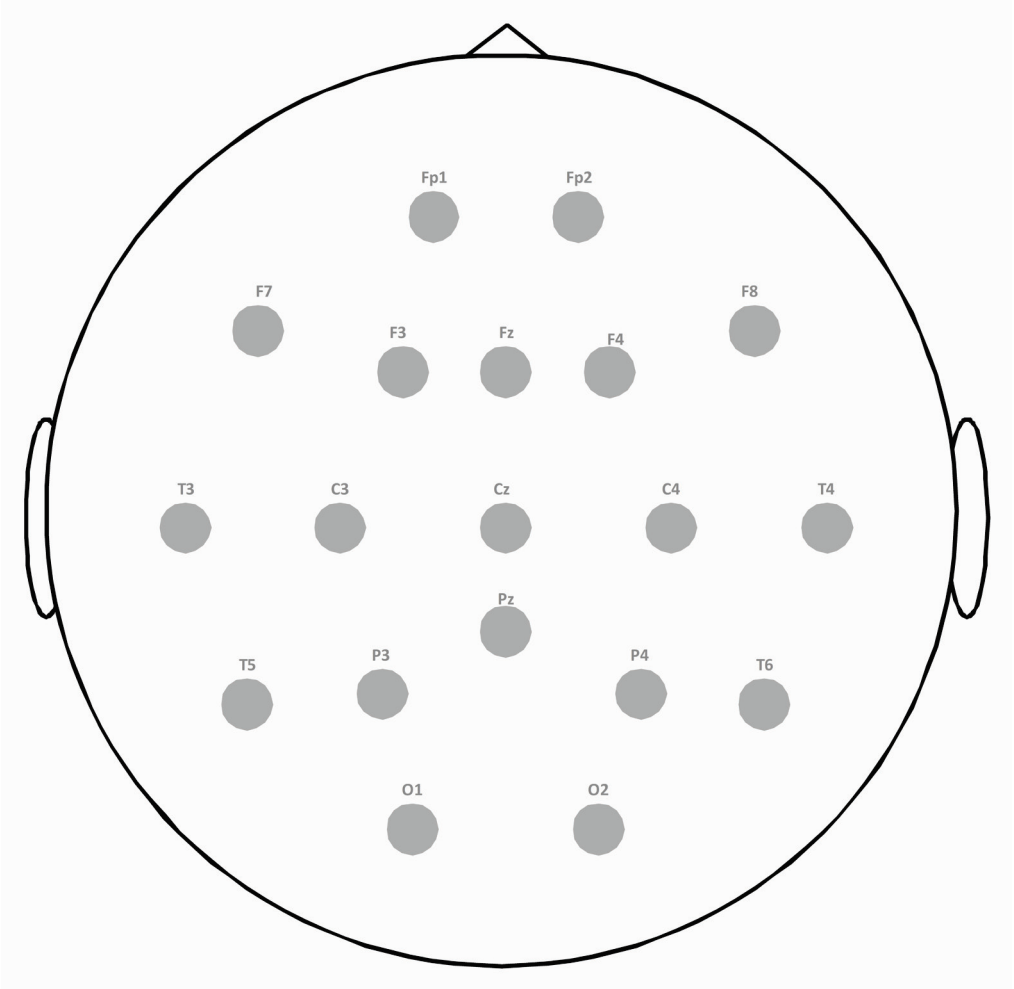

Figure 1.8 A schematic of the 10-20 EEG system

The data are digitised and recorded on a personal computer in real time with the applicable software. Newer systems have an increased number of electrodes (up to 256 or more) to get a higher resolution of the EEG signal over the scalp [32, 176, 230]. There are also needle electrodes which can be inserted into the brain to measure EEG [149, 257, 291]. This methodology might be used to study, for example, seizure or epilepsy patients [260, 288], or to determine individual neuron activity in animal studies [149, 257, 314]. This procedure is invasive and is not suitable for mechanistic type studies on healthy people. Therefore as this methodology was not used in this thesis it will not be discussed in further detail here.

1.6.3.2 Artefacts

Since the voltage signals produced by the brain are of low amplitude the signals are very susceptible to be corrupted by stronger electrical activity with higher signal amplitudes. Artefacts can be created by internal (subject specific) or external (technical) sources.

1.6.3.2.1 *Internal (subject related) artefacts*

The subject related artefacts are caused by muscular activity like eye blinking, jaw movements, tensed shoulder muscles or the heart beat [183, 286, 416]. Therefore artefacts need to be avoided during testing and data needs to be inspected during signal analysis to reduce the effect of artefacts on the data. There are several processes to minimize the effect of internal artefacts on the recorded data [109, 158, 288, 308].

While recording:

- Focus eyes on one point or close eyes
- Use eye blink channels on the EEG setup to monitor eye movement
- Record EMG and/or ECG simultaneously to monitor effect of muscle contractions and heart beat

During analysis:

- Use of eye blink channel recordings to remove or dampen eye blink and movement artefacts from recordings with the supplied software or Matlab

- Use of amplitude thresholds to reject data epochs which contain eye blinks or other muscular artefacts

- Manual removal of epochs in which data cannot be corrected

1.6.3.2.2 *External (technical) artefacts*

There are also external or technical artefacts which can influence EEG recordings. These include interference by the electrical mains as well as the impedance of the recording electrodes [260].

To record EEG as accurately as possible a standard protocol has to be followed. The impedance of the electrodes needs to be kept as low as possible to record accurate signals (or data needs to be recorded with high impedance amplifiers) and data needs to be filtered to remove any non physiological artefacts. These artefacts include those produced by the electrical mains [260, 288].

While recording:

- Subject to be prepared accurately (i.e.. with hair washed and wet)

- Electrodes to be supplied with enough conduction gel or salt solution to last for the entire testing period

- Use of correct size EEG net

- Electrodes adjusted and set on head correctly

- Impedance of electrodes set correctly and tested

During analysis:

- Filter data, especially apply a 50 Hz notch filter to remove effect of the electrical mains

- Use of a built in bad channel replacement function or use an appropriate software to remove channels with bad recordings [260].

1.6.3.3 EEG components

The measurements of an electrode on the surface of the scalp (EEG) represent an average of the oscillations in the region of the brain located beneath the electrode [3, 52, 199, 234, 260]. An EEG signal can be broken down into three components: 1) amplitude or magnitude, 2) frequency or oscillations per second and 3) phase or lag in time [260]. Therefore the recorded EEG data presents with different activity patterns of which different components are emphasized depending on the method of analysis.

1.6.4 Activity patterns

1.6.4.1 Spontaneous activity

Spontaneous activity is defined as brain activity in the absence of an explicit task, such as sensory input or motor output, and is therefore usually considered to be noise by those evaluating EEG recordings. However, spontaneous activity is considered to play a crucial role during brain development, such as neurogenesis, synaptogenesis and cortical plasticity [155]. Spontaneous activity may be informative regarding the current mental state of the person (e.g. wakefulness, alertness) [28, 138, 212] and is therefore often used in sleep research [28, 138, 212, 260]. Certain types of oscillatory activity, based on certain frequency ranges, can be part of spontaneous activity [28, 139, 212]. The term *ongoing brain activity* is used in electroencephalography for the signal components which are not associated with the processing of a stimulus or the occurrence of specific events; such as movement or attention to a task or stimulus. Therefore they form part of the evoked potentials/evoked fields, event-related potentials, or induced activity but are rather related to the underlying basic functionality of the brain which is not stimulus related [28].

Most neuroscience studies have focused on the brain's response to a task or stimulus. However, the brain is very active even in the absence of explicit input or output. Research on spontaneous fluctuations of oscillatory activity has shown that especially in the β frequency range a correlation between EEG and fMRI activity seems to exist [212]. Research on spontaneous activity led to the hypothesis that specific brain regions constitute a network supporting a default mode of brain functioning. These areas are especially active while the brain is in a resting mode but activity is attenuated when cognitive tasks are performed [28].

1.6.4.1.1 *Evoked activity*

The EEG recordings can change in signal strength and amplitude correlated to certain events. These time-locked changes in the activity of neuronal populations are generally called event-related potentials or evoked potentials [282]. These events can be motor or somatosensory evoked; they can be associated with movement, pain, temperature, visual or auditory tasks [23, 106, 379]. It is hypothesised that they are of cortical as well as subcortical origin [304]. These event related potentials (ERPs) are phase and time locked to an event and frequency independent.

The ERPs can only be extracted from the ongoing electroencephalographic recording by averaging large numbers of trials [106, 282]. As a consequence, those signal components that are the same in each single measurement are conserved and all other signals are averaged out. Evoked activity is often considered to be independent from ongoing spontaneous and induced brain activity although this is an ongoing debate [237]. Examples for ERPs are the Bereitschaftspotential[178, 302] or the P300 [174, 180, 304].

1.6.4.1.2 *Induced activity*

Induced activity refers to changes in ongoing brain activity brought on by processing of stimuli or event preparation (i.e.. medicated versus non-medicated or painful versus non-painful [322]). A well-studied type of induced activity is the change in amplitude in oscillatory activity. For example, the γ activity amplitude increases during increased mental activity such as during object representation [35], implying that a higher number of neurons are firing [35, 225]. Induced responses may have different phase angles across measurements and would cancel out during averaging, therefore they can only be obtained using time-frequency analysis [260].

The changes in the amplitude of the different frequency components are compared (i.e.. γ activity increasing during mental activity) [282]. It is a useful tool to compare changes in EEG frequency activity over longer periods or when comparing the reactions to different situations which are not necessarily events but longer lasting stimuli (i.e.. meditation, medication, pain etc [67, 69, 93, 175, 207, 255]). If the values are normalised to a baseline reading they are referred to as being relative and if they are used without a baseline they are called absolute values. The data are segmented into epochs which are then averaged. As mentioned before, the epoch length decides the frequency resolution [260].

Induced activity generally reflects the activity of numerous neurons and amplitude changes in oscillatory activity and is believed to arise from the synchronization of neural activity, e.g. synchronization of spike trains or membrane potential fluctuations of individual neurons [163, 227, 314, 325, 328, 345, 363].

1.6.4.1.3 *Event related synchronization and desynchronization*

Event related phenomena are frequency specific changes of the ongoing electroencephalographic activity. They consist either of decreases or of increases in signal amplitude in a given frequency band [259, 282, 282, 420], referenced to a baseline before the stimulus occurred [282]. This is called event related synchronization (increase of the number of neurons firing at the same time) and event related desynchronization (decrease in oscillatory activity related to internally or externally paced events) [259, 282].

An event related desynchronization can be interpreted as an electrophysiological correlate of activated cortical areas involved in processing of sensory or cognitive information or production of motor behaviour [281], while an event related synchronization is most commonly associated with the neuronal networks being in an idle state [279]. However recent research shows, that the activity in certain frequency bands increases with attention and cognition [274].

Event-related synchronization/desynchronization is usually topographically localised, phasic in its behaviour and as mentioned before, frequency specific [10, 151, 217, 359]. It can further be assumed that event related, as well as induced activity, or power changes represent the responses of cortical neurons due to changes in afferent activity (an event). To measure spontaneous induced activity, or “state” induced changes, a study of longer lasting (e.g. three minutes) spectral field powers is more advisable rather than measuring transient event-related activities in the EEG [28, 73].

1.6.4.1.4 *Summary of activity*

While evoked activity is phase locked induced activity is not phase locked. Evoked potentials are a good measure for stimuli with repetitive onsets (i.e.. evoked pain or movement) but induced activity has been shown to be superior at detecting the effect of a physiological state (i.e.. state of pain or EIMD)[84].

1.6.4.2 Prominent frequency bands within the EEG recording

As mentioned above an EEG signal is an average of neuronal oscillations with frequencies between 0.1 Hz and 100 Hz. The frequencies are grouped into frequency

ranges: delta (δ) (0.1 -3.5 Hz), theta (θ) (4.0- 7.5 Hz), alpha (α)-1 (8.0-10.0 Hz), alpha (α)-2 (11.0-13.0 Hz), beta (β)-1 (13.5-19.5 Hz), beta (β)-2 (20.0-35.0 Hz), the rolandic mu rhythm (μ) (10 Hz) gamma (γ) (>35 Hz) [30, 260, 325] (see also Table 1.1).

Table 1.1 The different EEG frequency bands and their most common recording locations and function

Frequency (Hz)	Recording location	Proposed origin	Function
0.1 – 3.5 δ	Frontal in adults [238]	Thalamus and cortex [238]	Large scale cortical integration [325] Attention [195] Sleep [168, 254]
4.0 – 7.5 θ	Frontal and central midline [231, 324]	Hippocampus [184, 225]	Memory [225, 324], mental activity and cognition [196, 225]
		Parietal-temporal areas of the cortex [254]	Observed during sleeping, waking and drowsiness [260] Increased during meditation [207]
8.0 – 10.0 α-1	Global distribution [260]	Cortex with thalamic pace maker [59, 260]	In eyes closed and relaxed state as well as during waking conditions [28, 41, 59]
11.0 – 13.0 α-2	Strongest posterior and occipital (bilateral) [59, 260]		Responds to induced activity i.e.. auditory [84, 333]
11.0 – 13.0 μ	Contralateral precentral/central (motor cortex) [10, 32, 283, 368]	Motor area and somatosensory area of the cortex [10, 283]	Usually blocked by α- and β- waves during movement [10, 32, 283, 368]
13.5 – 19.5 β-1	Frontal and parietal (bilaterally) [254]	Cortical origin (locally restricted) [316, 325]	Associated with sensory and motor activity [48, 316, 318]
0.0 – 30.0 β-2	Symmetrically over entire head [48]	Wide spread cortical β networks [48]	Coherent firing with motor units [199, 316, 318]
>35.0 γ	Central, somatosensory cortex [55, 225, 260]	Cortex [388, 389]	Memory and cognitive processes [166, 225] Neural synchronization or phase lock of firing activities [225], for example visual, sound, linguistic, attention, working memory, object recognition [35, 145, 254, 337]

1.6.4.3 Fast Fourier transform

To obtain the frequency content of the EEG activity the frequency power spectrum is calculated by applying mathematical calculations and assumptions called a Fast Fourier Transform (FFT). This is similar to the processing done with an EMG signal to gain a frequency power spectrum. In the FFT the EEG wave is displayed in the different frequency components of which the original EEG wave consists. The data are converted from a wavelike display of voltage changes over time to a display of grand overall voltage power (amplitude^2) at any given frequency and time point [6, 75, 126, 254, 288]. Therefore the frequency spectrum can have several peaks at different frequencies. In the frequency power spectrum there is always a trade off between the time and frequency resolution. The larger the data epoch length the better the frequency resolution will be and vice versa [288]. Therefore, when the data are processed, a decision has to be made whether it is more important to have a better frequency or time resolution of the recorded data.

Depending on the frequency the signal can be distributed widely over the entire scalp area (α) or be very localised (μ rhythm) [278, 283]. To keep the time and frequency effect one can also do wavelet analysis, which shows changes in the frequency spectrum without losing the time effect [75, 126, 312].

The EEG changes associated with voluntary movement start in the mesial fronto-ventral area and move towards the pre-motor area [151, 217]. However, they are mostly seen in the motor area anterior to the central gyrus, and in the primary somatosensory area on the side contralateral to the performed movement [151, 217], where the limb movements are usually represented on the motor homunculus (see Figure 1.9, page 42) [248].

Of special interest in the field of movement and pain research are the activities of α and β frequencies. Both α and β frequencies are known to be influenced by somatosensory and motor changes. Therefore the last part of this review will focus on the effects of pain and movement on these two activity bands.

1.6.4.4 Alpha activity

1.6.4.4.1 *Introduction to alpha activity*

Alpha was the first frequency band to be discovered by Berger in 1929 [30]. The α band has a frequency of between 8-14 Hz, which is not always clearly defined in the upper

limit (some researcher say 7-12 Hz other 8-14 Hz) [102, 274, 306, 358]. The α- band gets divided into the lower (α- 1) (8-10 Hz) and upper α (α- 2) (11-13 Hz) band [102, 306] due to the different responsiveness of the higher and lower α-activity to events and task.

Alpha shows large inter and intra individual differences, as some people display the α frequency bands prominently, whereas others hardly display them at all [3, 102, 104] and therefore the responses can be grouped into high and low α responders [3]. The waves can also display a high day to day variation within each person [3]. This makes the comparing of recorded data between subjects difficult as often the standard deviations for recorded α frequency bands are relatively large.

1.6.4.4.2 *Alpha-1 and -2 activity*

Generally there is a decrease in α activity in response to a task, especially if the task is related to movement, memory or learning [196, 420]. Alpha activity is known to decrease with the preparation and onset of motor or cognitive tasks as well as when the sensation of pain is experienced [67-70]. Alpha-1 reacts to processes related to attention to a task while α-2 reacts selectively to sensory-semantic memory processes especially related to long term memory systems [196, 327].

1.6.4.4.3 *Physiological meaning of alpha*

Berger was the first person to detect that certain events, such as eye opening can attenuate the ongoing α rhythm (the so called eyes open and closed paradigm), also called α blocking or desynchronization [30, 31]. Since then α desynchronization, has been associated with visual, motor and sensory evoked events [261, 270, 274].

Alpha activity is at its maximal in the occipital region (back of the head, the visual cortex), in the left lateral temporal cortical regions, the parietal area and the hippocampus and is blocked for a short time interval with the opening of the eyes and the accompanying influx of light [260, 408]. However, the α frequency activity can spread as far forward as the central areas (Cz) and even the superior frontal electrodes (F3, F4 and Fz) [20, 419]. The so called spontaneous α frequency, a smaller amplitude α wave, which does not respond to the eyes open and closed paradigm, is seen more globally especially in the prefrontal cortical region as well as the thalamus [28, 131].

It has been hypothesised that the α frequency band is of cortical origin [59, 260, 261]. However, it has also been suggested that there is a thalamic pacemaker and that corticocortical and thalamocortical modulatory systems and feedback loops play a part

in the generation of the α frequency [196, 260]. Therefore the exact location of the origin of the α frequency and the underlying neuronal mechanism is not well determined.

1.6.4.4.4 *The effects of movement on the alpha band*

Pre-movement

It has been shown that α activity is associated with movement planning [10, 278]and decreases before the onset of movement (event related desynchronization) over the central, posterior-parietal and occipital areas [10, 57, 101, 102, 280, 401].

During movement: Representation of alpha activity in electrodes overlying the motor and somatosensory homunculus

Movement itself is known to lead to a decrease of motor evoked α activity in the contralateral central and parietal areas. These areas are associated with motor control, learning and memory [196, 327, 420]. Changes in α activity, which are related to limb movements, are recorded in electrodes placed topographically over the homunculus representation on the precentral motor area, i.e. a shoulder movement is represented superior to a finger movement on the somatosensory and motor areas (Figure1.9) [151, 358, 358, 420].

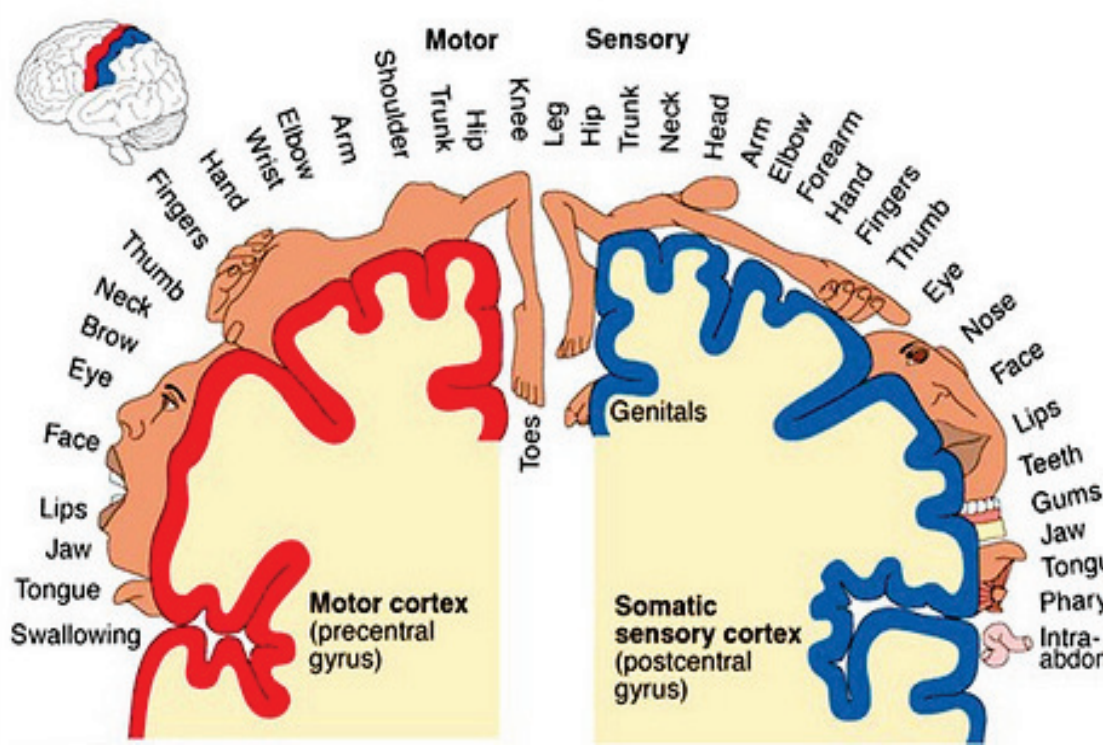

Figure 1.9 A graphical display of the homunculus in the motor and somatosensory areas of the cortex. *Copied from: ://www.vgmuseum.com/mrp/multi/Essays/enemies-kev/homunculus2.jpg*

Elite athletes even show increased synchronization in the 8-12 Hz oscillations, and therefore increased α-1 and -2 activity compared to non-athletes during movements [100, 102, 104]. Alpha-1 and -2 activity has been linked to a relaxed focus or mental activity [160, 421] and it is believed that an increased α activity during exercise increases focus and attention and therefore sporting performance [374].

P***ost-movement***

Alpha activity is enhanced again after a movement has ended [334]. The increase in α activity could be related to the intensity or fatiguing potential of the exercise involved and is especially pronounced in the frontal areas of the brain.

1.6.4.4.5 *Effect of pain on the alpha activity*

Pain leads to a decrease in activity in the α frequency band. However the decrease in activity is seen at different brain locations depending on the type of pain stimulus. While cutaneous pain, for example, stimulates activity in the contralateral prefrontal and ipsilateral pre-motor area, muscular pain does not [367].

Painful stimuli induced by either heat, capsaicin or hypertonic saline lead to decreases in α-1 activity in the frontal and temporal areas as well as the parietal and occipital areas of the scalp, contralateral to the side on which the stimulus was induced [66, 69, 70, 175]. Alpha-1 also decreases in the central and parietal areas with an injection which does not lead to the long term sensation of pain (injection of isotonic saline).

Changes in α-2 activity are less noticeable and only occur in the central and parietal area [66, 67, 69, 175] during movement, while the painful stimulus is experienced. This is followed by an increase in α-2 activity in the parietal area after the painful stimulus ends [66, 67, 69]. Following heat induced pain, the α-2 activity increases globally, except in the most anterior electrodes (Fp) after the end of the stimulus [67].

Muscular pain as induced by EIMD also causes changes in α-1 and -2 activity in the cortex. Alpha-1 and -2 activity decrease in the posterior parietal and occipital region when muscular pain is evoked in the brachioradialis[68, 74] and are negatively correlated to intensity of pain.

Interestingly it has been shown that meditation increases the global activity of α-2 activity while decreasing the feeling of pain [186, 207, 412].

1.6.4.4.6 *Combined effect of movement and pain on the alpha activity, as a model for EIMD*

The combined effect that movement and acute pain, as seen in EIMD, have on the electroencephalographic activity is different to that of movement and pain individually. In a study with laser induced pain the feeling of pain and unpleasantness was reduced when the subjects had to move a finger at the same time as the pain was induced in the arm [103]. The decrease in the feeling of unpleasantness occurred together with a smaller than usual decrease in α activity. In the case of α-2 (10-12 Hz) there was an increase in α-2 activity before the painful stimulus. The changes were maximal in the fronto-central midline area[103].

1.6.4.4.7 *Recent developments*

Until recently it was believed that increased α activity was related to decreased neuronal processing or an "idle state" of the brain. Therefore when neuronal assemblies display coherent activity and the α frequency band shows increased activity, it was assumed that the underlying cortical neuronal populations are less active in information processing [279].

Studies have shown that only about 15% of all cortical neurons are inhibitory instead of excitatory neurons [45, 260]. However, as inhibition is a crucial part of controlling neural networks, Klimesch *et al.* [198] suggested a top-down control [198, 326, 393] where an increase in α activity in cortical areas causes an inhibition of retrieval of information in the same areas. Thus uninhibited areas are able to process relevant information more efficiently [197, 326, 393], due to the "idling" of inessential neural pathways. However, in contrast to the findings of Klimesch *et al.*, recent research by Palva and Palva has shown that α activity increases with attention and cognitive processes [274]. Synchrony across the frequency ranges of α, β and γ might be needed during working memory tasks, perception and consciousness to select and maintain neural pathways [198, 274].

Von Stein *et al.* found in animal studies that synchronous interactions between frontal and parietal cortical areas lead to an increase in α-2 activity and the interactions appear to act as a top-down regulator [393]. Palva and Palva [274] and Halgren *et al.* [161] proposed that this fronto-parietal α synchrony is associated with focused attention, working memory, conscious perception, cognition and action. Chen *et al.* [72] suggested a similar cortical top-down regulator for pain in which the α activity could be involved.

1.6.4.5 Rolandic mu rhythm

The rolandic μ rhythm is, as the name implies measured by electrodes covering the rolandic area (motor cortex). Its distribution is confined to the motor and somatosensory area during rest [11, 317], and is associated with attention and execution [32, 283], especially of movements [10, 321]. The rolandic μ rhythm can be attenuated by active, passive or even reflexive movement [283]. The decreased activity is usually seen bilaterally over the motor cortex [11, 283], but is more pronounced on the side contralateral to the moving limb [282]. It is most commonly a 10 Hz frequency and is one of the several different α rhythms mentioned in Table 1.1.

1.6.4.6 Beta activity

1.6.4.6.1 *Introduction to beta activity*

The β activity has a frequency between 13 and 35 Hz [13, 64, 83] and like the α frequency band has often been divided into β-1 (13-24.5 Hz) and β-2 (25-35 Hz) [25, 26, 68, 70, 175]. The lower β range activity overlaps with the α-2 activity and therefore similar functionality can be assigned at times. The amplitude of the β frequency seldom exceeds 30 μV [260, 282]. It is mostly recorded in the frontal, central and parietal areas [68, 175, 310, 334], but has also been shown to exist as a globally distributed frequency [70] and is associated with arousal [116], attentiveness [13] and motor and sensory function [116, 208, 284, 305].

1.6.4.6.2 *Physiological function of beta*

The β activity in different areas has been linked to different underlying physiological functions, as it is unlikely that any given frequency subserves a single cognitive function in the brain [116]. Frontal β activity is common but has no proven relationship with any physiological function. The slow central 20 Hz β activity which can be attenuated by movement is believed to be an amplification of the rolandic μ rhythm [260, 306]. The posterior β rhythm, for similar reasons, is assumed to be an amplification of the posterior α frequency band [260, 306].

It has been established that the β activity is a dominant basis for information flow in the somatosensory area [208, 417] . This suggests that β activity is important to “maintain the *status quo*” in the motor and somatosensory area [12, 22, 116]. Beta activity is important for the interaction of afferent proprioceptive and neuromuscular information with the existing motor plan and execution of a new movement [292, 369].

Overall there is only limited knowledge on β oscillations which appear in a sustained state during high arousal and attentiveness [13, 116] and during somatosensory processing [116].

1.6.4.6.3 *The effects of movement on the beta band*

The motor and somatosensory cortex of monkeys displays an intermittent oscillatory activity at 25-35 Hz when animals are performing motor tasks [257]. This is especially pronounced when the animal has to focus its attention such as during fine control movements and during demanding somatosensory processes. Similar activity is seen in the pre-motor, motor and somatosensory area of humans [151] and it is suggested that the synchronous activity of cortical areas in the β frequency aids in attention and motor and somatosensory information integration and execution [104, 119, 145, 217, 247, 249, 257, 270].

There are two types of β oscillations related to movement, which have been described in humans. The first well known β oscillation is longer lasting and simultaneous with motor tasks, during activation of motor cortex networks [151, 358, 359]. The β activity decrease is usually more pronounced in the area contralateral to the movement but also appears in the ipsilateral brain area [359].

Several studies have shown that the motor cortex and peripheral motor units fire in β activity coherence during steady state contractions [64, 83, 199]. These coherent oscillations in the β activity during a steady state muscle contraction or a dynamic movement are observed in the motor and pre-motor cortex, the basal ganglia, supplementary motor area, thalamus and even subthalamic structures and the cerebellum as well as the peripheral motor units [22, 49, 199, 316]. These areas are involved in movement generation and execution. As β activity is increased during strong isometric contractions it seems that the correlation between force output and synchronised firing of cortical neurons and motor units decreases during the transition from an isometric contraction to movement [316].

The second type of β oscillation is made up of short lasting bursts of activity after the termination of a movement or somatosensory stimulation. In this case the β oscillation is increased due to an increased firing synchrony of the oscillating neurons, especially in the central electrodes [334, 358, 359]. This rebound of β activity after movement can be large (24-245%) in some subjects [358]. It was assumed to be an inhibitory activity, which increased to stop processes such as motor output or might just return the brain to its idling state [63, 279, 284].

1.6.4.6.4 *Recent beta activity research*

Recent research proposes that the rebound might be linked to the closure of cortical processes needed when processing movement sequences [119]. The authors suggested that the β activity is more closely related to the execution of a continuous movement than is α activity [119]. An alternative explanation is that the increased synchrony is reflecting on somatosensory processing after a movement as somatosensory feedback is needed for this β rebound to occur [63, 305].

1.6.4.6.5 *Beta activity and peripheral feedback and proprioception*

More recent studies suggest that increased β activity is not only important for the maintenance of steady state contractions but is needed for the efficient processing of peripheral feedback required to maintain the *status quo* and constant recalibration of the motor and somatosensory system [22, 49, 152, 199, 208], especially to maintain low force contractions [12, 13, 22, 62, 64, 300, 339, 398]. As a result of these associations with the motor and somatosensory system, β activity is not only represented in the central, frontal and temporal areas but is also strongly represented in the parietal area [104, 249], which together with the pre-motor area is associated with movement perception, proprioception, motor planning and learning [5, 208, 249, 386].

1.6.4.6.6 *The effect of pain on the beta band*

The findings on the reactivity of the β band to pain are not conclusive. Studies conducted with the injection of hypertonic saline into the brachioradialis have shown no effect on β frequency [66]. Furthermore, an auditory input also had no effect on the β frequency during the same study [66]. Tonic heat pain in the arm leads to an increase in β-1 activity in the frontal and ipsilateral temporal scalp region, but no or minimal changes in β-2 activity were observed[68, 74, 175].

However, other studies have shown that β-2 activity increases over the entire area of the head with induced muscle pain [69, 70] and β-1 increases in the frontal and ipsilateral temporal areas to the painful stimulus [68, 74, 175]. In patients with neurogenic pain the β activity is increased even during resting measurements [322]. Therefore there is no conclusive evidence about how the β band activity is influenced by painful stimuli.

1.7 SUMMARY AND HYPOTHESIS: POSSIBLE EFFECTS OF EIMD ON THE BRAIN MEASURED BY EEG

Exercise-induced muscle damage is a well studied phenomenon in exercise physiology in which the peripheral changes have been well described over the last 30 years. These studies show that EIMD disturbs body homeostasis as it leads to increases in serum creatine kinase activity, and muscular swelling as well as a decrease in muscle length.

Interestingly another measure of this disturbed homeostasis is the decrease in force output and EMG activity, both of which are influenced by neuromuscular function. Pain, which is known to be an emotional response to a disturbed homeostasis, only increases twelve hours after the damage was induced and peaks 48 hours after the damage occurred. From an evolutionary perspective it can be argued that acute pain is a protective mechanism aligned to survival. The mechanism of the delayed pain and explanations for its purpose are not so clear.

It has also been hypothesised that the changes in EMG activity associated with EIMD might be regulated by an upstream, central control mechanism which may be located as high up as the brain stem, thalamus and even cortical regions. Leading on from this, two fundamental questions arise. The first question is to determine the effects of EIMD on the periphery, especially the neuromuscular function while the muscle tissue is recovering from the induced damage. The second question is to determine where there are central effects or even a central regulating mechanism which contributes to reinstate homeostasis after it was disturbed by EIMD.

One of the methodologies which can be used to study central regulation is EEG as it measures the electrical activity on the scalp which is associated with the physiological states on the brain, mainly the cortex. Measuring induced cortical activity has been particularly useful in detecting cortical changes due to changes in various physiological states.

In recent years advances have been made with EEG methodology in explaining the cortical control of movement as well as cortical integration of pain. Of special interest in the area of movement and pain research are the activities of the α- and β frequency bands, as both are known to be related to motor and somatosensory activity of the

cortex. Movement and pain individually lead to decreased activities of central α-1 and α -2, but the combination of a pain state and movement, as often seen during EIMD, leads to an increased α activity over the central areas of the cortex. Beta activity on the other hand is associated with somatosensory feedback and integration and might therefore play an important role in the neurophysiologic feedback about neuromuscular adaptations, which occur as a result of EIMD.

Therefore the aim of this thesis is to investigate the effect of the symptoms of EIMD on neuromuscular changes and cortical activity in the α and β frequency bands during steady-state and low force contractions.

1.8 STUDY OBJECTIVES

1) To determine the effect of a protocol designed to induce symptoms of EIMD, on EMG activity during maximal and submaximal biceps movement tasks for 132 hours following the damage inducing protocol.

2) To measure the effect of EIMD and the associated neuromuscular changes and delayed pain on cortical α activity within the first 132 hours following the damage inducing protocol.

3) To measure the effect of EIMD and the associated neuromuscular changes and delayed pain on cortical β activity within the first 132 hours following the damage inducing protocol.

1.9 HYPOTHESES

1) Exercise-induced muscle damage and its associated pain and neuromuscular changes lead to changes in EMG activity measured during maximal and submaximal isometric actions as well as during a low force flexion extension biceps action lasting for 132 hours after exposure to the damage inducing protocol.

2) Cortical α- activity increases in the motor and somatosensory area while experiencing the symptoms of EIMD.

3) Cortical β activity in an experimental group experiencing the symptoms of EIMD for 132 hours will be significantly different to cortical β activity in a control group.

These hypotheses will be discussed in more detail in the subsequent experimental chapters.

The study objectives are addressed in three experimental sections (chapter 2, 3 and 4). Each experimental section has been written as a self-contained unit with its own introduction, methods, results and discussion; however the data was conducted as one study. While there may be some overlap and repetition, particularly in the methods, this compartmentalized approach was deemed more appropriate for interpreting and analysing these data than having a single methods section. The final chapter (chapter 5) integrates the data from the three studies and provides an overall summary and conclusion.

CHAPTER 2

DISSOCIATION IN CHANGES IN EMG ACTIVATION DURING MAXIMAL ISOMETRIC AND SUBMAXIMAL LOW FORCE DYNAMIC CONTRACTIONS WHILE EXPIRIENCING EXERCISE-INDUCED MUSCLE DAMAGE

2.1 INTRODUCTION

Exercise-induced muscle damage is a well described phenomenon which often occurs after exercise during which the muscle is lengthened under tension. The symptoms of EIMD include a lowered pain threshold in the affected area, a decreased range of motion of the affected joint, increased circumference of the affected area due to swelling and increased serum creatine kinase activity. The clinical symptoms usually appear twelve hours after the exercise, peak at 48 hours and subside about seven days later [18]. In addition to these symptoms there is also impaired performance during the recovery from muscle damage, especially during low force shortening and lengthening actions of the biceps brachii [385]. Several studies have shown that EMG activity during a submaximal isometric contraction increases with EIMD [95, 339, 385, 398]. Similarly, increased EMG activity also occurs during isokinetic elbow flexion under constant load [385] with the greatest increase in EMG activity occurring during muscle shortening (90-60°) compared to isometric or lengthening action (90-120°). The changes in the submaximal EMG can be ascribed to changes in motor unit or muscle fibre recruitment, changes in neuromuscular firing rates or increased synchronization of the firing units [331]. Muscle fibre recruitment changes are possibly a result of the type II muscle fibres being more prone to damage causing a recruitment shift towards type I fibres [141, 143]. Therefore to achieve the same submaximal force output when the muscle is damaged, compared to before the damage, more type I fibres are recruited increasing EMG activity.

While EMG activity during submaximal exercise increases after EIMD, the activity during maximal exercise is reduced [201]. This could either be due to changes in recruitment patterns as a result of damage to the muscle fibres or as a compensatory mechanism designed to protect the muscle fibres from further damage which might occur during maximal exercise [220, 296].

The time course of the changes and recovery of the neuromuscular function during submaximal and maximal exercise is not as clearly defined as the manifestation of the clinical symptoms described earlier. It has been shown that maximal EMG and neuromuscular function can be altered during a maximal contraction for up to seven to ten days after an EIMD exercise [43, 105], while the symptoms of EIMD subsided seven days after the protocol [105]. However most EMG studies following EIMD have been conducted up to 24 hours [339, 385] or 48 hours [398] after the exercise protocol, therefore the time course of changes have not been well described.

Therefore, the aim of this study was to determine how the symptoms of EIMD influence the neuromuscular recruitment patterns, measured by the means of EMG, during maximal and submaximal isometric and submaximal flexion–extension contractions over a 132 hours period after the muscle damage was induced.

Therefore we hypothesize that exercise-induced muscle damage and its associated pain and neuromuscular changes lead to changes in EMG activity measured during maximal and submaximal isometric actions as well as during a low force flexion extension biceps action lasting for 132 hours after exposure to the damage inducing protocol.

2.2 METHODS

Thirty-two right-handed male participants, aged 21-40 years, were recruited for this study. This sample size was based on similar research previously conducted and published in this department [210]. Handedness was determined by the *Edinburgh* handedness inventory (Appendix 1) [271]. Participants were recruited in groups of six and allocated to the control or experimental group matched for age, height, weight, body fat and skinfold thickness measured at the biceps and triceps as the thickness of the fat layer can influence the EMG signal [263]. None of the participants had any chronic or acute upper body pain or injuries (Appendix 2 and 3) and did not do any upper body training, which included lengthening the muscle under tension, within the twelve weeks before the study (Appendix 4) . They were informed about the study design, familiarized with the equipment and signed the consent form before starting the study (Appendix 5). The study was approved by the Human Ethics Committee of the Faculty of Health Science, University of Cape Town (Appendix 6), while the principles outlined by the Declaration of Helsinki were adopted in this study [410].

2.2.1 Study design

Before the start of the study all participants were familiarized with the different testing protocols. Data collection occurred over seven consecutive days (Figure 2.1). To minimize the effect of circadian rhythm on any of the outcome measures, all testing was scheduled at the same time of the day (within 60 minutes), except for the measurements at twelve hours.

Twelve hours before the exercise protocol, stature, body mass, body fat percentage [112] and skinfold thickness [311] of each participant was measured. In addition a blood sample was

taken and elbow muscle function, pain scores, biceps girth and resting elbow angle were measured for comparative reasons. During the different muscle function tests (MVC, isometric low force contraction test and active flexion and extension movement test) electromyography activity was captured of the musculus (M.) biceps brachii, M. triceps brachii and M. trapezius. Except for EMG measurements (at -12, 12, 36 and 132 hours) all other measurements occurred at -12, 12, 36, 60, 84, 108 and 132 hours (Figure 2.1).

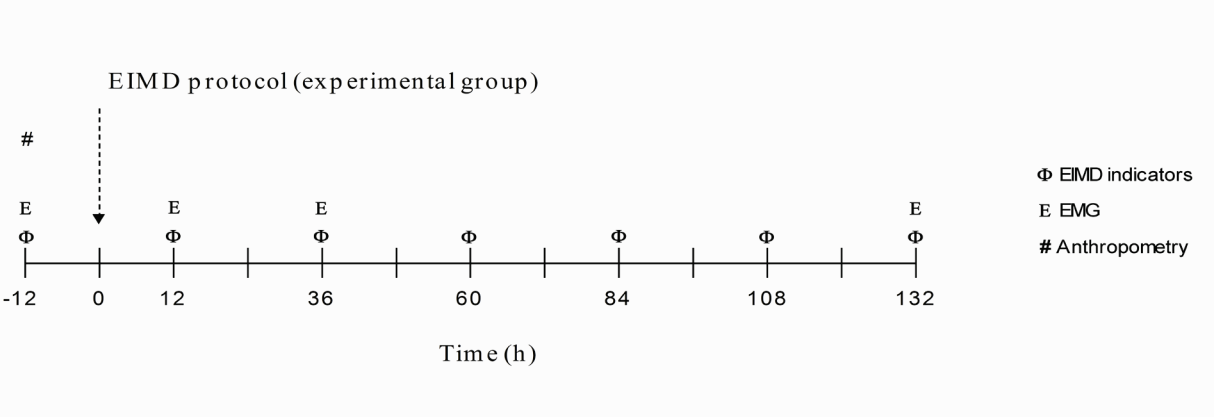

Figure 2.1 Timeline of measurements. The EIMD indicators include, pain, arm circumference, elbow angle, serum creatine kinase activity

2.2.2 Exercise protocol

The experimental group participated in an exercise intervention twelve hours after baseline testing. As the aim of the study was to induce muscle soreness, a previously described exercise protocol, which has been shown to induce EIMD [210],was adopted in this study. In brief, participants were asked to resist the lengthening movement of the left biceps (5 sets of 25 movements; see more details in 'muscle function tests' section). The resistance to these movements was set on a Biodex dynamometer at 80% of each subject's maximum isometric contraction torque [210].

2.2.3 Muscle function tests

The MVC for the left elbow flexion was determined by a Biodex dynamometer (Biodex pro 3, New York, USA). To assess MVC, participants sat on the Biodex chair with their upper body and left upper arm securely strapped to the machine while the left forearm rested freely on the arm rest. In this position, participants were able to freely flex and extend their elbow over a range of approximately 120°, without hyper-extending the elbow. The rotation axis of the dynamometer was aligned with the lateral epicondyle of the humerus, while the forearm was fixed into a fully supinated position. This ensured that the flexion-extension movement was carried out in the transversal axis and sagittal plane. Participants were asked to perform

three five second isometric elbow contractions at maximal effort, with a fixed dynamometer arm angle of 45°. This set-up results in an elbow angle at about 60°, which is within the optimal length tension curve range [71, 330], while also allowing for optimal EMG activity [221]. The three measurements were interspaced with 60 second recovery periods, while the last two measurements were used to determine an average MVC.

In addition to the MVC, an isometric low force contraction test and active low force flexion and extension movement test were performed. Within the isometric low force contraction test participants were placed on a normal chair and asked to relax their upper arm. The left arm was relaxed and hanging by their side, while the right arm was resting on the participants lap. For the submaximal EMG measurements participants were asked to move at an average angular velocity of $30°\cdot s^{-1}$ for three seconds with a 1kg wrist weight, with a fully supinated forearm placed at 90° and the fist closed. This measurement was also repeated three times interspaced with ten second intervals. Active flexion and extension movements of the elbow from to 90° and back to 180° were performed in the sagittal plane, while the upper and lower arm were kept as described in the MVC method. Participants were asked to perform three flexion (3 seconds) - extension (3 seconds) movements which were controlled by an auditory signal, while EMG data were captured continuously.

2.2.4 Electromyographic measurements

Electromyographic data were collected during muscle function tests at -12, 12, 36 and 132 hours. For measurements two circular surface electrodes (Blue Sensor, Medicotest, Denmark) were placed on the muscle belly of the M. biceps brachii, the M. triceps brachii of the left arm and the M. trapezius with a reference electrode being placed on the clavicle bone. Data were captured at a rate of 2000 Hz (Telemyo 900, Noraxon USA, Inc., Arizona, USA). To ensure accurate EMG readings, the skin where the electrodes were placed, was shaved and vigorously rubbed with ethanol [4]. To prevent skin irritations during the continuous measurements sandpaper was not used to abrade the skin. To ensure electrode placement remained the same on the four testing days over a period of seven days a permanent marker was used to circle the electrodes on the first day of EMG testing [43, 303]. EMG measurements of the M. triceps brachii and M trapezius were monitored closely to corroborate that participants did not use compensation techniques to produce more force during the exercises. To minimize the possible effect of skinfold thickness on the EMG measurements [263], the control and experimental groups were matched for these parameters.

2.2.5 Electromyographic data analysis

EMG data were analyzed with the Noraxon Myoresearch software (Version 2.11, Arizona, USA). The recorded data were filtered (50 Hz notch) to remove signal interference from external electrical sources (Myoresearch 2.11). Thereafter the data were filtered using a 15-500 Hz band pass filter and smoothed using root mean squared analysis which was calculated for a 50 ms window. Within the MVC measurement, the average EMG was calculated by averaging the 2, 3 and 4th second and excluding the data from the 1 and 5th second. In contrast to EMG data from MVC, expressed absolutely, the EMG data from the isometric low force and flexion and extension measurements were normalized to the peak EMG amplitude during the maximal isometric contraction.

Neuromuscular efficiency was calculated as torque produced during MVC/ divided by peak EMG during MVC[340].

To make the graphs of EMG activity more easily understandable the isometric data are displayed as percentage changes of the isometric data normalized to the EMG during MVC [339]. The flexion–extension data are displayed as normalized EMG (submaximal EMG normalized to EMG during MVC).

2.2.6 Other measurements

Blood samples, biceps girth, resting elbow angle and a pain score (VAS) (Appendix 7) were also measured and completed on a daily basis. Blood samples (5 ml) were taken from the right antecubital vein every testing day before the muscle function test. Samples were stored (-20°C) and later analyzed (Beckman DU-62, Beckman Instruments, Fullerton, California, USA) for the determination of serum creatine kinase (CK) activity as described previously [210]. The girth of the left biceps was measured with a tape measure midway between the acromion and radiale bony landmarks, which was marked with a permanent marker for repeatability purposes [210]. Resting elbow angles, and by implication the resting length of the biceps muscle were measured with a goniometer [210]. Current pain perception was measured on a daily basis before the muscle function test with the use of a 10 cm visual analogue scale (VAS) [366].

2.2.7 Statistical analysis

Statistical analysis was performed using STATISTICA 8.0 data analysis software (StatSoft, Inc. Tulsa, OK, USA). An independent t-test was used to compared the descriptive data between experimental and control group. As some of the data sets in this study had an unequal variance, determined using Levene's test of homogeneity of variance, we decided to use non parametric statistical tests. A Kruskal-Wallis test (H) compared the differences between the control and experimental group on each of the testing days. A Friedman's test (X^2) was used to compare changes within each group over the repeated testing days. A Dunn's test was used for p*ost-hoc* analysis. Statistical significance was accepted as $p < 0.05$.

2.3 RESULTS

2.3.1 Characteristics of subjects

Two subjects did not finish their entire trials. Due to incomplete EMG data sets and equipment failure another five subjects were excluded from further analysis. The general characteristics of the remaining 25 subjects are shown in Table 2.1. No significant differences were found between the experimental and control group in age, weight, height or skin fold measurements.

Table 2.1: Descriptive data for the control (n = 13) and experimental groups (n = 12). Data are expressed as mean ± SD.

Variable	Control	Responders
Age (years)	24 ± 5	23 ± 5
Body mass (kg)	74.4 ± 12.8	73.4 ± 12.9
Stature (cm)	173.9 ± 6.1	178.5 ± 8.3
Body fat (%)	16.0 ± 5.4	12.9 ± 6.1
Skinfolds (mm)	83 ± 50	66 ± 41

2.3.2 Muscle soreness

The difference in pain in the left arm in the experimental and control group measured by the means of the VAS scale is shown in Figure 2.2a. Peak pain in the experimental group was observed at 36 hours after the exercise protocol (X^2 = 43.03, p < 0.0001) (Figure 2.2a). A difference in pain between the two groups can be observed at 12 (H = 9.13, p = 0.0025), 36 (H = 14.67, p = 0.0001), 60 (H = 12.93, p = 0.0003), 84 (H = 10.13, p = 0.0015) and 108 (H = 10.13, p = 0.0015) hours after the exercise protocol. Significant changes in pain were also found over time in the experimental group compared to the baseline value at 36, 60 and 84 hours (X^2 = 43.03, p < 0.01).

2.3.3 Arm circumference

There was a significant increase in the difference in girth between the exercised and rested arm in the experimental group compared to the control group which lasted for the duration of the experiment 12 (H = 5.16, p = 0.0231), 36 (H = 6.99, p = 0.0082), 60 (H = 8.01, p = 0.0046), 84 (H = 7.79, p = 0.0053), 108 (H = 4.66, p = 0.031), 132 (H = 5.1, p = 0.0239) hours (Figure 2.2b). Significant changes in arm circumference were also found over time in the experimental group compared to the baseline value at 36, 60, 84 and 108 hours (X^2 = 32.29, p < 0.01). The difference between the left and right biceps girth of the control group did not change throughout the experiment.

2.3.4 Resting elbow joint angle (muscle length)

There was a significant decrease in elbow joint angle in the experimental group compared to pre-exercise values at 12, 36, 60 and 84 hours (X^2 = 34.70, p < 0.0001) (Figure 2.2c). The difference in joint angle increased in the experimental group compared to the control group at 12 (H = 5.34, p = 0.0208), 36 (H = 7.16, p = 0.0075), 60 (H = 7.01, p = 0.0081), 84 (H = 11.02, p = 0.0009) and 108 (H = 6.16, p = 0.0131) hours after the exercise protocol. No changes in the resting elbow joint angle over time were observed in the control group.

2.3.5 Serum creatine kinase activity

The serum creatine kinase activity increased significantly after the baseline measurement in the experimental group compared to the control group and peaked at 84 (H = 7.69, p = 0.0056), 108 (H = 8.84, p = 0.003) and 132 (H = 9.47, p = 0.0021) hours after the EIMD protocol. Increases over time were seen 108 and 132 hours after the induction of EIMD in

the experimental group (X^2 = 22.17, p < 0.0011) (Figure 2.2d). The serum creatine kinase activity in the control group did not change during the experiment.

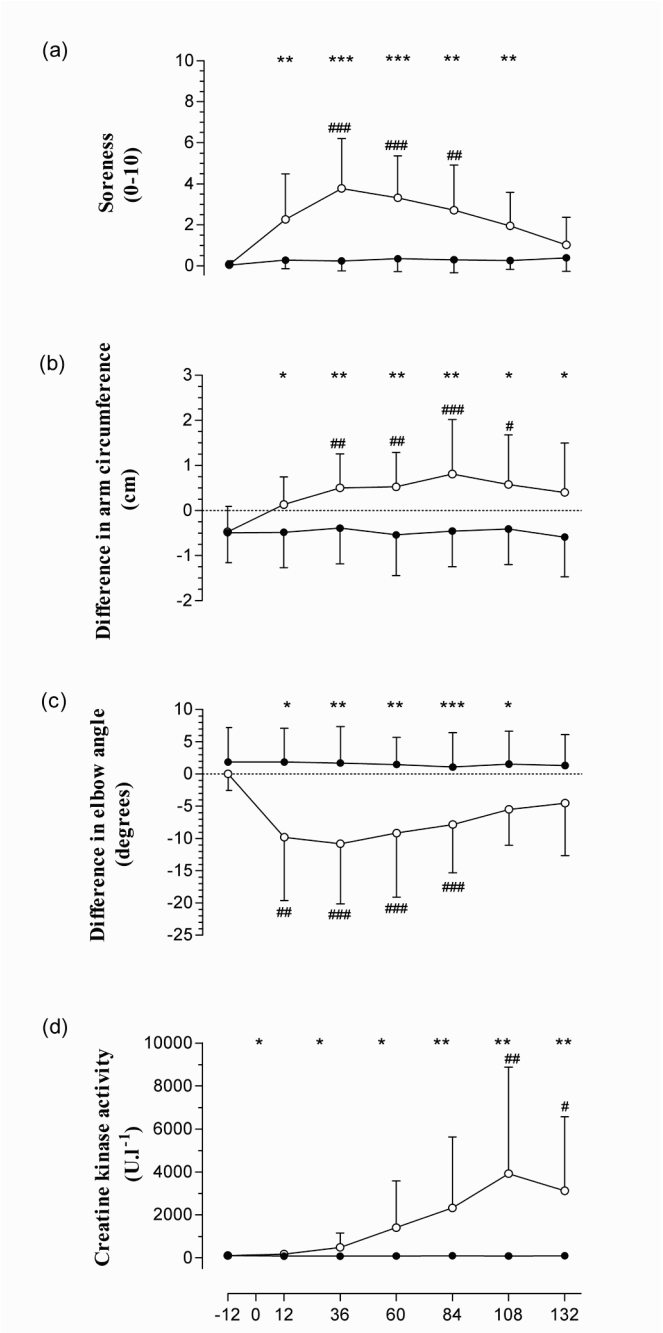

Figure 2.2
(a) The change in current pain measured with the VAS scale over seven days is shown the control (●) and experimental (○) group.

p < 0.01 at 84 hours post versus pre in the experimental group
p < 0.001 at 36 and 60 hours post versus pre in the experimental group

(b) The change in the difference in relaxed elbow girth (cm) between the left and right arm of the control (●) and experimental (○) group over seven days.

 # p < 0.05 at 108 hours post versus pre in the experimental group
p < 0.01 at 36 and 60 hours post versus pre in the experimental group
p < 0.005 at 84 hours post versus pre in the experimental group

(c) The change in the difference in elbow angle (degrees) between the left and right arm of the control (●) and experimental (○) group over seven days.

p < 0.01 at 12 hours post versus pre in the experimental group
p < 0.001 at 36, 60 and 84 hours post versus pre in the experimental group

(d) The change in creatine kinase activity ($U.l^{-1}$) over seven days is shown the control (●) and experimental (○) group.

 # p < 0.05 at 132 hours post versus pre in the experimental group
p < 0.01 at 108 hours post versus pre in the experimental group
---
(indicates results of the Kruskal-Wallis non-parametric test, # indicates results of the Friedman's non parametric test)*

** p < 0.05 control versus experimental group.*
*** p < 0.01 control versus experimental group*
**** p < 0.001 control versus*

2.3.6 Muscle function

Muscle function as measured by MVC (Figure 2.3a) decreased significantly in the experimental group compared to the control group on all but one visit to the laboratory after the exercise protocol (p < 0.05). The largest decrease in maximal force output was observed within the first twelve hours after the EIMD protocol in the experimental group (H = 14.50, p

= 0.0001). The force output in the experimental group did not recover completely for the duration of the trial at 36 (H = 10.30, p = 0.0013), 60 (H = 7.70, p = 0.0055), 84 (H = 6.82, p = 0.009) and 132 (H = 6.26, p = 0.0123) hours. The control group did not show any changes throughout the experiment.

A difference was also observed in the force output of the experimental group compared to the baseline measurement at 12, 36, 60 and 84 hours (X^2 = 38.41, p < 0.0001). There were no changes observed in the control group over time.

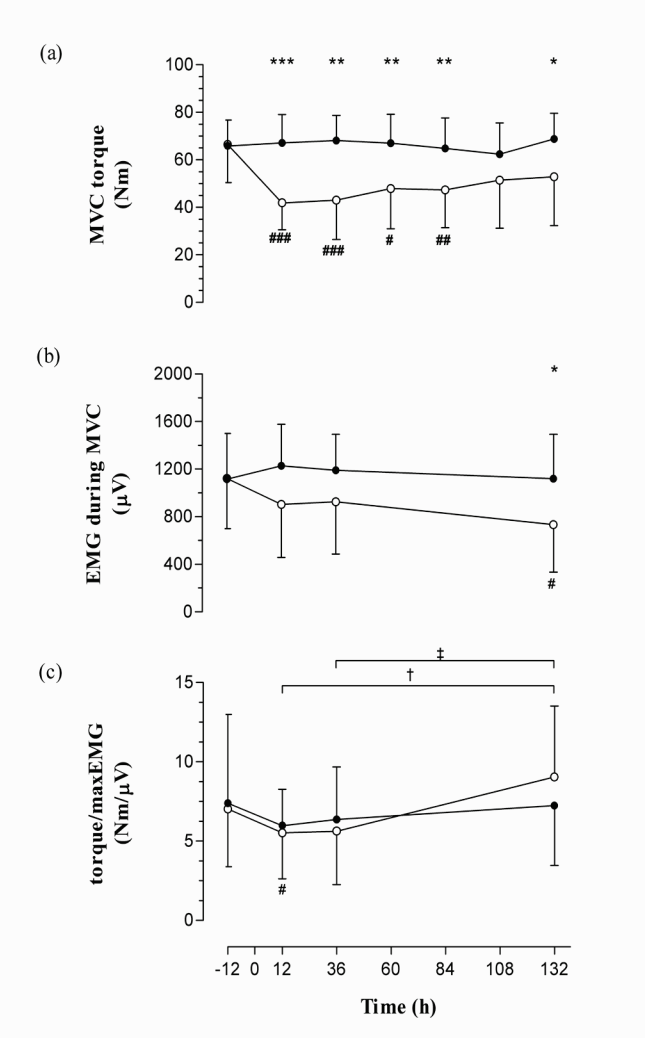

Figure 2.3
(a) The maximal force output produced on seven consecutive days is shown in the control (●) and experimental (○) group.

 # p < 0.05 at 60 hours post versus pre in the experimental group
p < 0.01 at 84 hours post versus pre in the experimental group
p < 0.005 at 12 and 36 hours post versus pre in the experimental group

(b) Change of maximal EMG amplitude on four different occasions

** p < 0.05 control group versus experimental group.*
 # p < 0.05 at 132 hours post versus pre in the experimental group

(c) Change in neuromuscular efficiency measured as a ratio of torque/EMG during the MVC in the control (●) and the experimental (○) group.

 # p < 0.05 at 12 hours post versus pre in the experimental group
† p < 0.001 at 132 hours post versus 12 hours post in the experimental group
‡ p < 0.01 at 132 hours post versus 36 hours post in the experimental group

---
(indicates results of the Kruskal-Wallis non-parametric test, # indicates results of the Friedman's non parametric test)*
** p < 0.05 control group versus experimental group.*
*** p < 0.01 control versus experimental group*
**** p < 0.001 control versus experimental group*

2.3.7 EMG activity

2.3.7.1 EMG activity during maximal force output

The electromyographic activity during a maximal force output is displayed as raw data in µV (Figure 2.3b). There was a significant decrease in EMG activity in the experimental group at

132 hours following the EIMD protocol (X^2 = 8.40, p < 0.05). The control group did not show any changes over time.

There was also a significant difference between the control and experimental group at 132 hours (H = 5.22, p < 0.0223) and a nearly significant difference at 36 hours (H = 3.83, p < 0.0502) after the EIMD protocol.

2.3.7.2 Neuromuscular efficiency measured as torque/EMG during the MVC

The neuromuscular efficiency during the maximal isometric contraction is displayed as the ratio torque/EMG. There was a significant decrease in this ratio at twelve hours compared to the pre-exercise value in the experimental group (X^2 = 21.70, p = 0.05). There was a significant increase in neuromuscular function at 132 hours compared to 12 hours (X^2 = 21.70, p = 0.001) and at 132 hours compared to 36 hours (X^2 = 21.70, p = 0.01). No changes were observed in the control group (Figure 2.3c).

2.3.7.3 EMG activity during an isometric low force contraction

The electromyographic activity during a submaximal isometric low force contraction is shown in Figure 2.4. The data are displayed normalized to the EMG during the maximal force output and as a percentage change compared to before the EIMD protocol. There was a significant increase in the experimental group at twelve hours (X^2 = 10.9, p < 0.0123) after the EIMD protocol. There was also a difference between the groups at twelve hours (H = 6.54, p < 0.0106). The control group showed a significant difference to the baseline values at 132 hours (X^2 = 9.24, p = 0.03).

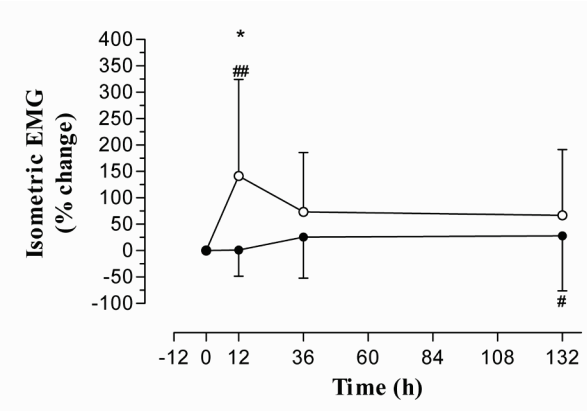

Figure 2.4 Percentage change of EMG amplitude produced during a low force isometric contraction with a 1kg wrist weight normalized to the maximal EMG amplitude measured on four different occasions in the control (●) and the experimental (○) group.

(indicates results of the Kruskal-Wallis non-parametric test, # indicates results of the Friedman's non parametric test)*
** p < 0.05 control group versus experimental group.*
 # p < 0.05 at 132 hours post versus pre in the control group, ## p < 0.01 at 12 hours post versus pre in the experimental group

2.3.7.4 EMG activity during a submaximal force output

EMG data measured during the submaximal flexion-extension movements are shown in Figure 2.5. The lightly grey shaded area represents the flexion while the dark grey shaded area represents the extension. The data are represented as a percentage of the EMG recorded during maximal force contractions. No differences were observed on the first day of testing (Figure 2.5a).

Twelve hours after the exercise protocol (Figure 2.5b) the muscle activity during the submaximal movement was significantly higher in the experimental compared to the control group during the last two seconds of the flexion as well as the first two seconds of the extension movement (s2: H = 9.61, p = 0.0019; s3: H = 11.01, p = 0.0009; s4: H = 8.00, p = 0.0047; s5: H = 4.05, p = 0.0442). The largest difference in muscle activation between the two groups occurred in the last or third second of the flexion movement (H = 11.01, p = 0.0009), where the experimental group had a higher EMG activity than the control group.
At 36 hours after the exercise protocol the muscle activity was still significantly increased in the experimental group during the entire flexion movement and the first second of the extension movement (s1: H = 4.73, p = 0.0296; s2: H = 7.4, p = 0.0065; s3: H = 8.62, p = 0.0063; s4: H = 8.00, p = 0.0047) (Figure 2.5c).

At 132 hours after the exercise protocol the muscle activity in the experimental and control group differed during the last two seconds of the flexion movement (s2: H = 4.74, p < 0.0295, s3: H = 4.27 p < 0.0387) (Figure 2.5d).

The increase in EMG activity was most visible twelve hours after the exercise protocol (Figure 2.5b), but could still be measured 36 and 132 hours after EIMD (Figure 2.5c and d). At twelve hours a marked increase occurred in the flexion, but also in the first two seconds of the extension movement (Figure 2.5b). At 36 and 132 hours the increases in EMG activity occurred especially in the flexion movement.

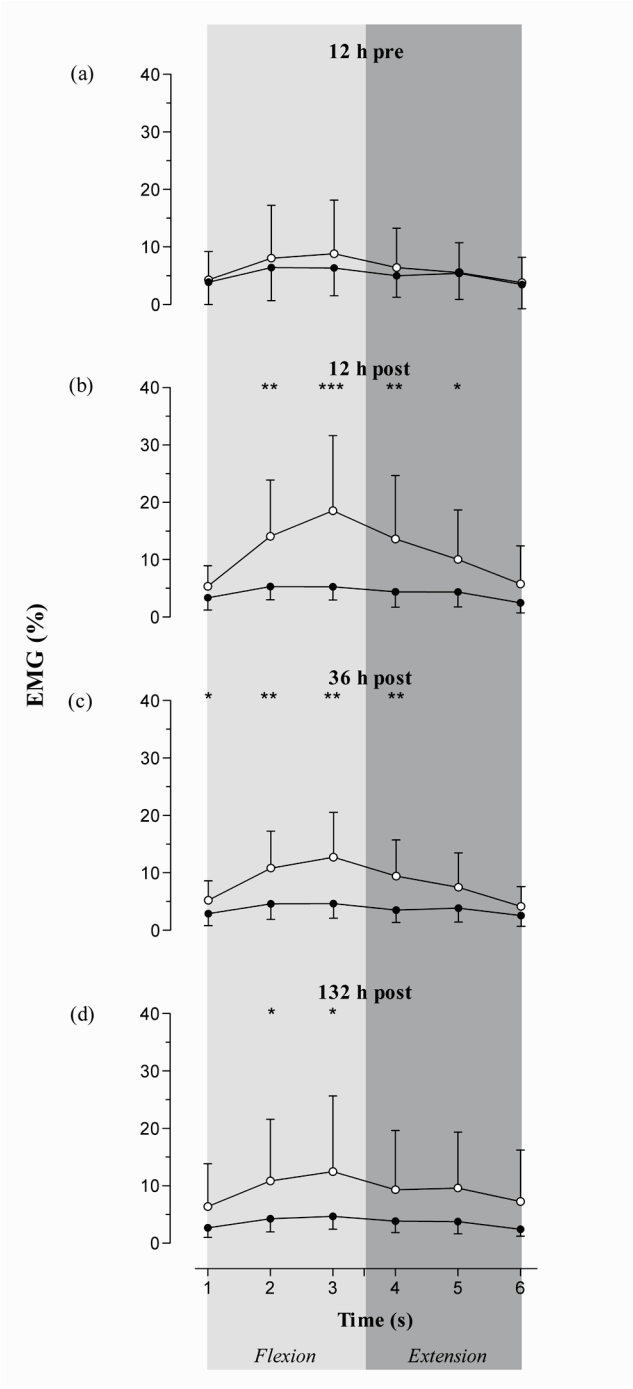

Figure 2.5: The EMG amplitude of the biceps brachii during a six second submaximal dynamic contraction normalized to the EMG amplitude during a maximal contraction measured on four different days in the control (●) and experimental (○) group.

(* indicates results of the Kruskal-Wallis non-parametric test)

* $p < 0.05$ control group versus experimental group, ** $p < 0.01$ control group versus experimental group

*** $p < 0.001$ control group versus experimental group

2.4 DISCUSSION

The first finding of this study was that the EIMD exercise protocol resulted in similar physiological responses reflecting muscle damage in the experimental group as has been reported previously in studies in this and other laboratories [80, 210, 236, 264, 267].

In particular the symptoms of EIMD (soreness, swelling, muscle shortening and creatine kinase activity) changed in the typical way for the duration of the experiment (Figure 2.2a-d). Also, muscle function measured as force output was impaired immediately after the EIMD protocol [296, 331] and gradually recovered but was still significantly different to the control group at 132 hours (Figure 2.3a). Maximal EMG activity and neuromuscular efficiency showed persistent signs of down regulation over the course of 132 hours. A significant decrease to pre-exercise values was seen at twelve hours in neuromuscular efficiency (Figure 2.3c) while the EMG during a MVC only showed a significant decrease to pre-exercise values and between groups 132 hours after the exercise protocol (Figure 2.3b).

Previous studies by Turner *et al.* [385] and Semmler *et al.* [339] have shown that an EIMD inducing protocol is followed by changes in the electromyographic activity in the 24 hours following the protocol. Both studies show that the EMG activity increases and force steadily decreases in the biceps brachii during submaximal isometric and flexion-extension contractions after an EIMD protocol, therefore showing alterations in neuromuscular recruitment within the first 24 hours. We could not find other published studies which measured neuromuscular function during maximal and submaximal contractions of the biceps for 132 hours after EIMD and therefore believe that this provides novel insight into neuromuscular recruitment while experiencing EIMD.

Our data shows that force output was decreased in the EIMD group until the end of the study at 132 hours (Figure 2.3a). The EMG during the maximal contraction was decreased at 132 hours, while neuromuscular efficiency was decreased at 12 and 36 hours suggesting that there was a down regulation of contractile function (explained in further detail in the following paragraphs) (Figure 2.3b and c).

Muscle function could be disturbed due to several factors. Based on the clinical symptoms it may be assumed that the EIMD protocol would have caused damage of the myofibrillar structure, including disruption of the contractile machinery, inexcitability of the muscle or changes in the release of Ca^{2+} and the sensitivity of cells to it [251, 403]. The damage could also

have caused changes in the excitation-contraction coupling rates, which in turn, would lead to a disturbance in the contractile properties of the individual muscle fibres [54]. EIMD protocols are known to damage type II, especially type IIB muscle fibres in preference to type I fibres [133, 219]. In contrast to type I fibres, type II fibres generate more force but are not as resistant to fatigue [222]. They also tend to tear when lengthened under tension [141]. Therefore, the type I fibres need to increase their firing frequency, or more fibres need to be recruited to attain the same force output reached before [39, 132].

This increased firing would lead to an increased EMG activity during submaximal contractions while the EMG during the MVC would be decreased due to the damaged overall fibre population. Therefore the entire muscle fibre population would not be available for activation during a MVC, while experiencing EIMD, and some fibres which are activated might not be capable of full contraction. The recovery of the muscle from EIMD usually takes a week or even longer [79, 80] and can therefore explain the decreased force output and EMG activity for 132 hours following the study. The maximal EMG/force ratio shows the dissociation of the recovery rates in MVC force output and the maximal EMG recorded at the same time. By 132 hours after the EIMD protocol the force output has started recovering while the maximal EMG recorded at the same is significantly decreased compared to pre measurements. This observation could be explained by adaptations in neuromuscular function including a down regulation of muscular activity during maximal voluntary lengthening actions [397].

Studies with motor nerve and transcranial stimulation have also shown that the expected decline in force output during a maximal isometric contraction while experiencing fatigue can be reduced. Transcranial magnetic stimulation increases the voluntary force output by about 25%, therefore it was suggested that there is an upstream down regulator during fatigue (termed central fatigue) [377]. We suggest that the same down regulation could occur for 132 hours after the EIMD protocol (Figure 2.3b). The results need to be interpreted with caution because the repeated daily measuring of the MVC could have lead to a repeated fatiguing or re-damaging of the already damaged muscle in the experimental group.
The opposite activation pattern to the maximal EMG can be seen in the low force isometric and flexion-extension contractions (Figures 2.4 and 2.5a-d). The low force isometric and flexion-extension data show similar significant changes when displayed as raw data, normalized to the EMG during MVC and when percentage normalized. During both the low force isometric and flexion-extension contraction there was an increase in EMG activity in the EIMD subjects as also shown in other studies of shorter duration[339, 385].

The increases in EMG amplitude during the submaximal contractions could be explained by several mechanisms. For example, there could be increased recruitment of motor units especially larger motor units [94, 132, 391], increased firing rates of the already active motor units, increased discharge variability and increased synchrony in the firing of motor units [95, 331, 414]. Any of the changes in muscle activation, measured as EMG activity, could manifest as a change in neural firing rates or in overall motor unit recruitment [39, 132]. A change in firing rates would be due to a change in either central activation or at the level of the motor nerve or the neuromuscular junction [240, 349] and EIMD could have an influence at any of these levels.

A change in firing rate and recruitment patterns can lead to an increase in rectified EMG amplitude and average EMG during submaximal contractions as well as a decrease in the mean power frequency [96, 356]. As EMG amplitude increases during submaximal exercise to fatigue, the same is true for submaximal exercise while experiencing the symptoms of EIMD. Therefore an interpretation of these data is that non-damaged muscle fibres start firing with more synchrony to produce the same force output which the undamaged muscle fibre population produced before the EIMD protocol. Due to the firing synchrony the submaximal EMG signal increases. It has been shown before that especially during low force contractions after an EIMD protocol, motor unit synchrony and the motor unit discharge rate increases [414].

Excitation-contraction coupling failure could also lead to an increase in EMG activity during the submaximal contraction. Due to the action potentials not being able to stimulate the release of adequate amounts of Ca^{2+} from the sarcoplasmic reticulum, a loss in force output is experienced [209, 289]. This is especially apparent at a shorter muscle length, when more Ca^{2+} release is needed to achieve the same force output as seen at longer muscle length [276, 289].

Therefore we hypothesize that an adaptive response of the central nervous system could be to up regulate the firing frequency at a shorter muscle length to achieve the same force output as seen at longer length. This study supports this hypothesis by the fact that the submaximal EMG in the experimental group shows significant increases in the later stage of the flexion (last 2-3 seconds of flexion) and early stage of extension (first 1-2 seconds of extension action). This hypothesis is in line with the suggested centrally mediated neural adaptation as described by Starbuck and Eston [360].

If one assumes that changes in especially the submaximal EMG are at least partially due to changes in neural firing rates, motor unit synchrony or recruitment changes then there must

be an upstream regulator which receives the afferent input (e.g. pain, disturbed functionality, changed proprioception, swelling etc) caused by EIMD and provides efferent output which regulates the muscle activation. This upstream regulator could probably be situated in the lower brain structures, but one can assume that especially the efferent output to the muscle could be guided by the motor cortex [83]. However this hypothesis needs to be confirmed in future research studies.

2.4.1 Conclusion

In conclusion this study shows that EIMD disturbs neuromuscular function during maximal isometric and submaximal shortening-lengthening exercises but these changes seem to not be directly related to the clinical symptoms. These changes suggest that central regulation influences the neural firing patterns and motor unit activity.

The next study examines the effect of EIMD and the associated neuromuscular changes and delayed pain on cortical α activity.

CHAPTER 3

THE RESPONSE OF CORTICAL ALPHA ACTIVITY TO PAIN AND NEUROMUSCULAR CHANGES CAUSED BY EXERCISE-INDUCED MUSCLE DAMAGE

INTRODUCTION

Exercise-induced muscle damage is a phenomenon which occurs after unaccustomed exercise, especially exercise where the muscles are lengthened under tension. Symptoms include structural damage to the muscle [141], development of pain and changes in neuromuscular function including electromyography activity (EMG) [339] and force production [339], as described in the previous chapter.

Interestingly, whilst there is a decrease in EMG activity during a maximal voluntary contraction, submaximal EMG is increased within the first twelve hours after an EIMD protocol. This is of interest since the sensation of pain is only perceived 12- 24 hours after an EIMD protocol (chapter 2). Collectively these data suggest that the changes observed in the neuromuscular function after EIMD are driven by an upstream regulator situated in the brain, which is guided by peripheral bottom-up input (i.e. noxious stimulus, inflammation, intramuscular enzyme leakage and tissue damage) [339].

It has been shown previously (fMRI and PET) that a noxious stimulus activates several areas of the brain, the primary somatosensory cortex (SI), secondary somatosensory cortex (SII), pre-motor cortex and the cingulate gyrus (limbic system) contralateral to the stimulus[14] [392]. These areas integrate the information arising from the periphery to create an emotional, behavioural and motor response, depending on the circumstances [241, 392].

As fMRI does not allow for movement during the measurement or precise time resolution of the data [323], electroencephalography (EEG) is a preferred method to measure brain activity during movement [374]. EEG is measured on the scalp with multiple low-amplitude sensitive electrodes and measures electrical activity produced by the neuronal firing in cortical and subcortical areas of the brain [260, 374]. An outcome of the EEG measurement is spectral power in different frequencies, also known as frequency band activity. The 8-13 Hz band which represents the α activity, is known to be influenced by pain [68, 69], movement [358], attention [327] and arousal [67].

The most common response of α activity to a painful, cognitive or motor stimulus is a decrease in activity at the onset of the stimulus, followed by an increase in activity once the stimulus has lasted for several minutes [68, 358] or ended [10, 69]. However recent research has shown that α activity can also increase during focused attention and cognitive processes [274]. Von Stein *et al.* showed that the increase in α activity especially α-2 activity was due to

interactions between the frontal and parietal areas and that the increase in activity appears to be acting as a top-down regulator [393]. A similar top-down regulatory process has been suggested by Chen and Herrmann [74] to control the sensation of pain. The notion of the top-down suppression of pain is further supported by studies showing that meditation increases the global α-activity whilst decreasing the subjective sensation of pain [186, 207].

EIMD provides an interesting model for investigating the effect of structural damage and acute pain on α-activity, particularly in the first twelve hours after EIMD when neuromuscular changes occur with minor symptoms of pain (chapter 2). Therefore the aim of this study was to investigate the effects of EIMD and determine whether there is a relationship between the peripheral symptoms, the neuromuscular changes and delayed pain sensation and cortical α-1 and α-2 activity.

We hypothesize that a compensatory increase in α-1 activity will be displayed over the motor and somatosensory cortex of the experimental group compared to the control group in response to changes in neuromuscular function. We further hypothesize that an increase in α-2 activity is associated with the suppression of the sensation of pain experienced within the first twelve hours after the EIMD inducing protocol was performed.

3.1 METHODS

Thirty-seven right-handed male participants, aged 21-40 years, were recruited for this study. Handedness was determined by the *Edinburgh* handedness inventory (Appendix 1) [271]. Participants matched for age, height, weight, body fat and skinfold thickness, were allocated to the experimental or control group. All participants had to be free of any upper body injuries and were not participating in any upper body training during the last twelve weeks before the study. This included the engagement in exercises involving specific muscle lengthening under tension movements.

Prior to being informed about the study design and familiarization with the equipment, participants had to sign an informed consent form (Appendix 5) and complete a Physical Activity Readiness Questionnaire (Par-Q) (Appendix 2) [7]. They were also asked to complete questionnaires about their injury and training history (Appendix 3 and 4). Participants were informed about the study design, familiarized with the equipment and signed the consent form before starting the study. The study was approved by the Human Ethics Committee of

the Faculty of Health Science, University of Cape Town (Appendix 6). The principles outlined by the Declaration of Helsinki for the use of Humans were adopted in this study [410].

3.1.1 Study design

Before the start of the study all participants were familiarized with the testing equipment and different test protocols. Figure 3.1 is a time line depicting the order of tests performed over the seven day testing period. To minimize the effect of circadian rhythm on any of the outcome measures, all tests were scheduled at the same time of the day (within 60 minutes). This however was not possible for the measurement at twelve hours after the exercise

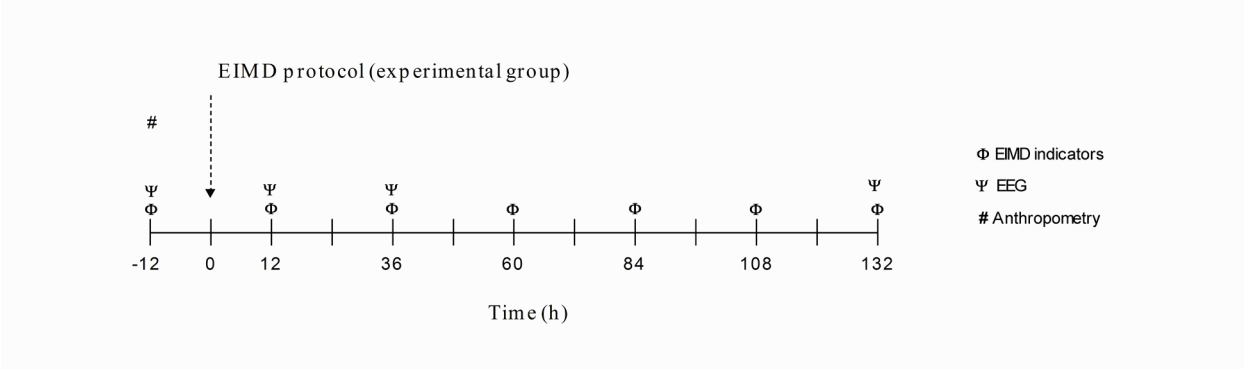

Figure 3.1 Timeline of measurements. The EIMD indicators include, pain, arm circumference, elbow angle, creatine kinase activity

Twelve hours before the start of exercise protocol (see also Figure 3.1), stature, body mass, body fat percentage and skinfolds of each participant was measured. In addition resting elbow angle, elbow muscle function (measured by MVC), biceps girth and pain scores were measured. A blood sample was taken to determine serum creatine kinase activity.

Electroencephalographically activity was measured during a self-initiated self paced flexion-extension movement. In contrast to all the above mentioned measurements that were conducted at -12, 12, 36, 60, 84, 108 and 132 hours EEG measurement were only captured at -12, 12, 36 and 132 hours (Figure 3.1). These measurements were time consuming for the participants and there was concern about poor compliance if they were required to be tested more frequently.

3.1.2 Exercise protocol

Twelve hours after baseline testing, the subjects in the experimental group completed an exercise protocol designed to induce muscle damage (EIMD protocol). In brief, participants

were asked to resist the lengthening movement of the left biceps (5 sets of 25 movements; see also 'muscle function tests' section for set up of the Biodex (Biodex pro 3, New York, USA)). The resistance to these movements was set on a Biodex dynamometer at 80% of each subject's maximum isometric contraction torque, as this has been shown to induce EIMD. The control group did not perform this exercise protocol. The set up of the Biodex dynamometer is explained in detail in the methodology section of chapter 2, page 55.

3.1.3 Muscle function tests

The muscle function tests consisted of a MVC measurement and a self-paced submaximal flexion-extension movement. The experimental and the control group performed these muscle function tests.

The MVC was measured using a Biodex dynamometer while performing elbow flexion of the left arm. For this, the participant sat in the chair of the Biodex dynamometer with their upper body and left upper arm securely strapped to the dynamometer, while the left forearm was only able to move in the sagittal plane (flexion-extension), In this position, participants were able to freely flex and extend their elbow over a range of approximately 120°, without hyper-extending the elbow. The rotation axis of the dynamometer was aligned with the lateral epicondyle of the humerus, while the forearm was fixed into a fully supinated position. This ensured that the flexion-extension movement was carried out in the transversal axis and sagittal plane. Participants were asked to perform three five second MVC's interspaced by 60 second recovery periods, as previously described in chapter 2, page 55.

The flexion-extension movement was not performed on the Biodex, but rather the participants were seated on a standard armless-chair. The participants' left arm was relaxed and hanging by their side, while the right arm was resting on the participants lap. For the submaximal self-paced flexion-extension movements all participants wore a 1kg wrist strap and movements were performed in the sagittal plane between elbow angles of 180° and 90°. During the movements subjects were ask to look at a fixed point at the wall to reduce the interference of eye movements on the EEG measurement (see also EEG evaluation). In addition, the upper body and upper arm were positioned as described in the MVC set-up for standardization purposes. Participants were asked to perform 75 repetitions, which were interspaced by 5-10 second recovery periods with slightly longer rest periods (during which EEG data were not captured) after each 25 repetitions, while EEG data were captured.

3.1.4 Electroencephalographic study procedure

The EEG data were obtained in a darkened, sound-attenuated, temperature controlled room to minimize the effect of confounding factors. Participants were instructed how to perform the self paced flexion-extension movements. EEG activity was measured during the 75 submaximal, fast self paced flexion- extension movements. In addition, subjects were asked to keep their eyes open and focused on a fixed spot on the wall during the submaximal self paced movements.

3.1.5 Electroencephalographic recording

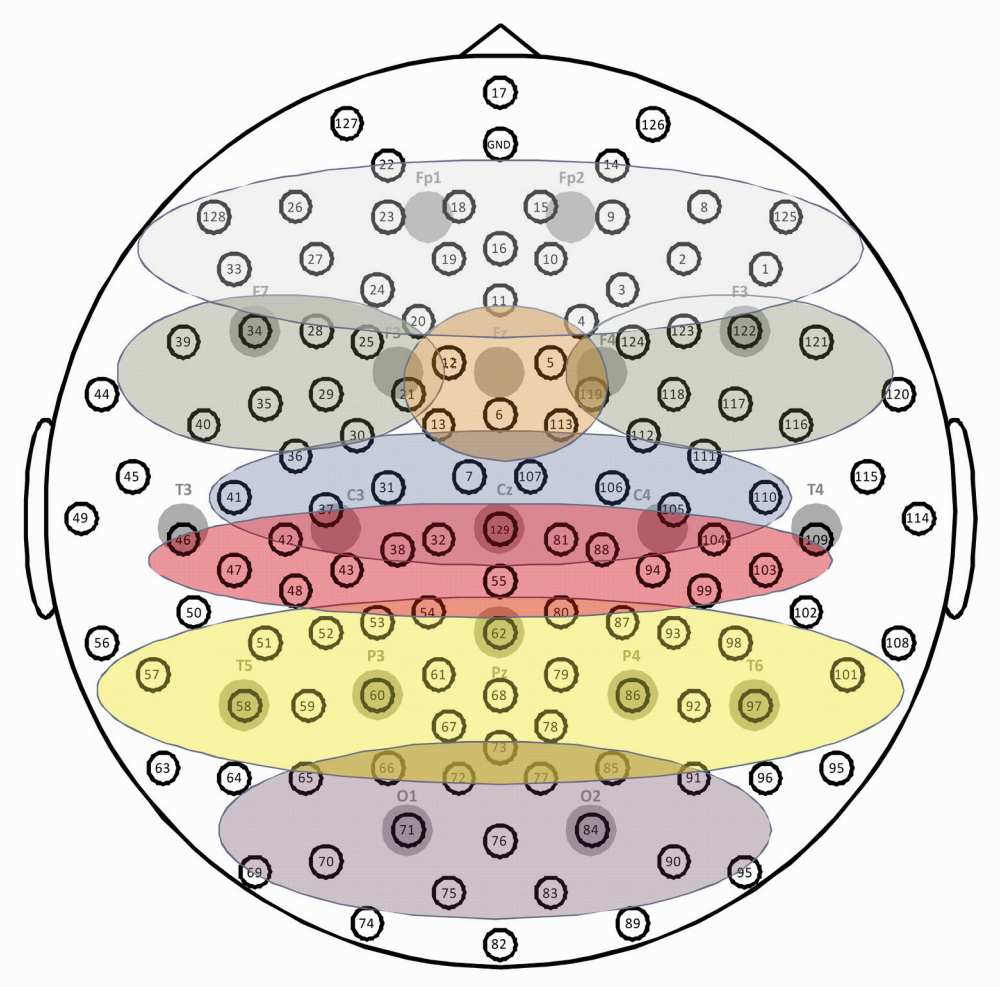

Figure 3.2 A layout of the EGI 129 channel system overlaid by the 10:20 electrode system (dark grey circles). Ellipses represent the following gross cortical areas: grey (Frontal), green (Pre-motor), orange (Supplementary Motor), blue (Motor), red (Somatosensory), yellow (Parietal), purple (Occipital).

An Electrical Geodesic ™ dense-array system (EGI) EEG net with 128 recording sites plus a vertex reference electrode (electrode 129) (Electrical Geodesics, Inc, Oregon, USA) [32],

which measures the electrical activity on the surface of the scalp, was fitted onto each participant (see Figure 3.2 for an electrode layout). The impedance of all electrodes was maintained below 50 kΩ as suggested by the manufacture of the EGI system and different technical references [32, 190, 256] due to the high input resistance of the EEG amplifier. Specially designed amplifiers processed the high impedance signal. EEG was recorded using a 0.1–50 Hz bandpass filter (3 dB attenuation) [256]. The signals were sampled at 250 Hz [32, 256]. All recordings were initially referenced to the central reference electrode (Cz/129) [32, 256]. The EEG system was connected to an experimental workstation (Net Station software, Apple Inc desktop) [32].

3.1.6 Electroencephalographic data analysis

Data were collected continuously during the pre-movement, movement and rest phase and therefore represent the common state of the brain during a biceps brachii movement protocol rather than only a movement induced change.

The EEG data were analyzed offline with the Net Station Waveform Tools (a component of Net Station software version 4.2.3, (Electrical Geodesics, Inc, Oregon, USA)). The data-analysis path recommended in the Net Station Waveform Tools Technical Manual (http://www.egi.com/ accessed January 2008) was followed. The raw EEG data were 50 Hz notch filtered as well as 1-40 Hz band pass filtered. Following this the data for each trial were segmented into three second epochs [374].

The EEG was re-referenced against an average reference [32, 34, 190], which made data recorded in the reference electrode (Cz/129) available for analysis. The EEG recording was monitored for movement, eye movement, blink artefacts, and noise by an amplitude threshold criterion. Epochs were excluded if the eye blink threshold exceeded 140 μV and the eye movement threshold exceeded 55 μV [167]. Ocular artefacts were also detected by a slope threshold, if the slope of a channel increased more than 14 $\mu V \cdot ms^{-1}$ that channel was discarded within that epoch [418]. The amplitude of a given channel was excluded in that epoch if it exceeded 150 μV, and in such a case replaced by an estimate calculated of the values of the surrounding channels. This was performed by computer algorithms built into the Net Station software version 4.2.3, (Electrical Geodesics, Inc, Oregon, USA) [256].

After the automated artefact rejection algorithm all epochs were also visually inspected offline and the recordings in which artefacts could not be removed were manually discarded

before further data analysis took place.

While shorter epochs might reveal the relationship between, for example, spectral estimates or time-locked events, our intent was to characterize background brain states (induced activity) rather than specific components of event related processing [382]. Subjects who had less than 40 usable, artefact-free epochs per trial day were excluded from further analysis. A Fast Fourier Transform (FFT) with a Welchen window was performed to obtain the spectral information of an epoch of each subject. Hereafter the data were exported to Microsoft Excel© and each subject's data were averaged before further analysis.

The different frequency bands used in this study were as follows: α-1 (7.81–9.77 Hz) and α-2 (10.74–12.7 Hz). Thereafter the relative power (activity) for each frequency on each day was calculated with the following formula: ((Power (12h or 36h or 132h) - Power (-12h)) / Power (-12h))*100. The different relative power values for each subject on the different testing days were used to calculate the statistical differences between the two different groups on the four different testing days.

Matlab 6.5 (The Mathworks Inc., Massachusetts, USA) and EEGlab v 5.02 (SCCN, University of California, San Diego, USA) were used to create topographical maps of the relative power on each day in each frequency.

Recorded data are represented based on the 10:20 system. All electrodes are grouped according to electrode on the 10:20 system which represents the same area. For example the 10:20 electrode Fz is represented by electrodes 5, 6, 11 and 12 in the Net Station system (Figure 3.2).

3.1.7 Other measurements

Blood samples, biceps girth, resting elbow angle and a pain score were measured daily as previously described in chapter 2. For the blood sample 5 ml of blood were drawn from the right antecubital vein. These samples were stored (-20° C) and later analyzed to determine the serum creatine kinase (CK) activity in the blood (Beckman DU-62, Beckman Instruments, Fullerton, California, USA) as described previously [210]. The girth of the left biceps was measured with a tape measure midway between the acromion and radial bony landmarks, that was marked with a permanent marker for repeatability purposes [210]. Resting elbow angles, and by implication the resting length of the biceps muscle were measured with a

goniometer [210]. Current pain perception was measured on a daily basis before the muscle function test with the use of a 10 cm visual analogue scale (VAS) [366].

3.1.8 Statistical analysis

An independent t-test was used to compare the descriptive data between experimental and control group, using STATISTICA 8.0 data analysis software (StatSoft, Inc. Tulsa, OK, USA). As some of the data sets in this study had an unequal variance, determined using Levene's test of homogeneity of variance, it was decided to use non parametric statistical tests instead of the parametric ANOVA test. A Kruskal-Wallis test (H) compared the differences between the control and experimental group on each of the testing days in each electrode separately. A Friedman's test (X^2) was used to compare changes within each group over the repeated testing days in each electrode separately. A Dunn's test was used for p*ost-hoc* analysis. Statistical significance was accepted at $p < 0.05$.

3.2 RESULTS

3.2.1 Characteristics of subjects

One participant did not finish the entire trial and was excluded from the study. Seven other participants were also excluded because they did not have sufficient EEG data epochs for further analysis. The remaining twenty eight participants were divided into two groups similar in weight, height, age, skinfold thickness and handedness (Table 3.1).

Table 3.1: Descriptive data for the control (n = 12) and experimental groups (n = 16). Data are expressed as mean ± SD.

Variable	Control	Experimental
Age (years)	23 ± 4	23 ± 3
Body mass (kg)	71.1 ± 8.8	72.7 ± 11.3
Stature (cm)	171.7 ± 6.8	177.4± 8.0
Body fat (%)	15.9 ± 4.9	13.4 ± 5.4
Skinfolds (mm)	79 ± 37	69 ± 38
Handedness (%)	73 ± 20	79 ± 19

3.2.2 Muscle soreness

The difference in pain in the left arm in the experimental and control group measured by the VAS scale is shown in Figure 3.3a. Peak pain in the experimental group occurred 36 hours after the EIMD inducing protocol (X^2 = 53.66, p = 0.0001). A difference in pain between the two groups occurred at 12 (H = 7.48, p = 0.0062), 36 (H = 14.32, p = 0.0002), 60 (H = 10.21, p = 0.0014), 84 (H = 8.03, p = 0.0046) and 108 hours (H = 8.37, p = 0.0038). Significant changes in pain occurred in the experimental group compared to the baseline value at 12, 36, 60 84 and 108 hours (X^2 = 53.66, p = 0.0001) (Figure 3.3a).

3.2.3 Arm circumference

During the experiment there was a significant increase in the difference in girth between the exercised and rested arm in the experimental group compared to the control group at 36 (H = 7.23, p = 0.0072), 60 (H = 6.97, p = 0.0093), 84 (H = 5.36, p = 0.0207) and 108 hours (H = 5.04, p = 0.0248). Significant changes in arm circumference were also found over time in the experimental group compared to the baseline value at 36, 60 and 84 hours (X^2 = 27.04, p = 0.01). The difference between the left and right biceps girth of the control group did not change throughout the experiment. The difference between the left and right biceps girth of the control group did not change throughout the experiment (Figure 3.3b).

3.2.4 Resting elbow joint angle (muscle length)

The difference in resting joint angle between the left and right arms in the control and the experimental group are shown in Figure 3.3c. There was a significant decrease in elbow joint angle in the experimental group until 84 hours after the EIMD inducing protocol (X^2 = 42.46, p = 0.001). The difference in joint angle decreased in the experimental group compared to the control group from 12 hours and reached its minimum 36 hours (H = 7.34, p = 0.0067) after the EIMD inducing protocol. It remained decreased until 108 hours (H = 6.71, p = 0.0096) after the EIMD protocol. No changes in the resting elbow joint angle over time were observed in the control group (Figure 3.3c).

3.2.5 Serum creatine kinase activity

The serum creatine kinase activity in the experimental group increased at 36 hours (H = 3.90, p = 0.0484) in the experimental group and reached its highest values compared to the

control group at 84 (H = 3.99, p = 0.0456), 108 (H = 4.87, p = 0.0274) and 132 hours (H = 5.27, p = 0.0217) after the EIMD protocol. Creatine kinase activity in the experimental group was only significantly increased compared to baseline at 108 and 132 hours (X^2 = 20.27, p = 0.0025). The serum creatine kinase activity in the control group did not change during the experiment (Figure 3.3d).

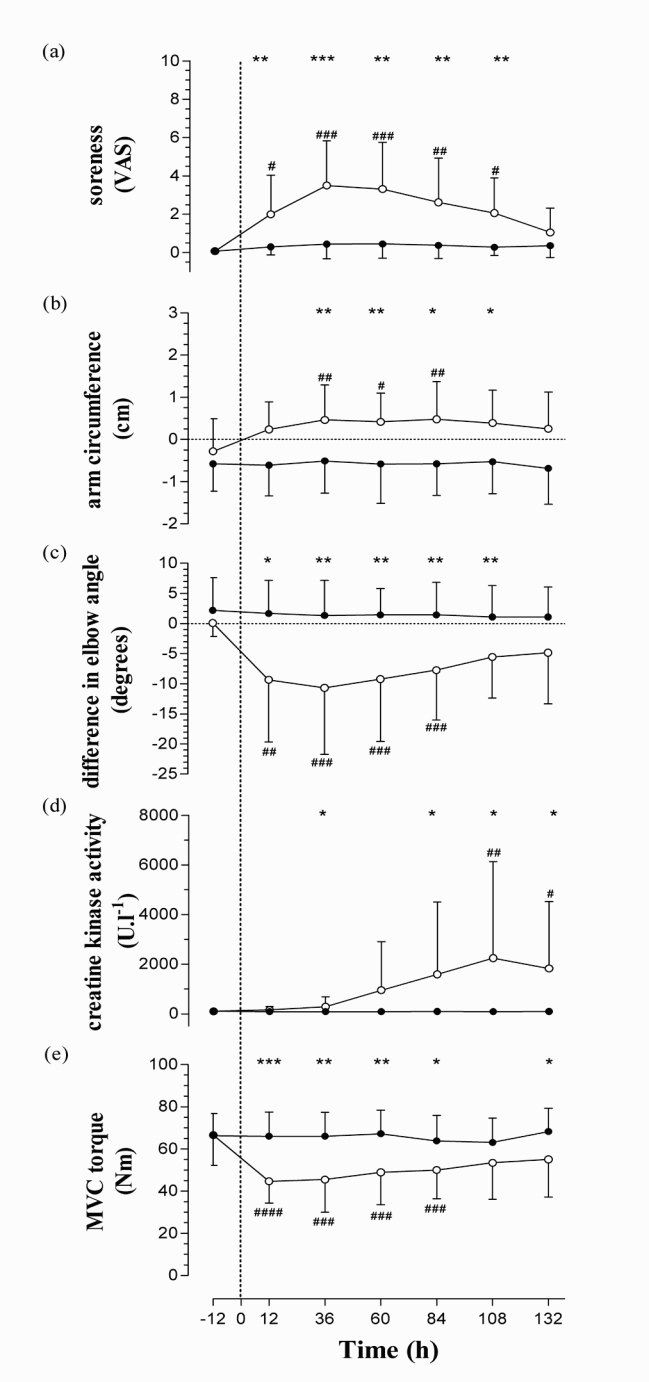

Figure 3.3

(a) The change in current pain measured with by VAS over seven days is shown the control (●) and experimental (○) group.

(b) The change in the difference in relaxed elbow girth (cm) between the left and right arm of the control (●) and experimental (○) group over seven days.

(c) The change in the difference in elbow angle (degrees) between the left and right arm of the control (●) and experimental (○) group over seven days.

(d) The change in creatine kinase activity ($U.l^{-1}$) over seven days is shown the control (●) and experimental (○) group.

(e) The maximal force output produced on seven consecutive days is shown in the control (●) and experimental (○) group.

(** indicates results of the Kruskal-Wallis nonparametric test, # indicates results of the Friedman's non* p*arametric test)*
Data are presented as averages with standard deviations.

* p < 0.05 control group versus experimental group, ** p < 0.01 control versus experimental group, *** p < 0.001 control versus experimental group.
# p < 0.05 post versus pre in the experimental group, ## p < 0.01 post versus pre in the experimental group, ### p < 0.005 post versus pre in the experimental group, #### p < 0.001 post versus pre in the experimental group

3.2.6 Muscle function

Muscle function, as measured by MVC (Figure 3.3e), decreased significantly in the experimental group compared to the control group on all but one visit (108 hours) to the laboratory after the EIMD inducing protocol ($p < 0.05$). The largest decrease in maximal force output was observed within the first twelve hours after the EIMD protocol in the experimental group ($H = 14.14$, $p = 0.0002$) while there were no changes in the control group throughout the experiment. The force output in the experimental group remained different to that of the control group until the end of the trial ($H = 5.61$, $p = 0.0179$) (Figure 3.3 e).

A difference was also observed in the force output of the experimental group over time at 12, 36, 60 and 84 hours compared to the baseline measurement ($X^2 = 48.3$, $p = 0.001$). There were no changes in the control group over time.

3.2.7 Alpha-1

Figure 3.4 and 3.5 show significant differences in α-1 activity between the control and experimental group at 12 and 36 hours after the exercise protocol. The greatest number of electrodes showing significant increases are seen 12 hours after the exercise protocol although changes remain at 36 hours especially in the electrodes overlying the motor and somatosensory areas (C3, Cz, and C4).

3.2.7.1 Twelve hours

At twelve hours after the EIMD protocol there was a widespread increase in α-1 activity over the motor and somatosensory area in the experimental group compared to the control group.

This is seen especially at electrodes representing the areas surrounding C3, Cz and C4. To simplify the understanding of the results, electrodes have been placed into subgroups and labelled with the title of the closest electrode represented on the 10:20 system.

Fz and F4

Electrodes 6 ($H = 4.97$, $p = 0.0259$) and 113 ($H = 4.56$, $p = 0.0327$) placed on the frontal area of the cortex show significant differences between the groups.

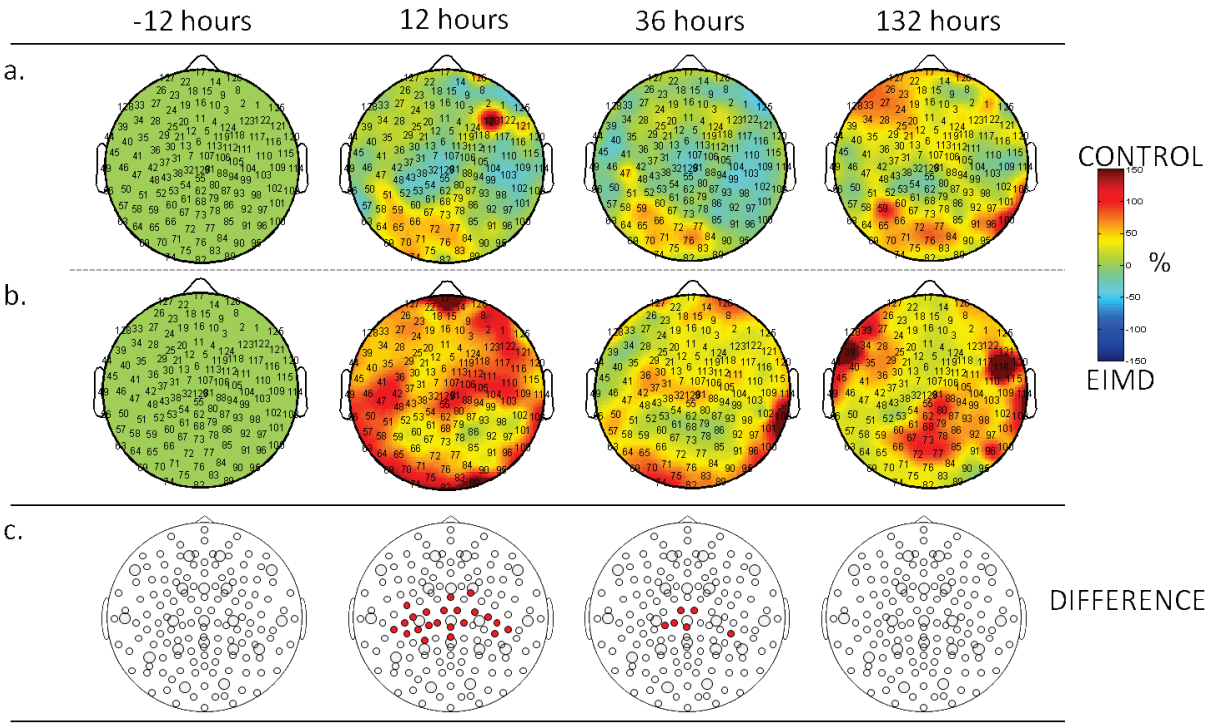

Figure 3.4 The global change (%) of α-1 activity measured with 129 electrodes over the scalp is shown in the control (a) and experimental (b) group. An outline of the electrodes showing significant differences between the two groups (c) at each time point is also shown.

T3

A significant differences was found in electrode 47 (H = 4.56, *p* = 0.0327) which is located between C3 and T3.

C3

In the ipsilateral central area surrounding electrode C3 significant differences were seen in electrodes, 31 (H = 4.97, *p* = 0.0259), 32 (H = 6.29, *p* = 0.0122), 36 (H = 4.17, *p* = 0.0411), 37 (H = 4.17, *p* = 0.0411), 38 (H = 8.02, *p* = 0.0046), 42 (H = 4.97, *p* = 0.0259), 43 (H = 7.76, *p* = 0.0053) and 48 (H = 5.61, *p* = 0.0179) where α-1 activity increases were significant in the experimental versus the control group.

Cz

Significant changes between the two groups were also seen over the vertex of the head in electrodes representing Cz. These electrodes are 7 (H = 6.28, *p* = 0.0122), 81 (H = 7.76, *p* = 0.0053) and 129 (H = 8.28, *p* = 0.004).

C4

Significant differences occurred in the central area contralateral to the moving arm in electrodes 99 (H = 5.61, *p* = 0.0179), 104 (H = 8.28, *p* = 0.004), 105 (H = 5.61, *p* = 0.0179),

106 (H = 7.00, *p* = 0.0081) and 111 (H = 7.00, *p* = 0.0081).

P3 and Pz

There were significant differences in electrode 53 (H = 3.99, *p* = 0.0459), representing P3 and also electrode 55 (H = 8.28, *p* = 0.004) representing Pz.

T4

Further down in the temporal areas there was a significant difference between groups (electrode 103; H = 7.77, *p* = 0.0053).

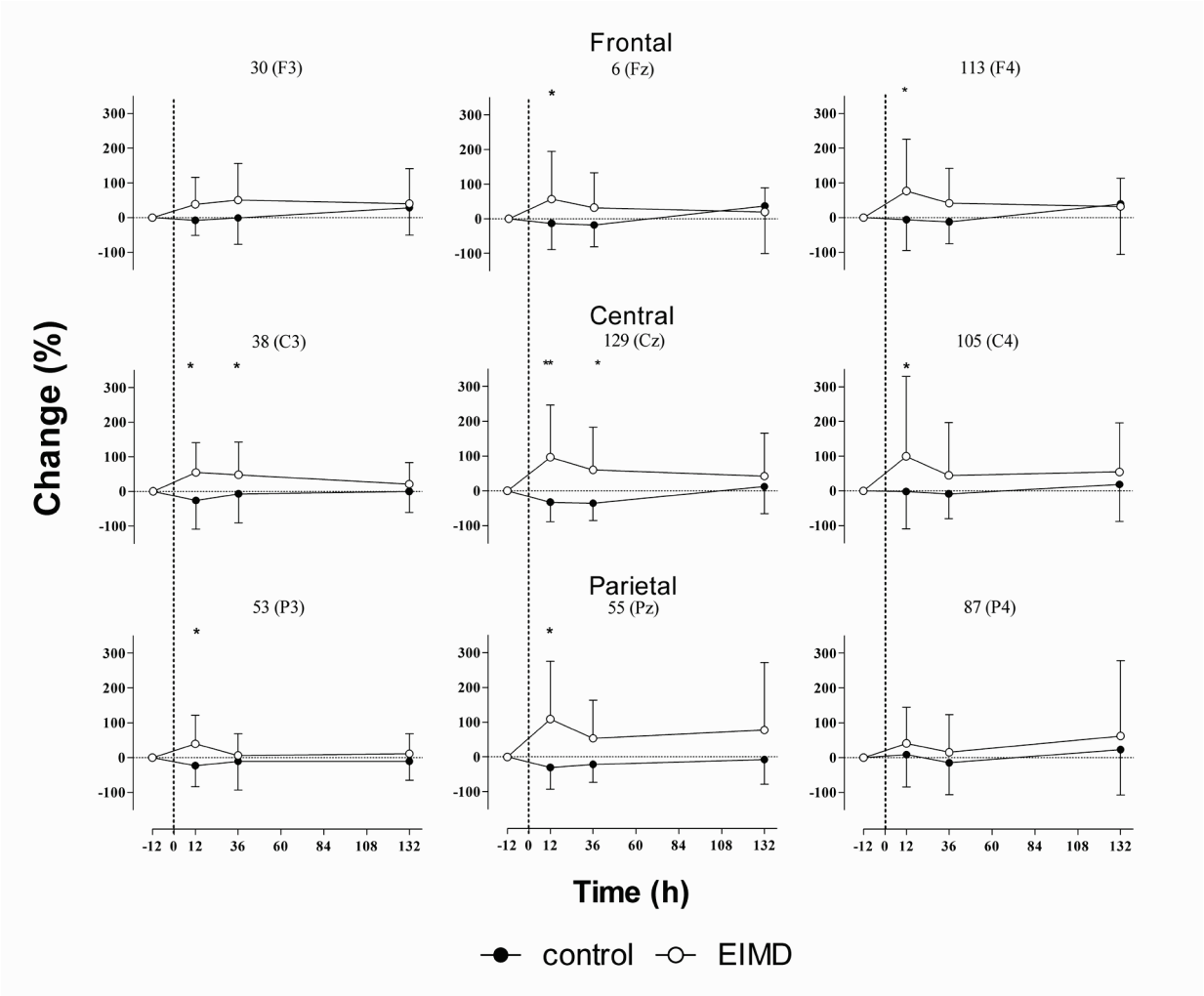

Figure 3.5 Nine different electrodes representative of the change (%) of α-1 activity in the frontal, central and parietal areas of the brain. Each of the electrodes represents a location on the 10:20 system.

(indicates results of the Kruskal-Wallis nonparametric test)*

** p < 0.05 control group versus experimental group, ** p < 0.01 control versus experimental group*

3.2.7.2 Thirty-six hours

Thirty-six hours after the EIMD inducing protocol differences were still observed between the experimental and control group, especially in the electrodes overlying the medial motor area.

Cz

The largest differences were recorded in the three electrodes surrounding the vertex area, electrodes 7 (H = 4.56, p = 0.0327), 107 (H = 4.17, p = 0.0411) and 129 (H = 5.83, p = 0.0158).

C3

Differences occurred between the experimental and the control group at electrodes 32 (H = 5.83, p = 0.0158) and 38 (H = 4.76, p = 0.0291) in the motor area ipsilateral to the movement (between C3 and Cz).

C4

Differences occurred between the groups in electrode 99 (H = 6.76, p = 0.0093).

3.2.7.3 One hundred and thirty-two hours

No differences were observed in α-1 activity between the two groups at 132 hours.

3.2.8 Alpha-2

Figures 3.6 and 3.7 show that there are significant differences in the α-2 activity between the control and experimental group at 12 and 36 hours after the induction of EIMD.

3.2.8.1 Twelve hours

At twelve hours after the EIMD protocol the differences between control and experimental group were seen on the contralateral frontal side of the cortex as well as on the ipsilateral side along the F3, C3 and P3 axis.

F3

In the ipsilateral frontal area only one electrode was significantly different, electrode 29 (H = 4.56, p = 0.0327).

Fz

In the medial frontal area electrodes 4 (H = 4.56, p = 0.0327), 6 (H = 4.17, p = 0.0411) and

12 (H = 3.99, p = 0.0459) were significantly different between the two groups.

F4

In the contralateral frontal area electrodes 112 (H = 6.28, p = 0.0122), 113 (H = 7.25, p = 0.0071), 118 (H = 9.97, p = 0.0016), 119 (H = 4.76, p = 0.0291) and 124 (H = 5.61, p = 0.0179) there were significant differences between the groups while in the ipsilateral frontal area only one electrode (29) showed significant differences (H = 4.56, p = 0.0327).

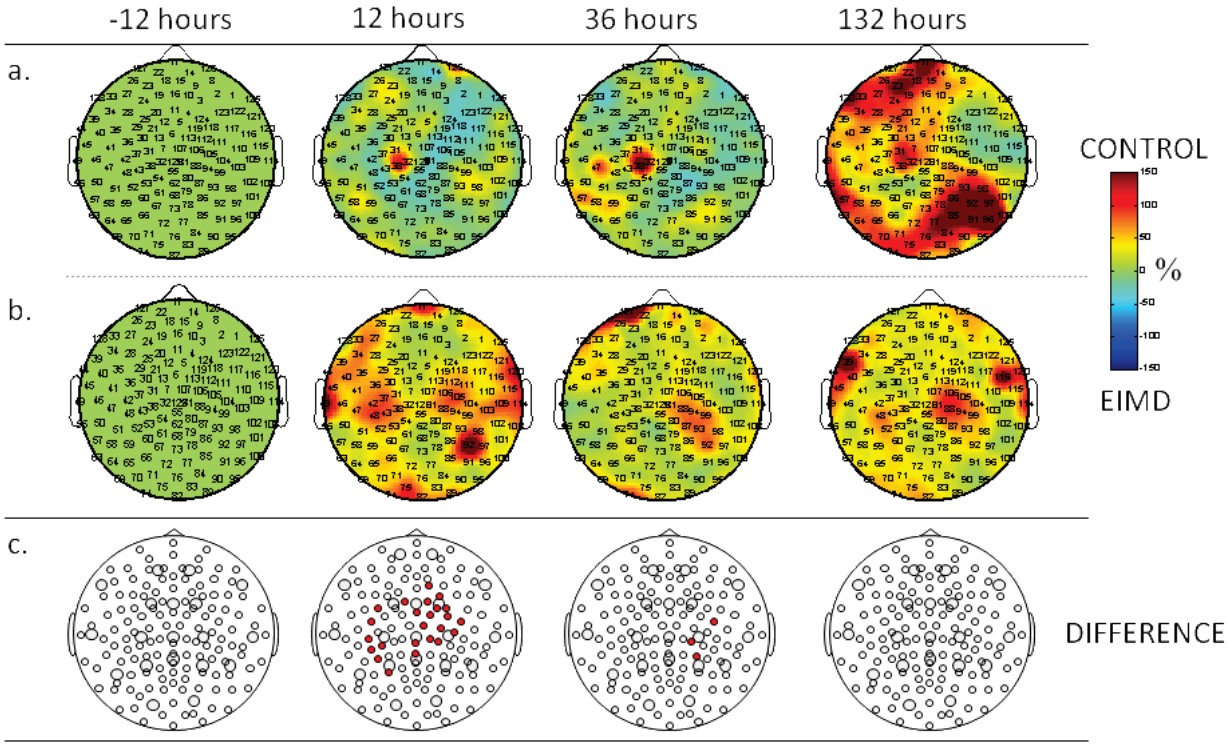

Figure 3.6 The global change (%) of α-2 activity measured with 129 electrodes over the scalp is shown in the control (a) and experimental (b) group. An outline of the electrodes showing significant differences between the two groups (c) at each time point is also shown.

C3

Differences between groups occurred in the ipsilateral central area at electrode 42 (H = 6.52, p = 0.0107), 43 (H = 4.97, p = 0.0259) and 48 (H = 6.52, p = 0.0107).

Cz

In the medial central area at electrodes 107 (H = 6.52, p = 0.0107) and 129 (H = 4.76, p = 0.0291) there were differences between the groups.

C4

In the contralateral central area surrounding C4 there were differences between groups at

electrodes 88 (H = 5.17, *p* = 0.0229), 105 (H = 6.52, *p* = 0.0107) and 106 (H = 11.80, *p* = 0.0006).

P3

The difference between groups occurred in the parietal area at 52 (H = 7.50, *p* = 0.0062) and 60 (H = 4.17, *p* = 0.0411).

Pz

Of the electrodes representing Pz only electrode 55 (H = 5.61, *p* = 0.0179) showed significant differences between the groups.

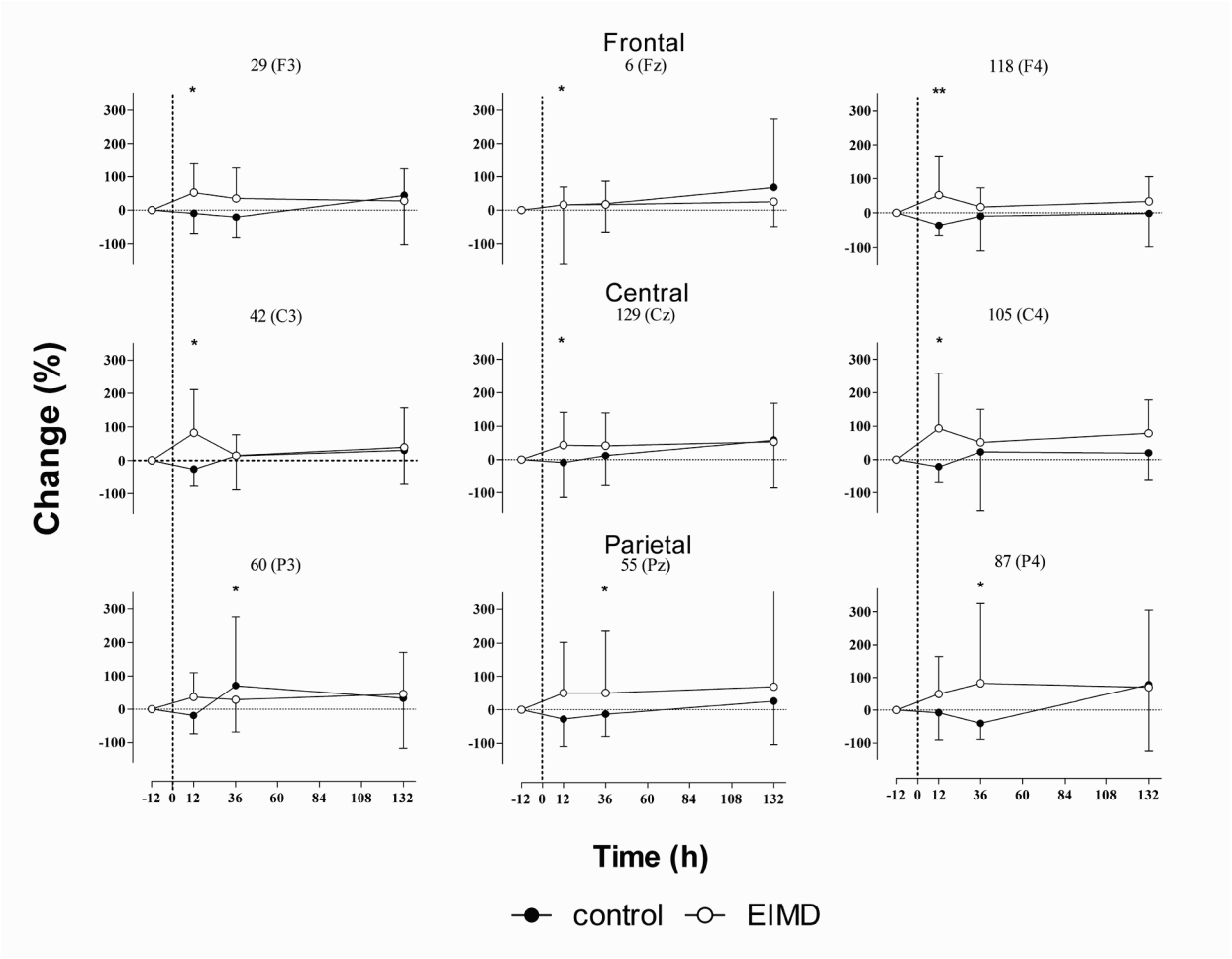

Figure 3.7 Nine different electrodes representative of the change (%) of α-2 activity in the frontal, central and parietal areas of the brain. Each of the electrodes represents a location on the 10:20 system.

(indicates results of the Kruskal-Wallis nonparametric test)*

** p < 0.05 control group versus experimental group, ** p < 0.01 control versus experimental group*

3.2.8.2 Thirty-six hours

At 36 hours after the exercise protocol significant changes between the experimental and

control group were localized and only found in the electrodes contralateral to the movement.

C4

Significant differences between groups occurred in electrode 88 (H = 4.17, p = 0.0411), 105 (H = 6.52, p = 0.0107) and 111 (H = 4.97, p = 0.0259).

P4

There were significant differences between groups in electrode 87 (H = 6.28, p = 0.0122).

3.2.8.3 One hundred and thirty-two hours

No differences were observed in the α-2 activity between the two groups at 132 hours.

3.3 DISCUSSION

The first finding of this study was that the EIMD protocol resulted in similar physiological responses (Figure 3.3) reflecting muscle damage in the experimental group as previously reported in chapter 2 (page 59).

In particular the symptoms of EIMD (swelling, muscle shortening and CK activity) changed in the typical way for the duration of the experiment (Figure 3.3a-d). Also, muscle function (force output) was impaired immediately after the EIMD protocol [339] and gradually recovered, but did not return to baseline by 132 hours (Figure 3.3e). Pain on the other hand progressively increased, peaking around 36 to 60 hours and was decreasing at 132 hours.

The following sections will discuss α-1 and α-2 activity separately, as the two bands have shown to be active in different cortical areas. It is acknowledged that deducing functional and anatomical associations from EEG data is challenging, but the clustering of significant differences in certain areas does support the association with areas and functionality.

3.3.1 Alpha 1

The participants with symptoms of EIMD experienced an increase in cortical α-1 activity (Figure 3.4 and 3.5) while performing a series of brisk biceps brachii contractions. The increase in the experimental group was most noticeable at twelve hours after EIMD induction (Figure 3.4b) and activity had decreased, although it was still significantly different between

the two groups, by 36 hours post (Figure 3.4b and c). The difference in activity between the groups was most pronounced in electrodes overlying the motor and somatosensory cortex (Figure 3.4c).

Heightened α-1 activity in the somatosensory and motor cortex has previously been associated with movement planning [10], as well as increased consciousness and perception [274]. Altered perception is suggested to lead to the increased α-1 activity as a response to changed peripheral bottom-up feedback. Feedback about changes in peripheral neuromuscular function (greater movement unsteadiness, increased submaximal EMG activity and decreased force output [339]) is integrated and processed by the central nervous system, including the cortical areas.

The increase in α-1 activity could also be a result of precise movement planning and execution in the motor and somatosensory cortex to compensate (feed-forward) for the loss in neuromuscular function whilst experiencing the symptoms of EIMD.

As previously shown in chapter 2 submaximal EMG activity increases within the first 12 to 36 hours after the induction of EIMD, while maximal EMG and force output decrease. It has been suggested that the increased submaximal EMG activity is due to increased neural drive initiated by the central nervous system [240, 339]. Thus our current findings of increased cortical α-1 activity in electrodes overlying the motor and somatosensory area together with the previous findings of increased submaximal and decreased maximal EMG activity at 12 and 36 hours post-EIMD, support our hypothesis that the motor and somatosensory cortex act as a compensatory upstream regulator of motor control while experiencing EIMD.

While it cannot be clearly stated what causes the increases in α-1 activity over the motor and somatosensory area in the EIMD group it is suggested that increased cortical α-1 activity might be necessary to counteract the loss of movement steadiness and force output whilst experiencing EIMD. Due to the recording of α-1 activity in this proximal location this leads to the assumption that this may be a cortical top- down regulator of peripheral function. Therefore the increased α-1 activity could be part of an upstream regulatory mechanism of motor perception, activation and neuromuscular function.

3.3.2 Alpha 2

Whilst α-1 activity increased in the motor and somatosensory areas, α-2 activity increased in the ipsilateral fronto-parietal area as well as in the contralateral fronto-central areas.

Alpha-2 activity peaked at twelve hours (Figure 3.6 and 3.7), when neuromuscular function was already disturbed although the main sensation of pain was yet to develop. At 36 hours post-EIMD induction α-2 activity remained elevated in the contralateral centro-parietal area. Pain peaked at 36 hours whilst α-2 activity decreased again towards pre-EIMD values.

It has previously been shown that α-2 activity increases due to interactions between the frontal and parietal cortical areas. Palva and Palva [274] and Halgren *et al.* [161] proposed that this fronto-parietal α synchrony is associated with focused attention, working memory, conscious perception, cognition and action. As this is assumed to be the most proximal level of control, this fronto-parietal α synchrony is known to act as a top-down regulator for subcortical and peripheral information integration processes [393].

Klimesch *et al.* [198] have further suggested that increased α-2 activity in cortical areas causes an inhibition of information retrieval from the involved areas. Hence the authors suggested the increase in α activity to be an inhibitory top-down control mechanism of information integration processes. A similar cortical top-down regulator has been suggested for pain [74], stating that the painful signal is perceived and incorporated at different frequency levels and areas of the cortex, with the somatosensory and the frontal cortex playing an important part.

Further research by Kakigi and Lagopoulos [186, 207] showed that meditation increased α-2 activity whilst simultaneously decreasing the sensation of pain. Following this trend Babiloni *et al.* [19] showed that α-2 activity decreases in the contralateral hemisphere with the induction of a combined stimulus of pain and movement [19]. In addition the perception of pain, especially limb pain, has been further localized to the dorsolateral prefrontal, the primary somatosensory, motor and supplementary motor cortex [228].

The above findings support the existence of a relationship between increased α-2 activity in the contralateral pre-motor, motor and somatosensory cortex and the subsequent inhibited perception of pain twelve hours post-EIMD induction [114, 228, 274, 393]. Therefore an increased α-2 activity in our cohort might be responsible for the dissociation between the sensation of pain and the changes in neuromuscular function caused by EIMD. This is of clinical importance as pain during the first twelve hours after the induction of EIMD does not reflect on the amount of damage caused.

Therefore we propose that an increased contralateral fronto-central α-2 activity acts as a cortical top-down regulator of the perception of pain twelve hours post-EIMD induction and

therefore leads to the delayed–onset pain response associated with EIMD. There was a visible increase in α-2 activity in the control group at 132 hours (Figure 3.6b), but this was not significant. We suggest that these changes are a consequence of a learning or familiarization phenomenon, although we have no descriptive data to confirm this. As a result of repetitive testing sessions control participants probably had a lower attentiveness and increased movement automation [420]. An increase in α-1 and -2 is associated with this lower attentiveness [327].

3.3.3 Limitations and future research

This study explored the relationship between the neuromuscular changes and pain induced by an EIMD protocol and associated changes in cortical α-1 and α-2 activity. However our study investigated changes in induced α activity, rather than event related α activity and therefore includes, pre movement, movement and post movement recordings. The aim was to investigate the influence of pain and changed neuromuscular function on α activity during a movement task. Also our data were not baseline corrected but rather compared to pre-EIMD protocol values to identify differences in activity between the groups post- versus pre-EIMD induction. Therefore it is acknowledged that other factors such as changes in pain pathways or inflammatory processes could have lead to the dissociated response of neuromuscular changes and the delayed pain response, but the interest of this study was how EIMD and its associated symptoms affected the α-1 and α-2 activity measured over the cortical areas. Further research is needed to integrate not only the pain and neuromuscular response with the EEG recordings but also possible inflammatory changes and adaptations in the pain pathways. Future studies should consider correlations between EMG and EEG, as well as look at a broader spectrum of EEG frequencies (including β, θ and γ).

3.3.4 Conclusion

Therefore it is proposed that the increase in α-1 activity, twelve hours after the EIMD protocol, may be part of a neurocognitive top-down regulator of neuromuscular function [186, 198, 300, 326], and that α-2 activity may be a cortical top-down regulator that suppresses the sensation of pain during the first twelve hours of experiencing EIMD [186, 198, 326].

CHAPTER 4

THE RESPONSE OF CORTICAL BETA ACTIVITY TO PAIN AND NEUROMUSCULAR CHANGES CAUSED BY EXERCISE-INDUCED MUSCLE DAMAGE

4.1 INTRODUCTION

As has been shown in the previous chapters EIMD leads to a decrease in force and EMG output during a MVC, and an increase in EMG activation during submaximal contractions with these changes lasting up to 132 hours post-EIMD induction [300, 301, 339, 385]. The change in neuromuscular function, immediately after EIMD induction, is caused by a reduction of voluntary activation either at the level of the spinal cord or motor cortex [296, 300]. These changes occur independently of the soreness caused by EIMD [300].

During voluntary movement, efferent output from the brain to the muscle is guided by the motor cortex [22, 49, 83, 148, 157, 300]. One of the frequencies in the brain associated with voluntary movement is the 15–35 Hz band [13, 64, 83]. This frequency, known as β activity, is usually pronounced in the motor and somatosensory areas of the cortex and has been linked to motor performance [104, 119, 145, 217, 247, 249, 270] due to the measurement of synchronized 15–30 Hz activity in the muscle, peripheral and central nervous system [12, 13, 64, 83] during isometric and dynamic muscle contractions. Due to the large frequency range covered by the β activity, the band has been divided into two sub bands, the β-1 activity at 15–20 Hz and the β-2 activity at 21–35 Hz, which display different responses to external stimuli [68-70, 73, 250].

Studies have shown that the motor cortex and motor units fire in coherence at a frequency between 15–30 Hz (i.e. in the β-1 and -2 activity range) during a steady state contraction [64, 83, 199]. These coherent oscillations are observed in the motor, somatosensory and pre-motor cortex during a steady state muscle contraction [22, 49, 199]. Beta activity is usually decreased before and during movement action [285] and increases after the movement has ended or as an isometric contraction is maintained over several seconds [63, 83, 119, 191, 280, 285].

However, recent studies suggest that increased β-1 and -2 activity is not only important for the maintenance of steady state contractions but is also required for the efficient processing of peripheral feedback required to maintain the *status quo* by means of constant recalibration of the motor and somatosensory system [22, 49, 152, 199, 208], especially while maintaining low force contractions [12, 13, 22, 62, 64, 300, 339, 398]. Further it has been shown that the coherence between the EEG and EMG activity in the 15–30 Hz range is positively correlated to force output [64, 193, 194].

During EIMD this peripheral feedback might be disturbed, as it has been shown that EIMD, as well as muscular pain in the biceps brachii, lead to a loss of proprioception, motor perception as well as neuromuscular function and motor recruitment [301], which in turn could lead to a compensatory increase in β activity in the associated cortical areas [5, 13, 208, 249, 386].

Movement perception, proprioception, motor planning and learning [5, 208, 249, 386] are associated with the pre-motor, supplementary motor and parietal area of the brain, all of which display strong β-1 and -2 activity during movement [104, 249].

EIMD not only causes changes in neuromuscular function and proprioception but also induces muscular pain. It has been shown previously that tonic muscle pain, which is comparable to EIMD induced pain, leads to an inhibition of motor evoked potentials in the contralateral motor cortex and therefore leads to a reduction in cortical and spinal motor neuron excitability [214]. It has been found that tonic heat pain in the arm leads to an increase in β-1 activity in the frontal and ipsilateral temporal region [68, 74, 175], while β-2 activity increases globally with non-exercise-induced muscle pain [69, 70]. However, other studies have not shown conclusive evidence that pain has an impact on β-1 or β-2 activity at all [66].

To clarify the inconsistencies in these studies we aim to investigate the relationship between the symptoms of EIMD and cortical β-1 and -2 activity during a submaximal movement for up to 132 hours following an exercise protocol design to cause EIMD. Of special interest is not only the effect of the neuromuscular changes, but also the effect of the sensation of pain on the β-1 activity and β-2 activity. We hypothesise that β-1 and -2 activity in electrodes overlying the frontal and parietal area will be increased during EIMD to compensate for loss of neuromuscular function and to integrate the increased sensation of pain.

4.2 METHODS

Thirty-seven right-handed male participants, aged 21-40 years, were recruited for this study. Handedness was determined by the *Edinburgh* handedness inventory (Appendix 1) [271]. Participants matched for age, height, weight, body fat and skinfold thickness, were allocated to the experimental or control group. All participants had to

be free of any upper body injuries and were not participating in any upper body training during the last twelve weeks before the study. This included the engagement in exercises involving specific muscle lengthening under tension movements.

Prior to being informed about the study design, participants had to sign an informed consent form and complete a Physical Activity Readiness Questionnaire (Par-Q) (Appendix 2) [7]. They were also asked to complete questionnaires about their injury (Appendix 3) and training history (Appendix 4). Participants were informed about the study design, familiarized with the equipment and signed the consent form before starting the study (Appendix 5). The study was approved by the Human Ethics Committee of the Faculty of Health Science, University of Cape Town (Appendix 6). The principles outlined by the Declaration of Helsinki for the use of Humans were adopted in this study [410].

4.2.1 Study design

Before the start of the study all participants were familiarized with the testing equipment and different test protocols. Figure 4.1 is a time line depicting the order of tests performed over the seven day testing period. To minimize the effect of circadian rhythm on any of the outcome measures, all tests were scheduled at the same time of the day (within 60 minutes). This however was not possible for the measurement at twelve hours after the exercise protocol.

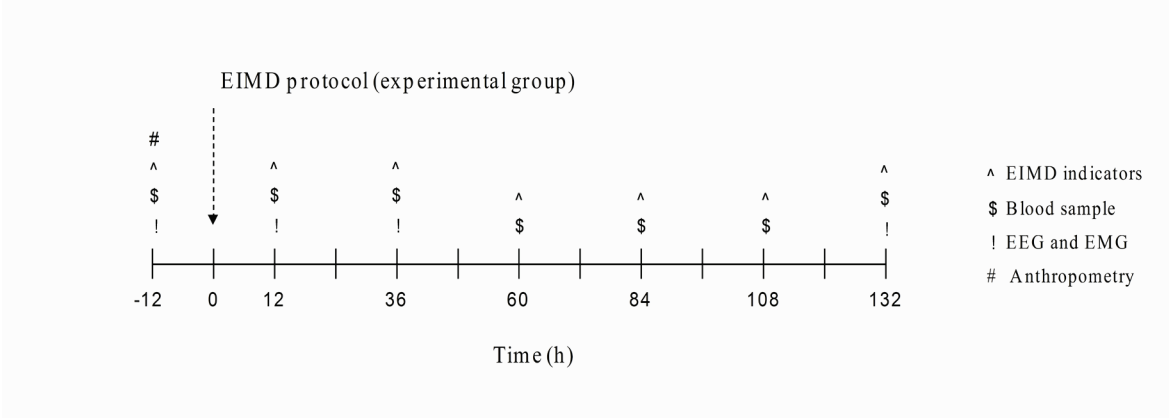

Figure 4.1: Timeline of measurements. The EIMD indicators include, pain, arm circumference, elbow angle, serum creatine kinase activity

Twelve hours before the start of exercise protocol (see also Figure 4.1), stature, body mass, body fat percentage and skinfolds of each participant was measured. In addition

resting elbow angle, elbow muscle function (MVC), biceps girth and pain scores were measured. A blood sample was taken to determine creatine kinase activity.

EEG activity was measured during a self-initiated self-paced flexion-extension movement. In contrast to all the above mentioned measurements that were conducted at -12, 12, 36, 60, 84, 108 and 132 hours EEG measurement were only captured at -12, 12, 36 and 132 hours (Figure 4.1). These measurements were time consuming for the participants and there was concern about poor compliance if they were required to be tested more frequently.

4.2.2 Exercise protocol

Twelve hours after baseline testing, the subjects in the experimental group completed an EIMD inducing protocol. In brief, participants were asked to resist the lengthening movement of the left biceps (5 sets of 25 movements; see also 'muscle function tests' section for set up of the Biodex (Biodex pro 3, New York, USA)). The resistance to these movements was set on a Biodex dynamometer at 80% of each subject's maximum isometric contraction torque, as this has been shown to induce EIMD in the previous two chapters. The control group did not perform this exercise protocol.

4.2.3 Muscle function tests

The muscle function tests consisted of a MVC measurement and a self-paced submaximal flexion-extension movement. The experimental and the control group performed these muscle function tests.

The MVC was measured using a Biodex dynamometer while performing elbow flexion of the left arm. For this, the participant sat in the chair of the Biodex dynamometer with their upper body and left upper arm securely strapped to the dynamometer, while the left forearm was only able to move in the sagittal plane (flexion-extension). In this position, participants were able to freely flex and extend their elbow over a range of approximately 120°, without hyper-extending the elbow. The rotation axis of the dynamometer was aligned with the lateral epicondyle of the humerus, while the forearm was fixed into a fully supinated position. This ensured that the flexion-extension movement was carried out in the transversal axis and sagittal plane. Participants were asked to perform three 5 second MVC's interspaced by 60 second

recovery periods, as previously described in chapter 2.

The submaximal flexion-extension movements were not performed on the Biodex, but rather the participants were seated on a standard armless-chair. The left arm was relaxed and hanging by their side, while the right arm was resting on the participants lap. For the submaximal self-paced flexion- extension movements all participants wore a 1kg wrist strap and movements were performed in the sagittal plane between elbow angles of 180° and 90°. In addition, the upper body and upper arm were positioned as described in the MVC set-up for standardization purposes. Participants were asked to perform 75 repetitions, which were interspaced by 5–10 second recovery periods with slightly longer rest periods after each 25 repetitions (during which EEG data were not recorded).

4.2.4 Electroencephalographic study procedure

The EEG data were obtained in a darkened, sound-attenuated, temperature controlled room to minimize the effect of confounding factors. Participants were instructed how to perform the self-paced flexion and extension movements. EEG activity was measured during the 75 submaximal, fast self-paced flexion and extension movements. In addition, subjects were asked to keep their eyes open and focused on a fixed spot on the wall during the submaximal self-paced movements.

4.2.5 Electroencephalographic recording

An EEG net with 128 recording sites plus a vertex reference electrode (electrode 129) Electrical Geodesic ™ system (Electrical Geodesics, Inc, Oregon, USA) [32], which measures the electrical activity on the surface of the scalp, was fitted onto each participant (see Figure 4.2 for an electrode layout). The impedance of all electrodes was maintained below 50 kΩ as suggested by the manufacture of the EGI system and different technical references [32, 190, 256] due to the high input resistance of the EEG amplifier. Specially designed amplifiers processed the high impedance signal. EEG was recorded using a 0.1–50 Hz bandpass filter (3 dB attenuation) [256]. The signals were sampled at 250 Hz [32, 256]. All recordings were initially referenced to the central reference electrode (Cz/129) [32, 256]. The EEG system was connected to an experimental workstation (Net Station software, Apple Inc desktop) [32].

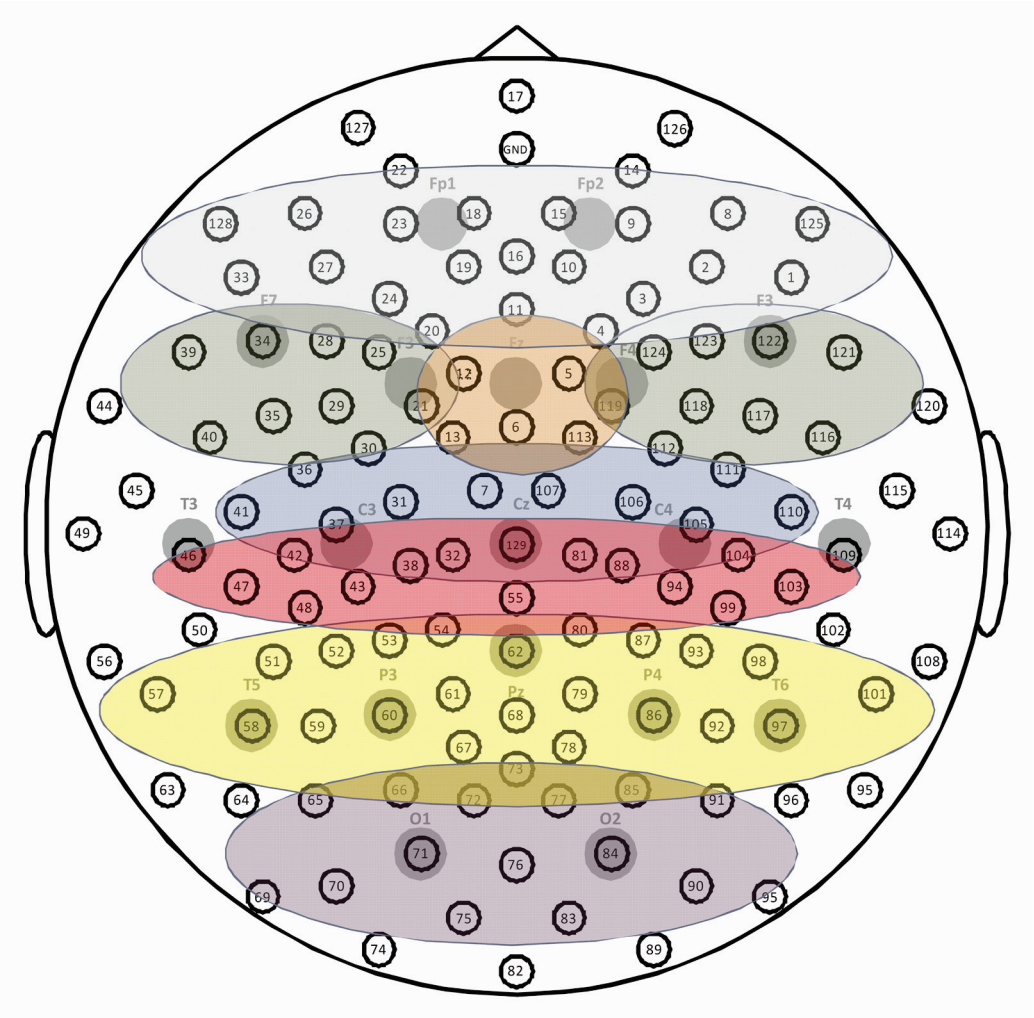

Figure 4.2 A layout of the EGI 129 channel system overlaid by the 10:20 electrode system (dark grey circles). Ellipses represent the following gross cortical areas: grey (Frontal), green (Pre-motor), orange (Supplementary Motor), blue (Motor), red (Somatosensory), yellow (Parietal), purple (Occipital).

4.2.6 Electroencephalographic data analysis

The raw EEG data were 50 Hz notch filtered as well as 1-40 Hz band pass filtered. Following this the data for each trial were segmented into three second epochs [374]. Data were collected continuously during the pre-movement, movement and rest phase and therefore represent the common state of the brain during a biceps brachii movement protocol rather than a movement induced change.

The EEG was re-referenced against an average reference [32, 34, 190], which made data recorded in the reference electrode (Cz/129) available for analysis. The EEG recording was monitored for movement, eye movement, blink artefacts, and noise by an amplitude threshold criterion. Epochs were excluded if the eye blink threshold

exceeded 140 μV and the eye movement threshold exceeded 55 μV. Ocular artefacts were also detected by a slope threshold, if the slope of a channel increased more than 14 $\mu V.ms^{-1}$ the channel was discarded within that epoch [418]. The amplitude of a given channel was excluded in that epoch if it exceeded 150 μV, and in such a case replaced by an estimate calculated of the values of the surrounding channels. This was performed by computer algorithms built into the Net Station software version 4.2.3, (Electrical Geodesics, Inc, Oregon, USA) [256].

After the automated artefact rejection algorithm all epochs were also visually inspected offline and the recordings in which artefacts could not be removed were manually discarded before further data analysis took place.

While shorter epochs might reveal the relationship between, for example, spectral estimates or time-locked events, our intent was to characterize background brain states (induced activity) rather than specific components of event related processing [382]. Subjects who had less than 40 usable, artefact-free epochs per trial day were excluded from further analysis. A FFT with a Welchen window was performed to obtain the spectral information of an epoch of each subject. Hereafter the data were exported to Microsoft Excel© and each subject's data were averaged before further analysis.

The different frequency bands used in this study were as follows: β-1 (13.67–18.55 Hz) and β-2 (19.35–35.16 Hz). Thereafter the relative power (activity) for each frequency on each day was calculated with the following formula: ((Power (12h or 36h or 132h) - Power (-12h)) / Power (-12h))*100. The different relative power values for each subject on the different testing days were used to calculate the statistical differences between the two different groups on the four different testing days.

Matlab 6.5 (The Mathworks Inc., Massachusetts, USA) and EEGlab v 5.02 (SCCN, University of California, San Diego, USA) were used to create topographical maps of the relative power on each day in each frequency.

Recorded data are represented based on the 10:20 system. All electrodes are grouped according to electrode on the 10:20 system which represents the same area. For example the 10:20 electrode Fz is represented by electrodes 5, 6, 11 and 12 in the Netstation system (Figure 4.2).

4.2.7 Other measurements

Blood samples, biceps girth, resting elbow angle and a pain score were measured daily as previously described in chapter 2. For the blood sample 5 ml of blood were drawn from the right antecubital vein. These samples were stored (-20° C) and later analyzed to determine the serum creatine kinase (CK) activity in the blood (Beckman DU-62, Beckman Instruments, Fullerton, California, USA) as described previously [210]. The girth of the left biceps was measured with a tape measure midway between the acromion and radial bony landmarks, that was marked with a permanent marker for repeatability purposes [210]. Resting elbow angles, and by implication the resting length of the biceps muscle were measured with a goniometer [210]. Current pain perception was measured on a daily basis before the muscle function test with the use of a 10 cm visual analogue scale (VAS) [366].

4.2.8 Statistical analysis

An independent t-test was used to compare the descriptive data between experimental and control group, using STATISTICA 8.0 data analysis software (StatSoft, Inc. Tulsa, OK, USA). As some of the data sets in this study had an unequal variance, determined using Levene's test of homogeneity of variance, it was decided to use non parametric statistical tests instead of the parametric ANOVA test. A Kruskal-Wallis test (H) compared the differences between the control and experimental group on each of the testing days in each electrode separately. A Friedman's test (X^2) was used to compare changes within each group over the repeated testing days in each electrode separately. A Dunn's test was used for p*ost-hoc* analysis. Statistical significance was accepted at $p < 0.05$.

4.3 RESULTS

4.3.1 Characteristics of subjects

One participant did not finish the trial and was excluded from the study. Seven other participants were also excluded because they did not have sufficient EEG data epochs for further analysis. The remaining twenty eight participants were divided into two groups similar in weight, height, age, skinfold thickness and handedness (Table 4.1).

Table 4.1: Descriptive data for the control (n = 12) and experimental groups (n = 16). Data are expressed as mean ± SD.

Variable	Control	Experimental
Age (years)	23 ± 4	23 ± 3
Body mass (kg)	71.1 ± 8.8	72.7 ± 11.3
Stature (cm)	171.7 ± 6.8	177.4± 8.0
Body fat (%	15.9 ± 4.9	13.4 ± 5.4
Skinfolds (mm)	79 ± 37	69 ± 38
Handedness (%)	73 ± 20	79 ± 19

4.3.2 Muscle soreness

The difference in pain in the left arm in the experimental and control group measured by the VAS scale is shown in Figure 4.3a. Peak pain in the experimental group occurred 36 hours after the EIMD inducing protocol (X^2 = 53.66, p = 0.0001). A difference in pain between the two groups occurred at 12 (H = 7.48, p = 0.0062), 36 (H = 14.32, p = 0.0002), 60 (H = 10.21, p = 0.0014), 84 (H = 8.03, p = 0.0046) and 108 hours (H = 8.37, p = 0.0038). Significant changes in pain occurred in the experimental group compared to the baseline value at 12, 36, 60 84 and 108 hours (X^2 = 53.66, p = 0.0001) (Figure 4.3a).

4.3.3 Arm circumference

During the experiment there was a significant increase in the difference in girth between the exercised and rested arm in the experimental group compared to the control group at 36 (H = 7.23, p = 0.0072), 60 (H = 6.97, p = 0.0093), 84 (H = 5.36, p = 0.0207) and 108 hours (H = 5.04, p = 0.0248). Significant changes in arm circumference were also found over time in the experimental group compared to the baseline value at 36, 60 and 84 hours (X^2 = 27.04, p = 0.01). The difference between the left and right biceps girth of the control group did not change throughout the experiment (Figure 4.3b).

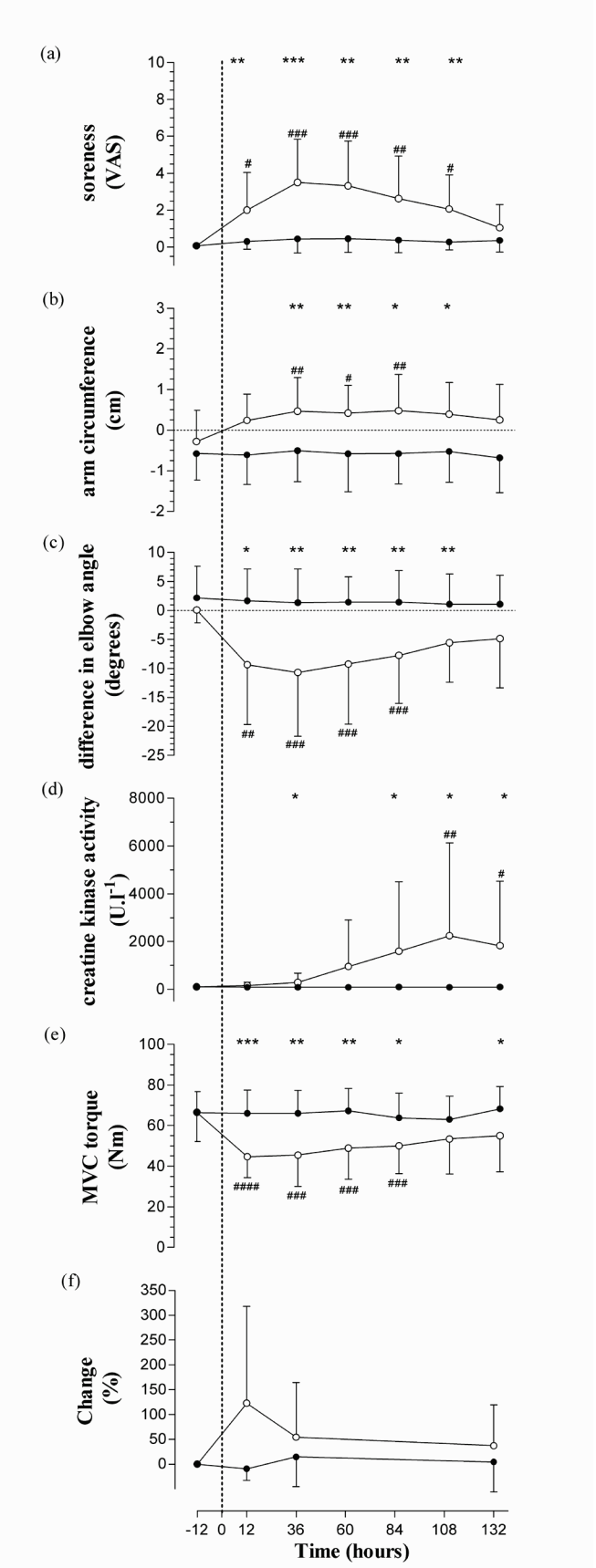

Figure 4.3

a: The change in current pain measured with the VAS scale over sevendays is shown the control (●) and experimental (○) group.

b: The change in the difference in relaxed elbow girth (cm) between the left and right arm of the control (●) and experimental (○) group over seven days.

c: The change in the difference in elbow angle (degrees) between the left and right arm of the control (●) and experimental (○) group over seven days.

d: The change in creatine kinase activity ($U.l^{-1}$) over seven days is shown the control (●) and experimental (○) group.

e: The maximal force output produced on seven consecutive days is shown in the control (●) and experimental (○) group.

f: Percentage change of EMG amplitude produced during a low force isometric contraction with a 1 kg wrist weight normalized to the maximal EMG amplitude measured on four different occasions in the control (●) and the experimental (○) group.

(** indicates results of the Kruskal-Wallis nonparametric test, # indicates results of the Friedman's non parametric test)*

Data are presented as averages with standard deviations.

* $p < 0.05$ control group versus experimental group, ** $p < 0.01$ control versus experimental group, *** $p < 0.001$ control versus experimental group, # $p < 0.05$ post versus pre in the experimental group, ## $p < 0.01$ post versus pre in the experimental group, ### $p < 0.005$ post versus pre in the experimental group, #### $p < 0.001$ post versus pre in the experimental group

4.3.4 Resting elbow joint angle (muscle length)

The difference in resting joint angle between the left and right arms in the control and the experimental group are shown in Figure 4.3c. There was a significant decrease in elbow joint angle in the experimental group until 84 hours after the EIMD inducing protocol (X^2 = 42.46, p = 0.001). The difference in joint angle decreased in the experimental group compared to the control group from 12 hours and reached its minimum 36 hours (H = 7.34, p = 0.0067) after the exercise protocol. It remained decreased until 108 hours (H = 6.71, p = 0.0096) after the exercise protocol. No changes in the resting elbow joint angle over time were observed in the control group (Figure 4.3c).

4.3.5 Serum creatine kinase activity

The serum creatine kinase activity in the experimental group increased at 36 hours (H = 3.90, p = 0.0484) and reached its highest values compared to the control group at 84 (H = 3.99, p = 0.0456), 108 (H = 4.87, p = 0.0274) and 132 hours (H = 5.27, p = 0.0217) after the EIMD inducing protocol. Creatine kinase activity in the experimental group was only significantly increased compared to baseline at 108 and 132 hours (X^2 = 20.27, p = 0.0025). The serum creatine kinase activity in the control group did not change during the experiment (Figure 4.3d).

4.3.6 Muscle function

Muscle function, as measured by MVC (Figure 4.3e), decreased significantly in the experimental group compared to the control group on all but one visit (108 hours) to the laboratory after the EIMD inducing protocol (p < 0.05). The largest decrease in maximal force output was observed within the first twelve hours after the EIMD protocol in the experimental group (H = 14.14, p = 0.0002) while there were no changes in the control group throughout the experiment. The force output in the experimental group remained different to that of the control group on all days but one day until the end of the trial (132 hours; H = 5.61, p = 0.0179) (Figure 4.3e).

A difference was also observed in the force output of the experimental group over time at 12, 36, 60 and 84 hours compared to the baseline measurement (X^2 = 48.3, p = 0.0001). There were no changes in the control group over time.

4.3.7 EMG activity

The electromyographic activity during a submaximal isometric low force contraction is shown in Figure 4.3f. The data are displayed as normalized to the EMG during the maximal force output and as a percentage change compared to twelve hours pre-EIMD protocol. There was a tendency towards a significant difference between the groups at twelve hours ($H = 3.81$, $p < 0.051$).

4.3.8 EEG results

4.3.8.1 Beta-1

Beta-1 activity was different between the experimental and control group in the frontal, central and parietal area at twelve hours post EIMD. The central differences are represented stronger on the ipsilateral compared to the contralateral side. These differences can still be seen 36 hours after the EIMD inducing protocol but were not as widespread and mainly focused in the contralateral frontal, ipsilateral central and contralateral parietal areas. Differences persisted 132 hours post-EIMD induction in the contralateral frontal and the parietal areas (Figures 4.4 and 4.5). To simplify the understanding of the results, electrodes have been placed into subgroups and labelled with the title of the closest electrode represented on the 10:20 system.

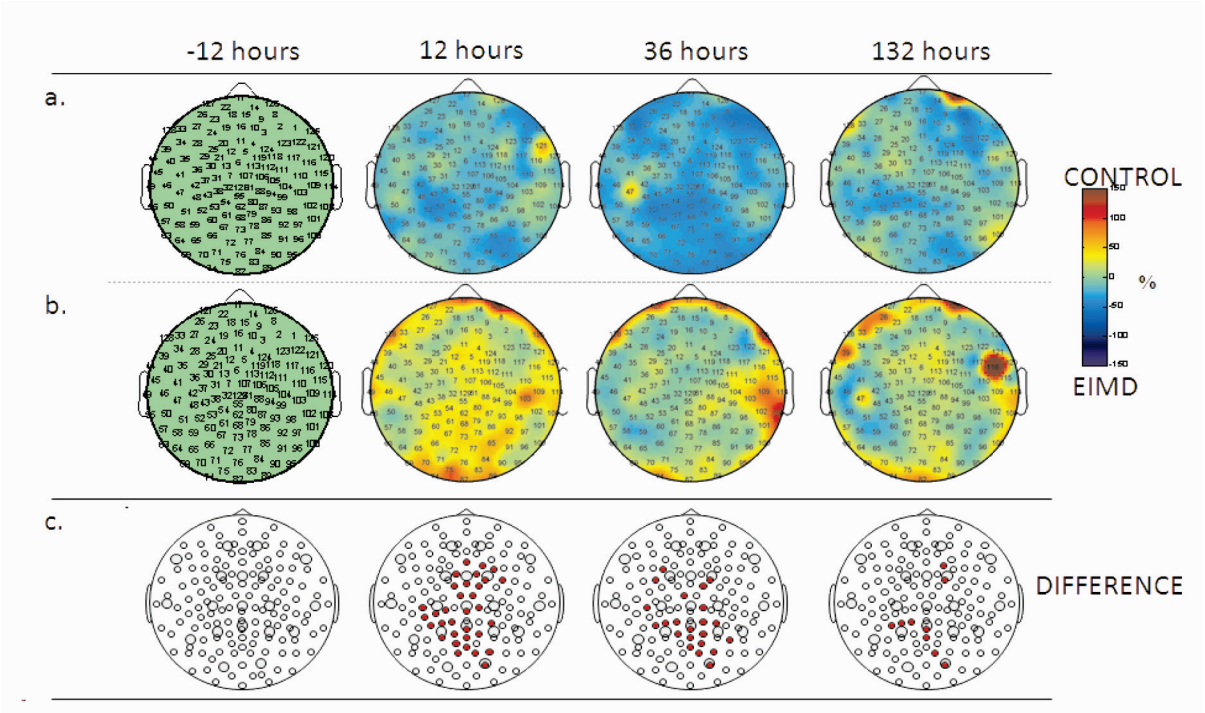

Figure 4.4 The global change (%) of β-1 activity measured with 129 electrodes over the scalp is shown in the control (a) and experimental (b) group. An outline of the electrodes showing significant differences between the two groups (c) at each time point is also shown.

4.3.8.1.1 *Twelve hours*

At twelve hours post-EIMD induction the differences between the control and experimental group were widespread over the frontal, ispilateral central and parietal areas (Figure 4.4 and 4.5) of the cortex. In particular the changes recorded in the frontal electrodes were widespread and recorded over the supplementary and pre-motor areas.

Fz

Differences between groups were very pronounced in the electrodes surrounding the Fz area. The electrodes showing significant differences caused by an increase in activity in the experimental group were electrodes 4 ($H = 5.39$, $p = 0.0203$), 5 ($H = 6.52$, $p = 0.0107$), 6 ($H = 7.25$, $p = 0.0071$), 11 ($H = 5.61$, $p = 0.0179$), 12 ($H = 4.56$, $p = 0.0327$) and 13 ($H = 4.36$, $p = 0.0367$), all directly surrounding the Fz area and therefore representing electrical activity changes above the supplementary motor area.

F4

Differences between groups are also seen in the in the contralateral frontal area in electrodes 113 ($H = 8.02$, $p = 0.0046$), 118 ($H = 4.76$, $p = 0.0291$), 119 ($H = 7.76$, $p = 0.0053$) and 124 ($H = 5.61$, $p = 0.0179$), which overlay the contralateral pre-motor area.

C3

Differences in the ipsilateral central area occurred in electrodes 38 ($H = 4.56$, $p = 0.0327$), 42 ($H = 6.05$, $p = 0.0139$), 43 ($H = 4.76$, $p = 0.0291$) and 48 ($H = 3.99$, $p = 0.0459$). These electrodes overlay the motor and somatosensory areas and differences between groups are caused by a decrease in activity in the control group rather than an increase in activity in the experimental group.

Cz

The electrodes covering the vertex of the head, such as electrodes 7 ($H = 6.05$, $p = 0.0139$), 81 ($H = 5.61$, $p = 0.0179$), 107 ($H = 7.00$, $p = 0.0081$) and 129 ($H = 4.76$, $p = 0.0291$) also showed significant differences between the groups at twelve hours after the EIMD protocol.

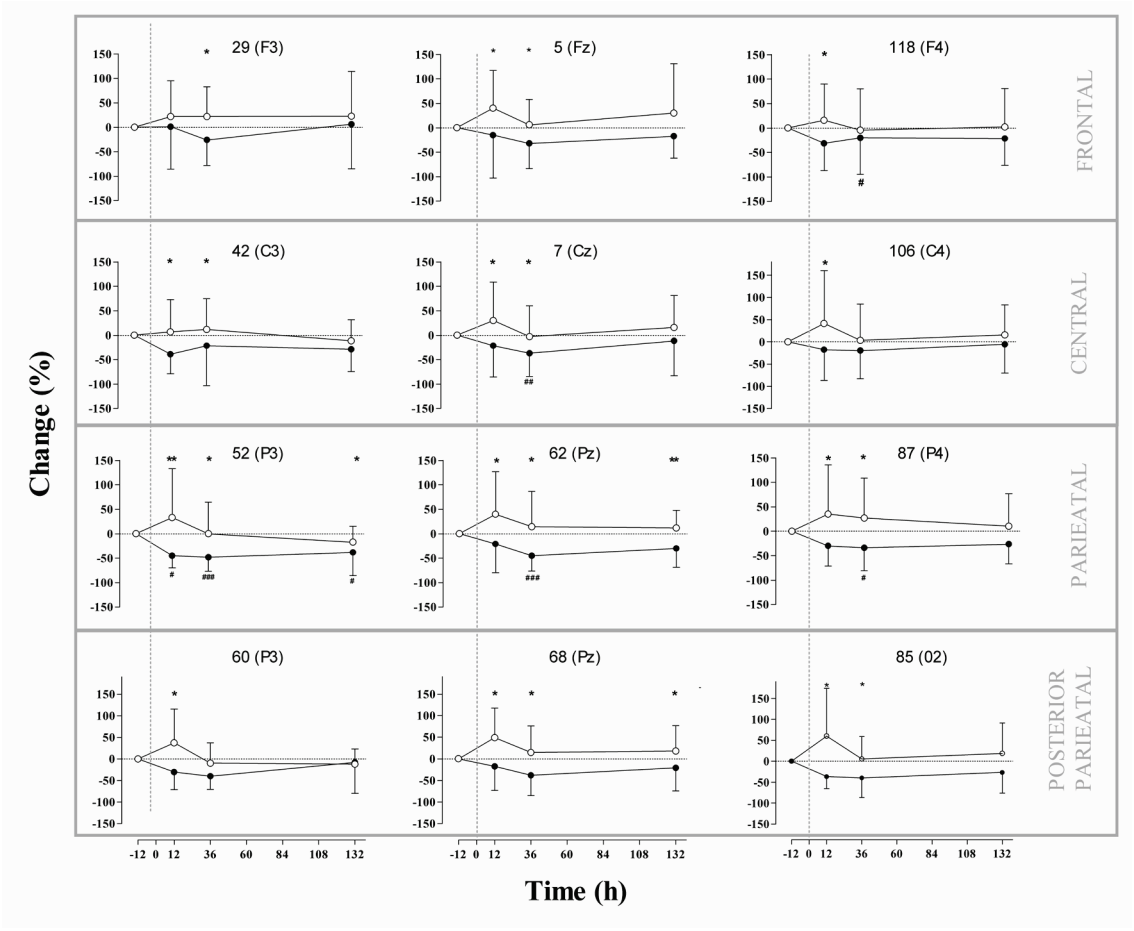

Figure 4.5 Twelve different electrodes representative of the change (%) of β-1 activity in the frontal, central and parietal areas of the brain in the control (●) and experimental (○) group. Each graph is labelled with the corresponding electrode number. The vertical dotted line marks the time of the EIMD inducing protocol in the experimental subjects.

(indicates results of the Kruskal-Wallis nonparametric test, # indicates results of the Friedman's non parametric test)*
** p < 0.05 control group versus experimental group, ** p < 0.01 control versus experimental group*
 # p < 0.05 at 12 and 36 hours post versus pre in the control group, ## p < 0.01 at 12 hours post versus pre in the control group,### p < 0.001 at 36 hours post versus pre in the control group

P3

Differences in activity between the groups also occurred in the ipsilateral parietal area seen in electrodes 52 (H = 7.76, p = 0.0053), 53 (H = 8.55, p = 0.0034), 60 (H = 6.52, p = 0.0107), 61 (H = 6.05, p = 0.0139) and 67 (H = 4.17, p = 0.0411).

Pz

Differences were further observed in electrodes surrounding the parietal area represented by Pz. Electrodes 62 (H = 4.56, p = 0.0327), 54 (H = 4.17, p = 0.0411) and 68 (H = 5.61, p = 0.0179) showed significant differences between groups.

P4

Differences between groups also occurred in electrodes on the contralateral side to the movement in electrodes 87 (H = 4.76, *p* = 0.0291) and 79 (H = 3.99, *p* = 0.0459).

O1 and O2

There were significant differences in the occipital region at electrodes 72 (H = 4.76, *p* = 0.0291), 73 (H = 5.83, *p* = 0.0158) representing O1 as well as 77 (H = 6.05, *p* = 0.0139), 84 (H = 9.67, *p* = 0.0019), 85 (H = 10.86, *p* = 0.0010) representing O2.

Differences over time in the control group

P3

A decrease in the β-1 activity was also observed in the ipsilateral parietal area of the control group compared to the baseline measurement in electrodes 48 (X^2 = 13.2, *p* = 0.05), 52 (X^2 = 16.3, *p* = 0.05), 53 (X^2 = 13.5, *p* = 0.05), 54 (X^2 = 13.1, *p* = 0.05) and 61 (X^2 = 12.7, *p* = 0.05).

Differences over time in the experimental group

There were no changes in the experimental group over time.

4.3.8.1.2 *Thirty-six hours*

F3 and F4

At 36 hours after the exercise protocol significant changes between the experimental and control group were more localized and mainly found in the ipsilateral (F3) electrodes 25 (H = 4.36, *p* = 0.0367) and 29 (H = 4.36, *p* = 0.0367) and over the contralateral pre-motor area (F4) in electrodes 113 (H = 4.56, *p* = 0.0327) and 119 (H = 4.17, *p* = 0.0411).

Cz and C3

Significant differences were also found around the vertex 7 (H = 4.56, *p* = 0.0327) and 81 (H = 4.17, *p* = 0.0411) and also in electrode 42 (H = 4.97, *p* = 0.0259) representing C3.

P3 and Pz

Differences between groups occurred in the ipsilateral parietal area (P3) at electrodes 52 (H = 5.61 *p* = 0.0179), 53 (H = 5.39, *p* = 0.0203) and 61 (H = 5.61, *p* = 0.0179) as well as the central parietal area (Pz) at electrodes 54 (H = 6.28, *p* = 0.0122), 62 (H = 6.52, *p* = 0.0107), 68 (H = 5.83, *p* = 0.0158), 73 (H = 5.83, *p* = 0.0158) and 80 (H =

6.52, p = 0.0107).

P4

In the contralateral parietal electrodes 78 (H = 3.99, p = 0.0459) and 79 (H = 5.83, p = 0.0158), 87 (H = 5.39 p = 0.0203) and 92 (H = 6.76 p = 0.00093) showed significant differences between groups.

O2

Significant differences occurred in the occipital region in electrodes 77 (H = 4.76 p = 0.0291), 84 (H = 4.36, p = 0.0367) and 85 (H = 5.61, p = 0.0179).

Differences over time in the control group

F4

The control group also showed a significant decrease compared to baseline in the contralateral frontal area in electrodes 113 (X^2 = 8.5, p = 0.05), 117 (X^2 = 9.7, p = 0.05), 119 (X^2 = 11.8, p = 0.01) and 123 (X^2 = 10, p = 0.05).

C3

Changes over time occurred in the ipsilateral central area of the control group in electrode 48 (X^2 = 13.2, p = 0.05).

Cz

Electrodes 7 (X^2 = 10.1, p = 0.05), 107 (X^2 = 8.5, p = 0.05) and 129 (X^2 = 9.3, p = 0.05) overlying the vertex area showed significant differences over time between the baseline value and 36 hours after the EIMD protocol.

P3

Decreases in activity in the control group were also recorded in electrodes overlying the ipsilateral parietal area. These were electrodes 51 (X^2 = 7.9, p = 0.05), 52 (X^2 = 16.3, p = 0.001), 53 (X^2 = 13.1, p = 0.05) and 61 (X^2 = 12.7, p = 0.01).

Pz

Decreases in activity compared to baseline also occurred in electrodes 54 (X^2 = 13.1, p = 0.05), 55 (X^2 = 12.4, p = 0.01), 62 (X^2 = 15, p = 0.001) and 80 (X^2 = 9.3, p = 0.05), overlying the medial parietal area of the control group.

P4

Decreases in activity in the control group spread into the contralateral parietal area and were detected in electrodes 77 (X^2= 10.3, p = 0.05), 79 (X^2 = 10.1, p = 0.05), 81(X^2 = 9.0, p = 0.05) and 87 (X^2 = 7.9, p = 0.05).

Differences over time in the experimental group

There were no changes in the experimental group over time.

4.3.8.1.3 *One-hundred and thirty-two hours*

F4

At 132 hours after the exercise protocol significant changes in β-1 between the experimental and control group were more localized and only found in two electrodes overlying the contralateral pre-motor cortex in electrodes 4 (H = 4.76, p = 0.0291) and 119 (H = 3.99, p = 0.0459).

P3 and Pz

The parietal area shows significant differences between the groups at electrodes representing P3 and Pz. Changes in the P3 area were observed in electrodes 52 (H = 5.18, p = 0.0229), 53 (H = 7.75, p = 0.0053) and electrodes 54 (H = 6.29, p = 0.0122), 62 (H = 7.75, p = 0.0053) and 68 (H = 4.36, p = 0.0367) which represent changes in the Pz area.

O2

The occipital region also shows significant differences around the O2 area at electrodes 77 (H = 4.36, p = 0.0367) and 84 (H = 5.83, p = 0.0158).

Differences over time in the control group

C3, P3 and Pz

A difference was also observed in the β-1 activity of the control group over time compared to the baseline measurement in electrodes representing the area around C3, P3 and Pz, these are electrodes 48 (X^2 = 13.2, p = 0.05), 52 (X^2 = 16.3, p = 0.01), 53 (X^2 = 13.5, p = 0.05) and 54 (X^2 = 13.1, p = 0.05).

O2

Significant decreases were also seen in electrode 77 (X^2 = 10.3, p = 0.05) in the control group.

Differences over time in the experimental group

No differences occurred in the experimental group over time.

4.3.8.2 Beta 2

Differences between the control and experimental group in the β-2 activity at twelve hours were represented in slightly different areas than β-1 activity. The contralateral pre-motor area (F4) showed no differences between groups in the β-2 activity. Differences did occur between the two groups in the ipsilateral pre-motor (F3), supplementary motor (Fz), motor (C3, Cz, C4) and parietal areas (P3, Pz).

While no differences in β activity occurred between the experimental and control group on the contralateral side, differences on the ipsilateral side are visible at twelve hours.

At 36 hours differences are only seen in the ipsilateral central and parietal areas and at 132 hours changes have attenuated (besides one significant difference in an ipsilateral frontal electrode) (Figures 4.6 and 4.7).

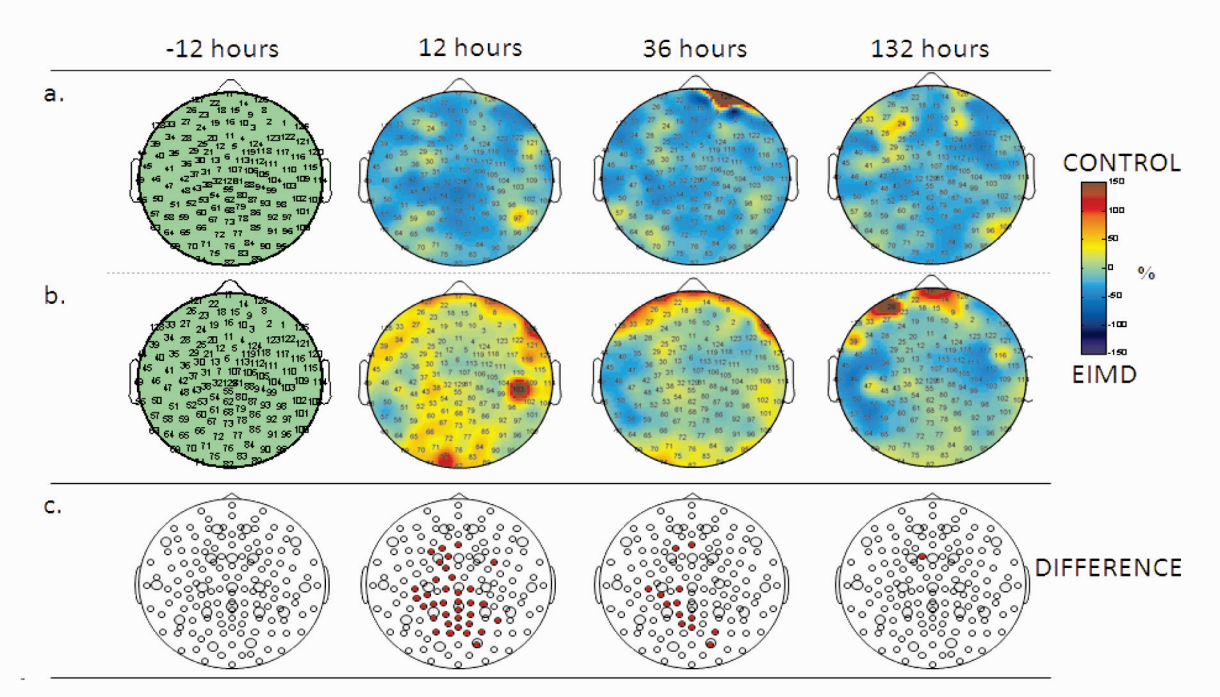

Figure 4.6 The global change (%) of β-2 activity measured with 129 electrodes over the scalp is shown in the control (a) and experimental (b) group. An outline of the electrodes showing significant differences between the two groups (c) at each time point is also shown.

4.3.8.2.1 *Twelve hours*

At twelve hours after the EIMD protocol the differences between control and experimental group were widespread from the vertex to the occipital area of the head and to a smaller extend in the ipsilateral frontal area.

Fz

Significant differences between the experimental and control group occurred in the frontal area electrodes 11 (H = 5.61, p = 0.0179), 12 (H = 6.05, p = 0.0139), 13 (H = 5.61, p = 0.0179), 20 (H = 5.39, p = 0.0203), 21 (H = 4.56, p = 0.0327), 25 (H = 4.36, p = 0.0367) and 118 (H = 3.99, p = 0.0459).

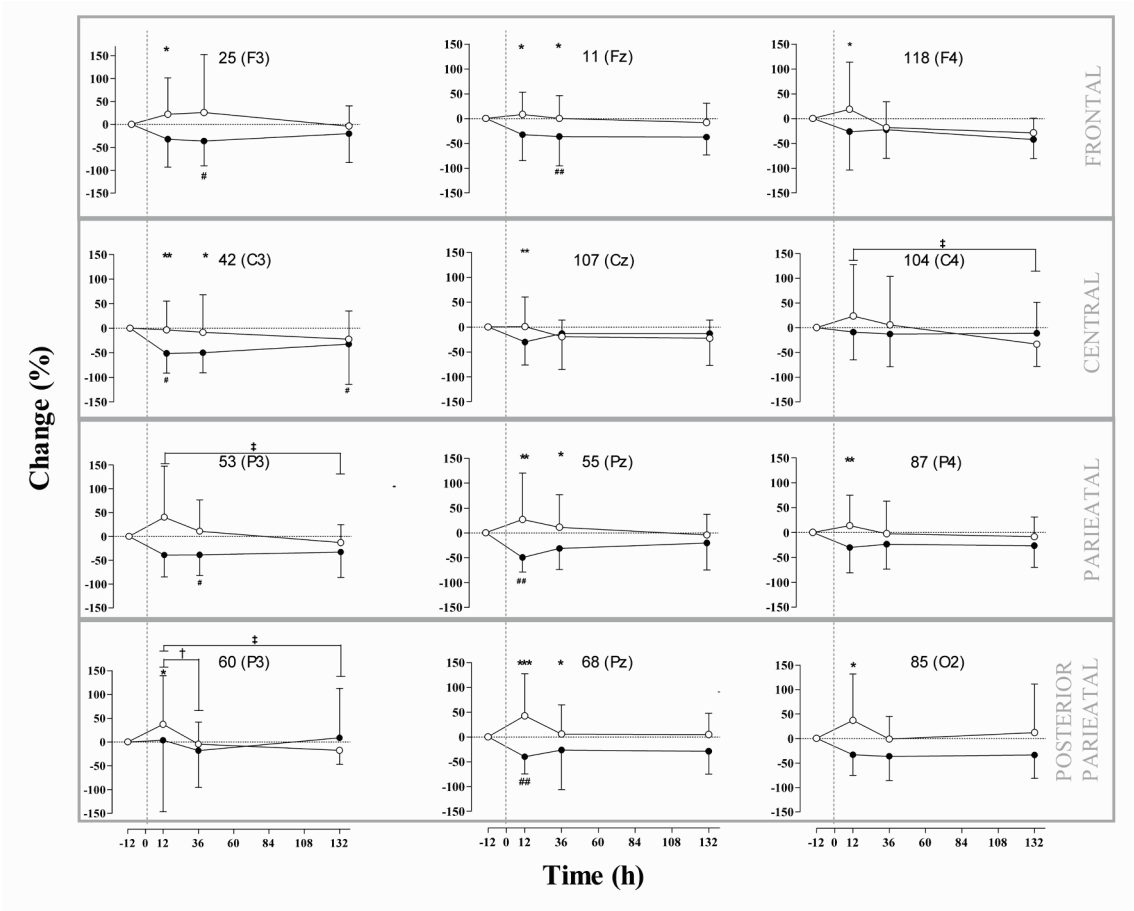

Figure 4.7 Twelve different electrodes representative of the change (%) of β-2 activity in the frontal, central and parietal areas of the brain in the control (●) and experimental (○) group. Each graph is labeled with the corresponding electrode number. The vertical dotted line marks the time of the EIMD inducing protocol in the experimental subjects.

(* indicates results of the Kruskal-Wallis nonparametric test, #, †, ‡ indicate results of the Friedman's non parametric test)
** p < 0.05 control group versus experimental group, ** p < 0.01 control versus experimental group, *** p < 0.001 control versus experimental group. # p < 0.0512 and 36 hours post versus pre in the control group, ## p < 0.01 at 12and 36 hours post versus pre in the control group.† p < 0.05 at 36 versus 12 hours post in the experimental group, ‡ p < 0.05 at 132 versus 12 hours post in the experimental group*

C3

In the ipsilateral motor area electrodes 31 (H = 5.39, *p* = 0.0203), 32 (H = 5.39, *p* = 0.0203), 42 (H = 7.50, *p* = 0.0062), 43 (H = 4.97, *p* = 0.0259) and 48 (H = 3.99, *p* = 0.0459) differences occurred between the experimental and control groups at twelve hours after the induction of EIMD.

Cz

Over the vertex area of the head differences between the groups were seen in electrodes 7 (H = 4.17, *p* = 0.0411), 81 (H = 7.00, *p* = 0.0081) and 129 (H = 4.76, *p* = 0.0291).

P3

In the ipsilateral parietal area the electrodes 52 (H = 7.50, *p* = 0.0062), 53 (H = 7.50, *p* = 0.0062), 60 (H = 4.17, *p* = 0.0411) and 61 (H = 7.50, *p* = 0.0062) showed significant differences.

Pz

Differences occurred in electrodes 54 (H = 8.55, *p* = 0.0158), 55 (H = 8.83, *p* = 0.0030), 62 (H = 11.17, *p* = 0.0008), 68 (H = 10.86, *p* = 0.0010), 73 (H = 9.39, *p* = 0.0022) and 80 (H = 7.00, *p* = 0.0081) representing the parietal area.

P4

Electrodes 78 (H = 5.83, *p* = 0.0158), 79 (H = 9.11, *p* = 0.0025), 87 (H = 7.25, *p* = 0.0071) and 92 (H = 4.76, *p* = 0.0291) were significantly different between the experimental and control group.

O1

Electrodes 66 (H = 5.61, *p* = 0.0179), 67 (H = 8.28, *p* = 0.040), 72 (H = 4.76, *p* = 0.0291) show significant differences between the groups in the ipsilateral occipital area.

O2

Significant differences also occurred in the contralateral occipital area in electrodes 77 (H = 5.83, *p* = 0.0158), 84 (H = 5.61, *p* = 0.0179) and 85 (H = 5.39, *p* = 0.0203).

Differences over time in the control group

Differences were observed in the β-2 activity of the control group over time compared to the baseline measurement at twelve hours post –EIMD induction. These mainly occurred in the areas of F3, Cz and Pz.

F3

Electrodes 20 (X^2 = 11.8, p = 0.01) and 21 (X^2 = 8.7, p = 0.05) overlying the pre-motor area showed significant decreases between the baseline and twelve hour post measurement in the control group.

C3

A significant decrease also occurred in electrode 42 (X^2 = 11.5, p = 0.05) overlying the ipsilateral motor area.

Cz

Significant decreases occurred in electrodes 7 (X^2 = 9.9, p = 0.05) and 81 (X^2 = 8.7, p = 0.05) representing the vertex of the head.

Pz and P4

Electrodes 62 (X^2 = 13.5, p = 0.05), 68 (X^2 = 12.2, p = 0.01), 73 (X^2 = 13.3, p = 0.05), 79 (X^2 = 13.0, p = 0.01) located over the parietal area all recorded significant decreases compared to baseline in the control group.

O2

Electrode 77 (X^2 = 11.5, p = 0.05) showed significant decreases in the control group.

Differences over time in the experimental group

No differences were seen in the experimental group over time.

4.3.8.2.2 *Thirty-six hours*

Fz

At 36 hours after the EIMD protocol the only difference in activity between groups in the frontal area occurred in electrode 11 (H = 6.28, p = 0.0122) and 20 (H = 4.36, p = 0.0367).

C3

Differences between the two groups were found in the ipsilateral motor area in

electrodes 32 (H = 5.18, *p* = 0.0229), 42 (H = 5.18, *p* = 0.0229) and 43 (H = 3.99, *p* = 0.0459).

P3

In the ipsilateral parietal area electrodes 53 (H = 5.83, *p* = 0.0158), 61 (H = 4.97, *p* = 0.0259) and 67 (H = 4.76, *p* = 0.0291) displayed differences between the groups.

Pz

The area around Pz at electrodes 54 (H = 7.50, *p* = 0.0062), 68 (H = 4.17, *p* = 0.0411) and 73 (H = 4.76, *p* = 0.0291) showed significant differences between the control and experimental group at 36 hours after the induction of EIMD.

O2

Differences between groups occurred in electrodes 84 (H = 3.99, *p* = 0.0459) and 85 (H = 3.99, *p* = 0.0459) overlying the occipital area.

Differences over time in the control group

Fz and F3

Decreases were observed in the frontal β-2 activity of the control group compared to the baseline measurement in electrodes 5 (X^2 = 8.7, *p* = 0.05), 11 (X^2 = 10.8, *p* = 0.01) and 12 (X^2 = 8.7, *p* = 0.05) all representing (Fz) as well as electrode 25 (X^2 = 7.9, *p* = 0.05) representing F3.

C3 and P3

The Ipsilateral central and parietal area each had one electrode in which a significant decrease occurred over time; these were electrodes 48 (C3) (X^2 = 8.4, *p* = 0.05) and 52 (P3) (X^2 = 8.7, *p* = 0.05).

Pz

Decreases, compared to baseline, occurred in electrodes 54 (X^2 = 10.1, *p* = 0.05), 55 (X^2 = 13.5, *p* = 0.01) and 68 (X^2 = 12.2, *p* = 0.05) in the medial parietal area.

Differences over time in the experimental group

T3

A difference was observed in the β-2 activity of the EIMD group over time compared to the baseline measurement in electrode 41 (X^2 = 15.23, *p* = 0.05).

4.3.8.2.3 *One hundred and thirty-two hours*

Fz

At 132 hours the only differences between groups was in the frontal electrode 12 (H = 3.99, $p = 0.0459$).

Differences over time in the control group

Differences were observed in the control group over time compared to the baseline measurement in electrodes along an ipsilateral anterior- posterior axis. In electrodes 35 (F3) ($X^2 = 9.0$, $p = 0.05$), 42 (C3) ($X^2 = 11.5$, $p = 0.05$), 53 (P3) ($X^2 = 11.8$, $p = 0.01$) and 73 (Pz) ($X^2 = 13.3$, $p = 0.05$).

Differences over time in the experimental group

Fz and F4

In the frontal area of the experimental group decreases occurred in electrodes 6 (Fz) ($X^2 = 11.1$, $p = 0.01$) and 117 (F4) ($X^2 = 10.13$, $p = 0.05$).

T3 and T5

Difference were observed in the β-2 activity of the experimental group over time compared to the baseline measurement in electrodes running along an anterior-posterior line in the ipsilateral temporal area 35 (F3) ($X^2 = 10.35$, $p = 0.05$), 41 (T3) ($X^2 = 15.23$, $p = 0.01$), 51 (T5) ($X^2 = 12.15$, $p = 0.01$), 59 (T5) ($X^2 = 9.25$, $p = 0.05$).

P3

Differences in the EIMD group were also seen in electrode 52 between 12 and 132 hours ($X^2 = 10.35$, $p = 0.05$).

As well as in electrode 60 between 12 and 36 hours ($X^2 = 15.23$, $p = 0.05$) and between 12 and 132 hours ($X^2 = 15.23$, $p = 0.01$).

C4

Differences occurred in electrode 104 ($X^2 = 10.28$, $p = 0.01637$) in the experimental group at 12 and 132 hours while experiencing the symptoms of EIMD.

4.4 DISCUSSION

Typical symptoms of EIMD were experienced by the experimental group (Figure 4.3). These included the symptoms of pain as well as decreased neuromuscular function as measured by force output (Figure 4.3).

The study also showed a trend in increased submaximal EMG activity in the biceps brachii in the first twelve hours (p = 0.051) after the induction of EIMD (Figure 4.3f). This is in agreement with chapter 2 which found submaximal EMG increased twelve hours after the induction of EIMD with the same protocol and a smaller cohort (25 participants of the 37). In the same chapter we could show that a submaximal flexion-extension movement lead to increased EMG activity until 132 hours after the EIMD-inducing protocol.

It has been shown that EIMD, as well as muscular pain in the biceps brachii, not only leads to altered proprioception and motor-perception but also changes in neuromuscular function and motor recruitment [301]. Several studies [62, 300, 339, 398] have previously shown that movement steadiness and force output, both associated with motion perception and proprioception, are difficult to maintain during low force contractions whilst experiencing symptoms of EIMD [339], as observed in this study.

The novel finding of this study was the significant changes in cortical β activity which occurred for up to 132 hours whilst experiencing symptoms of EIMD. Due to the β frequency range (from 13.67 to 35.16 Hz) overlapping with the α-2 frequency in the lower range and γ frequency in the upper range, we divided β into two different frequency bands, β-1 (13.67 to 18.55 Hz) and β-2 (19.35 to 35.16 Hz) activity respectively, for analysis and discussion.

4.4.1 Beta 1

The most pertinent β-1 finding was that differences between the experimental and control groups peaked at twelve hours after the induction of EIMD, these were predominantly evident in the frontal and parietal areas of the cortex (Figures 4.4 and 4.5). Interestingly β-1 activity peaked at twelve hours post-EIMD in the experimental group. This was evident in the ipsilateral frontal and temporal areas as well as the entire central area. These differences were only seen at 12 and 36 hours after the

induction of EIMD, and not at 132 hours post. However, in the contralateral frontal and the entire parietal area these changes were visible over the entire duration of the trial (132 hours post-EIMD) (Figure 4.4 and 4.5).

The increase in β-1 activity in the cortical areas, together with the above-mentioned similar changes in EMG activation during the same time period, suggest a link between the EEG and EMG activity patterns. Changes in the frontal and parietal cortical areas are often associated with movement perception, proprioception and movement integration [5, 208, 249, 386]. These concurrent changes in cortical and neuromuscular measurements indicate a top–down regulatory mechanism [119, 152, 175, 250, 256, 257, 285], where cortical β activity regulates neuromuscular recruitment whilst the symptoms of EIMD are experienced.

This hypothesis is supported by previous studies which have shown that a coherent firing of the motor cortex and peripheral motor units in a 15 Hz activity range is evident, especially during steady state contractions [64, 83, 199]. These studies suggest a regulating interaction between the motor cortex and the motor units innervated. However, further substantiation by means of a cortico-muscular coherence study, would help determine if this is indeed a predominantly top-down regulatory mechanism between β-1 activity and motor units during biceps brachii contractions.

Interestingly, these changes in neuromuscular function, seen as changes in EMG activity and force output, could also act as a bottom up control mechanism which would lead to a compensatory increase in β-1 activity in the associated frontal and parietal areas seen in our study [5, 208, 249, 386]. This hypothesis is supported by previous studies which have not only shown cortico-motor coherence in the β frequency range [13, 157], but also that β-1 activity measured in the frontal, somatosensory and parietal area is associated with bottom up sensory signalling from the peripheral motor system [22, 209, 344] to the brain. As the β-1 activity links afferent sensory information to motor planning, it has been suggested that this networking aids the decision making process before the execution of movement [48, 107, 116, 209, 257, 344, 344, 394].

These studies support the findings of our experimental group (EIMD) which had increased β-1 activity in the parietal and frontal areas. Therefore the findings of this study also lead us to suggest that increased β-1 activity is associated with a bottom up feedback of sensory information (proprioception, altered neuromuscular function, motor perception, movement unsteadiness and force output) to cortical areas associated with

motor planning and movement execution.

Hence we propose that an increase in β-1 activity in the parietal area during brisk biceps brachii contractions is due to altered bottom-up input as a result of the disturbed neuromuscular function (increased submaximal EMG and decreased maximal EMG) and decreased force output associated with EIMD. This increased β-1 activity in the parietal area may be involved in the integration of neuromuscular information movement learning and planning in the frontal and prefrontal areas as well as an top-down feed-forward mechanism to the periphery.
Interestingly at 132 hours post-EIMD differences between the groups can still be seen in frontal and parietal β-1 activity as well as in force output and serum creatine kinase activity, while all others markers of EIMD have already returned to baseline values. This strongly suggests that neuromuscular function (bottom-up signal) and movement planning (top-down) are still altered between the control and experimental group at 132 hours. Therefore we propose that there could be a modification in the upstream regulator integrating movement perception and proprioception [47, 300] whilst experiencing the symptoms of EIMD.

4.4.2 Beta 2

In contrast to β-1 activity, β-2 activity differences are marked between the groups at 12 and 36 hours but not at 132 hours after the exercise protocol. This difference is mainly seen in the frontal and parietal area and is due to a concomitant increase in activity in the experimental group and decrease in activity in the control group.

This is the first study to show decreases in β-2 activity in the control group during repeated trials over 132 hours. This decrease was most pronounced at 12 and 36 hours after the exercise protocol and was seen globally except for the contralateral motor area (Figure 4.7; electrode 104).

During testing session two (12 hours) the decrease in β-2 activity was most notable in the parietal area whilst the most prominent decrease during testing session three (36 hours) was observed in the frontal area. This is unlikely to be explained by circadian rhythm as both groups responded differently at the same time points.

It is known that an increased β activity is related to maintaining the *status quo* of a

movement whilst new information is being integrated [116]. A decrease in β activity, as seen in the control group and the contralateral motor area of the experimental group (Figure 4.7; electrode 104), is possibly a learning or familiarization response to repeated tasks. In this study the testing protocol was repeated over several days and the participants became familiarized with the voluntary movements as the experiment progressed. This is further supported by the decrease in β-2 activity in the frontal and parietal areas; both are brain areas associated with motor learning, attention and anticipation [5, 208, 249, 386].

In the experimental group it was found that β-2 activity did not decrease but rather increased within the first twelve hours, suggesting that familiarization may have been delayed while EIMD symptoms were peaking. Therefore it seems that the EIMD protocol lead to changes in either afferent signals influencing β-2 activity or in efferent β-2 activity signalling to the muscle [22, 208, 209, 217, 359].

This increase in β-2 activity was most pronounced in the parietal and somatosensory area (Figure 4.6 and 4.7) as shown by Rougeul *et al.* [313]. Previous animal models have shown this increased activity is due to increased synchrony in the neuronal firing [363], which is known to be augmented by focused attention as well as anticipation [12, 13, 68, 314, 363]. Previous research has also shown that the inferior parietal area is activated during the experience of muscle pain and is associated with the localization of the painful stimulus [205, 367]. Therefore β-2 activity in this current study appears to be due to anticipation of movement or pain on the focused attention.

This is supported by studies by Chang *et al.* and Worthen *et al.*, which showed that β-2 activity is positively correlated to pain intensity, especially muscle pain [68, 411]. Our study also noted an increased β-2 activity in the experimental group while they experienced muscular pain associated with EIMD. Therefore the increased β-2 activity in our study could be due to the recognition or anticipation of pain (Figure 4.6 and 4.7) induced by the EIMD protocol. This is supported by non-significant differences in β-2 activity between the groups at 132 hours where there were no differences in either pain, swelling or changes in elbow angle as these had returned to pre-EIMD values by 132 hours.

In summary we found that β-2 activity in the control group decreased over time due to familiarization of the testing protocol. In contrast, this familiarization appeared delayed in the experimental group reflected by an initial increase in β-2 activity. It may be

surmised from this that the β-2 activity increases due to the cognitive evaluation of, and attention to, both pain and neuromuscular changes caused by EIMD. This is supported by previous research that has shown that β-2 activity appears to be a common mode of long-range communication in cognitive networks [203, 204]. Therefore we conclude that β-2 activity may be associated with the anticipation of pain induced by movement and the neuromuscular changes associated with EIMD.

4.4.3 Limitations

This novel study, conducted over 132 hours, explored the relationship between pain induced by an EIMD protocol and β-1 and β-2 activity measured by EEG. Although our study investigated changes in induced β activity, rather than event related β activity, the aim was to investigate the influence of pain and neuromuscular changes induced EIMD on β activity over the entirety of a movement task. Also our data were not normalised to baseline but rather compared to pre-EIMD protocol values to identify percentage changes in β activity post- versus pre-EIMD. We also measured EMG and EEG during the same testing protocol but not at similar time points and therefore correlations and coherences can only be postulated and further research is needed to confirm our hypothesis.

4.4.4 Conclusion

In conclusion this novel study showed an increased β-1 activity in the parietal area, possibly due to a bottom-up and top-down interaction to integrate information about disturbed neuromuscular function, decreased force output, increased submaximal and decreased maximal EMG. These data suggest that there could be a change in activity in the upstream regulator integrating movement perception and proprioception [47, 300] after a bout of exercise as a result of EIMD. Additionally our data suggest that an increased β-2 activity might be associated with the anticipation of pain induced by movement and the cognitive evaluation of neuromuscular changes associated with EIMD.

Further motor-cortical coherence research is required to elucidate, firstly the association between β-1 activity and motor perception and proprioception, and secondly β-2 activity and the anticipation of pain and changes in neuromuscular functioning (loss of force, stiffness and changes in EMG activation) while experiencing the symptoms of EIMD.

CHAPTER 5
SUMMARY AND CONCLUSIONS

5.1 OVERVIEW

Exercise–induced muscle damage is experienced by many athletes after periods of long inactivity or after strenuous unaccustomed exercise. It has been documented that EIMD leads to a disruption of peripheral homeostasis. The understanding thereof has been a topic of continuous research over the last few decades, including research on micro- and macroscopic tissue damage, the delayed pain response, clinical symptoms and gross recovery of the muscle. However, the dissociation of the clinical symptoms from the perception of pain and changes in neuromuscular function associated with EIMD has not been well reported. Further no convincing explanations for the association between the delayed, and over exaggerated pain response, and the structural damage of the muscle have been presented. Countless studies have reported cellular and sub-cellular changes due to EIMD, however there are a lack of data on the neuromuscular changes and altered cortical upstream regulation due to EIMD. This lack of information can, among other things, be attributed to the difficulty of measuring upstream regulation.

Interestingly studies have investigated the cortical anticipation and regulation of evoked pain as well as the cortical involvement in voluntary movement control. However, this methodology has not been applied to study the regulation of EIMD and associated symptoms, pain response and neuromuscular changes.

Therefore this thesis intended to document the changes in muscular and cortical activation for 132 hours following the induction of EIMD. A summary of the main findings of the studies follows:

1. The experimental group, which underwent the EIMD inducing protocol, had increased serum creatine kinase activity, swelling of the M. biceps brachii, a decrease in elbow angle at rest, a loss of force during a maximal voluntary contraction and the sensation of pain, whereas the control group did not display symptoms of EIMD.

2. There was a significant decrease in EMG activity during a maximal voluntary contraction at 132 hours after the EIMD inducing protocol, whilst during a submaximal isometric contraction EMG activity increased twelve hours after the EIMD protocol in the experimental group. Furthermore, EMG activity increased during a submaximal flexion-extension protocol in the experimental group from 12 until 132 hours whilst

experiencing EIMD. These dissociations in EMG activation in the experimental group suggest the possibility of an upstream regulator of neuromuscular function as a result of EIMD.

3. An increase of α-1 activity in the motor, pre-motor and supplementary motor areas of the cortex was seen in the experimental group compared to the control group for the first 36 hours while experiencing EIMD. Together with the location and the previous findings of increased submaximal EMG twelve hours after inducing EIMD, this could imply the involvement of α-1 activity in the up-regulation of the neuromuscular function post EIMD.

4. Alpha-2 activity increased, in the contralateral frontal area, twelve hours after inducing EIMD in the experimental compared to the control group. The location and frequency of the activity suggest an inhibition of the painful stimulus within the first twelve hours of experiencing EIMD.

5. A decreased β-1 activity was observed in the control group for the duration of the study. This is associated with the familiarisation of the participants to the movement protocol over the days of the experiment, suggesting less anticipation and necessity of conscious control during the movement tasks.

6. An increase in β -1 activity occurred in the frontal and parietal area at twelve hours post EIMD in the experimental group. The increase in β-1 activity especially in the parietal and posterior parietal area proposes a feedback link about EIMD induced peripheral changes in proprioception, force steadiness and neuromuscular function in the experimental participants.

7. Significant differences were evident between the experimental and control groups and over time in the ipsilateral frontal, central and parietal areas at 12 and 36 hours post EIMD. In the experimental group there were significant increases in β-2 activity in the ipsilateral parietal area at 12 hours and a consistent decrease towards 132 hours. This change in activity, from an increase to a decrease, suggests that β-2 might be associated with the anticipation of movement and/or pain which explains its peaking at 12 hours and its continuous decrease until the end of the study at 132 hours.

A future research model, describing how to investigate further the central upstream regulation in response to the neuromuscular changes and delayed pain caused by the

disturbance of system homeostasis due to EIMD, has been proposed. This research model has been developed from the findings of the experiments described in the previous three chapters as well as previous research as discussed in chapter 1.

The next section will explain how this model was developed based on the findings of this study.

5.2 SUMMARY OF CHAPTER 2

Chapter 2 shows that changes in neuromuscular recruitment and delayed pain are associated with the clinical symptoms of EIMD.

Particularly interesting is the finding that neuromuscular changes during maximal isometric and submaximal isometric and flexion-extension contractions are dissociated. The decrease of EMG activity during a maximal contraction is caused by damage to the contractile machinery and the down regulation of neural drive to the muscle. The increased EMG activity during submaximal neuromuscular activity suggests that more muscle fibres are recruited to compensate for the disturbed motor perception and altered proprioception. Both the up and down regulation of EMG activity during contraction suggests that an upstream (central) regulator might be located within the central nervous system.

It was also noted during this study that the pain response is delayed and dissociated from the neuromuscular responses. The neuromuscular changes are most pronounced twelve hours after the induction of EIMD while the pain response is delayed and the symptoms of pain peak only 48 hours after the induction of EIMD.

This suggests that there is a central regulator overriding the pain within the first 12 to 24 hours. This could be explained using evolutionary principles as the delayed onset of the symptoms made a “fight or flight” response possible, after which the damage to the tissue could heal. Therefore it may be argued that the delayed onset of pain is an evolved mechanism, acquired for survival, as it provided the human being time to escape from danger before severe pain forced them to rest to give the damaged tissue time to recover.

An overview of the findings of chapter 2 is shown in Figure 5.1.

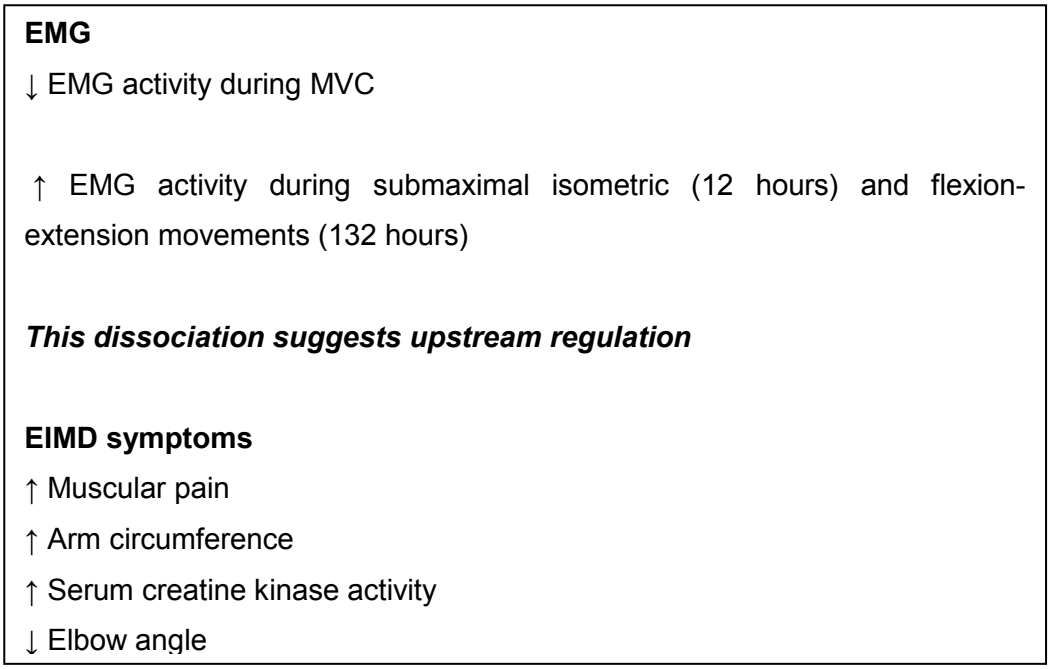

Figure 5.1 Schematic overview of the main findings of chapter 2

5.3 SUMMARY OF CHAPTER 3

The second study was designed to determine the involvement of the cortex in neuromuscular control and pain perception following EIMD. The cortex was selected as the area of the brain for this research, rather than the lower lying brain areas as it is easier to measure changes with EEG and also due to the fact that the motor and somatosensory control areas are to a large extent based in the cortex.

To determine whether the cortex was affected by EIMD the EEG was measured during a similar movement protocol in which the EMG was measured. EEG was measured during a movement sequence as the effect of movement was assumed to heighten the sensation of pain caused by EIMD.

The study found increased α-1 activity in the electrodes overlying the motor and somatosensory area during a submaximal flexion–extension contraction within the first 36 hours after the induction of EIMD. Previous research has shown that these areas are concerned with the integration of proprioceptive and motor perceptive feed back as well as the control of voluntary movement.

As these areas are associated with sensory stimuli as well as motor control it was concluded that the increased α activity was associated with the changed neuromuscular function measured with EMG in chapter 2. This conclusion was based on the fact that α-1 activity was elevated during the same time period during which neuromuscular disturbances had been measured in chapter 2. Therefore it was proposed that the increased activity was due to increased movement planning, to maybe counteract the altered proprioceptive feedback reaching the brain.

Alpha-2 activity, in contrast, increased in the pre-motor and motor cortex contralateral to the movement. These areas are to be part of the medial pain system and are associated with the perception and integration of a painful stimulus. Therefore it is proposed that an increased contralateral fronto-central α-2 activity acts as a cortical top-down regulator of the perception of pain twelve hours after the induction of EIMD which consequently leads to the delayed onset pain response associated with EIMD.

As hardly any significant changes are seen in α-2 activity 36 and 132 hours after EIMD induction it is proposed that α-2 activity suppresses the sensation of pain at the cortical

level even if painful stimuli are received from the periphery. This suppression is attenuated at 36 hours and therefore allows the sensation of pain to develop. It might even be attenuated earlier, as the sensation of pain is often felt 24 hours after the induction of EIMD. However this was not tested in this study and follow up studies would need to be conducted to show this. It is suggested that this is the mechanism by which the delayed onset of pain, possibly necessary for survival as mentioned earlier, is provided. This hypothesis needs to be tested further.

A summary of the main findings of chapter 3 are shown in Figure 5.2.

FEED-FORWARD

Cortical alpha activity

↑ alpha-1 activity in the motor area at 12 hours, decreasing at 36 hours and disappearing at 132 hours.

Suggests that α-1 activity is associated with motor response and execution of movement.

↑ alpha-2 activity in the contralateral frontal and pre-motor area at 12 hours post, no significant changes at 36 and 132 hours.

Suggests that α-2 activity is associated with suppressing the sensation of pain.

Figure 5.2 Schematic overview of the main findings of chapter 3

5.4 SUMMARY OF CHAPTER 4

The findings of the experiment described in Chapter 3 lead to the question of how the information about the neuromuscular changes and the painful stimulus are sent and perceived by the cortex before the feed-forward response can be formed.

The sensory information about the clinical changes due to EIMD (increased serum CK activity, oedema) and the proprioceptive information (shortened muscles, decreased force output, changes in neuromuscular function) are relayed to and integrated in the brain in the 14–35 Hz frequency.

Recent research has shown that proprioceptive information is not only received and integrated in the cerebellum but also in the parietal area of the cortex. In this study β-1 activity is increased particularly in the parietal and frontal areas of the cortex while experiencing EIMD. Therefore it is proposed that there is a link between the changed neuromuscular functioning, disturbed proprioception and the increased β-1 activity in the parietal area. This suggests that the increased β-1 activity in the parietal and frontal area is directly related to the feedback about proprioceptive changes and adaptations.

Beta-2 on the other hand seems to decrease in both groups over the time course of the study (132 hours) and we therefore propose that an increased β-2 activity is related to anticipation either of the movement or pain felt during the movement.

A summary of the main findings of chapter 4 are shown in Figure 5.3.

FEEDBACK and FEED-FORWARD

Cortical beta activity

↑ Beta-1 activity in the medial frontal, central and parietal area. Subsiding in frontal and central areas, but still different from control group in the parietal area at 132 hours.

Suggests that β-1 activity is linked to proprioception and motor perception. Bottom-up feedback from periphery to parietal area as well as top-down control from cortical areas to periphery

↑ Beta-2 activity at 12 hours in the ipsilateral frontal, central and parietal areas, subsiding towards 36 hours and differences between the groups are mainly seen in ipsilateral parietal area at 36 hours. This is the only frequency in which changes are seen over time in the experimental group between 12 and 132 hours post.

Suggests that β-2 could be associated with the anticipation of movement and pain, which could explain the over-compensatory increase in submaximal neuromuscular activity (top-down control) between 12 and 136 hours in the experimental group

Figure 5.3 Schematic overview of the main findings of chapter 4

5.5 LIMITATIONS

This novel study, conducted over 132 hours, explored the relationship between pain and neuromuscular changes induced by an EIMD protocol and cortical α-1 and α-2 activity as well as β-1 and β-2 activity measured by EEG. Although the study investigated changes in induced cortical activity, rather than event related activity, the aim was to investigate the influence of pain and neuromuscular changes induced by EIMD on cortical activity as well as EEG over the entirety of a movement task. Also our data were not normalised to baseline but rather compared to pre-EIMD protocol values to identify percentage changes in cortical activity post- versus pre-EIMD. We also measured EMG and EEG during the same testing protocol but not at similar time points and therefore correlations and coherences can only be postulated and further research is needed to confirm our hypothesis. We acknowledge that other factors such as changes in pain pathways or inflammatory processes could have lead to the dissociated response of neuromuscular changes and the delayed pain response, but the interest of this study was how EIMD and its associated symptoms affected the cortical α and β activity measured over the cortical areas. Further research is needed to integrate not only the pain and neuromuscular response with the EEG recordings but also possible inflammatory changes and adaptations in the pain pathways. Future studies should consider correlations between EMG and EEG, as well as look at a broader spectrum of EEG frequencies (including β, θ and γ).

Further limitations are that the distribution of the data needs to be seen with caution as the recorded signal source localization data was corrupted and could therefore not be used for further analysis. Signal source localization would have been of integral help in confirming the brain areas involved in the response to pain and changes in neuromuscular function. Also the additional recording of fMRI data would increase the interpretability of the data.

There was also a large amount of intra and inter subject variability especially in the EEG recordings leading to large standard deviations and the use of the non-conservative non-parametric statistical tests which might have lead to accepting false assumptions, although we tried to counteract this phenomenon by presenting data at $p<0.05$ but only discussing data at $p<0.01$ as calculated by an Bonferoni adjustment.

5.6 PROPOSED RESEARCH MODEL

Based on these three findings described in the preceding chapters and previous research summarized in the review of the literature the following proposed research model integrating the information is put forward (Figure 5.4 and 5.5).

This proposed model suggests that information supplied to the brain by the pain specific and proprioceptive pathways is received, integrated and processed in the cortical areas to generate an appropriate motor and somatosensory response to restore system homeostasis and neuromuscular function.

The first proposed line of communication is the sensation of pain during movement tasks which is assessed and integrated in the somatosensory structures. The inflammatory response (guided partially by the brain as mentioned in chapter 1) in the areas affected by the pain caused by EIMD is integrated and a response can be formed.

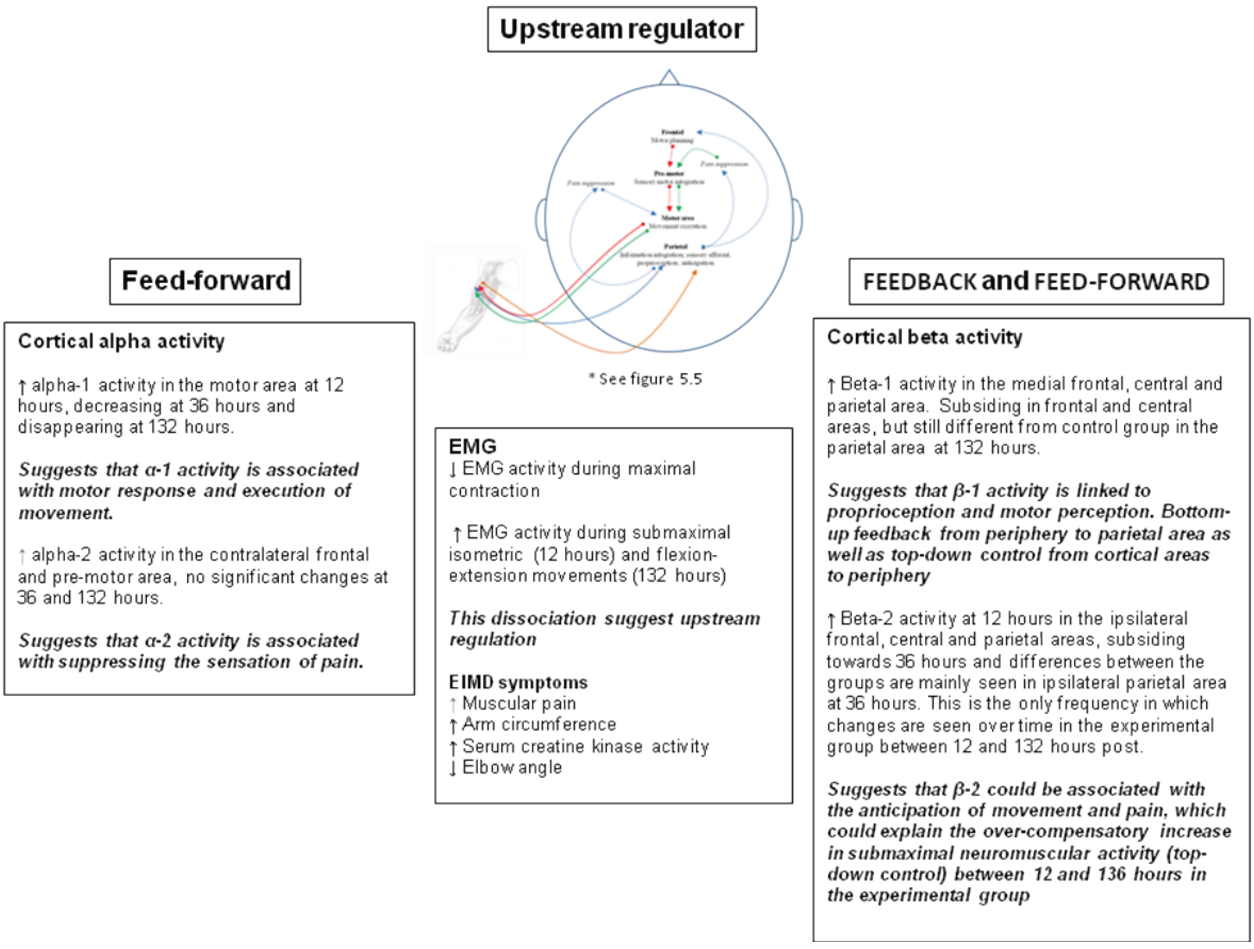

Figure 5.4 Schematic overview of the hypothesized model.

The second proposed communication pathway is the interaction between the periphery and the brain with information about the damaged muscle fibres being sent to the brain for assessment and processing. To be able to execute the identical submaximal movement task the brain is required to feed-forward this information to augment the contraction and recruitment patterns of the damaged muscle fibres to counteract the associated force loss.

The studies conducted for this thesis were the first studies to investigate the effect of EIMD on cortical activity. Therefore the approach was kept very broad and only general concomitant activity patterns in the muscle and cortical areas were investigated. Therefore in this last paragraph a model is suggested on which further research could be based to investigate the interaction between peripheral and cortical changes associated with EIMD in more detail.

In summary the proposed research model suggests that a feed-forward –feedback loop exists between the periphery and the cortical brain areas while the symptoms of EIMD are experienced. Information about pain and neuromuscular changes is transmitted to the brain via a feedback mechanism leading to increases in parietal β-1 and β-2 activity. The parietal area is known to be involved in the integration of movement and proprioception; therefore it is an entry point of feedback information. From the parietal area β-1 and -2 activities propagate information to the frontal areas of the brain where movement planning takes place. The pre-motor area relays planning information to the motor cortex. Within the motor cortex increased α-1 activity acts as a feed-forward mechanism to co-ordinate submaximal neuromuscular function detected as submaximal EMG activation. Concomitantly α-2 activity is increased in the frontal and pre-motor area leading to a suppressed pain sensation. Further activity in this area may also act as an additional feedback to control neuromuscular function, as moving the painful limbs might be inhibited.

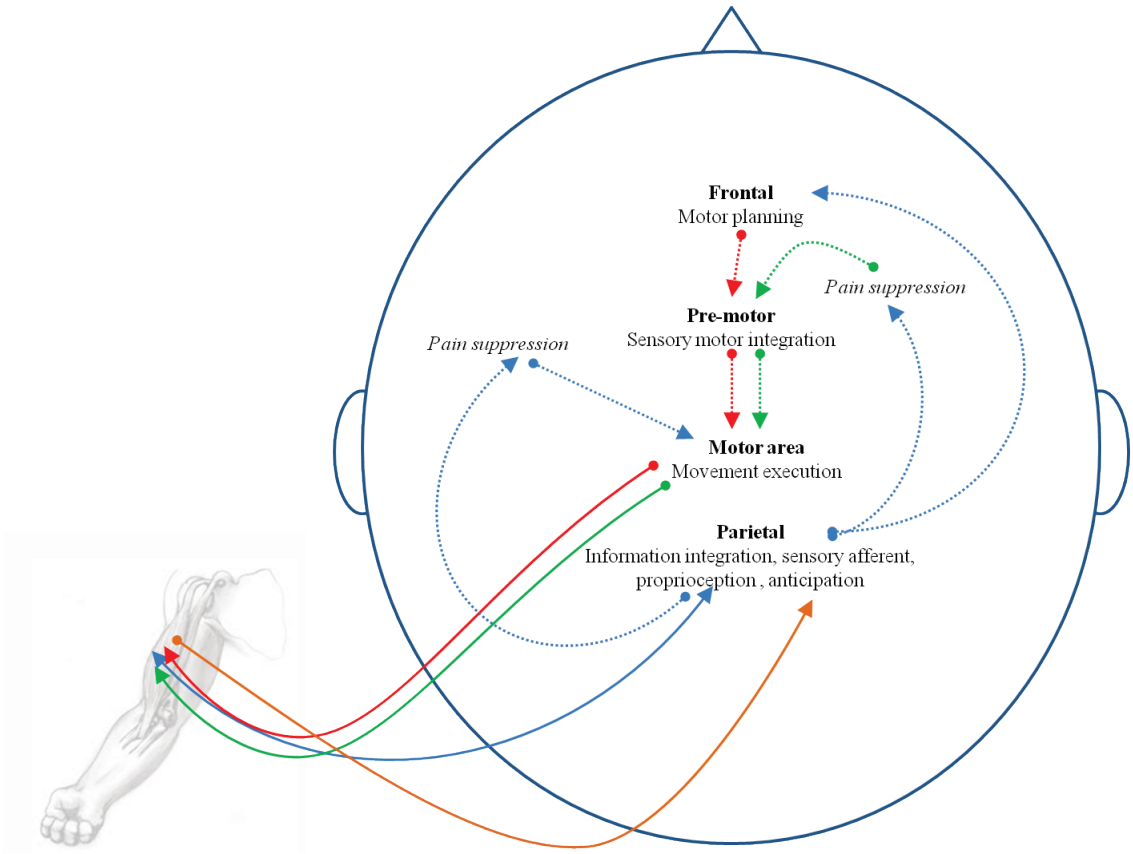

Figure 5.5 An abstract model of the communication pathways between the peripheral muscle and the cortical areas. Each colour represents a frequency. Blue = β-1, green = α-2, red = α-1 and yellow = β-2. The solid lines connect areas in which activity was found during the mentioned studies, while the dotted lines represent suggest interactions. The direction of the arrows is hypothetical.

5.7 FUTURE DIRECTIONS

This thesis has provided the foundation for understanding the complex interactions between the periphery and the brain during EIMD and exercise-induced muscular pain. The feed-forward and feedback loops as well as the information transmitting pathways in the proposed model, which was developed based on the experimental findings of this thesis, needs to be researched extensively in future studies to prove their proposed existence. Future research will have to confirm this proposed model by measuring coherences in changes in frequency and time between EMG and EEG (cortico-muscular) as well as EEG changes between brain areas (cortico-cortical). Further the investigation of changes in θ and γ activity as a result of EIMD could reveal a greater understanding of cortico-muscular communication.

This is the first study to document changes in cortical activity using EEG methodology over 132 hours (six days) after the induction of EIMD. This and other studies could further broaden the knowledge on the treatment of EIMD as well as the incurrence of the repeat bout effect.

Future studies should also consider evoked potentials, temporal spectral [270] and wavelet analysis should be further used to elucidate the signal propagation velocity and location within the cortical networks (i.e.. if the signal travels front to back or back to front in the fronto-parietal network). This increase in the body of knowledge will lead to the further understanding of pain during movement tasks and perhaps the use of biofeedback with different brain frequencies to control acute and exercise-induced pain states.

It would also be interesting in future to use fine-wire EMG electrodes as well as nerve stimulation or nerve conduction velocity measurements to gain more information about the neuromuscular changes within the muscle as well as to maybe determine in more detail if sensation of pain associated with EIMD is an emotional response to the nociceptive signal or an actual mechanical or physiological disturbance of the nociceptors or the nociceptive pathways.

In conclusion, these are the first series of studies illustrating the involvement in the cortical areas and documenting the time course of central and peripheral interactions

which co-ordinate movement tasks while experiencing pain associated with EIMD. This is the first study to illustrate the involvement of the cortical areas and document the time course of central and peripheral changes in relation to EIMD. The model contributes to the understanding of the physiological changes as a result of EIMD and the understanding of pain associated with it. These findings could contribute to better management of EIMD and acute muscular pain.

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APPENDIX

APPENDIX 1

THE ASSESSMENT AND ANALYSIS OF HANDEDNESS

(The Edinburgh inventory)

Name:_____

Have you ever had any tendency to left-handedness?

YES	NO

Please indicate your preferences in the use of hands in the following activities by putting + in the appropriate column. Where the preference is so strong that you would never try to use the other hand unless absolutely forced to, put ++. If in any case you are really indifferent put + in both columns. Some of the activities require both hands. In these cases the part of the task, or object, for which hand-preference is wanted is indicated in brackets. Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

		R	L
1	Writing		
2	Drawing		
3	Throwing		
4	Scissors		
5	Comb		
6	Toothbrush		
7	Knife (without fork)		
8	Spoon		
9	Hammer		
10	Screwdriver		
11	Tennis Racket		
12	Knife (with fork)		
13	Cricket Bat (lower hand)		
14	Golf Club (lower hand)		
15	Broom (upper hand)		
16	Rake (upper hand)		
17	Striking match (match)		
18	Opening box (lid)		
19	Dealing cards (card being dealt with)		
20	Threading needle (needle, thread according to which is moved)		
21	Which foot do you prefer to kick with?		
22	Which eye do you use when using only one?		

APPENDIX 2

MODIFIED PHYSICAL ACRIVUTY READINESS QUESTIONNAIRE (PAR-Q)

Name			**Date**
DOB	**Age**	**Home Phone**	**Work Phone**

Regular exercise associated with many health benefits, yet any change of activity may increase the risk of injury. Completion of this questionnaire is a first step when planning to increase the amount of physical activity in your life. Please read each question carefully and answer every question honestly and if applicable:

Yes	**No**	**1) Has a physician ever said you have a heart condition and you should only do physical activity recommended by a physician?**
Yes	**No**	**2) When you do physical activity, do you feel pain in your chest?**
Yes	**No**	**3) When you were not doing physical activity, have you had chest pain in the past month?**
Yes	**No**	**4) Do you ever lose consciousness or do you lose your balance because of dizziness?**
Yes	**No**	**5) Do you have a joint or bone problem that may be made worse by a change in your physical activity?**
Yes	**No**	**6) Is a physician currently prescribing medications for your blood pressure or heart condition?**
Yes	**No**	**7) Do you have insulin dependent diabetes?**
Yes	**No**	**8) Are you 45 years of age or older?**
Yes	**No**	**9) Do you know of any other reason you should not exercise or increase your physical activity?**

If you answered yes to any of the above questions, talk with your doctor BEFORE you become more physically active. Tell your doctor your intent to exercise and to which questions you answer yes.

If you honestly answered no to all questions, you can be reasonably positive that you

can safely increase your level of physical activity gradually.

If your health changes so you then answer yes to any of the above questions, seek guidance from a physician.

Participant Signature	**Date**

APPENDIX 3

MEDICAL QUESTIONNAIRE

MRC/UCT Research Unit for Exercise Science and Sports Medicine

Questionnaire

Name: _____

Date of Birth: _____

Age: _____

Medical and Surgical History (last 2 years):

Present/previous injuries to cervical spine or upper limbs:

Medication:

Are you currently receiving any massage, soft tissue or physiotherapy treatment?

Y	N

If “yes”, please state details of treatment:

Are you able to visit the laboratory at the Sports Science Institute (Boundary Road, Newlands) for the eccentric exercise protocol, and again at 12, 36, 60, 84, 108 and 132 hours after the eccentric exercise bout designed to induce muscle damage

Y	N

Signature: _____

Date: _____

Thank-you for your co-operation in completing this questionnaire.

APPENDIX 4

TRAINING HISTORY

Name: _____

Please complete the following table. List any sport/activity in which you have regularly participated, and estimate for how long and how often you participated.

Sport/Activity	**year started**	**number of years**	**months/yr**	**hrs/week**	**level ***	**HIT ** (yes/no)**

* competitive or social

** interval or high-intensity training (HIT) included; yes or no

Examples of sporting activities include:

- jogging - tennis - aerobic dance/step
- swimming - badminton - dancing
- cycling - volleyball - skating
- walking - netball - hiking
- rugby - squash - rock climbing
- football/soccer - canoeing - martial arts
- hockey - golf - strength and resistance training

APPENDIX 5

INFORMED CONSENT FORM

Dear Volunteer,

The MRC/UCT Research Unit for Exercise Science and Sports Medicine will be conducting a trial on the effect of exercise-induced muscle stiffness on brain cortical encephalographic patterns.

The clinical trial will involve the following tests:

1. Weight and height measurements
2. Anthropometric assessment of body composition
3. An eccentric exercise bout designed to induce muscle stiffness of the biceps muscle in the non-dominant arm. The magnitude of this induced muscle stiffness is of similar magnitude to that experienced after a bout of vigorous or unaccustomed exercise (people in the control group are not required to do this exercise bout).
4. Repeated assessments of muscle function (peak isometric, concentric and eccentric force), using a Biodex isokinetic dynamometer. These measurements will occur before the muscle is exercised, and again at 12, 36, 60, 84, 108, and 132 hours after the exercise.
5. Resting biceps girth measurements and elbow joint angles will be measured before the bout of exercise, and again at 12, 36, 60, 84, 108, and 132 hours after the exercise.
6. Muscle soreness will be measured using a visual analogue scale, and subjectively according to a “rating of perceived pain” scale. Both procedures will be explained to you in detail.
7. Blood samples will be collected from an antecubital vein prior to each test of muscle function to determine plasma creatine kinase activity (This is a marker of muscle damage.). This is the only invasive technique used in this study and standard medical practice and sterile procedures will be strictly adhered to.
8. The measurement of electrical activity of the elbow flexor and extensor muscles using an electromyography (EMG). This involves the marking of the skin of the upper arm with a washable body marker and 7 hypo-allergic sticky disks will be placed on these markings on the skin. To improve the stickiness, the skin will be wiped with an alcoholic wipe and if necessary body hair will be shaved with a disposable razor. The EMG will be measured before and again 12, 36 and 132 h after the bout of exercise.
9. The measurement of electroencephalographic (EEG) activity. Before the EEG net is

fitted on the head, the hair will be washed with Johnson's Baby Shampoo. This procedure will ensure optimal conduction of the EEG electrodes. The EEG measurements will be performed before and again 12, 36 and 132 h after the bout of exercise. During this test the subjects will perform several movements with the left arm either on command or self paced.

10. Immediately after the EEG testing, the position of EEG electrodes and anatomical landmarks will be scanned using a Polhemus™ 3D digitizer. This system uses a transmitter which generates a magnetic field for use as a reference frame. A stylus with an electromagnetic coil set is then used to tap on leads and reference points. A receiver then detects the position of the stylus in the reference frame and allows the leads and reference points to be marked in 3D.
11. During the trial, subjects will be required to refrain from alcohol consumption, strenuous physical activity, and from taking any other form of medication, including non-steroidal anti-inflammatory agents.

Risks

There will be a small risks for the subjects associated with the drawing of blood from the antecubital vein. Risks associated with the drawing of blood include discomfort, infection (wound sepsis), muscle bruising (haematoma formation) and numbness (peripheral subcutaneous nerve injury). The risks will be minimized by cleaning the area around the antecubital vein with a disinfectant alcohol swap before the blood will be drawn. Immediately afterwards a protective plaster will be applied.

The subjects will be instructed on the usage of the Biodex dynamometer. The risks associated with the Biodex are similar to the risks associated with training with weights. The bout of eccentric exercise on the Biodex dynamometer is designed to cause moderate muscle stiffness which disappears after about 3 days (Rec.Ref.075/99). The muscle stiffness might lead to a slight feeling of discomfort or pain which will disappear within 3 days after the exercise protocol and will not influence the subjects' daily life in any way.

The EEG and EMG will only be used for receiving electrical impulses from the brain and muscle respectively and as such will place no inherent risk on the subjects. Neither the EEG nor the EMG is being used as a diagnostic tool, but only as a research tool in this study. If any abnormalities show during either of the recordings the subjects will be referred to a qualified neurologist.

A qualified medical doctor will be available to treat any side effects that might occur during the study.

Benefits

The subjects will receive their results when the study has been completed. This feedback will contain their anthropometrical data including an estimate of their body fat percentage and the maximal power output of their biceps muscle.

The study will contribute to the better understanding of the mechanisms associated with a decline in muscle function after a bout of exercise which causes muscle stiffness.

This trial is undertaken for research purposes and is not designed to treat any medical condition.

I confirm that the nature, purpose, testing procedures, and the likely duration of the clinical trial study have been fully explained to me. I understand that I may ask questions at any time during the testing procedures. I agree to comply with any instruction given during the study and to co-operate with the project co-ordinator. I realise that I am free to withdraw from the study without prejudice at any time, should I choose to do so. I have been informed that the personal information required by the researchers will be held in strict confidentiality. In addition, I know that the information derived from the testing procedures will remain confidential and will be revealed only as a number in statistical analyses. I understand that this study has been reviewed by the Ethics and Research Committee of the University of Cape Town Medical School.

I have carefully read this form. I understand the nature, purpose and procedure of this study. I agree to participate in this study of the MRC/UCT Unit for Exercise Science and Sports Medicine.

Name (in full) of volunteer: _____

Signature of volunteer: _____

Name (in full) of witness: _____

Signature of witness: _____

Date: _____

APPENDIX 6

UNIVERSITY OF CAPE TOWN

Research Ethics Committee
Faculty of Health Sciences
OMB E46 Room 26, GSH
Queries : Xolile Fula
Tel : (021) 406-6492 Fax: 406-6411
E-mail : Xfula@curie.uct.ac.za

30 April 2004

REC REF: 090/2004

Prof M Lambert
Human Biology
Sports Science Institute

Dear Prof Lambert

THE EFFECT OF EXERCISE INDUCED MUSCLE STIFFNESS ON BRAIN CORTICAL ENCEPHALOGRAPHIC PATTERNS

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has ***formally approved*** *the above-mentioned study on the 29th April 2004.*

Your comments and revisions have been noted with thanks.

Please quote the Reference number in all correspondence.

Yours sincerely

PROF T. ZABOW
CHAIRPERSON

APPENDIX 7

VAS SCALE

10 cm Visual Analogue scale (10 cm VAS score)

Name_____ **Date**_____

Experiment day_____

1. Rate the average level of pain you have experienced in your left biceps brachii in the past 24 hours by making a mark on the line below.

No pain _____ Excessive pain

2. Rate the level of pain that you are experiencing currently in your left biceps brachii by making a mark on the line below.

No pain _____ Excessive pain

3. Rate the level of pain that you experienced while performing a single left biceps curl with a 3 kg weight by making a mark on the line below.

No pain _____ Excessive Pain