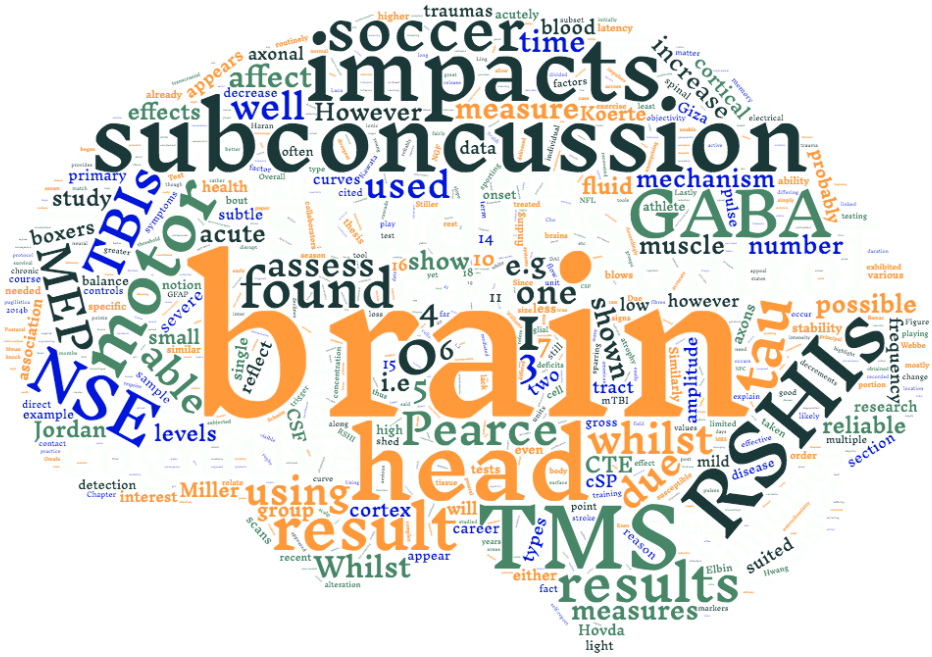
UNIVERSITY of STIRLING

ON THE HEAD: THE TRUE IMPACT OF ROUTINE HEAD STRIKES IN SPORT



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A thesis submitted to the University of Stirling in partial fulfillment for the degree of

Doctor of Philosophy

Faculty of Health Sciences and Sport; Psychology Division, Faculty of Natural Sciences

Physiology, Exercise and Nutrition Research Group

University of Stirling

January 2018

If we knew what it was we were doing, it would not be called research

Albert Einstein (allegedly)

Theoretical physicist and overall smart guy

(1879 – 1955)

Declaration

I declare that this thesis was composed by myself and that all the data were collected and analysed by myself, under the supervision of Dr Angus Hunter and Dr Magdalena Ietswaart. Neither the thesis, nor the original work contained therein have been submitted to this or any other institution for a higher degree.

Thomas George Di Virgilio

Stirling, 26/01/2018

Submission for Viva examination: 26/01/2018

Viva examination date: 29/03/2018

Thesis approved for final submission: 19/04/2018

Acknowledgements

First and foremost I would like to thank my supervisors **Dr Angus Hunter** and **Dr Magdalena Ietswaart**, whose tireless efforts throughout the past 4 years have helped me grow as an individual, and given me the tools needed to succeed in the big world that is Academia. Thank you also to **Mr Chris Grigson**, PENRG's mighty Chief Technician who's always been available to lend a helping hand, or a sympathetic ear. Cheers to **Dr Stuart Goodall**, **Prof Glyn Howatson** and **Prof Lindsay Wilson** for lending us key equipment and teaching me how to use it, and also thank you to **Prof David Donaldson** and **Dr Willie Stewart** for their guidance and suggestions throughout this 3 year experience.

I would also like to acknowledge **Dr Lewis Macgregor, Mr Adam Wade**, **Mr Niklas Jannesson**, **Dr Phyllis Windsor**, **Mr Zacharias Nicolaou** and **Mr Jamie Ross** for their invaluable help during the piloting phases and data collection process, as well as thanking **all the participants** that let me shave their leg, zap their brain and do other weird stuff physiologists are so very fond of. Without you, this thesis would have probably been a much shorter affair.

Thank you also to the present and past members of the **Physiology, Exercise and Nutrition Research Group**, your advice, friendliness, expertise and good humour were highlights of my postgraduate career. Special mention goes to **Ms Gillian Dreczkowski** for her help in anything lab-related, as well as **Ms Barbara Kettlewell**, **Ms Liz Harris**, **Ms Stella Turner** and the rest of the admin staff within the **Faculty of Health Sciences and Sport** and the **Faculty of Natural Sciences** for their support, help and advice during these stressful times.

A big thumbs up to those fellow postgrads who were lucky enough to share an office with the bearded wonder that is me; so thank you **Dr Lindsay**, **Dr Stuart**, **Ross**, **Dr Nidia**, **Jordan**, **Dr Lewis** (again) and **Adam** (again) for the sorely needed advice and distractions.

I am grateful to my parents, **Bruno Di Virgilio** and **Sarah Nicholson** and my sister **Daisy Di Virgilio** for their unwavering support (both moral and financial) and faith throughout my whole University studies, as well as my **grandparents** who allowed me to begin my postgraduate studies.

Although almost at the end of the page, I would like to thank the most important person in my life: my partner **Erin Brisco** for being my anchor during this journey, and always providing encouragement, support and comfort during the darker times.

General Abstract

Published research suggests a potential link between repetitive subconcussive head impacts and alterations in brain function. The results, however, are ambiguous due to the lack of direct evidence for this relationship. A non-invasive brain stimulation technique (transcranial magnetic stimulation, TMS) can detect changes to brain function following mild, moderate and severe brain trauma; yet, no data exist on its effectiveness in detecting subconcussion-related alterations. As such, the overall aim of this thesis is to determine whether TMS is able to detect acute changes in brain function as a result of repetitive subconcussive head impacts (RSHIs). Chapter 1 highlights techniques with potential to detect brain alterations following subconcussive, and concussive head impacts. TMS was identified as a possible tool for detecting such changes. Chapter 2 demonstrated primary and secondary outcome measures corticomotor inhibition and corticospinal excitability, respectively, to have good overall day-to-day reliability. Subsequently, corticomotor inhibition was transiently increased following two separate RSHI models (soccer heading in chapter 3, and sparring in chapter 4). Further, motor unit recruitment was also altered following sparring. Corticospinal excitability and postural control were unchanged in both studies, whilst parameters of cognitive function appeared altered in the immediate follow-ups. These data indicate that RSHIs are associated with measurable, albeit transient alterations to brain function. Chapter 5 further corroborates the notion that brain alterations in chapters 3 and 4 were due to RSHIs, by showing that corticomotor inhibition and corticospinal excitability are largely unaffected by the sole act of performing exercise. No alterations were also observed in the last experimental chapter; the pilot study explored the feasibility of using TMS in detecting alterations following cumulative exposure to RSHIs. This thesis is the first to provide direct evidence for a relationship between RSHIs and brain alterations. The data suggest that subconcussive head impacts, routine in a number of sports, may impair the brain's ability to control the body (in turn increasing the risk of injury) as well as affecting overall brain health.

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Publication(s)

Di Virgilio, T.G., Hunter A.M., Wilson, L., Stewart, W., Goodall, S., Howatson, G., Donaldson, D.I., Ietswaart, M., 2016. Evidence for acute electrophysiological and cognitive changes following routine soccer heading. Ebiom. 13, 66-71. **(Chapter 3)**

List of Conference presentations

Di Virgilio, T.G., Hunter, A.M., Goodall, S., Howatson, G., Wilson, L., Iestwaart, M. The effect of repeated soccer ball heading on corticospinal excitability and inhibition. *Poster session, American College of Sports Medicine (ACSM) Annual Conference, Boston, Massachusetts, USA, May-June 2016.*

Di Virgilio, T.G., Hunter, A., Wilson, L., Stewart, W., Goodall, S., Howatson, G., Donaldson, D.I., Ietswaart, M., Using transcranial magnetic stimulation (TMS) to identify acute brain changes following subconcussive head impacts. *Oral presentation, Scottish Imaging Network: A platform for scientific excellence (SINAPSE) Annual Scientific Meeting, Stirling, Scotland, UK, June 2016. [1st Prize, Best Proffered Talk]*

Di Virgilio, T.G., Hunter, A.M., Wilson, L., Stewart, W., Goodall, S., Howatson, G., Donaldson, D.I., Ietswaart, M., Acute electrophysiological and cognitive changes following routine soccer heading. *Oral presentation, NIHR Brain Injury Healthcare Technology Co-operative BIT think tank, London, UK, November 2016.*

Di Virgilio, T.G., Hunter, A.M., Jannesson, N., Wilson, L., Stewart, W., Donaldson, D.I., Iestwaart, M., Acute brain changes following routine subconcussive impacts: evidence from boxing. *Oral presentation, European College of Sport Science Annual Congress, Metropolis Rhur, Germany, July 2017*

List of abbreviations

aMT	Active motor threshold
APB	Abductor pollicis brevis
APH	After-hyperpolarization period
AST	Attention switching task
BDNF	Brain derived neurotrophic factor
Ca^{+}	Calcium ion
CANTAB	Cambridge neuropsychological test automated battery
Cho	Choline
CI	confidence interval
CSF	Cerebrospinal fluid
CSP	Cavum septum pellucidum
cSP	Corticosilent period/corticomotor inhibition
CT	Computed tomography
CTE	Chronic traumatic encephalopathy
CVR	Cerebrovascular reactivity
DAI	Diffuse axonal injury
dEMG	Precision decomposition EMG
DTI	Diffusor tensor imaging
EMG	Electromyography
ES	Effect size
FDI	First dorsal interossei
GABA	Gamma-aminobutyric acid
$GABA_a$	GABA type A receptor
$GABA_B$	GABA type B receptor
GFAP	Glial fibrillary acidic protein
GSH	Glutathione
Hz	Hertz
I-O	Input-output curves (recruitment curves)

ICC	Intra-class correlation coefficient
K^+	Potassium ion
M1	Primary motor cortex
MEP	Motor evoked potential
mI	Myo-inositol
Mmax	Maximal excitability of the muscle
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MT	Motor threshold
mTBI	Mild traumatic brain injury
MU	Motor unit
MUAP	Motor unit action potential
MVC	Maximal voluntary contraction
Na^+	Sodium ion
NFL	Neurofilament light polypeptide
NGF	Nerve growth factor
NPC	Near point of convergence
NSE	Neuron specific enolase
p-pTMS	Paired-pulse TMS
PAL	Paired associate learning
RF	Rectus femoris
RSHIs	Repetitive subconcussive head impacts
RTI	Reaction time
rTMS	RepetitiveTMS
RVP	Rapid visual processing
S100B	S100 calcium-binding protein B
sEMG	Surface EMG
SENIAM	Surface electromyopgraphy for the Non-invasive assessment of muscles
SWM	Spatial working memory

TBI	Traumatic brain injury
TMS	Transcranial magnetic stimulation
VL	Vastus lateralis

Chapter 1

Introduction and Aims

1.1 Introduction

"It's nothing, just walk it off" are words that most athletes and recreational players have heard at least once in their lifetime, probably more. Words often said by coaches following what is thought to be a mild and harmless head knock, yet we are starting to realize that this may not be the case. Like an iceberg floating serenely in the ocean, there is probably a lot more going on underneath the surface than what meets the eye.

The deleterious (and sometimes catastrophic) effects of traumatic brain injuries have been well established in the last few decades, both acutely (Giza and Hovda, 2014) and longitudinally (Hay et al, 2016; Maas et al, 2017). However, the relationship between head strikes that do not result in any visible symptoms or signs of concussion, and changes to brain function, is less straightforward. Head impacts of this nature are often referred to as "subconcussive", and are most commonly seen in sporting environments (ball heading in soccer, tackling in rugby and american football etc.), but can also be applied to the general public (trips and falls, certain types of manual labour, victims of domestic abuse).

Subconcussion is difficult to detect. Likely due to the subtle and possibly transient nature of the effects it has on the brain, particularly when assessing the acute time course. Transiency, however, is not the only issue that surrounds repetitive subconcussive head impacts (RSHIs). Whilst there are a number of techniques that may be able to detect functional changes to the various systems at work in our body, there is some ambiguity on their effectiveness (in terms of sensitivity) in doing so. Parameters such as cognitive function and postural control, often seen as a gold standard in concussion detection (McCrory et al, 2013), have in fact been applied in RSHI research with varying degrees of success (Broglio et al, 2004; Haran et al, 2013; Kaminski et al, 2007; Putukian et al, 2000). For this reason there has been a

push by the research community to identify other markers of brain dysfunction (discussed later on in this chapter) that may be more appropriately applied to a RSHI context. Accordingly, the overarching theme of this thesis is to explore the validity of a non-invasive brain stimulation technique (transcranial magnetic stimulation – TMS) in detecting functional brain changes in participants exposed to a bout of repetitive subconcussive head impacts (RSHIs). This chapter will discuss the literature behind subconcussive head impacts, as well as highlighting the various techniques that are used to detect concussed states and that are applicable to subconcussion.

1.2 Subconcussion

1.2.1 RSHIs and chronic traumatic encephalopathy

It is difficult to pinpoint when researchers began studying the contributions of RSHIs to cerebral decline. Certainly, as early as the 1920s academics suspected that repetitive head impacts were associated with impaired brain health. In 1928 Dr. Harrison Martland published the seminal paper on what he described as “punch drunk”, a disease characterized by hemorrhages in the brains of prize fighters. In the article he notes that the first symptoms of this “dementia pugilistica”, or what we now refer to as chronic traumatic encephalopathy (CTE), is an impairment of motor control. Martland also reports that the disease was commonly associated with those boxers who were not very good and so received “considerable head punishment”. Although he mostly refers to multiple concussive blows as the potential mechanism for the onset of the disease, it is likely that impacts of a subconcussive nature may have also contributed. Whilst he does not explicitly name subconcussion as a cause, Martland mentions that this punch drunk phenomenon was frequently seen in second-rate boxers. Athletes of this type who were not deemed good enough to compete were often used for training purposes, and were knocked down several times a day. It is therefore reasonable to postulate that it was a

combination of both concussive and subconcussive head impacts that probably dictated the onset of the disease.

Although the disease was initially termed “dementia pugilistica”, more recent studies have shown that it affects much broader populations, some of which are obvious (e.g. American football players), and some which are not (e.g. a dwarf circus performer who participated in dwarf-throwing competitions) (Omalu et al, 2005; Omalu et al, 2006; Williams and Tannenberg, 1996). The majority of research on this topic concentrated on the association between CTE and repetitive mild traumatic brain injuries (mTBIs), and as such will not be discussed in this section; however, a few studies found pathological signs of CTE in brains of athletes who had a low number of diagnosed concussions but were exposed to repetitive subconcussive hits over the course of their career (McKee et al, 2009; Ling et al, 2017). The paper by Ling et al., (2017) is of particular interest as it describes signs of CTE in former soccer players who were prolific ball headers and all only had 1 diagnosed concussion; although it is limited by a small sample size (6 brains, 4 of which had CTE) the findings suggest that CTE may not only occur as a result of repetitive mTBIs, but also from prolonged exposure to RSHIs occurring from ball heading and head-to-ground or head-to-player collisions. Therefore, it is critical that we concentrate our efforts on understanding the relationship between subconcussion and brain health and shed light on the potential consequences of this common practice.

Sport-related subconcussive head impacts occur in a variety of environments, ranging from contact sports (e.g. rugby and american football) to sports where there is little intentional contact between players (e.g. soccer). Actually studying subconcussive-related changes to the brain can be quite difficult, particularly under an ethical standpoint: it is not acceptable to expose participants from a general

population to a potentially harmful protocol to which they may not be familiar with. As such, it is important that studies are conducted using populations that are used to receiving, and routinely undergo head impacts. Two best examples of sports that are suited for this type of analysis are soccer and boxing. Soccer is particularly well suited for laboratory-based research since the act of heading a ball can be easily and consistently replicated with dedicated equipment. As such it is unsurprising that there are a number of studies, discussed below, assessing possible brain changes following repeated soccer ball heading.

1.2.2 RSHIs and cognitive function

Neuropsychological (or cognitive) performance appears to be the preferred mode to quantify brain function following both acute and cumulative traumas to the head. Commonly used tests (Wechsler Adult Intelligence Scale, Trail Making Test, Stroop Test, Wisconsin Card Sorting Test, and many more) have been designed to assess individual parameters of cognitive function, such as memory, attention, reaction time etc. Because such parameters have been shown to be reliable in detecting impairments following traumatic brain injuries (TBIs) (Draper and Ponsford, 2008; Kinnuenen et al, 2011), it is reasonable to postulate they would also be able to detect minor disruptions to brain function resulting from subconcussive impacts.

Most studies assessing cognitive function and medium to long-term exposure to head impacts found that repeated blows to the head over the course of a career impaired neuropsychological performance in soccer players and boxers (Tysvaer and Løchen, 1991; Jordan et al, 1996; Matser et al, 1998; Matser et al, 1999; Witol and Webbe, 2003; Straume-Naeshein et al, 2009; Lipton et al, 2013; Stiller et al, 2014; Koerte et al, 2016). The findings of reduced brain function were found in both professional (Matser et al, 1998; Koerte et al, 2016) and amateur (Matser et al, 1999; Lipton et al, 2013) soccer athletes, as well

as professional boxers (Stiller et al, 2014; Jordan et al, 1996). Further, assessing the contribution of heading frequency to cognitive decline showed that athletes with a higher exposure to head impacts had lower cognitive scores when compared to both a control group and a low exposure frequency group in both athlete populations (Witol and Webbe, 2003; Stiller et al, 2014; Jordan et al, 1996). Straume-Naesheim and colleagues (2009) assessed soccer players both in an acute (discussed below) and a long term context; interestingly, they found that athletes who experienced 1 or more head impacts exhibited small impairments in cognitive function when assessed one year following the event(s).

Conversely, other studies found no association between the number of soccer ball headers and declines in memory performance and concentration over one (Kaminski et al, 2007; Kaminski et al, 2008) or multiple (Rutherford et al, 2009) playing seasons. The results from Kaminski and collaborators may not be comparable to earlier studies as they relate to a female population, which had not been yet studied. However, a possible explanation for the different results is that previous studies assessed males who played with a heavier ball, abused alcohol and had already suffered mild head traumas; all factors that contribute to observable declines in brain health (Kaminski et al, 2007). Kontos and colleagues (2011) also show no relationship between low, moderate and high heading exposure and cognitive function in male and female youth soccer players (13-18 years old). A possibility for the lack of findings may be due to the age of the participants, younger individuals may be less susceptible to the subtle effects of RSHIs, and moreover may affect only a certain subset of athletes.

The papers cited thus far in this section relate to chronic, or long-term exposure to RSHIs. Parameters of cognitive function, however, have also been used to probe the acute effects of RSHIs (Putukian et al,

2000; Straume-Naesheim et al, 2009; Gutierrez et al, 2014; Elbin et al, 2015). All of these studies assessed players before and after soccer heading impacts, some following a match (Straume-Naesheim et al, 2009) and others using a laboratory-based, before-and-after controlled design (Putukian et al, 2000; Gutierrez et al, 2014; Elbin et al, 2015). The results demonstrated either measurable cognitive deficits following head impacts not diagnosed as concussion (Straume-Naesheim et al, 2009), or no effects associated with heading the ball (Gutierrez et al, 2014; Elbin et al, 2015). Where others show no interaction between soccer heading and cognitive performance, Elbin and colleagues (2015) report counterintuitive results; their aim was to assess the effectiveness of protective headgear in mitigating any potentially negative effects of repeated ball heading. They demonstrated significant verbal memory decrements in the headgear group (who were supposed to be protected) when compared to the non-headgear one.

Overall, it is clear that whilst there may be an association between RSHIs and cognitive deficits, the evidence to date is far from definitive. The reasons for the conflicting results may stem from the modalities used to collect impact data; the main limitation of most studies cited in this section, in fact, is that they use self-report methods to record concussion and subconcussion exposure (Kontos et al, 2011; Jordan et al, 1996; Witol and Webbe, 2003). The resulting inference of heading exposure, for example, can therefore only be a gross estimate either under- or over representing the amount of impacts participants were actually subjected to. Furthermore, a few studies, particularly those published in the 1990s and early 2000s, do not account for concussive brain traumas that players received during their career that may affect the outcome of the measures. As such, results need to be treated cautiously as the individual contributions of concussive and subconcussive impacts cannot be untangled.

Although neuropsychological tests can be good and reliable indicators of brain health, they require thorough familiarization in order to achieve a true and reliable measure. A few of the cited studies did not perform this process with their participants (i.e. Kaminski et al, 2007; Putukian et al, 2000), and consequently show an improvement in their pre- to post- heading cognitive performance, which may have masked any small decrements caused by RSHIs. Lastly, and probably more importantly, it is possible that whilst measures of neurocognitive function are able to detect brain changes following TBIs, they may not be sensitive enough to detect changes occurring from subconcussive impacts. This is likely because neuropsychological tests are designed to detect abnormal, or gross changes in cognitive function. Whilst these alterations are evident following severe head traumas, the subtle changes that possibly occur following RSHIs may not be large enough to be detected by the tests. It is therefore encouraging that other techniques with greater capacity to detect subtle and transient changes (discussed below) are being developed that may be able to provide more concrete evidence of the potential dangers of these head impacts.

1.2.3 RSHIs and fluid biomarkers

Biomarker sampling is a useful tool in detecting brain dysfunction in more severe types of brain injury (Anto-Orach et al, 2017), and is also showing promising results in the assessment of concussion (Papa et al, 2015). The appeal of this technique in concussion-related injuries stems from its objectivity. Tools such as cognitive function and postural control (discussed below), whilst useful and sensitive to brain changes following mTBI, are subjective. This is particularly important in a sporting environment because athletes can manipulate their results, that is, they purposefully perform poorly during baseline testing so that if they receive a concussion the test will not detect the impairment (Erdal, 2012). It then follows that a more objective measure of brain dysfunction to determine whether an athlete can safely return

to play would be desirable. The concentration of a certain brain-related chemical in the blood or cerebrospinal fluid (CSF) is independent to the motivations of an athlete; the alterations in the quantity of blood and CSF-derived biomarkers should therefore reflect biochemical changes in the brain as a result of concussive injuries. As will be seen below, researchers have also begun to apply this technique in subconcussive models, to assess whether it can complement other measures of brain function such as balance and cognitive performance.

Fluid-borne biomarkers can be divided in two major groups, blood-derived and CSF-derived. Both methodologies have advantages and limitations, which will be discussed below. Moreover, each group of proteins reflects damage or dysfunction of a particular portion of the brain. The CSF-derived proteins identified as the best indicators of axonal damage are total tau and neurofilament light polypeptide (NFL) (Zetterberg et al, 2013). Tau proteins are tasked with stabilizing axonal microtubule structures and are found predominantly in cortical interneurons, characterized by thin, non-myelinated axons. Interestingly, deposits of hyperphosphorylated tau proteins, as a result of repetitive exposure to mTBI, have been linked with neurodegenerative diseases, such as CTE (McKee et al, 2009; McKee et al, 2014). Similarly to tau, the primary purpose of NFL is to provide support to the neuron's axon. However, this protein is mostly expressed in bigger myelinated axons that project into deeper cortical areas and spinal cord (Friede et al, 1970; Zetterberg et al, 2013). Due to their location within axonal structures, an increase in the concentration in the CSF of these two proteins would suggest axonal damage, possibly through the shearing or stretching action the neurons are subjected to during a concussive injury.

Proteins have also been shown to reflect neuronal, rather than axonal, damage. For example, neuron-specific enolase (NSE) is an enzyme found in great numbers in neuronal bodies. Due to the location where it's predominantly found, an increase of NSE in the CSF would indicate neurodestructive or

neurodegenerative processes taking place following injury (Scarna et al, 1982). However, care should be taken when using this particular protein as it is highly sensitive to hemolysis: NSE levels can increase following sample collection due to *in vitro* lysis of red blood cells that may have contaminated the sample (Zetterberg et al, 2013). Other proteins have been linked with glial injury: S100 calcium-binding protein B (S100B) is part of a family of neurotrophic proteins responsible for regulating intracellular levels of calcium, and are found mostly in astrocytes and oligodendrocytes (although they are also found in some non-cerebral tissue such as adipocytes). Elevated levels of S100B in the CSF or blood are usually indicative of damage to the nervous system. Similarly, elevated levels of glial fibrillary acidic protein (GFAP), a CNS specific protein almost exclusively found in astroglia, have also been reported following mTBI (Zetterberg et al, 2013). Other neurotrophic proteins, such as nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) may also have diagnostic value in TBI detection. Because of their neuroprotective role, an increase in these proteins post-injury suggests a healing mechanism, designed to aid in cell proliferation and survival (Bamac et al, 2011).

A few studies (Stalnacke et al, 2004; Stalnacke et al, 2006; Kawata et al, 2017) showed that blood serum biomarkers of brain damage, S-100B and NSE, were increased following a single competitive soccer game; furthermore, S-100B appeared to correlate with the number of headers players received (Stalnacke et al, 2004; Stalnacke et al, 2006). Similarly, a single boxing match increases blood-derived S-100B and NSE, suggesting that repetitive blows to the head trigger mild neuronal damage (Graham et al, 2011). Another study assessed BDNF and NGF after a bout of soccer heading, finding significant increases in both parameters (Bamac et al, 2011). All these studies point towards repetitive microtrauma (from head impacts) as a probable mechanism. However, a caveat to the findings is that most of these proteins (S-100B, NSE, BDNF, NGF) are also found extracerebrally and can be released either as a result of traumas to the body (and not head) or as a result of exercise (Unden et al, 2005;

Koh and Lee, 2014). Therefore, the interpretation of the data becomes problematic, as it is impossible to discern whether the alterations in the biomarkers are due to the repeated heading, other types of small traumas to the body, or exercise (Zetterberg et al, 2007). In addition, it has been suggested that the optimal time window in which fluid biomarkers reflect alterations due to heading is 7-10 days post, based on a study on stroke dynamics (Zetterberg et al, 2007); to corroborate this notion, serum samples taken at those time-points show no association between heading and changes in S-100B (Zetterberg et al, 2007).

As already mentioned, the best markers for neuronal injuries are those found in the CSF, as the proteins are in direct contact with the brain interstitial fluid and not separated by blood-brain and blood-cerebrospinal barriers (Zetterberg et al, 2007). Biomarkers of neuronal tissue damage (NFL, tau, GFAP, amongst others) taken from the CSF were found to be acutely (7-10 days) increased following a boxing bout (with no knock out hits) compared to healthy controls (Zetterberg et al, 2006). All parameters were transiently increased, and there were no differences between boxers and controls after three months of rest from sparring (Zetterberg et al, 2006). However, when assessing CSF-related changes in amateur soccer players, tau, GFAP and S-100B show no alterations as a result of heading the ball (Zetterberg et al, 2007).

Whilst fluid-borne biomarkers are an appealing tool in concussion and subconcussion detection due to their objectivity and apparent sensitivity, more evidence on which specific protein(s) are best indicated for quantifying brain dysfunction is needed, particularly when assessing alterations as a result of subconcussion. Blood-derived markers would be ideally suited for the task as the sampling technique is relatively easy and pain free; yet most of the proteins of interest increase as a result of exercise, or

other non-head related traumas. It is therefore possible that the increases in serum-derived proteins are a "false positive" and do not reflect any direct changes due to RSHIs. On the other hand, though samples taken from CSF may directly relate to brain alterations, the procedure to obtain the samples is invasive, painful, requires specific training, and cannot be routinely performed. Therefore, whilst fluid biomarkers may be appealing for their objectivity, they need to be developed more before the techniques can be routinely used as an accurate indicator of brain changes as a result of RSHIs.

1.2.4 RSHIs and neuroimaging

Neuroimaging techniques such as magnetic resonance imaging (MRI), diffused tensor imaging (DTI), magnetic resonance spectroscopy (MRS) and computed tomography (CT) have been widely used to assess structural and functional changes to the brain following mild, and more severe types of TBIs (Prabhu, 2011). Due to their apparent sensitivity in detecting brain injuries, researchers have also applied these techniques in studies assessing cumulative exposure to subconcussive head impacts.

The first few studies assessed how frequent abnormal CT scans appeared in active boxers following a career in the sport. They showed that although the frequency of abnormal scans was relatively low (5-7% abnormal; 22-40% borderline abnormal) they were associated with a higher history of loss by knock-out or technical knock-out (Jordan et al, 1992a; Jordan et al, 1996). Grey and white matter densities, as well as cortical thickness, have also been shown to be abnormal in soccer players with repeated exposure to head impacts (Adams et al, 2007; Koerte et al, 2012; Lipton et al, 2013; Koerte et al, 2016). These findings are of interest because whilst they appear independent to concussion history, the alterations in brain structure are similar to those seen in patients with TBIs (Koerte et al, 2012; Lipton et al, 2013). Neuroimaging tools are not only limited to brain structure. When assessing neurochemistry

biomarkers using MRS, Koerte and collaborators (2015a) showed that soccer players with a high frequency to heading exposure exhibited higher levels of choline (Cho) and myo-inositol (mI). Increased levels of Cho are indicative of membrane damage; whist mI reflects glial activity (Koerte et al, 2015a). Additionally, glutathione (GSH) (responsible for removing damaging metabolites in the brain) was also positively correlated with exposure to RSHIs. All together, the findings from this study suggest that RHSIs trigger neuroinflammatory processes in the brain without any evidence of degeneration (Koerte et al, 2015a,b).

Assessing serial CT scans of active boxers also highlighted progressive changes in brain structure (Jordan et al, 1992b). The scans, although being a gross measure of structure, showed that boxers displayed progressive atrophy. Further, a small subset of participants also developed cavum septum pellucidum (CSP), suggesting ventricular dysfunction. One particular boxer, as well as developing severe ventricular atrophy also developed bilateral parietooccipital encephalomalacia (i.e. a softening of cerebral tissue, probably due to haemmorhages or inflammation) (Jordan et al, 1992b).

The findings suggest that repeated exposure to blows to the head may be associated with neurological impairment (Jordan et al, 1992b). Similarly, a more recent study used functional MRI to assess cerebrovascular reactivity (CVR - a compensatory mechanism to regulate cerebral blood flow) in female high school soccer players following a playing season, demonstrating quantifiable decreases in the activity of this mechanism, similar to those observed in mTBI (Svaldi et al, 2016). The results from this and previous studies suggest that athletes exposed to repetitive head impacts could actually be experiencing very low levels of brain injury, in the absence of externally visible symptoms (Svaldi et al, 2016). One study assessing brain structures of soccer players at baseline, and after 5 years of playing

found no brain abnormalities, implying that soccer heading does not affect brain structure (Kemp et al, 2016). However, as well as having a limited sample size for a longitudinal study, the athletes were young and at the beginning of their career. It is possible that they may not have been exposed to enough head impacts to develop any actual changes in cerebral structure. Furthermore, the neuroimaging technique used (T1 and T2 weighed MRI) may not have the required resolution needed to detect small brain abnormalities.

Although promising, the limiting factor of most neuroimaging studies is that they are either cross-sectional, as in they quantify brain structure or neurochemistry at one time point, or that they choose arbitrary time points to assess athletes. Using one time point cannot really predict or shed light on whether the observed alterations are permanent or can be reversed with a period of rest from head impacts; multiple time points circumvent this limitation, however, the chosen follow ups are not based on hypothesis or evidence as to why a change would be expected at that particular point in time. The majority of the studies cited also report a low number of participants, which may have affected the statistical outcome of the analyses. Furthermore, the preferred mode to assess heading or sparring frequency was self-report which may not give a true representation of RSHI exposure. Lastly, whilst neuroimaging techniques are well established in the detection of structural changes in the brain (e.g. atrophy, CSP, changes in grey and white matter), they are unable to quantify encephalopathy-related alterations (Shetty et al, 2016); this notion is particularly important when trying to quantify the contributions of subconcussive head impacts to the onset of CTE and it suggests that they may not be best suited for assessing cumulative head impacts in the context of long term complications to brain health.

1.2.5 RSHIs and motor control

The majority of the studies described thus far assessed brain function only in the context of neurocognitive performance, cerebral imaging or by using fluid biomarkers. In order to gain a more comprehensive understanding of brain dysfunction following concussive injuries, researchers integrated cognitive function with measures of postural stability; Guskiewicz et al (1997) even reported that postural control combined with neuropsychological tests may be more reliable in detecting brain impairments following sport-related head injury than one technique alone. It is therefore unsurprising that researchers began applying this parameter of motor control to subconcussive models. Assessing postural deficits following a soccer ball heading protocol showed that RSHIs either did not affect balance (Mangus et al, 2004; Broglio et al, 2004; Schmitt et al, 2004) or impaired it (Haran et al, 2013; Hwang et al, 2017). Whilst the findings appear contradictory, the diverging results are probably due to different study designs, as balance was quantified using different techniques and ball speeds differed between studies. Furthermore, in some instances participants were not familiarized with the testing procedures (e.g. Broglio et al, 2004) and ball speeds were not standardized across participants (e.g. Schmitt et al, 2004), factors that may have contributed to the differing results.

Postural stability is a fairly gross measure of motor control (Jacobson and Shepard, 2016). Whilst it may be useful in detecting more serious trauma to the head it is possible that it is not sensitive enough to detect small changes due to subconcussive impacts. In fact, the two studies that found an association between head impacts and postural control implemented more precise techniques, using a combination of dynamic, static and virtual environments (Haran et al, 2013) or vestibular function (Hwang et al, 2017) to quantify balance. A different subset of motor control, oculomotor control, also showed promise in detecting changes associated with subconcussive impacts. Near-point of

convergence (NPC), a measure of binocular function, has been shown to worsen following routine soccer heading (Kawata et al, 2016). Since NPC is controlled through the oculomotor system, a change following RSHIs suggests an alteration to motor control. Although promising, these findings are preliminary and require further corroboration.

Regardless of whether postural control is effective in detecting brain alterations or not, a decrease in balance following a blow (may that be concussive or non-) to the head suggests, at the very least, a slight impairment of the control the brain has over the muscles. This decrement in muscle control is feasibly mediated by an alteration in motor unit recruitment. As such, tools that provide information on individual motor units should shed light on the mechanisms linking head impacts and motor control impairments. Whilst there have been no studies assessing the effect of RSHIs (or TBI for that matter) on motor unit behaviour, such a parameter should be mentioned as it may prove useful in the context of brain injuries: using techniques such as precision decomposition electromyography (dEMG), researchers would able to assess motor unit function following concussion, or RSHIs, by extracting the information from surface (sEMG) techniques (De Luca et al, 1982; Adam and De luca, 2005; Nawab et al, 2010; Kline and De Luca, 2014).

1.2.6 Interim conclusion

Overall, the effects of RSHIs on brain function are difficult to interpret. Even when using similar methodologies and protocols, different studies report divergent results. Postural stability, for example, appears either to not be affected (Mangus et al, 2004; Broglio et al, 2004) or affected (Haran et al, 2013; Hwang et al, 2017) by RSHIs; similarly fluid biomarkers appear increased (Stalnacke et al, 2004; Kawata et al, 2017) or unchanged (Zetterberg et al, 2007) following subconcussive head impacts.

Furthermore, measures such as cognitive function and postural stability, whilst useful, are not truly objective as they depend on the motivation and ability of the participant or athlete. Regardless, of interest is the notion that when assessing brain changes using more objective and unbiased techniques (e.g. specific parameters of motor control or blood and cerebral biomarkers), changes following RSHIs are more prominent, suggesting that indeed there is an association between subconcussion and acute brain alterations.

The next portion of this chapter will discuss a technique that has already proved itself useful in detecting brain alterations following TBIs, and that may be effective in quantifying subtle changes as a result of RSHIs: transcranial magnetic stimulation (TMS). The appeal of this technique is its sensitivity in detecting brain changes (De Beaumont et al, 2007); furthermore, its ability to potentially highlight neurochemical changes within the brain would shed light on the processes underlying the relationship between RSHIs and cerebral function.

1.3 Transcranial magnetic stimulation (TMS)

Transcranial magnetic stimulation (TMS) is a technique based on the principle of electromagnetic induction, first described by Michael Faraday in 1831. A changing magnetic field generated by a current passing through a coil of wire creates a concomitant electrical field, able to penetrate the bony structures of the skull and induce currents to flow in the brain (Figure 1.1). Through adequate stimulation of the primary motor cortex (M1) researchers can trigger pyramidal neurons (primary excitation units of the corticospinal tract) to induce efferent volleys down the corticospinal pathways. The volleys, in turn, generate responses in the target muscles contra-lateral to the stimulation site, referred to as motor evoked potentials (MEPs). The MEPs can be easily assessed by integrating TMS with electromyographic (EMG) recordings and allow us to reliably measure the excitability and inhibition of the pyramidal tract, as well as its cortico-cortico and cortico-spinal mechanisms (Ferreri et al, 2011).

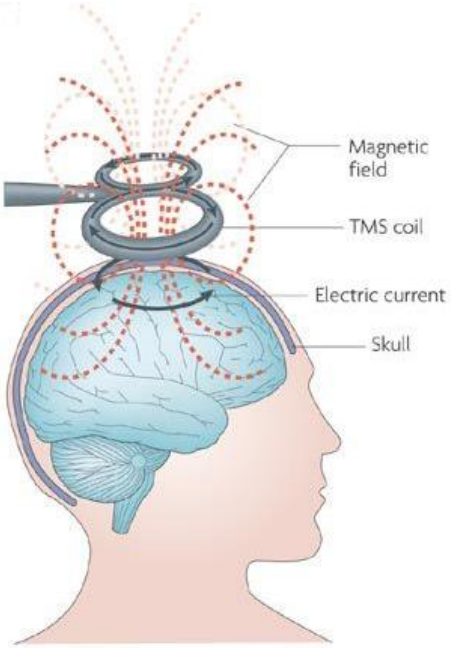


Figure 1.1. Illustration of the electromagnetic induction principle, the magnetic field generated by the coil induces an electric current that is able to activate the underlying neuronal pool. Adapted from Ridding and Rothwell, 2007.

TMS is an extremely versatile technique: it can be applied in neurocognitive studies to temporarily interfere with, or induce transient changes in the function of the motor cortex and other brain areas (Robertson et al, 2003). In physiology TMS can be used to quantify the contributions of spinal and supraspinal mechanisms to muscle fatigue (Gandevia, 2001; Taylor and Gandevia, 2001), neural adaptations as a result of strength training (Jensen et al, 2005; Gruber et al, 2009) or even cortical excitability and inhibition in a range of movement disorders (Hallett and Rothwell, 2011).

TMS can be divided in three "sub-techniques", depending on the number (or frequency) of the pulses delivered over the scalp: single pulse TMS, paired-pulse TMS (p-pTMS) and repetitive TMS (rTMS). Very briefly, p-pTMS allows researchers to assess inhibitory and facilitatory mechanisms within the motor cortex by combining a subthreshold conditioning stimulus followed by a suprathreshold test stimulus at varying interstimulus intervals (hence the term 'paired'). Repetitive TMS can disrupt, inhibit or facilitate cortical function depending on the frequency (low v high) at which trains of TMS pulses of the same intensity are delivered over the brain area (Kobayashi and Pascual-Leone, 2003). The section below will highlight the principal parameters that can be assessed with single pulse TMS, as well as discussing their use in the detection of TBIs.

1.3.1 Single pulse TMS

As the name suggests, single pulse TMS is the application of one pulse over the primary motor cortex (M1), with the concomitant MEP recorded in the target muscle. A number of measures can be obtained by stimulating M1, the most commonly used are: motor threshold (MT), corticospinal excitability, corticomotor inhibition (or corticosilent period – cSP).

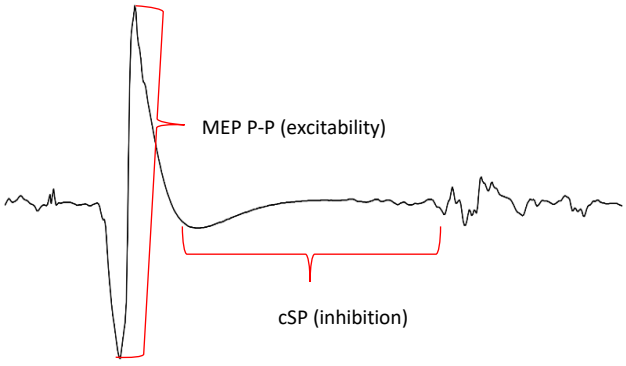


Figure 1.2. Trace of a motor evoked potential recorded in the RF muscle. The peak-to-peak amplitude of the potential reflects a parameter of the excitability of corticospinal pathways, whilst the duration of the silent period following the MEP is an indication of the levels of corticomotor inhibition.

Motor threshold refers to the lowest stimulus intensity needed to produce a detectable MEP in the target muscle. It is thought to reflect membrane excitability of interneurons and corticospinal neurons within the motor cortex, as well as the excitability of neurons in the spinal tract, neuromuscular junction and the muscle itself (Ziemann

et al, 1996; Kobayashi and Pascual-Leone, 2003). Corticospinal excitability can be quantified as the amplitude (peak-to-peak) of the MEP waveform (Figure 1.2); it reflects the integrity of the corticospinal tract, and the excitability of the motor cortex and nerves. Furthermore, it also provides an understanding of how well electrical impulses can propagate along said corticospinal tract (Kobayashi and Pascual-Leone, 2003). Even in healthy individuals MEP amplitude exhibits great inter- and intra-subject variability, making interpretation of raw values difficult (Kobayashi and Pascual-Leone, 2003). For this reason it is not uncommon for the parameter to be expressed as a percentage of a given maximal response. In physiology studies the maximal excitability of the muscle (Mmax – assessed through peripheral nerve stimulation) is commonly used to assess the proportion of the motor unit (MU) pool TMS is able to activate (Goodall et al, 2012b).

Corticomotor inhibition is quantified as the duration of the cSP, a period of EMG inactivity of a few hundred milliseconds following the MEP (Figure 1.2). The first part of the cSP reflects spinal inhibitory mechanisms, whilst the latter part (>50ms) is thought to be due to intracortical inhibitory mechanisms, mediated by the neurotransmitter gamma-aminobutyric acid (GABA) and its type B receptor ($GABA_B$) (Inghilleri et al, 1993; McDonnell et al, 2006). Whereas MT and excitability can both be assessed whilst the muscle is at rest or during a contraction, due to its inherent methodology corticomotor inhibition can only be assessed whilst the muscle is activated.

1.4 TMS and TBI

One of the hallmarks of TBIs is an impairment of motor control, usually characterized by a decrease in postural stability and deficiencies in gait (Sosnoff et al, 2011; Williams et al, 2009). Since TMS had been shown to be reliable in assessing the function and integrity of the brain-to-muscle pathways (Goodall,

et al, 2012), it was only a question of time before researchers started investigating its effectiveness in quantifying brain dysfunction as a result of TBIs. Furthermore, there was the knowledge that ischaemic cortical damage caused by strokes and neurodegenerative diseases such as Parkinson's were quantifiable when using TMS (Catano et al, 1997; Classen et al, 1997; Valzania et al, 1996; Young et al, 1997). Therefore it was reasonable to hypothesize that TMS would be able to detect brain alterations as a result of traumatic injuries.

1.4.1 Corticomotor inhibition and TBI

When assessed in the context of acute TBI, corticomotor inhibition has been observed increased compared to a cohort of healthy, non-injured controls (Pearce et al, 2015; Miller et al, 2014). Increased duration of the silent period acutely following injury suggests that TBIs affect the normal functioning of GABA-mediated mechanisms, with impairments persisting for weeks following the trauma (Chistyakov et al, 2001). Inhibition also appears increased in longitudinal assessments ranging from months (De Beaumont et al, 2011; Miller et al, 2014) to years (Tremblay et al, 2011) and even decades (De Beaumont et al, 2009) following a traumatic injury.

Cumulative traumatic injuries also affect corticomotor inhibition, as studies report that cSP is elongated in athletes who had experienced multiple concussions (De Beaumont et al, 2007; Tremblay et al, 2011). Furthermore, athletes who received another concussion following a first bout of testing showed an even greater increase in inhibition (De Beaumont et al, 2007). Similarly, a case study of 3 Australian rules football athletes who received 2 concussions in one season showed a cumulative effect on cSP (Pearce et al, 2014a). However, the results from Pearce et al, (2014a) should be treated with caution as the very small sample size makes it difficult to generalize to a greater population.

The complex neurochemical alteration processes resulting from mechanical stretching of neuronal axons have been thoroughly described, most notably by Giza and Hovda (2014). In brief, biomechanical injury within the brain causes alterations in ionic gradients (sodium, potassium and calcium) across cell membrane, as a result of damage to the phospholipid layers. Furthermore, disruption of neuronal cell membranes also releases excitatory and inhibitory neurotransmitters (e.g. glutamate and GABA) (Giza and Hovda, 2014; Pearce et al, 2014a). In an attempt to restore homeostasis, ATP-dependent pumps in the membrane increase their activity causing a state of hyperglycolysis; this hyperglycolytic state quickly turns into one of hypoglycolysis due to the disparity between metabolic demand and supply (probably due to shunted cerebral blood flow following injury) (Giza and Hovda, 2014). Ion fluxes (particularly calcium) can also damage neuronal supporting structures, and axons are particularly vulnerable to mechanical stretching (Giza and Hovda, 2014). Studies also show that myelinated and unmyelinated axons respond differently to brain injuries, with the latter ones being more vulnerable to damage (Reeves et al, 2005). The data from studies showing an acute increase in corticomotor inhibition following injury (Pearce et al, 2014a; Pearce et al, 2015; Miller et al, 2014) suggest that this inhibitory mechanism may reflect the neurometabolic cascade that occurs in the brain: post-concussive alterations in neurotransmitter activity (e.g. glutamate and more importantly GABA) as a result of cell membrane disruption may affect measures of cortical inhibition (Pearce et al, 2014a).

A different mechanism for increased corticomotor inhibition has been proposed following observation of the silent period of patients suffering from hemiparetic stroke (Classen et al, 1997). The authors reported that those stroke patients exhibited abnormally high cSP values following stimulation of their affected side, when compared to measures recorded from the unaffected portion of the brain, as well as from healthy controls. They suggest that although the lesions did not primarily affect, or involve M1 they effectively caused a loss of afferentiation to the motor cortex: damage to thalamo-, striato- and

cortico-cortical nerve fibres could decrease the excitatory drive to intracortical inhibitory interneurons. This pathological loss of excitatory input would result in overactivity of the inhibitory interneurons, resulting in an increase in corticomotor inhibition (Classen et al, 1997). Although these findings were reported in stroke-related brain damage, it is possible that the same mechanism is applicable to injuries of a traumatic nature.

However, increased GABA acutely following injury may not necessarily indicate solely brain dysfunction; In addition to the blockade of glutamate receptors, the acute efflux of the inhibitory neurotransmitter following TBI may be a mechanism the brain uses to protect itself from the excitotoxic results of glutamate release from the cells (O'Dell et al, 2000). Using a rat-based study, O'Dell and colleagues (2000) showed that rats treated with GABA-receptor agonists performed better in cognitive tasks than the control group following fluid percussion brain injury. The findings suggest that enhanced inhibitory function following head injury is beneficial for survival and to maintain cognitive function (O'Dell et al, 2000). Whilst initially beneficial, it is possible that this protective mechanism becomes maladaptive if repeatedly triggered (i.e. cumulative TBIs), with excess GABA creating a toxic environment within the cortex and ultimately hindering brain health (Demirtas-Tatlidede et al, 2012).

Not all studies show increased corticomotor inhibition as a result of TBIs, a couple showed no difference between a concussed group and a healthy control neither acutely (Powers et al, 2014) nor longitudinally (Tremblay et al, 2014; Bernabeu et al, 2009). Several factors could explain the occurrence of these divergent results. The number of concussions varied between studies, which could influence the results. Further, the time between the injury and the assessment also differed greatly between studies; if enough time passed from injury, the inhibitory dysfunction may have already recovered. Lastly, it

may simply be that alterations to the inhibitory system may not be a stable symptom of the neurological response to brain injury (Tremblay et al, 2014). It is also possible that the diverging results are due to methodological factors: different levels of muscle activation and different stimulation intensities may have influenced the results.

1.4.2 Corticospinal excitability and TBI

Corticospinal excitability appears to be less able to detect TBIs, with most studies showing no alterations (Chistyakov et al, 1998; De Beaumont et al, 2007; De Beaumont et al, 2009; Miller et al, 2014; Pearce et al, 2015), and less showing a decrease in the parameter following injury (Bernabeu et al, 2009; Chistyakov et al, 2001; Livingston et al, 2010; Livingston et al, 2012). The reason for the diverging results within the studies assessing corticospinal excitability may be due to the different methodologies implemented for assessing corticospinal excitability. Whilst corticomotor inhibition is fairly straightforward when measured using single pulse TMS (one measure, cSP), there are a number of ways in which the excitability of the corticospinal pathways can be quantified. Such parameters include MEP amplitude, MEP area, MEP latency, input-output curves and many more. For this reason it is possible that whilst one parameter is able to detect concussed states, another may not.

The parameters commonly used to assess corticospinal excitability in a TBI context are MEP amplitude, MEP latency and input-output curves. Assessing MEP peak-to-peak amplitude has shown significant decrements in the ability of the corticospinal pathways to transmit electrical impulses (Chistyakov et al, 2001; Livingston et al, 2010) whilst other studies show no change (Pearce et al, 2014b; Miller et al, 2014; Pearce et al, 2015). As mentioned in section 1.3.1, standard practice when measuring the amplitude of the MEPs (at least in physiology-based studies) is to normalize the averaged mean to the

maximal response of the muscle (Mmax) in order to account for some of the inter-subject variability. It is of interest that the two studies that found a significant interaction between TBI and MEP amplitude expressed their data as a MEP/Mmax ratio (Chistyakov et al, 2001; Livingston et al, 2010), whilst two of the three studies that found no changes presented raw values instead of normalized percentages (Pearce et al, 2014b; Miller et al, 2014). This could explain the lack of significant findings, as the increased variability of the MEPs could have masked any effects. Whilst Pearce and colleagues (2015) do normalize their MEP data, they still show no effects of TBI on excitability (whilst observing increased corticomotor inhibition – discussed in section 1.4.1) and are unable to explain why one parameter appears affected and the other does not (Pearce et al, 2015).

MEP latency is thought to reflect the integrity of fast-conducting fibres along the corticospinal tract, and is quantified as the time difference between stimulus delivery over the scalp and the onset of the MEP (Pearce et al, 2009; Miller et al, 2014; Livingston et al, 2010). When assessed following TBI, MEP latencies appear prolonged compared to a control group (Livingston et al, 2010; Livingston et al, 2012). Such an increase suggests a reduction in the neural conduction speed along the corticospinal pathways, as a result of demyelination of axons; furthermore, the complex neurometabolic cascade that occurs in the brain following TBI (refer to Appendix B for more information) triggers changes in ionic concentrations (e.g. K^+, Na^+, Ca^+) and the release of excitatory neurotransmitters (e.g. glutamate) (Giza and Hovda, 2014). Combined with axonal damage, they are also likely to affect the conduction of the MEPs down the corticospinal tract (Livingston et al, 2012). Other studies, however, found no relationship between MEP latency and TBI even though they used similar stimulation intensities (120-130% MT) (Chistyakov et al, 1998; Miller et al, 2014; Pearce et al, 2015). An explanation for the diverging results (that may also account for the results found by Pearce et al, 2015) is that TBIs may

affect different neurophysiologic mechanisms (i.e. inhibitory rather than excitatory), or simply that TBIs do not disrupt the conduction ability of the motor pathways.

Input-output curves (I-O - also referred to as recruitment curves) are obtained by consecutively stimulating M1 with increasing stimulator intensities, leading to higher MEP amplitudes with each stimulation. The slope of the MEP curve generated by this test is thought to be indicative of the excitability of the corticospinal pathways, a steeper slope indicating increased excitability (Lefebvre et al, 2015). Of the four available studies, one showed a rightwards shift in the I-O curve suggesting decreased excitability (Bernabeu et al, 2009) and three showed no changes (De Beaumont et al, 2007; De Beaumont et al, 2009; Pearce et al, 2014b) following TBIs. Bernabeu's cohort of participants was made up of patients with diffuse axonal injury (DAI); moreover, the significant difference was found in those patients with severe DAI, suggesting that only more serious types of injury affect this parameter. The notion is further corroborated by the finding that the I-O curve was not different in young asymptomatic athletes with less severe injuries when compared to a control group (De Beaumont et al, 2007). The studies published by De Beaumont et al, (2009) and Pearce et al, (2014) assessed retired athletes in order to determine the effect of repeated concussion exposure on motor function, finding no alterations in the I-O curves. These findings suggest that recruitment curves are not affected by cumulative exposure to very mild TBIs over the course of a career.

Overall, evidence suggests that TMS is able to detect concussion and more severe types of TBIs with varying degrees of success. Whilst both excitability and inhibition parameters have been assessed in the context of brain injury, it appears that inhibitory mechanisms are better suited as they have been shown to be more susceptible to alter as a result of TBI. However, the differing methodologies

implemented by various research groups (i.e. using different stimulator intensities, varying quantifications of MTs, different parameters to assess excitability) result in diverse, often conflicting findings. It is possibly for this reason that two recent systematic reviews on the use of TMS in assessing brain changes following TBIs concluded that although TMS may have prognostic value in detecting neurophysiological alterations, the lack of research does not allow to draw any final conclusions (Major et al, 2015; Lefebvre et al, 2015). Nevertheless, Lefebvre and colleagues do suggest that amongst all the available measures, corticomotor inhibition may be the most reliable in detecting TBIs (Lefebvre et al, 2015).

Also of interest is the notion that when alterations in the brain, as a result of TBIs, are studied using multimodal approaches (for example neuropsychologic and electrophysiologic) the results show different recovery rates, with cognitive performance returning to baseline levels faster than electrophysiological parameters (Pearce, 2015; Livingston et al, 2012). The finding suggests that whilst cognitive function may appear recovered, there are nevertheless dysfunctions still present in the brain. This is particularly important in a sporting context where the majority of post-concussive assessment is based on neuropsychological performance. If an athlete returns to play with apparently normal cognitive function, but still has motor impairments as a result of a concussion they may be more prone to other types of injuries; recent studies have in fact shown that players with a history of concussion are more susceptible to musculoskeletal injuries than a healthy cohort (Nordstrom et al, 2014; Lynall et al, 2015; Cross et al, 2017).

1.5 Conclusion

This chapter discussed RSHIs and how they are thought to affect brain health, whilst being seemingly innocuous in the acute stages. The techniques that may be effective in detecting brain alterations, following cumulative head impacts, have limitations that prevent them from being fully reliable; some may not be sensitive enough to detect subtle changes following RSHIs (i.e. postural control) and others can be manipulated by patients or athletes, providing "fake" baseline data (i.e. cognitive function and postural control). What is needed is a technique that is fast, reliable, affordable, relatively painless, and able to provide direct evidence of brain alterations as a consequence of repetitive head impacts.

Consequently, the contention at the core of this thesis is that TMS is well suited for assessing brain alterations as a result of RSHIs, as it can provide a direct link between alterations at a cortical and neuromuscular level (i.e. motor control). Other techniques (i.e. cognitive function, fluid biomarkers, brain scans), whilst useful, are not able to provide us with information of how head impacts affect the brain, and are translated in changes in peripheral systems. Not all parameters of motor control are able to do so; for example, postural control appears to be too much of a gross measure to reliably detect imbalances following RSHIs. Although there have been no studies to date assessing the effectiveness of TMS in identifying such brain alterations, we believe it to be an ideal tool for the task. Whilst TMS is still an indirect measure of neurotransmitter activity in the brain, it nevertheless provides an indication of GABA activation, as well as providing an accurate and sensitive measure of brain-to-muscles pathways. Since one of the principal symptoms of concussion/TBI is a decrease in the brain's control of the musculoskeletal system, and subconcussive impacts are sometimes referred to as very minor brain injuries, it is conceivable to hypothesize that TMS would be able to assess changes as a result of RSHIs.

Amongst the various TMS parameters available, we chose corticomotor inhibition as our primary outcome measure because, as shown above, it is thought to be the most reliable parameter in detecting TBIs and thus may be best suited for subconcussive head impacts. Accordingly, the aims of this thesis are:

- To determine the inter- and intra-subject reliability of the primary outcome measure corticomotor inhibition using baseline data from the experimental projects – Chapter 2.
- To assess whether TMS is able to detect acute alterations in motor control following RSHIs from two different sports (i.e. soccer and boxing) – Chapters 3 and 4.
- To examine whether TMS measures are affected by (control) exercises replicating the metabolic demands of soccer heading and sparring, in the absence of RSHIs – Chapter 5.
- To explore the feasibility of using TMS to detect brain alterations following a season of soccer play – Chapter 6.

The overall hypotheses of the thesis are:

1. That corticomotor inhibition would be transiently increased as a result of RSHIs, and unchanged following the control exercises.

2. That the increased inhibition would be combined with decreases in the secondary outcome measures of a) corticospinal excitability, b) cognitive function and c) postural control.

Chapter 2

The reliability of TMS-derived indices of corticomotor inhibition and corticospinal excitability in two distinc populations

Abstract

Introduction: TMS has been shown to be effective in detecting brain alterations following mild (concussive), moderate and severe types of brain injuries. Recent evidence also suggests that corticomotor inhibition is better able to detect traumatic injuries than corticospinal excitability. It is possible, therefore, that the technique may be able to assess small functional changes to the brain as a result of RSHIs. The aim of chapter 2 is to assess the reliability of corticomotor inhibition and corticospinal excitability using methodologies relevant to the thesis. **Methodology:** Thirty-nine participants were separated in "Population A" (soccer players, N=19, 5 females, age 22 ± 3 y) and "Population B" (recreationally active, N= 20, 4 females, age 24 ± 4 y). Corticomotor inhibition and corticospinal excitability were recorded using TMS during two testing sessions, spaced 1-2 weeks apart. Inter-day reliability was quantified by using intra-class correlation coefficients (ICC) and coefficients of variation (CV). **Results:** Corticomotor inhibition showed good overall reliability (ICC= 0.68 ± 0.07; CV= 6.78 ± 3.23), furthermore, the parameter was not different between Population A and Population B ($p > 0.05$). Corticospinal excitability also demonstrated good reliability (ICC = 0.66 ± 0.07; CV = 21 ± 5.23 %), however, Population A was significantly lower than Population B (45.1 ± 20.8 vs 81.3 ± 39.7 %Mmax, $p < 0.0001$). **Conclusion:** Corticomotor inhibition and corticospinal excitability are stable and maintain a good degree of reliability when assessed over different days. Corticomotor inhibition was confirmed as the primary outcome measure because it appears better suited to assess brain changes following head trauma, as well as having slightly greater reliability values than corticospinal excitability.

2.1 Introduction

In the General Introduction we discussed the various techniques that can be used to assess brain function following subconcussive and concussive head impacts. We also identified TMS as a potential tool for detecting such changes to neurological performance. Before evaluating the consequences of RSHIs on brain function using TMS, it is important to establish the reliability of the parameters and methodology we propose to use.

As mentioned in chapter 1, single pulse TMS allows researchers to quantify the cortico-cortico and corticospinal excitatory and inhibitory mechanisms that govern our motor skills. Various factors have been shown to affect the repeatability of these parameters, including the positioning of the coil over the scalp, the target muscle being investigated, number of stimuli applied over M1 and the stimulator intensity (Kobayashi and Pascual-Leone, 2003). Studies assessing the day-to-day reliability of corticomotor inhibition and corticospinal excitability show fair-to-excellent reliability (ICC values ranging from 0.52 to 0.92) (Bastani et al, 2012; Brown et al, 2017; Cacchio et al, 2009; Christie et al, 2007; Fisher et al, 2013; Lewis et al, 2009; Liu et al, 2014; O'Leary et al, 2015; Ngomo et al, 2012). Such a diverse array of results is most likely due to the different methodologies implemented when using TMS; the stimulator intensity varied between studies, ranging from 110% of the motor threshold (MT) to 175%MT. Furthermore, the contraction intensity of the target muscle also differed, with the majority of studies using very low percentages (10-30%MVC).

For the experimental projects of this thesis we decided to assess corticomotor inhibition at 100%MVC and corticospinal excitability at 20%MVC, both taken from the rectus femoris (RF); this methodology has already been implemented in previous studies (Goodall et al, 2009; 2012a,c). By measuring

corticomotor inhibition at 100%MVC we ensure to recruit a motor unit pool large enough to show an effect, even though this limits the number of feasible repetitions. Measuring cSP at lower intensity may not be as sensitive since a smaller pool of motor units is recruited, reducing relative effect sizes of GABA inhibitory mechanisms on EMG signals. In turn, this would make cSP measurements less sensitive in detecting subtle and transient corticospinal changes. The decision to record TMS parameters from the lower limbs was made because our intention was to try and relate any potential alterations in corticomotor inhibition and corticospinal excitability, as a result of RSHIs, to changes in postural control. As such, measures assessed from the lower, rather than upper limbs would have greater physiological validity in this context. We also chose to assess the brain-to-muscle pathways with a stimulator intensity of 130%MT; this commonly used supra-threshold output was applied to all participants to ensure they received the same relative intensity of stimulation (Goodall et al, 2012b).

The aim of this first experimental study was to explore the inter-day reliability of our primary outcome measure corticomotor inhibition measured at 100%MVC, and secondary measure corticospinal excitability measured at 20%MVC. This study will enable us to assess whether the inhibitory and excitatory parameters have the reliability required to detect potentially small and transient changes to the brain-to-muscle pathways. The analysis will be carried out on two separate groups, one sport specific (soccer players) and a more general population; these groups were chosen as we wanted to determine whether there were group-dependent differences in terms of reliability when comparing a cohort with a similar level of training to a less homogenous one. Based on previously published data we hypothesized that both parameters of motor control would exhibit fair-to-excellent reliability values. Furthermore, we expected the soccer population to show greater degrees of reliability when compared to the general population group, as they are the more homogenous population.

2.2 Methodology

2.2.1 Approvals and recruitment

A total of 39 participants were recruited for the study via advertisements on university noticeboards and social media. Individuals participating in soccer were sorted in a group referred to as "Population A" (N= 19; 5 females; age 22 ± 3 y; mass 72.9 ± 8.3 Kg; height 175.4 ± 10.2 cm), and the general public in "Population B" (N= 20; 4 females; age 24.3 ± 4 y; mass 76.4 ± 13.6 Kg; height 174.9 ± 9.8 cm). All participants were screened prior to taking part; potential candidates were excluded if they presented with any of the following: 1) history of brain injury resulting in loss of consciousness; 2) history of a neurological condition; 3) history of concussion in the 12 months prior to taking part; 4) family history of epilepsy; 5) current use of psychoactive recreational or prescription drugs. The local Research Ethics Committee approved the study and procedures conformed to the guidelines set out by the Declaration of Helsinki. Written informed consent was obtained from all participants prior to taking part.

2.2.2 Study design

Data for this study were collected as part of a bigger project. Participants were asked to refrain from vigorous physical activity, consuming alcohol, and caffeine or smoking for 24h prior to each study session. Participants were also required to present to the laboratory fasted where they were provided with a standardized breakfast. Prior to commencing data collection, participants reported to the laboratory for a familiarization session, during which they completed all outcome measures to acquaint them with the assessment procedures and minimize later learning effects. Following the practice session, participants reported to the laboratory for two further experimental days, spaced a minimum of one and a maximum of two weeks apart.

2.2.3 Transcranial magnetic stimulation

Motor evoked potentials (MEPs) were elicited in the rectus femoris of the dominant leg via single pulse TMS and assessed using electromyographic (EMG) recordings (see section 2.2.4). Single magnetic stimuli of 1 ms duration where applied over the contralateral primary motor cortex using a magnetic stimulator (Magstim 2002 unit, The Magstim Company Ltd., Whitland, UK) and a 110 mm double cone coil. Optimal coil location for generating MEPs was determined by placing the coil over the motor cortex, laterally to the vertex; the area where the largest MEP peak-to-peak amplitudes occurred was identified and marked on the scalp with ink (Goodall et al., 2009). The active motor threshold (aMT) for the quadriceps femoris was determined by increasing stimulator output from 10% by 5% increments, while the participant held a ~20% maximal voluntary isometric contraction (MVC) until discernible MEPs were visible (Wilson et al., 1995). Once this individual level was established, subsequent stimulations were delivered at 130% of aMT.

MEPs, alongside all other EMG measures, were recorded with participants sitting with their dominant leg secured to a calibrated load cell of an isokinetic dynamometer (Kin-Com, Chattecx Corp, Chattanooga Group Inc., Tennessee). Knee angle was set at 60° (0° being fully extended limb) and the arm of the dynamometer was set such that the axis of rotation was aligned with the participant's lateral femoral condyle. To assess the primary outcome measure corticomotor inhibition participants were required to perform MVCs of 5s duration while a single TMS stimulation was delivered over the motor cortex. This was repeated three times with 60s rest between contractions, as is common practice. Corticomotor inhibition was quantified as the cSP duration, taken from the stimulation artefact to the resumption of discernible, uninterrupted EMG activity from the muscle (Figure 2.1).

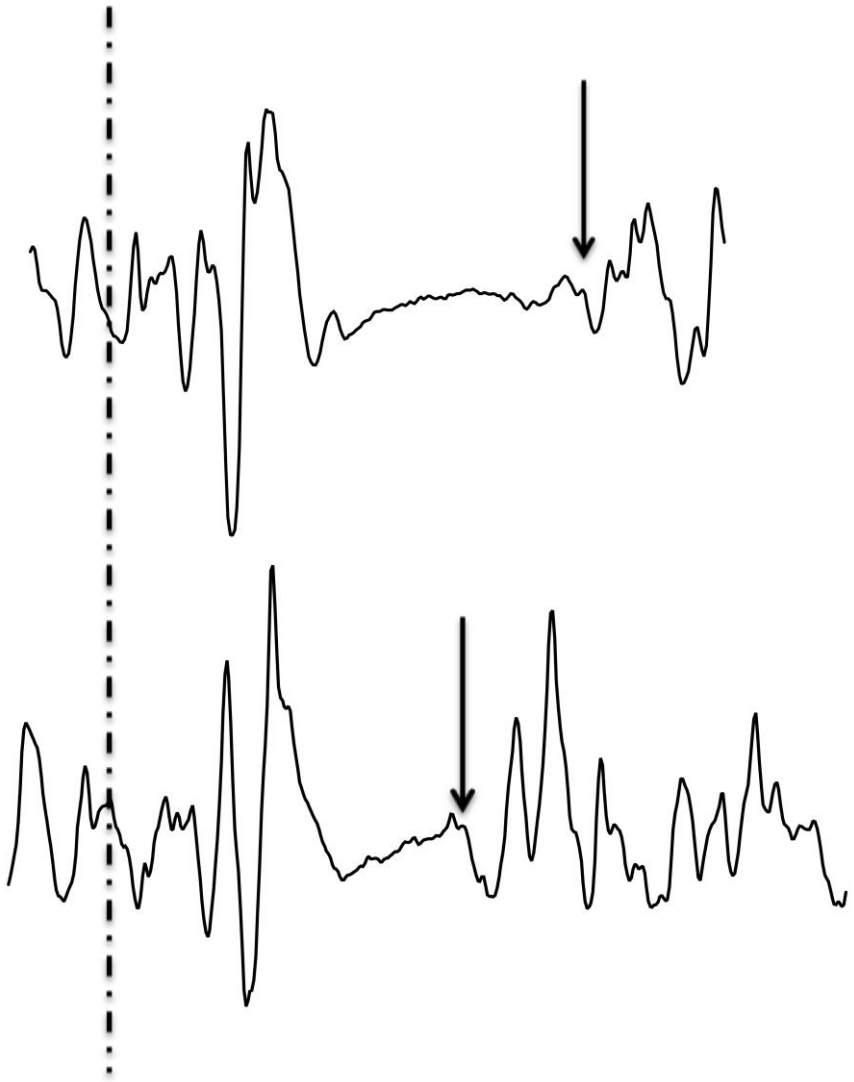


Figure 2.1. Raw cortical silent period (cSP) of two participants. The cSP was quantified as the period of time between the delivered TMS pulse (dashed line) and the resumption of uninterrupted EMG activity (arrows)

During the assessment of secondary outcome measure corticospinal excitability, participants maintained a 20% MVC isometric contraction while 20 single TMS pulses, separated by 6 s, were delivered over the motor cortex. Corticospinal excitability was determined as the average MEP amplitude normalized to the maximal response elicited by motor nerve stimulation (%Mmax, see below). We chose to assess cortical excitability and inhibition in the lower limbs rather than in the

upper limbs because of its functional relevance; in a sporting environment, changes in lower limb may be more valid as they relate directly to gait and performance.

2.2.4 Electromyography and femoral nerve stimulation

Electromyographic activity was recorded using a wireless system (Biopac Systems, Inc. Goleta, CA, USA). Data were sampled at 2 kHz, and filtered using 500 Hz low and 1.0 Hz high band filters. Signals were analyzed offline (Acqknowledge, v3.9.1.6, Biopac Systems, Inc. Goleta, CA, USA). EMG activity was assessed using Ag/AgCl surface electrodes (Vermed, Devon, UK) with an intra-electrode distance of 2 cm positioned over rectus femoris; prior to electrode placement, the area of interest was shaved and abraded as per Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) guidelines. The position of each electrode was marked with permanent ink to ensure consistent placement during subsequent visits.

Peripheral stimulation of the femoral motor nerve was administered using an electrical stimulator (Biopac Systems, Inc.). The stimulation site was identified by locating the femoral artery and placing a self-adhesive surface electrode (cathode) lateral to it, high over the femoral triangle, with the anode on the gluteus maximus. Single stimuli were delivered to the muscle while participants maintained a 20% MVC isometric contraction, and the intensity of stimulation was increased until a plateau in twitch amplitude and rectus femoris M-wave (Mmax) occurred. Supramaximal stimulation was delivered by increasing the final stimulator output intensity by a further 30%.

2.2.5 Statistical analysis

Data collected from the 2 laboratory visits were organized based on the time point at which they were recorded (Population A timepoint 1, Population A timepoint 2 – 2 week interval; Population B timepoint 1, Population B timepoint 2 – 1 week interval). Reliability of the measures within each population was quantified using intra-class correlation coefficients (ICC) computed by SPSS (v21; IBM Corporation), and coefficients of variation (CVs). ICC values were defined as following: ≤0.39 = poor; 0.4 – 0.59 = fair; 0.6 – 0.74 = good; 0.75 – 1 = excellent, as outlined by Cicchetti (1994). The CV values were calculated using the formula: **(σ / μ)*100**; where **σ** is the standard deviation, and **μ** is the mean of the sample. Analysis for statistical differences between groups was carried out using repeated measures ANOVAs on Graphpad Prism 6, comparing both populations at all time points. If significant differences were observed, Tukey's post hoc tests were used to further explore effects. Statistical significance was set at $p \leq 0.05$; data are expressed as means (± standard deviation) unless otherwise stated.

2.3 Results

2.3.1 Corticomotor inhibition

Corticomotor inhibition appeared stable across timepoints, as ICC analysis showed good reliability (0.68 ± 0.07) accompanied with moderately low CVs (6.78 ± 3.23%) (Table 2.1). Furthermore, no group differences were observed between populations ($p > 0.05$) (Figure 2.2).

2.3.2 Corticospinal excitability

Similarly to corticomotor inhibition, corticospinal excitability showed good reliability (ICC= 0.66 ± 0.07), however, with high CVs (21 ± 5.23%) (Table 2.1). The excitatory parameter was also different between populations ($p< 0.0001$; $F_{(3, 72)}= 11.6$; $\eta^2= 0.32$) (Figure 2.3), population A1 was significantly lower than

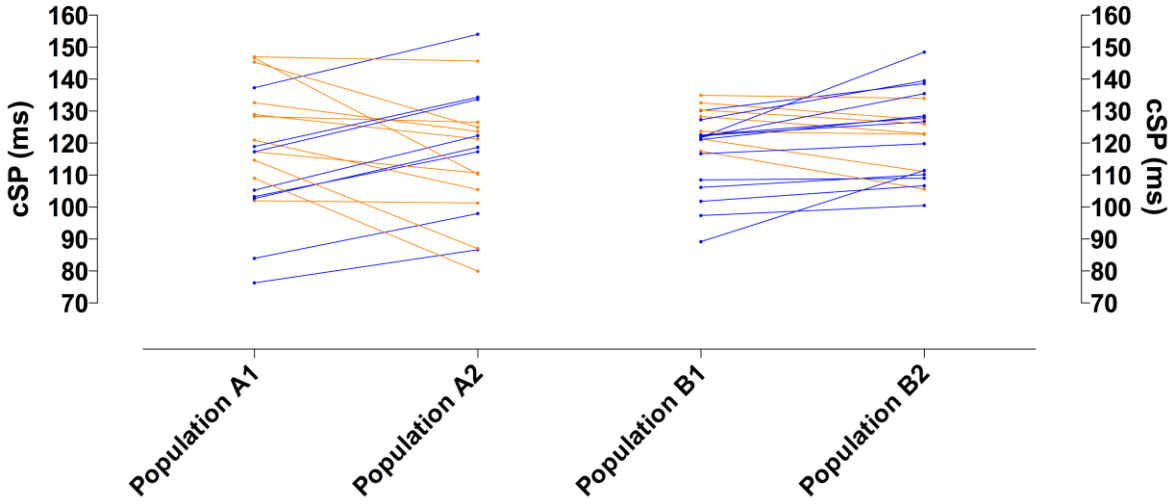


Figure 2.2. Corticomotor inhibition taken 100%MVC for each individual during each testing session.

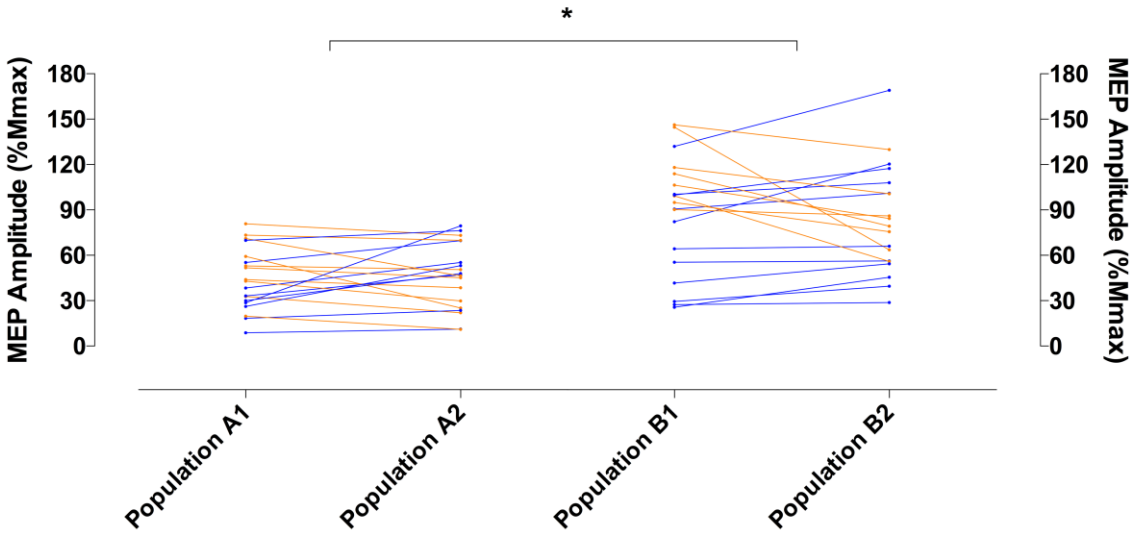


Figure 2.3. Corticospinal excitability taken at 20%MVC for each individual during each testing session. *denotes significant differences between groups ($p< 0.05$)

Population B1 (p= 0.0002; CI= 17.95 to 68.98), and B2 (p=0.0007; CI= 13.68 to 64.72). Population A2 was also significantly lower than population B1 (p= 0.0003; CI= 15.95 to 66.98), and B2 (p= 0.001; CI= 11.68 to 62.72).

Table 2.1. Intraclass correlation coefficients and coefficient of variations for corticomotor inhibition and corticospinal excitability.

	ICC (95%CI)	CV
Corticomotor inhibition		
Population A	0.63 (0.33-0.82)	9.07
Population B	0.73 (0.49-0.86)	4.49
Corticospinal excitability		
Population A	0.61 (0.30-0.80)	24.66
Population B	0.71 (0.47-0.86)	17.30

2.4 Discussion

We have shown that inhibitory and excitatory parameters, more specifically corticomotor inhibition and corticospinal excitability show good inter-day reliability across 2 different populations when assessed at one, and two-week intervals. The findings are in line with a number of studies showing good correlations when assessing the same parameters (Hermsen et al, 2016; Badawy et al, 2011; O'Leary et al, 2015; Cacchio et all 2009). Further, we also report both low and high CV values for corticomotor inhibition (<10%) and corticospinal excitability (>15%) respectively, also seen in the literature (O'Leary et al, 2015; Ngomo et al, 2012). The results from this study suggest that both our

protocol to record inhibitory and excitatory measures provide stable and reliable data when assessed day-to-day. The different levels of excitation in the two populations also suggest that, unlike corticomotor inhibition, corticospinal excitability may be only better suited for intra-subject comparisons, and not to quantify the excitability of distinct populations.

Whilst some of the available literature shows similar results to the ones in the current study, other data show poor (Van Hedel et al, 2015; Sankarasubramanian et al, 2015), fair (Brown et al, 2017; Christie et al, 2007) or excellent (Cacchio et al, 2009; Liu et al, 2014; Cacchio et al, 2011) reliability values associated with corticomotor inhibition and corticospinal excitability. In the current study, the values shown for corticospinal excitability are normalized to the Mmax of the same muscle, whilst previous studies presented raw values (Bastani et al, 2012; Cacchio et al, 2009; Christie et al, 2007; Fisher et al, 1997). Corticospinal excitability, quantified as MEP amplitude, is usually expressed as a ratio of the maximal excitability of the muscle. TMS is not able to activate the whole motor neuron pool in M1, therefore it is useful to know what proportion of the pool is being activated when the stimuli are delivered over the scalp (Goodall et al, 2009). It is unclear whether normalized values are comparable to raw values. However, it is preferable to express data as ratios especially when recording measurements over the course of multiple days as it is impossible to determine whether the same pool of MU are being recorded at each session when removing and replacing electrodes.

One of the limitations of TMS is that there is no consensus on the specific methodology to be used for the recording of excitatory and inhibitory parameters, as can be noted by observing the wide range of procedures implemented in the available literature. Most studies use differing stimulator output intensities, and furthermore, the level of muscle activation is not kept constant: some use very

submaximal intensities (10-30%MVC) (O'Leary et al, 2015; Hermsen et al, 2016), others perform excitability testing whilst the muscle is at rest (as mentioned in chapter 1 it is impossible to assess corticomotor inhibition without pre-activating the target muscle prior to stimulation) (Pearce et al, 2012). Lastly, the target muscle also differed from study to study, ranging from small muscles in the upper limbs (First dorsal interossei - FDI, Abductor pollicis brevis - APB) (Hermsen et al, 2016; McDonnell et al, 2006) to large muscles in the lower limbs (Vastus lateralis - VL, Gastrocnemius) (O'Leary et al, 2015; Pearce et al, 2012). Combined, these factors may affect the stability of the measure, resulting in the wide range of reliability values seen in the literature.

2.5 Conclusion

Our data indicate that parameters of cortical inhibition and excitability are stable when assessed over different days, and therefore are suitable in quantifying subsets of brain health. Although both inhibitory and excitatory parameters in this chapter show similar degrees of reliability, we confirmed corticomotor inhibition as our primary outcome measure because it appears slightly more reliable than excitability, as well as better suited to assess functional changes following head traumas (Lefebvre et al, 2015). To ensure replicable and comparable results within this thesis, we chose to record all TMS parameters from the lower limbs, in order to relate any potential changes in the brain-to-muscle pathways to changes in postural control. Furthermore, the chosen supra-threshold stimulator intensity has been previously used in TMS-related studies (Goodall et al, 2012a,c). Lastly, since corticomotor inhibition appears more reliable when assessed by one investigator, as opposed to multiple (Cacchio et al, 2009), we chose to use a single researcher to perform data analysis in order to reduce the variability

Chapter 3

Evidence for acute electrophysiological and cognitive changes following routine soccer heading

Abstract

Introduction: There is growing concern around the effects of RSHIs on brain health. However, little and ambiguous data exist regarding the consequences of this activity. Soccer is of interest in a subconcussive context as players routinely perform ball headers during practice and in matches. Chapter 3 will assess the immediate outcomes of repetitive soccer heading using direct and sensitive measures of brain function. **Methods:** Nineteen amateur football players (5 females; age 22 ± 3 y) headed machine-projected soccer balls at standardized speeds (~40 Km/h), modelling routine soccer practice. Corticomotor inhibition, alongside corticospinal excitability, postural control and cognitive function were assessed prior to heading and repeated immediately, 24h, 48h and 2 weeks post-heading. **Results:** Corticomotor inhibition increased immediately following heading (117.8 ± 19.8 to 123.1 ± 17.6 ms, p= 0.04); further, measurable reductions in memory function were also found. Specifically, spatial working memory (SWM) and paired associate learning (PAL) decreased immediately following the heading protocol (SWM: 11 ± 12 to 16 ± 16 errors, p= 0.03; PAL: 3 ± 4 to 5 ± 5 errors; p= 0.007). No alterations were observed in other cognitive, as well as balance parameters **Conclusion:** Repetitive head impacts, routine in soccer, are associated with immediate, measurable electrophysiological and cognitive impairments. The alterations in brain function were transient (with values normalizing 24h post-heading), and the magnitude of the effects small. Regardless, the effects may highlight direct consequences of routine soccer heading on brain health. Moreover, this chapter also suggests that measures of motor control previously used in subconcussion (i.e. postural control) may lack the sensitivity needed to detect subtle changes as a result of RSHIs.

3.1 Introduction

Having highlighted the potential of TMS as a detection tool for RSHIs in chapter 1, and established the reliability of the primary and secondary outcome measures corticomotor inhibition and corticospinal excitability in chapter 2, this chapter will turn to applying this parameter to RSHIs by using soccer heading as a model for subconcussion. Although soccer is often seen as a non-contact sport there has been growing concern regarding its safety, particularly in the context of RSHIs. Ball heading is a skill regularly included in training sessions even from a young age, resulting in athletes being exposed to routine, intentional and repetitive impacts to the skull over the course of a playing career. Whereas rates of concussion are relative low in soccer compared to other contact sports such as rugby union or American football (Pfister et al., 2016), participation rates and the incidence of intentional subconcussive impacts through heading in training and match play are such that the safety of heading in soccer has been questioned in some quarters (Patlak and Joy, 2002).

Though accepted as part of routine gameplay, emerging evidence suggests that exposure to repeated subconcussive impacts in soccer may be associated with measurable changes in brain structure and function, and perhaps with late neurodegenerative disease (as discussed in chapter 1). Rotational headers may prove of particular interest as they are often performed in training drills and matches (i.e. corner kicks). These types of headers are believed to be more injurious compared to linear accelerations (Cantu and Hyman, 2012), due to the additional stretching action the axons are subjected to. Imaging studies over the course of a season in active soccer players report evidence of white matter microstructural changes with associated impaired cognition (Lipton et al., 2013). Further, imaging of former professional soccer players aged 40–65 demonstrates evidence of cortical thinning, again with associated cognitive impairment (Koerte et al., 2016). Regarding longer term outcomes, recent

identification of a form of dementia known as chronic traumatic encephalopathy (CTE) in athletes from a range of contact sports including soccer (Geddes et al., 1999; McKee et al., 2014; Ling et al, 2017) has drawn attention to the possibility that head impacts in soccer might be associated with increased risk of neurodegenerative disease.

Nevertheless, despite growing evidence of risks from the cumulative effects of sport-related head impacts and anxieties around the safety of ball-heading, little data exist demonstrating the direct consequences of heading on brain function. As discussed in the introductory chapter, transcranial magnetic stimulation (TMS) can be used to assess a variety of indices of function in the brain to muscle pathways (Goodall et al., 2014). Given its apparent high sensitivity in identifying alterations to the brain, TMS could potentially be used to detect acute changes in brain function following subconcussive head impacts. The relative novelty of TMS used in this context makes interpretation in terms of clinically meaningful effects difficult, but its appeal is sensitivity in detecting direct brain changes (De Beaumont et al., 2007). The use of TMS could potentially highlight relevant neurochemical changes (Demirtas-Tatlidede et al., 2012) that can be used to direct routes of investigation into the effects of subconcussive impacts on the brain.

Soccer is an interesting sport to use as a model for subconcussion due to the high number of players who engage with the discipline across the globe. As previously stated many will intentionally and routinely undergo heading drills during training, and perform headers during a match. It is important, therefore, to fully understand the relationship between a seemingly innocuous practice such as soccer heading and alterations in brain health. Even from a methodological standpoint soccer, and more specifically an experimentally controlled evaluation of heading, is ideally suited for assessing the acute

brain-dependent effects of RSHIs: the controlled environment of a laboratory setting allows researchers to account for factors that may otherwise affect results (e.g. having access to a ball throwing machine enables for the standardization of both the height of the trajectory and the speed of the ball, allowing for accurate and repeatable throws), thus isolating the effects of heading on brain function from confounding variables. Heading a soccer ball also requires a relatively low level of skill and is time- and cost-effective, further lending to its appeal for use in research. Furthermore, whilst we are specifically using soccer heading as a model for subconcussion, the findings can be applicable to other sporting populations, and members of the general public that are nevertheless routinely exposed to RSHIs.

Therefore, the aim was to study the use of corticomotor inhibition in the lower limb in detecting acute changes to brain function from repetitive subconcussive head impacts. As discussed in chapter 1, corticomotor inhibition appears to be the best parameter for detecting brain alterations as a result of head impacts. As such, we hypothesized that there would be a (transient) increase in our primary outcome measure corticomotor inhibition following a standardized bout of soccer heading; possibly accompanied by measurable changes in secondary outcome measure corticospinal excitability, and other established but less sensitive or less objective indexes of brain function and injury, such as cognitive tests and postural control.

3.2 Methodology

3.2.1 Approvals and Recruitment

Twenty-three healthy, amateur football players (5 females; age 22 ± 3 y; weight 72.9 ± 8.3 Kg; height 175.4 ± 10.2 cm) were recruited for study via advertisement on university noticeboards and meetings with local football clubs. Participants were excluded from taking part if they presented with any of the following: 1) history of brain injury resulting in loss of consciousness; 2) history of a neurological condition; 3) history of concussion in the 12 months prior to taking part; 4) family history of epilepsy; 5) current use of psychoactive recreational or prescription drugs. Data from one participant could not be analyzed and three more participants withdrew from the study for personal reasons. The final cohort included a total of nineteen participants. The local Research Ethics Committee approved the study and procedures conformed to the guidelines set out by the Declaration of Helsinki. Written informed consent was obtained from all participants, prior to taking part.

3.2.2 Study Design

Participants were asked to refrain from vigorous physical activity, consuming alcohol and caffeine or smoking for 24 h prior to each study session. Furthermore, participants were required to present to the laboratory fasted where they were provided with a standardized breakfast. Following a familiarization day, baseline measures for cognitive function, postural control, corticospinal excitability and corticomotor inhibition were recorded in this order. Following baseline testing, participants underwent the heading protocol and then repeated the measures at 4 follow-up time points (taking measures in reverse order from baseline, starting with the corticomotor inhibition): immediately post-heading and at 24 h, 48 h and 14 days following the heading protocol. The decision to include the 48 h follow-up was to assess the transient nature of the effects of heading, and the 14 day follow-up was intended as

a time point at which complete “wash out” would have occurred following heading impact. Prior to commencement of study data collection participants attended the laboratory for a familiarization session, during which they completed all outcome measures to acquaint them with the assessment procedures and minimize later learning effects.

3.2.3 Heading Protocol

The heading protocol consisted of heading a standard football (400 g; 70 cm circumference; 8 psi) projected at a speed of 38.7 ±2.1 Km/h from a football delivery device (JUGS sports, Tualatin, USA) positioned 6 m from participants, simulating routine soccer game-play (Haran et al., 2013; Broglio et al., 2004). Participants were instructed to perform a rotational header, redirecting the football perpendicularly to the initial trajectory, with each session consisting of 20 consecutive head impacts over a 10 min period, replicating typical heading practice. A custom-built accelerometer placed at the back of the participant's head recorded linear g-force of the head during impact. Ball speed was determined based on the participants' perceived ability to head the ball with a minimum speed of 30 km/h and maximum speed of 50 km/h.

3.2.4 Transcranial Magnetic Stimulation

Measures of corticomotor inhibition and corticospinal excitability were recorded using techniques and methodologies previously described in chapter 2 (section 2.2.3).

3.2.5 Electromyography and Femoral Nerve Stimulation

All EMG parameters were recorded using protocols described in section 2.2.4 of chapter 2.

3.2.6 Cognitive Function

Secondary outcome measure cognitive function was assessed in a quiet room using the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computer based cognitive assessment tool and neuropsychological standard. The following standard CANTAB tasks were included, as they mostly relate to the frontal areas of the brain, and therefore may be more susceptible to soccer heading: Reaction Time (RTI; divided attention); Paired Associate Learning (PAL; long-term memory); Spatial Working Memory (SWM; short- term memory); Attention Switching Task (AST; executive function); and Rapid Visual Processing (RVP; sustained attention).

3.2.7 Postural Control

Secondary outcome measure postural control was assessed using the Biodex Balance System SD (BBS; Biodex Medical Systems, Inc. NewYork, USA). Participants stood on a circular dynamic platform and average sway score was determined by measuring the degree of tilt on anterior-posterior and medial-lateral axes during three, 20 s trials (using dedicated Biodex software, v1.08, Biodex Inc.).

3.2.8 Statistical Analysis

Immediate post-heading responses were analyzed using a paired t- test, comparing measures before and immediately after the heading protocol. Recovery was analyzed for individual growth curves using

the SPSS MIXED model with the restricted maximum likelihood method in keeping with standards for analysis of longitudinal data (Singer and Willett, 2003; Peugh and Enders, 2005). To achieve this, individual curves were analyzed to examine change over time. The following time points were included: immediate post, 24 h post, 48 h post, two weeks post. Data was checked for skewness and kurtosis and three cognitive measures (SWM, PAL, and RVP) and the balance variable were normalized using log transformation. The 95% lower and upper confidence intervals (CIs) were also calculated from difference of the mean values. Effect sizes (ES) were calculated for non-transformed differences using Cohen's d formula and were quantified as follows: 0.2 = small; 0.5 = medium; 0.8 = large. Statistical significance was set at $p \leq 0.05$. Each measure was separate in relation to the hypotheses and therefore no correction for multiple comparisons was necessary. Data are expressed as means (± standard deviation) unless otherwise stated.

Table 3.1. Mean impact values for each individual recorded using a linear accelerometer. Data for 2 participants were not recorded due to hardware malfunction.

Force of head impact (g) for each participant (mean±SD)
12.8±2.0
12.0±2.1
14.0±2.1
15.3±3.2
12.7±2.1
14.6±2.5
11.7±2.7
11.3±1.9
10.5±1.9
12.4±1.8
17.0±4.1
11.7±2.9
11.9±2.2
12.3±2.7
11.8±2.0
14.6±2.5
16.7±4.1
Mean±SD: 13.1±1.9

3.3 Results

Overall, each participant performed 20 headers, achieving a mean force of impact of 13.1 ± 1.9g (Table 3.1), with a coefficient of variance of 18%(±3%).

3.3.1 Effect of heading on corticomotor inhibition and corticospinal excitability

Immediately after ball heading there was a measurable increase in the primary outcome measure cSP within 74% (14 out of 19) of participants (Figure 3.1). The cSP duration increased from 117.8(±19.8)ms at baseline to 123.1(±17.6)ms ($t_{(18)}$= -2.11, p= 0.049; ES= 0.28), representing an average increase of 5.4(±4.8)% in cSP duration, reflecting increased corticomotor inhibition. This increase in cSP proved transient with apparent normalization to baseline in subsequent follow-up assessments at 24h, 48h and 14 days ($F_{(1,18)}$= 4.23, p= 0.04) (Figure 3.2). There was a moderate, but not significant, relationship between these acute increases in cSP and g-force on impact with the ball (r = 0.37, p= 0.07 one-tailed).

No changes were found on the secondary TMS outcome measure corticospinal excitability; MEP amplitude demonstrated no change in the acute phase immediately after ball heading, nor in the follow-up assessment time-points (Table 3.2). There was no notable change in knee extensor MVC after heading the ball (Table 3.2), suggesting the participants did not experience significant muscular fatigue that might interfere with TMS measurement.

3.3.2 Altered cognitive function following heading

Immediately after the heading protocol there was a reduced performance compared to baseline in two CANTAB sub-tasks assessing accuracy on different aspects of memory. Specifically, SWM error scores were significantly higher ($t_{(18)}$= -2.28, p= 0.03, ES= 0.3) immediately after the heading protocol, compatible with impairment in short-term memory (Figure 3.3A). Furthermore, total adjusted error score on the PAL task immediately after heading increased by 67% ($t_{(18)}$= -3.05, p= 0.007, ES= 0.5), compatible with a reduced long-term memory function (Figure 3.3B). These disturbances in short- and

long-term memory proved transient, with normalization to baseline performance in SWM ($F_{(1,18)}$= 10.28, p= 0.002) and PAL ($F_{(1,18)}$= 11.14, p= 0.002) in the subsequent follow-up assessments at 24h, 48h and 14 days (Table 3.2).

Heading only significantly affected memory function; the remaining CANTAB tasks assessing aspects of attention and processing speed did not show significant heading-associated decrements compared to baseline assessments (Table 3.2). No change was detected on the RVP task, with RVP A' scores close to ceiling/maximum, making the measure insensitive to change. There was a marginal improvement on the median corrected latency scores of the executive function AST ($t_{(18)}$= 2.52, p= 0.021), possibly due to practice. On the Choice RTI measure there was no effect of heading on decision times ($t_{(18)}$= 0.69, p= 0.5) (Table 3.2).

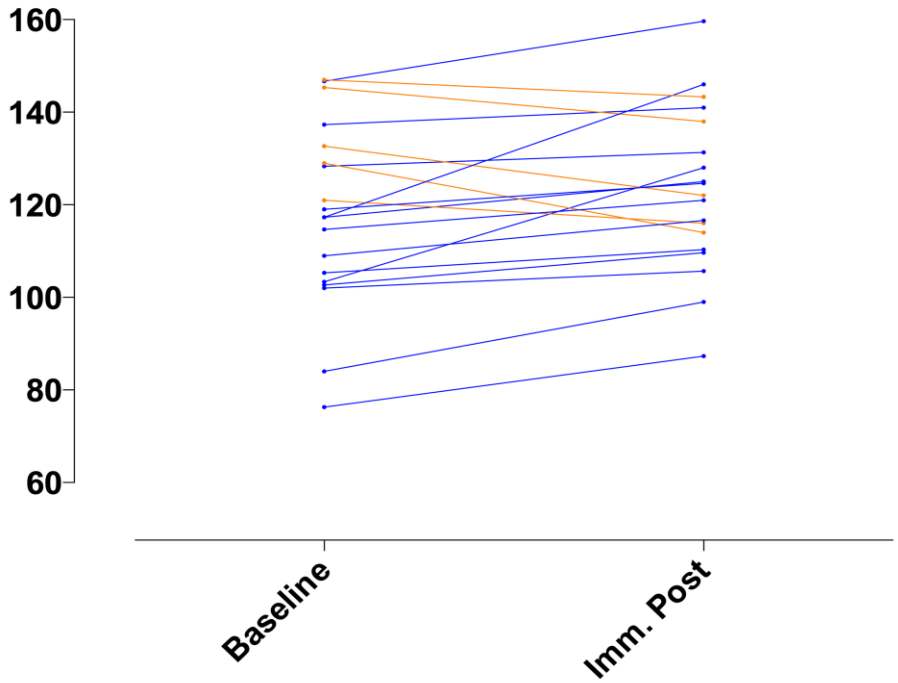


Figure 3.1. Change in cortical silent period (cSP) duration for each participant from baseline to immediately following the heading protocol.

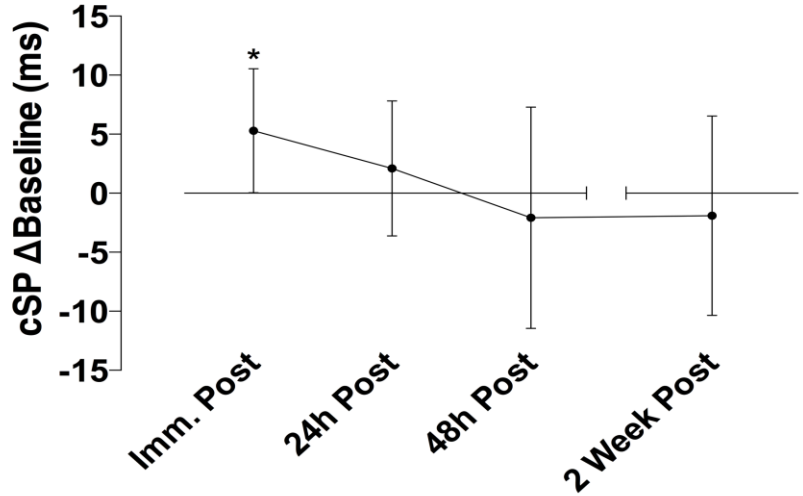
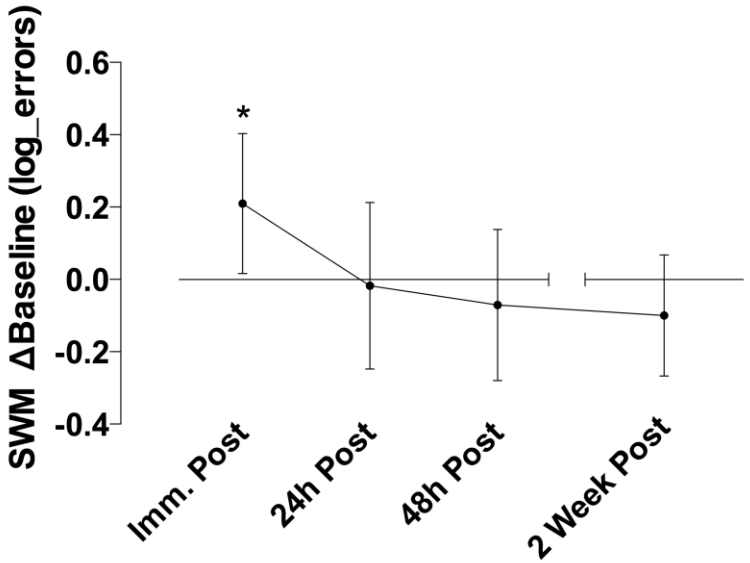


Figure 3.2. Difference in cSP in ms after heading relative to baseline. Immediately after heading cSP duration increased on average by 5.3(±5·7)ms (*p< 0.05) which within participants is an 5.4(±4.8)% average increase from baseline values. This increase detectable immediately after heading normalized over the four follow-up timepoints (p< 0.05) with values apparently returning to baseline level. Error bars indicate the 95% confidence

A



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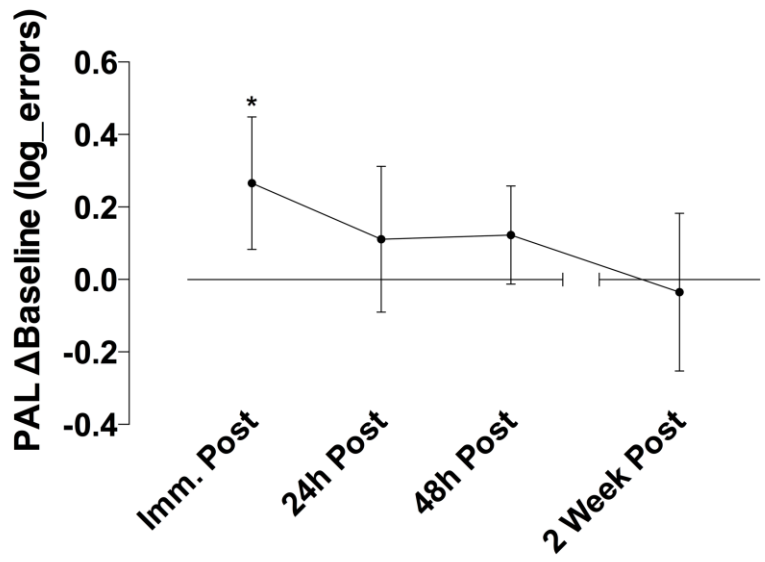

Figure 3.3. Difference in memory performance (log transformed error score difference) after heading relative to baseline. Immediately after heading, errors were higher compared to baseline on both the Spatial Working Memory SWM (*p< 0.05 - A) and Paired Associated Learning PAL (*p< 0.01 - B) tasks. This increase evident immediately after heading normalized over the four follow-up timepoints (p< 0.01) with error scores apparently returning to baseline level. Error bars indicate the 95% confidence intervals.

Table 3.2: Mean (standard deviation) values for each of the outcome measures: corticomotor inhibition (cortical silent period in ms) and corticospinal excitability (MEP amplitude normalized to femoral nerve M-wave, %Mmax), Spatial Working Memory (SWM errors), Paired Associate Learning (PAL errors), Rapid Visual Processing (RVP A' score), Attention Shifting Task (AST median corrected latency), Reaction Time (Choice RTI decision times) and Postural control (Balance, SI stability index deviation from the horizontal baseline) measured at each time point, and 95% lower and upper confidence intervals (CIs) for the difference in means before and immediately after heading. *denotes significant difference from baseline; §denotes significant recovery growth curve

Variable	Assessment Time Post Heading Exposure					
	Baseline	Immediately	24 h	48 h	2 Weeks	Δ mean Pre v Imm Post (95% CI)
TMS						
Inhibition (ms)	117.8 ± 19.8	123.1 ± 17.6*	119.9 ± 19.8	115.7 ± 20.6	115.9 ± 19.7§	5.3 (0.02 to 10.54)
Excitability (%Mmax)	44.1 ± 20.6	47.4 ± 22.3	48.0 ± 24.0	44.4 ± 22.5	46.1 ± 22.5	3.3 (-5.03 to 11.72)
Cognitive Function						
SWM (errors)	11 ± 12	16 ± 16*	12 ± 13	10 ± 14	10 ± 15§	5 (-9.49 to -0.41)
PAL (errors)	3 ± 4	5 ± 5*	3 ± 3	3 ± 3	2 ± 2§	2 (0.08 to 0.44)
RVP A'	0.95 ± 0.05	0.95 ± 0.04	0.95 ± 0.04	0.97 ± 0.02	0.96 ± 0.03	0.0(-0.01 to 0.02)
AST (ms)	396 ± 58	376 ± 67*	369 ± 64	370 ± 66	373 ± 82	-19.1 (-35.01 to -3.20)
RTI (ms)	295 ± 29	301 ± 35	295 ± 33	297 ± 32	297 ± 31	6 (-6.13 to 19.24)
Postural Control						
Balance (SI)	0.8 ± 0.4	0.7 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	-0.1 (-0.16 to 0.03)

3.4 Discussion

Following a standardized session of football heading designed to simulate routine soccer practice we demonstrate immediate alterations in brain electrophysiological and cognitive function compared to baseline assessments in a cohort of healthy, young soccer players. Specifically, using TMS there was a measurable increase in corticomotor inhibition after just 20 consecutive headers. Furthermore, in cognitive assessments, the data demonstrate decreases in measures of both short- and long-term memory immediately following heading. Notably, in this single exposure experimental evaluation, the alterations in corticomotor inhibition and cognitive function appeared short-lived, with the effects apparently normalizing in follow-up assessments from 24h onwards. In contrast to previous studies assessing athletes and patients with confirmed concussion or mild TBI (De Beaumont et al., 2007; Chistyakov et al., 2001; Bernabeu et al., 2009; Livingston et at., 2010) these novel observations demonstrate, for the first time, detectable alterations in brain function in footballers exposed to 'routine' head impacts not associated with clinically recognizable brain injury.

The prolonged silent period of neuromuscular recruitment found in this study is a sign of increased inhibition in the motor system and is thought to reflect GABA activity (Inghilleri et al., 1993; McDonnell et al., 2006), the most powerful inhibitor in the motor system. Although the mechanisms behind corticomotor inhibition are not fully understood (Chen et al., 1999), increased inhibition following repeated subconcussive head impact may reflect protective mechanisms against minor injury. A concern, however, is that such protective mechanisms could become maladaptive when stimulated repeatedly, as occurs during soccer heading practice. Albeit apparently transient, the acute increases in corticomotor inhibition following football heading could trigger a pathological process damaging brain health through the accumulative effect of subconcussive head impact. Increased corticomotor inhibition has been found to be associated with pathophysiology in brain damage suggesting a link

between functional deficits and hyperactivity of cortical inhibitory interneurons (Classen et al., 1997). Further study into the dynamic metabolic processes as a direct result of soccer heading is required. As researchers, it is important we understand the complex interplay between functional, metabolic, and structural brain changes following repeated subconcussive head impact; only then we can begin to establish the link to accumulative and long-term consequences. At present, the current findings at least suggest acute brain changes occur as a direct consequence of soccer heading.

Further to increased corticomotor inhibition, parameters of memory function were altered following the heading protocol, consistent with a recent report of a relationship between memory function and history of heading in soccer (Lipton et al., 2013). Furthermore, a study of retired Australian Rules footballers found that elite players performed worse on the PAL test than amateurs (Pearce et al., 2014). Practical limitations of cognitive-based tests to detect impairment in athletes are due to reliability: in high performance sports athletes have been recognized to purposely produce low baseline performances on cognitive tests to allow them to avoid removal from play, or to reduce return to play intervals (Erdal, 2012).

For completeness postural control (balance) was included as a secondary, albeit indirect, outcome measure as concussion has been shown to result in impaired balance (McCrory et al., 2013; Powers et al., 2014), yet the participants in the current study were able to maintain their balance despite an increased level of corticomotor inhibition. And while one study has shown a decrease in postural control following bouts of soccer heading (Haran et al., 2013), another study has not (Broglio et al., 2004); and now our own show no change in postural control. A reasonable explanation for the lack of findings in this regard (as already discussed in chapter 1) may be that postural control is not sensitive enough to detect the transient and subtle changes observed in motor control in the current chapter.

Cortical excitability has previously been shown to decrease following TBI (De Beaumont et al., 2007; Chistyakov et al., 2001; Bernabeu et al., 2009; Livingston et al., 2010), yet we demonstrated no such change following ball heading. The reason why changes were seen in cortical inhibition and not cortical excitability may be due to the different levels of muscle contractile force applied during recording of the two parameters (20% MVC for excitability vs. 100% MVC for inhibition). Furthermore, it should be noted that measuring cortical excitability is a less straightforward procedure than corticomotor inhibition as it requires MEP normalization to maximal motor nerve response (Goodall et al., 2009). Lastly, corticomotor inhibition was thought to be most sensitive to quantifying electrophysiological changes based on a recent systematic review (Major et al., 2015), and is a direct measure of changes to brain function.

There are some factors to account for when considering the results from this chapter, some of which will be addressed at a later stage in the thesis, and some which will be recommendations for future work. First, whilst participants acted as their own control in a pre-to-post study design, the project did not account for the effect that whole body movements (without head impact) would have on the parameters measured. However, the current pattern of results leaves little doubt that the changes in brain function were related to head impact rather than physical activity. Furthermore, the force of maximal knee contraction was not reduced after heading, suggesting that muscular fatigue did not affect data interpretation. Nevertheless, the effect of exercise on TMS-derived parameters is accounted for in chapter 5.

Further study into the dynamic metabolic processes as a direct result of soccer heading is required. Implementing the use of magnetic resonance spectroscopy in future studies could help determine short-term alterations in GABA and glutamate responses. With regards to changes in GABA, because of the use of single-pulse TMS, this study is only able to report on the activity of $GABA_B$, while the use of paired-pulse TMS in future work can distinguish modulation of $GABA_A$ and $GABA_B$.

3.5 Conclusion

The current study is the first to show direct evidence for acute changes to corticomotor function and changes to memory function following routine soccer heading. It is also the first study to show that corticomotor inhibition, measured by TMS, is able to detect acute transient changes in brain function following subconcussive head impacts. Although the magnitude of the acute changes observed was small, it is the presence of the effect that is of interest. Cortical Inhibitory mechanisms are altered in confirmed concussion; the acute changes in the same measure (accompanied by decrements in cognitive performance) following the subconcussive impact of a soccer heading drill raises concerns that this practice, routine in soccer, may affect brain health.

A further interesting notion from this study is that whilst corticomotor inhibition increased, another parameter of motor control (postural control) was unaffected by RSHIs. As stated before, it is likely due to the latter's lack of sensitivity; however, it raises questions regarding how small corticospinal alterations translate to the periphery. Relating these findings to previous literature is difficult as this is the first and only study to apply transcranial magnetic stimulation to a subconcussive model. As such, there are no available sources to corroborate (or disprove, as it may be) our findings. The next logical step is, therefore, to provide more empirical evidence for the relationship between RSHIs, brain

function and motor control; having already established that a soccer heading drill may affect the normal functioning of the brain, we now turn to a sport that is well known for the short and long term consequences it may have on brain health: boxing.

Chapter 4

Routine subconcussive head impacts alter motor unit behaviour, corticomotor inhibition and cognitive function in boxers

Abstract

Introduction: Repetitive concussions are associated with risks of long-term sequelae, and RSHIs may have similar consequences. Preliminary data demonstrates that soccer ball heading results in acute changes to brain function, but whether similar effects exist following other contact sports is unclear. Therefore, the aim of chapter 4 is to assess whether subconcussive impacts from boxing sparring result in similar alterations to those previously observed. **Methods:** Twenty amateur boxers (2 females, age 21.1 ± 1.5 y) performed a sparring bout (3 x 3 minute rounds) modelling a routine training session. All parameters (corticomotor inhibition, corticospinal excitability, motor unit recruitment behaviour and postural control) were assessed prior to sparring and again immediately, 1h and 24h post-sparring. **Results:** Corticomotor inhibition significantly increased 1h following sparring (124.5 ± 30.6 to 132.0 ± 32.9 ms, p= 0.03), with values returning to baseline levels by the 24h follow up. Corticospinal excitability decreased significantly 24h post-sparring when compared to the 1h follow up (55.7 ± 19.0 to 42.2 ± 19.3 %Mmax, p=0.01), but did not differ significantly from baseline. PAL was decreased following sparring (3 ± 3 to 6 ± 5 errors, p=0.018) and remained in a similar state at the 24h follow up, though not significant when compared to baseline (p=0.07). Motor unit (MU) behaviour was also altered 1h following sparring, with early recruited MUs being activated later, and later recruited MUs activated earlier compared to baseline. Other cognitive function subtasks, as well as postural control remained unchanged. **Conclusion:** Boxing sparring resulted in acute and transient electrophysiological and cognitive changes similar to that of soccer heading. In addition, increased inhibition 1h post-sparring likely caused MU recruitment strategies to be affected. Altered MU behaviour and by extension motor control may put athletes at greater risk of musculoskeletal injuries, but further study is needed for this to be established.

4.1 Introduction

In the previous chapter we have shown that a bout of soccer ball heading results in measurable albeit small changes in corticomotor inhibition, assessed using transcranial magnetic stimulation. These findings are interesting because they show, for the first time, direct changes in motor control following RSHIs. The alterations, whilst transient, may nevertheless translate in more severe issues, such as an increased risk of injury (through a decrease in motor control) and the onset of neurological disorders (as a result of chronic exposure to RSHIs).

A number of studies have found a particular association between RSHIs and acute changes in motor control (Hwang et al., 2017; Kawata et al, 2016; Haran et al, 2013), for more information please refer to section 1.2.5 in chapter 1. These findings are in line (albeit to a lesser extent) with data from concussion patients (Pearce et al, 2015; Pearce et al, 2014a,b,c; Davidson and Tremblay, 2016; De Beaumont et al, 2011; Livingston et al, 2010), showing both acute and long-term impairments of motor function as a result of the injuries. The notion of altered motor control following concussion has also been extensively supported by data observing changes in balance (McCrory et al, 2013; Powers et al, 2014; Rochefort et al, 2017) and gait (Parker et al, 2006; Doherty et al 2017; Oldham et al, 2016); moreover, a decrease in the brain's capability to control muscle activation (i.e. neuromuscular control) may explain why patients who sustained a concussion are more susceptible to musculoskeletal injuries than a healthy population (Nordstrom et al, 2014; Lynall et al, 2015; Cross et al, 2017).

Having shown brain changes following a soccer heading drill, seen as harmless by most people, the next step was to corroborate the findings using another type of impact to the head. The detrimental brain-

related effects of boxing have been well established for decades, particularly when discussing the cumulative effects over the course of a career or lifetime (Jordan et al., 1996; Stiller et al, 2014). Whilst there is some evidence on the acute effects of boxing competitions on brain health (Zetterberg et al, 2006), little to no data exist on the acute and direct consequences of sparring sessions in training. This is a particularly important topic as boxers routinely perform the sessions multiple times a week, exposing themselves to impacts to the head that, whilst not concussive, may nevertheless impact the functioning of the brain.

Whilst corticomotor inhibition is an indication of intracortical inhibitory mechanisms, it translates in alterations in the brain's ability to control the musculoskeletal system. It is possible that increased GABAergic activity in the brain inhibits proper functioning of motor units. To date, however, there have been no studies exploring the relationship between corticomotor inhibition and motor unit recruitment behavior, in both a healthy and concussed cohort. To gain a better understanding of how RSHIs alter inhibitory mechanisms in the brain, in turn affecting the normal functioning of the motor system we included a measure of motor unit recruitment strategy in this study. Neuromuscular strategies can be quantified by studying the behavior of individual motor unit action potentials (MUAP) extracted from surface electromyography (sEMG) (De Luca et al, 1982; Adam and De luca, 2005; Nawab et al, 2010; Kline and De Luca, 2014). Precision decomposition EMG (dEMG) allows for the assessment of MU firing properties over the course of a submaximal contraction, providing an understanding of the brain's ability to govern the proper functioning of muscles. To the best of our knowledge, no study has assessed MU properties in the context of concussion/subconcussion.

The principal aim of this study was, therefore, to assess whether a different type of subconcussive impact (in this case a sparring session in boxing) would give rise to a similar change to motor function

to the one observed following soccer heading in chapter 3. Our secondary aim was to examine motor unit recruitment patterns following RSHIs, as we predicted any changes in motor control to be translated in alterations in motor unit recruitment behavior. We hypothesized that, similarly to the study in chapter 3, RSHIs would be associated with transient alterations in motor control, expressed by an increase in corticomotor inhibition, as well as temporary decreased in cognitive function. Furthermore, we also expected MU recruitment patterns to be altered, as a result of increased corticomotor inhibition dampening the neural drive to the muscles and reducing the firing rate of the MUs.

In addition to the immediately post- follow up, we chose to include a data collection time point 1 hour following the sparring session. In this way, if corticomotor inhibition was significantly increased immediately following the sparring session we could have a clearer indication of how transient the effect is. Furthermore, we also made the decision to limit the follow up assessment to immediately post-, 1h post-, and 24h post-sparring, since we showed in chapter 3 that corticomotor inhibition returned to baseline values by the 24h follow-up.

4.2 Methodology

4.2.1 Approvals and recruitment

Twenty-three healthy, amateur boxers and Muay Thai athletes (age 22 ± 1.7 y; mass 76 ± 7.5 Kg; height 178 ± 8.4 cm) were recruited for the study via advertisements on university notice boards and social media. Participants were excluded from taking part if they presented with any of the following: 1) history of brain injury resulting in loss of consciousness; 2) history of a neurological condition; 3) history

of concussion in the 12 months prior to taking part; 4) family history of epilepsy; 5) current use of psychoactive recreational or prescription drugs. Two participants could not take part in the study due to their medication; a further individual experienced a syncopal episode during baseline testing and withdrew. The final cohort included a total of 20 participants. The local research ethics committee approved the study, and procedures conformed to the guidelines set out by the Declaration of Helsinki. Written informed consent was obtained from all participants prior to taking part.

4.2.2 Study design

Prior to the first experimental trial participants attended a familiarization session, during which they completed all outcome measures to acquaint themselves with the assessment procedures and minimize the possibility of learning effects.

Participants were asked to refrain from vigorous physical activity, consuming alcohol and caffeine or smoking for 24h prior to each trial. During the first experimental session baseline measures for cognitive function, postural control, corticospinal excitability, corticomotor inhibition and motor unit firing instances were recorded. Following baseline testing participants completed the sparring session and then repeated the measures immediately post-sparring and at 1h and 24h following sparring.

4.2.3 Sparring session

The sparring session consisted of three, three-minute rounds with two minutes rest in between each round. Participants provided their own sparring partner, boxing equipment and were instructed to spar as they would normally do so in a training session.

4.2.4 Transcranial magnetic stimulation

Measures of corticomotor inhibition and corticospinal excitability were recorded using techniques and methodologies previously described in chapter 2 (section 2.2.3).

4.2.5 Electromyography and femoral nerve stimulation

All EMG parameters were recorded using protocols described in section 2.2.4 of chapter 2.

4.2.6 Precision decomposition EMG

Rectus Femoris (RF) surface EMG was measured during a 60%MVC isometric contraction using a modified Bagnoli 16-channel EMG system (Delsys, Boston, USA). A five-pin sensor was applied to the belly of the muscle in compliance with SENIAM guidelines to record bipolar surface EMG signals, and a reference electrode was placed over the patella. The sensor is made up of five cylindrical metal probes 0.5 mm in diameter, placed at the four corners and centre of a 5 x 5 mm square. The sensor was applied so that the pins were pressed into the skin, without perforating it. The dEMG software (EMGworks

Acquisition V4.3.0) recorded four separate EMG signals from the 5-pin sensor, at a sampling rate of 20 kHz, filtered with a bandwidth of 20-1750 Hz.

The isometric contraction implemented to record dEMG signals consisted of a 3 second quiescent period, a linear 7 second ramp up in force from 0% to 60% of baseline peak MVC force, 10 seconds of constant contraction force at 60%MVC, a linear ramp down from 60% to 0% and a final 3 second quiescent period. Participants were required to follow the required trapezoid trace via visual feedback on a computer screen. The signals collected during this task were analysed using dedicated software (dEMG Analysis, v 1.1.3) and Precision Decomposition III (PD III) algorithms (first described by De Luca & Adam, 1999) to decompose the raw EMG signals into individual motor unit action potential trains. The algorithms have been shown to reliably and accurately quantify motor unit behaviour by an extensive number of publications (De Luca and Hostage, 2010; Hu et al, 2013a, b, c; Hu et al, 2014). In order to accurately calculate the mean firing rate of active motor units, a long enough epoch of the plateau phase of the isometric trapezoid trace is needed, however, excessively long epochs may cause unwanted fluctuations in force and EMG amplitude. Our research group has previously deemed that a 3-second portion at the distal end of the plateau phase is the window with the greatest reliability (Balshaw, 2013). Motor unit action potentials were separated in three equal groups (if the number of MUAPs was not divisible by 3, the third group contained any extra MUs), allowing the MUAPs to be divided in tertiles (early recruited, mid recruited and later recruited MUs – Figure 4.1). This is an accepted method allowing us to quantify the order of recruitment of the different motor units, as already shown by in our laboratory by Balshaw et al, (2017). In order to assess the accuracy of the decomposing algorithm, a reconstruct-and-test procedure (Nawab et al, 2010; De Luca & Contessa, 2012) was performed over the chosen 3-second window showing any signals the software

misinterpreted using the following formula: Accuracy = 1 – N_{error} / N_{truth} (where N_{error} is the number of erroneous events, and N_{truth} is the number of true events). To minimize the risk of low accuracy MUs affecting the results, we included only MUs with a decomposition accuracy of ≥90% (Balshaw et al, 2017). To the best of our knowledge, this is the method best suited to validate the decomposition process of the sEMG data (De Luca et al, 2015).

The level of common drive (i.e. the degree of synchronized activity of different MUs during the same contraction) was determined via cross-correlation analysis of all the mean firing rates during the 3-second window. All possible combinations of motor units where cross-correlated with one another using previously described methods (Beck et al, 2012) and common drive was quantified as the peak cross-correlation coefficient calculated from each cross-correlation.

4.2.7 Cognitive function

Cognitive function was assessed in a quiet room using the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computer based cognitive assessment tool and neuropsychological standard. The following CANTAB tasks were included: Reaction Time (RTI; divided attention); Paired Associate Learning (PAL; long-term memory) and Spatial Working Memory (SWM; short-term memory).

4.2.8 Postural control

Postural control was assessed by means of a force platform (Bertec forceplate model 6090-15, Bertec Corporation, Columbus, OH, USA) connected to in house build software using raspberry pi. The interface was composed of a cross hairs display with the centre of the display being the centre of the force platform. The device returned a value indicating the participants' centre of pressure (COP). Participants completed 4, 20s conditions: 2 legs eyes open (2LEO), 2 legs eyes closed (2LEC), 1 leg eyes open (1LEO) and 1 leg eyes closed (1LEC). Each condition was performed twice, for a total of 8 measurements per test.

4.2.9 Statistical analysis

Statistical analysis and graph creation was carried out using GraphPad 6 Prism (v 6.0; GraphPad Software, Inc.). Data were checked for normality and $\log_e$ transformations were used on cSP, PAL and SWM as they appeared skewed. Non-transformed values (mean±SD) can be found in table 4.1. Repeated measures ANOVAs were used to explore the effect of sparring on corticomotor control, cognitive function, balance, common drive, and MUFR (group, 3 x time, 4 for the MUFR). If a significant difference was observed, Tukey's post hoc test was used to further explore the effect. Statistical significance was set at a p-value ≤ 0.05; where significant, eta squared (η^2) values were calculated with the formula: Sum of $Squares_{groups}$/Sum of $Squares_{total}$, and 95% upper and lower confidence intervals were established relative to the difference of means. Eta squared values were interpreted as: 0.02= small; 0.13= medium; 0.26= large. Data are presented as means (±standard deviation) unless otherwise stated.

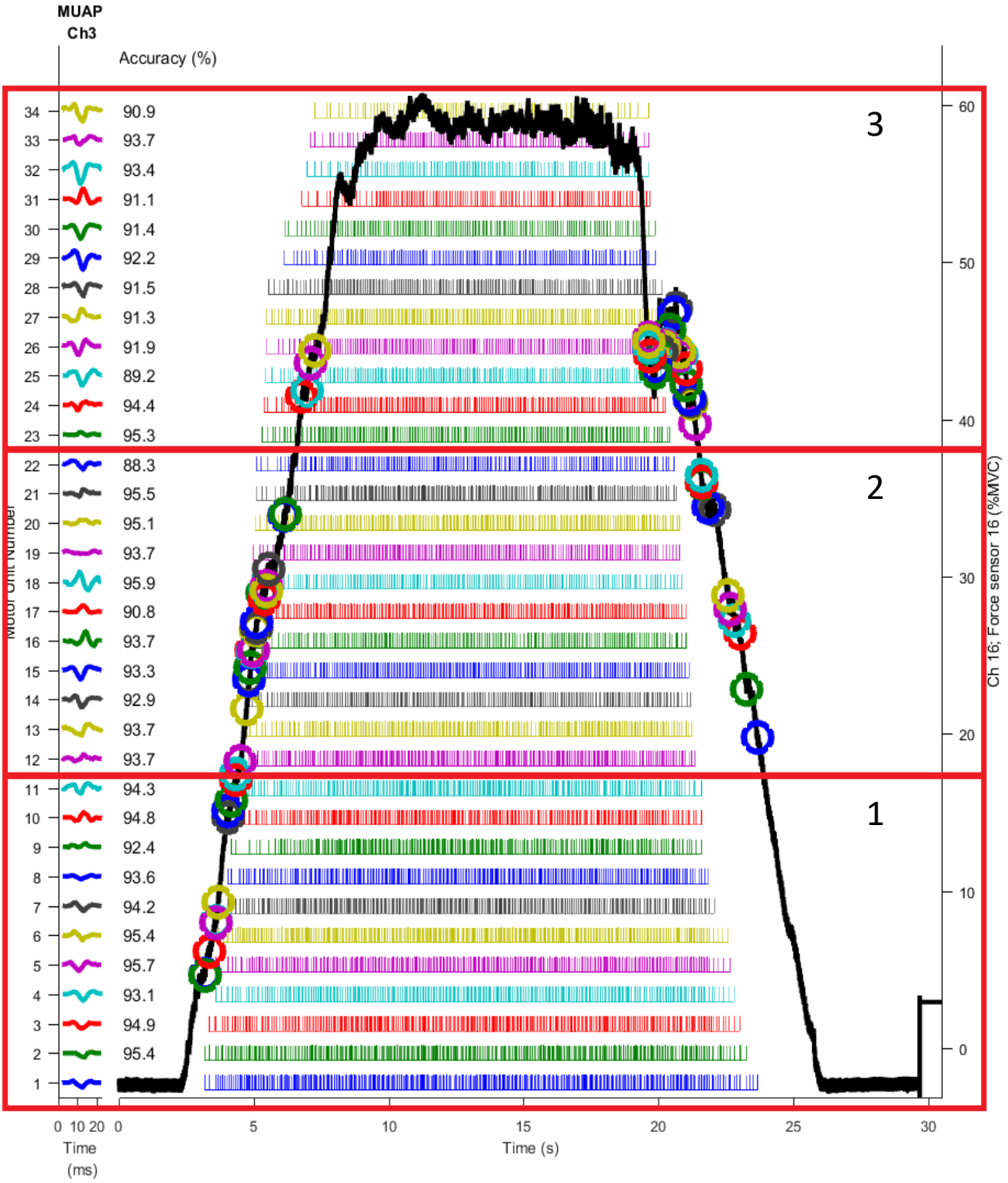

Figure 4.1. Example of one participant's MU firing rate data. Each vertical bar represents the firing of each motor unit with the black line indicating the force trajectory. The red boxes indicate tertile groupings: early recruited (1); mid-recruited (2); and later-recruited (3) motor units.

4.3 Results

4.3.1 Effect of sparring on corticomotor inhibition and corticospinal excitability

Corticomotor inhibition showed a significant effect over time (p= 0.012; $F_{(2.76, 49.74)}$ = 4.15 – figure 4.2) with inhibition significantly (p= 0.036; CI= 0.0029 to 0.1107; η^2= 0.01) increasing from baseline by 7.5ms 1h post sparring. Inhibition returned to baseline levels by 24h. Similarly, a significant time effect was also observed for corticospinal excitability (p= 0.0006; $F_{(2.74, 52.05)}$= 4.83 – figure 4.3), decreasing 24h post sparring compared to the 1h follow up (p= 0.014; CI= 2.40 to 24.64; η^2= 0.06). There were no changes when compared to baseline.

4.3.2 Precision decomposition EMG

There was a significant effect over time for the relationship of the slope coefficient and y-intercept (p= 0.028; $F_{(2.21, 39.79)}$ = 3.76 and p= 0.035; $F_{(2.88, 51.85)}$= 3.13, respectively – figures 4.4 and 4.5) between average MUFR and the recruitment threshold. This time effect was caused by a decline in the slope coefficient 1h post sparring compared to baseline (p= 0.011; CI= -0.29 to -0.03; η^2= 0.08) and 1h after sparring which returned to baseline by 24h (p= 0.008; CI= -0.27 to -0.03; η^2= 0.08). Whereas the y-intercept mirrored this by increasing 1 hour post sparring, compared to baseline (p= 0.026; CI= -11.68 to -0.61; η^2= 0.05) and returning to baseline by 24 hours. Mean values can be found in table 4.2.

4.3.3 Alterations to cognitive function following sparring

The total adjusted error score of the PAL task significantly increased by 47% (p= 0.013; $F_{(1.91, 36.32)}$= 4.97 – figure 4.6) following the sparring session. (p= 0.018; CI= 0.10 to 1.22; η^2= 0.12). Furthermore, the parameter also demonstrated a tendency (p= 0.072) to remain elevated at the 24h follow-up. No significant differences were observed for RTI and SWM (p> 0.05) (Table 4.1).

4.3.4 No alterations to postural control following sparring

There were no changes observed in postural control in all four conditions (2LEO, 2LEC, 1LEO, 1LEC) (p> 0.5) (Table 4.1).

4.3.5 Maximal voluntary contraction (MVC)

MVC significantly (p= 0.006; CI= -26.42 to -3.89; η^2= 0.01 – figure 4.7) decreased 1h following the sparring protocol, with an average decline of 15.1±17.4 Nm from baseline. No significant effects were observed for the other time-points (p> 0.1).

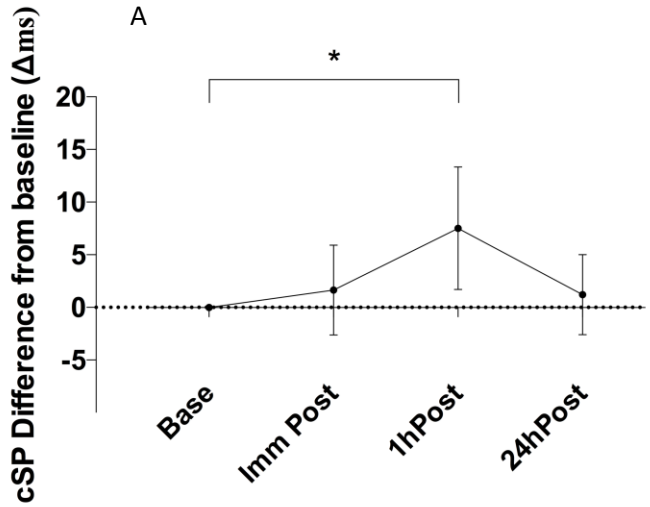

Fig 4.2A. Difference of cSP relative to baseline. Inhibition appeared increased following sparring, peaking at the 1h mark with a 6% increase, and returning to pre-values by the 24h follow up. *p= 0.03; error bars indicate 95% CI.

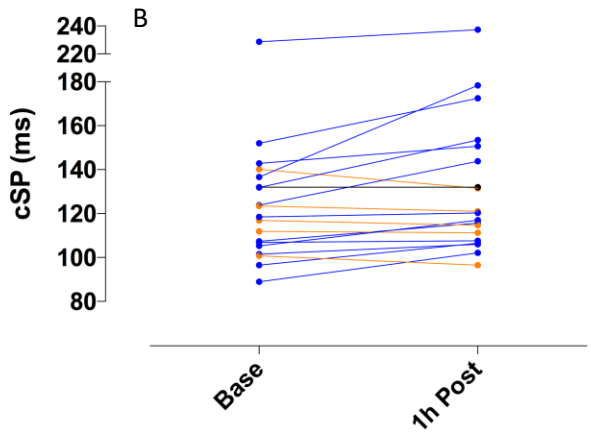

Fig 4.2B. Change in cSP duration for each participant from baseline to 1h following sparring.

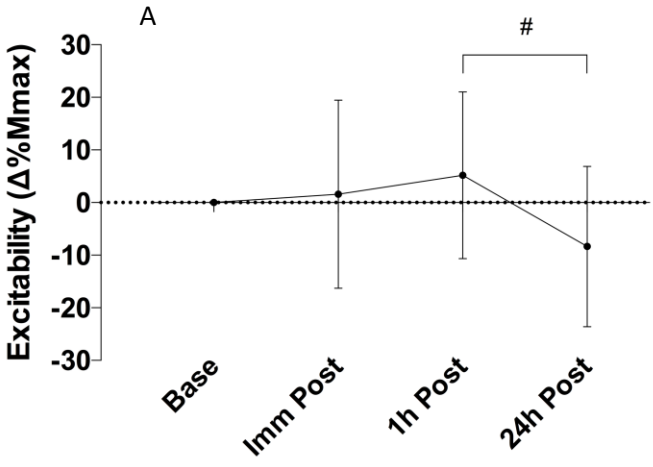

Fig 4.3A. Difference of corticospinal excitability relative to baseline. Excitability appeared slightly increased immediately and 1h post sparring (though not significant) and decreased 24h after sparring, when compared to the 1h post follow up. #p= 0.014; error bars indicate 95% CI.

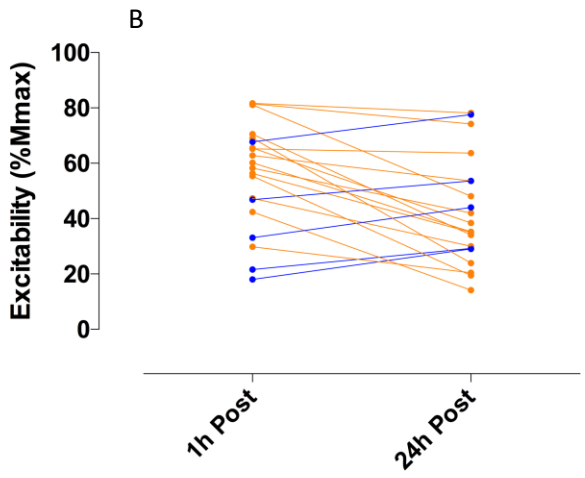

Fig 4.3B. Change in excitability for each participant from 1h to 24h following sparring.

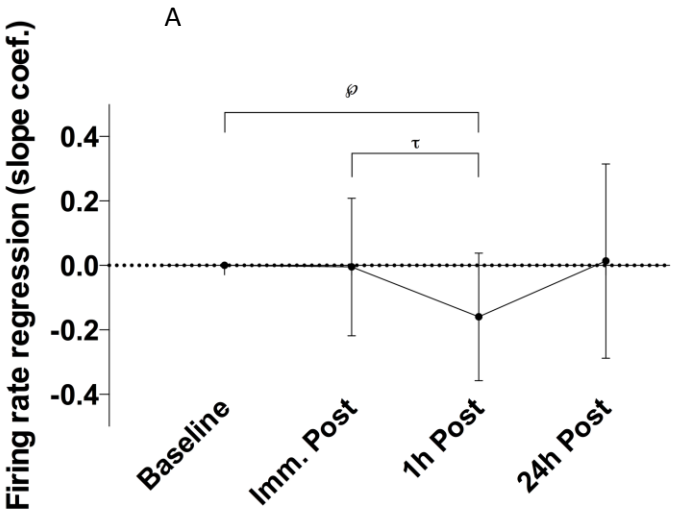

Fig 4.4A. Difference of the slope coefficient of the firing regression relative to baseline. There appeared to be negative shift in the regression's slope following sparring, peaking at the 1h time-point. ℘p= 0.011; τp= 0.008 error bars indicate 95% CI.

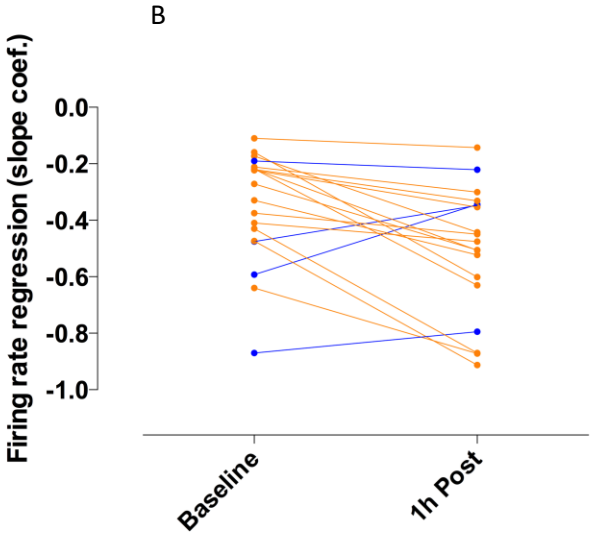

Fig 4.4B. Individual data-points showing the change between baseline and 1 hour post-sparring.

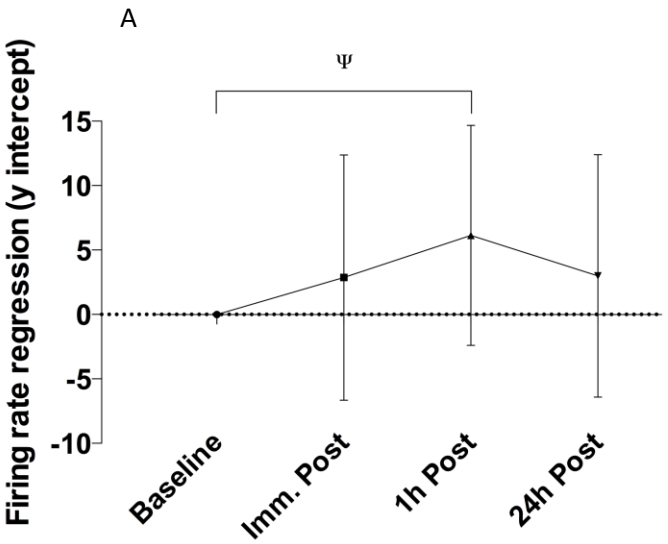

Fig 4.5A. Difference of the y intercept of the firing regression relative to baseline. There appeared to be an upwards shift in the regression, peaking at the 1h time-point. ψp= 0.026; error bars indicate 95% CI.

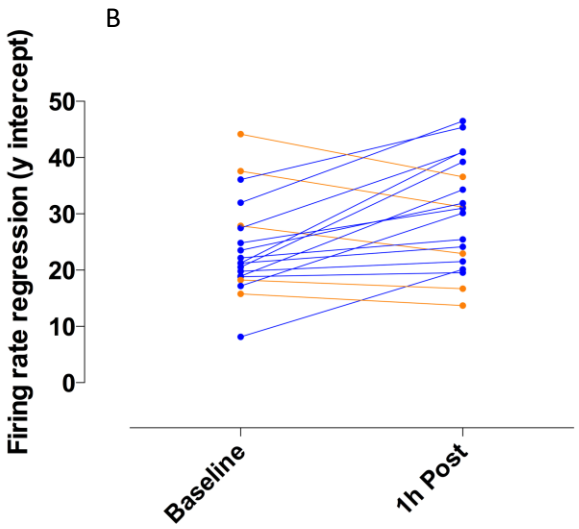

Fig 4.5B. Individual plots showing the change between baseline and 1 hour post

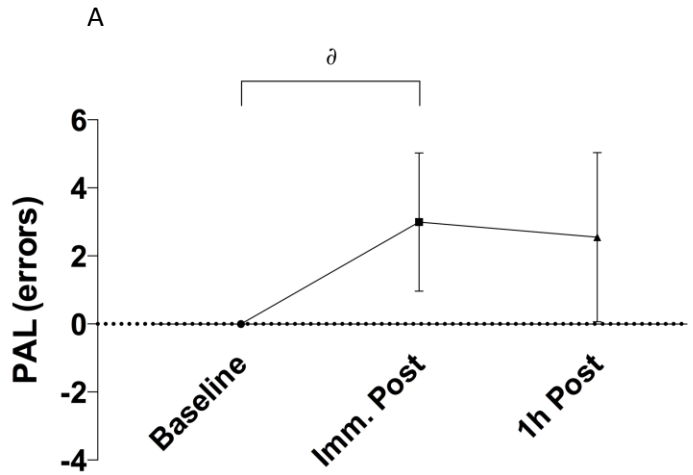

Fig 4.6A. Difference in the PAL subtask relative to baseline. Memory performance (number of errors made) appeared to decrease immediately following the sparring, and remain in a similar state at the 24h follow up (though not statistically significant). ∂p= 0.018; error bars indicate 95% CI.

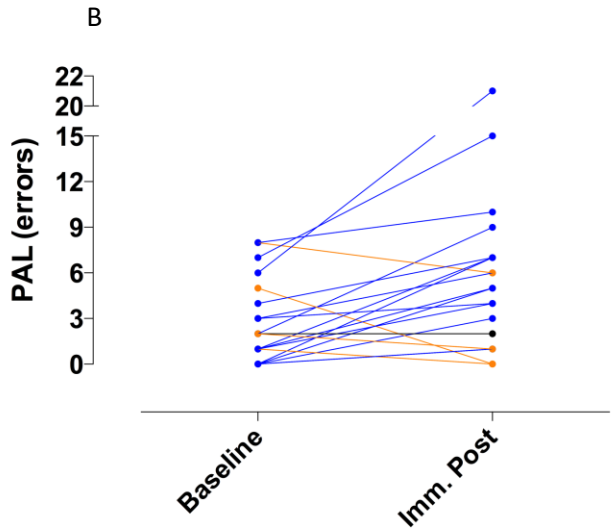

Fig 4.6B. Individual data-points showing the number of errors made on the PAL subtask before and after the sparring session.

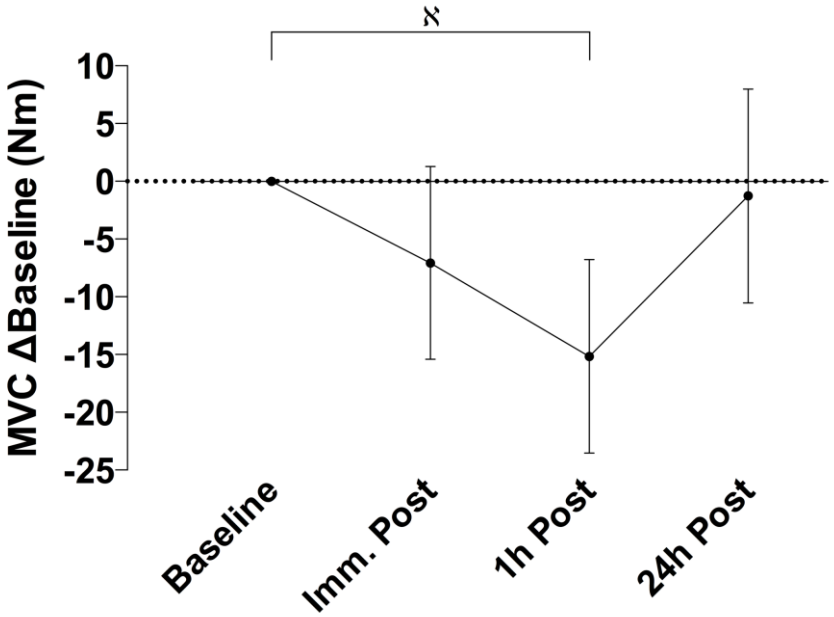

Fig 4.7 Difference in MVC relative to baseline. Force production declined following the sparring protocol, reaching statistical significance at the 1h follow-up. χp< 0.006; error bars indicate 95% CI.

Table 4.1: Mean (±SD) values for each of the outcome measures: corticomotor inhibition (cortical silent period in ms) and corticospinal excitability (MEP amplitude normalized to femoral nerve M-wave, %Mmax), paired associate learning (PAL errors), spatial working memory (SWM errors), reaction time (choice RTI decision times ms) and postural control (centre of pressure for each condition) measured at each time point. *p=0.03 Baseline v 1h Post; #p=0.01 1h Post v 24h Post; ∂p=0.01 Baseline v Imm. Post

Variable	Assessment Time Post Sparring			
	Baseline	Immediately	1 h	24 h
TMS				
Inhibition (ms)	124.5 ± 30.6	126.2 ± 34.1	132.0 ± 32.9*	125.7 ± 29.4
Excitability (%Mmax)	50.5 ± 20.6	52.1 ± 18.8	55.7 ± 19.0	42.2 ± 19.3#
Cognitive Function				
PAL (errors)	3 ± 3	6 ± 5∂	-	5 ± 4
SWM (errors)	6 ± 9	10 ± 10	-	9 ± 9
RTI (ms)	304.1 ± 33.9	308.0 ± 35.5	-	305.7 ± 33.0
Postural Control				
2LEO (COP)	48.3 ± 18.6	44.8 ± 23.4	-	50.1 ± 18.2
2LEC (COP)	46.6 ± 16.5	48.8 ± 22.9	-	50.0 ± 18.1
1LEO (COP)	29.2 ± 14.8	34.6 ± 12.8	-	35.1 ± 14.3
1LEC (COP)	34.1 ± 15.4	38.3 ± 19.7	-	34.5 ± 15.2

Table 4.2: Mean (±SD) values for precision decomposition EMG measures: recruitment threshold and motor unit firing rate regression (slope coeff., y-intercept and R^2), common drive (correlation coeff. of individual motor units) and motor unit firing instances (pulses per second – divided in early, mid and late recruited MUs) measured at each time point. $\wp$p=0.01 Baseline v 1h Post; τp=0.008 Imm. Post v 1h Post; ψp=0.026 Baseline v 1h Post

Variable	Assessment Time Post Sparring			
	Baseline	Immediately	1 h	24 h
Rec. Thresh. Vs MUFR				
Slope coefficient	-0.35 ± 0.20	-0.35 ± 0.16	-0.51 ± 0.22$^{\tau}_{\wp}$	-0.33 ± 0.32
Y-intercept	23.99 ± 8.60	26.86 ± 7.92	30.13 ± 9.79ψ	26.98 ± 10.95
R^2	0.72 ± 0.20	0.75 ± 0.12	0.77 ± 0.09	0.78 ± 0.14
Common drive				
Cross correlation coeff.	0.40 ± 0.03	0.41 ± 0.05	0.39 ± 0.03	0.40 ± 0.03
MUFR				
Early recruitment	17.81 ± 1.81	17.96 ± 2.82	17.91 ± 3.03	18.58 ± 2.53
Mid recruitment	14.62 ± 1.77	14.43 ± 2.40	14.76 ± 3.34	15.22 ± 2.02
Late recruitment	11.29 ± 2.38	11.33 ± 2.59	11.86 ± 3.52	11.97 ± 2.81

4.4 Discussion

We have shown that a single sparring session, routinely performed in boxing and a number of other martial arts disciplines, is associated with measurable changes in electrophysiological and cognitive parameters. Corticomotor inhibition increased alongside an alteration in motor unit recruitment behaviour; the negative shift in the linear regression suggested that thresholds of the early recruited motor units were delayed whereas recruitment thresholds of later recruited motor units occurred sooner (please refer to appendix A for a graphical illustration). Furthermore, visual memory and new learning (PAL) (Arndt, 2012) were also decreased following sparring.

The current study strengthens the notion that subconcussive head impacts affect motor control, as we observed similar increases in corticomotor inhibition following routine soccer heading, described in chapter 3 (also published - Di Virgilio et al, 2016). Although the exact mechanisms of action are not yet fully understood, corticomotor inhibition is a reflection of gamma-aminobutyric acid (GABA) activity within the motor system (Inghilleri et al, 1993); an elongation of the silent period measured by EMG is indicative of an increase of levels of GABA within the synapse. Within this context, our results are of interest because such an increase in GABA activity has been previously shown in patients with diagnosed concussion or mild TBI (De Beaumont et al, 2007; Chistyakov et al, 2001; Livingston et al, 2010). It has been suggested that with more severe brain trauma, lesions affecting thalamocortical, corticocortical and striatocortical nerve fibres alongside impairments in $GABA_B$ receptor activity could increase cortical inhibition (Chistyakov et al, 2001). While we do not think that the force of head impacts in our study were high enough to cause such lesions, it may well be that they were forceful enough to disrupt the normal functioning of the GABAergic inhibitory neurons, in turn eliciting and increase in cortical silent period duration.

The increase in corticomotor inhibition in the current study was observed in conjunction with decreased maximal force production and altered motor unit behaviour. The decrease in force production may be due to a number of reasons. Cortical inhibition is thought to modulate the force generating ability of muscles, particularly in the relaxing phase of a contraction, by dampening corticospinal input (Motawar et al, 2012). In our study, the increase in GABA activity caused by the sparring protocol may have diminished the descending neural drive to the muscles. The end result is a possible impairment in the participants' ability to maintain maximal force levels observed during baseline testing.

A less intriguing explanation for the observed decline in MVC may simply be due to neuromuscular fatigue, as the sparring session was moderately intense and the testing period relatively long. We believe this to be unlikely, as we would have observed significant declines in force production immediately following the sparring protocol. Furthermore, due to the design of the study there was a ~40 minute interval between the immediately post- and 1h post- neuromuscular measures which would have allowed participants to recover from any residual fatigue.

Decomposition EMG (dEMG) is a widely validated technique used to assess motor unit recruitment properties (Nawab et al, 2010). By using dEMG we are able to gain a more intimate understanding of how brain and muscles work together to produce movement. We found that RSHIs alter the recruitment strategies of the neuromuscular system. The decrease in the slope coefficient of the regression, alongside an increase in the y-intercept can be interpreted in a number of ways. The early recruited motor units may have shifted to the left, or the late recruited motor units shifted to the right of the regression suggesting an alteration in the speed at which action potentials propagate onto the sarcolemma. However, if that were the case the tertile analysis of the mean firing rates of early, mid and late recruited motor units would have reflected such a change. It is then more likely that the point at which the motor units were recruited would be affected (recruitment threshold), with the early recruited motor units being activated later, and the late recruited motor units being engaged earlier compared to baseline. The most intriguing finding is perhaps that these effects are seen 1h following sparring, matching the time-course of corticomotor inhibition. Altered motor unit behaviour generally reflects diminished descending neural drive from the CNS during times of stress or danger (Davis and Bailey, 1997; Nybo and Nielsen, 2001; Hunter et al, 2011), possibly mediated by GABA activity. Motor unit behaviour in the current study, coupled with increased corticomotor inhibition, suggests that the

GABAergic protective mechanism (seen in concussed patients) may be triggered following subconcussive blows to the head. To the best of our knowledge, this is the first study to integrate the use of TMS and dEMG in the context of head impacts, showing a direct link between changes at the cerebral level translating to changes at a muscular level.

Contrarily to our previous findings of increased inhibition immediately after soccer heading, the current study shows significant effects 1 h post sparring. Unfortunately, a direct comparison between the two studies is not possible, as the first study did not include a 1h follow up. We speculate that if we had included an assessment 1h following soccer heading we would have observed an even greater increase in inhibition, possibly mediated by more consistent (in both force and location) head impacts, as opposed to the more random ones observed during sparring. Nevertheless, the current findings suggest that the onset of alterations within the brain following repetitive impacts to the head may not be as immediate as we previously believed. It remains to be seen if concussive episodes follow a similar time-course; an interesting notion, particularly in sporting environments where concussion assessment tests are usually administered immediately after a suspected concussion.

Nowadays it is well established that alterations caused by head impacts are not only limited to the brain. Applying magnetic stimulations over M1 can be extremely useful in this framework as it provides an indication of both cortical and spinal mechanisms (i.e. neuromuscular). A number of studies have found an association between blows to the head and changes in the neuromuscular system. Increased corticomotor inhibition as a result of a concussive injury could translate into impaired postural control and gait, both hallmarks of mTBI (McCrory et al, 2013; Parker et al, 2006). Decrements in motor control,

and vestibular function in particular, have also been shown following RSHIs (Kawata et al, 2016; Haran et al, 2013; Hwang et al, 2017). The limiting factor of these studies is that whilst they show that RSHIs trigger changes in motor control, they are unable to discern what exactly causes these alterations within the neuromuscular system. Furthermore, postural control is a fairly indirect measure of motor control, and may not be sensitive enough to pick up on subtle changes to brain function as studies show diverging results (Haran et al, 2013; Di Virgilio et al, 2016; Broglio et al, 2004). It is not surprising, therefore, that postural control appeared unchanged across the time-points in the current study. The novelty of the current study and the findings in chapter 3 are that the alterations in motor control, shown by an increase in corticomotor inhibition, were until now only found in relation to more severe types of brain injury. We show that although transient, RSHIs nevertheless induce an increase in the activity of GABAergic mechanisms.

RSHIs were also associated with changes in parameters of cognitive function. More specifically, PAL – a measure of new learning and visual memory appeared decreased following the sparring session. This parameter has been found to be impaired in elite Australian Rules footballers when compared to amateur players (Pearce et al, 2014c), and overall declines in cognitive function are reported in soccer and other sports (Lipton et al, 2013; Echemendia et al, 2001)

Future work should include more assessments between the 1h and 24h time points to further understand the time course of these alterations. Due to methodological limitations we were unable to record force of impact data during the sparring sessions, future extensions of this study should incorporate the use of accelerometers to monitor the extent of the impacts the athletes are receiving.

Whilst single pulse TMS allows us to infer alterations in GABA activity, it is only limited to mechanisms mediated by $GABA_B$ receptors; future studies could differentiate between $GABA_A$ and $GABA_B$ receptor modulation by using paired pulse TMS.

4.5 Conclusion

The results from this chapter provide further evidence that RSHIs are associated with acute, transient electrophysiological and cognitive changes. These findings are similar to what we have previously shown in soccer players, indicating that alterations brought about by subconcussive impacts are not sport-specific. Although temporary, these alterations in the neuromuscular system (including an impairment in force generating abilities) following an exercise that is routinely performed in many martial arts may result in an increased risk of injury and long-term complications to brain health, if exposed to repeatedly over the course of a lifetime.

Chapter 5

Stability of corticomotor inhibition ad corticospinal excitability following two exercises with differing metabolic demands

Abstract

Introduction: This thesis has previously shown measurable alterations in brain-to-muscle pathways and cognitive function following subconcussive head impacts. Whilst of interest, interpretation of the data is limited by the lack of control exercise groups, to account for the possible effect of exercise alone on inhibitory and excitatory parameters. Accordingly, the aim of chapter 5 is to assess corticomotor inhibition and corticospinal excitability following two types of exercise. The tasks are designed to simulate soccer heading and sparring (boxing), without any head impacts. **Methodology:** Twenty healthy boxers and muai thai athletes (5 females, age 22 ± 3 y) performed either a jumping exercise or a mock-sparring session in a randomized, cross-over design. Corticomotor inhibition, corticospinal excitability and cognitive function were assessed prior to, immediately following, and 1h following both exercises. These data were tested for an interaction effect against the results from chapters 3 (jumping v soccer heading) and 4 (mock-sparring v sparring) **Results:** Corticomotor inhibition showed significant interaction effects both when the control (heading) group was compared to the data from chapter 3 ($p= 0.01$; $F_{(1, 36)}= 7.33$; $\eta^2= 0.20$), and when the control (sparring) group was compared to the sparring data in chapter 4 ($p= 0.04$; $F_{(2, 72)}= 3.14$; $\eta^2= 0.08$). No changes in cognitive function occurred following the jumping exercise, whilst reaction time was increased following the mock-sparring exercise (307.7 ± 50.4 to 323.3 ± 61.1 ms, $p= 0.04$). **Conclusion:** Corticomotor inhibition and corticospinal excitability are largely unaffected by non-contact exercises simulating soccer heading and sparring, reinforcing the notion that the alterations observed in chapters 3 and 4 are most likely due to RSHIs.

5.1 Introduction

In chapters 3 and 4 we have shown that parameters of motor control, more specifically corticomotor inhibition and corticospinal excitability appear altered following RSHIs. Whilst of interest, one of the limitations of both projects is the lack of a control group to account for any effects caused by muscular fatigue, and other factors associated with physical exertion.

Fatigue in the context of physical exercise refers to reduced muscle capacity to produce or maintain force/power (Gandevia, 2001), a process that can occur distally to the neuromuscular junction (peripheral fatigue) and within the central nervous system (central fatigue) (O'Leary et al, 2017). Peripheral fatigue is brought about mostly by inhibited muscle contraction mediated by intramuscular metabolic factors, such as reduced pH (Hunter et al, 2009) and a failure of the excitation-contraction coupling mechanisms, from impaired Ca^{2+} release from the SR (Kent-Braum, 1999). Contrastingly, central fatigue is a decline of the neural drive originating from the brain, most likely due to neurochemical changes and inhibitory mechanisms within the cortex (Roelands et al, 2015). These two mechanisms of muscle fatigue do not work independently of each other; rather, they work together, through afferent and efferent signaling in modulating force/power decline of skeletal muscle typically responsible for performance.

Central or more specifically; spinal and supraspinal contributions to the development of muscle fatigue can be effectively measured with TMS (Gandevia et al, 1996; Weavil et al, 2016; Suruagy et al, 2017). The response of the corticospinal tract to exercise is dependent on its type and intensity; studies using single-joint nonfatiguing tasks have shown that increasing muscle activation facilitates the excitability

of the corticospinal pathway, even in the presence of peripheral fatigue (Weavil et al, 2016; Sidhu et al, 2009; Levenez et al, 2008). Conversely, fatigue induced by maintaining muscle activation (i.e. EMG) at a constant level during submaximal contraction appears to impede excitability; the mechanism is possibly mediated through modulation of the afterhyperpolarization period (APH), as it has been shown that increasing the APH results in a decrease in the firing rate of the motoneurons (McNeil et al, 2011; Martin et al, 2006; Mathews 1999). Increases in corticospinal excitability through muscle activation have also been shown in non-fatiguing whole body exercises (i.e. cycling), whereas fatiguing whole body exercise does not affect excitability (Sidhu et al, 2012; O'Leary et al, 2016). It is possible that the facilitatory effect of muscle activation is cancelled out by fatigue-related depression in the corticospinal pathways, resulting in overall unchanged corticospinal excitability (Weavil et al, 2016).

Corticomotor inhibition, quantified as the duration of the EMG silent period (cSP) following stimulation over the motor cortex, increases during single-joint fatiguing contractions (Taylor and Gandevia, 2001). This increased inhibition appears transient, as it returns to pre-exercise levels within 10-30s of recovery (Taylor et al, 1996; Taylor and Gandevia, 2001). Contrariwise, cSP is unaffected following moderate, and suppressed following high intensity whole body exercises (O'Leary et al, 2016).

The aim of this experimental project, therefore, was to assess corticomotor inhibition and corticospinal excitability following two types of exercises, without head impacts, to simulate: 1) soccer ball heading and; 2) boxing sparring. The subconcussive protocols implemented in the previous chapters were of light (soccer heading) and moderate (sparring) intensity; as such, we hypothesized that corticomotor

inhibition and corticospinal excitability in the current study would remain unchanged following both types of exercise.

5.2 Methodology

5.2.1 Approvals and Recruitment

Twenty-three healthy males and females (4 females, age 22±3 y, mass 76.4±13.6 Kg; height 174.8±9.8cm) were recruited for the project by means of advertisements on notice boards and social platforms. Potential candidates were excluded if they presented with any of the following: 1) history of brain injury resulting in loss of consciousness; 2) history of a neurological condition; 3) history of concussion in the 12 months prior to taking part; 4) family history of epilepsy; 5) current use of psychoactive recreational or prescription drugs. Two participants withdrew their consent before completing the study, and one participant withdrew due to injury; the final cohort included a total of 20 participants. The local Research Ethics Committee approved the study, and all procedures conformed to the Declaration of Helsinki. Written informed consent was obtained from all participants prior to taking part.

5.2.2 Study design

Participants were asked to refrain from vigorous physical activity, consuming alcohol, and caffeine or smoking for 24h prior to each study session. Participants were also required to present to the laboratory fasted where they were provided with a standardized breakfast. Prior to commencing data collection, participants reported to the laboratory for a familiarization session, during which they completed all outcome measures to acquaint them with the assessment procedures and minimize later

learning effects. In addition to the familiarization session, the study design comprised two experimental sessions spaced 1 week apart (Figure 5.1).

For each experimental session baseline measures for cognitive function, corticospinal excitability and corticomotor inhibition were recorded; following baseline testing participants completed one of two exercises tasks, in a randomized order. Exercise 1 simulated a soccer heading drill: participants were asked to complete 20 maximal jumps whilst keeping head movement to a minimum, with 30 seconds rest in between each jump. Exercise 2 simulated a sparring session; participants completed three, 3-minute rounds with one minute rest between each round. To ensure all participants performed a similar amount of exercise, each sparring round started with a 10 second period of moving about, followed by a 20 second bout of pad hitting using a 3-punch combo (Right, Left, Right; Left, Right, Left; Right, Left, Right; etc.); cues for each combo were given by a metronome set at 30bpm. Participants alternated between moving about and pad hitting until the 3-minute round was up. The same measures as baseline were recorded immediately following both types of exercises, with a further assessment of corticomotor inhibition and corticospinal excitability 1h following the exercises.

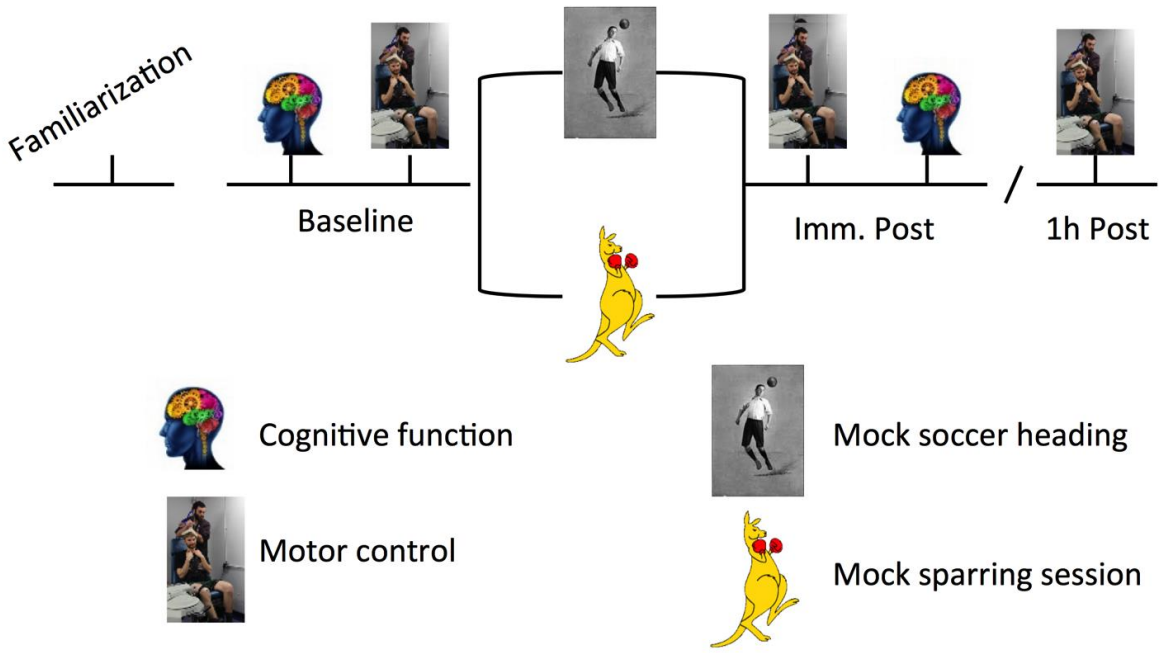

Figure 5.1. Experimental timeline of the study

5.2.3 Transcranial magnetic stimulation

Motor evoked potentials (MEPs) were elicited in the Rectus Femoris of the dominant leg via single pulse TMS and recorded using EMG. For more in depth methodology please refer to section 2.2.3 in chapter 2)

5.2.4 Electromyography and Peripheral nerve stimulation

All EMG activity was recorded using previously described methods (section 2.2.4 in chapter 2)

5.2.5 Cognitive Function

Cognitive function was assessed in a quiet room using the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computer based cognitive assessment tool. Paired associate learning (PAL), Spatial working memory (SWM) and Reaction time (RTI) were assessed.

5.2.6 Statistical analysis

Graphpad Prism 6 was used for statistical analysis and graph creation. Because our intention was to relate the TMS-related measures in the current study to the ones previously recorded for chapter 3 and 4, we used two-way ANOVAs (time x group) comparing the jumping exercise to the soccer heading dataset (Ch. 3), and mock-sparring to the actual sparring (Ch. 4) for any interaction effect. To account for the groups being independent, time and groups were respectively set as within- and between-subject factors. Furthermore, we also analyzed the datasets in the current chapter using one way repeated measures ANOVAs (corticomotor inhibition, corticospinal excitability and MVC); if a main effects significant difference was observed, Tukey's post hoc test was used to further explore the effect. PAL, SWM and RTI were analysed using paired t-tests, comparing pre- to post- exercise timepoints for any differences. Furthermore, paired t-tests were also performed between the baseline measures of each test to determine their stability from one week to the next. Statistical significance was set at a p-value ≤ 0.05. Data are presented as means (± standard deviation) unless otherwise stated.

5.3 Results

Comparing the measures taken at baseline from the control (heading) and control (sparring) groups showed no significant differences ($p > 0.05$) (Table 5.1).

5.3.1 Effect of exercise on corticomotor inhibition and corticospinal excitability

Although corticomotor inhibition in the control (heading) group was unchanged, as predicted, immediately following jumping ($p > 0.05$), the measure appeared significantly increased 1h following the exercise in comparison to baseline ($p = 0.01$; CI= 0.84 to 6.92), and immediately after the exercise ($p=0.002$; CI= 2.43 to 11.22) by 3.3% and 5.9% respectively (Figure 5.2A). Conversely, the parameter remained unchanged in the control (sparring) group, following the mock-sparring exercise ($p > 0.05$) (Figure 5.2B).

Furthermore, corticomotor inhibition showed significant interaction effects between control (heading) group and heading group (chapter 3) ($p = 0.01$; $F_{(1, 36)} = 7.33$; $\eta^2 = 0.20$) (Figure 5.3A) and between control (sparring) and sparring (chapter 4) ($p = 0.04$; $F_{(2, 72)} = 3.14$; $\eta^2 = 0.08$) (Figure 5.3B). These interactions occurred from a 4.5% increase in corticomotor inhibition following the heading protocol (chapter 3), compared to a 2.5% decline in the control (heading) group. Similarly, the inhibitory parameter increased by 6% at the 1h measure in the sparring group (chapter 4), compared to 0.08% increase the control (sparring) group.

No significant effects were observed on corticospinal excitability following both types of exercise ($p>0.2$) (Figure 5.2, 5.3 C and D).

5.3.2 Cognitive function following exercise

There were no changes observed for all cognitive measures following the jumping exercise ($p>0.05$) (Table 5.2). A significant increase was found in RTI following the exercise task in the control (sparring)

group (p=0.043; CI= -30.57 to -0.5076), whilst the remaining measures remained unchanged following the same task (p>0.05) (Table 5.2).

5.3.3 Maximal voluntary contraction (MVC)

MVC was unchanged following control (heading) (p> 0.05) but declined following control (sparring) (p= 0.01; $F_{(1.94, 36.92)}$= 4.68; η^2= 0.006) (Figure 5.4). More specifically, MVC declined by 6.7% 1h post sparring when compared to baseline (p=0.01; CI= -22.51 to -1.984) (Figure 5.4B).

Table 5.1. Mean (±SD) values for each of the outcome measures assessed at baseline for both types of exercise (jumping and mock-sparring). No differences where observed between groups (p>0.05).

Variable	**Exercise type**	
	Control (heading)	**Control (sparring)**
Excitability (%Mmax)	83.3 ± 41.4	79.2 ± 38.9
Inhibition (ms)	118.8 ± 12.3	122.6 ± 13.1
PAL (errors)	3 ± 2	3 ± 3
SWM (errors)	11 ± 9	8 ± 7
RTI (ms)	299.5 ± 40.3	307.7 ± 50.4

Table 5.2. Mean (±SD) values of cognitive function parameters for both types of exercise (jumping and mock-sparring). *p<0.05.

Variable	**Exercise type**			
	Control (heading)		**Control (sparring)**	
	Baseline	Post	Baseline	Post
PAL (errors)	3 ± 2	6 ± 6	3 ± 3	5 ± 5
SWM (errors)	11 ± 9	7 ± 7	8 ± 7	6 ± 5
RTI (ms)	299.5 ± 40.3	307.9 ± 56.5	307.7 ± 50.4	323.3 ± 61.1*

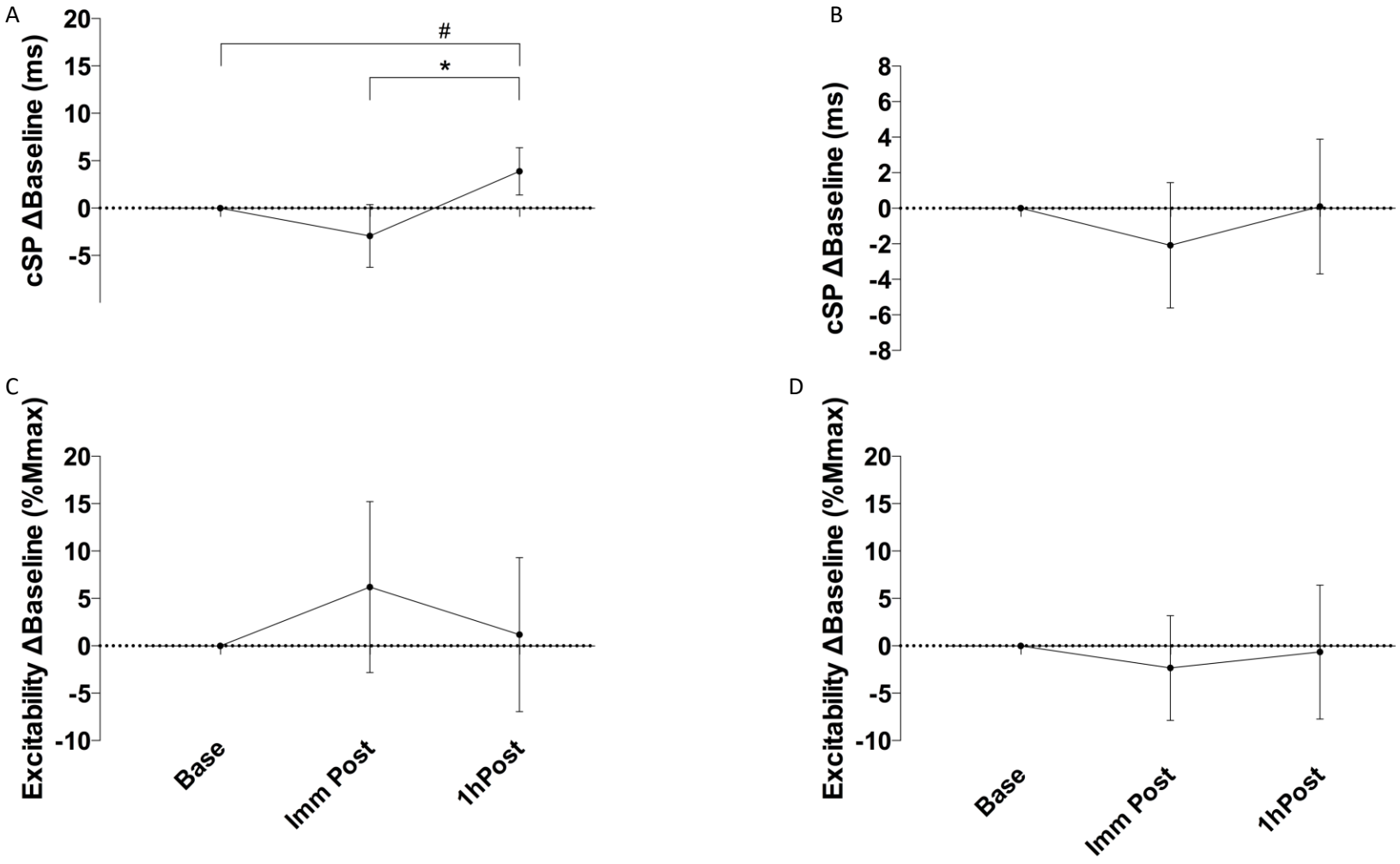

Figure 5.2. Corticomotor inhibition was increased 1h following the jumping exercise (A) and unaltered following mock-sparring (B). # and * $p < 0.05$ from baseline and immediately post, respectively. No differences were observed in corticospinal excitability when following both exercises (C and D). Error bars denote 95% CI.

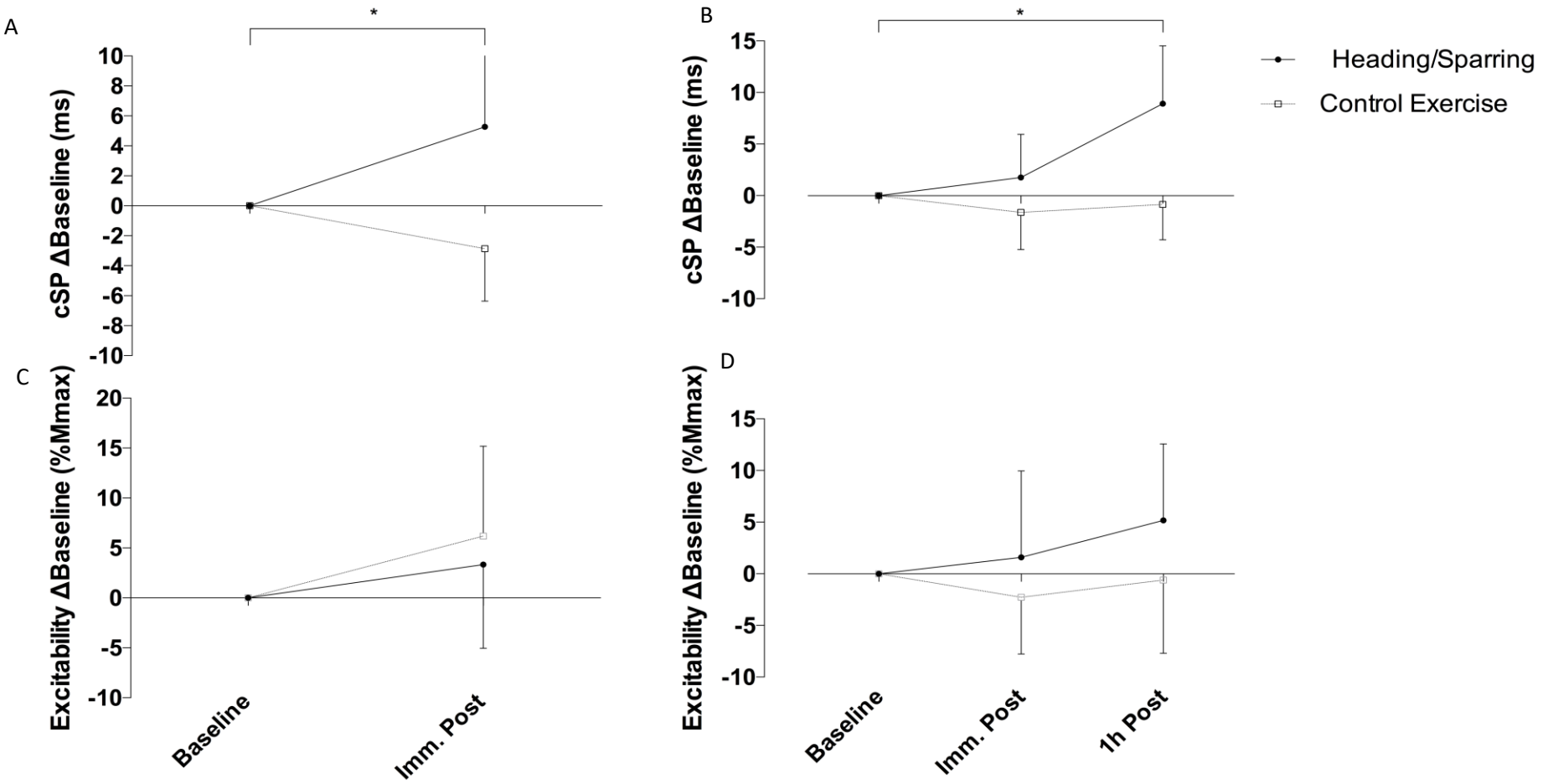

Figure 5.3. Corticomotor inhibition showed a significant interaction effect when comparing jumping to heading (A) and mock-sparring to sparring (B). *p<0.05. No differences were observed in corticospinal excitability when comparing jumping to heading (C) and mock-sparring to sparring (D). White squares represent control groups (jumping/mock-sparring); black circles represent actual heading/sparring. Error bars denote 95% CI.

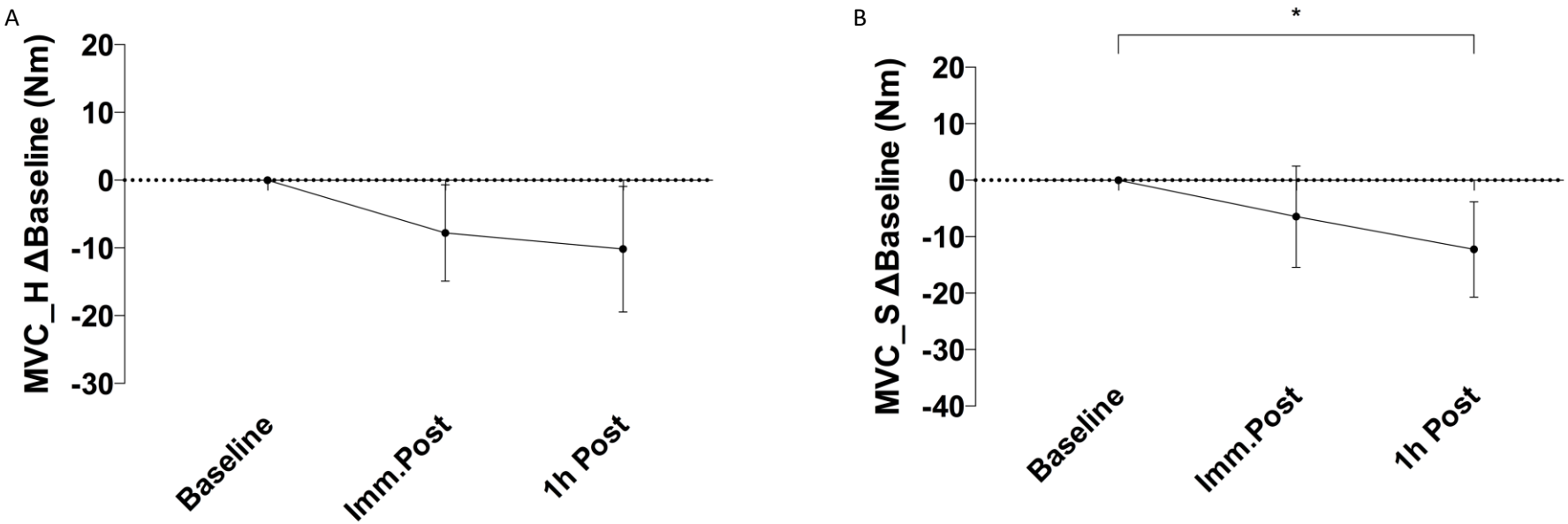

Figure 5.4. MVC was unchanged following the jumping exercise (A) ($p>0.05$). Force production was decreased 1h following the mock-sparring exercise (B) ($p<0.05$). *Significantly different from baseline. Error bars denote 95% CI.

5.4 Discussion

The aim of this study was to assess whether specific tasks not involving RSHIs affected motor control, through exercise-dependent modulation of corticomotor inhibition and corticospinal excitability. The control (heading) task was designed to simulate the heading protocol implemented in experimental chapter 3, and the control (sparring) protocol replicated the sparring session in chapter 4. Corticomotor inhibition following control (heading) remained unchanged in comparison to the increase following heading (chapter 3) over the same time points. However, it is interesting to note that 1 hour following control (heading) we did show increased corticomotor inhibition. Similarly, corticomotor inhibition measured in the control (sparring) group also showed a significant interaction effect when compared to the actual sparring data (chapter 4). Corticospinal excitability remained unchanged following both types of exercise, alongside PAL, SWM. Reaction time (RTI) was also unchanged following the jumping exercise, however, it was slower in the control (sparring) group, following the exercise.

Corticomotor inhibition showed to respond differently when measured following a subconcussive protocol, or following control exercises without head impacts: the parameter was increased following RSHIs, whilst it remained stable immediately following the jumping exercise, and 1h following the mock-sparring exercise. The exercises in the current study were designed to have similar physiological demands on the body as the protocols in the previous chapters, as such, this is evidence that the increased inhibition associated with the heading and sparring protocol in the previous chapters is most likely due to the RSHIs, and not an effect of the exercise itself.

Interestingly, when analyzing corticomotor inhibition for just control (heading) over 3 time points we found it increased 1h following the exercise in comparison to baseline. The cSP increases when measured during a fatiguing exercise or contraction (Taylor et al, 1996; Taylor et al, 2000; Søgaard et al, 2006), returning to pre-exercise values almost immediately upon completion of the exercise (Taylor et al, 1996; Søgaard et al, 2006; Taylor and Gandevia, 2001). Increased corticomotor inhibition following the jumping exercise would suggest muscular fatigue. However, MVC was maintained; since fatigue is, by definition, an impairment in one's ability to maximally generate force (Hill et al, 1924) it should follow that the jumping exercise in the current study did not induce a level of fatigue great enough to impair MVC and affect inhibition.

Furthermore, instructing participants to jump as high as they could, coupled with landing on a hard surface possibly caused the brain to undergo similar (albeit less severe) mechanisms to what is referred to as "brain sloshing" (acceleration-deceleration forces resulting in a coup-contrecoup type of injury) (Smith et al, 2012), compounding the effects of whole body movements, and resulting in a delayed increase in inhibition. Unfortunately it is impossible to directly relate the current findings to the soccer heading ones in chapter 3, as the 1h follow up time point was added when that study was already completed. We argue that had we included a 1h follow up in study design in chapter 3, we would have observed an even greater increase in the inhibitory measure, thus showing a similar time-course to the one observed following sparring in chapter 4.

Corticospinal excitability is either facilitated or not affected by whole body movements, depending on whether the exercise is non-fatiguing or fatiguing (Sidhu et al, 2012; O'Leary et al, 2016). Our finding of

unchanged excitability following both exercises is in line with previous studies, although MVC declined 1h following the mock-sparring exercise. This finding suggests that a combination of exercise and the actual testing protocols contributed to the development of fatigue, in turn exerting a disfacilitating effect on the corticospinal tract, effectively suppressing the facilitatory effects of exercise on the corticospinal pathways (Weavil et al, 2016).

PAL, SWM and RTI were unchanged following the jumping exercise, although cognitive function has been shown to improve following physical exertion (Ferris et al, 2007; Brisswalter et al, 2002). The data suggest that in the current study exercise levels were too low to induce any changes; similarly, no effects were seen on PAL and SWM following control (sparring). RTI, however, was decreased after the same task; the decrease in reaction time is most likely due to the nature of the cognitive test and the actual exercise. RTI was quantified as the speed at which participants were able to touch a circle on a tablet following a visual stimulus; since they were required to throw punches against pads for the mock-sparring exercise, it is likely that fatigue in the upper limbs following the protocol would have negatively impacted reaction time.

These findings should be considered alongside the following limitations: the jumping exercise may have not replicated the soccer ball heading protocol in chapter 3 and therefore may not have been an objective control. Participants in this study were instructed to jump as high as they could, so to standardize the exercise across individuals; therefore, by jumping to their maximal capacity, participants subjected their lower limbs to a greater workload than in the protocol we were replicating, which may have affected the results. Furthermore, as described earlier, the maximal jumps in the

current study may have further influenced corticomotor inhibition through a brain sloshing action not present in chapter 3. The likelihood of the same mechanism occurring in the soccer heading protocol is minimal, as participants did not jump to their maximal capacity. Brain sloshing may still have occurred in the participants in chapter 3, however, it would likely be due to the act of heading the soccer ball, rather than the jumping itself. It is, nevertheless, reassuring that no differences in inhibition where observed immediately following the jumping exercise, rather 1 hour post, suggesting that inhibition measured in chapter 3 was affected by the repetitive head impacts and not the exercise. The notion is further confirmed by the unaltered corticomotor inhibition following the higher energy demands of the control (sparring) exercise.

5.5 Conclusion

The current study showed that corticomotor inhibition is largely unaffected by exercise, and therefore the transient increases observed following soccer heading in chapter 3 and sparring in chapter 4 are likely due to RSHIs. Unfortunately the jumping exercise may not have objectively replicated the heading protocol in chapter 3, as contrary to our hypothesis, corticomotor inhibition appeared increased 1h following the exercise. However, the suggestion that RSHIs caused inhibition to increase in the previous chapters is strengthened by the fact that the parameter was differently affected when measured following subconcussive protocols, or in an exercise only scenario.

Chapter 6

Using corticomotor inhibition to quantify brain changes following a season's worth of exposure to RSHIs: a field study

Abstract

Introduction: The previous chapters in this thesis show an acute relationship between RSHIs and brain function. The findings demonstrate small yet measurable effects, suggesting that routine head strikes are indeed associated with slight motor and cognitive impairments. The occurrence of these transient effects also raise questions regarding long-term exposure, as an accumulation of head impacts over the course of a season, or lifetime may also affect brain health. As such, the aim of chapter 6 was to explore the feasibility of using TMS to detect alterations to corticomotor inhibition following a cumulative exposure to soccer heading over a playing season. **Methodology:** Nineteen male soccer players (age 24.1 ± 3.5 y) were recruited from a semi-professional club. Baseline measures for corticomotor inhibition and cognitive function were recorded during the team's pre-season training period (Aug-Sept), follow-up assessments were performed as the playing season was coming to a close (May). **Results:** Sixty percent of the initial cohort was lost to follow up. Multiple single case analysis of 8 participants showed no significant effects ($p > 0.05$), bar one athlete whose cSP appeared significantly increased compared to normative data ($p < 0.0001$). PAL and SWM were also unchanged following the playing season ($p > 0.05$); RTI appeared significantly faster in the post-season follow up, when compared to baseline data ($p = 0.006$). **Conclusion:** Due to the lack in statistical power, group analysis of the data was not possible. Comparing single athletes to a cohort of normative data, however, also showed no pattern of change to brain function. The findings suggest that there may be an exposure threshold beneath which brain function is unaffected; further studies with greater statistical power are needed to provide evidence on the medium and long-term effects of RSHIs on brain function.

6.1 Introduction

Chapter 1 discussed the potential detrimental effects of RSHIs on brain function, highlighting both the acute and long-term complications that can stem from repeated impacts to the head. The subsequent chapters concentrated on the acute timeline, using TMS to observe how brain health is affected immediately following subconcussive events. The data suggest that corticomotor inhibition appears sensitive enough to detect subtle alterations to brain function. Whilst transient, these findings are of importance because they show, for the first time, direct evidence for impairments in both central and peripheral systems following head impacts that athletes are routinely exposed to.

The existences of small and acute effects following RSHIs also raise questions regarding long-term outcomes. Acute exposure to repetitive heading may bring about cognitive and motor impairments (the latter possibly increasing the risk of musculoskeletal injuries), therefore accumulation of these impacts over the curse of a career - or lifetime - may also affect brain function. The long term sequelae of subconcussion exposure are somewhat corroborated by the existing literature, with studies showing both cognitive and structural-related neural changes following RSHI exposure over the course of a season (Lipton et al, 2013) and a career (Tysvaer and Løchen, 1991; Stiller et al, 2014; Koerte et al, 2016). Furthermore, pathological studies have found evidence of CTE in the brains of American football athletes (McKee et al, 2009) and soccer players (Ling et al, 2017) with little-to-no exposure to concussive injuries. It is difficult to accurately ascertain the frequency and intensity with which the athletes in these studies experienced TBIs (as the injury can present as asymptomatic and therefore not be diagnosed), as such, there is still the possibility that the observed alterations are due to the concussive injuries rather than the exposure to RSHIs. Nevertheless, these pathological (Ling et al, 2017), structural (Lipton et al, 2013) and cognitive (Tysvaer and Løchen, 1991; Stiller et al, 2014; Koerte

et al, 2016; Lipton et al, 2013) changes as a result of cumulative subconcussive (and likely concussive) exposure are interesting because they show, at the very least, that the combination of RSHIs and concussive injuries can lead to chronic, and potentially fatal complications to brain health.

Unfortunately, there is a dearth of information regarding the long-term alterations that RSHIs may have on motor control. Whilst TMS has been shown effective in detecting longitudinal impairments to the cortico-cortico and corticospinal mechanisms as a result of TBIs (Pearce et al, 2015; Miller et al, 2014; Chistyakov et al, 2001), no study has explored its use in the detection of long term sequelae as a result of RSHIs. Accordingly, the aim of this pilot study was to assess the feasibility of TMS in detecting motor control alterations following a soccer playing season. We chose to work with a semi-professional team, as they would have a higher exposure to soccer heading due to the skill level. As such, to reach an agreement with the team manager we had to minimise the battery of assessment tests to just the primary outcome measure, corticomotor inhibition, and cognitive function as secondary outcome measure. Cognitive assessments were included as previous evidence linked a season's worth of exposure to soccer heading to cognitive impairments (Lipton et al, 2013). We hypothesized that RSHIs exposure over a season would increase corticomotor inhibition relative to a pre-season baseline assessment; additionally, we expected impaired cognitive performance, again as a result of a season's cumulative exposure to subconcussive head impacts. These predictions were made in the context of a preliminary study with just a single team to assess the feasibility of such an outcome evaluation.

6.2 Methodology

6.2.1 Approvals and recruitment

Nineteen male athletes (age 24.1±3.5 y) from a local sub-elite soccer team were recruited for the study; 9 athletes were defenders, 7 played midfield, 2 were forwards, and 1 goalkeeper. Potential candidates were approached by the team's medic and asked if they would undergo a short battery of tests prior to a training session. Participants were excluded from taking part if they presented with any of the following: 1) history of brain injury resulting in loss of consciousness; 2) history of a neurological condition; 3) history of concussion in the 12 months prior to taking part; 4) family history of epilepsy; 5) current use of psychoactive recreational or prescription drugs. Of the 19 participants recruited, 11 were lost to follow up, due to transfer in 8 cases, injury in 2 cases and withdrawal from the study in 1 case. Of the 8 players retained for the study, 6 were defenders and 2 were midfielders. The local ethics committee approved the study, and procedures conformed to the guidelines set out by the Declaration of Helsinki.

6.2.2 Study design

Due to the location of the club's facilities, and the training demands, testing was carried out at the training grounds of the soccer team to accommodate more athletes. All baseline testing was carried out over the course of three weeks during the team's pre-season training in the months of August and September 2016, and follow up testing was performed as their playing season was coming to an end in May 2017. Measures of cognitive function and corticomotor inhibition were recorded during both experimental sessions. The research team did not have access to video analysis to track heading

exposure; as such, the team medic tracked heading frequency from 4 randomly chosen athletes over the course of 4 matches.

6.2.3 Transcranial magnetic stimulation

Corticomotor inhibition was recorded using the same methodology described in section 2.2.3 in chapter 2.

6.2.4 Electromyography and force recordings

EMG data were collected using methodologies described in section 2.2.4 in chapter 2. All EMG measures were recorded with participants sitting on a portable dynamometer chair. Their dominant leg secured to a calibrated load cell, connected to the data collection hardware (Biopac Systems, Inc. Goleta, CA, USA) at a 60° (with 0° being fully extended limb).

6.2.5 Cognitive function

Parameters of cognitive function (PAL, RTI and SWM) were recorded as described in section 3.2.6 in chapter 3.

6.2.6 Statistical analysis

Graphpad Prism 6 was used to perform the statistical analysis and create graphs. All parameters were analyzed using paired t-tests, comparing means before and after the playing season. However, due to the low sample size at follow up, comparisons solely relying on group analysis would not be

appropriate. Therefore, software developed by Crawford J.R. (Crawford and Garthwaite, 2007) was used to compare a single case (in this case individual athletes) to normative data taken from the baseline assessments of previous experimental studies reported in the thesis. The normative data used for the comparison relate to measurements taken on two time points, not associated with head impacts, which can therefore act as a control group (n=59). Data are expressed as means (± standard deviation) unless otherwise stated.

6.3 Results

The average number of headers during a match was 11 ± 5 (range 6-18), all recorded from athletes in the defender position.

6.3.1 Corticomotor inhibition

Corticomotor inhibition was unchanged after a playing season compared to baseline ($p > 0.05$) (Figure 6.1). Furthermore, comparing single individuals to normative data also showed no significant effects ($p > 0.05$), bar one athlete whose corticosilent period appeared significantly increased compared to the normative data ($p < 0.0001$ - red line in figure 6.1).

6.3.2 Cognitive function

PAL and SWM were unchanged following the playing season ($p > 0.05$) (Figure 6.2A-B). However, Reaction times (RTI) appeared significantly faster post-season, when compared to baseline data ($p = 0.006$) (Figure 6.2C).

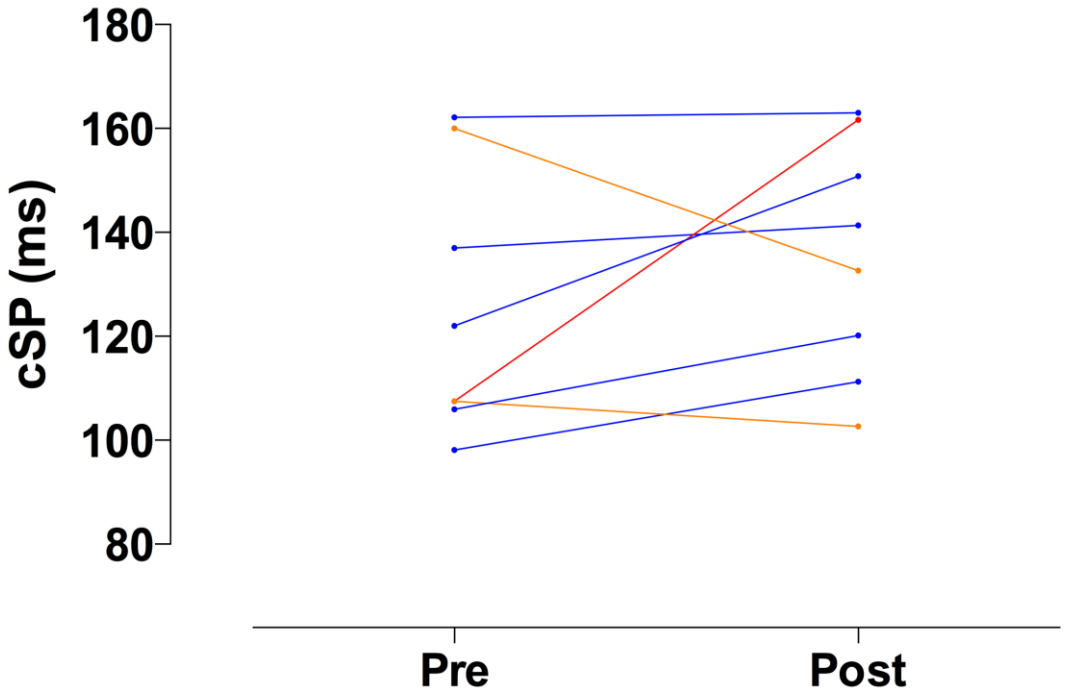

Figure 6.1. Individual data for corticomotor inhibition (cSP) measured before and after a playing season. No significant differences were seen between time points.

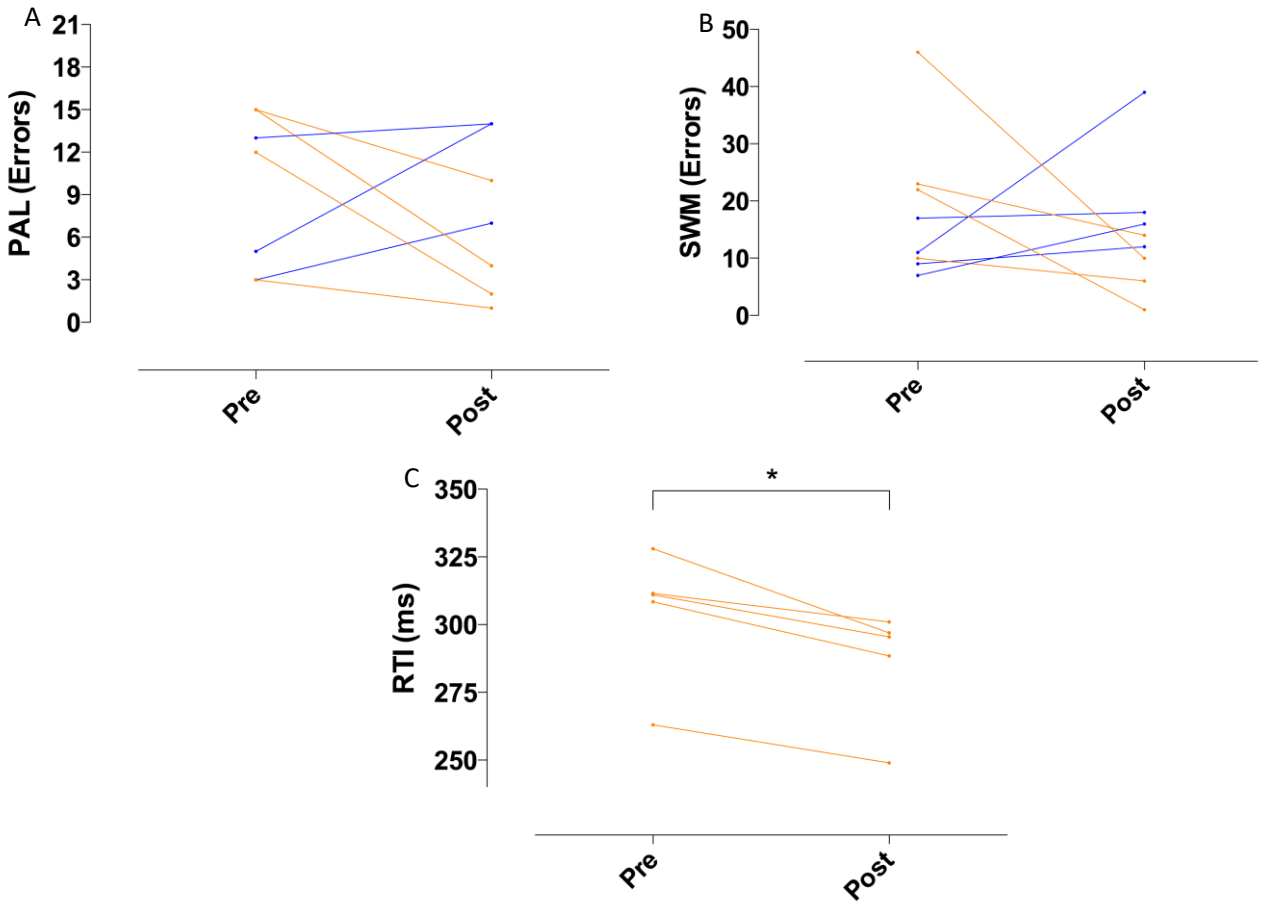

Figure 6.2. Individual participant changes pre- to post-season for all cognitive tasks. No changes were observed for PAL (A) and SWM (B), whilst RTI appeared significantly faster following the playing season (C) (p= 0.006)

6.4 Discussion

The data appear to suggest that corticomotor inhibition and cognitive performance are unaffected by a season's worth of subconcussive exposure. Using single-case analysis statistics, all but one of the eight cases showed no post-season changes to corticomotor inhibition. Moreover, the athlete with a significant difference post-season compared to control data was characterized by a 50% increase in corticomotor inhibition compared to baseline. Such an increase should not occur in healthy individuals not suffering from neurological issues, and therefore is possibly due to measurement error. Whilst no

significant alterations were observed in PAL and SWM (parameters of learning and memory), RTI appeared faster following the playing season, most likely due to a learning effect.

The hypothesis of this chapter was that, upon completion of the playing season, corticomotor inhibition would be increased. Together with a decrease in cognitive ability, the findings would have reflected a cumulative effect of RSHIs on brain capability. However, the group analysis of the cohort shows no such effect, likely due to the low sample size, furthermore, also comparing single athletes to a sample of control data showed that most of them did not differ from normative values. This pattern of results may be due to a number of factors; first, it is possible that the number of headers (heading exposure) the athletes performed over the one season where not enough to elicit any detectable changes. Whether an exposure threshold (beneath which RSHIs do not affect brain function) exists or not is unclear, as the literature provides mixed evidence. Some suggest that RSHIs over the course of a season result in measurable changes to brain function (Matser et al, 2001; Lipton et al, 2013), whilst others show no alterations following cumulative exposure to RSHIs (Kaminski et al, 2007; Kaminski et al, 2008). Interestingly, the studies showing no relationships between RSHIs and brain impairments also reported very low incidences of heading exposure per game (1-3 headers/game), whilst Matser et al, (2001) report values similar to the ones in the current study (10 headers/game). It is possible, therefore, that players in the Kaminski papers did not perform enough cumulative headers to elicit any detectable changes. However, observing functional changes following an exposure of 10 headers/game (Matser et al, 2001) would mean that the athletes in the current study should exhibit some degree of impairment. The absence of such alterations to brain function suggests there are other mechanisms at play that would affect the results.

Another explanation for the lack of changes in this chapter proposes that athletes can be divided in two groups, depending on how vulnerable they are to RSHIs: those who are affected by RSHIs (responders) and those who are less likely to suffer from subconcussive exposure (non-responders). As such, an individual's status as responder or non-responder would dictate brain alterations following RSHIs, with the latter group being less susceptible to head impacts. Athletes in the non-responder group would then exhibit smaller, or no impairments as a result of subconcussive head impacts. Because of the semi-professional level of the team in this study, there is the possibility that non-responders where (unintentionally) preferentially selected to play for the team, as they would arguably outperform athletes who are affected by repetitive heading. It is then reasonable to suggest that the few participants retained at follow up may be non-responders, and therefore exposure to head impacts would not affect their cerebral function.

The project has also taught us valuable lessons in how to best conduct a field study to evaluate the consequences of heading, particularly when dealing with semi-professional or professional sports clubs. First and foremost, the feasibility aspect of this study demonstrates the extent of drop-out rates to be expected and helps understand the number of athletes needed for a full scale field study. Therefore, in order to ensure a cohort large enough to account for eventual drop-outs, future studies should sign up more than double the number of players recruited in the current study. The enlarged group of participants would help maintain the statistical power required to demonstrate any effects that may be present at the outcome assessment. The fast turnaround players are sometimes subjected to when playing at medium-to-high levels means that if the drop-out rate is similar amongst different teams (~60%), at least 60 players should be recruited in a field study in order to account for eventual drop-outs and maintain an appropriate number of athletes in the sample.

Secondly, it is of pivotal importance to accurately track the exposure each player has to both concussive and subconcussive impacts to the head. Video analysis of league games may be beneficial in this regard, allowing the coaching staff to count the numbers of headers players received offline. Head impacts received during training sessions may be a bit more difficult to track, however, players can be asked to keep a diary or complete a short questionnaire at the end of each training session in order to gain an estimation of heading exposure.

Lastly, it would perhaps be best to tailor data collection and the interpretation of the results to individual players, similar to what is shown in this chapter, accounting for the directly relevant aspects with regards to heading in individual players. Taking multiple baseline assessments during pre-season and calculating the range of the measures, alongside their coefficient of variance, can further strengthen the analysis of results. By doing so, “normal” values for each player can be determined, so to more accurately track what changes repeated exposure to heading may have on the brain health of individual players.

6.5 Conclusion

This experimental study set out to assess the feasibility of using TMS to gain longitudinal data on the relationship between RSHIs and brain function. Due to the lack in statistical power, group analysis of the data was not possible. Comparing single athletes to a cohort of normative data, however, also showed no pattern of change to brain function after a season. The findings suggest that there may be an exposure threshold under which brain function is unaffected; alternatively, the small cohort maintained at follow up might be made up of non-responders to RSHIs and as such show no

impairments following a season of soccer heading. Regardless, further studies with larger cohorts are needed to provide further evidence on the medium and long-term relationship between RSHIs and brain function.

Chapter 7

Summary, conclusion and implications

7.1 General discussion

The main theme of this thesis was to investigate the effectiveness of non-invasive brain stimulation (TMS) in detecting acute alterations following single bouts of RSHIs. Our main hypotheses were that RSHI exposure would result in acute and transient alterations to brain function. More specifically, we proposed that corticomotor inhibition (parameter of motor control, and an indicator of neurotransmitter activity) would be increased, and cognitive function would be decreased following subconcussive head impacts.

RSHIs have become a "hot topic" in the past years, as these impacts may contribute to long term sequelae to brain function, an effect initially solely associated with more severe head injuries in sports such as boxing and American football (Martland, 1928; Omalu, 2005; McKee et al, 2009). Whilst there have been a number of studies exploring the potentially detrimental nature of repetitive exposure to subconcussive head impacts, the results are somewhat ambiguous; some show an association between RSHIs and alterations in cognitive and motor function (Tysvaer and Løchen, 1991; Lipton et al, 2013; Haran et al, 2013), whilst others suggest the opposite (Broglio et al, 2004; Kaminski et al, 2008; Rutherford et al, 2009). An explanation for the lack of definitive evidence is that the parameters measured and techniques implemented were not sensitive enough to detect subtle changes. It is, therefore, important that researchers find or develop a technique that is sensitive and reliable enough to reveal any acute alterations in the proper functioning of the brain.

The results from chapter 2 show TMS to be reliable in detecting indices of corticomotor inhibition and corticospinal excitability. Establishing reliability of TMS-based parameters was important to ensure the

technique we proposed to use was appropriate to fulfill the objectives of the thesis. Both parameters were shown to be sufficiently reliable in day-to-day measurements in healthy participants, both at one, and two-week intervals. As such, TMS measures were able to detect any changes to brain function as a result of RSHIs. Subsequently, we applied the technique to our first subconcussive protocol which was soccer heading. The results from this experimental project agree with our hypothesis by showing, for the first time, measurable (albeit small) increases in corticomotor inhibition following RSHIs. Furthermore, we also found that parameters of memory (PAL and SWM) were also negatively affected by RSHIs. Following these results, we were interested in finding out whether the transient effects were reproducible in a sport where the long-term adverse health effects of RSHI are known. One such sport is boxing, whereupon athletes routinely expose themselves to RSHIs, both in competition and during training. We explored the acute effects of sparring and also found increased corticomotor inhibition, however, our largest (and statistically significant) effect was observed 1h following the protocol. The findings support the theory of a more delayed, potentially protective response of the brain to counteract a glutamate-dependent excitotoxic environment, resulting from impacts to the head. This notion is speculative, more evidence is required to fully understand the exact time course of concussive injuries, and subconcussive responses to head impacts.

The results reported in chapter 4 differ from chapter 3, a possible reason for the diverging data is due to the addition of an assessment 1h following the subconcussive protocol in the sparring project. This follow up was not present in the soccer heading study, and we argue that had we added it in, we may have seen a greater effect size in corticomotor inhibition resulting from ball heading. Effects in the heading and boxing study may also differ due to the participants in the two studies being exposed to different types and intensities of head impacts; the consistent nature of the soccer heading drill, as well

as the fact that participants knew when the ball was arriving allowed them to brace themselves for impact. In the sparring protocol the impacts to the head were more randomized, and it is likely some caught the participants off guard. It is possible that the head impacts subjected the cerebral matter of the two groups to undergo different mechanical stresses, which may have altered corticomotor inhibition in a dissimilar manner. This suggestion, however, is speculative; future work would benefit from using biomechanical models to highlight how direction and force of impact from these two subconcussive drills (heading and sparring) propagate from the skull into deeper brain tissue. Regardless, the fact remains that we observed an increase in inhibition immediately following the heading protocol, and a delayed onset as a result of the sparring drill. The delayed alteration to brain function is of particular interest sporting environments, should concussive episodes follow a similar time-course; concussion assessment tests are usually administered immediately following the suspect injury, the player may pass the tests and resume playing whilst developing symptoms at a later stage.

In addition to the 1h measurements following the sparring protocol, we also included another parameter of motor control to test the hypothesis that RSHIs would alter motor unit recruitment behavior. The application of dEMG in a RSHI context is novel as, to the best of our knowledge, no other data are available to explore this relationship. Interestingly, corticomotor inhibition increased in tandem with early recruited motor units being activated later, and late recruited motor units being activated earlier compared to baseline. The shift in the recruitment thresholds is possibly due to a diminished descending neural drive, mediated by increased GABA activity in M1. Decreased neural drive is thought to be a protective mechanism of the CNS to slow the body down during times of stress or danger to the body (e.g. exercise-induced fatigue/hyperthermia) (Davis and Bailey, 1997; Nybo and Nielsen, 2001; Hunter et al, 2011). Similarly, the alteration in motor unit behaviour observed following

RSHIs may reflect said protective mechanism, in this case triggered following blows to the head. However, this protective response may have subsequent detrimental effects; if the motor system is not functioning optimally, there could be increased risks of sustaining further (non-brain related) injuries. A prime example of this phenomenon are rugby players returning to play following a concussion; they have a higher risk of musculoskeletal injury than non-injured players (Cross et al, 2017), suggesting that the neural drive-dampening affects control of movement.

Corticomotor inhibition and motor unit recruitment patterns were not the only parameters of motor control that where implemented for the experimental projects of this thesis. Because postural control is one of the hallmarks of concussion, and is also often used in post-concussion assessment, we decided to include it in our methodology. Furthermore, evidence on the effectiveness of balance in detecting acute brain alterations following RSHIs is ambiguous, with studies showing no change (Broglio et al, 2004), and other showing decreased postural stability following head impacts (Haran et al, 2013). Results from both studies, implementing a subconcussive protocol (chapters 3 and 4), suggest that postural control is unaffected by RSHIs, even when assessed using different conditions (eyes open/closed; one/two legs) as was the case for the sparring project. Therefore, only using balance as a parameter of motor control may not be appropriate, as we show measurable alterations in motor unit behaviour, in the absence postural stability changes. This finding is of importance because it suggests there may be underlying alterations in motor control not detected by postural measurements, but that could nevertheless affect performance and, ultimately, health. As such, we propose that balance is not sufficiently sensitive to detect subtle changes to motor control following subconcussive head impacts; future work should implement the use of more sensitive and direct parameters that provide a closer indication of brain signaling, such as dEMG.

A recurring theme in feedback received through peer-review, in regards of the experiments outlined in chapter 3 and chapter 4, was the lack of a control group: participants acted as their own controls in the before-and-after design of the projects. However, reviewers pointed out that we could not definitively ascertain whether the increase in inhibition following the subconcussive protocols was due to the actual impacts to the head or simply due to the exercise. The decision to design a before-after study, rather than a randomized crossover design was due to the time constraints the research team was under. Such constraints where further compounded by the (at the time) unknown washout period that participants would require to ensure they were free from any subconcussion-related symptoms. For this reason in chapter 5 we designed a study that would replicate the metabolic demand of the heading and sparring protocols, without exposing the participants to any head impacts. Comparing the results from this control study to the data from chapters 3 and 4 showed a clear interaction effect over time, with corticomotor inhibition taken following RSHIs increasing, compared to the same measure, unchanged following the control tasks. The interaction effect, as well as the unchanged inhibitory parameter following the mock heading and sparring tasks, strengthen the suggestion that RSHIs and not exercise and/or fatigue increased corticomotor inhibition in chapters 3 and 4. Furthermore, these results also validate the relevance of corticomotor inhibition as an outcome measure of RSHI-induced brain changes.

A secondary aim of the thesis was to pilot the use of TMS in detecting brain alterations following accumulative exposure to RSHIs over the course of a playing season. Although a considerable portion of the initial cohort was lost to follow up, comparing individual athletes to a control cohort showed no discernible pattern of alteration to brain function. There are multiple reasons to explain the lack of significant findings: an exposure threshold may exist, beneath which brain function is not affected by

RSHIs; alternatively, the cohort assessed following the playing season may have been made up of non-responders to subconcussion. As such, the players would not exhibit impairments following cumulative exposure to RSHIs. The number of athletes lost to follow-up during the season also suggests that greater numbers need to be recruited in order to account for the rate at which players are transferred to other teams.

Corticomotor inhibition is an indirect measure of GABAergic function within the brain (Inghilleri et al, 1993); an elongation of the cSP following stimulation of M1 indicates increased quantity of this particular neurotransmitter. In most studies measuring the use of TMS in mTBI patients it was found that corticomotor inhibition increased as a result of the injury. Mechanisms underlying increased coticomotor inhibition are not fully understood, however, up-regulation of GABA activity may be compensating for excitotoxic actions of glutamate (Guerriero et al, 2015). Therefore, if a patient or athlete receives a low number of concussive injuries, this protective mechanism is beneficial, as it aids brain recovery. It is likely the real problem arises when players are subjected to multiple head injuries over the courses of their careers; the initial protective action of GABA may eventually become harmful, with the accumulation of GABA creating a toxic environment within the brain. In a similar fashion, increased GABAergic responses triggered by subconcussive impacts, in the absence of injury, may harm brain homeostasis, eventually affecting its optimal functioning.

7.2 Conclusion and implications

The novelty of this thesis is the integration of a non-invasive brain stimulation technique with EMG recordings to assess whether, and how RSHIs affect brain function and motor control. In addition, our laboratory and experimental design is able to safely and ethically elicit routine levels of subconcussion in accustomed participants. The novel integration of standardized subconcussive protocols, and techniques used (TMS and dEMG), allowed us to primarily assess what changes RSHIs elicit in the brain, and subsequently relate them to alterations in the musculoskeletal system. Overall, the results have shown that RSHIs are associated with transient, albeit small, electrophysiological and cognitive alterations. Namely, corticomotor inhibition was increased, and cognitive parameters of memory and learning (PAL following both subconcussive protocols, and SWM following soccer heading) were decreased following both heading and sparring drills, although the inhibitory parameter in chapters 3 and 4 followed slightly different time-courses. As previously described, corticomotor inhibition's behaviour following soccer heading and sparring is likely due to the number of acute follow ups implemented in the two studies (1 for soccer heading, and 2 for sparring). The delayed GABAergic increase observed following sparring may reflect a brain mechanism to counteract a hyper-excitability status brought about by increased glutamate release, and also highlights the relevance of this inhibitory parameter to our understanding of the effects RSHIs have on the brain.

The results discussed throughout this thesis provide insight into the reliability of particular TMS-based techniques. We show that inhibitory and excitatory assessments taken within one, or two weeks show good degrees of reliability; however, corticomotor inhibition, our primary dependent measure, appeared slightly more reliable than corticospinal excitability. Furthermore, we also demonstrate that inhibitory and excitatory parameters are largely unaffected following low and moderate intensity

exercise. Other studies show corticomotor inhibition increases when measured during a contraction and corticospinal excitability to either increase, or be suppressed during and following contractions, depending on exercise intensity (Taylor and Gandevia, 2001; Sidhu et al, 2012; O'Leary et al, 2016). As such, researchers should bear these data in mind when designing future studies around subconcussion.

Whilst the reliability and control aspects of the thesis are significant, the most novel, and arguably more interesting findings are those detailed in chapters 3 (soccer heading) and 4 (sparring). This thesis is the first to use TMS and dEMG to highlight potential alterations to both cognitive, and motor function following routine exposure to subconcussive head impacts. Previous studies suggest associations between RSHIs and motor impairments (Haran et al, 2013; Kawata et al, 2017), whilst others do not (Broglio et al, 2004; Kaminski et al, 2007); as previously discussed, the techniques used (postural control – balance) may be at the root of the diverging results. Our data corroborate the notion of altered brain function following RSHIs, and further add to the literature by showing direct evidence for GABAergic alterations as a result of RSHIs. These findings will facilitate much needed research into RSHIs, having highlighted that commonly used techniques (e.g. balance) may not be appropriate for detecting subtle changes following subconcussion, and suggesting more sensitive, and direct methods (e.g. TMS and dEMG) as a valid alternative.

Although transient, the importance of the current findings lies in the fact that the alterations are observed after only one time exposure to training drills, routinely performed by soccer players and boxers. Impaired motor control as a result of head impacts may expose individuals to a greater risk of other non-brain related injuries (e.g. musculoskeletal); the notion is corroborated by studies assessing

the incidence of other types of injuries following a diagnosed concussion (Nordstrom et al, 2014; Lynall et al, 2015; Cross et al, 2017). Whether a similar scenario is seen following subconcussive head impacts is speculative, as there are no published studies looking at this particular interaction. A further interesting notion stemming from this thesis is that the observed alterations are specifically related to corticomotor inhibition, MU behaviour and memory/new learning. Studies have shown that inhibitory (GABAergic) systems are related to both movement (motor control, in this case corticomotor inhibition and MU recruitment), and learning/memory (Davey et al, 1994; Schuler et al, 2001). As such, alterations in these parameters following subconcussive models, suggest that RSHIs indeed elicit an increase in GABA activity. Moreover, altered cognitive function suggests that subconcussive effects on GABA are not limited to M1, rather, they are widespread also encompassing more frontal areas of the brain. This finding is particularly relevant to sporting communities, where athletes routinely undergo RSHIs in training, during matches and competitions (e.g. soccer and boxing); as mentioned previously, repeatedly triggering a GABAergic response in the absence of actual injury may create a toxic environment within the brain, ultimately negatively affecting brain health of individuals partaking in those disciplines.

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Appendix A

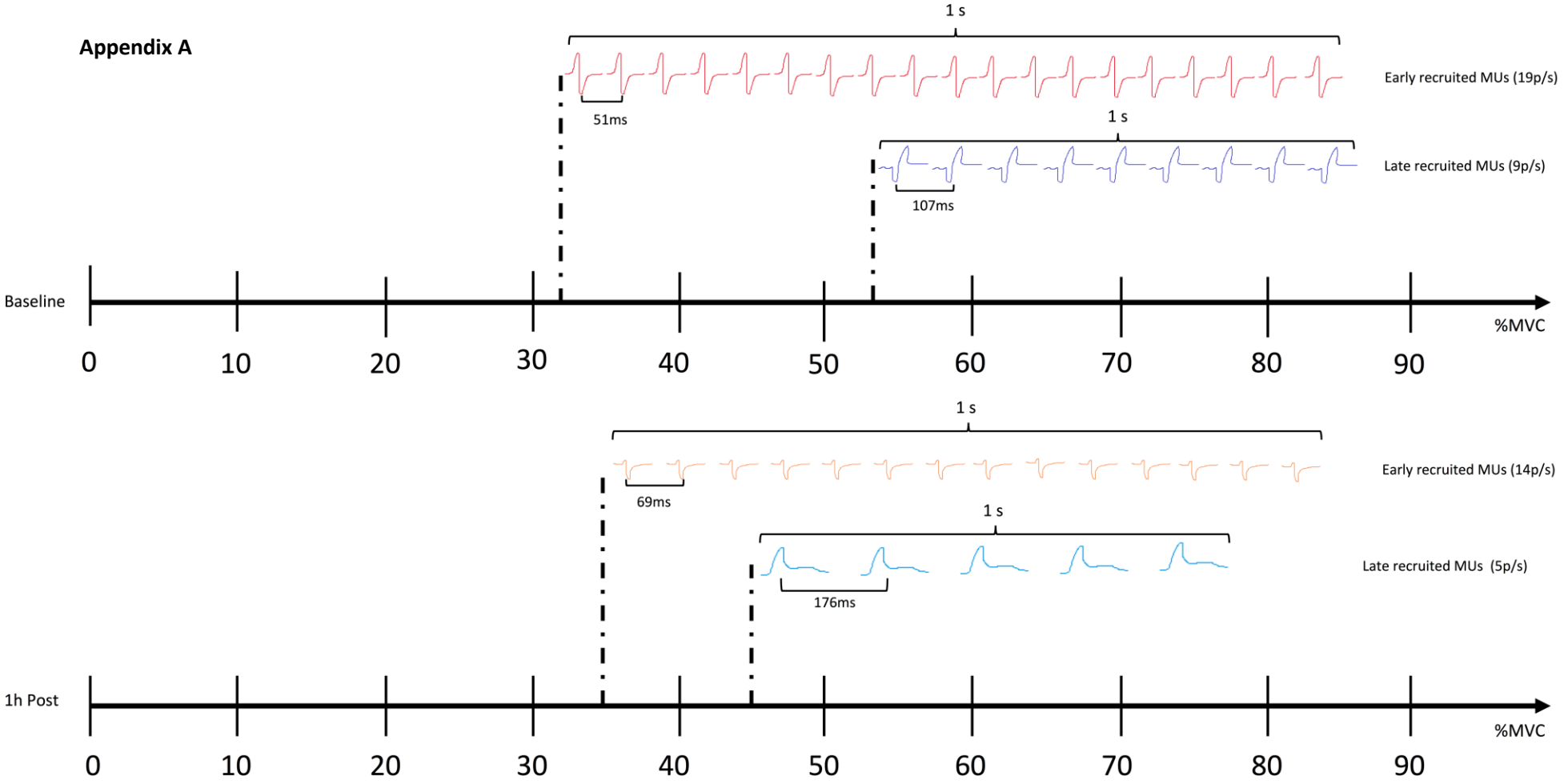
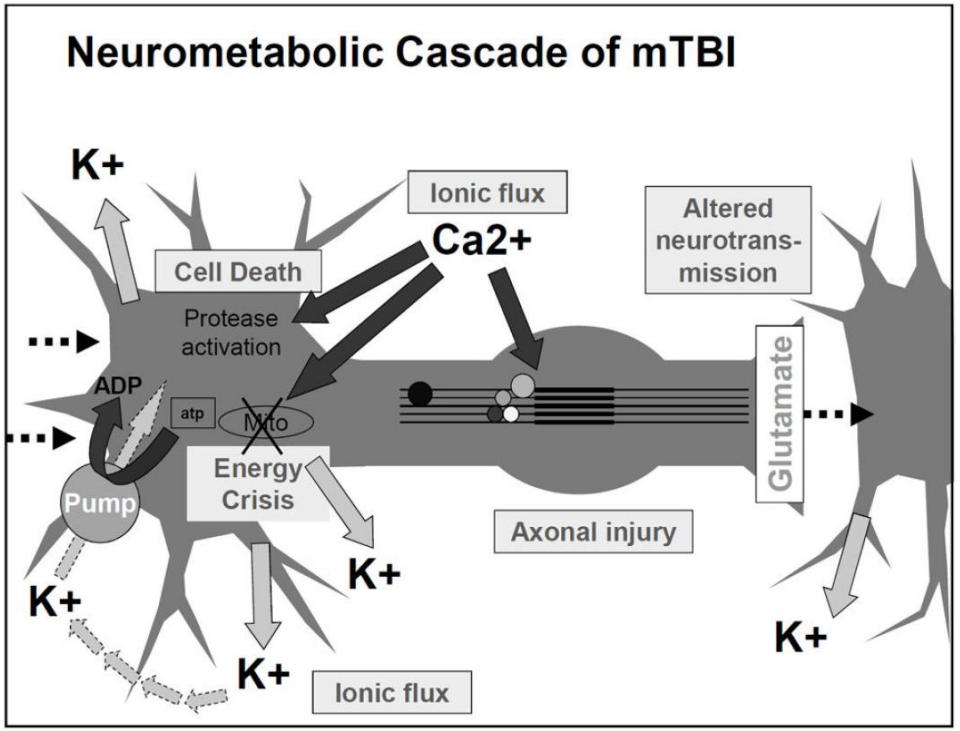


Figure A. Comparing the recruitment threshold (%MVC) of the individual motor units (MUs) against their average firing rate (pulses per second – p/s) using a linear regression shows a negative shift in the regression line 1h following sparring. The finding suggests that the recruitment patterns and the firing rates of the MUs as a whole are altered; the early recruited, small motor units are activated later compared to baseline, and the frequency with which they propagate on the sarcolemma of the muscle is slowed down (showed by an increase in the average inter-pulse interval – IPI, and a decrease in how many pulses are discharged per second). The later recruited, big motor units are activated earlier compared to baseline, yet also show an increase in IPI and a decrease in the pulses discharged per second.

Appendix B

1



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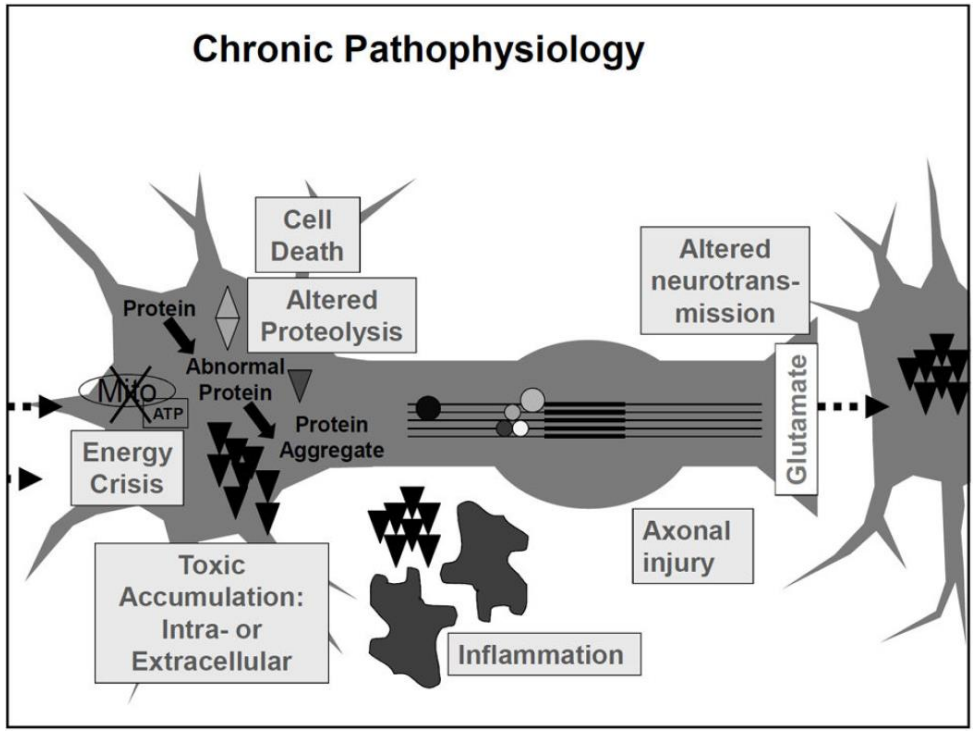

Figure B. Graphical representation of the complex neurometabolic cascade triggered by TBI. B1 illustrates the acute timecourse, with ionic fluxes caused by physical damage resulting in altered neurotransmission and cell death. B2 shows the chronic sequelae of TBI, similar to those seen in the acute phase, with the addition of intra- and extra-cellular accumulation of toxic metabolites, and inflammatory responses. Figure taken from Giza and Hovda, 2014

Publications

EBioMedicine 13 (2016) 66–71

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Evidence for Acute Electrophysiological and Cognitive Changes Following Routine Soccer Heading

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ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form 18 October 2016
Accepted 18 October 2016
Available online 23 October 2016

Keywords:
Sports concussion
Transcranial magnetic stimulation
Sub-concussion
Traumatic brain injury

ABSTRACT

Introduction: There is growing concern around the effects of concussion and sub-concussive impacts in sport. Routine game-play in soccer involves intentional and repeated head impacts through ball heading. Although heading is frequently cited as a risk to brain health, little data exist regarding the consequences of this activity. This study aims to assess the immediate outcomes of routine football heading using direct and sensitive measures of brain function.

Methods: Nineteen amateur football players (5 females; age 22 ± 3 y) headed machine-projected soccer balls at standardized speeds, modelling routine soccer practice. The primary outcome measure of corticomotor inhibition measured using transcranial magnetic stimulation, was assessed prior to heading and repeated immediately, 24 h, 48 h and 2 weeks post-heading. Secondary outcome measures were cortical excitability, postural control, and cognitive function.

Results: Immediately following heading an increase in corticomotor inhibition was detected; further to these electrophysiological alterations, measurable reduction memory function were also found. These acute changes appear transient, with values normalizing 24 h post-heading.

Discussion: Sub-concussive head impacts routine in soccer heading are associated with immediate, measurable electrophysiological and cognitive impairments. Although these changes in brain function were transient, these effects may signal direct consequences of routine soccer heading on (long-term) brain health which requires further study.

1. Introduction

With increased awareness of immediate and late complications of head injuries in sport, in particular the proposed association between exposure to repetitive concussion and late neurodegenerative disease (Hay et al., 2016), there have been considerable efforts to reduce risk of injury and better manage concussions when they do arise (McCrory et al., 2013). Soccer (association football) is acknowledged as the most popular participation sport globally, with routine game-play in soccer involving intentional and repeated head impacts through heading the ball; a skill regularly included in training sessions and from a young age. Therefore, although rates of concussion are relative low in soccer compared to other contact sports such as rugby union or American football (Pfister et al., 2016), participation rates and the incidence of intentional sub-concussive impacts through heading in training and match play are such that the safety of heading in soccer has been questioned in some quarters (Patlak and Joy, 2002).

Though accepted as part of routine game-play, emerging evidence suggests exposure to repeated sub-concussive impacts in soccer may be associated with measurable changes in brain structure and function, and perhaps with late neurodegenerative disease. Rotational headers may prove of particular interest as they are often performed in training drills and matches (i.e. corner kicks) and are believed to be more injurious compared to linear accelerations (Cantu and Hyman, 2012). Imaging studies over the course of a season in active soccer players report evidence of white matter microstructural changes with associated impaired cognition (Lipton et al., 2013). Further, imaging of former professional soccer players aged 40–65 demonstrates evidence of cortical thinning, again with associated cognitive impairment (Koerte et al., 2015). Regarding longer term outcomes, recent identification of a

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http://dx.doi.org/10.1016/j.ebiom.2016.10.029

form of dementia known as chronic traumatic encephalopathy (CTE) in athletes from a range of contact sports (for review see Smith et al., 2013) including soccer (Geddes et al., 1999; McKee et al., 2014) has drawn attention to the possibility that head impacts in soccer might be associated with increased risk of neurodegenerative disease.

Nevertheless, despite growing evidence on risks from the cumulative effects of sport-related head impacts and anxieties around the safety of ball-heading, little data exists demonstrating direct consequences of heading on brain function. Transcranial magnetic stimulation (TMS) can be used to assess a variety of indices of function in the brain to muscle pathway (Goodall et al., 2014). Further, TMS has demonstrated utility in quantifying electrophysiological changes in concussion (Major et al., 2015). The most consistent TMS marker of concussion (both acutely and longitudinally) appears to be corticomotor inhibition (Major et al., 2015; Pearce et al., 2015; Miller et al., 2014), expressed by a longer period of electromyographic silence (cortical silent period – cSP), after a motor evoked potential (MEP) is delivered to the primary motor cortex during contraction. Given the apparent high sensitivity to identify alterations in brain function, TMS could potentially be used to detect acute changes in brain function following sub-concussive head impacts. The relative novelty of TMS used in this context makes interpretation in terms clinically meaningful effects difficult, but its appeal is sensitivity in detecting direct brain changes (De Beaumont et al., 2007). It potentially highlights neurochemical changes that can be used to direct routes of investigation into the effects of sub-concussive impacts on the brain. Therefore, the aim was to study the use of TMS corticomotor inhibition in the lower limbs as primary outcome measure to detect acute changes in brain function from repetitive sub-concussive head impacts simulating routine soccer heading. We hypothesized that there would be a (transient) increase in corticomotor inhibition following a standardized bout of soccer heading, which may be accompanied by measurable changes in other established but less sensitive or less objective indexes of changes to brain function and brain injury as secondary outcome measures such as cognitive tests.

2. Methods

2.1. Approvals and Recruitment

The study was approved by the local Research Ethics Committee and procedures conformed to the guidelines set out by the Declaration of Helsinki. Written informed consent was obtained from all participants, prior to taking part. Twenty-three healthy, amateur football players (5 females; age 22 ± 3 y; weight 72.9 ± 8.3 kg; height 175.4 ± 10.2 cm) were recruited for study via advertisement on university noticeboards and meetings with local football clubs. Participants were excluded from taking part if they presented with any of the following: 1) history of brain injury resulting in loss of consciousness; 2) history of a neurological condition; 3) history of concussion in the 12 months prior to taking part; 4) family history of epilepsy; 5) use of psychoactive recreational or prescription drugs. Data from one participant could not be analyzed and three more participants withdrew from the study for personal reasons. The final cohort included a total of nineteen participants.

2.2. Study Design

Participants were asked to refrain from vigorous physical activity, consuming alcohol and caffeine or smoking for 24 h prior to each study session. Furthermore, participants were required to present to the laboratory fasted where they were provided with a standardized breakfast. At the first experimental session baseline measures for cognitive function, postural control, corticospinal excitability and corticomotor inhibition were recorded in this order; assessments thereafter recorded in the same order at each time point during study. Following baseline testing, participants underwent the heading protocol and then repeated the measures at 4 follow-up time points (taking measures in reverse order from the baseline order, starting with the TMS primary outcome measure); immediately post-heading and at 24 h, 48 h and 14 days following the heading protocol. The decision to include the 48 h follow-up was to assess the transient nature of the effects of heading, and the 14 day follow-up was intended as a time point at which complete "wash out" would have occurred following heading impact. Prior to commencement of study data collection participants attended the laboratory for a familiarization session, during which they completed all outcome measures to acquaint them with the assessment procedures and minimize later learning effects.

2.3. Heading Protocol

The heading protocol consisted of heading a standard football (400 g; 70 cm circumference; 8 psi) projected at a speed of 38·7 ± 2.1 kph from a football delivery device (JUGS sports, Tualatin, USA) positioned 6 m from participants, simulating routine soccer game-play (Haran et al., 2013; Broglio et al., 2004). Participants were instructed to perform a rotational header, redirecting the football perpendicularly to the initial trajectory, with each session consisting of 20 consecutive head impacts over a 10 min period, replicating typical heading practice. A custom-built accelerometer placed at the back of the participant's head recorded linear g-force of the head during impact. Ball speed was determined based on the participants' perceived ability to head the ball with a minimum speed of 30 kph and maximum speed of 50 kph.

2.4. Transcranial Magnetic Stimulation

Motor evoked potentials (MEPs) were elicited in the rectus femoris of the dominant leg via single pulse TMS and assessed using electromyographic (EMG) recordings (see below). Single magnetic stimuli of 1 ms duration where applied over the contralateral primary motor cortex using a magnetic stimulator (Magstim 200^2 unit, The Magstim Company Ltd., Whitland, UK) and a 110 mm double cone coil. Optimal coil location for generating MEPs was determined by placing the coil over the motor cortex, laterally to the vertex; the area where the largest MEP peak-to-peak amplitudes occurred was identified and marked on the scalp with ink (Goodall et al., 2009). The active motor threshold for the quadriceps femoris was determined by increasing stimulator output from 10% by 5% increments, while the participant held a 10% maximal voluntary contraction (MVC) isometric contraction until discernible MEPs were visible (Wilson et al., 1995). Once this individual level was established, subsequent stimulations were delivered at 130% of active motor threshold.

MEPs, alongside all other EMG measures, were recorded with participants sitting with their dominant leg secured to a calibrated load cell of an isokinetic dynamometer (Kin-Com, Chattecx Corp, Chattanooga Group Inc., Tennessee). Knee angle was set at 60° (0° being fully extended limb) and the arm of the dynamometer was set such that the axis of rotation was aligned with the participant's lateral femoral condyle.

To assess the primary outcome measure corticomotor inhibition participants were required to perform maximal knee extensor voluntary contractions (MVCs) of 5 s duration while a single TMS stimulation was delivered over the motor cortex. This was repeated three times with a minute's rest between each contraction, as is common practice. Corticomotor inhibition was quantified as the cortical silent period (cSP) duration, taken from the stimulation artefact to the resumption of discernible, uninterrupted EMG activity from the muscle (Fig. 1). By measuring cSP at MVC, even though this limits the number of repetitions feasible, we ensure to recruit a motor unit pool large enough to show an effect. Measuring cSP at lower intensity may not be as sensitive since a smaller pool of motor units is recruited, reducing the relative effect size of GABA inhibitory mechanisms on the EMG signal. In turn, making the cSP measurements less sensitive in detecting subtle and transient cortico-spinal changes. During the assessment of secondary

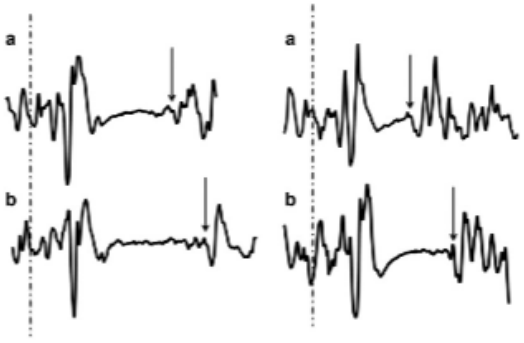

Fig. 1. Snapshots of the cortical silent period (cSP) of two participants measured by TMS before (a) and immediately after heading (b) illustrating a typical lengthening in cSP immediately following heading. The cSP was quantified as the period of time between the delivered TMS pulse (dashed line) and the resumption of uninterrupted EMG activity (arrows).

outcome measure corticospinal excitability, participants maintained a 20% MVC isometric contraction while 20 single TMS pulses, separated by 6 s, were delivered over the motor cortex. Corticospinal excitability was determined as the average MEP amplitude normalized to the maximal response elicited by motor nerve stimulation (%Mmax, see below). We chose to assess cortical excitability and inhibition in the lower limbs rather than in the upper limbs because of its functional relevance; in soccer, changes in lower limb may be more valid as they relate directly to gait and performance.

2.5. Electromyography and Femoral Nerve Stimulation

Electromyographic activity was recorded using a wireless system (Biopac Systems, Inc. Goleta, CA, USA). Data were sampled at 2 kHz, and filtered using 500 Hz low and 1.0 Hz high band filters. Signals were analyzed offline (Acqknowledge, v3.9.1.6, Biopac Systems, Inc. Goleta, CA, USA). EMG activity was assessed using Ag/AgCl surface electrodes (Vermed, Devon, UK) with an intra-electrode distance of 2 cm positioned over rectus femoris; prior to electrode placement, the area of interest was shaved and abraded as per Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) guidelines. The position of each electrode was marked with permanent ink to ensure consistent placement during subsequent visits.

Peripheral stimulation of the femoral motor nerve was administered using an electrical stimulator (Biopac Systems, Inc.). The stimulation site was identified by locating the femoral artery and placing a self-adhesive surface electrode (cathode) lateral to it, high over the femoral triangle, with the anode on the buttock. Single stimuli were delivered to the muscle while participants maintained a 20% MVC isometric contraction, and the intensity of stimulation was increased until a plateau in twitch amplitude and rectus femoris M-wave (Mmax) occurred. Supramaximal stimulation was delivered by increasing the final stimulator output intensity by a further 30%.

2.6. Cognitive Function

Secondary outcome measure cognitive function was assessed in a quiet room using the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computer based cognitive assessment tool and neuropsychological standard. The following CANTAB tasks were included: Reaction Time (RTI; divided attention); Paired Associate Learning (PAL; long-term memory); Spatial Working Memory (SWM; short-term memory); Attention Switching Task (AST; executive function); and Rapid Visual Processing (RVP; sustained attention).

2.7. Postural Control

Secondary outcome measure postural control was assessed using the Biodex Balance System SD (BBS; Biodex Medical Systems, Inc. New York, USA). Participants stood on a circular dynamic platform and average sway score was determined by measuring the degree of tilt on anterior-posterior and medial-lateral axes during three, 20 s trials (using dedicated Biodex software, v1.08, Biodex Inc.).

2.8. Statistical Analysis

Immediate post-heading responses were analyzed using a paired *t*-test, comparing measures before and immediately after the heading protocol. Recovery was analyzed for individual growth curves using the SPSS MIXED model with the restricted maximum likelihood method in keeping with standards for analysis of longitudinal data (Singer and Willett, 2003; Peugh and Enders, 2005). To achieve this, individual curves were analyzed to examine change over time. The following time points were included: immediate post, 24 h post, 48 h post, two weeks post. Data was checked for skewness and kurtosis and three cognitive measures (SWM, PAL, and RVP) and the balance variable were normalized using log transformation. The 95% lower and upper confidence intervals (CIs) were also calculated from difference of the mean values. Effect sizes (ES) were calculated for non-transformed differences using Cohen's d formula and were quantified as follows: 0.2 = small; 0.5 = medium; 0.8 = large. Statistical significance was set at $p \leq 0.05$. Each measure was separate in relation to the hypotheses and therefore no correction for multiple comparisons was necessary. Data are expressed as means (±standard deviation) unless otherwise stated. The funding source had no input in the study other than suggesting at the design stage to add a full recovery assessment time-point.

3. Results

Overall, each participant performed 20 headers, achieving a mean force of impact of 13.1 ± 1.9 g (Table 1), with a coefficient of variance of 18%(±3%).

3.1. Effect of Heading on Corticomotor Inhibition and Corticospinal Excitability

Immediately after ball heading there was a measurable increase in the primary outcome measure cSP within 74% (14 out of 19) of participants (Fig. 2). The cSP duration increased from 117.8(±19.8)ms at baseline to 123.1(±17.6)ms ($t_{(18)} = -2.11$, $p = 0.049$; ES 0.28),

Table 1
Mean impact values for each individual recorded using a linear accelerometer. Data for 2 participants was not recorded due to hardware malfunction.

Force of head impact (g) for each participant (mean ± SD)
12.7 ± 2.02
11.9 ± 2.1
13.9 ± 2.1
15.3 ± 3.2
12.7 ± 2.1
14.6 ± 2.4
11.6 ± 2.6
11.3 ± 1.7
10.5 ± 1.8
12.3 ± 1.7
16.9 ± 4.0
11.7 ± 2.9
11.9 ± 2.1
12.3 ± 2.6
11.8 ± 1.9
14.6 ± 2.5
16.7 ± 4

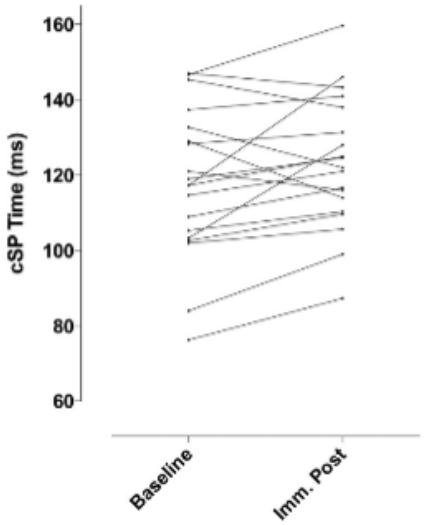

Fig. 2. Change in cortical silent period (cSP) duration for each participant from baseline to immediately following the heading protocol.

representing an average increase of 5.4(±4.8)% in cSP duration, compatible with increased corticomotor inhibition. This increase in cSP proved transient with apparent normalization to baseline in subsequent follow-up assessments at 24 h, 48 h and 14 days ($F_{(1,18)} = 4.23$, $p = 0.04$) (Fig. 3). There was a moderate, but not significant, relationship between these acute increases in cSP and g-force on impact with the ball ($r = 0.37$, $p = 0.07$ one-tailed).

To determine the reproducibility of the primary outcome measure the intraclass correlation coefficient (ICC) was calculated between the baseline and the 2 weeks post-measure. ICC for the two measures was 0.764, suggesting excellent agreement (Cicchetti, 1994). Furthermore, no within-participant differences were found between the two time points using a paired *t*-test ($t_{(18)} = -0.47$, $p < 0.63$; ES 0.09; CI −10.36 to 6.53).

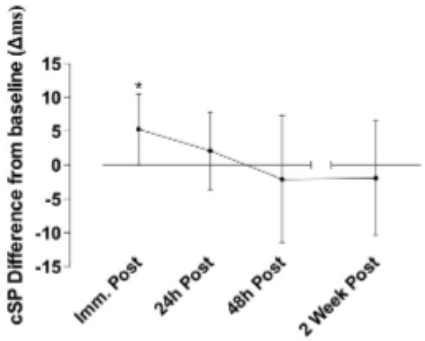

Fig. 3. Difference in cSP in ms after heading relative to baseline. Immediately after heading cSP duration increased on average by 5.3(±5.7)ms (*$p < 0.05$) which within participants is an 5.4(±4.8)% average increase from baseline values. This increase detectable immediately after heading normalized over the four follow-up timepoints ($p < 0.05$) with values apparently returning to baseline level. Error bars indicate the 95% confidence intervals.

No changes were found on the secondary TMS outcome measure corticospinal excitability; MEP amplitude demonstrated no change in the acute phase immediately after ball heading, nor in the follow-up assessment time-points (Table 2). There was no notable change in knee extensor MVC after heading the ball (Table 2), suggesting the participants did not experience significant muscular fatigue that might interfere with TMS measurement.

3.2. Altered Cognitive Function Following Heading

Immediately after the heading protocol there was a reduced performance compared to baseline in two CANTAB sub-tasks assessing accuracy on different aspects of memory. Specifically, Spatial Working Memory (SWM) error scores were significantly higher ($t_{(18)} = -2.28$, $p = 0.03$, ES 0.3) immediately after the heading protocol, compatible with impairment in short-term memory (Fig. 4a). Furthermore, total adjusted error score on the Paired Associated Learning (PAL) task immediately after heading increased by 67% ($t_{(18)} = -3.05$, $p = 0.007$, ES 0.5), compatible with a reduced long-term memory function (Fig. 4b). These disturbances in short- and long-term memory proved transient, with normalization to baseline performance in SWM ($F_{(1,18)} = 10.28$, $p = 0.002$) and PAL ($F_{(1,18)} = 11.14$, $p = 0.002$) in the subsequent follow-up assessments at 24 h, 48 h and 14 days (Table 2).

Heading only significantly affected memory function; the remaining CANTAB tasks assessing aspects of attention and processing speed did not show significant heading-associated decrements compared to baseline assessments (Table 2). No change was detected on the Rapid Visual Processing task, with RVP A′ scores close to ceiling/maximum, making the measure insensitive to change. There was a marginal improvement on the median corrected latency scores of the executive function Attention Shifting Task ($t_{(18)} = 2.52$, $p = 0.021$), possibly due to practice. On the Choice RTI measure there was no effect of heading on decision times ($t_{(18)} = 0.69$, $p = 0.5$) (Table 2).

4. Discussion

Following a standardized session of football heading designed to simulate routine soccer practice our data demonstrate immediate alterations in brain electrophysiological and cognitive function compared to baseline assessments in a cohort of healthy, young soccer players. Specifically, using TMS we found a measurable increase in cortical silent period (cSP) after just 20 consecutive headers. Furthermore, in cognitive assessments, our data demonstrate evidence of decreases in measures of both short- and long-term memory immediately following heading. Notably, in this single exposure paradigm, these alterations in brain corticomotor inhibition and cognitive function appeared short-lived; the effects apparently normalizing in follow-up assessments from 24 h onwards. In contrast to previous studies in athletes and patients with confirmed concussion or mild TBI (De Beaumont et al., 2007; Chistyakov et al., 2001; Bernabeu et al., 2009; Livingston et al., 2010) these novel observations demonstrate, for the first time, detectable alterations in brain function in footballers exposed to 'routine' head impacts not associated with clinically recognizable brain injury.

The prolonged silent period of neuromuscular recruitment found in this study is a sign of increased inhibition in the motor system and is thought to reflect GABA activity (Inghilleri et al., 1993; McDonnell et al., 2006) which is the most powerful inhibitor in the motor system. Although the mechanisms behind corticomotor inhibition are not fully understood (Chen et al., 1999), increased inhibition following repeated sub-concussive head impact may reflect protective mechanisms against minor injury. What is a concern however is that such protective mechanisms could become maladaptive when stimulated repeatedly, as occurs during soccer heading practice. Albeit apparently transient, the acute increases in corticomotor inhibition following football heading could trigger a pathological process damaging brain health through the accumulative effect of sub-concussive head impact. Increased

Table 2
Mean (standard deviation) values for each of the outcome measures: corticomotor inhibition (cortical silent period in ms) and corticospinal excitability (MEP amplitude normalized to femoral nerve M-wave, %Mmax), Spatial Working Memory (SWM errors), Paired Associate Learning (PAL errors), Rapid Visual Processing (RVP A' score), Attention Shifting Task (AST median corrected latency), Reaction Time (Choice RTI decision times) and Postural control (Balance, SI stability index deviation from the horizontal baseline) measured at each time point, and 95% lower and upper confidence intervals (CIs) for the difference in means before and immediately after heading.
*p < 0.05 Baseline v Imm. Post; ‡p < 0.05 change over time growth curve analysis.

	Assessment time post-heading exposure					
Variable	Baseline	Immediately	24 h	48 h	2 weeks	Δ mean Pre v Imm Post (95% CI)
TMS						
Inhibition (ms)	117.8 ± 19.8	123.0 ± 17.6*	119.9 ± 19.8	115.7 ± 20.6	115.9 ± 19.7‡	5.28 (0.017 to 10.54)
Excitability (%Mmax)	44.1 ± 20.6	47.4 ± 22.3	47.9 ± 24.0	44.4 ± 22.5	46.5 ± 22.1	3.34 (−5.03 to 11.72)
Cognitive function						
SWM (log_errors)	0.79 ± 0.59	1 ± 0.51*	0.77 ± 0.62	0.72 ± 0.57	0.69 ± 0.57‡	0.2 (0.016 to 0.40)
PAL (log_errors)	0.38 ± 0.41	0.65 ± 0.29*	0.49 ± 0.32	0.51 ± 0.32	0.35 ± 0.32‡	0.26 (0.08 to 0.44)
RVP A'	0.952 ± 0.052	0.959 ± 0.040	0.958 ± 0.044	0.971 ± 0.028	0.962 ± 0.038	0.0007 (−0.005 to 0.021)
AST (ms)	396 ± 58	376 ± 67*	369 ± 64	370 ± 66	373 ± 82	−19.11 (−35.01 to −3.20)
RTI (ms)	295 ± 29	301 ± 35	295 ± 33	297 ± 32	297 ± 31	6 (−6.13 to 19.24)
Postural control						
Balance (SI)	0.76 ± 0.36	0.71 ± 0.21	0.67 ± 0.23	0.63 ± 0.25	0.72 ± 0.18	−0.06842 (−0.164 to 0.028)

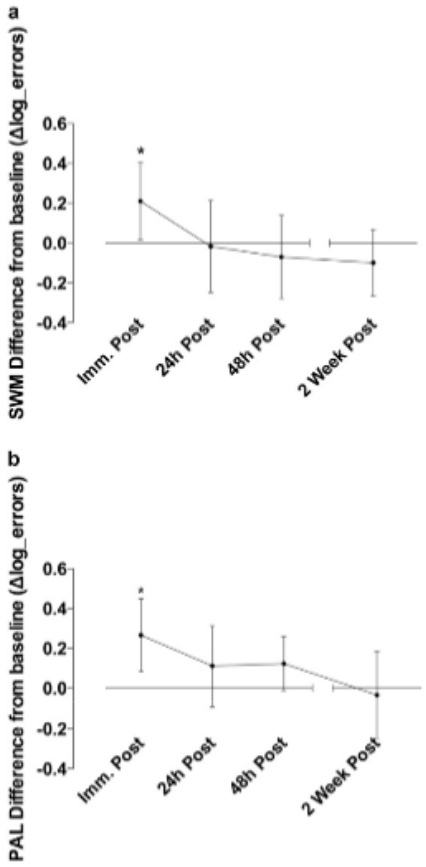

Fig. 4. Difference in memory performance (log transformed error score difference) after heading relative to baseline. Immediately after heading, errors were higher compared to baseline on both the (a) Spatial Working Memory SWM (*p < 0.05) and (b) Paired Associated Learning PAL (*p < 0.01) tasks. This increase evident immediately after heading normalized over the four follow-up timepoints (p < 0.01) with error scores apparently returning to baseline level. Error bars indicate the 95% confidence intervals.

corticomotor inhibition silent period has been found to be associated with pathophysiology in brain damage suggesting a link between functional deficits and hyperactivity of cortical inhibitory interneurons (Classen et al., 1997). Further study into the dynamic metabolic processes as a direct result of soccer heading is required. When we understand the complex interplay between functional, metabolic, and structural brain changes following repeated sub-concussive head impact, we can establish the link to accumulative and long-term consequences. At present, the current findings at least suggest acute brain changes occur as a direct consequence of soccer heading.

As well as increased corticomotor inhibition, parameters of memory function were altered following the heading protocol, consistent with a recent report of a relationship between memory function and history of heading in soccer (Lipton et al., 2013). Furthermore, a study of retired Australian Rules footballers found that elite players performed more poorly on the Paired Associate Learning test than amateurs (Pearce et al., 2014). Practical limitations of cognitive-based tests to detect impairment in athletes are due to reliability: in high performance sports athletes have been recognized to purposely produce low baseline performances on cognitive tests to allow them to avoid removal from play, or to reduce return to play intervals (Erdal, 2012).

For completeness postural control (balance) was included as a secondary, albeit indirect, outcome measure as concussion has been shown to result in impaired balance (McCrory et al., 2013; Powers et al., 2014), yet the participants in the current study were able to maintain their balance despite an increased level of corticomotor inhibition. And while one study has shown a decrease in postural control following bouts of soccer heading (Haran et al., 2013), another study has not (Broglio et al., 2004); and now our own show no change in postural control.

Secondary TMS outcome measure cortical excitability has previously been shown to decrease following TBI (De Beaumont et al., 2007; Chistyakov et al., 2001; Bernabeu et al., 2009; Livingston et al., 2010), yet we demonstrated no such change following ball heading. The reason why changes were seen in cortical inhibition and not cortical excitability may be due to the different levels of muscle contractile force applied during recording of the two parameters (20% MVC for excitability vs. 100% MVC for inhibition, see the introduction for its justification). Furthermore, it should be noted that measuring cortical excitability is a less straightforward procedure than corticomotor inhibition as it requires MEP normalization to maximal motor nerve response (Goodall et al., 2009). Primary outcome measure TMS corticomotor inhibition was thought to be most sensitive to quantifying electrophysiological changes based on a recent systematic review (Major et al., 2015), and is a direct measure of changes to brain function.

Future work should include a control activity, such as body movement without head impact. However, the current pattern of results

leaves little doubt that the changes in brain function were related to head impact rather than physical activity. The force of maximal knee contraction was not reduced after heading, therefore the absence of a physical exercise control group it is highly unlikely to explain the effect on corticomotor inhibition or memory function. Nevertheless, a future extension of this work can focus on the acute effects of heading now that the transience of the effect has been established, and would be well placed to reveal the mechanisms underlying these brain changes through a cross-over design that includes a control activity. Furthermore, because it is likely that sub-concussive impacts are more general in nature (i.e. they do not affect single muscles) future work should assess corticomotor inhibition in a larger number of muscles, possibly encompassing both upper and lower limbs. Further study into the dynamic metabolic processes as a direct result of soccer heading is required. Implementing the use of magnetic resonance spectroscopy in future studies could help determine short-term alterations in GABA and glutamate responses. With regard to changes in GABA, because of the use of single-pulse TMS in this study, we were only able to report on the activity of $GABA_B$, while the use of paired-pulse TMS in future work can distinguish modulation of $GABA_A$ and $GABA_B$. Critically, however, the sensitivity of the current primary outcome measure suggests that corticomotor inhibition through future dose-response studies has the potential to provide the evidence-base to guide safe engagement in contact-sports, such as soccer.

5. Conclusion

The current study is the first to show direct evidence for acute changes to corticomotor function and changes to memory function following routine soccer heading. It is furthermore the first study to show that corticomotor inhibition, measured by TMS, is able to detect acute transient changes in brain function following sub-concussive head impacts. And although the magnitude of the acute changes observed was small, it is the presence of the effect that is of interest. This measure was previously shown to be altered in confirmed concussion, but the acute changes in corticomotor inhibition, accompanied by cognitive changes, following the sub-concussive impact of football heading raise concerns that this practice, routine in soccer, may affect brain health.

Funding and Acknowledgements

This work was supported by the National Institute for Health Research (NIHR) Brain Injury Healthcare Technology Cooperative. This work was supported by existing funding awarded to L.W. as part of Framework 7 programme of the European Union (CENTER-TBI, Grant number: 602150-2). The work made use of a TMS coil to which the company Smartfish contributed £1500 for purchase of. T.DiV.'s postgraduate study is support by the research office of Stirling University. W.S. is supported by a NHS Research Scotland Career Researcher Fellowship. D.I.D. and M.I. are members of SINAPSE – see www.sinapse.ac.uk.

The funders had no input in the conception or design of the study, other than NIHR suggesting, at the design stage, to add a full recovery assessment time-point. The funders also had no input in the interpretation or presentation of the results.

Declaration

None of the authors have a competing interest to declare.

Author Contributions

TDV, A.H., L.W., W.S., D.I.D, and M.I. conceived of the study, TDV, A.H., L.W., W.S., S.G., G.H., D.I.D, and M.I. designed the study; TDV executed the study under the guidance of A.H., L.W., S.G., G.H., and M.I.; TDV analyzed data; TDV, A.H., L.W., W.S., S.G., G.H., D.I.D, and M.I. interpreted the results; TDV prepared figures; TDV, A.H. and M.I. drafted manuscript; TDV, A.H., L.W., W.S., S.G., G.H., D.I.D, and M.I. edited and revised manuscript; TDV, A.H., L.W., W.S., S.G., G.H., D.I.D, and M.I. approved final version of manuscript.

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