THE INFLUENCE OF INTERMITTENT EXERCISE AND HEAT EXPOSURE ON NEUROMUSCULAR AND COGNITIVE FUNCTION

Rachel Malcolm

A thesis submitted in partial fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy

September 2018

Copyright Statement

Abstract

Performance in team sport exercise requires athletes to maintain cognitive and neuromuscular function whilst completing intermittent bouts of exercise, at varying intensities. This ability enables athletes to perform skills effectively throughout a match, whilst achieving a high physical output. However, the influence of this type of exercise, particularly in the heat, on neuromuscular and cognitive function is not well known. Therefore, the aim of this thesis was to establish the neuromuscular and cognitive responses to intermittent exercise in the heat, providing potential mechanisms involved for any changes.

Chapter 4 highlights the detrimental cognitive response to passive heat exposure, mediated largely by negative perceptual feelings. Due to the short duration (60 min) of heat exposure (40º C & 50 % Rh), core temperature did not show a physiologically significant increase during passive heat exposure (baseline: 37.1 ± 0.3º C vs end: 37.6 ± 0.4º C). This allowed the effect of skin temperature and perceptual feeling upon cognition to be established. Response times were slower in the hot trial on the simple (main effect of trial, $P<0.001$) and complex (main effect of trial, $P<0.01$) levels of the Stroop test (Hot: 872 ± 198 ms; Moderate: 834 ± 177 ms) and the simple level of the visual search test (Hot: 354 ± 54 ms; Moderate: 331 ± 47 ms) (main effect of trial, $P<0.01$). Participants demonstrated superior accuracy on the simple level of the Visual Search test in the hot trial (Hot: 98.5 ± 3.1%; Moderate: 97.4 ± 3.6%) (main effect of trial, $P=0.04$). Participants also demonstrated an improvement in accuracy on the complex level of the visual search test following 1 h passive heat exposure (Pre: 96.8 ± 5.9 %; Post: 98.1 ± 3.1 %), whilst a decrement was seen across the trial in the moderate condition (Pre: 97.7 ± 3.5; Post: 97.0 ± 5.1 %) (trial*time interaction, $P=0.029$). The findings of chapter 4 suggest that response times for perception and executive function tasks are the most likely to be altered when exposed to heat stress.

Chapter 5 found that a competitive hockey match facilitated response times for simple perception tasks at full-time (trial*time interaction, $P<0.01$). Response times on the complex executive function task also improved from baseline to half-time (Pre: 827 ± 168; HT: 787 ± 163ms) (trial*time interaction, $P<0.01$). However, working memory declined at full-time on the match trial (Pre: 6.3 ± 1.0; FT: 5.9 ± 1.1 %) (trial*time interaction, $P<0.01$). The beneficial effects were likely a result of higher serum BDNF (Control: 23787 ± 899; Match: 28113 ± 2115 pg/ml) (main effect of trial, $P=0.03$) on the match trial, as well as increased arousal, demonstrated by the increases in noradrenaline shown during the match (Pre: 329 ±

82; Post: 451 ± 156 pg/ml) (trial*time interaction, P<0.01). The study was the first to highlight the beneficial effect a competitive team sports match can have on response times in the domains of perception and executive function, whilst the detriment in working memory may influence tactical recall.

Chapter 6 assessed the reliability of a number of the neuromuscular function measures to be used in chapter 7. Maximal cortical voluntary activation was not different between-day (94.2±4.1% versus 93.4±4.6%, *P*=0.06) or within-day (93.1±5% versus 92.9±4.5%, *P*=0.45). Systematic error (95% limits of agreement) for maximal cortical voluntary activation was −0.78% (−4.92%, 3.36%) for between-day and -0.28% (−4.12%, 3.57%) for within-day. ICC and CV values demonstrated high reliability between-day (ICC=0.927, CV=2.32%) and within-day (ICC=0.953, CV=2.19%). These results indicate that TMS can reliably estimate the output of the motor cortex to the knee extensors, both between-day and within-day.

Chapter 7 found that both cortical (Hot: 89.0 ± 5.8 %; Moderate 91.7 ± 4.1 %) (main effect of trial, P = 0.04) and peripheral (Hot:84.2 ± 6.7 %; Moderate: 87.7 ± 4.3 %) (main effect of trial, P<0.01) voluntary activation were worse in the heat, likely as a result of the increase in core temperature (trial*time interaction, P<0.01). A positive effect of intermittent exercise in the moderate condition was seen for the simple perception task, however this effect was reversed in the heat where response times slowed (Pre: 303 ± 28; Full-time (FT): 333 ± 56 ms) (trial*time interaction, P = 0.01), and accuracy was worse throughout the hot trial (Main effect of trial, P<0.01). Response times improved across the moderate trial for the complex executive function task (Pre: 865 ± 162; FT: 813 ± 174 ms), however no positive effect was seen in the hot (Pre: 902 ± 171; FT: 906 ± 222 ms) (trial*time interaction, P = 0.02).

The findings of this thesis suggest perception and executive function are the most vulnerable cognitive domains in response to heat and exercise, where heat has a negative effect and exercise has a positive one. However, the final study suggests that when combined intermittent exercise and heat stress detrimentally influence performance in these domains, reversing the beneficial effect seen with exercise. Similarly, both cortical and peripheral voluntary activation are worse in the heat, which will limit muscular function of athletes competing in hot conditions. Therefore, protective strategies must be investigated, focusing specifically on the protection of cognition and neuromuscular function during intermittent exercise in the heat.

Acknowledgements

First and foremost, I would like to thank my Director of Studies Caroline Sunderland. Caroline's unwavering confidence in my ability as a researcher and infectious can-do attitude made the perfect companion to my stressy/worrying personality. She was always on hand for help and advice, whether it be related to my PhD or sporting aspirations, and very quickly became someone I could always rely on during the last five years. Her support in enabling me to combine completing my PhD and achieving my goals in sport, has allowed me to achieve far beyond any of my aspirations, which I will always be grateful for.

Secondly, I would like to thank Simon Cooper, one of my co-supervisors. Simon was always on hand to keep things light hearted and convince me "it can't be as bad as the time I …" He was always there to offer an extra pair of hands, despite being the busiest man on campus. I also owe him a massive thank you for all his help and expertise on the cognitive aspects of this thesis.

I would like to thank Chris Tyler and Jonathan Folland who have supervised from afar and provided me with invaluable input in their specialist areas. Additionally, thank you to Martin Lewis, John Morris and Ricci Hannah who have all provided knowledgeable input into aspects of the analysis for various parts of the thesis.

Thank you to all of the amazing colleagues I have been lucky enough to work alongside at NTU during the last 5 years. In particular, my fellow PhD students, a special thanks to Dan and Bex who were there from day one and were always on hand to help with data collection and for the constant supply of tea, coffee, gin and emotional support. Also, a massive thanks to Steph and Liv who have kept me sane during my final year.

I cannot thank my amazing participants enough. They have given up their time, energy and sweat to carry out far from enjoyable protocols and kept me entertained in the process. You have made this thesis possible.

Finally I would like to thank all of my family, friends, team mates and coaches. I have the most amazing and understanding support system and for that I am so so thankful. A special thanks to my Chapel Rise gals, who have picked me up more times than I can count. Also, to our newest member Justo, thank you for always telling me I can, regardless of how much I try to convince you I can't.

Publications and conference proceedings

Some of the results presented within this thesis have been published or presented as detailed below:

Publications

Malcolm, R. A., Cooper, S., Folland, J. P., Tyler, C. J., & Sunderland, C. (2018). Passive heat exposure alters perception and executive function. *Frontiers in Physiology*, *9*, 585. DOI: 10.3389/fphys.2018.00585.

Conference proceedings

Malcolm, R., Cooper, S., Folland, J., Tyler, C., Hannah, R. and Sunderland, C. (2015) The reliability of transcranial magnetic stimulation in the determination of voluntary activation of the knee extensors. *Journal of Sports Sciences*, **33:sup1**, s36. DOI: 10.1080/02640414.2015.1110321.

Malcolm, R. A., Cooper, S., Folland, J. P., Tyler, C. J., & Sunderland, C. (2017). The influence of intermittent exercise in the heat on neuromuscular function and cognitive function. The International Conference of Environmental Ergonomics. Japan.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

LIST OF FIGURES

CHAPTER 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of abbreviations

AMT- Active motor threshold

BDNF – Brain derived neurotrophic factor

BF – Biceps femoris

BRUMS – Brunel mood scale

CAR – Central activation ratio

CRT – Choice reaction time

CNS – Central nervous system

CV – Coefficient of variation

EEG - Electroencephalogram

EMG – Electromyography

ERT – Estimated resting twitch

FAS – Felt arousal scale

FS – Feeling scale

FSINT – Football specific intermittent treadmill protocol

FT – Full-time

GPS- Global positioning system

HT – Half-time

ICC – Intraclass correlation coefficient

ITT- Interpolated twitch technique

MEP – Motor evoked potential

MO –Maximum output

MVC – Maximal voluntary contraction

MVF – Maximal voluntary force

PASSAT - Paced auditory serial addition test

PVT – Psychomotor vigilance test

RF – Rectus femoris

RMT- Resting motor threshold

Rh – Relative humidity

RPE – Rating of perceived exertion

RT – Resting twitch

RVIP – Rapid visual information processing

Tc – Core temperature

Trec – Rectal temperature

TS –Thermal sensation

TTE – time to exhaustion

Tsk – skin temperature

TMS – Transcranial magnetic stimulation

SIT - Superimposed twitch

USC-REMT - repeated episimodic memory test

VA – Voluntary activation

VO_{2max} – Maximal oxygen consumption

VO_{2peak} – Peak oxygen consumption

VL – Vastus lateralis

VM – Vastus medialis

CHAPTER 1: GENERAL INTRODUCTION

Heat exposure is inevitable in almost every person's lifetime. The influence of heat exposure on physiological responses, perceptual feelings and exercise capacity has been extensively researched e.g.: (Sunderland & Nevill, 2005; Tatterson, Hahn, Martin, & Febbraio, 2000; Tucker, Rauch, Harley, & Noakes, 2004; Tucker, Marle, Lambert, & Noakes, 2006; Wendt, Van Loon, & Lichtenbelt, 2007). However, a more detailed mechanistic understanding of the changes to aspects of human functioning in certain populations such as athletes, under heat stress, are less well known.

Cognitive function enables us as humans to utilise information from either internal or external sources, process that information and make an informed decision or action as a result (Schmitt, Benton, & Kallus, 2005). Therefore, cognitive responses are important in all occupations, however a consensus as to how they respond to certain stressors, including both exercise and heat exposure, is not well known due to a number of methodological issues (Gaoua, 2010). Specifically, vocations regularly exposed to heat such as the military, firemen and athletes, as well as the general public exposed to high external temperatures, are the main populations who provide the basis for the research within this thesis.

Both moderate (Gaoua, Grantham, Racinais, & El Massioui, 2012; Tucker et al., 2004) and extreme heat stress (Gaoua, Racinais, Grantham, & El Massioui, 2011; Racinais, Gaoua & Grantham, 2008) brought about by passive (Liu et al., 2013; Schlader et al., 2015) and active hyperthermia (McMorris et al., 2006b; Morley et al., 2012) are known to alter cognition, as a result of their influence on body temperature and perceptual feeling, amongst other factors. Exercise, in the absence of heat stress, has also been recognised as a moderator of cognition (Bandelow et al., 2010; Chang, Chu, Wang, Song, & Wei, 2015; Chang, Labban, Gapin, & Etnier, 2012a; McGregor, Nicholas, Lakomy, & Williams, 1999), predominantly in a beneficial manner. Hence, combining heat and exercise presents a complex relationship with regards to cognition. Understanding the mechanisms which underpin this relationship, particularly in a sport specific environment, will enable greater practical application and ultimately more specific protective strategies to be established for athletes.

Vocational heat stress, if not effectively managed, can put lives at risk, via unsafe behaviours and acute injuries (Chang, Bernard, & Logan, 2017), often as a result of a detriment in cognitive function (Hancock & Vasmatzidis, 2003). Hence, understanding how heat stress in isolation influences cognition can enable more effective coping strategies to be implemented. From a sport specific standpoint, the cognitive contribution to skill performance in sport has

previously been highlighted (McMorris & Graydon, 1996; McMorris & Graydon, 1997; Starkes, 1987; Sunderland & Nevill, 2005). The body produces additional heat during exercise, via metabolic heat production, which requires dissipation to protect both health and performance (Sawka & Wenger, 1988; Wendt et al., 2007). Hence, when exercise is coupled with high external temperatures the removal of heat may be challenged (Wendt et al., 2007). Heat storage, and the resulting increase in core temperature, is known to jeopardise cognitive capacity, hence understanding the mechanisms associated with these alterations is essential for optimising performance.

At present no study has accurately detailed the acute cognitive responses to team sport specific exercise, in isolation, e.g. in the absence of any other stressor. These sports have arguably the greatest practical application when considering exercise and cognitive function, where exercise is repeatedly coupled with skill and decision making. Hence, providing information regarding this relationship and the mechanisms involved will again provide a basis to investigate methods of optimising cognitive performance and thus skill and overall performance. For example, the inability of an athlete to select the correct pass, complete an action quickly and accurately enough to beat an opponent or correctly recall tactical information will directly influence performance. These aspects of skill performance are tightly related to domains of cognition such as visual perception, executive function and memory. Therefore, if heat stress, exercise or the two in combination can influence these domains of cognition, then it is more than likely we will see a negative effect on overall exercise performance.

In addition to the need for a greater understanding of cognitive responses, the mechanisms underpinning physical fatigue in team sport athletes exposed to high external temperatures are also unclear. A limited body of literature has now highlighted the decline in physical output when high intensity intermittent exercise is completed in the heat (Sunderland & Nevill, 2005). Recent literature has established how the brain to muscle pathway is affected by football specific exercise (Goodall et al., 2017), however the neuromuscular response when heat is also present is not yet known. The assessment of neuromuscular function highlights any changes in the brain to muscle pathway (Gandevia, 2001). Previous literature has highlighted the negative effect of both heat stress (Morrison, Sleivert, & Cheung, 2004; Thomas, Cheung, Elder & Sleivert, 2006; Todd, Butler, Taylor, & Gandevia, 2005) and exercise (Bentley, Smith, Davie, & Zhou, 2000; Lepers, Hausswirth, Maffiuletti, Brisswalter, & Van Hoecke, 2000; Lepers, Millet, & Maffiuletti, 2001) on neuromuscular function. These

studies allow for a greater understanding of the sites in the brain to muscle pathway which are causing a decrease in physical output. However, similar to cognitive function, there is a lack of understanding regarding the changes in function in response to intermittent team sport exercise in the heat. Once an understanding of the changes in the brain to muscle pathway have been established, the practical applications and protective strategies for these athletes can be further developed, optimising performance.

Therefore, this thesis aims to affirm how cognitive function and neuromuscular function are influenced by heat and intermittent exercise. With this in mind, this thesis is made up of eight chapters which are detailed below:

Chapter

1. General introduction provides a brief overview of the topic as a whole, setting the scene for the thesis.
2. The review of the literature provides an insight into the previously investigated areas within each of the specific areas of this research, providing a rationale for the studies included in this thesis.
3. The general methods offer a more detailed description of a number of the more complex methods used within the experimental chapters (4 – 7).
4. Chapter 4 assesses the cognitive responses to 1 h of passive heat exposure.
5. Chapter 5 assesses the cognitive responses to a competitive hockey match, assessing performance at baseline, half-time and full-time.
6. Chapter 6 assesses the reliability of the transcranial magnetic stimulation technique in the determination of voluntary activation of the knee extensors.
7. Chapter 7 assessed the influence of high intensity intermittent exercise in the heat on cognitive function and neuromuscular function.
8. The general discussion critically evaluates the overall findings of the thesis, addressing the aims and hypotheses and providing an overall conclusion.

CHAPTER 2: REVIEW OF THE LITERATURE

2.1. Introduction

This chapter will provide an overview of the literature concerning neuromuscular and cognitive function in response to exercise, and heat, in isolation, followed by their combined effect.

2.2. Neuromuscular function

2.2.1. Muscular contraction and muscular fatigue

Muscular contraction, initiated by a sequence of events starting in the brain and ultimately ending at the muscle, is essential for both movement and the production of force (Taylor, Todd, & Gandevia, 2006). This sequence is initiated by supra-cortical structures and the ability to carry out movements or generate force may be impaired due to a decrement at any point in the pathway (Figure 2.1). Changes occurring within this pathway can influence an athlete's ability to perform. Definitions of fatigue have varied across the literature (Enoka & Duchateau, 2008), encompassing motor deficits, declines in mental function, declines in force capacity or alterations in electromyography activity. Therefore to provide clarity throughout this thesis, fatigue is defined as "any exercise-induced reduction in the ability to exert muscle force or power regardless of whether or not the task can be sustained" (Gandevia, 2001). Fatigue is a complex and multifaceted phenomenon, traditionally spilt into two groups, peripheral and central, with a number of mechanisms highlighted to influence these different aspects. The ultimate cause of fatigue is that one, or more, of the physiological processes that allow the contractile proteins to produce force are compromised (Enoka & Duchateau, 2008). However, the site within the neuromuscular pathway which is responsible for this decrement in force production allows the determination of fatigue to be either peripheral or central in origin, with decrements happening from the central nervous system down to the intramuscular contractile filaments (Kent-Braun, 1999).

2.3. Peripheral fatigue

Peripheral fatigue occurs due to a change in processes occurring at or near to the neuromuscular junction causing a loss of force. Primary mechanisms include disruption of action potential propagation, excitation-contraction coupling or cross bridge cycling (Ross, Gregson, Williams, Robertson, & George, 2010), often elicited through metabolic inhibition (Kent-Braun, 1999). During low intensity prolonged exercise the contribution appears to primarily from failure within the excitation-coupling, whereas peripheral fatigue during high

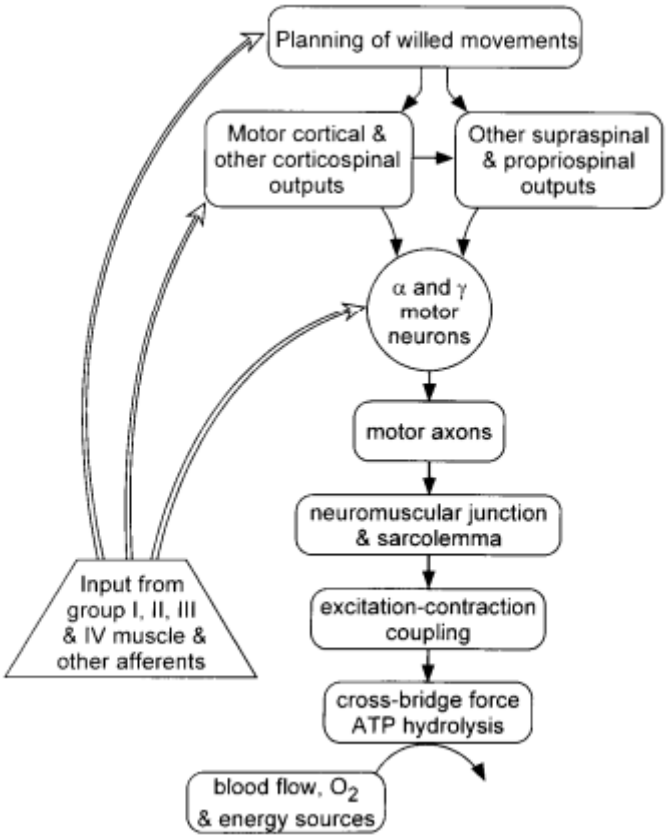


Figure 2. 1 Diagram highlighting the steps involved in producing a voluntary contraction. Copied from Gandevia (2001).

intensity exercise has been attributed to the accumulation of metabolites (Baker, Kostov, Miller, & Weiner, 1993; Cady, Jones, Lynn, & Newham, 1989; Kent-Braun, 1999; Miller, Boska, Moussavi, Carson, & Weiner, 1988; Weiner, Moussavi, Baker, Boska, & Miller, 1990). However, research into the influence of exercise which is both prolonged and intermittent, similar to that in team games, is currently lacking. The primary mechanisms contributing to peripheral fatigue are thought to be extracellular accumulation of potassium, the accumulation of hydrogen ions causing a decrease in muscle pH and the disruption of calcium handling (Kent-Braun, 1999).

2.3.1. Sodium-Potassium pump

Action potentials activate the skeletal muscle via an efflux of sodium, immediately followed by an influx of potassium in the muscle cells. This process is referred to as the sodium-potassium pump, which is altered relative to the intensity of exercise, with the movement of sodium and potassium increasing with increasing intensity of exercise. Therefore at very high exercise intensities potassium becomes concentrated in extracellular spaces (Medbø & Sejersted, 1990), which can cause excitation to be inhibited, and consequently reducing muscle force production.

2.3.2. Acidosis

Lactic acid is known to accumulate in response to high intensity exercise as a result of anaerobic respiration (Fitts, 1994). The simultaneous increase in hydrogen ions results in a lowering of pH at the muscle which has been shown to reduce the muscles ability to produce force or power due to the disruption in contractile function (Miller et al., 1988; Westerblad, Allen, & Lannergren, 2002). However, there is a lot of contrasting research within this area, debating the contribution of hydrogen ions to muscle fatigue, suggesting it is most likely not the primary cause of muscle fatigue.

2.3.3. Calcium handling

Calcium ions released from the sarcoplasmic reticulum drive the contractile process required to produce muscle force (Berchtold, Brinkmeier, & Muntener, 2000; Hill, Thompson, Ruell, Thom, & White, 2001). Therefore disruption to the handling of calcium ions can influence muscle function (Hill et al., 2001). This is generally a result of a decrease in free calcium concentration, limiting the release of calcium and increasing the rate at which it may be uptaken. As a result, cross bridge formation is restricted, directly influencing the muscle's ability to produce force. The accumulation of free phosphate during muscular contraction as a result of the hydrolysis of creatine phosphate further hinders muscle contraction as phosphate limits the release of calcium from the sarcoplasmic reticulum.

2.4. Central fatigue

Central fatigue occurs due to failure of the central nervous system, resulting in a decrease in the neural drive to the muscle, causing a reduction in force produced (Taylor et al., 2006). However, the two components of fatigue (central and peripheral) are intrinsically linked, with motoneuron recruitment relying on drive from supraspinal sites, which is determined via feedback, either excitatory or inhibitory, from a number of sites such as muscles and tendons (Figure 2.1)(Gruet et al., 2013). Therefore central fatigue can occur due to impairments in the cerebral cortex, spinal cord and motoneuron properties. Central fatigue also enables the body to protect muscles against over exertion and injury, by using feedforward mechanisms of altering pacing strategies (Noakes, St Clair Gibson, & Lambert, 2005). This feedforward process prevents any major disruption to the muscle, and ultimately the body's state of homeostasis by preventing over exertion at the muscle and therefore preventing the body achieving its absolute capacity. This system is also influenced by a person's perceived exertion, sending feedback to the brain which further dampens the drive to exercising muscle. However, a lowering of perceived exertion is also known to influence exercise capacity via

methods unrelated to neuromuscular function (Castle, Maxwell, Allchorn, Mauger, & White, 2012).

2.4.1. Supraspinal fatigue

Supraspinal fatigue occurs as a result of inadequate output from the motor cortex (Gandevia, 2001). Changes in supraspinal fatigue, and resulting voluntary activation, can be influenced by the excitability of the motor cortex, the strength of the connections within the pathway, the excitability of the motoneurons and the properties of the action potential. The responses in muscle twitch force and excitability and contractility of the muscle, measured via EMG activity is used to assess supraspinal fatigue.

2.5. Assessment of neuromuscular function

The brain both initiates muscular contraction and dictates the strength of contraction, however research suggests that even during maximal contractions the brain does not utilise all its available resources to drive the muscle (Gandevia, Allen, Butler, & Taylor, 1996). This has been shown via the interpolated twitch technique, which demonstrates that if an additional stimulus is applied within the brain to muscle pathway then it is often possible to achieve a greater amount of force and electrical activity in the muscle, indicating suboptimal activation of a muscle and untapped resources (Figure 2.2). Hence, an individual is often unable to achieve maximum force due to suboptimal output at some stage within the pathway. The application of stimuli at different points within the pathway has allowed greater understanding regarding the brain to muscle pathway and the mechanisms involved in muscle fatigue, in terms of the sites of suboptimal output to the muscle (Gandevia, 2001).

2.5.1. Voluntary activation

Voluntary activation refers to the proportion of neural drive to muscle fibers and motoneurons (Gandevia, 2001). Merton, (1954) found that the increment in force added by a twitch was inversely related to the level of voluntary effort, hence if a maximal voluntary effort was achieved no additional force would be produced. The interpolated twitch technique involves comparing the force response to stimulation at rest to the response when a stimulus is superimposed on top of a maximal voluntary contraction. Given that the superimposed twitch will be potentiated, the resting twitch used for comparison should also be potentiated e.g. (approximately 5s post contraction) (Gandevia, 2001). This technique is based on the assumption that any added force seen with stimulation is equal to the shortfall in activation (Goodall, González‐Alonso, Ali, Ross, & Romer, 2012).

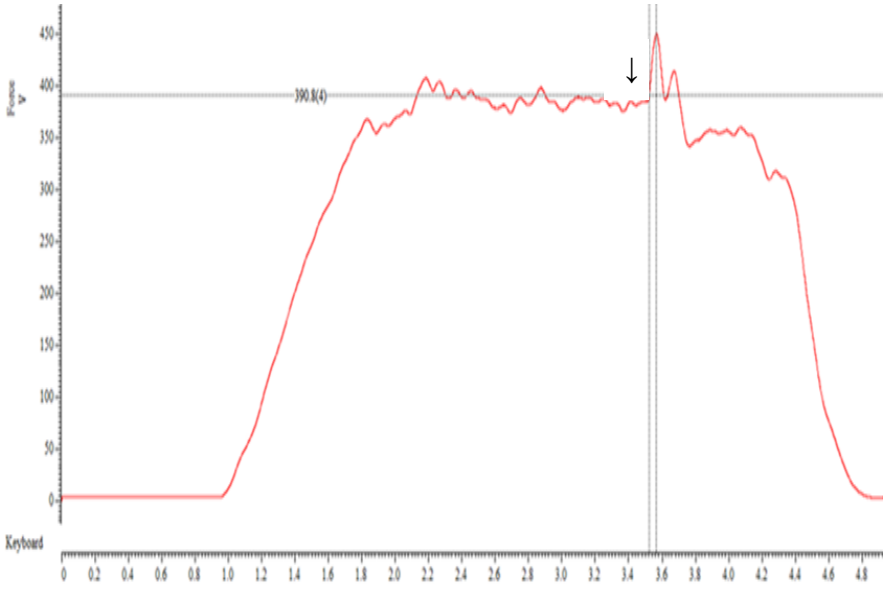


Figure 2. 2 Example force trace in response to peripheral nerve stimulation of the femoral nerve. Taken from a participant in the study described in chapter 7. ↓ indicates the input of the stimulus.

Motor nerve stimulation can identify a suboptimal level of activation, however the site of failure within the pathway remains unclear, with a twitch suggesting the failure must be at or above the motor axons (Todd, Taylor, & Gandevia, 2004). Transcranial magnetic stimulation (TMS) provides more specific information regarding the site of failure, with a twitch demonstrating suboptimal output from the motor cortex (Taylor et al., 2006). These measures allow us to establish the level of activation at different points within the pathway, and provide information regarding the peripheral and supraspinal contribution to the production of force. Therefore, motor nerve stimulation provides an indication of peripheral voluntary activation and TMS provides an indication of cortical voluntary activation. Voluntary activation allows the changes that occur with training, ageing, fatigue and injury to be assessed, with respect to neural activation (Harridge et al., 1999; Urbach et al., 1999).

2.5.2. Peripheral nerve stimulation

Peripheral factors causing fatigue can include metabolic inhibition of contractile processes and excitation-contraction coupling disruption (Kent-Braun, 1999). Metabolic accumulation which accompanies high intensity exercise can also impact the muscles ability to produce force. Electromyography (EMG) data enhances the understanding of neuromuscular junction and muscle membrane excitability (Kent-Braun, 1999).

2.5.3. Peripheral voluntary activation

Merton (1954) introduced the peripheral nerve stimulation technique in order to assess decrements in force production at the peripheral level. A resting twitch is often used as an estimation of peripheral fatigue, providing information regarding the muscle's contractility and excitability. A decrement in twitch force suggests reduced contractility at the muscle. The maximum rate of force development also suggests cross-bridge formation has been altered. Merton (1954) was the first to demonstrate that an inverse relationship existed between voluntary force and superimposed twitch amplitude, which could be used to assess peripheral fatigue. Therefore, when a muscle is fatigued, voluntary activation is unlikely to be complete and a larger twitch will be seen during a maximal voluntary contraction (MVC). During the interpolated twitch technique, the stimulus is believed to drive all motor units synchronously; hence, if a maximal tetanus is produced any shortfall in activation will be shown. Therefore, peripheral nerve stimulation can be used to assess a change within a muscle group in response to stress, such as exercise (Bentley et al., 2000; Lepers et al., 2000; Lepers et al., 2001; Lepers, Maffiuletti, Rochette, Brugniaux, & Millet, 2002). However, the site within the brain to muscle pathway responsible for a decrement cannot be reliably differentiated with this technique alone.

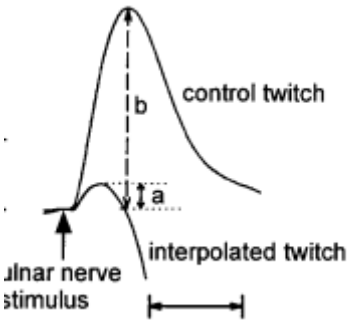


Figure 2. 3 Example of the difference between a control twitch completed at rest, and a superimposed twitch upon a maximal voluntary contraction. From Gandevia (2001).

2.5.4. Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) has been used extensively over recent years to stimulate areas of the motor cortex, providing insight into pathways existing between the brain and specific muscles (Goodall et al., 2012; Goodall, Howatson, Romer, & Ross, 2014; Goodall, Charlton, Howatson, & Thomas, 2015a; Goodall et al., 2017; Ross, Middleton, Shave, George, & Nowicky, 2007; E. Z. Ross et al., 2010; Sidhu, Bentley, & Carroll, 2009;

Temesi et al., 2014; Temesi et al., 2017; Thomas et al., 2015; Thomas, Elmeua, Howatson, & Goodall, 2016). TMS can activate specific neurons in order to evoke short latency responses in a specific muscle group, associated with a specific region of the motor cortex (Figure 2.4) (Taylor et al., 2006). The loss of force as a result of decreased output from the motor cortex can be assessed using TMS, which is known as supraspinal fatigue (Taylor & Gandevia, 2008). Two categories contribute to supraspinal fatigue (Taylor et al., 2006). Firstly, factors that result in a decrease in the output from the motor cortex. Secondly, factors that influence the effectiveness of that output in any force generating process. This could include changes in responsiveness of the motoneurons, therefore affecting the amount of descending input required to maintain the same muscular force. Similarly any difference in contractile properties of a target muscle can affect the motor unit firing rates required to maintain a desired level of force. Cortical voluntary activation provides an indication of the level of drive to a muscle from the cortical and subcortical structures to the motor cortex (Goodall et al., 2014; Goodall et al., 2015a; Goodall et al., 2017; Temesi et al., 2014; Temesi et al., 2017; Thomas et al., 2015; Thomas et al., 2016).

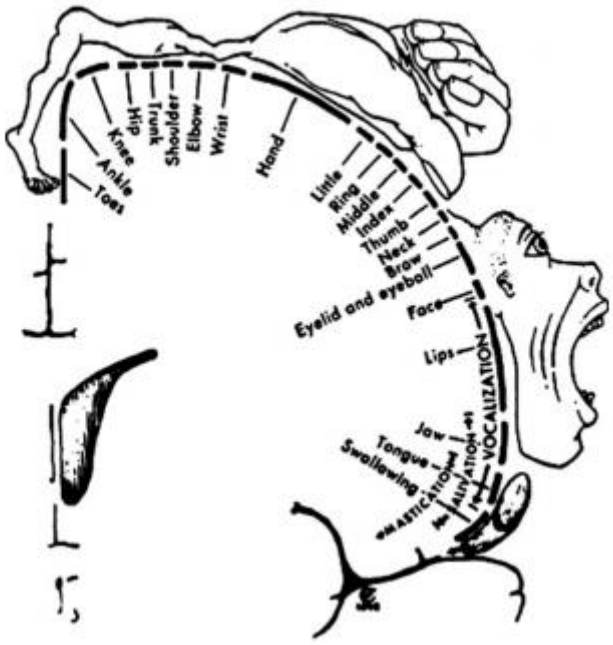


Figure 2. 4 Mapping of the human homunculus. Reprinted from Goodall et al (2014), with permission.

2.5.5. Cortical Voluntary Activation

TMS relies on the belief that currents sent through a coil placed on the scalp can cause the magnetic field to continuously change resulting in neural tissue stimulation (Goodall et al., 2012), and involuntary contraction of the targeted muscle. TMS is more frequently

superimposed on an already activated muscle, in order to recruit any available motor units. The interpolated twitch technique, when combined with TMS, must create a linear regression whereby the superimposed twitch (SIT) force is plotted against voluntary force (Goodall, Howatson, Romer, & Ross, 2014). This allows an estimate of resting twitch force to be established by extrapolating the regression for y=0 (e.g. the twitch response when 0 N of force is produced). The need for an estimation of resting twitch is due to the corticospinal excitability being less at rest than during activity, and fewer motoneurons will be activated, providing an under representation of true resting twitch (Goodall et al., 2014). If TMS causes a further increase in muscle force, resulting in a SIT during an MVC, then voluntary activation is less than maximal as a result of inadequate motor cortical output (Gandevia, 2001; Goodall et al., 2012; Taylor et al., 2006). The method of assessing voluntary activation for TMS is identical to that used with peripheral nerve stimulation, however the estimated resting twitch is utilised instead of a true resting twitch.

Combining peripheral nerve stimulation and transcranial magnetic stimulation allows simultaneous quantification of the contribution of peripheral and supraspinal fatigue to muscle function.

2.5.6. Reliability of Transcranial Magnetic Stimulation

A number of studies have assessed the reliability of TMS on the knee extensors, however all of these studies have used an inadequate number of participants to assess reliability (Goodall, Romer, & Ross, 2009; Sidhu et al., 2009). Goodall et al (2009) and Sidhu et al (2009) assessed SIT responses on two separate occasions in the knee extensors using TMS during isometric contractions. TMS was found to be reliable, both between-day and within-day, in the determination of cortical voluntary activation, however a maximum of 9 participants were used in both studies. Small subject numbers has recently been highlighted as a significant issue in neurophysiology (Héroux, Taylor, & Gandevia, 2016), in addition to its being highlighted as a critical issue in reliability research (Atkinson & Nevill, 1998). Therefore, clarification of the reliability of this technique is warranted, in order to support the existing research.

High within-day (CV = 10.2%, ICC = 0.82) and between day (CV = 2.2 %, ICC = 0.87) reliability for cortical voluntary activation has been found by Goodall et al (2014) and Goodall et al (2017), respectively. However, similar to the aforementioned studies, the participant number do not satisfy the requirements set by Atkinson & Nevill (1998). Therefore, the reliability of the TMS technique in the determination of cortical voluntary

activation will be further investigated within experimental chapter 6. The key aim of this chapter was to establish the reliability of the technique in accordance with the requirements set by Atkinson & Nevill (1998), whilst overcoming a number of issues currently present within the neurophysiology research, highlighted by Heroux et al (2016).

2.5.7. Electromyography: M-wave and motor evoked potential

The M-wave and motor evoked potential (MEP) refer to the electrical potential, measured via EMG activity, detected in the target muscle in response to electrical stimulation and TMS, respectively. TMS activates corticospinal neurons to produce an MEP (Taylor et al., 2006), therefore the size of the MEP provides information regarding corticospinal excitability, the excitability of motoneurons and the characteristics of the muscle fiber action potential (Gandevia, 2001). As motoneuron firing rates increase, the size of EMG may decrease as a result of motoneurons' decrease in responsiveness to drive, hence incurring an increase in drive or due to a change in the amount of excitatory or inhibitory drive (Taylor & Gandevia, 2008). This change may have been caused by motoneuron membrane properties being negatively affected by exercise, or inhibition of the motoneuron pool (Taylor et al., 2006). However, an increase in EMG activity suggests an increase in excitatory input has occurred in order to maintain the same force, suggesting a decrease in responsiveness of motoneurons. In order to normalise data into a format which can be compared between participants, EMG data is generally normalised to Mmax, which is defined as the maximum M-wave potential seen within a testing session (Gandevia, 2001).

The silent period, which directly follows the surge in EMG activation, represents the inhibitory response, and therefore suppression of EMG activity, as a result of the inhibition of intracortical circuits (Gandevia, 2001). The duration of the silent period provides information regarding the excitability of cortical neurons, with increased duration indicating increased excitability (Taylor et al., 2006). Duration of the silent period increases in response to greater stimulus intensity (Gandevia, 2001).

When interpreting the MEP data, the latency (which is a measure of the time elapsed between a neural signal and muscular response) can be considered an indication of the central motor conduction time. Corticospinal excitability is measured via changes in the amplitude of an MEP signal. Finally the total MEP area, can be used to identify the fraction of the motoneuron pool recruited in response to a TMS signal. This can be done via a direct comparison between the area achieved in response to TMS and the area seen in response to motor nerve stimulation (Goodall et al., 2012).

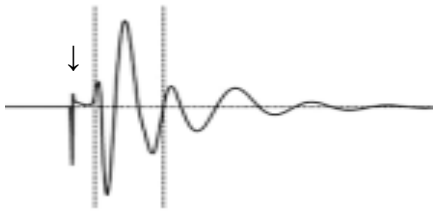


Figure 2. 5 Example of an EMG trace detailing the motor evoked potential following transcranial magnetic stimulation. Copied from Goodall et al (2014). ↓ indicates the input of the stimulus.

2.6. Neuromuscular function and exercise performance

Table 2.1 details the key findings within the literature which has assessed the influence of exercise on neuromuscular function.

Neuromuscular function encompasses all the processes involved in the motor drive to active muscles, ultimately leading to the production of force or tension (Gibson, Lambert, & Noakes, 2001). Therefore changes in neuromuscular function can influence athletic performance, with different methods of assessment providing information regarding the mechanisms involved in these changes (Racinais et al., 2008), as previously described in section 2.5.

2.6.1. Prolonged exercise

Decrements in neuromuscular function have been strongly linked to long duration exercise (Bentley et al., 2000; Lepers et al., 2000; Lepers et al., 2001; Lepers et al., 2002). Lepers et al (2002) investigated the effect of a long duration cycle at 55% of maximal aerobic power on neuromuscular function and the time course that changes occur in the quadriceps utilising the twitch interpolation technique with peripheral nerve stimulation. This study demonstrated that voluntary activation was markedly reduced as the 5 h cycle progressed, however only reaching significance at the end of the 5 h bout. The impact on excitability, measured via M-wave properties, and central drive were also only affected towards the end of the cycle. However, excitability remained impaired following exercise as a result of changes in ionic processes including the alterations in potassium and sodium. Contrastingly, contractile properties were impaired much sooner into the cycle, with all contractile properties (contraction time, total twitch area, maximal twitch torque) showing a decrease from baseline by the end of the exercise bout apart from half relaxation time. Maximal voluntary torque, measured via both eccentric and concentric movements on an isokinetic dynamometer, diminished throughout the protocol (18%). Vastus lateralis and vastus medialis demonstrated differential changes in drive, indicating variability in monoarticular agonist muscles as a result of submaximal, long duration exercise to fatigue. The exact mechanism causing fatigue,

whether spinal or supraspinal cannot be determined using this protocol as the motor unit firing rate cannot be assessed, however it can be said that a reduced excitability of motoneurons was present, as the superimposed twitch technique showed an increase in twitch size following exercise. The findings of Lepers et al (2002) were in agreement with the previous findings of Lepers et al (2000) who found MVC to decrease substantially (11-15% decrease across different contraction types) following a 2 h cycle, likely due to peripheral mechanisms, shown by the decrease in potentiated twitch force (11-15% decrease). Bentley et al (1999) also found that exhaustive cycling exercise, lasting 30 min, resulted in significant decrements in the force generating capacity of the knee extensor muscles. This study indicated that decrements were still seen up to 6 h post exercise, despite exercise being considerably shorter, indicating a detriment in neuromuscular function occurs after shorter duration exercise.

As previously mentioned, using only electrical stimulation to assess neuromuscular function prevents detail surrounding the site of failure within the brain to muscle pathway being established. Ross et al (2010) and Todd, Taylor & Gandevia (2003) overcame this issue assessing neuromuscular function using electrical stimulation and TMS in response to multiple consecutive bouts of prolonged cycling exercise and following repetitive fatiguing elbow flexor contractions. Ross et al (2010) found that both central and peripheral fatigue of the knee extensors were increased following exercise, with peripheral twitch responses and M-wave activity recovered within 2 days however corticomotor function remained below baseline following 2 days of rest. The extent of the reduction in both peripheral and central fatigue were not relative to the amount of time spent cycling or the distance cycled. This supports the suggestion that changes in neural drive are predominantly a protective mechanism to prevent peripheral damage (Gibson et al., 2001). Todd et al (2003) investigated the effects of having either fresh or fatigued muscles on voluntary activation across a range of stimulus intensities. This study used both TMS and motor nerve stimulation in order to identify the site of failure, but also to compare the two methods of assessment. Both methods identified the fatigued muscle to show reduced voluntary activation during maximal efforts.

Temesi et al (2014) assessed the cortical contribution to changes in central changes during ultra-trail running (110 km race). Both peripheral and cortical activation decreased following the 110km race (-26% and -16%, respectively). These findings imply the cortical motoneurons are significantly affected by long duration exercise, influencing firing potential which can therefore be identified as a cause of a drop off in intensity in ultra-endurance

running. The majority of research with TMS and endurance exercise has been completed with cycling protocols, however the findings cannot necessarily be applied to running due to the greater central fatigue associated with running (Lepers et al., 2002). Temesi et al (2014) was one of the first studies to highlight the supraspinal conrtibutions to ultra-running exercise. The use of TMS has been used to demonstrate a decrement in voluntary activation as a result of suboptimal cortical output following prolonged running exercise (Ross et al., 2007) and incremental, exhaustive running exercise (Verin et al., 2004). However, at present very few studies have assessed the cortical output in response to intermittent high intensity exercise, replicating team sport performance (Goodall et al., 2017).

2.6.2. High intensity exercise and neuromuscular function

The effect of prolonged exercise on neuromuscular function has been extensively researched, however high intensity, short duration exercise has received limited attention in comparison. Fernandez-del-Olmo et al (2013) assessed the effect of two 30 s Wingate tests against 7.5% of body mass, with 30 min of rest between them on knee extensor activation. The control session involved two sessions of 30 s at 25% of maximal power output. The wingate bout caused a significant reduction in MVC (16%) and voluntary activation declined by 34%. However, the recovery of the motor cortex ability to stimulate motoneurons was similar to that of the recovery of MVC, hence suggesting the majority of fatigue was a result of supraspinal mechanisms, however some amount of peripheral fatigue is also present as a result of "intramuscular impairment", shown by decreased potentiated twitch amplitude likely as a result of the accumulation of metabolites interfering with the excitation-contraction coupling. Similarly only a small number of studies have assessed neuromuscular function in conjunction with intermittent exercise (Girard, Lattier, Maffiuletti, Micallef, & Millet, 2008; Perrey, Racinais, Saimouaa, & Girard, 2010). Girard et al (2008) found torque to decrease significantly following exercise. Perrey et al (2010) investigated the impact of repeated sprint activity on neuromuscular activity. This study found MVC torque of the plantar flexors and voluntary activation assessed via twitch interpolation were both depressed following the intermittent sprint exercise. The fatigue seen in this study is believed to be predominantly peripheral in nature as a result of recovery of force replicating the recovery of contractile properties, with little contribution being found from central fatigue.

2.6.3. Intermittent exercise and neuromuscular function

Goodall et al (2015a) was one of the first studies to assess the supraspinal response to intermittent exercise. This study implemented a repeated sprint protocol of 12 sets of 30m

maximal sprints, interspersed with 30 s of rest. During the rest period peripheral voluntary activation of the knee extensors was estimated, whilst both peripheral and cortical voluntary activation was estimated prior to and immediately following the entire protocol. This research is the first to provide information regarding the mechanisms involved in fatigue during repeated sprint exercise, finding that both peripheral and central aspects of fatigue contribute to the drop off in power after only two sprints. The high intensity nature of this protocol provides greater application to competitive team sport unlike the majority of research utilising steady state exercise protocols (Lepers et al., 2000; Lepers et al., 2001; Temesi et al., 2014). However, it still does not provide a true reflection of team sport exercise and its influence on neuromuscular function.

More recently, Goodall et al (2017) has investigated both peripheral and cortical response to a simulated soccer match. This study demonstrated significant peripheral fatigue at half time, full-time and extra-time with potentiated twitch force significantly dropping (-15, -23 and -23 %, respectively). Peripheral voluntary activation was less at full-time and extra-time (-15% and -18%, respectively) and cortical voluntary activation was less at half-time, full-time and extra-time (-11%, -15% and -17%, respectively). This study highlights that both peripheral and central processes contribute to neuromuscular fatigue in response to intermittent exercise. The peripheral fatigue demonstrated in this study followed a biphasic pattern of change whereby a large drop off occurred in the initial stages of the match, followed by a plateau in the rate of change at full-time and extra-time. This is explained through the exhausting of faster fatiguing, high threshold motor units compared to the lower threshold and more fatigue resistant motor units which are preferentially recruited into the latter stages of a match. Mmax values were not influenced by the protocol, suggesting peripheral fatigue was a result of disruption to the excitation-contraction coupling, most likely due to inadequate calcium handling.

Rampini et al (2011) also utilised a competitive football match to assess changes in neuromuscular function. This is one of the first to do this with a true replication of a match. However measures were made 40 min following the match, 24 h post and 48 h post. This data provides interesting information with regards to the longer-term fatigue and reduction in performance. However the latency between the completion of exercise and assessments mean the acute and immediate effects of the exercise are unknown, therefore it is unknown what mechanisms are involved in changes within that game. Rampini et al (2011) found that both peak torque and voluntary activation were lower 40 min post-match and 24 h post-match,

however had recovered to baseline levels by 48 h post-match. These results have clear implications for multi-match tournaments in team sport competitions, and stresses the need to understand how the recovery of neuromuscular function can be enhanced, in order to optimise performance.

Team sports exercise results in metabolic, mechanical and perceptual stress (Goodall et al., 2017). It is known that fatigue in this type of exercise is associated with alterations in excitation-contraction coupling (Rampinini et al., 2011), depletion of endogenous fuel sources (Bendiksen et al., 2012), ionic disturbances (Bangsbo, Mohr, & Krustrup, 2006) and dehydration (Laitano, Runco, & Baker, 2014). However, despite the knowledge of the mechanisms contributing to fatigue, quantifying the neuromuscular contributions to changes in performance requires further investigation. The literature in this area has begun to highlight the underlying mechanisms causing performance decrements following intermittent exercise which can now enable better planning in terms of the days prior to and following a match in order to optimise both performance and recovery.

2.7. Neuromuscular fatigue and heat

Table 2.2 details the key findings from the literature which have assessed the effects of heat on neuromuscular function.

Small increases in both core and muscle temperature have previously been found to improve contractile function (Asmussen & Boje, 1945; Ball et al., 1999) via enhancing the speed of the muscle twitch response, relaxation rate and contractile rate processes as temperature directly influences the motor unit firing rate essential for tetanic fusion (Todd et al., 2005). However, larger increases in core temperature (e.g. 1 º C and above) have been found to impair neuromuscular performance (Morrison et al., 2004; Nybo & Nielsen, 2001a; Racinais, Gaoua, & Grantham, 2008; Thomas et al., 2006; Todd et al., 2005). Various studies have investigated the impact of hyperthermia alone and in combination with exercise on central fatigue, after the suggestion that heat may exacerbate it to a greater extent (Brück & Olschewski, 1987).

2.7.1. Peripheral nerve stimulation

Morrison et al (2004) isolated the effects of increasing core and skin temperature via passive heating to achieve an increase in rectal temperature from 37.4˚C to 39.4˚C, before using a liquid conditioning garment to lower rectal temperature back down to baseline (37.4˚C). This

Table 2. 1 Details the key findings within the current literature for the effects of exercise on neuromuscular function. MAP = maximal aerobic power, VA = voluntary activation, MVC = maximal voluntary contraction, EMG = electromyography, HT = half-time, FT = full-time, ET = extra-time.

Study	Participants	Exercise protocol	Measures	Findings	Notes
Leper et al (2002)	9 trained cyclists/triathletes	55 % MAP for 300 min (5h)	Peripheral VA of the knee extensors	Progressive ↓ in MVC torque and ↓ in VA after 5h. ↓ in EMG activity from 1h.	Contractile properties ↓ after 1h and excitability and VA are affected later in the exercise bout.
Ross et al (2010)	8 trained cyclists	20 Tour de France cycling stages (rest days 9 and 17)	Peripheral and cortical VA of the knee extensors	Sustained ↓ in peripheral and cortical VA post exercise. Peripheral fatigue ↓ for 2days.	Cortical VA remained below baseline after 2 days.
Temesi et al (2014)	25 ultra endurance trail runners (11 females & 14 males)	110km run/walk (elevation 5862m)	MVC, peripheral and cortical VA of the knee extensors.	↓ in cortical and peripheral VA.	MEP amplitude ↑, therefore showing ↑ in corticospinal excitability
Perrey et al (2010)	16 male team sport or racket sport athletes	12 x 40m sprints (30 s rest)	Peripheral VA and MVC in the plantar flexors	↓ in peripheral VA and MVC torque.	Recovery of MVC was rapid (30 min) via renewal of muscle contractile properties.
Goodall et al (2015a)	12 intermittent sprint sport male athletes	12 x 30m sprints (30 s recovery)	Sprint time, MVC, potentiated twitch, Peripheral and cortical VA	Sprint time ↓ from 3rd sprint onwards. MVC and potentiated twitch ↓ from 2nd sprint onwards. ↓ in peripheral and cortical VA post exercise.	Fatigue ensues following just 2 high intensity efforts in team sport athletes, as a result of both peripheral and supraspinal origins.
Goodall et al (2017)	10 male soccer players	2 x 45 min soccer simulated halves plus extra time (120 min)	MVC, Peripheral and cortical VA, potentiated twitch force	↓ Peripheral VA at FT and ET. ↓ Cortical VA at HT, FT and ET. Potentiated twitch ↓ at HT, FT and ET.	Biphasic change in peripheral fatigue – large drop off followed by a plateau in the rate of change.

study assessed voluntary strength and voluntary activation of the knee extensor muscles at regular intervals, coinciding with each 0.5˚C increase in rectal temperature using 10 s MVCs. In line with the work of Racinais et al (2008) heating caused voluntary activation to decrease by 11% and MVC to progressively decrease, eventually by 13%, which remained decreased despite skin cooling (1.3 º C decrease in skin temperature and 2 º C drop in head temperature). Consequently, this study supports the knowledge that core temperature (only 0.6 º C drop in Tc) is the main determinant of neuromuscular function during passive heat exposure. An argument exists in the literature, regarding whether drive decreases gradually or dramatically as a result of the attainment of a "critical temperature". However, this study suggests performance shows a gradual decline rather than a rapid decline on the attainment of a "critical temperature". The methods used by this study and the study by Thomas et al (2006) allow the differentiation of the effects of increases in core and skin temperature. Unlike Thomas et al (2006), this study utilised an MVC of 10 s and VA was again determined using percutaneous muscle stimulation and the interpolated twitch technique. Muscle temperature was not measured, which has previously been found to affect force production during isometric contractions as a result of a faster muscle twitch response in the heat (Ruiter & De Haan, 2000). Hence in order to maintain contraction strength, a greater rate of motor neuron firing must occur to result in summation (Enoka, 2002). However the findings of Nybo and Nielsen, (2001a) dispute this finding, again suggesting muscle temperature does not significantly affect functioning, and core temperature remains the primary driving force for neuromuscular function.

Thomas et al, (2006) utilised a similar protocol to that of Morrison et al (2004). However, this study expanded on the findings of Morrison et al (2004) as throughout the protocol one leg was kept in a thermoneutral state, and therefore the effects of peripheral temperature could also be assessed. Maximal voluntary force and voluntary activation of the plantar flexor muscles were similarly decreased in the heated and thermoneutral leg in response to heating. This study suggests changes in peripheral muscle temperature, which may cause contractile characteristics to change, marginally affect voluntary activation. When cooling was initiated the skin temperature dropped to baseline rapidly, however no change in torque or voluntary activation was seen until core temperature also returned to baseline. In agreement with Morrison et al (2004), this protocol clearly demonstrates that changes in core temperature appear to be the driving force for alterations in neuromuscular function and therefore voluntary activation. Correspondingly, performance did not become impaired upon the

attainment of a “critical temperature” but demonstrated a gradual decrease in drive as core temperature increased. Interestingly, hyperthermia resulted in a decrement in voluntary activation during brief contractions. Previously studies have found that an impairment was only seen in more sustained contractions (Nybo & Nielsen, 2001a; Todd et al., 2005). It may be possible that voluntary activation is not affected during brief contractions as the neuromuscular system can overcome the stress for a short period of time by increasing drive (Nybo & Nielsen, 2001a). However, differences seen may be as a result of Nybo & Nielsen (2001) utilising an exercise induced method of heating, and Todd et al (2005) only heating participants passively to 38.5˚C, hence inducing a lower level of thermal strain.

Various methods exist in order to heat and cool individuals to a desired level. Racinais, Gaoua and Grantham (2008) passively heated participants at 20˚C and 40% Rh, 50˚C and 50% Rh and 50˚C with a head cooling device for 2 h. Muscle action potentials (M-waves) were induced via electrical stimulation in order to test neuromuscular function in the plantar flexor muscles. Potentials were evoked at rest, during brief contractions and sustained contractions of 120 s. Torque was less during brief contractions in both the hot and head cooling conditions, however voluntary activation was only decreased in the hot condition. The changes seen in peripheral voluntary activation in the hot condition were a result of alterations at a spinal and neuromuscular junction level. Sustained contractions resulted in a greater decrement in voluntary activation during heating, and impairments were also seen in the head cooling condition. Additionally, results suggest supraspinal fatigue was also responsible for the decrement in activation seen during sustained contraction, as no additional failure was seen in M-waves despite a further decrease in voluntary activation, however this cannot be confirmed in the absence of TMS. Results proved that electrical stimulation of a set intensity failed to induce the same level of sarcolemmal action potential when participants were in a hyperthermic state, therefore indicating failures must have occurred in the synaptic transmission at the neuromuscular junction level. Further the extra force produced by the superimposed twitch implies the sarcolemma has greater opportunity to depolarize and α-motoneurons had the possibility of discharging at a greater rate, therefore suggesting some level of peripheral failure may have existed at the spinal level. Overall, head cooling had limited beneficial effect on neuromuscular function despite core, tympanic and head temperature all significantly decreasing. In accordance with previous findings (Morrison et al., 2004; Thomas et al., 2006) improvements were only seen once core temperature returned to baseline.

2.7.2. Transcranial magnetic stimulation

Todd et al (2005) utilised TMS in conjunction with heat and neuromuscular performance in order to identify the site of central fatigue in the elbow flexor muscles. This study used a passive heating protocol involving participants submerging themselves in a hot bath in order to induce an increment of core temperature to 39.5˚C. TMS and electrical stimulation were elicited separately during contractions of varying intensities, followed by a 2 min sustained contraction. This contraction protocol was repeated whilst a superimposed twitch was induced. Hyperthermia resulted in a decrease in both brief and sustained MVC torque. However, the reduction in torque during brief contractions was small (93.4 % vs 95.7%) and no significant decrease was seen in voluntary activation during brief contractions, in agreement with previous findings (Racinais et al., 2008). This provides support for the finding that the rate of motor unit discharge can be increased sufficiently to maintain voluntary activation during brief contractions. Contrastingly sustained contraction greatly decreased individuals' ability to drive the muscle in the heat, as the superimposed twitch amplitude during cortical stimulation increased by 50%, and MVC declined dramatically. This implies stimulated axons were working sub optimally, either via submaximal levels of recruitment or discharge. TMS allowed researchers to confidently declare that the decrease in voluntary activation is a result of a change at or above the cortical output, however excitability appeared unchanged in response to hyperthermia according to the EMG responses. Therefore although there is additional cortical output available, central fatigue resulted from descending voluntary drive failing to deal with the changes in muscle properties. Therefore a certain amount of the decrement in voluntary drive whilst in a hyperthermic state is as a result of a change at or above the level of motor cortical output, with no change in motor cortical excitability or intracortical inhibition, shown via EMG activity.

A more recent study by Ross et al (2012) utilised a passive heating protocol with TMS to assess changes in corticomotor function as a result of progressive heating. Similar to Thomas et al (2006) and Morrison et al (2004) a water perfused suit was utilised in order to manipulate body temperature. Changes were assessed at every 0.5 º C increment in core temperature, both at rest and during brief contractions of the knee extensor muscles at varying intensities. This study was the first to show that this method of passive heating resulted in a reduction in voluntary drive as a result of impairments in output at or above the level of the motor cortex when temperature increase was at or greater than 1 º C. However,

this study only assessed changes in one of the knee extensor muscles, the tibialis anterior, therefore there is scope for future research to assess changes in all the knee extensor muscles and the knee flexor muscles. Moreover, this study utilised the water perfused suit to heat all active muscles, therefore it may be potent to assess the comparative differences to heating and cooling different areas of the body.

The use of passive heating prevents cardiovascular strain associated with exercise induced hyperthermia, hence it can be said with considerable confidence that the change in neuromuscular function is a result of temperature changes. When utilising exercise induced hyperthermia additional metabolic, thermoregulatory and cardiovascular demands fluctuate depending on the type of exercise used (Todd et al., 2005). Hence, this must be considered.

2.8. Neuromuscular fatigue, exercise and the heat

Table 2.3 details the key findings from the literature assessing the influence of exercise in the heat on neuromuscular function. It is widely understood that exercise performance is influenced by changes in environmental temperatures; as a result of the influence such changes have on muscle temperature, skin temperature, core temperature, as well as perceptual changes which may influence motivation (Nybo & Nielsen, 2001a; Nybo & Nielsen, 2001b). The intensity and duration of both heating and exercise will determine the changes in performance observed, whether beneficial or detrimental. For example, warm muscle temperature has proven beneficial for sprint performance, whereas prolonged heating has proven to be detrimental for endurance performance as a result of changes in core temperature (Drust, Rasmussen, Mohr, Nielsen, & Nybo, 2005). The following section will review the literature where exercise and heat stress have been combined in order to underpin the mechanisms causing alterations in exercise performance. The use of peripheral nerve stimulation and TMS will be reviewed separately.

A number of studies have utilised TMS to assess central fatigue in response to exercise (Goodall et al., 2012; Ross et al., 2007; Ross et al., 2010; Sidhu et al., 2009; Verin et al., 2004), however at present combining TMS with exercise in the heat has received less attention. Central fatigue is thought to be affected via exercise in the heat as a result of the increased speed of both muscle contraction and relaxation in response to hyperthermia, therefore influencing motor-unit firing frequency and hence voluntary activation (Todd et al.,

Table 2. 2 Details of the current literature showing the effect of heat on neuromuscular function.

Study	Participants	Heat exposure/protocol	Neuromuscular measures	Findings	Notes
Morrison et al (2004)	22 males	35 º C (liquid conditioning garment to achieve Tc of 39.5 º C, prior to cooling). During cooling chamber was exposed to external temperatures of ~ 22º C.	Peripheral VA was measured at 0.5 º C increments in Tc, and following cooling.	Peripheral VA↓ by 11% and MVC progressively decreased, reaching 13%↓ in the heat. Skin Cooling did not counteract this effect.	Highlights core temperature > skin temperature as a key determinant of neuromuscular function.
Thomas et al (2006)	7 men & 2 woman	32 º C & 15% Rh (liquid conditioning garment @ 50 º C & 10 º C during cooling). One leg was kept thermoneutral throughout.	Peripheral VA of the plantar flexor muscles in both legs.	VA↓ in the heat in both legs. Cooling had no influence on performance until Tc reached baseline levels.	Peripheral muscle temperature only marginally affects voluntary activation.
Racinais et al (2008)	11 males & 5 females	Hot 50 º C & 50% vs Control 20 º C & 40 % & Head cooling (-14 º C) in 50 º C & 50% Rh.	Peripheral VA of plantar flexors.	VA ↓ in the hot, however was unaffected in the head cooling condition. VA ↓ during sustained contractions in hot.	Passive hyperthermia causes peripheral failures in the plantar flexors.
Todd et al (2005)	5 females & 2 males	Water bath @ 39.5 º ± 1.4 C – 40.9 º C ± 0.6 º C. Tc increased to 39.5 º C (average time = 19 ± 7 min).	Cortical VA and EMG responses in elbow flexors.	Brief and sustained MVC torque ↓. No change in VA during brief contraction but ↓ during sustained contractions.	Rate of motor unit discharge can ↑ to maintain voluntary contraction during brief contractions.

Ross et al (2012)	6 males & 2 females	Water perfused suit @50± 1 º C	Peripheral VA and cortical VA measured at 0.5 º C ↑ in Tc.	MVC began to ↓ after Tc increased by 1 º C. Cortical VA ↓ AT 1.5 º C.	Failure of the motor cortex is evident above 1.0 º C ↑ in Tc. May be a result of the hyperventilatory response to heating, causing hypocapnia, and influencing cortical output.

Tc = Core temperature, Rh = Relative humidity, VA = voluntary activation, EMG = electromyography, MVC = maximal voluntary contraction.

2005). However, highlighting the site in the brain to muscle pathway causing changes in voluntary activation requires the assessment of voluntary activation at a cortical level.

2.8.1. Peripheral nerve stimulation

Over the past two decades, the impact of hyperthermia on performance and more specifically, neuromuscular function, both alone and in conjunction with exercise has been given considerable interest. However, at present the majority of research involving exercise in conjunction with hyperthermia has utilised peripheral nerve stimulation in order to assess neuromuscular function at the site of the muscle, rather than upstream in the pathway. Nybo and Nielsen (2001a) were the first study to identify there was a central fatigue aspect contributing to hyperthermia induced fatigue during prolonged exercise in the heat. Nybo and Neilsen (2001a) also found that voluntary activation was unaffected following exercise for the first 5 s of a contraction, however after this point force began to decline during a sustained isometric contraction, at a greater rate in the hyperthermic state. This might suggest that if only brief contractions are required, they will be unaffected by hyperthermia, however sustained exercise has a more pronounced affect. This study allowed the effect of exercise in the heat on muscle function to be better understood, by assessing strength in both exercised and non-exercised muscles. Results suggested decrements in activation were not exercise specific.

The review by Racinais & Oska (2010) indicated the most influential factor in the study of exercise, hyperthermia and performance is exercise duration whilst exposed to heat. This review suggests performance lasting <1s to 30s may aid performance, whereas if exercise lasted more than 10 min with heat exposure, performance was negatively affected. Racinais & Oska (2010) also suggest that whether heat exposure results in no change or a decrease in neuromuscular performance may depend on the nature of the neuromuscular test, and whether the assessment was brief or sustained. Findings suggest that performance of a brief MVC following heat exposure may demonstrate no effect (Nybo & Nielsen, 2001a; Saboisky, Marino, Kay, & Cannon, 2003), whereas a sustained MVC may result in a more detrimental outcome.

The study by Saboisky et al (2003) exercised 13 participants to fatigue in the heat and assessed the central activation ratio (CAR) prior to and post exercise in both exercising and non-exercising muscles. The CAR is calculated using the difference between the force produced during an MVC and an MVC combined with a superimposed electrical stimulus. The main finding of this study was that the exercising muscles demonstrated a decrease in

their CAR, however the non-exercised muscles were unaffected. This implies the central nervous system (CNS) is able to differentiate between muscle groups and only reduce central drive to active muscles, hence preventing damage in these muscles. However, this finding contrasts a body of research (Gonzalez-Alonso et al., 1999; Nielsen et al., 1993; Nybo & Nielsen, 2001a) showing exercise in the heat reduces central drive to all muscles. This suggests that hyperthermia causes temperature-related reductions in central drive, rather than an exercise-specific reduction (Racinais & Oksa, 2010). Therefore, further exploration is required.

Team sports exercise is characterised by intermittent bouts of exercise of varying intensity and duration (Burke, 1997). Drust et al (2005) highlighted the reduction in cycling sprint performance when this type of exercise was performed in the heat. This study speculated that the decrements in performance seen were as a result of changes in central nervous system function, mediated by increases in core temperature. Therefore, despite the potential for improvements in high intensity exercise performance with increased muscle temperature, core temperature changes appear to override this mechanism. However, this study is limited by its lack of an ecologically valid exercise protocol, as well as not extensively investigating the changes in activation. Tennis (Periard et al., 2014a) and football (Girard, Nybo, Mohr, & Racinais, 2015; Nybo et al., 2013) simulations have provided information regarding the peripheral changes in the plantar flexors (Girard et al., 2015; Nybo et al., 2013; Periard et al., 2014a) and knee extensors (Periard et al, 2014a), when exercise is combined with a high external temperature. Each of these studies utilised the interpolated twitch technique using peripheral nerve stimulation, prior to and following a match. Collectively the findings demonstrate that a similar drop off in peripheral voluntary activation occurs in the plantar flexors following a match, however knee extensor voluntary activation has been shown to drop off to a larger extent in the heat (Periard et al, 2014a). This decrement may be as a result of a reduction in the drive from the motor cortex, however without the incorporation of transcranial magnetic stimulation, this cannot be established. The differing responses in the different muscle types could be explained by the higher percentage of type 2 muscle fibres in the knee extensors than the plantar flexors, causing a more prolonged recovery due to the higher recruitment threshold challenging the brain's ability to fully activate this muscle. Therefore, it is necessary to further investigate the phenomenon of intermittent exercise in the heat, particularly the knee extensors due to their importance for running, in order to assist a

more thorough understanding of the mechanisms influencing neuromuscular performance during intermittent running activity.

2.8.2. Transcranial magnetic stimulation

TMS in conjunction with exercise in the heat has only recently been used to assess changes in cortical function (Periard et al., 2014b). MVCs were carried out at intervals allowing for moderate (Tre 38.5 º C) and severe (Tre 39.5 º C) hyperthermia to be assessed. Brief and sustained MVC torque decreased in the extreme hyperthermic state in both passive and exercise induced hyperthermia. However during moderate hyperthermia, a decrease was only seen when hyperthermia was exercise induced. During sustained contractions the decrease in torque was greater in both exercise induced states than either passively-induced states. Following TMS stimulation muscle relaxation rate during the silent period increased in both exercise and passive hyperthermia, for brief and sustained contractions. This was further exacerbated as a result of severe hyperthermia, however with no lasting effect on muscle force, or voluntary activation suggesting the increase in rate of motor unit firing is adequate in order to overcome the increased relaxation rate, preventing any detriment to force. This study aimed to investigate whether exercise induced hyperthermia could diminish the increase in peak muscle relaxation rate, traditionally seen in response to passive hyperthermia, as a result of peripheral fatigue. However, voluntary muscle force production and cortical voluntary activation decreased to a similar extent in both conditions. Therefore this study found that relaxation rate was similar between the two conditions. Hence, the added peripheral fatigue associated with exercise induced hyperthermia did not prevent the increase in peak muscle relaxation rate. Further, the results from electrical stimulation and TMS suggest that a similar level of central fatigue occurred. However, the centrally mediated rate of activation appears sufficient to overcome even the largest increase in muscle relaxation rate, seen during severe hyperthermia which was passively induced.

The mechanisms associated with fatigue during exercise in the heat are not conclusive. However, a general consensus exists that sustained force production is impaired as a result of neural drive to the muscle decreasing (Nielsen & Nybo, 2003). Electroencephalogram (EEG) activity gradually decreases during hyperthermia, indicating significant changes in CNS activity on exposure to intense heats (Nybo & Nielsen, 2001a). Changes in EEG have been found in various brain areas, including the frontal motor areas, central motor cortex and the occipital cortex. Further, changes in brain temperature during exercise in the heat has the potential to significantly influence central activation (Deboer, 1998) as heat removal via the

jugular venous blood is negatively affected by hyperthermia, causing heat storage. Finally neurotransmitters, either via accumulation or depletion, can influence CNS fatigue during exercise in the heat. The existing changes seen in response to exercise in the heat could result in catastrophic outcome, hence the decrease in central drive may be a safety mechanism in order to maintain functioning within the various physiological systems (Nielsen & Nybo, 2003).

Goodall et al (2015b) assessed the supraspinal component to fatigue when constant load cycling was performed in the heat. This study found supraspinal fatigue was increased to a greater extent in the heat than remainder of fatigue measures. A number of mechanisms were suggested for this change, including elevated cerebral temperature, cerebral blood flow changes, and increases in hyperventilation. Ross et al (2012) similarly suggested that supraspinal fatigue was evident when core temperature rose by 1.5 º C and cerebral blood was reduced by approximately 18%. The findings from Goodall et al (2015b) suggest the excitability and responsiveness of the corticospinal tract did not contribute to the changes in supraspinal fatigue seen as the MEP/Mmax ratio was similar following exercise to that at baseline. This study also highlighted the time course of supraspinal fatigue in relation to exercise intensity. These findings suggest that short duration high intensity exercise (~10min) is adequate to prompt supraspinal fatigue.

2.9. Acclimation

In a number of the aforementioned studies the acclimation status of participants exposed to either passive hyperthermia (Morrison et al., 2004; Racinais et al., 2008; Todd et al., 2005) or exercise induced hyperthermia (Périard, Thompson, Caillaud, & Quaresima, 2013; Saboisky et al., 2003) is not mentioned. As a number of these studies take place in countries where ambient temperatures are relatively high, participants may be acclimatised to such a level that the stress imposed has less of an effect, potentially due to a slower rate of increase in core temperature (Nielsen et al, 1993). Consequently, results from such studies cannot be generalised to a population who are not acclimatised.

2.10. Conclusion

From the current research surrounding neuromuscular fatigue following intermittent exercise in the heat it appears a gap in the research exists in terms of where the exact site of failure exists. The current methodology used in the majority of studies assessing central fatigue in association with exercise in the heat utilise a superimposed/interpolated twitch technique using only electrical stimulation (Morrison et al., 2004; Nybo & Nielsen, 2001a; Thomas et

al., 2006). This technique clearly highlights there is suboptimal neuromuscular functioning following exercise in the heat, however cannot adequately assess the site of failure. As previous research into the effect of intermittent exercise alone on neuromuscular function has successfully identified the site of failure through TMS, suggests this is a possible area for further research in order to increase the mechanistic understanding of fatigue in the heat, hence providing greater practical application, and specific coping strategies as a result.

2.11. Cognitive function

Cognitive function refers to a range of functions and processes controlled by the brain which enables individuals to "perceive, evaluate, store, manipulate, and use information from external sources (i.e. our environment) and internal sources (experience, memory, concepts, thoughts), and to respond to this information" (Schmitt et al., 2005). Six main domains are incorporated in cognitive function, which can also be further divided into smaller domains; executive functions, memory functions, attention functions, perceptual functions, psychomotor function and language skills. However, there is some degree of overlap between the separate domains, therefore one function can affect the success of another. Hence, emphasising the importance of assessing cognitive function in a large range of domains in order to accurately estimate overall cognitive performance.

Cognitive performance is a product of internal and external stress causing an environment which either optimises or detracts from cognitive performance, largely thought to be as a result of its influence on physiological arousal. The loss of homeostasis within the body as a result of heat stress is widely known to incur significant decrements in cognitive performance (Færevik & Reinertsen, 2003; McMorris et al., 2006b; Simmons, Saxby, McGlone, & Jones, 2008), an important aspect of exercise performance and occupational health. However, stress may not always be limiting, with exercise stress often improving cognitive performance (Chang, Liu, Yu, & Lee, 2012a; Lambourne & Tomporowski, 2010). Hence the combination of exercise and heat stress has provided a complex phenomenon to investigate, however a necessary one due to the ever-growing number of sporting competitions/tournaments played in hot climates and vocations requiring workers to perform under these stressors.

2.12. Cognitive function and performance

Performance in sport relies on a variety of processes, not solely on physical ability. Psychological, tactical, technical and mental skills collectively determine overall performance (Starkes, 1987). A detriment to mental function can impact performance in a

Table 2. 3 Details of the key findings in the literature for the effects of exercise in the heat on neuromuscular function.

Study	Participants	Heat exposure/ Exercise protocol	Neuromuscular measures	Findings	Notes
Nybo & Nielsen (2001a)	14 endurance trained cyclists	TTE @ 60% VO_{2max} in the hot (40 º C) vs 1 h (18 º C)	Peripheral VA of knee extensors	Sustained MVC ↓ and sustained VA ↓. No effect on brief contraction.	Total muscle force is not affected by hyperthermia.
Periard et al (2014a)	12 Male tennis players	Simulated tennis match @ 36.8 ± 1.5 º C & 36.1 ± 11.3% Rh vs 21.8 ± 0.1 º C & 72.3 % Rh)	Peripheral VA of knee extensors.	Greater ↓ in knee extensor torque and VA in hot post-match and 24 h post-match. Knee extensor VA was lower throughout hot trial.	Lower limb fatigue was increased when exercise was in the heat.
Girard et al (2015)	17 Male football players	Simulated football match @ 43 º C & 12% Rh vs 21 º C & 55% Rh.	Peripheral VA of plantar flexors.	Tc = 39.2 vs 38.3 ˚ C. Similar drop off in PF VA in hot and moderate.	Similar neuromuscular changes.
Periard et al (2014b)	10 active males	60 % VO_{2max} @ 38 º C& 45% Rh vs passive @ 48 º C & 45% Rh.	Peripheral and cortical VA	Severe hyperthermia ↓brief MVC during passive and active hyperthermia. Rate of decline in force production was greater in severe exercise hyperthermia. Cortical VA ↓equally in passive and active hyperthermia.	Mod hyperthermia = Tc 38.5 º C and severe = 39.5 º C.
Goodall et al (2015b)	7 male endurance trained athletes	34 º C vs 18 º C & 20% Rh. Constant load cycling (range 7.1-15.2 min).	MVC, potentiated twitch, peripheral and cortical VA.	No significant difference in change in MVC or potentiated twitch. Greater ↓ in cortical VA in the heat, no difference peripheral VA.	Peak Tc = 38.36 ± 0.43 º C (hot) vs 37.86 ± 0.36 º C (mod). Similar profile of fatigue in both conditions, however greater supraspinal fatigue in the heat.

TTE = time to exhaustion, Rh = relative humidity, VA = voluntary activation, MVC = maximal voluntary contraction.

variety of sporting contexts, hence affecting the outcome of a game or competition. The physiological response to exercise, including the dopaminergic and serotonergic changes, are known influencers of cognitive function via their influence on arousal hence monitoring these changes may be a robust method of monitoring performance. Understanding the environmental limits associated with performance decrements and the physiological limit of performers ensures the protection of their mental skills and ultimately sporting performance. Once this is established protective strategies can be enforced to optimise cognitive performance.

2.13. Cognitive domains

Although 6 cognitive domains exist (perception, executive function, attention, memory, language and psychomotor function), this thesis will focus on specific domains and sub-domains related to sporting performance. The four major domains of interested are briefly described below.

Visual perception involves the identification and localisation of stimuli (Farah, 2003). Executive function refers to higher level functioning and can be defined as an ability to adaptively respond to a novel stimulus by developing new strategies in order to respond accurately and effectively (Strauss, Sherman & Spreen, 2006). Working memory refers to the ability to store specific information for a relatively short period of time (up to 2 min). This time is adequate to perform the necessary processes on this information prior to it being disregarded. Working memory also allows a certain amount of information to be stored prior to its storage within the long-term memory (Strauss, Sherman & Spreen, 2006). Attention allows the differentiation of relevant and irrelevant cues in order to modulate the response to a stimulus (Strauss, Sherman & Spreen, 2006), therefore attentional processes work together to filter information, hence selecting the information which will undergo processing (Banich, 2004).

2.14. Cerebral blood flow and cognitive function

Cerebral blood flow is defined as the volume of blood delivered to the brain in a set period of time (Cipolla, 2009). The brain has a high metabolic demand, utilising 20% of the body's available oxygen (Cipolla, 2009). Alterations in cerebral blood flow regulate the delivery of nutrients to the brain in order to mediate function, and the dilation of upstream vessels allows more specific allocation of resources to brain regions with a greater demand. Therefore, fluctuations in blood flow to the brain can influence its ability to process and respond to stimuli, impacting cognition.

2.15. Blood parameters and their influences on cognitive performance

Fluctuations in several stress hormones have been related to changes in cognitive performance (Liebermann et al., 2005; McMorris et al., 2006b). McMorris et al (2006b) studied the effects of wearing PVC clothing whilst intermittently exercising at a low intensity in the heat (36℃ and 75% Rh). Increases in core temperature resulted in an increase in Cortisol, 5-HT, adrenaline and noradrenaline concentration. This study also saw a decrease in random movement generation, a task involving executive function, therefore the increases in these hormones could explain alterations in executive function (previously described in section 2.13). Similarly, Lieberman et al (2005) also saw an increase in cortisol concentration in response to exercise in the heat, which correlated strongly with decrements in cognitive function.

2.15.1. Brain derived neurotrophic factor

Brain derived neurotrophic factor (BDNF) has been associated with improvements in cognitive function following chronic exercise programmes (Egan et al., 2003; Erickson et al., 2009; Grassi-Oliveira, Stein, Lopes, Teixeira, & Bauer, 2008; Rex et al., 2006). However more recently has been shown to increase in response to a single bout of exercise (Griffin et al., 2011) showing positive associations with memory function (Lee et al., 2014). BDNF can cross the blood-brain barrier, and increase the permeability of the blood-brain barrier, which is likely to enhance cognitive function via the actions of S100B, as this indicates that there has been disruption to the blood-brain barrier (Lee et al., 2014). Therefore, assessing changes in serum and plasma BDNF may provide understanding regarding changes in cognitive function in response to exercise in the heat.

2.15.2. Adrenaline and Noradrenaline

Heightened arousal has been commonly cited as a contributor to changes in cognitive function, in response to stress, which has been linked to the inverted-U hypothesis (Yerkes & Dodson, 1908) and Easterbrook's cue utilisation theory (Easterbrook, 1959). Increases in adrenaline as a result of the increased central nervous system activation with arousal has been linked with improved cognitive performance (Péronnet & Szabo, 1993), hence assessing the changes in this hormone may provide a mechanistic explanation for changes in cognitive performance. Further, training has been shown to enable increased sympathetic nervous system activity, resulting in higher levels of plasma adrenaline and noradrenaline at the same relative exercise training. This provides explanation for improved cognitive performance in trained individuals over untrained in response to exercise (Greiwe et al., 1999), however also

highlights the close relationship which exists between adrenaline concentration and cognitive function (Brisswalter, Collardeau, & René, 2002).

2.15.3. Cathepsin B

Cathepsin B is a muscle secretory factor which is known to influence brain health and plasticity (Moon et al., 2016). How cathepsin B responds to exercise and heat stress has not been researched to date, however emerging research has shown that cathepsin B increases in response to exercise (Moon et al., 2016). This study also suggests that cathepsin B is a mediator of the cognitive responses to exercise. As a relatively novel mediator of cognitive performance, it is necessary to investigate how this secretary factor may contribute to the cognitive response to both heat and exercise.

2.16. Heat and Cognitive Performance

Table 2.4 details the key findings from the literature assessing the influence of heat on cognitive function.

A lack of uniformity in the methodologies used within the passive heating literature exists, including the amount of heat stress, the method used to induce hyperthermia, the complexity and duration of cognitive tasks, the duration of heat exposure and the skill level of participants. This has hindered a consensus being established with regards to the impact of isolated heat stress (e.g. in the absence of exercise stress) on cognition (Gaoua, 2010). Increasing levels of stress can decrease an individual's scope to process information involved in the specific cognitive task. However, an optimal level of stress can actually result in a focusing of attention and improved performance. Hence, variability affects comparability between studies. A number of studies have shown varying responses to heat stress, including a positive effect on attention (Lee et al., 2014; Schlader et al., 2015; Simmons et al., 2008) and working memory (Bandelow et al., 2010; Lee et al., 2014). However a number of studies have also reported a negative effect on working memory capacity (Gaoua et al., 2011; Liu et al., 2013; Racinais et al., 2008). Whilst some studies show no effect on perception (Gaoua et al., 2011) and aspects of attention (Sun et al., 2013).

An elevation in core temperature above the homeostatic range (i.e. > 1 - 1.5 º C increase) has been highlighted throughout the research as a mediator of the heat induced effects on cognitive function, detrimentally influencing working memory (Lieberman et al., 2005; Morley et al., 2012), executive function (Schlader et al., 2015) and attention (Lieberman et al., 2005; Schlader et al., 2015; Simmons et al., 2008). However, the perceptual responses and

changes in skin temperature associated with passive heating protocols (Gaoua, Grantham, Racinais, & El Massioui, 2012), can also influence cognition. For example, changes in skin temperature (Racinais et al., 2008) and short duration exposure to heat (Ramsey & Kwon, 1992) can influence memory, regardless of their effect on core temperature. This was shown by Racinais et al (2008) where head cooling did not alter core temperature, however the change in skin temperature and resulting alteration in perceptual feelings attenuated the decrement in working memory seen in response to heating.

The influence of skin temperature on cognitive function may be due to an alliesthial effect, closely related to perceptual feelings (Cabanac, 1971). However, this response is not consistent across all cognitive tasks with a task dependant response to passive heat exposure and cognitive function demonstrated, often determined by the complexity of the task (Gaoua et al., 2011; Hancock, 1989). Figure 2.6 provides a diagrammatic explanation of how stress interacts with our ability to adapt, and therefore how stress influences cognitive performance. Complex tasks have generally demonstrated greater vulnerability to the effects of heat due to prior draining of neural resources (Hancock, 1989), whilst simple task performance can be maintained. However, improved reaction times have also been seen with passive hyperthermia, due to enhanced nerve conduction velocities (van den Heuvel, Anne MJ, Haberley, Hoyle, Taylor, & Croft, 2017), with a reduced ability to select the correct responses typically accompanying this, thus eliciting a speed-accuracy trade-off. However, a comprehensive assessment of the effects of passive heat exposure across a range of domains of cognitive function is lacking in the literature.

Simmons et al (2008) isolated the effects of both increasing skin and core temperature via passive hyperthermia (heating at 45 º C and 50 % Rh) on cognitive performance. A battery of cognitive tests were used and 3 conditions enforced in order to investigate the varying effects; low skin/low core; high skin/low core; and high skin/high core. Lower skin temperature was achieved via head and neck cooling. High core and skin temperature resulted in an improvement in reaction time in tasks assessing attention, however a decrease in accuracy. Although the findings imply that heat related fatigue can be attributed to changes in skin temperature and cardiovascular strain, the results suggest core temperature must exceed compensatory levels to incur a significant decrement, as a rise in skin temperature alone had a limited effect on cognition. The facilitation of reaction time has been linked to levels of arousal, associated with changes in thermoregulatory centres (Hancock & Vasmatzidis, 2003).

Improvements in reaction time during and following passive heat stress has been related to improvements in conduction velocity.

Faervik and Reinersten (2003) found that performance was unaltered until a significant change in deep body temperature was experienced. When core temperature increased by 1.2 º C, following 3 h passive heat exposure (40 º C, 19 % Rh) whilst wearing protective flight clothing, a decrease in accuracy on a vigilance test using the Vienna test battery was seen. Similarly, McMorris et al (2006b) found increases in core temperature resulted in impaired performance in random movement generation tasks (2 h at 36 º C, 75% Rh, 20 min cycle at 100W to initiate heat production). However, tasks controlled via other neurological pathways, including verbal and special recall tasks, were unaffected. Hence, central executive tasks, which require the deciphering of potentially incongruent responses and have a large contribution from the prefrontal cortex as shown in fMRI data from Liu et al (2013), are negatively affected. This implies various pathways have differential levels of demand placed upon them in response to heat exposure, hence certain tasks can be maintained whilst others may demonstrate a decrement.

Reducing cerebral temperature via head cooling has been shown to improve thermal comfort (Simmons et al., 2008), despite the maintenance of a high core temperature. This change may reduce the attentional resources lost in response to heat stress, aiding cognitive performance. A study by Racinais, Gaoua & Grantham (2008) manipulated head temperature following the attainment of whole body hyperthermia, utilising head cooling. The heating protocol involved participants walking on a treadmill for ~ 15 min before sitting for 45 min at 50 º C and 50 % Rh, therefore incorporating the confounding effect of low intensity exercise. This study found passive hyperthermia impaired working memory and visual recognition. However choice reaction time was unaffected, potentially as a result of improved nerve conduction velocity (Cian, Barraud, Melin, & Raphel, 2001; McMorris et al., 2006b). The impairment of working memory was attenuated in response to head cooling, returning to control values. Visual recognition also improved however not to the same extent. Working memory relies on frontal lobe control, whereas visual recognition is controlled by temporal and medial temporal areas of the brain, suggesting head cooling selectively preserves function in specific brain regions. However, Simmons et al (2008) found accuracy on attention and executive function tasks remained supressed in response to head and neck cooling despite cardiovascular and heat related fatigue significantly improving. This may be a result of different domains being tested,

however these inconsistencies suggest the mechanisms involved in cognitive performance changes during heat exposure require further investigation.

Research demonstrating a change in cognitive function in the absence of core temperature changes also exists, highlighting the perceptual contributions to this aspect of function. Gaoua et al (2011) found that a decrement in short term memory as a result of an increase in core temperature can be counteracted by the application of cold pack to reduce skin temperature, while core temperature remains high. This highlights the perceptual contribution to changes in cognitive function in a specific domain, suggesting attention can be refocused when participants feel cooler despite their body temperature not changing.

Hocking, Silberstein, Lau, Stough & Roberts (2001) utilised a functional brain imaging technique in order to assess electrical changes in the brain occurring during heat stress and cognitive performance. The group with higher core temperatures had increased amplitude of steady state visually evoked potentials, however a decrement in latency. This overall finding suggests that either participants are exerting more effort to maintain cognitive performance, or they are utilising a greater proportion of their neural resources. This response was greater in the frontal regions of the brain during the spatial working memory task, whereas the occipito-parietal regions were activated to a greater extent during the vigilance task. The alterations in brain activity in response to heating suggests individuals increase their utilisation of neural resources in order to perform in these conditions, and in response to specific task requirements. This implies some level of neural "reserve" exists that can be accessed during stress, and performance decrements occur when this reserve is drained. However, this study used a small number of participants (n = 11) and only tested cognitive function over a small range of domains. Hence, these findings cannot be used as a definitive conclusion regarding brain activity in the heat.

Liu et al (2013) also assessed brain activation, utilising functional magnetic resonance imaging, whilst participants completed tasks within the attention network, looking at alerting, orienting and executive function. The brain was able to increase activation in certain regions (superior frontal gyrus and temporal lobe) whilst depressing activity within others in order to maintain performance. Executive function showed the greatest decrement following passive heat exposure, which was shown to be a result of alterations in activation in the prefrontal cortex. This was only seen on the complex level of tests, suggesting as stimuli become more conflicting, they influence the brain's ability to effectively redistribute resources. The ability

to redistribute resources to maintain function within a specific domain is known as the hypofrontality theory (Hocking et al., 2001) and may explain the differing cognitive responses to different heating strategies, determined by whether resources can be adequately redistributed or not.

2.16.1. Heat stress and cerebral blood flow

As previously highlighted, tight regulation of cerebral blood flow is central to the maintenance of cognitive performance. Cerebral blood flow has been shown to decrease in response to heat stress, as a result of alkalosis via respiratory changes (Bain, Nybo, & Ainslie, 2015). Reducing cerebral blood flow limits convective heat loss at the brain, resulting in heat storage and an increase in brain temperature, which is exacerbated by the increase in metabolic heat gain. This response to heat stress can limit cerebral oxygenation and hinder nutrient availability at the brain, providing a mechanism for altered cognitive function in response to extreme heat stress. As blood flow becomes limiting during hyperthermia, it is essential for cerebral perfusion to be maintained and optimised in order to maintain function (Ogoh et al., 2014), as when perfusion is reduced simultaneously, function will be negatively affected. Cerebral blood flow is influenced by cerebral autoregulation, which is the intrinsic processes that allow the maintenance of cerebral blood flow to occur regardless of blood pressure changes (Robertson & Marino, 2017). Cerebral blood flow is also influenced by carbon dioxide concentrations (Ogoh et al., 2014), which correlate with changes in respiration. However, the carbon dioxide reactivity has been shown to differ in different regions of the brain, causing varied redistribution (Ogoh et al., 2014). This may help explain the variation in cognitive results across the cognitive domains, whilst potentially offering the advantage of maintaining autonomic nervous system function.

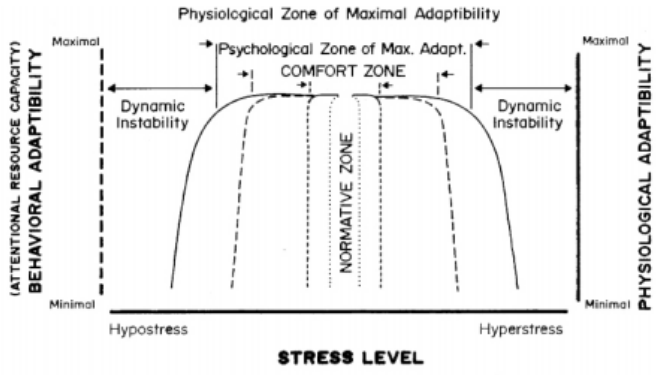


Figure 2. 6 The extended-U model, highlighting the influence of stress on cognitive performance. Copied from Hancock (1989).

Table 2. 4 Shows the key findings of existing literature regarding the influence of heat on cognition.

Study	Participants	Heat exposure/protocol	Cognitive measures	Findings	Notes
Hancock (1983)	6 males, 6 females.	Control, Placebo (unheated helmet), heat (heated helmet).	Simple mental addition task.	↑ no. of additions attempted in heat. ↔ in the no. of errors.	↑in Tc and Tsk in heat. Only Tsk was higher than placebo in control group.
Færevik & Eidsmo Reinertsen, (2003)	8 males.	3 h at 0 º C and 80% Rh, 23 º C at 63% Rh and 40 º C at 19% Rh. All whilst wearing protective aircrew clothing.	Vigilance test and DG test *(*Vienne determination unit test*)*.	No change in DG test. ↓ in vigilance test performance at 40 º C vs 23 º C.	↑Tc, ↑Tsk, ↑HR, ↑Body water loss at 40 º C vs 23 º C. Therefore heat stress correlates with performance↓.
Chase et al (2005)	34 students	60 min in either 20 º C or 35 º C.	Dual visual task was a shared attention task.	Performance ↓ in the 35 º C condition.	Negative effects on dual task performance and attention allocation due to heat.
Racinais, Gaoua & Grantham (2008)	16 subjects.	2 h exposure to: control 20 ° C, hot 50 ° C and hot with head cooling where cold packs were applied.	Three cognitive tests measuring attention and two measuring memory were performed.	↓ in both memory tests in the hot condition. No change in simple attention tests. Head cooling maintained memory capacity but not visual memory.	Suggests hyperthermia results in a decrement in frontal lobe activity.
Simmons et al (2008)	6 males, 4 females.	Low Tsk/low Tc; high Tsk/low Tc; high Tsk/ high Tc. 45 º C and 50% Rh. First test done when skin temp↑, 2nd when core↑. Wearing cooled balaclava vs control.	Simple RT, digit vigilance, choice RT, rapid visual info processing, morse tapping task, self-perception of mood & alertness.	↑Tc resulted in faster RT but decreased accuracy.	HR↑ and SV↓ with ↑Tc. When Tc↑, HC ↓HR. ↑Tc and Tsk = ↓contentment, alertness &calmness, however HC prevented this.

Gaoua et al (2011)	16 subjects (male and female)	Control (20 º C, 40% Rh), hot (50 º C, 50% Rh) and hot with the head kept cool. 10-15 min walking and 45 min passive heating.	Attention and memory task performance.	Hyperthermia resulted in a↓in working memory but no effect on simple attentional processes. Head cooling only benefitted short term memory, hence tasks related to the frontal area of the brain.	The detrimental effect of hyperthermia and the beneficial effect of head cooling on cognitive function are task specific.
Schlader et al (2015)	14 males	Water perfused suit at 48 ° C to increase Tc 1 - 1.6 º C above baseline	Attention, memory, executive function	Reaction time improved in the heat in young adults for RVP, however no change in accuracy. No other effects of heat were demonstrated.	Minimal detrimental effect of passive heat exposure on cognitive function when enforced via a water perfuse suit.
Hocking et al (2001)	11 males	25ºC & 65% Rh, 35 º C & 65% Rh, 35 º C & 65% Rh with raised Tc.	Working memory and psychometric tests	Decrements in working memory, information retention and information processing.	Thermal strain altered electrical activity of the brain, suggestions an increase in utilisation of neural resources occurs alongside additional effort applied to maintain performance.
Liu et al (2013)	26 males	50 ° C & 40% Rh vs 20° C and 40% Rh. Water perfused suit worn throughout scanning (28 ° C vs 50° C).	Attention Network task	No effect on alerting or orienting tasks. Executive function tasks, however executive function performance was impaired in the heat.	The effectiveness of participants at resolving conflict on executive function tasks is negatively influenced. Whereas brain activity was altered in order to maintain task performance in alerting and orienting tasks.

Rh=relative humidity, Tc = core temperature, Tsk = skin temperature, HR = heart rate, RT = response time, SV =stroke volume, HC=head cooling.

2.17. Exercise and Cognitive performance

Table 2.5 details the key findings of the literature regarding the influence of exercise on cognitive function.

Exercise increases cerebral blood flow, optimising the delivery of oxygen and nutrients to the brain to enable optimal brain functioning (van den Heuvel, Anne MJ et al., 2017). However the distribution of neural resources can vary depending on the type, timing and mode of exercise, therefore exercise stress can have varying influence on different aspects of cognitive function.

An acute bout of exercise can have a positive effect on both mood and affect, increasing levels of arousal (Tomporowski, 2003). The inverted-U hypothesis was first introduced by Yerkes & Dodson, (1908) and despite receiving some criticism surrounding it's simplistic concept, remains widely used when discussing the impact of arousal on cognition (Hancock, Ross, & Szalma, 2007). This theory implies an optimal level of arousal allows only relevant cues to be processed through the narrowing of attention, however beyond this point attention may narrow too dramatically resulting in the omission of relevant cues, causing cognitive performance to decrease (Brisswalter et al., 2002; Hancock & Vasmatzidis, 2003; Schmitt et al., 2005). Increases in plasma levels of adrenaline and noradrenaline with exercise, due to alterations in central nervous system function increase levels of arousal (McMorris & Graydon, 1997), hence are likely mediators of the "inverted-U" relationship.

Various researchers have attempted to overcome the limitations associated with the inverted-U (Hancock et al., 2007) following criticism as a result of failing to recognise arousal to be multi-dimensional, inadequately explaining the theory and for the lack of conclusive support that exists, suggesting that it cannot be reliably tested. Näätänen (1973) believed the descending arm of the inverted-U could be prevented if individuals focused attention on task-relevant cues alone. This updated model suggests performance would continue to improve and prevent any dramatic impairment. Whereas, Hancock (1989) describes an "extended U", where a plateau region exists, and performance remains unchanged, prior to more drastic decrements at the highest arousal levels (Figure 2.6). Uncertainty clearly remains within the literature as to the exact mechanisms associated with changes in cognitive performance, however a general consensus exists whereby arousal is a key mediator of performance. The magnitude of increase in arousal that influences cognition, either beneficially or detrimentally is yet to be confirmed.

2.17.1. Exercise intensity

Exercise encapsulates a wide variety of modes, intensities and durations, all of which must be considered when ascertaining the impact of exercise on cognition. Exercise intensity is of particular importance as it has been closely related to changes in arousal. Kamijo et al (2004) found that arousal was at its highest during moderate intensity cycling, when compared to low and high intensity cycling, implying an inverted-U exists. However, a number of studies have found improvements in cognition during, and after high intensity exercise. Hogervorst et al (1996) identified the acute effects of an intense bout of exercise on choice reaction time, 3-choice reaction time and stimulus response incompatible reaction time tasks, a finger tapping task and a Stroop test prior to and following a fatiguing bout of exercise at 75% of VO_{2max} in endurance trained athletes. Improvements in speed were seen in the simple reaction time task, the stimulus response task and the colour word interference on the stroop test. Therefore suggesting an improvement in cognitive performance can result from intense exertion, in both simple and highly complex cognitive tasks.

The training status of participants is important to consider, as the highly trained nature of the participants in this study may have enabled greater motivation and effort input on the more difficult tasks, such as the Stroop test. Similarly, McMorris and Graydon (1997) found an improvement in simple tasks, speed of visual search and speed of information processing at exercise intensities of 70% and 100% VO_{2max} compared to rest. The use of trained participants in this study is also worth considering and may contribute to response seen. Nonetheless, as cognition improves beyond moderate intensities, this research contradicts the inverted-U hypothesis, instead supporting more recent explanations (Hancock, 1989).

2.17.2. Exercise duration

Similar to exercise intensity, variations in exercise duration influence physiological responses, such as heart rate and heat production (Grego et al., 2005), known moderators of cognitive performance. The meta-regression analysis by Lambourne & Tomporowski (2010) summarised the main findings regarding acute exercise and cognitive function, which suggest that performance was negatively affected during the first 20 min of exercise, however showing facilitation thereafter. This study also found a significant post-exercise improvement, irrespective of the type of exercise, compared to pre-exercise values, even in protocols inducing fatigue. However, with very few studies requiring participants to exercise for a period longer than 2 h, the topic of prolonged exercise and cognitive function was not assessed.

Table 2. 5 Shows the key findings of the current literature regarding the influence of exercise on cognition.

Study	Participants	Exercise protocol	Cognitive measure	Findings	Notes
Hogervorst et al, (1996)	15 males triathletes and cyclists.	Cycling time trial lasting approximately 1 h.	Stroop test, CRT, finger-tapping test.	Exercise improved speed on simple tasks (CRT) but complex tasks were unaffected or facilitated in some cases.	Suggests intense exercise does not detrimentally influence cognition.
Brisswalter et al (1997)	10 males middle distance runners, 10 sedentary males.	Cycling at 20, 40, 60 and 80% of Pmax.	Simple RT task pre, during and post exercise.	No effect of work load in either group, RT slowed in both groups during exercise. However, RT was always faster in runners.	Greater difference seen between groups when there was a greater difference in the energetic constraints.
McMorris & Graydon (1997)	12 males.	At rest and while exercising at 70% and 100% of maximum power output.	Speed of search, visual search, speed of decision making following ball detection, overall speed of decision and accuracy of decision.	Visual search (simple task), and improvement in overall speed of information processing at maximum power output.	Suggests exercise-induced arousal cannot be predicted or explained by inverted-U theories.
Kamijo et al, (2004a)	12 males.	Low, medium and high intensity cycling. EEG and EMG allowed P300 and no-go P300 to be measured.	Go/no-go reaction time task.	P300 amplitude decreased following HIE and increased following MIE, suggesting attention impairment. RT was unaffected at all intensities.	Differences in exercise intensity influence the intensity of processing requirements for both go and no-go responses.
Kamijo et al (2007)	13 males.	At baseline and following light (RPE:11), moderate (RPE:13), and hard	Flanker task. ERPS measured – looking at P3 component.	P3 amplitude increased across task conditions except hard cycling. RT were faster during exercise	When tasks were incongruent and required greater executive control, P3

		(RPE:15) cycling exercise.		compared to baseline.	latency changed.
McMorris et al (2008)	12 active males.	Cycling at 40% and 80% of Wmax.	4-choice response time test and random number generation.	Concentrations of catecholamine metabolites (MHPG and HVA) ↑ linearly with exercise intensity. There was no difference between the exercise only group and the exercise plus cognition group.	Results do not support the catecholamine hypothesis. Results suggest the relationship is more complex.
Audriffren et al (2009)	16 males.	Before, during and after 35 min at 90% of ventilatory threshold.	Random number generation task.	Results showed a shift to a less effortful strategy during exercise, influencing certain executive functions and specific inhibitory processes.	The change in strategy was more obvious in the first part of exercise than later in order to maintain optimal performance.
McMorris et al (2009)	24 males.	At rest and whilst exercising at 50% and 80% of maximum aerobic power.	Flanker task (central executive task).	RT on both congruent and incongruent tasks was slower at 80% than other conditions. Physiological stress must be high to have a detrimental influence.	Exercise must be at a high intensity to influence flanker task.
Lambourne & Tomporowski (2010)	11 females and 8 males.	Pre, during and post 40 min of cycling at 90% ventilatory threshold.	Sensory (critical flicker fusion) and executive (PASAT) processing.	PASAT scores unaffected. Sensory discrimination improved during exercise and returned to baseline upon completion.	Exercise induced arousal facilitates sensory processes involved in stimulus detection but does not influence the updating

					component of executive processing.
Yanagisawa et al (2010)	17 males and 3 females.	Cycle ergometer at 50% of a subject's peak oxygen uptake.	Stroop test and multichannel near-infrared spectroscopy.	Reaction time on the Stroop test improved with exercise.	Activation of the left dorsolateral prefrontal cortex was enhanced with exercise and coincided with improved cognition.
Labelle et al (2013)	37 (19 male and 18 female) participants.	3 bouts of constant intensity cycling at 40%, 60% and 80% of participant's PPO.	Stroop test (test completed during exercise)	No effect of 40% or 60% on cognition. Cognition deteriorated at 80% PPO.	In agreement with the transient hypofrontality theory. Intense exercise disrupts executive control.
Martins et al (2013)	24 active males.	Cycled at between 60-90 rpm, generating between 60 -180 Watts.	PASAT to test working memory (test completed during exercise)	Improvement in performance when the task was not too simple and stimuli were presented at a relatively fast rate.	Moderate intensity cycling improved working memory.

PPO= peak power output, RPE = rating of perceived exertion, RT = response time, Rh=relative humidity, Tc = core temperature, Tsk = skin temperature, HR = heart rate, , SV =stroke volume, HC = head cooling, CRT = choice reaction time, RPM =revolutions per minute, Wmax = watt max, CNS = central nervous system, Pmax = power max. HIE = high intensity exercise, MIE= moderate intensity exercise, CRT = choice reaction time, ERPS = event related potentials, PASAT= paced auditory serial addition task, MHPG = 3-methoxy 4 hydroxyphenylglycol, HVA = homovanillic acid, EMG = electromyography, EEG = electroencephalograph

Despite the suggestion by Lambourne & Tomporowski (2010) that increasing exercise duration past 20 min provided a favourable cognitive response, Etnier et al (1997) found no relationship between exercise duration and cognition. Therefore, the effects of exercise duration on cognition remain unclear and warrant further investigation.

Chang et al (2012) aimed to improve the meta-analysis by Lambourne & Tomporowski (2010) by testing potential moderators of performance. Overall, findings suggest exercise improved cognitive performance to some extent at any time point (during, immediately post or following recovery). In contrast to Lambourne and Tomporowski (2010), no negative effect was found during exercise. However, the remaining findings agree with Lambourne and Tomporowski (2010), as cognitive performance improved following exercise. When testing was completed post exercise, lower intensity exercise had a superior effect on performance. Improvements following exercise diminished if a prolonged recovery was administered, with findings suggesting an optimal time for testing to be 11-20 min post-exercise. In agreement with Brisswalter et al (2002), the meta-analysis found short duration exercise had a limited effect on performance during exercise. The benefits of exercise began to increase following 20 min of exercise, allowing physiological responses time to adjust. Findings also suggested that individuals with a low level of fitness demonstrated poorer responses to exercise, requiring a greater amount of resources during exercise, hence limiting the resources they have available for cognitive processes. This may have also been hindered by the superior motivational characteristics of trained athletes. However, generally acute exercise demonstrated significantly positive effects for all fitness levels.

2.18. Exercise in the heat and cognitive performance

Table 2.6 details the key findings from the literature assessing the influence of exercise in the heat on cognitive function.

The contradictory findings regarding both isolated exercise and heat stress, where exercise generally improves cognitive performance and heat negatively affects it, provides little insight into the potential effects if the two stressors were combined. Decrements in cognitive performance following exercise in the heat have been shown (Lieberman et al., 2005; Morley et al., 2012), however some studies have found either no effect (Parker et al., 2013) or an improvement in cognitive performance (Serwah & Marino, 2006).

2.18.1. Low intensity exercise

The majority of the literature at present focuses on low intensity exercise in the heat combined with heat stress (Morley et al., 2012). Morley et al (2012) analysed the effect of walking in a hot room in firefighting clothing and equipment, preventing evaporative heat loss, on cognitive function. A large battery of tests were conducted at varying intervals, including paced auditory serial addition test (PASSAT), repeated episimodic memory test (USC-REMT) and pscyhomotor vigilance test (PVT), hence assessing the domains of executive function, memory and psychomotor function. Immediately following exercise there was no effect on any of the domains, however after 1hr there was a significant decrease in both recall (memory function) and psychomotor vigilance. Heart rate, core temperature (~39 º C) and skin temperature all peaked at the end of exercise, therefore suggesting there was no clear association between these variables and cognitive function. Despite no significant difference in mean reaction time 120 min post exercise, the mean of the 10 slowest reactions times did show significance (P=0.04), where reaction time progressively increased as recovery continued. REMT scores also decreased 60 and 120 min post exercise. Conversely, PASSAT scores showed facilitation immediately and 30 min post exercise. However, short-term memory appeared unaffected at any time point during the study. This study suggests individuals should be given adequate recovery time to ensure cognitive functioning is restored prior to re-entering a hot environment, as findings suggest there is some amount of delayed onset decrement in cognitive function. The contrasting findings seen across the different domains highlights the complex nature of the brain's response to stress. However, the facilitation shown in the PASSAT test suggest the added heat stress is not adequate to overcome the beneficial effects of exercise within this domain (Pontifex, Hillman, Fernhall, Thompson, & Valentini, 2009).

The combined effect of heat stress and exercise is particularly relevant to individuals in military vocations, where an individual's safety is based on their ability to carry out various cognitive processes whilst exerting themselves in extreme external temperatures, for a prolonged period of time. Lieberman et al (2005) used a cohort of U.S army recruits to assess the impact of prolonged heat exposure during exercise on cognitive function. In addition to the confounding influence of sleep deprivation and hunger, in order to mimic the real world conditions, the participants were exposed to temperatures between 19 º C and 31 º C for a period of 53 hrs. Significant decrements were seen in vigilance, reaction time, attention, memory, and reasoning, with clear associations between cognitive function and cortisol

levels. Results effectively highlight the vulnerability of both simple and complex task performance under prolonged exertional heat stress. Although ad libitum water intake was encouraged, participants became dehydrated due to the nature of the trial. Preventing dehydration in this type of study would be difficult and would require a lab based study to effectively monitor hydration throughout. This study is one of the few to assess cognitive function over such a prolonged period of time whilst exposed to heat, however the practicality of carrying out such a prolonged test in a laboratory, in order to control for all variables, raises significant issues.

The use of low intensity protocols provides a valid example for those individuals exposed to vocational heat stress, however provides minimal practical application to performance in sports where cognition influences performance. A study by Jiménez-Pavón et al (2011) investigated the influence of a running bout at 60% $VO_{2max.}$ Participants ran for an average of 52.4 ± 7.6 min in 35 º C and 60% Rh, and completed a number of cognitive tests from the Vienna Test System Battery (simple reaction time, choice reaction time, multiple reaction time and rate of correct/incorrect reactions and peripheral vision reaction time, field of vision, left visual angle and right visual angle) prior to and following the exercise bout. The findings suggested running in the heat improved the speed of response in complex tasks despite impairing perception function, and left and right visual angles, however only right visual angle actually proved to be significant. This is one of the only studies to investigate peripheral visual perception, which has fundamental relevance to sport and activity (Helsen & Starkes, 1999). Accuracy did decrease as a result of the running bout, suggested to be a result of the impairment in visual perception. Despite this change in cognitive function, this study failed to isolate the effects of hydration and heat, therefore this result is a combined effect of both. Moreover, this study used a single-group design, failing to use a control group. This style of study is considered particularly weak as it fails to control for extraneous factors and can result in the overestimation of the true effects of exercise on cognitive performance (Lambourne & Tomporowski, 2010).

Conversely, a number of studies have emerged demonstrating no effect or an improvement in cognitive function following exercise in the heat. Serwah and Marino (2006) investigated the impact of 90 min of cycling at 31 º C and 63% Rh on choice reaction time (CRT). This study manipulated the level of hydration in participants in a crossover style study, however found no significant decrement as a result of either heat or hypohydration on cognitive performance, finding CRT improved as exercise progressed. Further, Parker et al (2013) found no effect of

active heating on cognitive function. Parker et al (2013) aimed to simulate trekking and found that in response to 90mins of low intensity exercise (40-45% VO_{2max}) at 35-38 º C heat, no effect on performance was seen in the Wisconsin card sorting test (executive function) or psychomotor vigilance test despite increases in skin temperature and heart rate. However, the core temperature achieved was similar to that of the control group, exercising in a temperate environment, despite the negative perceptual responses seen.

Very few studies have assessed the effect of high intensity exercise in the heat on cognitive function, which is more applicable to team sport performance. Lee et al (2014) investigated the influence of exercise-induced hyperthermia on cognitive function by incorporating high intensity long duration exercise (75 min run at 70% of VO_{2peak}) with high external temperature (30.2 ± 0.3 º C, 71 ± 2 % Rh). This study assessed symbol digit matching, search and memory, digit span and choice reaction time; hence assessing in the domains of working memory, executive function and sustained attention. Results highlighted an improvement in reaction time in the symbol digit matching test and the psychomotor vigilance test. The maximum span was also increased in the digit span test. Gastrointestinal temperature was significantly increased (39.5 ± 0.4 º C), however had decreased significantly by the end of the cognitive test battery (37.6 º C), which is below the threshold found by Gaoua et al (2011) (38.7 º C) to negatively affect cognitive performance. These findings suggest the beneficial effects of exercise can overcome the negative effects of heat stress if core temperature is given adequate time to recover. Hence, there is no delayed effect of heat stress. This study also implemented a neck cooling condition, whereby the neck was cooled using a collar in the initial 5 min of running and immediately following the running protocol and throughout cognitive testing. The neck cooling improved performance on the most complex level of the search and memory task. This finding might suggest that neck cooling is effective at minimising the cognitive load and allowing improved performance via influencing the pre-frontal cortex, temporal lobe and the parietal cortex, hence improving executive function.

2.18.2. Intermittent exercise

Performance during intermittent team sports require a considerable degree of skill and decision making, and undoubtedly has the greatest ecological relevance when considering the impact of exercise in the heat on cognitive performance (MacLeod, Cooper, Bandelow, Malcolm, & Sunderland, 2018). Hence, any detriment to cognition can have a significant effect on competitive performance; therefore the lack of research in this area is surprising. Bandelow et al (2010) is one of the only studies to assess the effects of hydration, heat stress

and cooling on cognitive function during a football match. One of the main findings of this study was that following exercise, high core temperatures were associated with a decrease in speed on all cognitive function tests, including a finger-tapping task (assessing fine motor control), visual sensitivity test (assessing visuo-motor reaction time) and the Sternberg test (assessing working memory). Decrements were seen across the range of psycho-motor tests and in visual processing. This suggests a range of cognitive domains are supressed as a result of exposure to heat during intermittent exercise. However, improvements in accuracy were seen with increasing core temperature. This study speculates changes may be a result of changes in dopaminergic and serotonergic output, changes in blood-brain barrier permeability and regional blood flow alterations in the brain. However, plasma glucose was also highlighted as a potential contributor to changes in cognitive function. The findings of this study highlight the changes in cognition which can occur in a real-life competitive team sports environment in the heat. However, this study assessed a large number of variables, hence there is clear scope to isolate the effects of heat and intermittent exercise and elaborate on the mechanisms causing change, and provide a more complete understanding across the domains of cognitive function. Similarly, MacLeod et al (2018) recently assessed the cognitive responses to hockey specific exercise in the heat, however this study focused on the impact of hypohydration, hence the isolated effect of heat and intermittent exercise still requires further investigation.

The link between cognitive function and skill performance in sport is not well established; despite studies highlighting one may exist in athletes for a number of years due to the importance of fine motor control, perception and decision making (Starkes, 1987). Sunderland & Nevill (2005) assessed the influence of intermittent exercise in the heat, using the Loughborough intermittent shuttle test, on hockey skill performance. Results showed skill performance was worse following exercise in the hot condition. The mechanisms associated with this change were not established; however, how these changes in skill performance relate to cognitive function is a key area for future research to understand how performance may be influenced in the heat.

2.19. Hydration and cognitive performance

Hypohydration is a consequence of exercise in the heat if an adequate volume of fluid is not consumed. A limitation of various aforementioned studies is that the results cannot be isolated to heat changes if hydration is not maintained. This confounds findings as an impairment in aspects of cognitive function have been found when hypohydration reaches 1-

2% of body mass loss (Cian et al., 2001; Ganio et al., 2011; Lieberman et al., 2005). Gopinathan, Pichan & Sharma (1988) found a strong association between levels of hypohydration and the degree of impairment (1%, 2%, 3%, and 4%) in cognitive performance. This study implies that the effect of hydration on cognitive performance resides in the diversion of attention, with the stressor (heat/hydration) competing for attention hence reducing the amount of attention focused on cognitive tasks, implicating performance. Cian et al (2001) also found cognitive performance was impaired for perceptual tasks and short-term memory. However, long-term memory, reaction time and tracking were unaffected. Nevertheless, these studies clearly demonstrate the potential for hypohydration to affect cognitive performance.

2.20. Acclimation and cognitive function

The physiological adaptations and benefits associated with heat acclimation are well known (Lorenzo, Halliwill, Sawka, & Minson, 2010; Nielsen et al., 1993; Sunderland, Morris, & Nevill, 2008), however how cognitive processes adapt from an acclimation protocol is less well known. A study by Radakovic et al (2007) found that cognitive responses following exertional heat stress in both acclimatised males soldiers showed no detriment to attentional processes, whereas the unaclimmatised group showed a decrement in performance. Hence, considering the acclimation status of participants is important when interpreting changes in cognitive function. However, more research is required in this area in order to fully understand the implications of acclimatising participants on their cognitive function.

2.21. Conclusion

The research presented in this chapter highlights the influence that heat, exercise and the two in combination can have on both cognitive and neuromuscular function. Both these aspects of function contribute significantly to overall performance in team sports, skill performance from a cognitive perspective, and physical output from a neuromuscular perspective. For neuromuscular function, the responses to team sport exercise have been established, yet a gap in the area clearly exists in the area of team sport exercise in the heat. Establishing the neuromuscular responses to this type of exercise in the heat will enable an understanding of the mechanisms contributing to physical fatigue in the heat to be established, enabling more specific protective strategies to be produced. Whereas for cognitive function, both heat and exercise have been investigated, presenting contradictory findings. The cognitive responses to heat and exercise have also been established, however intermittent exercise both in moderate and hot conditions is yet to be determined. Hence to provide a mechanistic explanation for

changes in skill performance, and management strategies to improve performance, across a team sports match, both in moderate and hot conditions, the changes in cognitive function must be established.

Table 2. 6. Shows the key findings of the current literature regarding the combined influence of exercise and heat stress on cognition.

Study	Participant	Protocol/heat exposure	Cognitive measure/ component examined	Findings	Performance variables/notes
McMorris et al (2006b)	8 recreationally active males.	PVC in heat chamber (36 º C and 75% Rh) vs temperate conditions. 2h Intermittent low intensity cycling.	RMG tasks, verbal and short term memory tests, mood states.	Heat- RMG↓post, no effect on verbal or spatial recall.	↑Tc, ↑body mass loss. Cortisol, 5-HT, adrenaline, noradrenaline↑ post heat.
Bandelow et al (2010)	20 male footballers.	Played football for 90 min in 34 º C and 64% Rh (approx.). Cognitive tests were used pre, during and post-match.	Visual sensitivity, Finger TappingTask, Visual/auditory WM, visuo-spatial WM.	Decrease in speed across all tests, however improvements in accuracy were seen.	Changes in Tc were associated with decrements in speed in all tests.
Caldwell et al (2011)	9 males.	Walking for 2.5h (1.5h @ 2.0km/h and 1h @ 4.0 km/h) wearing 1. Combat uniform and cloth hat. 2. Torso armour with cloth hat and uniform 3.Torso armour, helmet and uniform. 36 º C and 60% Rh.	Vigilance test, three-term reasoning, filtering (modification of the Stroop test), verbal working memory, divided attention, perceptual reaction time.	All tests unaffected.	Tc didn't reach levels associated with exertional heat stress. However, greater stress with more clothing.
Jimenez-Pavon et al (2011)	22 physically active men.	Ran for approximately 60 min (average of 52.4 +/- 7.6 min) in 35 º C and 60% Rh.	Simple RT, CRT, multiple RT, peripheral vision RT, field of vision, left visual angle and right visual angle.	Running in the heat improved the speed of response in complex tasks, & impaired right visual angle.	Accuracy did decrease by 62% in the heat, suggested to be a result of the impairment in vision.

Morley et al (2012)	Study 1 – 10 males Study 2 -14 male, 5 females.	Walking in firefighter clothing in 33-35 º C for up to 50 min at 4.5km/h. Study 2 – 20 min at 4.5km/h, 3min at 2.5km/h, 4 min rest, 3min at 2.5km/h, remainder at 4.5km/h.	PASAT @ 90 + 120 mins, Repeated episimodic memory test (USC-REMT) @ 60 + 120 mins, Pscyhomotor vigilance test (PVT) @ Pre + 30 + 60 + 90 + 120 mins.	No change immediately post exercise. USC-REMT + PVT↓ 1 hr and 2 hrs post. PASAT scores improved immediately and 30 mins post-exercise.	HR↑, Tc↑, Tsk↑. Results indicate need for recovery following exposure to high heats with adequate cooling and rehydration.
Parker et al (2013)	40 males and females.	90 min intermittent Treadmill walking whilst wearing clothing and a weighted backpack (20% of BW) in hot (35-38 º C) or temperate conditions (22-24 º C). 40-45% VO2max. No crossover.	Wisconsin card sorting test (executive function), PVT.	No effect on cognitive function tests.	Heat = ↑HR, ↑Tsk. Tc increased the same in both groups. Findings suggest without an increase in Tc, cognition is unaffected.

Rh=relative humidity, Tc = core temperature, Tsk = skin temperature, HR = heart rate, RT = response time, SV =stroke volume, HC = head cooling, PVC = polyvinyl chloride, RMG = random movement generation, CRT = choice reaction time, 5-HT =5-hydroxytryptamine , WM = working memory .

CHAPTER 3: GENERAL METHODS

3.1. Introduction

This chapter is focused on the procedures used in the studies described in chapters 4, 5, 6 and 7.

3.2. Participants

The participants for the studies described in chapters 4, 6 and 7 were all recruited voluntarily. The participants for the study described in chapter 5 were recruited as a result of being a part of the hockey club who expressed interest in the research. All participants volunteered for the study and had the opportunity to drop out at any point. Prior to partaking in each study, participants were provided with written and verbal information about the study and given the opportunity to ask any questions. A health screen was also completed for all studies to ensure participants were suitable to be included in the relevant study.

3.3. Preliminary measures

3.3.1. Ethics

Ethics for all experimental studies was gained (Appendix B) from the Nottingham Trent invasive ethical committee prior to data collection. Participants were anonymised, and all data was kept in accordance with the Data Protection Act.

3.3.2. Familiarisation

For each of the studies described in this thesis extensive familiarisation procedures were employed, the details of which are described within each experimental chapter. However, for each of the studies described in this thesis, height, body mass and a full verbal and practical run through of all aspects of the protocol was completed in the familiarisation sessions.

3.3.3. Height

Height was measured using a stadiometer (Seca 123, Seca Ltd). Participants removed their shoes and stood with their back facing the stadiometer, and their heels and the back of their head touching it. Participants took and held a deep breath whilst the headboard was lowered until it touched the top of the head, allowing height to be read off the stadiometer.

3.3.4. Body mass

Nude body mass was measured to the nearest 0.1kg (GFK 150 AEADAM digital scale, Vitech scientific Ltd). Participants entered a locked bathroom and measured their nude body mass in privacy, in order to later relay their body mass to the investigator.

3.3.5. Pre-trial restrictions

For all the studies described in this thesis a number of pre-trial restrictions were put in place. Participants were asked to avoid exercise and caffeine in the 24 h prior to each main trial, to prevent muscle fatigue influencing the neuromuscular results between trials. Caffeine is known to influence cognition and exercise performance (Hogervorst et al., 2008), therefore this was needed to be standardised. Participants were also asked to complete a 24 h food diary prior to their first main trial, which was to be repeated prior to the remaining trials. This was done to minimise metabolic fluctuations between trials, which may have simultaneously influenced blood parameters, many of which are known to influence cognition (McMorris et al., 2006).

3.4. Main trials

3.4.1. Cognitive Function

Cognitive testing was completed in the studies described in chapters 4, 5 and 7. The details of the tests and analyser are listed below. The speed and accuracy of a response to a stimulus during cognitive testing is used as an indicator of performance (Schmitt et al., 2005). In order to give an accurate estimation of cognitive function and the effect of the intervention it is important to select the correct tests that may be affected by the specific stressor, whilst not over-exerting participants by utilising too many tests, as fatigue and motivation have been found to influence results (Ackerman & Kanfer, 2009).

The cognitive test battery lasted approximately 15 min and was administered via a laptop computer (Thinkpad T450, Lenovo PC HK Limited, China). The four cognitive tests used were Visual search test, Stroop test, Corsi Blocks and Rapid Visual Information Processing (RVIP) and were completed in that order on each occasion. The reliability of the cognitive tests has been previously established. This laboratory have found the Stroop test and visual search test to be highly reliable in an adolescent population (Stroop test: ICC = 0.976, CV: 4.32%; Visual search test: ICC = 0.915, CV = 6.68%). However, Bandelow et al (2011) assessed the reliability of all of the cognitive tests presented in this thesis in an elderly population. The tests demonstrated varying levels of reliability (Visual search simple: r = 0.78; visual search complex: r = 0.78; Corsi blocks: r = 0.61; Stroop baseline: r = 0.85; Stroop complex: r = 0.89; RVIP response time: r = 0.53; RVIP accuracy: r = 0.85). It was deemed that the tests selected were appropriate and provided adequate reliability for the current thesis.

All cognitive tests for each trial were completed in the allocated condition. Participants wore noise-cancelling headphones in order to eradicate any distracting stimuli (Figure 3.1). Prior to each test, 3-6 practice stimuli were presented during which feedback was provided on the accuracy of the response. The purpose of these practice stimuli was to re-familiarise participants with the task and eradicate any learning effect, and the results for practice stimuli were not included in analysis.

3.4.1.1. Perception (Visual search test)

Perception and visual processing were assessed using the Visual Search test, as used by Cooper, Bandelow, Nute, Morris & Nevill (2015). The test consisted of two levels, each containing 21 stimuli. On the simple level of this test participants were required to respond to the appearance of a bold, solidly outlined green triangle. The complex level of the test was also made up of 21 stimuli, however required participants to respond to the appearance of a triangle shape made up of a number of dots. The background was comprised of green dots covering the screen, which were redrawn every 250 ms to induce the visual effect of a flickering background. For both levels of the test the stimuli appeared at randomised locations across the screen at variable intervals. Participants were instructed to press the space bar in response to the stimulus as quickly as possible on both test levels. This test examines how well participants can filter distracting information from their surroundings and interpret specific cues, utilising visual processing and perception. The response time between the presentation of a stimulus and the response was recorded as well as the proportion of the correct responses achieved.

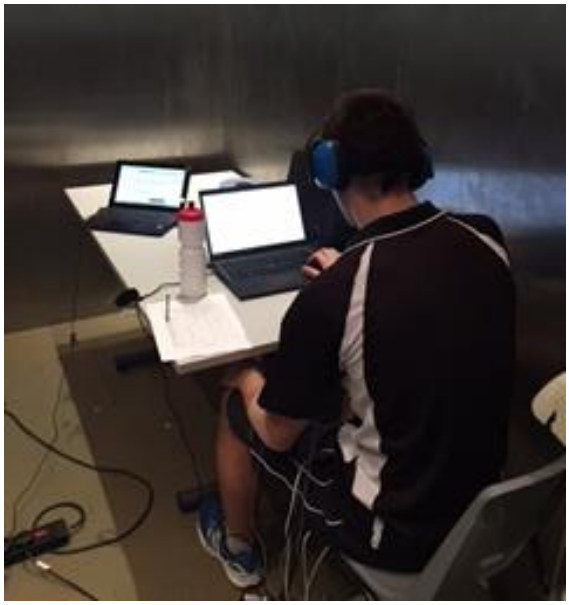


Figure 3. 1 Set up used for cognitive testing across the studies detailed in this thesis.

3.4.1.2. Executive Function (Stroop test)

The Stroop test is an executive function and selective attention task which measures frontal lobe function (Stroop, 1935) and the ability to suppress an automated response. The test is comprised of two levels, which have varying levels of interference. Each level involves a test word appearing in the centre of the screen, with a target word and a distractor either side of it. The target word's position was counterbalanced for the left and right side within each test level and the participant was asked to respond as quickly as possible, using the left and right arrow key, to identify the target word's position.

The simple level of the test had 20 stimuli and the complex level was made up of 40. On the simple level of the Stroop test, the target word was the word matching the word in the centre of the screen, with all words presented in white font. The colour interference complex level of the test required the participant to select the word which corresponds with the colour the word in the centre of the screen was written in, rather than the word itself (which was an incongruent colour). The inter-stimulus interval was 1 s, and choices remained on the screen until a response was selected. Response time between the presentation of the stimuli and the response was recorded and the proportion of correct responses was measured.

3.4.1.3. Working Memory (Corsi Blocks)

The Corsi Blocks test assessed visuo-spatial short term working memory (Corsi, 1972). A 3x3 grid filled the screen, where the squares randomly lit up. Participants were required to then replicate the order of the squares lighting up by clicking on the boxes. The sequence length started at three and with each correct response, the sequence got longer in length by

one unit. Where participants correctly recalled a sequence of 9 boxes, the grid increased in size to 4x4. Performance was determined by the mean of the 3 longest correctly remembered sequences (Cooper et al., 2015).

3.4.1.4. Attention (Rapid Visual Information Processing (RVIP))

The RVIP test assesses sustained attention as used by Hogervorst et al (2008). The test lasted 5 min and numbers 2-9 appear on the screen for the duration at 600 ms intervals, with 8 target sequences appearing per min. Participants were instructed to press the space bar each time a sequence of 3 odd or even numbers were shown e.g. "2-4-6", "3-5-7", "9-3-7", "8-4-2" and "4-6-8". Responses could be registered during the last digit sequence and the 1500 ms that followed. Response time from the presentation of the stimulus to the response was recorded, alongside the proportion of correct responses.

3.4.1.5. Data Analysis

The cognitive data (Stroop, visual search and RVIP) were analysed using R (www.r-project.org). Linear mixed effects models were used to analyse the data, with a random effect for each participant. Response time analyses were performed using the nlme package, which implements mixed effects models and produces t statistics. Accuracy analyses were performed with the lme4 package, to account for the binomial outcome data distribution, which produces z statistics. All analyses were conducted using a trial by session time interactions. Separate analyses for each test level on the Stroop test and the visual search test were completed to account for the varying cognitive demands of these tests (Miyake et al., 2000). Response times on all cognitive tests were log transformed to normalise the distribution, which demonstrated the right-hand skew expected of human response times. Minimum and maximum cut-offs were employed in order to exclude any responses which could be deemed anticipatory or delayed. Therefore, across the Visual Search and Stroop tests response times less than 100 ms were excluded and maximum cut off times were 1500 ms and 3000 ms, for the simple and complex levels, respectively. The minimum cut off time for RVIP was 200 ms and the maximum was 1500 ms. Only the response times of correct responses were used for response time analyses.

3.4.2. Mood Questionnaires

Mood questionnaires were completed prior to cognitive testing in the studies described in chapters 4, 6 and 7. Participants completed a shortened version of the Brunel Mood Scale (BRUMS) questionnaire (Terry, Lane, Lane, & Keohane, 1999) answering 24 items related to 6 aspects of mood; anger, confusion, depression, fatigue, tension and vigour. A scale of 1 to 5

was used to assess each of these items (where 1: “not at all”, 2 “a little”, 3: “moderately”, 4: “quite a lot”, 5: “extremely”), which enabled a score out of 20 to be calculated for each aspect of mood.

3.4.3. Neuromuscular Function

In the studies described in chapters 6 and 7 the following techniques were used to measure neuromuscular function.

3.4.3.1. Knee extension force

A custom-built dynamometer was used to measure knee extensor torque (Johnson et al., 2015). The participant was sat with hip and knee joints at 100˚(full extension=180˚). Participants were strapped to the chair across the chest and pelvis, in order to prevent displacement during contractions. The dominant leg was strapped to a strain gauge (615, Teda-Huntleigh, Herzliya, Israel), positioned perpendicular to the tibia, using an ankle cuff which was 2 cm proximal to the medial malleolus (Figure 3.2). The force signal was amplified (×1000) and sampled at 2000 Hz using an external A/D converter (1401; CED, Cambridge, UK), interfaced with a personal computer (PC) using Signal 5.08 software.

3.4.3.2. Electromyography (EMG)

Electromyographic signals were recorded form the vastus lateralis (VL), rectus femoris (RF), vastus medialis (VM) and biceps femoris (BF). Bipolar surface electrodes (2.5cm between-electrode distance; silver/silver chloride, 95mm^2 area, Ambu, Ballerup, Denmark) were attached to each muscle at distances based on percentages of thigh length measured from the knee joint space to greater trochanter: VL, 45%; RF, 55%; VM, 25%; BF, 50%. Electrodes were placed parallel to the assumed orientation of the muscle fibres and medio-laterally over the belly of the muscle. EMG signals were pre-amplified by active EMG leads (input impedance 100M, CMMR > 100 dB. Base gain 500, 1st order high pass filter set to 10 Hz; Noraxan, Scottsdale, U.S.A) connected in series to a custom-built junction box and subsequently to the same A/D converter and PC software that enabled synchronisation with the force data. The signals were sampled at 2000 Hz. EMG data was band-pass filtered in both directions between 20 and 450Hz using a fourth-order zero-lag Butterworth filter prior to analysis.

3.4.3.3. Electrical stimulation of the femoral nerve

Electrical stimuli were delivered via percutaneous stimulation over the femoral nerve. A cathode stimulation probe (1cm diameter,), complete with an anode (4 × 7cm carbon rubber

electrode, Electro Medcal Supplies), was coated in electrode gel and pressed into the femoral triangle to achieve stimulation. Square wave pulses, 100μs in duration were delivered via a constant current variable voltage stimulator (Model DS7AH, Digitimer, Ltd, Welwyn Garden City, UK), using a submaximal electrical current (approximately 40mA). The precise location of the femoral nerve was determined by the greatest twitch response to this current. In order to ensure supramaximal stimulation, the current was increased in a step-wise manner until the amplitude of twitch force and M-waves plateaued. The current at this plateau was multiplied by 1.2 and used for the remainder of the testing (Buckthorpe et al., 2012).

3.4.3.4. Transcranial magnetic stimulation of the motor cortex

TMS of the motor cortex was achieved using a double-cone coil (11cm diameter) and delivered via a magnetic stimulator (Magstim 200^2, Magstim Company Ltd, Dyfed, UK). The coil was held manually over the motor cortex on the side contralateral to the involved leg and oriented in order to induce a posterior-to-anterior current in the brain. The optimal coil position on the scalp (hotspot) to maximally stimulate the knee extensor muscles was located by initiating stimulation during submaximal voluntary contractions (20% MVF), with a stimulation intensity of approximately 80% of maximum output (MO). The optimal position was defined as the one producing the greatest SIT force, and was marked using indelible ink.

The resting motor threshold was determined by decreasing the stimulation intensity in 1% increments, starting at 100% of maximum output until the MEP amplitude of the knee extensors exceeded 50 μV in approximately 50% of stimuli across 5 stimuli. The active motor threshold was determined whilst the participant performed 20% MVF contraction, and by decreasing the stimulation intensity in 1% increments starting at the resting motor threshold. The active motor threshold was defined as the point where MEP amplitude of the knee extensors was just over 200μV in 50% of stimuli. The stimulus intensity at active motor threshold was multiplied by 1.4 and used throughout the remainder of the testing.

3.4.3.5. Preliminary measures

Once seated, the skin was prepared and EMG electrodes applied. The femoral nerve was located at rest and stimulation thresholds determined. Eight submaximal voluntary warm-up contractions were completed at 55%, 70%, 85% and 90% of self-determined MVF.

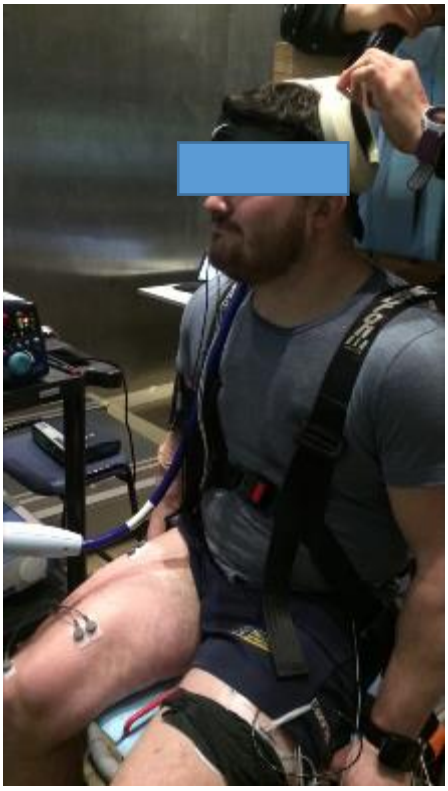


Figure 3. 2 The set-up for neuromuscular function testing during the studies described in chapters 6 and 7. In this example the participant is undergoing transcranial magnetic stimulation.

3.4.3.6. Maximal voluntary force

Prior to and following the 6 sets of voluntary contractions, 2 MVCs of the knee extensors, each separated by 20 s rest, were performed. Participants were instructed to contract as hard as possible for 3 s, whilst being provided with biofeedback and encouragement. This process, as well as changes in estimated resting twitch (ERT), allowed the assessment of whether the protocol had resulted in significant levels of fatigue, defined as a reduction in MVF.

Hotspot location was then determined prior to establishing motor threshold during active contraction (20% of MVF) and at rest.

3.4.3.7. Voluntary contractions with electrical stimulation and transcranial magnetic stimulation

Participants completed 6 sets of voluntary contractions, lasting approximately 3 s and separated by 10 s (Figure 1). Three sets (sets 1, 3 and 5) consisted of a single contraction at 100%, 85%, 70% and 55% MVF with magnetic stimulation superimposed on the peak of the contraction. The remaining 3 sets (sets 2, 4 and 6) consisted of one contraction at 100% MVF with electrical stimulation superimposed on the peak of the contraction and immediately after

the contraction (Figure 3.3). Each set was separated from the preceding set by 120s. Measurements included volitional force at the onset and peak of the superimposed twitch, in order to calculate twitch magnitude, and peak to peak amplitudes of the MEP (TMS) and M-wave (electrical stimulation). An average value amplitude was calculated across the 3 sets for each session. These values were normalised to Mmax, which is defined as the maximum M-wave potential seen within the session (Gandevia, 2001).

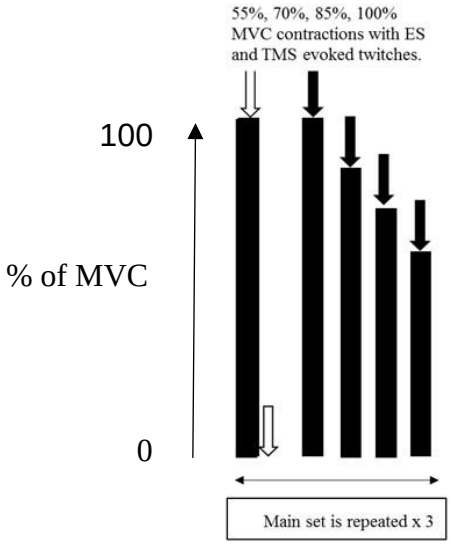


Figure 3. 3. Protocol diagram highlighting the order of contractions for the determination of cortical and peripheral voluntary activation.

3.4.3.8. Data and statistical analyses

Voluntary activation

Peripheral voluntary activation was quantified by measurement of the torque responses to electrical stimulation of the femoral nerve. SIT was determined by placing a cursor at the onset of the twitch and a cursor at the peak of the twitch force, the force at the onset of the twitch was then taken from the peak twitch force. RT was determined using the same method. These values were substituted into the following equation (Morrison et al., 2004):

$$\text{Peripheral voluntary activation} = 1 - (\text{SIT}/\text{RT}) * 100$$

M1 cortical voluntary activation was quantified by measurement of torque responses to magnetic stimulation of the motor cortex. Estimated resting twitch (ERT) was established by extrapolating the linear regression for SIT against torque, ranging between 55% and 100% of MVC. SIT for M1 cortical voluntary activation was determined using the same method as

above, utilising the TMS response during a maximal contraction (100% MVF). The y-intercept was deemed the ERT. Voluntary activation was determined using the equation (Todd et al., 2003):

$$\text{Cortical voluntary activation} = 1 - (\text{SIT}/\text{ERT}) * 100$$

Quadriceps MEP values, normalised to Mmax, were averaged across contraction sets and sessions. Mean and SDs of all MEP and M-wave amplitudes (p-p) were calculated from each six-repetition block. MEP values were normalised to the M-wave values at the same contraction intensity. MEP values at 100% MVF were also normalised to Mmax (e.g. MEP amplitude at 100% MVF/Mmax*100). BF and VL MEP values were normalized to the maximal voluntary EMG values during maximal voluntary knee flexion and extension, respectively.

MVF, measured at the beginning and end of each session, was the highest instantaneous torque achieved across two maximal contractions.

3.4.4. Physiological Measures

3.4.4.1. Rectal temperature

Core temperature was measured via rectal probe (MEAS 440 Series Temperature Probe, Measurement Specialities Inc, USA) self-inserted 10 cm beyond the anal sphincter. Core temperature was measured throughout the procedure using the core temperature logger (4600 Thermometer, Measurement Specialities Probe, Ohio, USA).

3.4.4.2. Heart rate

Heart rate monitor belts (Polar, T31 Coded Transmitter, Kempele, Finland) were worn for the duration of each trial and heart rate was recorded from a Polar watch (Polar, FT1 Polar, Finland).

3.4.4.3. Laser Doppler

The laser Doppler technique was utilised in the study described in chapter 7. This technique was used to monitor changes in cerebral and quadriceps blood flow (Laser Doppler Perfusion MonitorPeriFlux System 5000 Perimed Instruments Ltd., Hoddesden, Hertfordshire, England). The laser Doppler probe was placed in the belly of the quadriceps muscle, and 3 cm from the midline of the forehead and 4 cm above the eyebrow and held in place with double sided tape and covered with an adhesive bandage to remove any external light sources. The technique is based on the emission of a beam of laser light carried by a fiber-optic probe.

The light scatters and is partly absorbed, with moving blood cells altering the wavelength of the beam. The number and velocity of blood cells influences the change in wavelength which is detected by the returning fiber prior to this wavelength being converted into an electronic signal. The signal is presented in perfusion units (PU).

3.4.5. Perceptual scales

Rating of perceived exertion (RPE) (Borg, 1962) was measured on a 6 to 20 scale to measure perception of effort. Feeling was measured using a -5 (Very bad) to +5 (Very good) scale (Hardy & Rejeski, 1989). Arousal was measured using a 1 (low-arousal) to 6 (high-arousal) scale (Svebak & Murgatroyd, 1985). Thermal sensation (TS) was measured using a 0 (Unbearably cold) to 8 (Unbearably hot) scale (Young, Sawka, Epstein, Decristofano, & Pandolf, 1987).

3.4.6. Hydration

Urine osmolality was measured at the beginning of studies described in chapters 4, 5 and 7 using a handheld urine osmometer (Osmometer-Osmocheck™; Vitech Scientific Ltd, West Sussex, UK) as an indicator of hydration status. A sample of ultra-pure water was pipetted on to the refractometer prior to each measurement in order to recalibrate the osmometer. Following removal of the water, a sample of urine was pipetted on to the osmometer and then measured for osmolality. Analyses were performed in duplicate. Any value < 800 mosmol.kg^{-1} was considered hydrated (Perrier et al., 2015). If values exceeded 800 mosmol.kg^{-1}, participants were instructed to rest and consume water ad libitum and a sample was retaken to ensure hydration following 30 min of rest.

In order to confirm participants had remained euhydrated, in the studies described in chapters 4, 5 and 7, nude body mass was measured prior to beginning the trial and upon completion, correcting for fluid consumed and urine output.

3.4.7. Chamber temperature

In the studies described in chapter 4 and 7 a walk-in environmental chamber (Model WIR52-20HS; Weiss Technik, Gwent, UK) was utilised to create both a hot and moderate condition for participants. In the study described in chapter 4 the chamber was set at 40 º C and 50% Rh for the hot trial, whereas in study described in chapter 7 the chamber was set to 33 º C and 50% Rh for the hot trial. In the moderate condition for chapter 4, participants remained in the laboratory where an average temperature of 21.2 ± 1.8 º C existed. For the experimental study described in chapter 7 a temperature of 15 º C and 50% Rh was utilised.

3.4.8. Blood Collection, treatment, storage and analysis

Blood samples during the study detailed in chapter 5 were collected via the antecubital vein using a butterfly needle. Blood samples in the study detailed in chapter 7 were obtained via an indwelling cannula (Venfon 20G, Sweden) inserted into the antecubital forearm vein. Saline was frequently flushed through the cannula to keep it patent, hence preventing clotting. Any remaining saline was removed prior to a sample being taken.

Twenty ml samples were taken at each time point using 2, 10 ml syringes. Samples were all taken with the participant in the seated position, in order to prevent any influence of postural changes on plasma volume (Harrison, 1985). For serum samples, approximately 5ml of blood was pipetted into anticoagulant-free tubes (Sarstedt, Germany) and allowed to clot for 30 min. Following this the sample was spun in the centrifuge (accuSpin 1R, Fisher Scientific, Germany) for 10 min at 3000 g. The supernatant was removed using a pipette and dispensed into six eppendorphs and initially frozen at -20 º C and then at -80 º C until analyses were performed.

For plasma samples, approximately 5 ml of blood was pipetted into each of 2 lithium heparin tubes (Sarstedt, Germany). Tubes were inverted 5 times and immediately spun in the centrifuge (accuSpin 1R, Fisher Scientific, Germany) for 10 min, as described above. The supernatant was removed using a pipette and dispensed into three eppendorphs and initially frozen at -20 º C and then at -80 º C until analyses were performed.

3.4.8.1. Haematocrit

Haematocrit was analysed by filling Na heparinized haematocrit tubes with whole blood and microcentrifuging for 15 min (haematospin 1300). Haematocrit was then determined using a micro-haematocrit reader (Hawksley, England). Haematocrit was measured in triplicate and the mean value used.

3.4.8.2. Haemoglobin

Haemoglobin (B-HemoCue AB, Angelholm, Sweden) was measured in duplicate, and the mean value used, using haemoglobin photometry for chapter 7. Changes in plasma volume could then be calculated via the method of Dill & Costill (1974).

3.4.8.3. Glucose and lactate

Glucose and lactate were analysed from the whole blood using an automated analyser (Biosin c_line, EKF diagnostic) in chapter 4 and 7. 20 µL of whole blood was collected in sodium

heparinized plastic capillary tubes which was shaken in a pre-prepared Eppendorf filled with glucose/lactate hemolyzing solution.

3.4.8.4. BDNF

Plasma and serum BDNF were measured via immunoassay (R & D systems, bio-techne, UK) in chapter 5 and 7. Human free BDNF is pre-coated on a microplate, which samples and standards are then pipetted on to. Free BDNF present in samples becomes bound to an immobilized antibody. An enzyme –linked antibody is also added prior to a wash to remove any unbound antibody-enzyme reagent. Finally, a substrate solution is added which develops colour depending on the amount of free human BDNF, which is then measured.

3.4.8.5. Cathepsin B

Cathepsin B was measured in serum via an in vitro ELISA (enzyme-linked immunosorbent assay) (R & D systems, bio-techne, UK), which utilises the sandwich technique, in chapters 5 and 7. Following preparation, samples are added to pre-coated (monoclonal antibody) wells. Cells are incubated and any cathepsin B present becomes bound to the immobilised antibody. Washing removed any unbound substances, before an enzyme linked monoclonal antibody is added to the wells. A second wash then removed any unbound antibody enzyme reagent. Substrate solution caused the colour in the wells to develop relative to the amount of cathepsin B originally present. The colour development is stopped, and the intensity of the colour is then measured.

3.4.8.6. Adrenaline and noradrenaline

A manual ELISA was used to measure the concentration of adrenaline and noradrenaline in plasma samples (ibl international gmbh, Hamburg, Germany) for chapters 5 and 7. Following centrifugation plasma was snap frozen in liquid nitrogen to prevent the breakdown of either adrenaline or noradrenaline, hence giving a true value for the concentration. Prior to the ELISA an extraction step was performed to remove adrenaline and noradrenaline from the plasma sample. This ELISA is based on the sandwich principle. The wells are coated with goat anti rabbit antibody. The added liquid antibody, directed towards an epitope of an antigen molecule binds to the plate within the incubation time. The antigen of the sample is incubated in the coated well with enzyme conjugated second antibody (E-ab), directed towards a different region of the antigen molecule. After the substrate reaction the intensity of the developed colour is proportional to the amount of the antigen. Results of samples can be determined directly using the standard curve.

3.4.8.7. Cortisol

The assay for cortisol is based on competitive binding whereby cortisol competes with a fixed amount of HRP-labelled cortisol for sites on a mouse monoclonal antibody (R & D systems, bio-techne, UK). Samples in chapters 5 and 7 were analysed for cortisol. Samples are incubated, during which the monoclonal antibody becomes bound to the goat anti-mouse antibody pre-coated on to the plate. Excess conjugate is removed during a wash and substrate solution is added, causing colour to develop. Following this cortisol concentration can be measured.

3.4.8.8. Estradiol

Estradiol, the predominant female sex hormone, was measured via an assay based on the competitive binding technique (R & D systems, bio-techne, UK) in chapter 5. A monoclonal antibody specific for estradiol becomes bound to goat anti-mouse antibody coated on to the microplate. Two washes are completed to remove excess monoclonal antibody, then excess conjugate and sample, respectively. Between the two washes the estradiol present in the sample competes with HRP for sites on the monoclonal antibody. Substrate solution enables the determination of bound enzyme activity, allowing the concentration of estradiol to be assessed, whereby the intensity of the colour is inversely proportional to concentration.

3.4.8.9. Coefficient of Variation for Blood Samples

The inter and intra assay coefficient of variation was calculated for each of the assays completed for blood samples using the calculation listed below. The intra-assay CV is based on 8 – 10 samples. The results of this analysis can be found in Table 3.1.

$$\text{Coefficient of Variation} = (\text{SD}/\text{Mean})*100$$

Table 3. 1 Intra and Inter assay coefficient of variation values

Metabolite	No. of plates	Intra-assay CV	Inter-assay CV
Plasma Adrenaline	6	10.5	18.2
Plasma BDNF	7	7.3	7.7
Serum BDNF	6	12.1	9.6
Serum Cathepsin B	7	8.5	6.9
Serum cortisol	5	11.3	12.1
Plasma Noradrenaline	5	7.9	10.8

CHAPTER 4: PASSIVE HEAT STRESS ALTERS PERCEPTION AND EXECUTIVE FUNCTION

4.1. Introduction

Successful performance in many sports requires not only effective physical performance, but also effective cognitive performance (Schmit, Hausswirth, Le Meur, & Duffield, 2016; Simmons et al., 2008). While the negative effects of heat stress on physical performance are well known (Drust et al., 2005; Morris, Nevill, Boobis, Macdonald, & Williams, 2005), data regarding the effects of heat stress on cognitive performance are less well understood. An understanding of the effect that heat stress has on cognitive performance is essential for sportspeople as well as for professions such as firefighting and the military (Hemmatjo, Motamedzade, Aliabadi, Kalatpour, & Farhadian, 2017). It is important to understand the effects of heat stress across a range of domains of cognition, given that the components of cognition will interact to affect overall performance (Allard & Burnett, 1985).

The impact of heat stress on cognitive function remains equivocal, largely due to a number of methodological discrepancies across the research. Heat stress has been reported to have a positive effect on attention (Lee et al., 2014; Schlader et al., 2015; Simmons et al., 2008) and working memory (Bandelow et al., 2010; Lee et al., 2014), but a negative effect on working memory capacity (Gaoua et al., 2011; Liu et al., 2013; Racinais et al., 2008) and no effect on perception (Gaoua et al., 2011) or aspects of attention (Sun et al., 2013). The type and timing of cognitive test utilised and the mode, intensity and duration of heat stress (Gaoua, 2010) directly influence the heat stress, perceptual strain and physiological strain experienced by an individual and so these methodological differences likely explain much of the data. Additionally, the use of exercise-induced heating protocols prevents the isolation of heat as a stressor. A recent meta-analysis highlighted the small positive effect of exercise on executive function and a number of information processing tasks, such as visual search and Stroop test (Chang et al., 2012), hence incorporating exercise in heating protocols may confound the effect of heat. Further, the consequential increase in sweat rate in the heat can result in dehydration (Morgan, Patterson, & Nimmo, 2004), which is also known to negatively impact cognitive function (Grandjean & Grandjean, 2007).

The lack of consensus in the literature surrounding passive heating and cognitive function is a result of a number of factors including the change in core temperature, perception of changes in core temperature, thermal comfort which is closely related to skin temperature and the complexity or domain of the cognitive task. An elevation in core temperature has been highlighted as a mediator of the heat induced negative effects on cognitive function, detrimentally influencing working memory (Lieberman et al., 2005; Morley et al., 2012),

executive function (Schlader et al., 2015) and attention (Lieberman et al., 2005; Schlader et al., 2015; Simmons et al., 2008). However, the negative perceptual responses and large increases in skin temperature, without associated increases in core temperature (Gaoua et al., 2012), in response to passive heating, can also negatively influence cognition, hence studies have demonstrated impaired cognition without changes in core temperature (Racinais et al., 2008; Ramsey & Kwon, 1992). Further, when thermal strain is increased, performance on complex tasks have generally demonstrated greater vulnerability to the detrimental effects of heat (Hancock, 1989), whilst simple task performance can be maintained. Therefore, the current literature clearly highlights the complex range of factors that have the potential to influence cognitive function during hyperthermia. However, an overall consensus on how cognitive function is influenced when hyperthermia is induced passively and how factors such as skin temperature, perceptual changes and core temperature contribute to these changes, is unknown.

Consequently, the aim of this study was to examine the impact of passive heat exposure on cognitive function, eradicating the confounding influence of exercise evident in much of the literature to date. Assessing core temperature, skin temperature and a variety of perceptual measures allowed us to highlight how these measures contribute to cognitive changes across a broad range of domains and tasks of varying complexity. It is hypothesised that passive heat exposure will result in an increase in perceived thermal strain, which will adversely influence complex cognitive tasks, particularly those in the domains of executive function and attention, an effect mediated by negative perceptual responses. The findings have implications for sporting performers and professions who have to operate under heat stress.

4.2. Methods

Forty-one active males were recruited to take part. The mean (± SD) age, height and body mass of the 41 participants who completed the study were 21.3 ± 1.6 yrs, 181.0 ± 5.7 cm and 81.6 ± 9.8 kg, respectively. A full description of participant recruitment and ethics are provided in sections 3.2 and 3.3.

4.2.1. Study Design

All data was collected at Nottingham Trent University between the months of September and December (2015 and 2016) to prevent any heat acclimatisation effect (Nakamura & Okamura, 2001). Each participant completed a familiarisation trial, a control trial in a thermoneutral

environment (21.2 ± 1.8 ° C and 41.9 ± 11.4 % Rh) and a hot trial in the environmental chamber (39.6 ± 0.4 ° C and 50.8 ± 2.3 % Rh) in a randomised crossover design. Each experimental trial was separated by exactly 7 d and performed at the same time of day to eradicate any influence of circadian rhythm (Van Dongen & Dinges, 2005). A full description of pre-trial restrictions can be found in section 3.3.5. On the day of each main trial participants were asked to arrive at the laboratory at 9 am and 2 h postprandial, having abstained from caffeine and following the consumption of 500 ml of water ~ 2 h prior to arrival at the laboratory.

4.2.2. Protocol

4.2.2.1. Familiarisation

The familiarisation trial was completed 7 d prior to the first main experimental trial. A full description of familiarisation procedures, height and body mass measurements can be found in section 3.3.2., 3.3.3. and 3.3.4. Participants completed a full battery of the cognitive function tests.

4.2.2.2. Main trial

On arrival, participants recorded their nude body mass and self-inserted a rectal probe. In both trials the first physiological measures were taken in the laboratory (21.2 ± 1.8 ° C and 41.9 ± 11.4 % Rh) before participants entered their allocated condition. This set of measurements will be referred to as baseline measures. Participants then completed the first battery of cognitive function tests and a mood questionnaire, followed by 1 h seated rest in their allocated condition, prior to completing the second battery of cognitive function tests and mood questionnaire. Throughout all trials participants were allowed to drink water ad libitum. All water drank, and urine produced was weighed in order to establish body mass lost and estimated sweat rate. All perceptual and thermal measures were taken at baseline, every 10 min for the duration of the passive rest and on completion of each battery of cognitive tests (Figure 4.1).

4.2.3. Measurements

4.2.3.1. Mood questionnaire

Participants completed the Brunel Mood Scale (BRUMS) questionnaire (Terry et al., 1999). A full description of the mood questionnaire can be found in section 3.4.2.

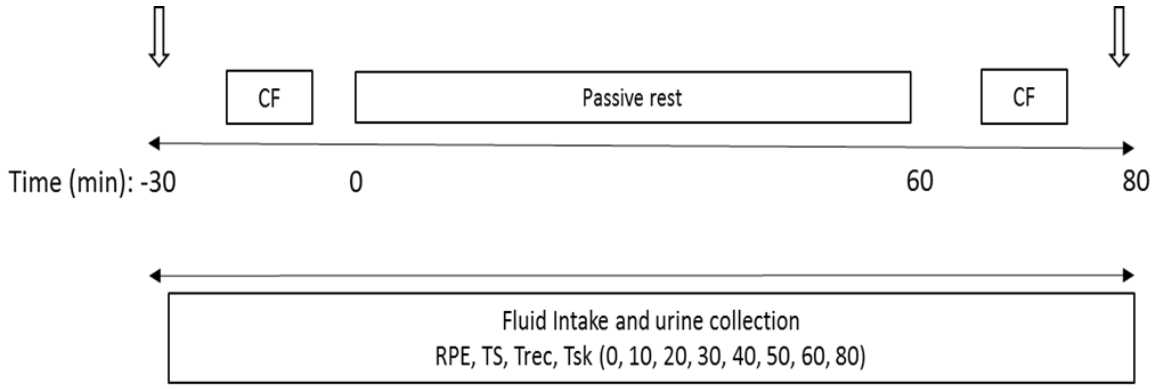


Figure 4. 1 Protocol diagram. RPE = rating of perceived exertion, TS = thermal sensation, Trec = core temperature, Tsk = skin temperature. The arrow indicates body mass and urine osmolality measurement.

4.2.3.2. Cognitive function tests

The cognitive test battery lasted approximately 15 min and was administered via a laptop computer (Thinkpad T450, Lenovo PC HK Limited, China) at -20 min (immediately on entering the allocated condition) and following 1 h passive rest in the allocated condition. A full description of cognitive testing procedures and analysis can be found in section 3.4.1.

4.2.3.3. Physiological Measures

All physiological measurements were taken at baseline, every 10 min during seated rest and at the completion of each cognitive test. Heart rate, core temperature and skin temperature were measured throughout. A full description of the collection of heart rate and core temperature data can be found in sections 3.4.4.2 and 3.4.4.1, respectively. Skin temperature was measured at the midpoint on the right thigh, using a hand held infrared gun (Standard ST-812 InfraRed Thermometer, CEM, Shenzhen, China). Urine samples were analysed for urine osmolality using a handheld osmometer (Pocket-Pal Osmo-Osmocheck™, 4595-E04, Vitech-Scientfic Ltd, Horsham, UK). A full description of urine osmolality analysis can be found in section 3.4.6.

4.2.3.4. Perceptual Measures

Rating of perceived exertion (RPE), feeling, arousal and thermal sensation were measured at baseline and throughout (every 10 min). A full description of the scales used to measure perceptual feelings can be found in section 3.4.5 and Appendix C.

4.2.3.5. Data analysis

Physiological data, perceptual measures and corsi blocks data were analysed using SPSS (Version 23, SPSS Inc., Chicago, Il, USA) via two-way repeated measures Analysis of Variance (ANOVA), using a trial by session time approach. Where paired comparisons were

required, paired samples t-tests with Bonferroni corrections were conducted for physiological measures only.

The cognitive data (Stroop, visual search and RVIP) were analysed using R (www.r-project.org). A full description of the analysis procedures for cognitive data can be found in section 3.4.1.5.

The effect size (Cohen's d) of all significant differences were calculated using trial pairings and interpreted using the following thresholds: <0.2 = trivial effect; 0.2- <0.5 = small effect; 0.5-0.8 = moderate effect and >0.8 = largest effect (Cohen, 1992). For all analysis, significance was set as $P<0.05$. Data are presented as mean ± standard deviation.

4.3. Results

Mean data for all cognitive tests are presented in **Table 4.1**.

4.3.1. Cognitive function

Response times on all cognitive tests were log transformed to normalise the distribution, which demonstrated the right-hand skew expected of human response times. Minimum and maximum cut-offs were employed to exclude any responses which could be deemed anticipatory or delayed. Therefore, across the Visual Search and Stroop tests response times less than 100 ms were excluded and maximum cut off times were 1500 ms and 3000 ms, for the simple and complex levels, respectively. The minimum cut off time for RVIP was 200 ms and the maximum was 1500 ms. only the response times of correct responses were used for response time analysis.

4.3.1.1. Visual Search

Response Times

Simple: Overall, response times were slower in the hot trial (main effect of trial, $t_{(1, 3687)} = 4.9$, $P<0.01$), and response times slowed over time (main effect of time, $t_{(1, 3687)} = 2.8$, $P < 0.01$). However, the pattern of change in response times across the hot and moderate trial were similar (trial*time interaction, $P = 0.85$).

Table 4. 1 Cognitive function data (mean±SD; range (min, max)) across the hot and moderate trials. Cohen's d effect size for the hot vs moderate condition. * P<0.05.

Test	Variable	Test level	Moderate		Hot		Trial effect	Time effect	Interaction	Effect size
			Pre	Post	Pre	Post				
Visual Search	*Response Time (ms)*	*Simple*	330±46 213 (222,457)	333±47 216 (243,439)	349±46 191 (274,465)	359±61 265 (262,527)	P<0.01*	P<0.01*	P=0.85	d = 0.46 small
		Complex	1213±268 1245 (902,2147)	1180±240 1010 (838,1848)	1270±281 1164 (791,1955)	1307±300 1257 (890,2147)	P=0.17	P=0.28	P=0.01*	d = 0.33 small
	Accuracy (%)	*Simple*	97.9±3.1 12.5 (87.5,100)	96.9±4.0 16 (84,100)	98.9±3.7 22.2 (77.8,100)	98.1±2.3 4.5 (95.5,100)	P=0.04*	P=0.53	P=0.53	d = 0.33 small
		Complex	97.7±3.5 12.5 (87.5,100)	97.0±5.1 22.2 (77.8,100)	96.8±5.9 27.6 (72.4,100)	98.1±3.1 12.5 (87.5,100)	P=0.14	P=0.25	P=0.03*	d = 0.0 trivial
Stroop test	*Response Time (ms)*	*Simple*	618±74 294 (488,782)	638±95 418 (501,919)	665±105 455 (460,915)	657±130 630 (500,1130)	P<0.01*	P<0.01*	P<0.01*	d = 0.33 small
		Complex	842±182 764 (567,1331)	826±174 683 (596,1279)	894±196 803 (614,1417)	851±200 1011 (565,1576)	P<0.01*	P=0.15	P=0.14	d = 0.20 small
	Accuracy (%)	*Simple*	98.9±3.0 14.3 (85.7,100)	97.0±4.5 14.3 (85.7,100)	97.9±4.3 14.3 (85.7,100)	97.3±4.4 14.3 (85.7,100)	P=0.23	P=0.03*	P=0.22	d = 0.10 trivial
		Complex	94.8±6.1 25 (75,100)	95.1±4.8 15 (85,100)	96.3±4.2 15 (85,100)	94.0±5.8 25 (75,100)	P=0.14	P=0.66	P=0.22	d = 0.04 trivial
Corsi Blocks	*Sequence length*		6.0±0.7 2.8 (4.4,7.2)	6.1±0.9 4.2 (4,8.2)	5.9±0.8 3.8 (3.6,7.4)	5.9±0.9 3.8 (4.2,8)	P=0.22	P=0.71	P=0.74	d = 0.13 trivial
RVIP	*Response Time (ms)*		496±82 419 (263,682)	486±61 264 (383,647)	494±77 433 (339,772)	506±57 236 (404,640)	P=0.99	P=0.20	P=0.57	d = 0.13 trivial
	Accuracy (%)		52.8±18.3 75 (15,90)	56.9±19.8 76 (17,93)	53.0±16.2 64 (26,90)	55.7±16.9 68 (15,83)	P=0.46	P<0.01*	P=0.27	d = 0.06 trivial

Complex: Response times were not different between the hot and moderate trial (main effect of trial, P = 0.17) and there was no change across time (main effect of time, P = 0.28). However the pattern of change across trials was different, whereby responses slowed in the heat, whereas they improved in the moderate condition (trial*time interaction, $t_{(3,3687)} = 2.5$, P = 0.01; Figure 4.2).

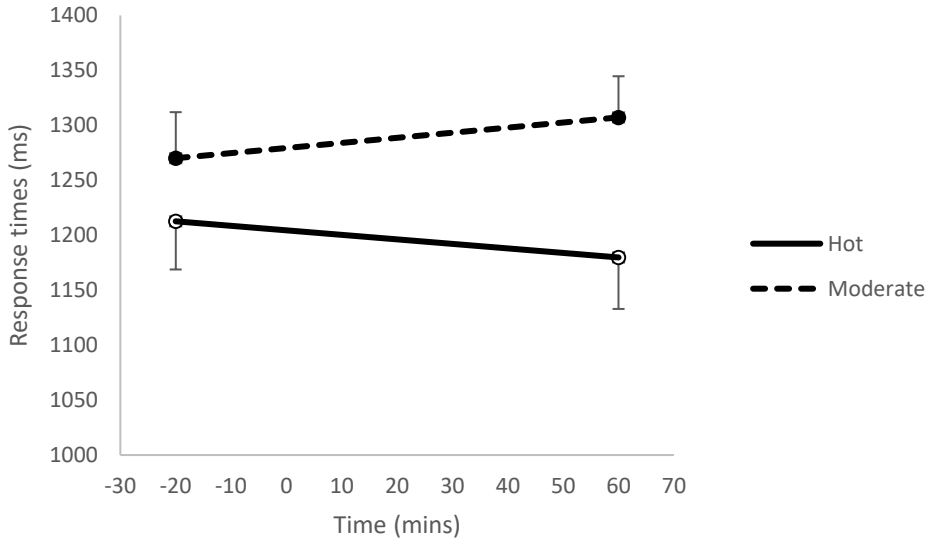


Figure 4. 2 Response time on the complex level of the Visual Search test. Trial*time interaction (P = 0.01).

Accuracy

Simple: Participants demonstrated superior accuracy on the simple level of the test in the hot trial (main effect of trial, $z_{(1, 3844)} = 2.1$, P = 0.04). However, there was no effect of time (main effect of time, P=0.53) on accuracy and the pattern of change in accuracy across the hot and moderate trials did not differ (trial*time interaction, P = 0.53).

Complex: Accuracy did not differ between trials, (main effect of trial, P = 0.14) or across time (main effect of time, P = 0.25). However, accuracy improved on the complex level of the test following 1 h of passive heat exposure, whereas a decrement occurred in the moderate condition (trial*time interaction, $z_{(3,3872)} = 2.2$, P = 0.03; Figure 4.3).

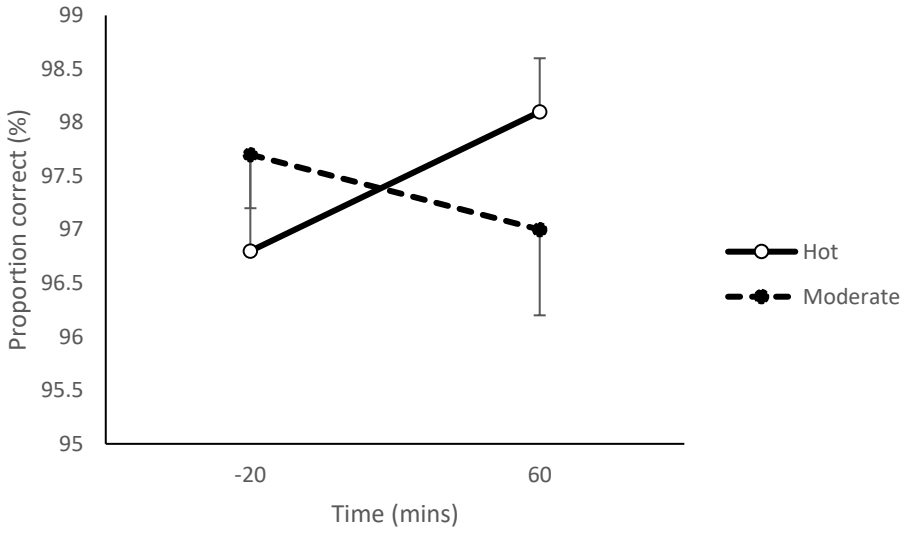


Figure 4. 3 Proportion correct on the complex level of the Visual Search test. Trial*time interaction (P = 0.03).

4.3.1.2. Stroop test
Response Times

Simple: Overall, response times were slower in the hot trial (main effect of trial, $t_{(1,2376)} = 5.8$, P<0.01) and changed across time (main effect of time, $t_{(1,2376)} = 2.9$, P< 0.01). The pattern of change differed between trials, where a marginal improvement in response time was seen following passive heating, while the control trial saw a slowing in response time following 60 min exposure to moderate conditions (trial*time interaction, $t_{(1,2376)} = -2.6$, P< 0.01; Figure 4.4).

Complex: Overall, response times were slower in the hot trial (main effect of trial, $t_{(1,3297)} = 4.7$, P<0.01), however response time did not change across time (main effect of time, P = 0.15) and the pattern of change between trials did not differ (trial*time interaction, P = 0.14).

Accuracy

For both the simple and complex levels of the Stroop test accuracy did not differ between the hot and moderate trials (simple - main effect of trial, P = 0.23; complex – main effect of trial, P=0.14). Accuracy decreased across time (main effect of time, P = 0.03) on the simple level of the test in both trials, however was unaffected in the complex level (main effect of time, P = 0.66). For both levels, the pattern of change across the hot and moderate trials was similar (simple - trial*time interaction, P = 0.22; complex – trial*time interaction, P = 0.22).

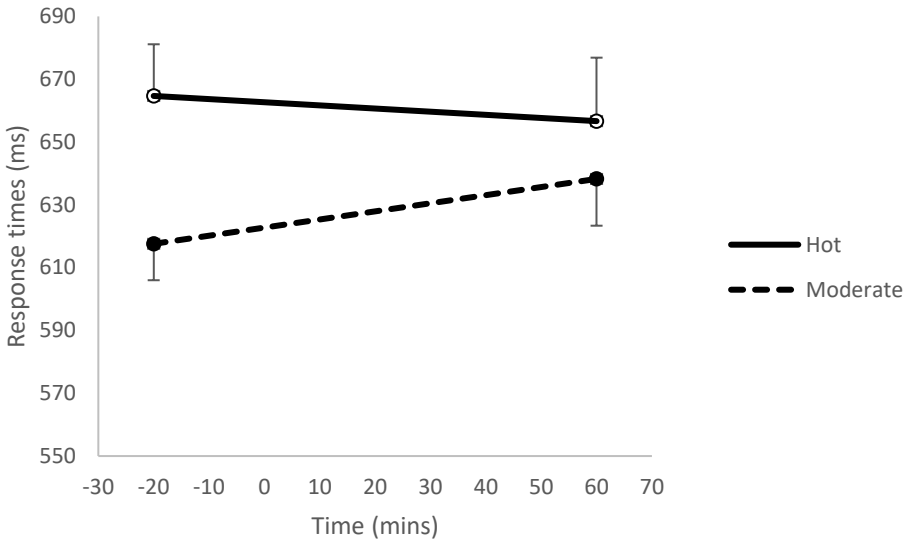


Figure 4. 4 Response time on the simple level of the Stroop test. Main effect of trial P<0.01; Main effect of time, P<0.01; Trial*time interaction, P<0.01.

4.3.1.3. Corsi blocks

The mean of the 5 longest remembered sequences did not differ between trials (main effect of trial, P= 0.22), or across time (main effect of time, P= 0.71). The pattern of change across the hot and moderate trials was similar (trial*time interaction, P= 0.74).

4.3.1.4. RVIP

Response times

Response times were not different between the hot and moderate trials (main effect of trial, P=0.99), and did not differ across time (main effect of time, P=0.20). The pattern of change across the hot and moderate trials was similar (trial*time interaction, P=0.57).

Accuracy

Overall, there was no effect of trial on accuracy (P=0.46), and the pattern of change across trials was not different (P=0.27). However, across time accuracy improves (main effect of time, $z_{(3,8790)} = 2.7$, P<0.01).

4.3.2. Physiological Data

4.3.2.1. Core temperature

Core temperature was greater in the hot trial (main effect of trial, $F_{(1,40)} = 11.1$, P<0.01) (figure 4.5), however no main effect of time was seen. A trial*time interaction was seen for core temperature ($F_{(4, 142)} = 28.7$, P<0.01). Core temperature was not different at baseline, 0

min and 15 min, however core temperature was greater in the heat at every time point thereafter (all P<0.05; Figure 4.5).

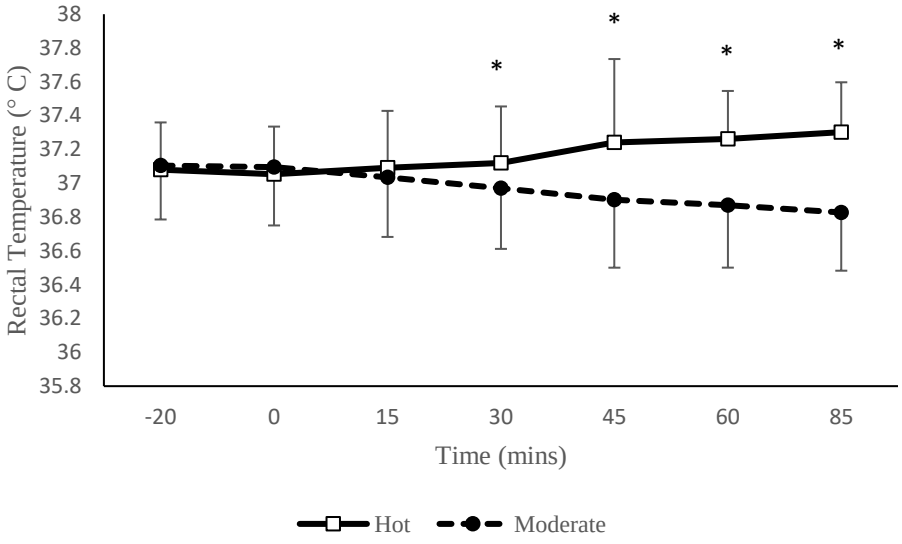


Figure 4. 5 Core temperature during the hot (□) and moderate (●) trials. Main effect of trial, P<0.01; main effect of time, P<0.01; trial*time interaction, P<0.05. *identifying the time point where the core temperature is greater in the hot trial.

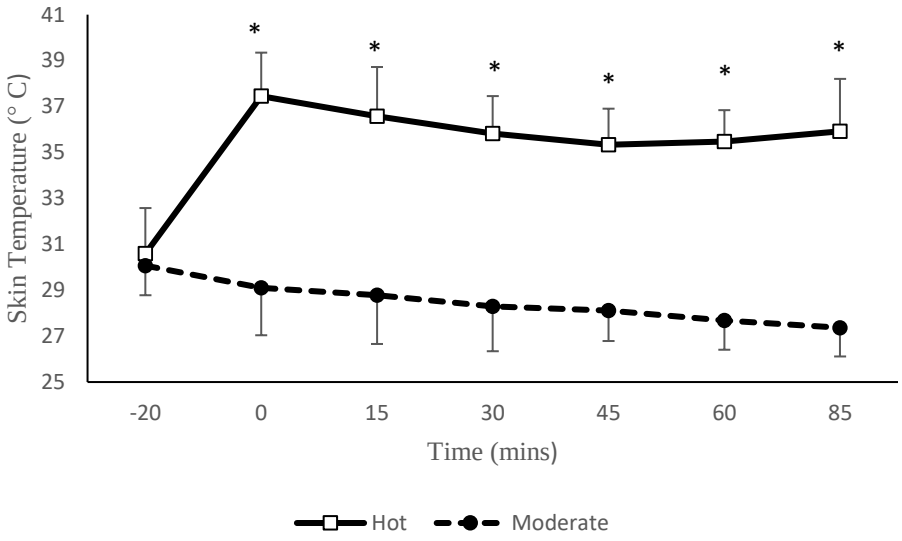


Figure 4. 6 Skin temperature during the hot (□) and moderate (●) trials. Main effect of trial, P<0.05; main effect of time, P<0.05; Trial*time interaction, P<0.05. *Identifying time points where skin temperature is significantly greater in the hot trial.

4.3.2.2. Skin temperature

Thigh skin temperature was greater in the hot trial (main effect of trial, $F_{(1,40)}$ = 996.7, P<0.01) (figure 4.6), increased across time (main effect of time, $F_{(4, 168)}$ = 31.0, P<0.01) and

the pattern of change differed between trials (trial*time interaction, $F_{(4, 168)} = 78.3$, $P<0.01$; Figure 4.6). Skin temperature did not differ at baseline ($T_{(40)} = 1.5$, $P=0.15$), however was greater in the heat at all other time points (all $P<0.01$).

4.3.2.3. Heart rate

Heart rate was greater during the hot trial (main effect of trial, $F_{(1,40)} = 139.0$, $P<0.001$, d=1.21, large effect; Figure 4.7), and increased throughout the trial in the hot trial whereas a gradual decrease was seen in the moderate trial (trial*time interaction, $F_{(6, 240)} = 27.4$, $P<0.001$). Heart rate did not differ at baseline ($t_{(40)} = -1.9$, $P=0.07$), however was greater at every subsequent time point in the hot trial (all $P<0.01$).

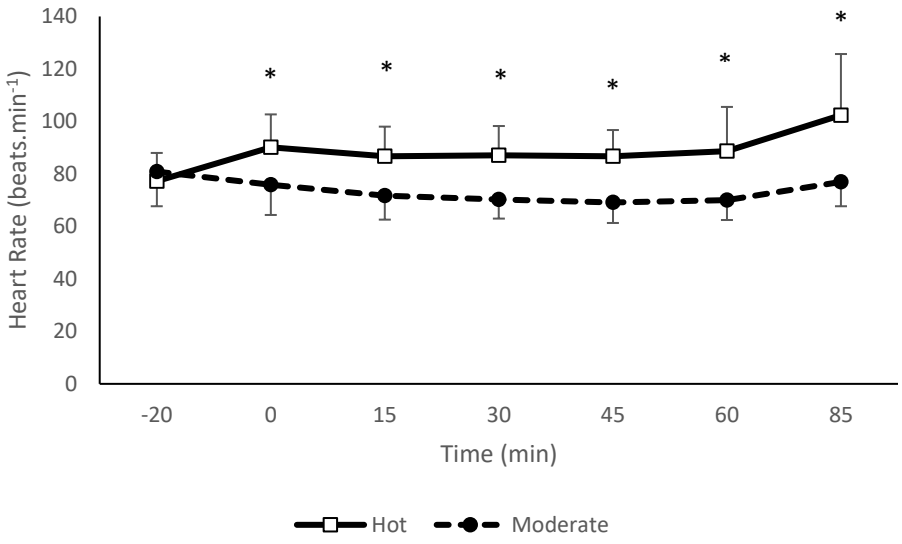


Figure 4. 7 Heart rate during the moderate (●) and hot (□) trials. Main effect of trial: $P<0.01$; Main effect of time: $P<0.01$; Trial*time interaction: $P<0.01$. *Identifying time point where heart rate is significantly greater in the hot trial.

4.3.4. Hydration Status

Participants were hydrated (<800 mosmol.kg^{-1}) at the beginning (Hot: 683 ± 297 mosmol.kg^{-1}; Control 630 ± 310 mosmol.kg^{-1}) and end (Hot: 394 ± 273 mosmol.kg^{-1}; Control: 387 ± 219 mosmol.kg^{-1}) of each trial. There was no effect of trial ($P=0.43$) or a trial*time interaction ($P = 0.38$) for urine osmolality, however participants became more hydrated across time (main effect of time, $F_{(1,40)} = 65.381$, $P<0.01$) in both trials. Ad libitum water intake was 444 ± 278 ml in the moderate trial and 1060 ± 553 ml in the hot trial.

There was no effect of trial (P=0.70), time (P = 0.91) or an interaction effect (P = 0.42) on body mass. Sweat rate was greater in the heat (hot : 0.56 ± 0.38 $l.hr^{-1}$, moderate: 0.25 ± 0.20 $l.hr^{-1}$, P<0.01), however body mass change, corrected for fluid intake and urine output, was maintained in both trials (hot : -0.94 ±0.83 %, control : – 0.31 ± 0.52 %).

4.3.5. Perceptual Measures

Rating of perceived exertion (main effect of trial, $F_{(1,40)}$ = 29.4, P<0.01), felt arousal (main effect of trial, $F_{(1, 39)}$ = 9.9, P<0.01) and thermal sensation (main effect of trial, $F_{(1,40)}$ = 156.0, P<0.01) were higher in the heat, whereas feeling (main effect of trial, $F_{(1,40)}$ = 16.7, P<0.01) was lower in the hot trial (Table 4.2).

A trial*time interaction was seen for rating of perceived exertion ($F_{(2, 90)}$ = 17.2, P<0.01) and thermal sensation ($F_{(2, 98)}$ = 44.5, P<0.01), however the pattern of change didn't differ between trials for feeling (P=0.10) and felt arousal (P=0.12). Thermal sensation and rating of perceived exertion did not differ at baseline, however were both greater in the heat at the remainder of time points (all P<0.01).

4.3.6. Mood questionnaire

Anger, tension and vigour were all unaffected by the heat. Confusion (main effect of trial, $F_{(1,31)}$ = 4.6, P=0.04), depression (main effect of trial, $F_{(1,31)}$ = 6.6, P=0.02) and fatigue (main effect of trial, $F_{(1,31)}$ = 4.2, P<0.05), were greater in the hot trial compared to the moderate trial. A significant trial*time interaction was seen for fatigue ($F_{(1,31)}$ = 21.433, P<0.01), whereby fatigue increased during the hot trial ($t_{(31)}$ = -4.6, P<0.01).

4.4. Discussion

The main findings of the present study were that passive heat exposure affects cognitive function in both simple and complex tasks where effects differ between the domains assessed. Passive heat exposure has a detrimental influence on response times in perception tasks; however, a trade-off appeared to occur whereby accuracy improves. Response times on the simple level of an executive function task marginally improved following 1 h heat exposure, suggesting an increase in conduction velocity. However, response times on the complex level of the executive function task were slower in the heat, suggesting an impairment of more complex cognitive functions. No change was observed in performance for the working memory or attention task.

The findings of the present study suggest that a physiologically meaningful increase in core temperature (peak core temperature 37.3 ± 0.3 ˚ C) is not necessary to influence cognitive

function, with cognitive effects potentially governed by changes in skin temperature and perceptual measures. These findings are in line with previous literature where decrements in complex cognitive tasks have been observed following 5 min of heat exposure, where skin temperature increased by ~ 3˚C but core temperature was unaffected (Gaoua et al., 2012). One factor that limits the interpretation of the effects of hyperthermia on cognition in the literature to date is the lack of consistency across studies in terms of heating strategies and cognitive tasks used. Therefore, this study is unique in that it utilises a passive heating approach (eliminating confounding factors such as dehydration and exercise) and tests across a large number of domains simultaneously, enabling the assessment of a wider range of cognitive domains under the same conditions.

There is limited research looking specifically at the effects of passive heating on visual perception, and particularly visual search. The findings of the present study suggest a slowing of response times for complex visual perception, alongside an improvement in accuracy. This is in contrast to the findings of Gaoua et al (2011) where a match to sample visual search test was unaffected by heat stress, but in line with the study of Hancock & Dirkin (1982), where accuracy improved at the expense of a slowing in response time when cortical temperature was increased. The slower response times in the heat for the complex level of the visual search test agrees with the research suggesting that more complex tasks may be affected by hyperthermia, whilst more simple tasks will be unaffected (Gaoua et al., 2011). fMRI data from Liu et al (2013) suggests hyperthermia increases activation in the temporal lobe during a visual perception task, whereas a decrease in activity was seen in neurons in the frontal lobe, parietal lobe and occipital lobe. These findings suggest the brain alters the distribution of resources in response to passive heat stress in an attempt to maintain performance in this domain. The speed-accuracy trade-off seen in the present study could thus be explained by alterations in these brain regions responsible for perception.

The alliesthesial effect, relating to perceptual feelings, provides support for increases in skin temperature influencing cognitive functioning (Cabanac, 1971; Gaoua et al., 2012). In the present study, participants reported lower levels of pleasure (feeling scale data) and greater confusion, depression and fatigue (BRUMS questionnaire data) in the hot trial, highlighting the negative effect of passive heating. This coincided with a significant increase in skin temperature and thermal strain, potentially explaining the discomfort leading to this mood response. This deterioration in feeling has the potential to distract participants from the

Table 4. 2 Perceived ratings of exertion, thermal sensation, feeling and felt arousal during the hot and moderate trials (mean ± SD).

Trial	**Time (min)**							**Trial Effect**	**Time Effect**	**Interaction**	**Effect size**
	-20	0	15	30	45	60	80				
Rating of perceived exertion (RPE)											
Moderate	6.0±0.2	6.2±0.6	6.1±0.3	6.2±0.5	6.1±0.5	6.2±0.7	6.3±0.8				
Hot	6.1±0.3	7.5±1.7*	7.6±2.0*	7.9±2.3*	8.0±2.4*	8.1±2.6*	8.7±2.8*	P<0.01	P<0.01	P<0.01	d = 0.92 large
Thermal Sensation (TS)											
Moderate	3.3±0.8	3.4±0.8	3.4±0.8	3.4±0.8	3.3±0.8	3.2±0.9	3.1±0.9				
Hot	3.5±0.9	5.6±0.6*	5.9±0.6*	6.1±0.7*	6.2±0.7*	6.2±0.8*	6.4±0.8*	P<0.01	P<0.01	P<0.01	d = 2.41 large
Feeling scale (FS)											
Moderate	1.8±1.6	1.5±1.5	1.7±1.5	1.8±1.4	1.8±1.4	1.7±1.5	1.6±1.4				
Hot	1.2±1.7	0.7±1.6	0.9±1.7	0.7±2.0	0.7±1.9	0.6±1.9	0.5±1.7	P<0.01	P<0.01	P=0.10	d = 0.58 moderate
Felt Arousal Scale (FAS)											
Moderate	1.8±1.1	1.9±1.1	1.9±1.1	1.8±1.1	1.8±1.1	1.7±1.1	1.9±1.1				
Hot	2.0±1.1	2.5±1.1	2.3±1.1	2.3±1.1	2.1±1.4	2.2±1.1	2.5±1.2	P<0.01	P<0.01	P=0.12	d = 0.42 small

cognitive task, influencing performance negatively. The compensatory responses, such as an increase in heart rate as shown in the present study, and an increase in sweat rate, that occur in response to heat to prevent large changes in core temperature may also influence perceptual feelings and contribute to decrements in cognitive function, rather than core temperature change itself (Gaoua et al., 2012).

In the present study, no effect of passive heat exposure was seen on visuo-spatial memory, measured using the mean of the three longest remembered sequences on the Corsi Blocks task. Gaoua et al (2011) and Racinais et al (2008) found a significant decrement on working memory when employing a more aggressive heating strategy (15 min walking followed by 45 min rest in 50 º C and 50 % Rh), which resulted in a greater increase in core temperature. The present study adopted a less aggressive heating strategy to remove the confounding effect of exercise stress that is incorporated in aforementioned studies. Physical exertion is known to influence levels of arousal, closely associated with cognitive function, which a number of studies have shown to have a positive effect on cognition (Hogervorst et al., 1996; McMorris & Graydon, 1997). The addition of physical exertion elicits greater thermal strain on participants. Therefore, despite inducing a greater increase in core temperature, the stress exerted on participants is not truly from a "passive" protocol, hence must be interpreted with caution given the potential for exercise to confound study outcomes (Gaoua et al., 2011; Simmons et al., 2008). The present study addresses this concern by using a passive heating protocol.

Response times on the complex level of the executive function (Stroop) task were slower in the heat in the present study. Research by Liu et al (2013) found that executive function was the primary domain to be influenced in the heat, which agrees with the slower response times on the complex level shown in the current study. The executive attention network allows an individual to decipher between potential incongruent responses, in order to select the correct response. This function involves the frontal lobe and lateral prefrontal cortex (Liu et al., 2013). Liu et al (2013), utilising fMRI imaging, found activation of the prefrontal cortex differed in the heat, an area related to the efficiency of resolving conflict between stimuli and responses. Hence this may explain the overall slower response times on the complex level of the Stroop task. In contrast to the slowing of responses on the complex level of the Stroop task, response times on the simple level of the Stroop task improved in the heat, demonstrating an interaction effect. The lack of conflicting stimuli on the simple level, combined with the increase in nerve conduction velocity associated with heat stress, may

explain the improvement in response time seen. In line with the previously discussed findings for visual perception, the findings of the present study also suggest that only complex executive functions are affected by passive heating, whilst more simple cognitive functions are unaffected or improved (Gaoua et al., 2011).

The findings of this study suggest that short duration exposure to heat stress does not influence sustained attention, as assessed using the RVIP task. Neave et al (2004) found exercise induced hyperthermia negatively impacted response times for attention, however both the current study and Gaoua et al (2011) found that in the absence of significant exercise stress this effect is not seen. Neave et al (2004) achieved a lower core temperature than Gaoua et al (2011), therefore the exercise stress is more likely the cause of changes in cognitive function, given that exercise alone has previously been shown to be beneficial for attention (Pesce, Capranica, Tessitore, & Figura, 2003). In line with the findings of the present study, Gaoua et al (2011) assessed RVIP performance, finding that heat stress had no effect on performance. The findings of Schlader et al (2015) also agree with those of the present study where heat stress, incurred through a water-perfused suit, had no effect on sustained visual attention. Similar to this study no meaningful change in core temperature was observed, however perceptual measures were detrimentally affected. Therefore, collectively these findings suggest that changes in attention may require a greater disruption to the body's state of homeostasis, suggesting resources can be redistributed to protect function within this domain. Liu et al (2013) found that brain areas associated with the alerting network of attention were more activated during passive hyperthermia (premotor cortex, middle temporal gyrus and superior parietal lobule) yet no change in performance was seen. This may suggest that in the present study increased activation within these areas has enabled the maintenance of attention during passive hyperthermia.

4.7. Conclusion

The main finding of the present study was that passive heat stress influenced responses for perception and executive function tasks, whilst having no influence on tasks involving visuo-spatial memory and sustained attention. This suggests that neural resources may be sacrificed in certain domains of cognition (e.g. perception and executive function) in order to maintain performance in others (e.g. memory and attention). The detrimental effects seen on perception and executive function may be explained by an alliesthesial effect caused by changes in perceptual measures (thermal sensation, feeling scale, rating of perceived exertion and felt arousal scale) and skin temperature. Future research is required which incorporates

brain imaging techniques in order to establish how activation and neural resource distribution is increasing in response to passive heat stress and to examine whether these changes can explain the effects on cognitive function. The present study provides novel findings regarding the effects of passive heat exposure (without the confounding effects of dehydration and exercise) across a range of domains of cognitive function.

CHAPTER 5: THE INFLUENCE OF A HOCKEY MATCH ON COGNITIVE FUNCTION

5.1. Introduction

Field hockey is a team sport, requiring players to complete intermittent bouts of high-intensity exercise whilst maintaining cognitive function and performing specific motor skills (Burke, 1997). Research has focused largely on the physical demands of this sport (Gabbett, 2010; Lothian & Farrally, 1992; Lothian, 1994; Lythe & Kilding, 2011; MacLeod, Bussell, & Sunderland, 2007; Reilly & Borrie, 1992; Spencer et al., 2004) however, the constantly changing environment associated with this type of activity (Starkes, 1987) demands a high cognitive requirement throughout (Allard & Burnett, 1985; Starkes, 1987) where players are limited in time to process information and respond (Williams, Hodges, North, & Barton, 2006). An acute bout of exercise is thought to improve cognitive function (Chang et al., 2012), however the precise mechanisms involved and the impact of different types of exercise is still under debate.

Skill, in its simplest form, is the capability to produce a set motor pattern (Williams et al, 2006). However, in open sports such as hockey, skill is determined by a player's ability to adapt and perform a skill despite the environmental constraints imposed upon them, by calling upon neural resources (Allard & Burnett, 1985). Williams et al (2006) assessed the perceptual-cognitive performance relationship in soccer players, however this study examined responses of these players at rest e.g. in the absence of exercise. Starkes (1987) found that elite hockey players demonstrate superior recall of game-structured information and an enhanced use of advanced visual cues, when compared to sub-elite hockey players. Therefore, elite games athletes have demonstrated superior perceptual-cognitive responses in sport specific tasks; however how this relationship is influenced by the physical demands of a match is yet to be assessed.

A number of studies have tried to mimic the demands of a game in a laboratory to investigate the influence of intermittent games type activity on skill performance in soccer (McGregor et al., 1999), hockey (Sunderland & Nevill, 2005) and tennis (Dawson, Elliott, Pyke, & Rogers, 1985). However, research investigating its influence on cognitive function remains sparse, limiting explanations relating to changes in skill performance, and therefore preventing practical application. Bandelow et al (2010) is the only available study that assesses the influence of a true competitive match on cognitive function, utilising a competitive football match. However, this study was performed at high external temperatures (33.8 ^{o}C – 34 ^{o} C), which presents a confounding variable due to the known effects of heat on cognition as demonstrated in chapter 4 alongside a number of previous studies (Gaoua, Racinais,

Grantham, & El Massioui, 2011; Liu et al., 2013; Racinais, Gaoua, & Grantham, 2008). Consequently, there is no study, to our knowledge, which has assessed the influence of an intermittent games type match in isolation on cognitive function, and no study to our knowledge which assesses this in field hockey.

The positive relationship between exercise and cognitive function is well-documented (Chang, Labban, Gapin, & Etnier, 2012b). However, the exact mechanisms associated with changes in cognitive function in response to exercise are unknown, despite heightened arousal via increased secretion of adrenaline and noradrenaline, being consistently named as a contributor (Brisswalter, Durand, Delignieres, & Legros, 1995; McMorris & Graydon, 1997). A number of studies show increased BDNF levels to be associated with improvements in cognitive function (Egan et al., 2003; Erickson et al., 2009; Grassi-Oliveira et al., 2008; Rex et al., 2006), however at present there is limited understanding as to the acute BDNF response to exercise, regardless of intensity. Contrastingly high levels of cortisol, a hormone released in response to stress (McMorris et al., 2006b), have been found to detrimentally influence cognitive function (Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996), via the action of glucocorticoid receptors in the prefrontal cortex (Oei, Everaerd, Elzinga, van Well, & Bermond, 2006). The relationship between these blood parameters contribute to the exercise induced changes in cognition, however their responses to intermittent exercise are currently unknown. Cathepsin B (a muscle secretory factor) has also recently been recognised to contribute to the positive cognitive alterations in response to exercise (Moon et al., 2016). At present this has yet to be investigated in humans in response to acute exercise, however mouse studies have shown cathepsin B to be a facilitator of the improvements in cognition following exercise (Moon et al., 2016). Therefore, this is the first study to assess the changes in cathepsin B in response to exercise, alongside the measurement of cognitive function.

The aim of the current study was to establish the influence of a hockey match on cognitive performance across a number of cognitive domains. It was hypothesised that response times would be positively influenced by the arousing effects of the hockey match, however accuracy would be sacrificed as a result.

5.2. Methods

Two female (1 national and 1 regional) and two male (1 national and 1 regional) hockey teams participated in the study. Twenty female athletes volunteered for the study (mean ± SD): age 19.6 ± 1.1 years, height 1.67 ± 0.03 m, body mass 64.7 ± 6.3 kg. Seventeen male athletes volunteered for the study (mean ± SD): age 19.8 ± 1.3 years, height 1.83 ± 0.07 m,

body mass 77.9 ± 6.3 kg. A full description of participant recruitment and ethics can be found in sections 3.2 and 3.3.1, respectively.

5.2.1. Study Design

5.2.1.1. Familiarisation

The familiarisation trial was completed at least one week prior to the first main trial. A full description of familiarisation procedures, height and body mass measurements can be found in section 3.3. Participants completed a full battery of the cognitive function tests.

5.2.1.2. Protocol

All data reported was collected during four competitive hockey matches and simulated control days. Cognitive data was collected at three time points, relative to the timings of the match (1 h prior to, at half time and immediately after full-time). All hockey matches took place in Loughborough, England between September 2015 and March 2016. The environmental conditions of each match can be found in table 5.1, which were not different between control and match trials (Mean: P = 0.7). Each participant undertook a familiarisation trial, a match trial and a control trial. The control trial and match trials were assigned in a randomised order. The control trial consisted of seated rest between measurements.

Each trial was completed 2 h post-prandial, following which participants were not permitted to eat until the trial was completed. Water was consumed *ad libitum* during both trials, however participants were encouraged to drink frequently in the day and evening prior to the trial to ensure euhydration, checked via osmometer. A full description of pre-trial restrictions can be found in section 3.3.5.

5.2.1.3. Match trial

Participants arrived 2 h prior to the start of the match (Figure 5.1). A urine sample was taken on arrival to measure urine osmolality (osmocheck, Pocket PAL-OSMO, Japan). Participants nude body mass was then collected in privacy, to the nearest 0.1kg (GFK 150 AEADAM digital scale, Vitech scientific Ltd). This was repeated following the completion of each trial. A full description of urine osmolality measurements and body mass measurements can be found in sections 3.4.6. and 3.3.4, respectively. The change in body mass was then corrected for urine output and fluid intake in order to determine sweat loss and estimate levels of hydration. On match day trials, participants were fitted with GPS (SPI Pro, GPSports,

Fyshwick, Australia) and HR (Polar Electro, Kemple, Finland) transmitters in order to assess their activity during the match.

Table 5.1. Environmental conditions for the control and match trials for each team.

Team	Control day	Match day
	Mean temp (℃)	Mean temp (℃)
Women's (National level)	12	12
Women's (Regional level)	9.5	6
Men's (National level)	7	10
Men's (Regional level	14.0	12

5.2.1.4. Control trial

The measures taken, and timing of measurements was identical in the control trial to the match trial (Figure 5.1). The match was replaced with seated rest. Heart rate data, body mass changes, fluid intake and urine output were measured throughout the control trial.

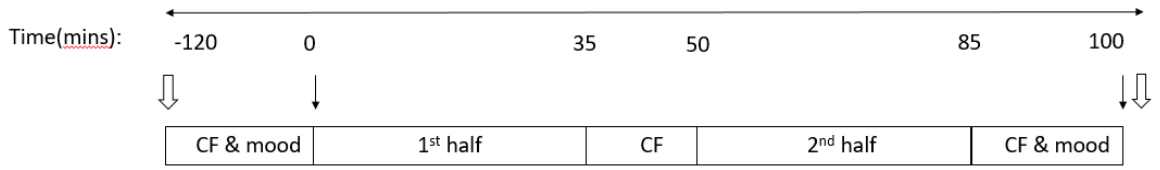


Figure 5. 1 Protocol diagram.⇩ = represents body mass and hydration measurement.↓ = represents blood sampling. CF = cognitive function test battery.

5.2.2. Measurements

5.2.2.1. Mood Questionnaire

The Brunel Mood Scale (BRUMS) questionnaire was completed by participants immediately prior to the first and final battery of cognitive function tests, a full description of the mood questionnaire is presented in section 3.4.2.

5.2.2.2. Cognitive function tests

A battery of cognitive function tests lasting approximately 15 min was administered using a laptop computer (Thinkpad T450, Lenovo PC HK Limited, China) 1 h prior to and following the competitive hockey match. A shortened version of the battery of tests was administered at half time due to the time constraints of a 10 min half time period, including the complex level

of the visual search and Stroop test, the Corsi blocks test and 3 min of the RVIP test. A full description of the cognitive tests and analysis can be found in section 3.4.1.

5.2.2.3. Blood analysis

Blood samples were taken immediately following pre-match cognitive function testing, and immediately after post-match cognitive function testing via venepuncture. A full description of blood collection, storage and analysis can be found in section 3.4.8.

5.2.3. GPS

Match analysis was based on data from a global positioning system (GPS, GPSports Ltd.). Participants wore GPS receivers throughout game play, which were fitted in place with a harness, assessing total distance ran and also distance ran at various speeds. Time spent off the pitch (e.g. during substitutions or following sending off) was not included in calculations of rest or low intensity activity. Distances covered were split into 6 different speed zones; 0 – 4 km/h, 4 – 7 km/h, 7 – 11 km/h, 11 – 15.5 km/h, 15.5 – 20 km/h, >20 km/h. Data was analysed using GPSports team AMS v.1.2.1.11 after being downloaded to a laptop computer. Previous literature has confirmed this GPS system is a valid tool for the measurement of speed and distance during hockey specific exercise (MacLeod, Morris, Nevill, & Sunderland, 2009).

5.2.4. Heart rate

Heart rate monitor belts (Polar Electro, Kemple, Finland) worn for the duration of the match and control trials provide HR data every 5 s, which was simultaneously downloaded alongside GPS data.

5.2.5. Data analysis

Physiological data, perceptual measures and corsi blocks data were all analysed using SPSS (Version 23, SPSS Inc., Chicago, Il, USA) via two-way repeated measures Analysis of Variance (ANOVA), using a trial by session time approach. Where paired comparisons were required, paired samples t-tests with Bonferonni corrections were conducted.

A full description of the cognitive function analysis procedures can be found in section 3.4.1.5. Due to the use of a different battery of tests at half-time, 2 analyses were run. The first analysis assessed changes from pre-match to half time, and the second analysis assessed changes from pre-match to full-time. For all analysis, significance was set as $P<0.05$. Data are presented as mean ± standard deviation

The effect size (Cohen's d) of all significant differences were calculated using trial pairings and interpreted using the following thresholds: <0.2 = trivial effect; 0.2- <0.5 = small effect; 0.5-0.8 = moderate effect and >0.8 = largest effect (Cohen, 1992). Effect sizes are based on main effect of trial.

5.3. Results

5.3.1. Cognitive data

Response times on all cognitive tests were log transformed to normalise the distribution, which demonstrated the right hand skew expected of human response times. Minimum and maximum cut-offs were employed in order to exclude any responses which could be deemed anticipatory or delayed. A full description of cut offs can be found in section 3.4.1.5.

Mean data for all cognitive tests are presented in table 5.2.

Cognitive Function – Pre-match to half-time analysis

5.3.1.1. Visual search

Response Times

Complex: Response times did not differ between trials (main effect of trial, P = 0.34; d = 0.12, trivial effect), or across time (main effect of time, P = 0.29). Hence, the pattern of change was similar (trial*time interaction, P = 0.99).

Accuracy

Complex: Participants accuracy was similar between trials (main effect of trial, P = 0.39; d = 0.00, trivial effect), with minimal change across time (main effect of time, P = 0.15). Hence no trial by time interaction was seen (trial*time interaction, P = 0.09).

5.3.1.2. Stroop test

Complex: Response times were similar across trials (main effect of trial, P = 0.72; d = 0.05, trivial effect), with an overall minimal change across time (main effect of time, P = 0.60). However, participants rate of change between pre-match and half-time differed between trials (trial by time interaction, $t_{(3, 4348)}$= -2.77, P < 0.01; Figure 5.2), with response time getting faster at half-time on the match trial by 40 ms, compared to a slowing in response time by 6 ms in the control trial.

Accuracy

Complex: Overall, no difference in accuracy was seen between trials (main effect of trial, P = 0.06; d = 0.26, small effect). No changes in accuracy were seen across time (main effect of time, P = 0.43), resulting in no interaction effect (trial*time interaction, P = 0.62).

5.4.1.3. RVIP

Response time

Response times were similar across trials (main effect of trial, P = 0.31, Table 5.2; d = 0.18, trivial effect), with no change across time (main effect of time, P = 0.40) or an interaction effect (trial*time interaction, P = 0.61).

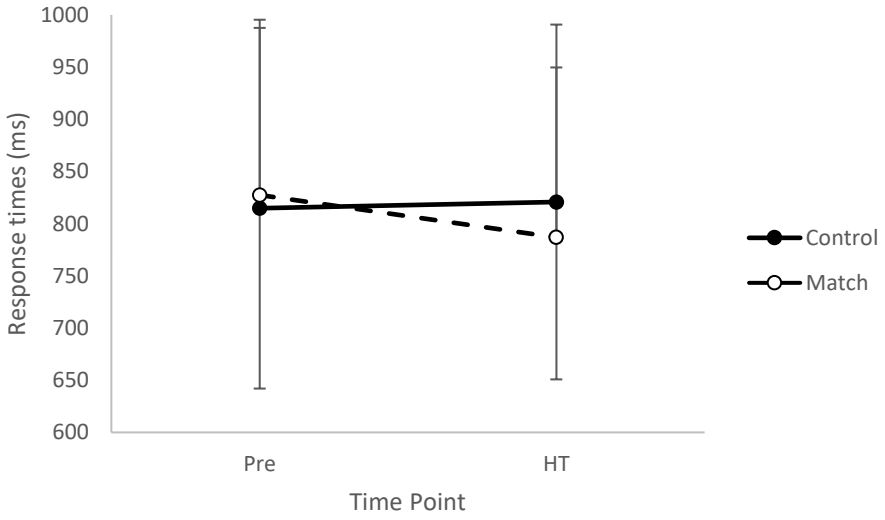


Figure 5. 2 Response times on the complex level of the Stroop test. Pre = prior to the match; HT = half-time. (Trial*time interaction, *P<0.01).

True positive rate

Overall, true positive rate was not different between the match trial and control trial (P = 0.09, Table 5.2; d = 0.03, trivial effect). Across time true positive rate was better at half-time (main effect of time, $z_{(3, 10654)} = 3.43$, P < 0.01). From baseline to half-time participants accuracy improved in the control trial by 6.3 %, where minimal change was seen on the match trial (0.3 % decrease) (trial by time interaction, P = 0.04, Figure 5.3).

Table 5. 2 Cognitive function data across the control and match day trials. Data is mean ± SD. Pre = Baseline, HT = half-time and FT = full-time. Where tests were completed at half-time and full-time, 2 P values are presented; pre to half-time and pre to full-time, respectively.

Test	**Variable**	**Test Level**	**Control**			**Match**			**Trial effect**	**Time effect**	**Interaction**	**Effect size**
			Pre	**HT**	**FT**	**Pre**	**HT**	**FT**				
Visual Search	Response Time (ms)	Simple	284 ± 24		302 ± 41	293 ± 25		298 ± 32	P<0.01*	P<0.01*	P<0.01*	0.06 trivial
		Complex	1114 ± 190	1079 ± 195	1079 ± 180	1080 ± 187	1074 ± 202	1051 ± 141	P=0.34/ 0.32	P=0.29/ 0.13	P=0.99/ 0.92	0.12 trivial
	Accuracy (%)	Simple	98.6 ± 2.5		97.5 ± 3.7	97.7 ± 3.6		97.3 ± 3.7	P=0.17	P=0.09	P=0.35	0.15 trivial
		Complex	97.1 ± 3.3	98.1 ± 4.3	96.4 ± 6.3	97.8 ± 5.3	97.2 ± 6.7	96.7 ± 5.2	P=0.39/ 0.11	P=0.15/ 0.40	P=0.09/ 0.69	0.00 trivial
Stroop Test	Response Time (ms)	Simple	610 ± 82		605 ± 79	608 ± 74		625 ± 100	P=0.51	P=0.86	P=0.09	0.10 trivial
		Complex	815 ± 173	821 ± 170	810 ± 186	827 ± 168	787 ± 163	807 ± 162	P=0.72/ 0.75	P=0.60/ 0.48	P<0.01*/ 0.66	0.05 trivial
	Accuracy (%)	Simple	98.1 ± 3.6		97.2 ± 4.9	97.5 ± 3.8		97.1 ± 4.3	P=0.60	P=0.31	P=0.77	0.08 trivial
		Complex	96.5 ± 4.2	95.8 ± 4.9	95.3 ± 4.9	94.5 ± 5.7	94.2 ± 5.3	95.0 ± 5.7	P=0.06/ 0.06	P=0.43/ 0.38	P=0.62/ 0.34	0.26 small

Corsi	Sequence	6.3 ±	6.2 ±	6.5 ±	6.3 ±	5.9 ±	5.9 ±	P=0.47/	P<0.01*/	P=0.09/	0.25
Blocks	Length	1.1	1.0	1.0	1.	0.9	1.1	0.03*	0.39	P<0.01*	small
RVIP	Response	471 ±	503 ±	482 ±	484 ±	466 ±	447 ±	P=0.31/	P=0.40/	P=0.61/	0.18
	Time (ms)	126	57	113	106	105	123	0.41	0.31	0.06	trivial
	Accuracy	49.6 ±	55.9 ±	50.7 ±	52.5 ±	52.2 ±	53.1 ±	P=0.09/	P<0.01*/	P=0.04*/	0.03
	(%)	18.4	17.5	19.3	18.6	20.1	19.3	0.06	0.53	0.64	trivial

5.3.1.4. Corsi

The mean length of the 3 longest remembered sequences was similar across trials from pre-match to half-time (main effect of trial, P = 0.47; d = 0.25, small effect). From pre-match to half-time sequence length decreased (main effect of time, $f_{(1,37)} = 7.72$, P<0.01), however no difference was seen between trials in the rate of change (trial*time interaction, P = 0.09).

5.3.2. Cognitive Function – Pre-match to full-time analysis

5.3.2.1. Visual Search

Response time

Simple: Overall, response times were worse on the match (main effect of trial, $t_{(1, 3219)} = 391.09$, P < 0.01; d = 0.06, trivial effect; Table 5.2) and globally slowed across time (main effect of time, $t_{(3, 3210)} = 5.57$, P < 0.01). Response time got worse in the control condition (trial*time interaction, $t_{(3,3210)} = -2.90$, P < 0.01; Figure 5.4), where response time slowed by 18 ms compared to only 5 ms in the match trial.

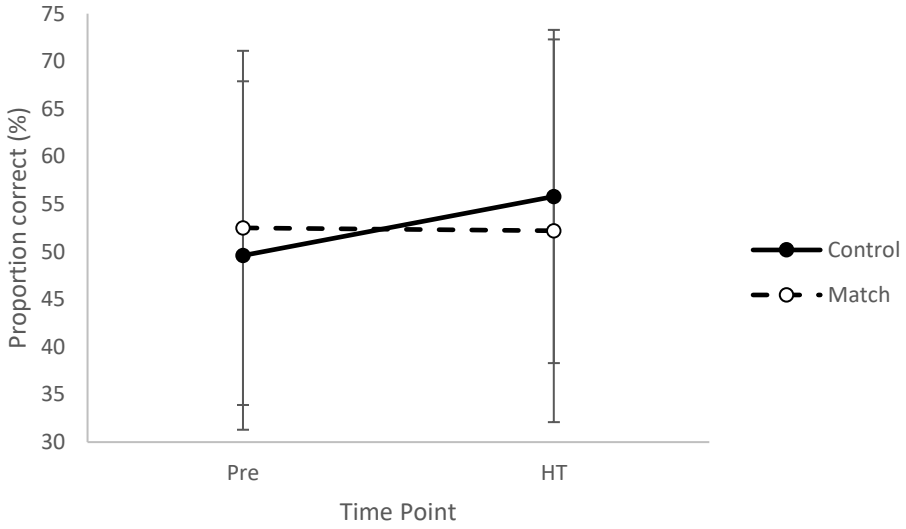


Figure 5. 3 Proportion correct on the RVIP test. Trial*time interaction, P = 0.04. Pre = baseline and HT = half-time. (Trial*time interaction, P = 0.04).

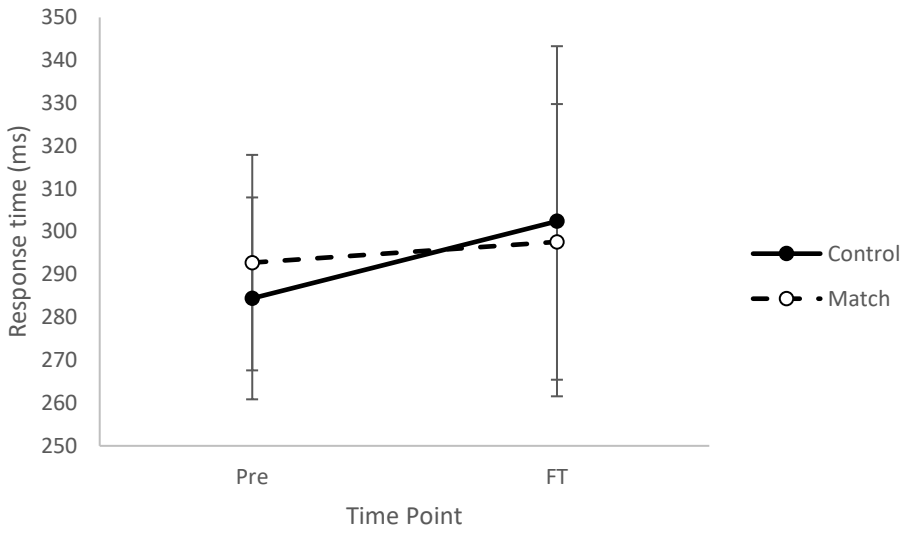


Figure 5. 4 Response time on the simple level of the Visual Search test. (Main effect of time, P<0.01; trial*time interaction, P<0.01). Pre = baseline; FT = full-time.

Complex

Complex: Response times did not differ between trials (main effect of trial, P = 0.92, Table 5.2; d = 0.12, trivial effect). Response times remained constant over time (main effect of time, P = 0.13), resulting in a similar pattern of change (trial*time interaction, P = 0.92).

Accuracy

Simple: Accuracy did not differ between trials (main effect of trial, P = 0.17; d = 0.15, trivial effect), with similar responses across time (main effect of time, P = 0.09). The pattern of change was similar across both trials (trial*time interaction, P = 0.35).

Complex: Participants accuracy was similar across trials (main effect of trial, P = 0.11, Table 5.2; d = 0.00, trivial effect), with no change across time (main effect of time, P = 0.40) and no trial by time interaction (trial*time interaction, P = 0.69).

5.3.2.2. Stroop test
Response times

Simple: Response times did not differ between trials (main effect of time, P = 0.51; d = 0.10, trivial effect) and there was no change across time (main effect of time, P = 0.86). No trial by time interaction was seen (trial*time interaction, P = 0.09).

Complex: No changes were seen between trials (main effect of trial, P = 0.75; d = 0.05, trivial effect) or across time (main effect of time, P = 0.48; d = 0.05, trivial effect). Hence the pattern of change between trials was not different (trial*time interaction, P = 0.66).

Accuracy

Simple: Overall, accuracy was not different between trials (main effect of trial, P = 0.60, Table 5.2; d = 0.08, trivial effect). No changes occurred across time (main effect of time, P = 0.31) and no trial by time interaction was seen (trial*time interaction, P = 0.77).

Complex: No effect of trial was seen on accuracy (main effect of trial, P = 0.06, Table 5.2; d = 0.26, small effect). Overall, accuracy did not change across time (main effect of time, P = 0.38), therefore the pattern of change between trials was also not different (trial*time interaction, P = 0.34).

5.2.2.3. RVIP

Response time

No effect of trial (main effect of trial, P = 0.41; d = 0.18, trivial effect) or time was seen (main effect of time, P = 0.31), resulting in a similar pattern of change (trial*time interaction, P = 0.06).

True positive rate

True positive rate did not differ between trials (main effect of trial, P = 0.06; d = 0.03, trivial effect) or across time (main effect of time, P = 0.53). Hence, no trial by time interaction was present (trial*time interaction, P = 0.64).

5.3.2.4. Corsi Blocks

The mean length of the 3 longest remembered sequences was greater in the control trial than the match trial from pre-match to full-time (Control: 6.4 vs match: 6.1) (main effect of trial, $F_{(1,37)}$ = 4.83, P = 0.03; table 5.2; d = 0.25, small effect). Across time performance did not change. The pattern of change differed from pre-match to full-time (trial*time interaction, $F_{(1,37)}$ = 11.89, P <0.01, Figure 5.5), whereby performance decreased in the match trial by 0.4 and increased in the control trial by 0.2.

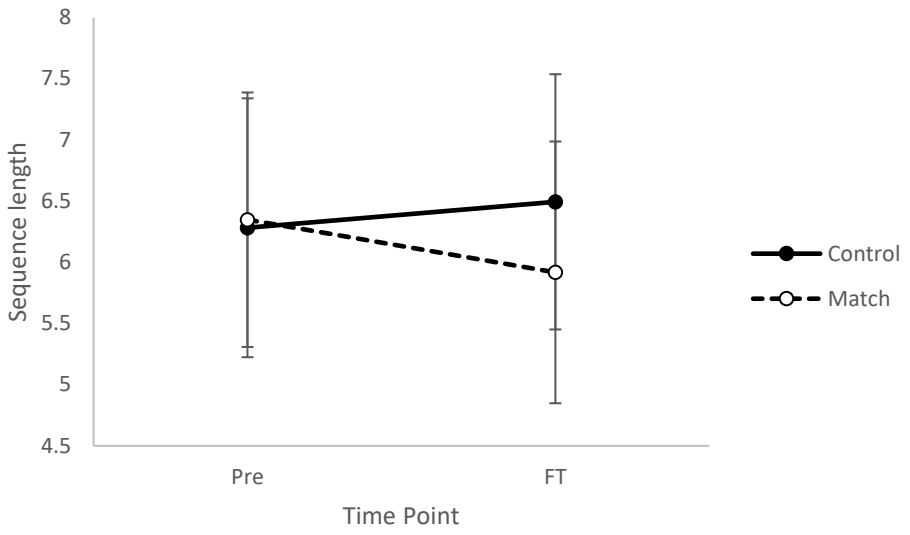


Figure 5. 5 Mean sequence length from pre-match to full-time for the Corsi Blocks test. Main effect of trial (P = 0.03), and trial*time interaction (P< 0.01).

5.3.3. Hydration status

Urine osmolality at the beginning of the match trial (567 ± 289 mosmol.kg^{-1}) and the control trial (548 ± 287 mosmol.kg^{-1}) both demonstrated participants were hydrated (<800 mosmol.kg^{-1}) at the beginning of each trial. There was no difference between trials (P = 0.73).

Body mass change, corrected for fluid intake and urine output, as a percentage of resting body mass, was well maintained in both trials (match *vs* control: 0.49 ± 1.14 vs 0.29 ± 0.96 %).

5.3.4. GPS data

Total distance ran for the male athletes was 6183 ± 1589 m and 5943 ± 1445 m for the female athletes. Total distance ran at high speed (> 20 km.h^{-1}) for the male athletes was 180 ± 120m in the first half and 136 ± 92 m in the second half. The distance ran by the female athletes in the first half was 29 ± 23 m in the first half and 41 ± 33 m in the second half. There was no difference in high speed distances run by the female athletes in the first half compared to the second half (P = 0.27), however the men ran less in the second half than the first (P = 0.02). However, there was no difference in the distances run in zone 5 (15.5 – 20 km.h^{-1}) between the first and second half for the men (P = 0.65) or the women (P = 0.81).

5.3.5. Heart rate data

Mean heart rates were higher throughout the exercise trial than the control trial (main effect of trial, P<0.001). Heart rate date for the match trial is shown in table 5.3.

Table 5. 3 Mean and maximum heart rates in the first and second half of the match trial.

	1st Half		**2nd Half**	
	Male	Female	Male	Female
Mean HR (beats.min^{-1})	169 ± 7	164 ± 19	164 ± 9	160 ± 18
Maximum HR (beats.min^{-1})	191 ± 6	195 ± 8	190 ± 6	195 ± 9

Overall mean heart rate for the first half was 166 ± 14 beats.min^{-1} and 162 ± 14 beats.min^{-1} for the second half.

Control

Mean heart rate for the male participants was 69 ± 5 beats.min^{-1} and 60 ± 11 beats.min^{-1} for the female athletes.

5.3.6. Mood

Anger was greater in the match trial (main effect of trial, $F_{(1,35)} = 22.90$, $P<0.01$; d = 0.62, moderate effect) and increased across time (main effect of time, $F_{(1,35)}=18.80$, $P<0.01$). This led to a significant trial by time interaction (trial*time interaction, $F_{(1, 35)}=18.80$, $P<0.01$), where anger was greater post-match trial than following the control trial (*post hoc*, $P<0.01$).

The pattern of change for confusions differed between trials (trial*time interaction, $F_{(1,35)}=6.60$, $P=0.02$), with a higher value for confusion being demonstrated post-match than following the control trial (5 ± 2 vs 4 ± 1, *post hoc* $P=0.05$).

Depression was greater in the match trial (main effect of trial, $F_{(1,35)}=17.80$, $P<0.01$; d = 0.62, moderate effect) and increased across time (main effect of time, $F_{(1,35)}=6.70$, $P=0.01$). This resulted in the pattern of change differing between trials (trial*time interaction, $F_{(1,35)}=14.10$, $P<0.01$), with a significantly higher value for depressions being demonstrated post-match than following the control trial (4 ± 1 vs 6 ± 3, *post hoc* $P<0.01$).

Fatigue increased across time (main effect of time, $F_{(1,35)}=19.80$, $P<0.01$) and the pattern of change differed between trials (trial*time interaction, $F_{(1,35)}=31.80$, $p<0.01$) , with a significantly higher value for fatigue following the match than following the control trial (8 ± 3 vs 11 ± 3, *post hoc* $P<0.01$). However fatigue was less at baseline ($t_{(36)} = 2.05$, $P<0.05$) on the match day trial.

Tension was greater on the match trial (main effect of trial, $F_{(1,35)}$= 8.00, P<0.01; d = 0.41, small effect), and decreased across time (main effect of time, $F_{(1,35)}$ = 8.00, P<0.01). However no trial*time interaction was present, with a similar pattern of change in both trials.

Vigour was greater in the control trial than the match trial (main effect of trial, $F_{(1,35)}$= 7.80, P<0.01; d = 0.37, small effect).

5.3.7. Blood Analysis

5.3.7.1. Adrenaline

Adrenaline was greater on the match trial (main effect of trial, $F_{(1,30)}$ = 4.44, P = 0.04; d = 0.31, small effect), however no change was seen across time (P = 0.39). The pattern of change differed between trials (trial*time interaction, $F_{(1,30)}$ = 5.47, P = 0.03, Figure 5.6), where adrenaline was greater at the end of the match trial (*post hoc* P<0.01).

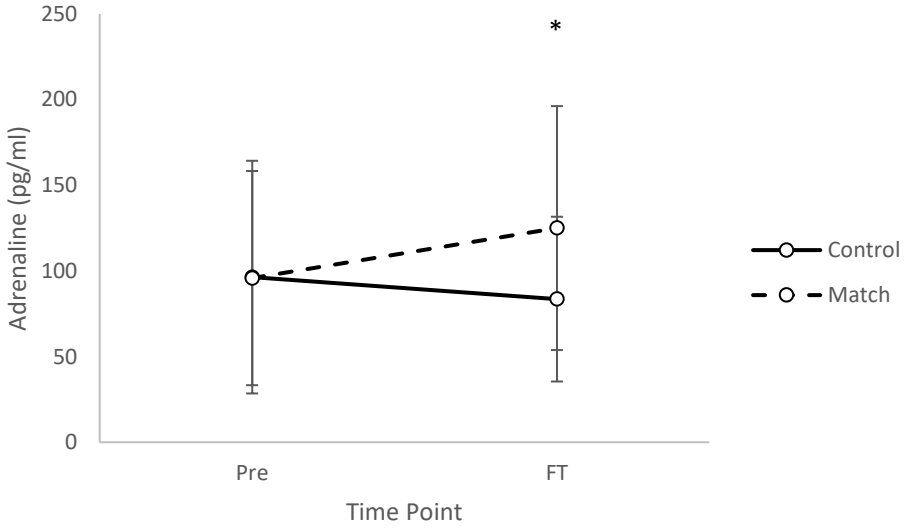


Figure 5. 6 Adrenaline concentrations across the match and control trials. Pre = pre-match and FT = full-time.

5.3.7.2. Noradrenaline

Noradrenaline was greater on the match trial (main effect of trial, $F_{(1,28)}$ = 13.04, P<0.01; d = 0.52, moderate effect), increased across time (main effect of time, $F_{(1,28)}$ = 15.05, P<0.01) and saw a greater rate of increase from baseline to full-time in the match trial (trial*time interaction, $F_{(1,28)}$ = 0.01, *post hoc* P<0.01, Table 5.3 & Figure 5.7).

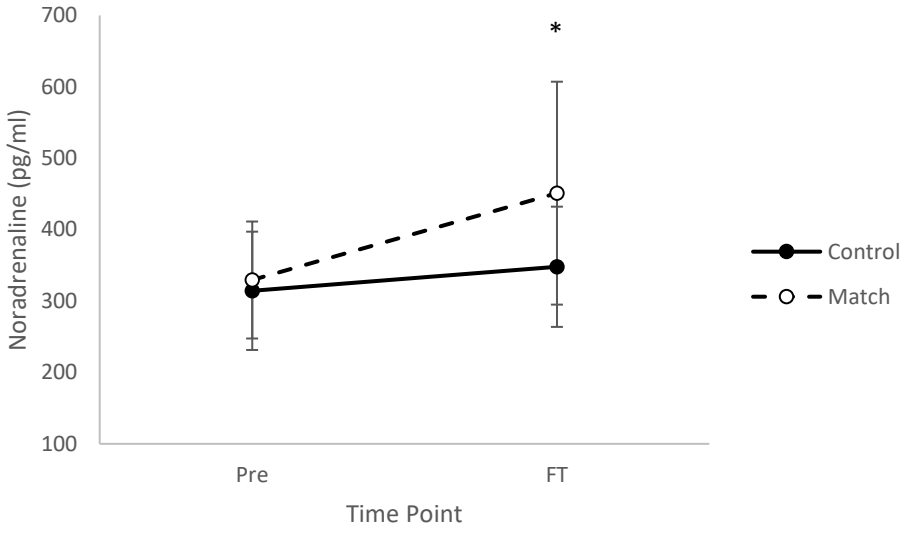


Figure 5. 7 Noradrenaline changes across the control and match trials. Pre = pre-match and FT = full-time.

5.3.7.3. Cortisol

Cortisol concentration was greater on the match trial (main effect of trial, $F_{(1,25)}$=9.30, P<0.01; d = 0.38, small effect). The pattern of change differed between trials, where cortisol concentration increased on the match trial and decreased on the control trial (trial*time interaction, $F_{(1,25)}$=22.10, P<0.01, Figure 5.8). Cortisol was greater post-match than following the control trial (*post hoc* P<0.01).

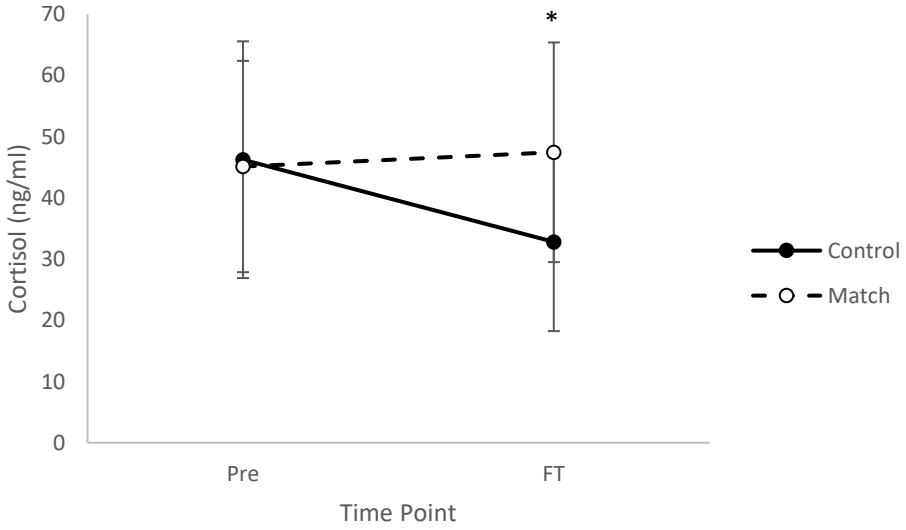


Figure 5. 8 Cortisol changes across the control and match trial. Pre = pre-match and FT = full-time.

5.3.7.4. BDNF (plasma)

Overall, plasma BDNF was not different between match and control trials (P = 0.09; d = 0.39, small effect) and did not change across time (P = 0.56), resulting in a similar pattern of change (P =0.67).

5.3.7.5. BDNF (serum)

Serum BDNF was greater on the match trial (main effect of trial $F_{(1,23)}$=5.70, p=0.03, table 5.3; d = 0.52, moderate effect). However, there was no change across time (P = 0.15), resulting in a similar pattern of change (P = 0.18).

5.3.7.6. Oestrogen

Oestrogen was measured to assess whether female participants were in different phases of the menstrual cycle on the match day and control day trials. There was no difference between trials (control: 767 ± 426 pg/ml vs match: 630 ± 339pg/ml, P = 0.31).

5.3.7.7. Cathepsin B

Cathepsin B did not differ between trials (P = 0.87; d = 0.02, trivial effect), across time (P = 0.80) and there was no trial*time interaction (P = 0.08).

5.4. Discussion

This study was the first to isolate the effects of a hockey match on cognitive function. The findings demonstrate that response times were improved on the match trial for executive function, whilst exercise helped maintain response time during a perception task. Working memory responded negatively to the stress associated with a hockey match whereas accuracy was better maintained at half-time for the attention task. The findings confirm the understanding that exercise can influence cognition (Chang et al., 2012), and the direction of that change is directly related to the complexity and domain of cognition assessed. The findings of this study provide important implications for how decision-making and skill performance may be influenced across a competitive hockey match, which could inform future training and timing of substitutions to optimise performance in this area.

Perception (Visual Search test)

The hockey match provides a protective influence on the simple perception test preventing the slowing in response time seen on the control trial. Hence on the less arousing tasks, the increment in arousal seen with exercise appears to protect against a drop off in response time, which could be a result of hypostress due to a minimally arousing task combined with rest

Table 5. 4 Blood parameter (mean ± SD) across the control and match day trials. Pre = Baseline and FT =full-time.

Blood parameter	Control		Match		Trial Effect	Time Effect	Interaction Effect	Effect size d
	Pre	**FT**	**Pre**	**FT**				
Adrenaline (pg/ml)	96 ± 68	84 ± 48	96 ± 62	125 ± 71	P=0.04*	P=0.39	P=0.03*	0.314 small
BDNF (plasma) (pg/ml)	476 ± 238	498 ± 537	325 ± 222	373 ± 352	P=0.09	P=0.56	P=0.67	0.387 small
BDNF (serum) (pg/ml)	23151 ± 9203	24423 ± 11183	26617 ± 5472	29608 ± 5933	P=0.03*	P=0.15	P=0.18	0.522 moderate
Cathepsin B (ng/ml)	67 ± 27	64 ± 24	64 ± 26	66 ± 28	P=0.87	P=0.80	P=0.08	0.023 trivial
Cortisol (ng/ml)	46 ± 19	33 ± 15	45 ± 17*	47 ± 18*	P<0.01*	P=0.03*	P<0.01*	0.379 small
Noradrenaline (pg/ml)	314 ± 83	348 ± 84	329 ± 82*	451 ± 156*	P<0.01*	P<0.01*	P=0.01*	0.517 moderate

(Hancock, 1989). Perception is particularly important in games type activities, where having the ability to pick up cues in various planes allow quick recognition of ball and player positions within the playing area. Hence the beneficial effect on the simple level in terms of response time and the trend towards an improved accuracy on the complex level, on the match trial, would likely promote effective performance. Similar results have been found following more complex, and sport specific visual search tasks (McMorris & Graydon, 1996), suggesting the overly simple task selection may have minimised the response. However, ultimately the minimal effect within this domain, shown by the lack of change in performance on the match trial, suggest perceptual skills are unlikely to be affected during competitive high intensity intermittent exercise.

Fleury, Bard, Jobin & Carrière (1981) found that neither high intensity running intervals, nor a running to exhaustion protocol had any influence on visual perception. However improved response speed has been seen on visual search tasks in the studies by Aks (1998) and Allard (1989), all of which provide some similarities to the present findings. The studies highlighted an improvement in response time following exercise was a result of an improvement in "preparation time" (Aks et al., 1998; Allard et al., 1989), suggesting exercise influences how participants prepare for the onset of a stimuli, enhancing response time. Similarly, McMorris & Graydon (1997) concluded that the effect of exercise on visual perception was based around its positive effect on information processing speed.

Executive Function (Stroop test)

For the simple simple level of the executive function tasks, no changes were seen in response times or accuracy. Whereas on the complex level of this test, response times were quicker on the match trial from baseline to half-time, when compared to the control trial. Despite not reaching significance, accuracy had a tendency to be worse for the complex task (small effect) in the match trial compared to the control trial, indicating a degree of speed-accuracy trade off. Byun et al (2014) found mild exercise (30% VO_{2peak} for 10 min) also improved reaction time on the Stroop test, mediated by increased arousal levels (measured via mood questionnaire) and an increase in cortical activations in the prefrontal cortex, an area of the brain closely related to executive performance. Although very different exercise intensities, executive function tasks are well known to be vulnerable to the effects of exercise, and thus arousal (Ferris, Williams, & Shen, 2007; Kamijo et al., 2004; Yanagisawa et al., 2010). The current study found noradrenaline increased across time in the match, which indicates greater

activation of central nervous system and increased levels of arousal (McMorris & Graydon, 1997). Therefore, the current study provides the first mechanistic explanation of the cognitive responses to this type of exercise, in a competitive environment.

Lemmink & Visscher, (2005) showed improvements in choice reaction time following exercise on the Vienna test battery, implying a narrowing of attentional focus. This study is one of the few that has used an intermittent exercise protocol (40 s high intensity and 20 s low intensity). This protocol still lacks ecological validity due to using cycle ergometer and only an 8 min protocol; however providing a physiological strain which may mimic the transient effects of a section of a match (e.g. time before a rolling substitute) provides some application to a games athlete. The present study demonstrated an increase in serum BDNF and serum cortisol in the match day trial, agreeing with previous literature where both increases in cortisol (Vega et al., 2006) and serum BDNF (Ferris et al., 2007; Vega et al., 2006) have been found following exercise. Therefore, the findings of this study and that of Ferris et al (2007) both provide important implications for neurological health, with the present study providing further evidence of the potential to utilise high intensity exercise to increase the amount of serum BDNF crossing the blood-brain barrier and thus enhance neuron health.

Working Memory (Corsi Blocks)

Similar to executive function, neurons within the lateral prefrontal cortex have been highlighted to contribute closely to both encoding and response related processes (D'Esposito, Postle, & Rypma, 2000). Previous literature has suggested exercise induced arousal can positively influence mental processing and memory function (Lambourne & Tomporowski, 2010; McMorris, Sproule, Turner, & Hale, 2011). However, the present study demonstrated a negative effect on working memory, when performance at half-time and full-time were compared to baseline. Martins et al (2012) found that moderate intensity exercise enhanced the number of correct responses in the "paced auditory serial addition task", whilst also improving the response latency for the Sternberg task. This exercise protocol was of a short duration and moderate intensity, suggesting that the higher intensity and more intermittent protocol in the current study negated the beneficial effect seen following moderate exercise stress, resulting in an overall detriment to performance.

Plasma cortisol is known to be closely related to levels of anxiety (McMorris et al., 2006a), which can influence cognition. Therefore, during a competitive real-world sporting event, this

could prove to be important for cognitive performance. The present study found cortisol was overall greater in the match trial, as well as increasing to a greater extent across the match trial than in the control trial. It is believed that in states of anxiety, when cortisol concentration is increased, the brain preferentially uptakes neurotransmitters associated with emotion over those related to cognitive neurons (McMorris et al, 2005), explaining the cognitive detriment in these states. Previous studies have highlighted the negative effect of high cortisol concentrations (salivary) on memory retrieval (De Quervain et al., 2003; Dominique, Roozendaal, Nitsch, McGaugh, & Hock, 2000). Cortisol's influence on working memory has been recognised and attributed to the actions of the glucocorticoid receptors in the hippocampus and prefrontal cortex (Oei et al., 2006). Hence the increment in cortisol in the current study may influence function within these brain areas, affecting cognitive performance. These initial findings may explain the detriment in working memory, however further investigation is required to confirm this relationship.

Attention (RVIP)

A number of studies have highlighted the importance of visual attention (amongst other domains) in soccer specific perceptual skills (Pesce, Tessitore, Casella, Pirritano, & Capranica, 2007). However, no studies we are aware of have completed this assessment in hockey players or utilising a truly sport specific exercise protocol.

There was no significant difference in the rate of change in response times; however there was a tendency for quicker response times from baseline to full-time. A number of studies have highlighted an association between high cortisol levels and poor attention and memory performance (Bohnen, Houx, Nicolson, & Jolles, 1990; Newcomer, Craft, Hershey, Askins, & Bardgett, 1994). However, the higher level of cortisol in the match trial in the present study did not appear to negatively affect attention, for either response time or accuracy. This implies the stress and resultant cortisol concentration experienced, has not reached a level great enough to influence performance within this domain, and potentially that the simultaneous increase in serum BDNF has prevented a decrement in attention.

5.5. Conclusions and future research

This study was the first to isolate the effects of a hockey match on cognitive function. The findings demonstrate that response times were improved on the match trial for executive function, whilst exercise helped maintain response time during a perception task. Working memory responded negatively to the stress associated with a hockey match. The effects seen

in response times are likely mediated by an increase in arousal, shown via the increment in noradrenaline, as well as the beneficial effect of serum BDNF. The negative responses for working memory are likely a result of this domain being unable to deal with the stress induced by the competitive hockey match, demonstrated by increases in cortisol concentration on the match trial, as well as worse mood responses for anger, confusion, depression and fatigue following the match trial. Future research should aim to demonstrate the correlation between these changes in cognitive function and their effect on skill performance, utilising sport specific examples alongside a sport specific exercise protocol. However, the present study provides more evidence that exercise has the potential to influence cognition in a domain and task specific manner.

CHAPTER 6: RELIABILITY OF TRANSCRANIAL MAGNETIC STIMULATION MEASUREMENTS OF MAXIMUM ACTIVATION OF THE KNEE EXTENSORS

6.1. Introduction

Voluntary muscle activation, a key factor in neuromuscular function and thus the changes that occur with training, ageing, fatigue and injury, is widely assessed with the interpolated twitch technique in order to quantify and express neural drive to the agonist muscles (Gandevia, 2001). The interpolated twitch technique involves comparing the force response to a post-contraction potentiated twitch at rest to the response when a stimulus is superimposed on top of a maximal voluntary contraction (Gandevia, 2001). A potentiated resting twitch makes for a more valid comparison as the superimposed twitch will also be influenced by post-activation potentiation effects as a result of the voluntary contraction prior to the stimulus (Behm et al., 1996). The stimulus can be delivered at either the peripheral or cortical level in order to measure peripheral or M1 cortical voluntary activation (Gandevia, 2001). M1 cortical voluntary activation is estimated using TMS and is used to assess the neural drive from the motor cortex, the region of the brain responsible for motor movement, and thus reveal any deficits in motor cortex output (Gandevia, 2001). Cortical voluntary activation provides an indication of the lack of drive from the cortical and subcortical structures to the motor cortex, which is known to be involved in volitional movement (Goodall et al., 2015a; Goodall et al., 2017; Goodall et al., 2014; Sidhu et al., 2009; Temesi et al., 2017). Specifically, M1 cortical voluntary activation of the knee extensors is of particular interest due to the role of this muscle in locomotion and weight bearing activities. Therefore, understanding the underlying mechanisms of fatigue for this muscle group is extremely important and has implications for exercise performance and health. Peripheral voluntary activation can be used to assess the level of neural drive at the spinal level (Gandevia, 1992).

Transcranial magnetic stimulation coupled with voluntary contractions has been used extensively in the assessment of cortical function and central fatigue in recent years (Goodall et al., 2015a; Goodall et al., 2017; Goodall et al., 2014; Klass et al., 2016; Ross et al., 2010; Ross et al., 2007., Sidhu et al., 2009; Temesi et al., 2014; Temesi et al., 2017). This technique utilises the SIT response upon contractions of various intensities to create a linear regression. The SIT represents the force difference between the peak twitch torque and the force immediately prior to stimulation (e.g. the voluntary contraction). Extrapolating the regression, using Y=0, provides an estimated resting twitch, to demonstrate the response if the muscle was at rest. Goodall et al (2014) found a high within day reliability for the estimation of cortical voluntary activation (CV=10.2%, ICC=0.82) and between day reliability at baseline (CV=2.2%, ICC=0.87) (Goodall et al. 2017). However, despite

demonstrating a good level of reliability, these studies used 7 and 8 participants, respectively. Therefore, according to the recent research by Heroux et al (2015), it is necessary to expand upon the current literature by using an appropriate participant number in order to guide future research. Two further studies have assessed the reliability of M1 cortical voluntary activation in this muscle group in greater detail (Goodall et al., 2009; Sidhu et al., 2009) and similarly, these studies had low participant numbers and did not familiarise participants or control for time of day. Sidhu et al (2009) only assessed between day reliability, whereas Goodall et al (2009) was the first study to assess between and within day reliability in this muscle group. A high degree of within day reliability (CV=3.1 - 3.7%) (Goodall et al., 2009; Sidhu et al., 2009) and between day reliability (Goodall et al., 2009) has previously been demonstrated. Similarly, Sidhu et al (2009) found electrical stimulation was reliable in the determination of peripheral voluntary activation within day (CV=7.8% and ICC=0.96). However, one issue prevailing across current TMS related reliability studies is the limited participant numbers (n=8-12) (Goodall et al., 2009; Heroux et al., 2015; Ngomo et al., 2012; Sidhu et al., 2009). This has been recently highlighted as a major flaw in neurophysiology research in the review by Heroux et al (2015) lending to irreproducible results. Thus, based upon the recommendations of Atkinson and Nevill (2001) a reliability study employing an appropriate sample size >20 is warranted in order to reflect the true variability of a technique.

Familiarisation can affect the reliability of a measurement and should therefore be evaluated in reliability studies; however, previous studies did not familiarise participants to the protocol (Goodall et al., 2009; Sidhu et al., 2009). Additionally, previous research has highlighted how isometric knee extensor torque varies depending on the time of day (Callard et al., 2000; Racinais et al., 2005), but time of day did not appear to be controlled in the previous reliability studies (Goodall et al., 2009; Sidhu et al., 2009). Therefore, to our knowledge no study currently exists in the knee extensors which utilises an adequate number of participants or that provides a familiarisation session and controls for time of day.

Electromyography (EMG) responses provide vital information regarding the stimulation conditions (Ngomo et al., 2012). The EMG responses to TMS show an MEP, which can provide further information regarding the corticospinal excitability of a muscle (Goodall et al., 2012). Therefore, documenting the reliability of the stimulation conditions may inform and explain variability in superimposed force responses and consequently activation.

The aim of the present study was to assess the between-day and within-day reliability of M1 cortical voluntary activation, peripheral voluntary activation, resting motor threshold, active motor threshold, and MEP characteristics. It was hypothesized that these measures would be highly reproducible between-days and within-days, providing support for the use of TMS and electrical stimulation for the determination of M1 cortical and peripheral voluntary activation, respectively, in the knee extensor muscles. An additional aim was to provide researchers with sample size and power analysis using the intra-class correlations from our study.

6.2. Methods

6.2.1. Participants

Twenty seven recreationally active males (age 23±4years, height 1.79±0.07m, body mass 74.31±10.78kg and body mass index 23.19±2.35kg.m^{-2}) participated in the study. A full description of participant recruitment and ethics has been presented in sections 3.2 and 3.3.1, respectively.

6.2.2. Study Design

Participants were required to attend the laboratory at the same time of day on 4 separate occasions to complete a habituation session, a familiarisation session and 2 experimental sessions (Figure 6.1). During the habituation session each participant was introduced to the two methods of stimulation and became familiar with producing force on the isometric dynamometer. The familiarisation session involved an identical protocol as the experimental sessions. Session 1 and session 2 were separated by exactly one week, with 1h rest between session 2 and session 3, assessing within-day reliability.

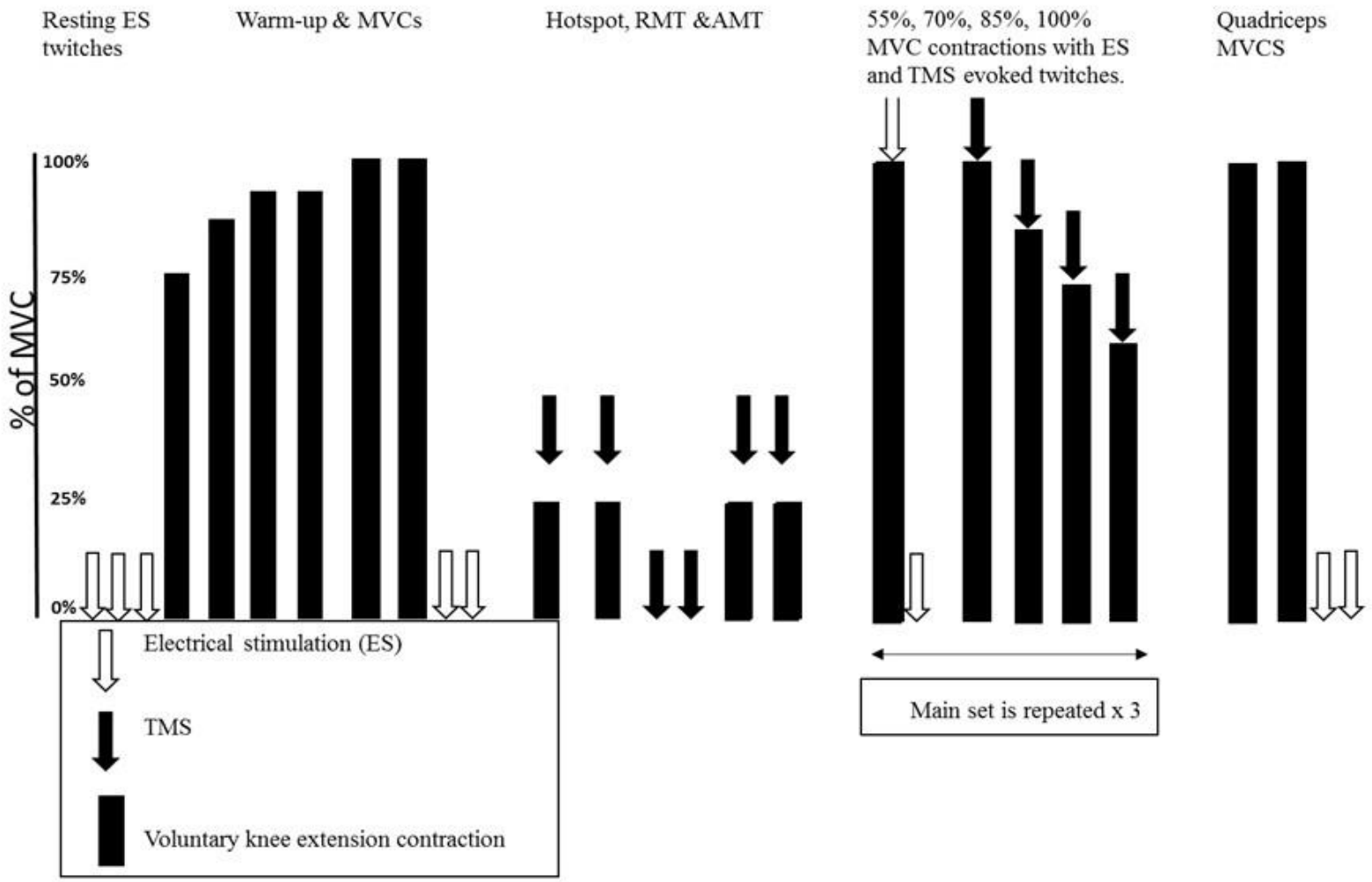


Figure 6. 1 Schematic of the protocol

6.2.3. Measurements and analysis

Participants completed neuromuscular testing in a custom built dynamometer, designed to measure knee extensor force (Johnson et al., 2015). A full description of the testing protocol and data analysis procedures can be found in section 3.4.3.

SPSS 22.0 software for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Data are presented as mean ± standard deviation (SD) and the level of statistical significance was set at $P \leq 0.05$. To determine any differences in values between one experimental session and the next, paired sampled t-tests were used. ICCs were applied to assess how well electrical stimulation and TMS measurements correlated within-day and between-day. 95% confidence intervals were established. In order to determine and compare the variability in measures between the two conditions, the coefficient of variation (CV) and 95% limits of agreement (Bland & Altman, 1986) were established. An ICC close to 1 demonstrates excellent reliability, with anything over 0.9 indicating high reliability and an ICC below 0.8 exhibiting questionable reliability (Atkinson & Nevill, 1998). A CV value of less than 10% is also required to demonstrate high reliability. A statistical simulation of 10,000 experiments (https://github.com/keithlohse/power_reliability) was run based upon the ICC to provide information regarding the statistical power for a range of study designs (independent t-test, paired t-test, within & between 2 x 2 ANOVA) and sample sizes. 6.3. Results

6.3.1. Reliability of MVF

Measures of reliability for MVFs can be found in Table 6.4. Between-day and within-day CV values for MVF were 5.8% and 6.1% respectively.

6.3.2. Familiarisation

The between-day reliability for the familiarisation session and the first experimental session show lower levels of reliability for cortical voluntary activation (91.1±6.5% vs 94.2±4.1%, *P=0.01*) than the between-day reliability for experimental session 1 and 2 (94.2 ± 4.1% versus 93.4±4.6%, *P=0.06*) (Table 6.1 & 6.2). The between-day CV value for cortical voluntary activation between the familiarisation session and the first experimental session was 6.1% and only 2.3% for experimental session 1 and 2. Therefore, subsequent sections will compare experimental session 1 and 2 to assess between day reliability.

6.3.3. Voluntary activation

There were no systematic differences in maximal M1 cortical voluntary activation either between-day (94.2±4.1% versus 93.4±4.6%, *P=0.06*) or within-day (93.4±5.0% versus 93.1±4.5%, *P=0.45*) (Table 6.1; Figure 6.2). There were no systematic differences in maximal peripheral voluntary activation either between-day (93.8±4% versus 93.8±4%, *P=0.82*) or within-day (93.8±4% versus 92.9±3.8%, *P=0.07*). CV values for M1 cortical voluntary activation were 2.3% (between-day) and 2.2% (within-day), and of similar magnitude for peripheral voluntary activation 2.2% (between-day) and 2.5% (within day).

6.3.4. Estimated resting twitch and potentiated twitch

The amplitude of the SIT-torque relationship decreased linearly as contraction intensity increased from 55% MVF to 100% MVF (trial1, r^2 =0.88±0.07; trial 2, r^2 =0.85±0.11; trial 3, r^2 = 0.86±0.09), indicating a strong linear relationship exists both within- and between-day. Between-day and within-day CV values for ERT were 20.4% and 16.8%, respectively. There was no systematic difference in ERT between-day (101±40 N versus 99±39 N, *P=0.56*) (Table 6.1). However, within-day ERT was significantly lower in the second session (95±39N versus 91±37N, *P=0.03*).

Potentiated resting twitch force demonstrated no systematic difference between-day (192±36N versus 194±37N, *P=0.42;* Table 6.1), however a significant difference was seen within-day, with a lower potentiated twitch in the second session (194±37N versus 180±38 N, *P=0.01*). Between-day and within-day CV values for potentiated twitch force were 9.3% and 14.0%, respectively.

6.3.5. MEP characteristics

Between-day and within-day CV values for MEP amplitude during a maximal voluntary contraction ranged from 19.6% to 37.10% of Mmax. Between-day and within-day CV values for Mmax amplitude ranged from 12.7% to 32.0% and Between-day and within-day CV values for Mmax area ranged from 13.4% to 50.4%.

Peak-to-peak MEP amplitude in all three quadriceps muscles at 55% of MVC was VM=63.9%, RF=74.3%, VL=64.9%. As contraction intensity continued to increase up to 100%, MEP amplitudes decreased. MEP amplitude at 100% MVC, when normalised to Mmax, did not differ between-day (VM *P=0.70*, RF *P=0.72*, VL *P=0.06*) or within-day (VM *P=0.05*, RF *P=0.64*, VL *P=0.12*) across any of the knee extensors.

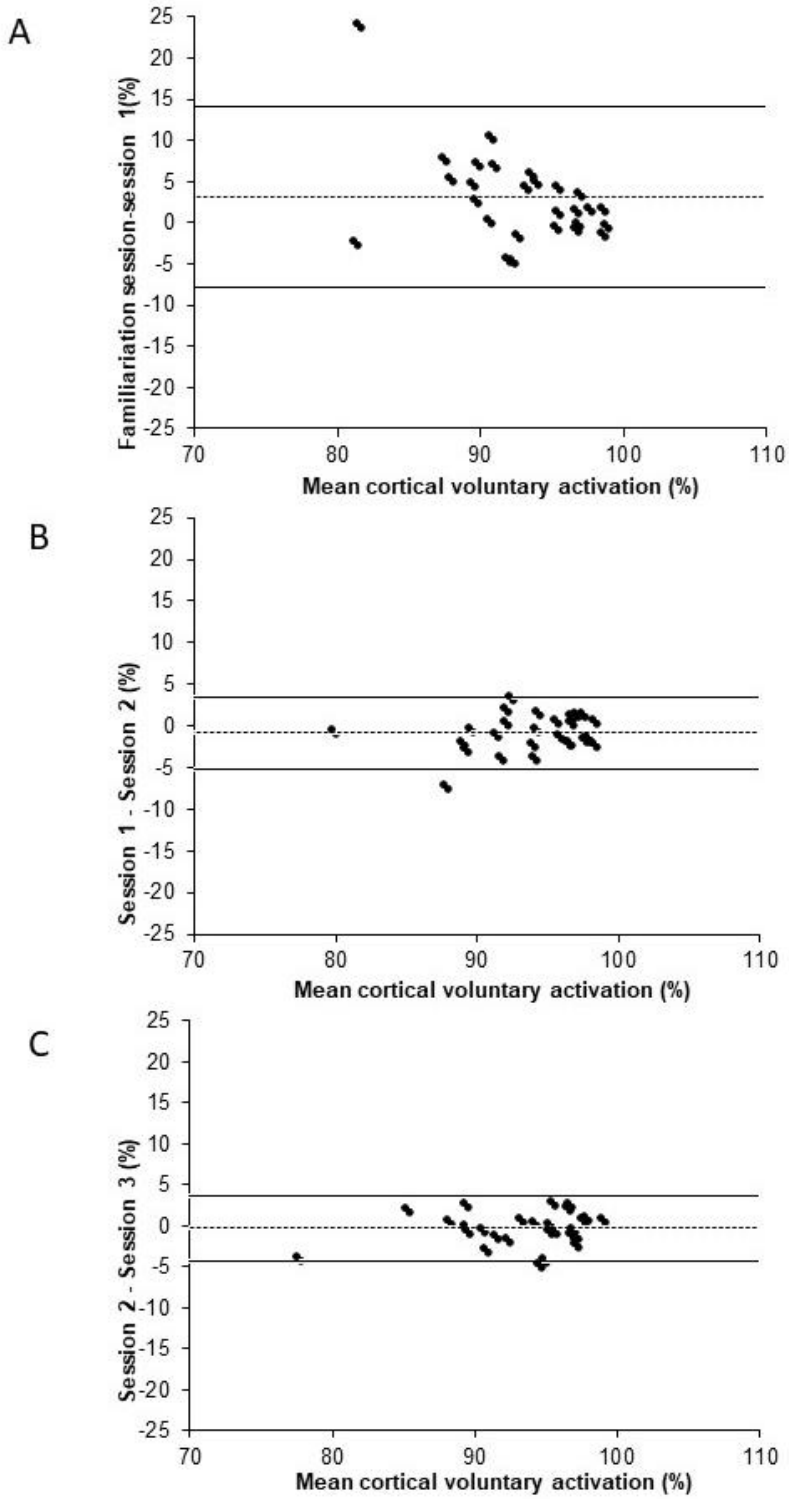
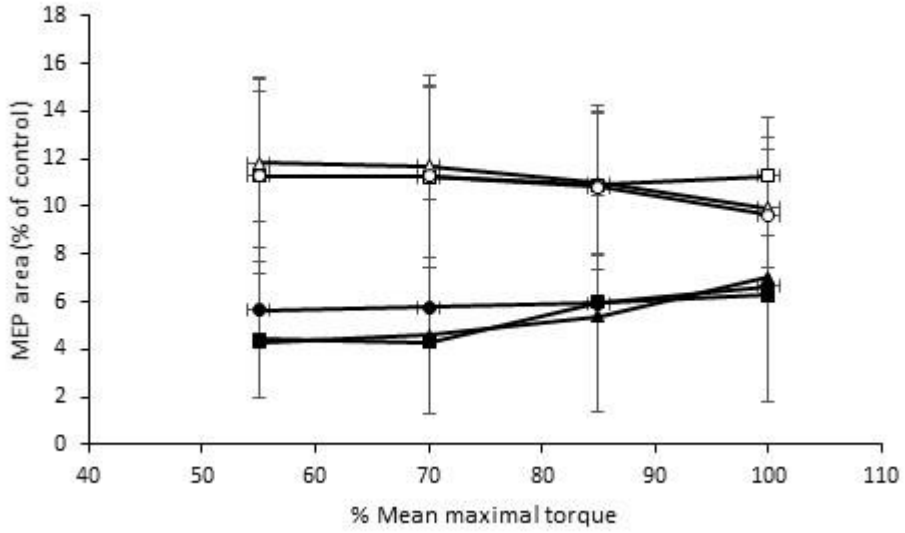


Figure 6. 2 Bland-Altman plots for maximal cortical voluntary activation, demonstrating (A) between-day reliability between the familiarisation session and the first experimental session (B) Between-day reliability between experimental session 1 & 2 and (C) within-day reliability between experimental session 2 & 3. The dotted line represents systematic bias and the bold line represents the limits of agreement.

Figure 6.3 demonstrates the minimal activation of the biceps femoris during stimulation in comparison to the vastus lateralis muscle.

a)



b)

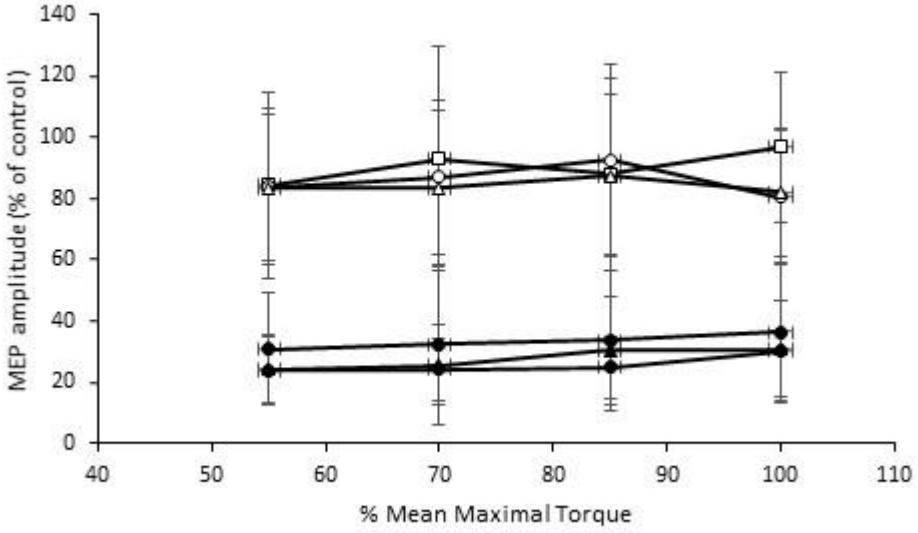


Figure 6. 3 Group mean ± S.D. MEP areas (a) and amplitudes (b) evoked from the vastus lateralis (open symbols) and the biceps femoris (closed symbols) by transcranial magnetic stimulation.

6.3.6. Thresholds

No systematic difference existed between-day or within-day for any RMT, AMT and ES threshold (Table 6.3).

6.3.7. MVF changes from the start to end of the protocol

Group mean MVF before the stimulation protocol when compared to after the protocol were not significantly different for experimental session 1, 2 or 3 (Table 6.4).

6.3.8. Computer simulation

Results of the computer simulations of 10,000 experiments can be found in tables 6.5 and 6.6.

Table 6. 1 Mean values for cortical and peripheral voluntary activation (VA), estimated resting twitch (ERT) and potentiated twitch and measures of between-day and within-day reliability. Mean values are displayed ± standard deviation. LoA= limits of agreement; ICC=intraclass correlation coefficient; CI=confidence intervals; CV=coefficient of variation.

Measure	Mean 1	Mean 2	t-test	Mean systematic bias	95% LoA	CI	ICC	CV (%)
Between-session reliability								
Cortical VA (%)	94.2 ± 4.1	93.4 ± 4.6	0.06	−0.8	−5.1, 3.4	92.9, 94.7	0.93	2.3
Peripheral VA (%)	93.8 ± 4.0	93.8 ± 4.0	0.82	− 0.1	−4.2, 4.0	93.0, 94.6	0.93	2.2
ERT (N)	100.8 ±40.1	98.5 ± 38.5	0.56	−2.3	−42.1, 37.5	91.8, 107.5	0.93	20.4
Potentiated twitch (N)	192 ± 36	194 ± 37	0.42	2.9	−32.3, 38.0	186, 200	0.94	9.3
Within-session reliability								
Cortical VA (%)	93.4 ±4.6	93.1 ±5.0	0.45	−0.3	−4.3, 3.7	92.4, 94.0	0.95	2.2
Peripheral VA (%)	93.8 ± 4.0	92.9 ± 3.8	0.07	−0.84	-5.4, 3.8	94.3, 92.4	0.89	2.5
ERT (N)	98.5 ± 38.5	91.2 ± 37.1	0.03	−7.30	−38.6, 24.0	88.6, 101.0	0.95	16.8
Potentiated twitch (N)	194 ± 37	180 ± 38	0.01	−14.37	−65.8, 37.0	177, 198	0.83	14.0

Table 6. 2 Mean values and between-day measures of reliability between the familiarisation session and the first experimental session for cortical and peripheral voluntary activation. Mean values are displayed ± standard deviation. LoA= limits of agreement; CI=confidence intervals; ICC=intraclass correlation coefficient; CV=coefficient of variation.

Measure	Familiarisation mean (%)	Session 1 mean (%)	t-test	Mean systematic bias (%)	95% LoA (%)	CI	ICC	CV (%)
Cortical voluntary activation (%)	91.1 ± 6.5	94.2 ± 4.1	0.01	3.1	−7.9, 14.1	90.4, 94.9	0.58	6.1
Peripheral voluntary activation (%)	92.8 ± 5.5	93.8 ± 4.0	0.16	1.1	−6.4, 8.5	91.8, 94.8	0.81	4.1

Table 6. 3 Mean values for electrical stimulation threshold, resting motor threshold and active motor threshold and between-day and within-day measures of reliability. Mean values are displayed ± standard deviation. LoA= limits of agreement; CI=confidence intervals; ICC=intraclass correlation coefficient; CV=coefficient of variation.

Measure	Mean 1	Mean 2	t-test	Mean systematic bias	95% LoA	CI	ICC	CV (%)
Between-session reliability								
Electrical stimulation threshold (mA)	78 ± 18	81 ± 17	0.16	−2.3	−42.1, 37.5	76, 83	0.93	20.4
Resting motor threshold (mA)	87 ± 11	87 ± 11	0.14	0.8	−4.6, 6.3	86, 88	0.98	3.2
Active motor threshold (mA)	66 ± 13	66 ± 13	0.81	−0.2	−7.9, 7.6	64, 67	0.98	6.0
Within-session reliability								
Electrical stimulation threshold (mA)	81 ± 17	81 ± 16	0.76	0.3	-8.4, 8.9	79, 83	0.98	5.45
Resting motor threshold (mA)	87 ± 11	88 ± 11	0.27	0.4	−2.9, 3.7	87, 88	0.99	1.9
Active motor threshold (mA)	66 ± 13	66 ± 13	0.75	0.1	−3.5, 3.7	65, 67	0.99	2.8

Table 6. 4 Mean values and between-day and within-day measures of reliability for MVF. Mean values are displayed ± standard deviation. LoA= limits of agreement; CI=confidence intervals; ICC=intraclass correlation coefficient; CV=coefficient of variation.

Measure	Mean 1 (N)	Mean 2 (N)	t-test	Mean systematic bias (N)	95% LoA (N)	CI	ICC	CV (%)
Between- session reliability								
Session 1 MVF	640 ± 131	626 ± 184	0.66	12.4	−64.2, 95.3	630, 660	0.97	5.77
Within-session reliability								
Session 1 MVF	626 ± 184	607 ± 194	0.02	-19.4	−197.2, 158.5	626, 657	0.97	6.09

Table 6. 5 Percentage of significant results out of 10000 simulations for between day and within day cortical VA for n=5-50 with a Cohen's d effect size set at either 0.5 or 0.8.

Cohen's d	n= 5 (%)	n= 8 (%)	n= 10 (%)	n= 12 (%)	n= 20 (%)	n= 50 (%)
Between day						
Paired Samples t test						
0.5	31.6	56.0	68.2	77.9	95.2	100
0.8	63.1	91.7	97.2	99.1	100	100
Independent t test						
0.5	9.6	13.3	16.6	19.5	29.6	62.6
0.8	17.6	28.1	34.8	40.5	62.5	95.4
ANOVA						
0.5	22.5	39.3	47.6	55.4	78.5	99.4
0.8	50.8	76.2	86.0	91.6	99.3	100
Within day						
Paired Samples t test						
0.5	46.5	75.2	87.2	93.1	99.8	100
0.8	83.8	98.7	99.9	100	100	100
Independent t test						
0.5	9.7	14.0	16.2	18.8	30.0	62.7
0.8	17.9	29.4	35.4	42.4	63.6	95.9
ANOVA						
0.5	31.4	53.1	62.9	71.4	91.6	100
0.8	66.3	89.5	95.4	98.3	100	100

Table 6. 6 Percentage of significant results out of 10000 simulations for between day and within day peripheral VA for n=5-50 with a Cohen's d effect size set at either 0.5 or 0.8.

Cohen's d	n= 5 (%)	n= 8 (%)	n= 10 (%)	n= 12 (%)	n= 20 (%)	n= 50 (%)
Between day						
Paired Samples t test						
0.5	31.6	56.0	68.2	77.9	95.2	100
0.8	67.3	93.2	97.9	99.5	100	100
Independent t test						
0.5	9.8	13.4	16.2	18.8	30.0	62.9
0.8	17.2	28.3	34.1	40.6	62.7	95.8
ANOVA						
0.5	25.6	39.3	46.9	55.3	78.3	99.4
0.8	51.2	75.8	85.4	91.7	99.3	100
Within day						
Paired Samples t test						
0.5	22.3	38.2	48.3	56.9	82.0	99.7
0.8	47.6	75.5	86.4	92.47	99.5	100
Independent t test						
0.5	9.3	12.7	14.6	17.5	26.3	56.5
0.8	16.1	25.5	30.4	38.0	57.4	93
ANOVA						
0.5	15.3	24.3	29.2	34.6	55.4	91.2
0.8	31.8	52.2	62.6	71.9	91.7	100

6.4. Discussion

The main findings of this study demonstrate that when participants are adequately familiarised and conditions, such as time of day and prior exercise, are tightly controlled, TMS provides a reliable between-day (CV = 2.3%) and within-day (CV = 2.2%) estimate of maximal M1 cortical voluntary activation for the knee extensors. The level of reliability seen for TMS was comparable to that of electrical stimulation (CV= 2.2 & 2.5%, between-day and within-day, respectively) providing rationale for its use in conjunction with electrical

stimulation to provide a more detailed understanding of muscle function and specifically the contribution of the motor cortex (Table 6.1). The incorporation of a between-day and within-day design also supports the use of this technique in acute and repeated-measures research designs. Secondary measures which were assessed showed variable levels of reliability, with motor thresholds demonstrating high within- and between-day reliability (Table 6.3), whereas M-wave and MEP responses demonstrated a substantial amount of between- and within-day variability. Therefore care must be taken when interpreting this data.

The CV values in previous reliability studies (3.7% & 3.1%), with participant numbers of 9 and 8, were higher than that of the present study (Goodall et al., 2009; Sidhu et al., 2009). The values demonstrate good reliability however by using a much greater sample size in the present study, which is essential for reliability research to ensure greater statistical power and prevent false discoveries greater reliability is demonstrated (Heroux et al., 2015).

Voluntary activation and ERT

The findings of the present study demonstrate that the determination of M1 cortical voluntary activation between-day (CV=2.3%) and within-day (CV=2.2%) is reliable (Table 6.1). The negative linear relationship seen between TMS-evoked SIT produced by the quadriceps following stimulation by TMS and increasing voluntary contraction resulted in a reliable between-day estimation of ERT; however, a difference was shown within-day (*P=0.03*) with a systematic bias of −7N; and random error of 31N (Table 6.1). The CV values for ERT were comparable to those of Sidhu et al (2009) for within-day difference (16.8% in the present study versus 15.3%), as were the ICC values (0.95 in the present study vs 0.98). Sidhu et al (2009) investigated the influence of using different intensities of voluntary contraction in order to create a linear regression and estimate resting twitch. As a result we utilised contraction intensities between 50% and 100% to create the linear regression, as this was found to be the most reliable method for estimation of resting twitch amplitude (Sidhu et al., 2009). Despite a significant difference for within-day ERT, the determination of maximal M1 cortical voluntary activation utilising TMS, the primary measure of supraspinal fatigue, demonstrated high within-day and high between-day reliability.

The random error component displayed in this study is smaller than that of Goodall et al (2009) therefore demonstrating that the TMS technique is more reliable at determining M1 cortical voluntary activation than initially indicated in a smaller unfamiliarised participant

group. Similarly, our reliability coefficients exhibit good levels of reliability with an ICC of 0.93 for between-day and 0.95 for within-day (Table 6.1). These values are similar to those found by both Goodall et al (2009) and Sidhu et al (2009) ICC=0.94 and ICC=0.95, respectively, for between-day M1 cortical voluntary activation. Collectively our findings, and those of Sidhu et al (2009) and Goodall et al (2009), indicate that the interpolated twitch technique using TMS can provide a reliable estimation of maximal M1 cortical voluntary activation of the knee extensors, therefore providing important implications for research involving locomotion or full-body exercise.

In agreement with Sidhu et al (2009) levels of voluntary activation measured with motor nerve stimulation were similar to those measured with TMS (between-day mean for M1 cortical voluntary activation was 93.8% versus 93.8% for peripheral and within-day mean for M1 cortical voluntary activation was 93.3% versus 93.4% for peripheral). Therefore, the high levels of reliability demonstrated for peripheral and M1 cortical voluntary activation both in the current study and in the literature (Amann et al., 2013; Goodall et al., 2015b; Sidhu et al., 2009) support the use of electrical stimulation and TMS in parallel to provide an extensive understanding of the diverse contributions to fatigue.

Table 6.5 and 6.6 provides information regarding the statistical power for a range of study designs and sample sizes. This information can be used to inform future studies on an appropriate participant number for a specific technique (e.g. the interpolated twitch techniques using either TMS or electrical stimulation) and study design (e.g. within-day or between-day).

Motor evoked potentials

The largest MEP amplitude was seen at a contraction intensity of 55% of MVF. In agreement with previous literature in the knee extensors (Goodall et al., 2009; Sidhu et al., 2009), elbow flexors (Todd et al., 2003; Todd et al., 2004) and wrist extensors (Lee et al., 2008) increasing the contraction intensity caused minimal changes in MEP amplitude beyond the value achieved at 55% MVF. The findings of this study suggest a large amount of variability exists for MEP characteristics both within-day and between-day. Between-day mean amplitude value for ICC was 0.61. Within-day mean amplitude ICC was 0.82. The values for systematic bias and random error in the current study suggest EMG both between-day and within-day may not provide a reliable indication of corticospinal excitability. The variation in MEP data

is greater than that seen in Goodall et al (2009), but similar to that of Sidhu et al (2009). These results demonstrate questionable levels of reliability (Atkinson & Nevill, 1998), as all values fall below the 0.9 threshold associated with high reliability. Mathur et al (2005) assessed the reliability of EMG measurements in the knee extensors and reported ICC values ranging from 0.58 to 0.99 for amplitude. The findings highlight variability exists and care must be taken when utilising EMG data to assess corticospinal excitability. This variability could be linked to the variability in Mmax shown in the current study, which may have influenced results during normalization of MEPs.

The size of an MEP can vary greatly between one stimulation and the next (Magistris et al., 1998). The variability may be due to the large number of contributing factors influencing MEP characteristics, including the excitability of the motor cortex and nerve roots and the conduction along the peripheral motor pathway of the muscles (Kobayashi & Pascual-Leone, 2003). Therefore, the intrinsic fluctuations in neural excitability at both the cortical and spinal levels may have resulted in highly variable MEP amplitudes (Rossini et al., 1994). Variation in EMG recordings has also been commonly attributed to changes in the orientation of the recording electrodes and minor difference in skin preparation (Mathur et al., 2005) and a number of other non-physiological factors such as subcutaneous tissue, motor unit synchronisation and signal cancellation (Buckthorpe et al., 2012). Despite effort being made to eradicate these issues in the present study, it is still possible that these factors may have influenced the reliability of the technique. Therefore, in agreement with Buckthorpe et al (2012) , individual EMG data is highly variable between measurement sessions, whether it has been normalised or not.

Limitations and future considerations

This study included all participants regardless of r^2 value for linear regression, with the aim of providing a truly random sample. Data was re-analysed, omitting 3 participants due to R squared<0.85 in accordance with Todd et al (2016), demonstrating lower, yet still good reliability for M1 cortical voluntary activation (ICC; between-day=0.87 vs 0.93 and within-day=0.89 vs 0.95). Therefore, this data supports the argument for analyzing data separately due to the differing findings, however still supports the technique as reliable in the determination of M1 cortical voluntary activation.

The TMS intensity in the present study is higher than that used in a number of other studies (Goodall et al., 2009; Sidhu et al., 2009). Participants in this study were not screened to ensure a low stimulation threshold, which may account for the higher stimulation intensity observed. Co-activation should not influence results as the thresholds are likely to be similarly high for both the quadriceps and hamstrings, hence not evoking a disproportionate amount of hamstring activity, as previously suggested with high stimulation intensities. Additionally, the intensity of thresholds may inevitably vary to some extent between stimulators.

6.5. Conclusions

The findings of the present study suggest that maximal M1 cortical voluntary activation of the knee extensors can be reliably estimated between-day (CV=2.3%) and within-day (CV=2.2%) using TMS following familiarisation, allowing the determination of supraspinal fatigue. Further, the incorporation of the computer simulation techniques enables the findings of this study to be applied to future research to enable justification of adequate participant numbers. The use of both a between-day and within-day design also supports the use of this technique in research which may require repeated measures during acute and chronic research protocols and designs. Therefore, TMS can be used reliably to estimate the extent to which output from the motor cortex influences muscle fatigue during lower body exercise.

CHAPTER 7: THE INFLUENCE OF A SIMULATED FOOTBALL MATCH ON NEUROMUSCULAR FUNCTION AND COGNITIVE FUNCTION IN THE HEAT

7.1. Introduction

Performance in team sports is dictated by the ability to frequently produce numerous skilful activities, whilst performing exercise of varied intensities interspersed with brief recovery intervals (Bishop & Girard, 2013). There are both physical (Bangsbo, Mohr & Krustrup 2006) and cognitive demands (Pesce et al., 2007) placed on an athlete, which presents a unique challenge in terms of optimising performance, particularly in extreme environments. Until recently no study had assessed the exact mechanisms contributing to changes in neuromuscular performance during intermittent exercise (Goodall et al., 2015a; Goodall et al., 2017). Output from the motor cortex can affect performance in football from a physical output standpoint (Goodall et al., 2017), however alterations in brain activity during this type of activity may also be important for optimising cognitive function. Therefore, the mechanisms involved in maintaining both of these aspects of function could prove crucial in both the preparation for, and the recovery from, high intensity intermittent exercise. This would be particularly true of competitions taking place in hot climates, where both physical (Tatterson et al., 2000; Tucker et al., 2004) and mental fatigue (Bandelow et al., 2010; Gaoua et al., 2011; Gaoua et al., 2012) can be exacerbated (Gonsalez-Alonso et al., 1999).

The contributions to neuromuscular fatigue during intermittent exercise could be a result of peripheral contractile failure, sub-optimal motor cortical output (supraspinal fatigue) and/or altered afferent inputs (spinal fatigue) innervating the active musculature (Gandevia, 2001; Minnett & Duffield, 2014; Goodall et al., 2015a; Goodall et al., 2017). A number of studies have assessed the neuromuscular response to intermittent exercise (Girard et al., 2015; Rampini et al., 2011) and intermittent exercise in the heat (Nybo et al., 2013; Periard et al., 2014a), focusing on the peripheral contribution to fatigue. Very few studies have assessed the supraspinal contribution to fatigue during intermittent exercise (Goodall et al., 2015a; Goodall et al., 2017). The study by Goodall et al (2015a) was the first to incorporate electrical stimulation and magnetic stimulation following repeated sprint running activity, hence assessing the contribution of supraspinal fatigue. However, it was not until Goodall et al (2017) utilised a simulated football protocol that true team sport exercise had been assessed. This study found a progressive increase in both peripheral and cortical voluntary activation during a football match, decreasing at half-time, full-time and extra-time. Hence this is the first study to highlight the central and peripheral contributions to fatigue in team sport exercise, detailing the time-course and magnitude of peripheral and central fatigue.

Understanding the relationship between intermittent exercise and both cognitive function and neuromuscular performance is essential, however the added effect of heat must also be considered. Athletes are frequently required to compete in hot climates, where changes in core and skin temperatures are likely (Gonzalez- Alonso et al., 1999; Sunderland & Nevill, 2005). Changes in core temperature are known to influence neuromuscular function (Racinais & Girard, 2012). However, both tennis (Periard et al., 2014a) and football (Girard et al., 2015; Nybo et al., 2013) matches in the heat have resulted in similar alterations in neuromuscular function of the plantar flexors (Periard et al., 2014a; Girard et al., 2015; Nybo et al., 2013) and knee extensors (Periard et al., 2014a) as following a match in moderate conditions. The influence of intermittent exercise in the heat on neuromuscular function, and more specifically suprapsinal fatigue, is warranted in the knee extensors. This is vital in terms of recovery strategies, due to the known contribution of delayed onset muscle soreness in altering neuromuscular recruitment or activation patterns (Cheung et al., 2003; Minnett & Duffield, 2014), therefore understanding the relative contributions to fatigue will enable clearer focus in terms of recovery strategies.

In addition to the limited understanding regarding the aetiology of neuromuscular fatigue in team sports, particularly in the heat, the changes seen in cognitive function and the resulting impact on skill performance are also unknown. At present only one study has implemented an exercise intervention which replicates true team sport performance in order to establish how cognitive function is affected (Bandelow et al., 2010). This study looked only at the response during a match in the heat, providing no suitable control condition. However, this study did demonstrate a relationship between higher core temperatures and a decrement in cognitive performance. Hence, the study we conducted, which is described in chapter 5, is the first to detail the cognitive alterations during a competitive hockey match. Our findings suggest this type of stress positively influences a number of cognitive domains. However, the impact of added environmental stress creates a greater issue for performance, due to the added challenge of maintaining homeostasis (Sunderland & Nevill, 2005). It is known that intermittent exercise in the heat detrimentally influences skill performance (Sunderland & Nevill, 2005), which is tightly linked to cognitive function (Starkes et al., 1987), however only recently has cognitive function been directly assessed in the heat (MacLeod et al., 2018), where a beneficial response was seen.

The mechanisms contributing to performance changes in cognitive function are extensive. Arousal levels have long been implicated in changes in cognitive function (Yerkes & Dodson,

1908; McMorris et al., 2006b). Concentrations of adrenaline, noradrenaline and cortisol are known to increase in response to stress (McMorris et al., 2006b), with research showing these hormonal changes significantly influence cognition. Additionally, Ferris et al (2007) showed BDNF concentration, a known contributor to neuron health, to increase in response to exercise, in an intensity dependant manner whereby higher intensities of exercise incurred greater increases in serum BDNF. This could be of direct relevance to team sport performance as shown in chapter 5 where increased BDNF levels are associated with improved cognitive function, which is in agreement with a number of other studies (Erickson et al., 2009; Rex et al., 2006; Egan et al., 2003; Grassi-Oliveira et al., 2008). Therefore it is possible that all of the discussed hormones contribute to changes in cognitive function in response to stress and therefore can provide additional information regarding the mechanisms involved.

The aim of this study was to establish how performing intermittent exercise in the heat will influence neuromuscular and cognitive function which contribute to overall performance in team sports. Analysing hormonal changes and measures of central and peripheral activation will allow us to understand the mechanisms involved in the changes in both cognitive function and neuromuscular function. It was hypothesised that both cognitive function and neuromuscular function would be detrimentally influenced by the combination of intermittent exercise and heat stress.

7.2. Methods

Participants

Seventeen well-trained, un-acclimatised male team sport players volunteered to participate and provided informed consent for this study. A health screen was completed to ensure all participants were in good health. The mean (± SD) age, body mass, height and VO_{2max} of the participants who completed the study were 22 ± 2yrs, 82.3 ± 11.9 kg, 180.7 ± 4.9 cm, and 52.6 ± 7.1 ml.kg.min^{-1}, respectively. A full description of participant recruitment and ethical approval are presented in sections 3.2. and 3.3.1.

7.2.1. Study Design

All data were collected at Nottingham Trent University. Each participant completed two familiarisation trials, a control trial in moderate conditions (15 º C, 50 % Rh) and a trial in hot conditions (33 º C, 50 % Rh). Both main trials were performed in a purpose built environmental chamber (Design environmental; UK) in a crossover order-balanced` design.

Each main trial was separated by exactly 7 d and performed at the same time of time of day to eradicate any influence of circadian rhythm. Pre-trial restrictions are detailed in section 3.3.5. Participants were requested to arrive at the laboratory 2 h post prandial, having consumed a minimum of 500 ml of water ~ 2 h prior to arrival.

Each main trial consisted of two 45 min bouts of the football specific treadmill protocol (FSITP) (Greig et al, 2006). Cognitive function and neuromuscular function testing was completed at baseline and immediately following each 45 min bout of exercise (Figure 7.1).

7.2.2. Protocol

7.2.2.1. Familiarisation

Familiarisation 1 was completed 2 weeks prior to the first main trial. Familiarisation 2 was completed 1 week prior to the first main trial.

Familiarisation 1. On the participants' first visit to the laboratory their height (Seca 213 portable stadiometer; UK) and nude body mass (Adam GFK 150; UK) were measured, and a speed lactate and VO_{2max} test was performed. Participants also became familiar with producing force on the isometric dynamometer. Additional details of familiarisation procedures can be found in section 3.3.2.

7.2.2.2. Speed lactate test

The speed lactate test was made up of 3 min stages performed at a 1% incline on a treadmill (h/p/cosmos Para Graphics, Nussdorf-Traunstein, Germany). The speed started at 9 km/h and increased in by 1 km/h at the beginning of each new stage. Expired air was collected in the final min of each stage, and a capillary blood sample for the determination of blood lactate was taken, at the completion of each stage (MacLeod et al., 2018). This test continued until the lactate concentration threshold was achieved, which was defined as the fastest speed with less than a 1 $mmol.L^{-1}$ increase in blood lactate concentration above the preceding levels (Astrand et al., 2003). Lactate was analysed using the methods described in section 3.4.8.3.

7.2.2.3. Maximal oxygen uptake test

Following 10-min static rest participants performed a VO_{2max} test (MacLeod et al., 2018). This test was completed at the speed preceding the lactate threshold. The test begun at a 0% gradient and increased by 1% every minute until the participant indicated they could only complete one more minute. An expired air sample was collected in a douglas bag for the final minute of exercise. Expired air was analysed using a digital gas analyser (Servomex Group Ltd, Sussex, UK). A Harvard dry gas meter (Harvard apparatus, Cambridge, UK) was used to

assess both volume and temperature of the expired air. The VO_{2max} values allowed a regression analysis to take place and therefore provide accurate speeds for the various stages of the FSITP (table 7.1).

Familiarisation 2. Participants completed a full run through of the first half of the protocol; neuromuscular function testing, cognitive function testing, and a 45 min bout of FSITP at the same conditions as the hot trial (33 º C & 50 % Rh).

7.2.2.4. Main trial

Participants arrived 2 h post-prandial and a urine sample was immediately collected and analysed to measure urine osmolality (Pocket-Pal Osmo-Osmocheck™, 4595-E04, Vitech-Scientfic Ltd, Horsham, UK). Following this, nude body mass (GFK 150 AEADAM digital scale, Vitech scientific Ltd) was measured in private. A full description of urine osmolality measurement and body mass measurements are presented in sections 3.4.6. and 3.3.4, respectively. These measures were completed following the completion of each trial, which combined with urine output and water intake, was used to estimate hydration status. Prior to entering the chamber participants self-inserted a disposable rectal probe (MEAS 440 Series Temperature Probe, Measurement Specialities Inc, USA) in order to monitor core temperature throughout the trial using the core temperature logger (4600 Thermometer, Measurement Specialities Probe, Ohio, USA). Full details of core temperature measurement can be found in section 3.4.4.3.

7.2.3. Physiological measurements

Figure 7.1 provides an overview of the main trial requirements. All physiological measurements were assessed at 14 time points throughout each main trial (baseline, 0mins, 10mins, 20mins, 30mins, 40mins, 45mins, Post CF during each half of the FSITP).

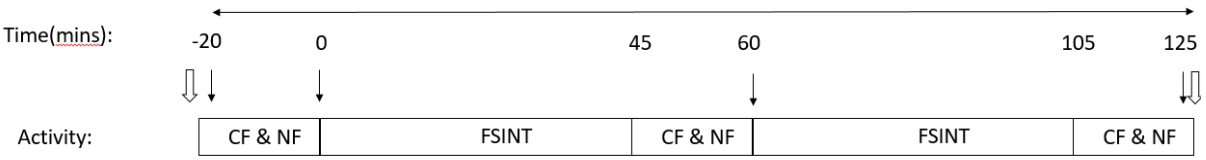


Figure 7. 1 Protocol diagram. CF = cognitive function, NF = neuromuscular function, FSINT = football specific intermittent treadmill protocol. ⇩= Body mass and urine osmolality measurement. ↓= Blood sample.

7.2.3.1. Skin temperature

Skin temperature was measured at the thigh using a skin thermistor (EUS-U-VL2-0, Grant instruments, UK). This was attached to the midpoint of the thigh and held in place with tape, adhesive strips and bandaging.

7.2.3.2. Oxygen saturation

Oxygen saturation was assessed using a portable pulse oximeter, which was attached to the left index finger during each measurement (Nonin Puresat; Minnesota, USA).

7.2.3.3. Blood flow

The laser Doppler technique was utilised to monitor changes in cerebral and quadriceps blood flow (Laser Doppler Perfusion MonitorPeriFlux System 5000 Perimed Instruments Ltd., Hoddesden, Hertfordshire, England). A full description of laser doppler procedures can be found in section 3.4.4.3.

7.2.4. Perceptual Measures

Rating of perceived exertion, thermal sensation, feeling and felt arousal were all measured throughout the protocol. A full description of the scales and analysis can be found in section 3.4.5. and appendix C.

7.2.5. Blood parameters

Blood samples were taken at 4 time points; -30, 0, 45 and 140 min. A full description of blood sample collection, storage and analysis can be found in section 3.4.8.

Plasma was analysed for adrenaline and noradrenaline using an immunoassay. Plasma and serum were analysed for brain derived neurotrophic factor using separate immunoassay procedures. Serum was analysed for cortisol and cathepsin B using an immunoassay. Full details of the analyses procedures can be found in section 3.4.8.

Glucose and lactate were analysed using an automated analyser (Biosin c_line , EKF diagnostic). 20 microlitres of whole blood was collected in sodium heparinized plastic capillary tubes which was shaken in a pre-prepared Eppendorf filled with glucose/lactate hemolyzing solution. Haematocrit was analysed in triplicate and haemoglobin was measured in duplicate. Full details of the procedures can be found in section 3.4.8. Changes in plasma volume could then be calculated via the method of Dill and Costill (1974).

7.2.6. Football Specific Intermittent Treadmill Protocol (FSITP)

Participants performed the FSITP in hot (33 º C & 50% Rh) or moderate (15 º C & 50% Rh) conditions. The FSITP was made up of 2 x 45 min blocks of activity, separated by a 15 min

rest period to simulate half-time. The entire protocol was completed on a motorised treadmill (h/p/cosmos Para Graphics, Nussdorf-Taunstein, Germany) at a 1% gradient in order to simulate outdoor running (Figure 7.2). The treadmill speeds were individualised and determined by participants VO_{2max} results. The various exercise bouts fell under 7 different categories, which we interspersed throughout the 45 min protocol in a manner which replicated a football match, more details regarding the intensities of the various bouts can be found in Table 7.1.

Measurements

7.2.7. Neuromuscular function

Participants completed neuromuscular testing on a custom-built dynamometer, designed to measure knee extensor force (Johnson et al., 2015). A full description of the testing protocol and data analysis procedures can be found in section 3.4.3.

Table 7. 1 Speeds and durations of the various levels of the football specific intermittent treadmill protocol

Activity	Speed	Mean duration (s)
Standing	0	7.8
Walking	4	6.7
Jogging	Speed before lactate threshold	3.5
Low speed	85% VO_{2max}	3.5
Moderate speed	100% VO_{2max}	2.5
Fast run	21	4.0
Sprint	25	4.0

Figure 7. 2 Participant completing the FSINT protocol.

7.2.8. Cognitive function

A battery of cognitive function tests were completed at baseline, half-time and full-time. The battery of tests took 15 min and consisted of the Visual Search test, the Stroop test, the Corsi blocks test and the RVIP test. All three batteries of cognitive tests were completed inside the environmental chamber. A full description of cognitive tests and analysis procedures are presented in section 3.4.1.

7.2.9. Data analysis

Neuromuscular data, physiological data, perceptual measures and corsi blocks data were all analysed using SPSS (Version 23, SPSS Inc., Chicago, Il, USA) via two way repeated measures Analysis of Variance (ANOVA), using a trial by session time approach. Where paired comparisons were required, paired samples t-tests with Bonferonni corrections were conducted.

Voluntary activation, Mmax and MEP were determined using the equations and methods detailed in section 3.4.3.

The cognitive data (Stroop, visual search and RVIP) were analysed using R (www.r-project.org). A full description of cognitive data analysis procedures is presented in section 3.4.1.5.

The effect size (Cohen's d) of all significant differences were calculated using trial pairings and interpreted using the following thresholds: <0.2 = trivial effect; 0.2-0.5 = small effect; 0.5-0.8 = moderate effect and >0.8 = largest effect (Cohen, 1992). For all analysis, significance was set as P<0.05. Data are presented as mean ± standard deviation.

7.3. Results

7.3.1. Cognitive Function

Mean data for all cognitive function tests can be found in table 7.2.

7.3.1.1. Visual Search

Response Times

Simple: Response times were slower on the hot trial (main effect of trial $t_{(1, 4195)}$ = -2.62, P<0.01, d = 0.06). Response times did not differ across time (main effect of time, P = 0.16). However response times slowed to a greater extent in the hot than the moderate trial, with a 30 ms slower time in the hot compared to a 5 ms slower time in the moderate trial from pre to full-time (trial*time interaction $t_{(1, 2096)}$ = 3.10, P<0.01; Figure 7.3).

Complex: The hot environment did not influence response times (main effect of trial, P = 0.30, d = 0.25), and there was no change in response time across time (main effect of time, P = 0.67). This resulted in a similar pattern of change between trials (trial*time interaction, P = 0.06).

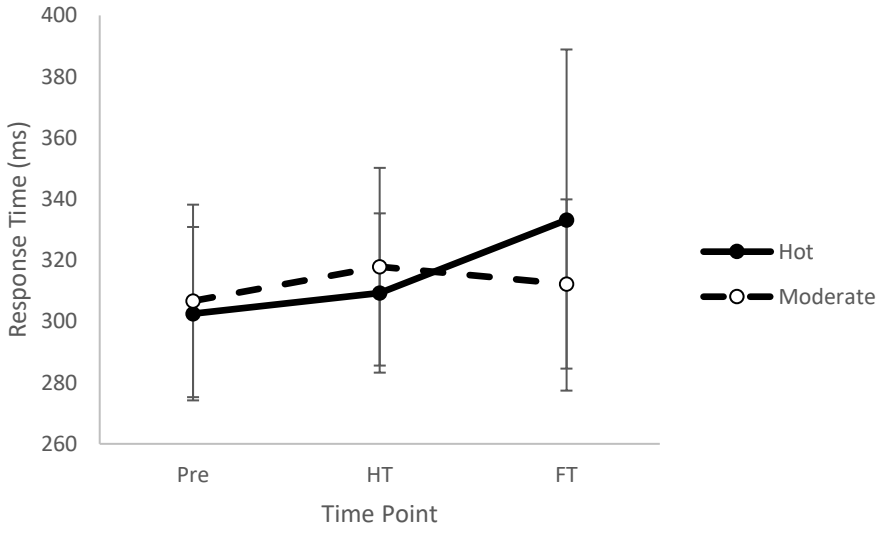


Figure 7. 3 Baseline level response times for Visual Search. Data are mean ± SD. Main effect of trial, P<0.01 and trial*time interaction, P<0.01.

Table 7. 2 Cognitive function data (mean ± SD) across the control and hot trials. Pre = baseline, HT = half-time and FT = full-time. * P <0.05.

Test	Variable	Test Level	Control			Hot			Trial effect	Time effect	Interaction	Effect size
			Pre	HT	FT	Pre	HT	FT				
Visual Search	Response Time (ms)	Simple	307 ± 31	318 ± 32	312 ± 28	303 ±28	309 ± 26	333 ±56	<0.01*	0.16	<0.01*	0.06 (trivial)
		Complex	1126 ± 144	1119 ± 157	1126 ± 170	1120 ± 209	1187 ± 209	1232 ± 256	0.26	0.67	0.06	0.25 (small)
	Accuracy (%)	Simple	97.4 ± 3.2	98.3 ± 3.2	98.2 ± 2.8	98.5 ± 3.3	97.7 ± 3.4	96.4 ± 3.9	<0.01*	0.01*	<0.01*	0.06 (trivial)
		Complex	96.1 ± 4.0	98.7 ± 2.1	96.4 ± 6.0	97.2 ± 5.0	95.8 ± 5.5	96.5 ± 4.2	0.93	0.98	0.70	0.08 (trivial)
Stroop Test	Response Time (ms)	Simple	641 ± 79	677 ± 147	691 ± 146	660 ± 125	658 ± 108	688 ± 122	0.68	<0.01	0.65	0.00 (trivial)
		Complex	865 ± 162	855 ± 158	813 ± 174	902 ± 171	920 ± 195	906 ± 222	0.85	<0.01	0.02	0.36 (small)
	Accuracy (%)	Simple	98.7 ± 2.8	96.4 ± 4.5	97.5 ± 3.5	98.3 ± 3.1	97.8 ± 3.4	97.3 ± 4.4	0.99	0.37	0.92	0.08 (trivial)
		Complex	97.1 ± 4.0	94.7 ± 5.1	96.8 ± 3.5	97.1 ± 4.7	97.2 ± 4.1	95.3 ± 6.4	0.38	0.84	0.44	0.06 (trivial)
Corsi Blocks	Sequence Length		6.3 ± 0.8	6.4 ± 0.9	6.6 ± 0.8	6.2 ± 1.0	6.2 ± 0.8	6.5 ± 0.7	0.21	0.44	0.89	0.17 (trivial)
RVIP	Response Time (ms)		471 ± 132	459 ± 132	489 ± 64	514 ± 67	518 ± 102	512 ± 83	0.26	0.29	0.93	0.42 (small)
	Accuracy (%)		50.2 ± 21.4	50.3 ± 18.9	51.2 ± 22.0	44.4 ± 21.3	52.4 ± 22.4	50.2 ± 23.9	0.08	0.51	0.22	0.10

Accuracy

Simple: Overall, accuracy was lower on the hot trial at 97.5 % (main effect of trial, $z_{(1, 2161)}$ = 2.92, P < 0.01; d = 0.06, trivial effect; table 7.2), compared to the moderate trial, with an accuracy of 98.0 %. Accuracy got worse across time (main effect of time, $z_{(1,2161)}$ = 2.47, P = 0.01). Accuracy decreased in the hot trial from pre to full-time by 2.1 %, whereas it improved in the moderate trial by 0.8 % (trial* time interaction $z_{(1,2161)}$ = -2.95, P < 0.01; Figure 7.4).

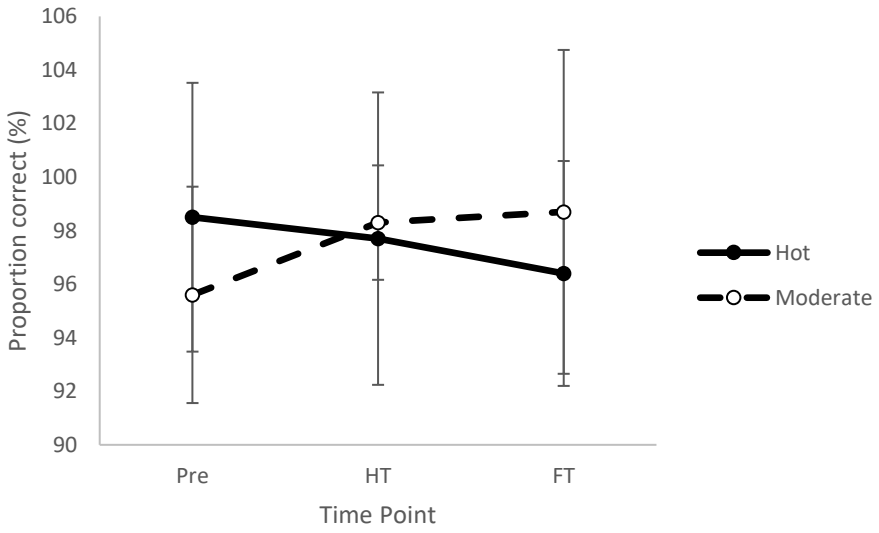


Figure 7. 4 Proportion correct on the baseline level of the Visual Search test. Data are mean ± SD. Main effect of trial, P<0.01. Main effect of time, P<0.01. Trial*time interaction, P<0.01.

Complex: Accuracy was similar between trials (main effect of trial, P = 0.93), across time (main effect of time, P = 0.98) and the pattern of change did not differ (trial*time interaction, P = 0.70).

7.3.1.2. Stroop test
Response Times

Simple: There was no effect of trial on response times (main effect of trial, P = 0.68). Response times got slower across time (main effect of time, $t_{(1,1332)}$ = 2.99, P < 0.01), however the pattern of change did not differ (trial*time interaction, P = 0.65).

Complex: Response times did not differ between trials (main effect of trial, P = 0.85; d = 0.36, small effect; table 7.2), however they got better across time (main effect of time, $t_{(1, 1354)}$ = -3.20, P < 0.01). Response times got worse in the hot trial across time by 18 ms from baseline

to half-time, whereas they improved in the moderate trial by 10 ms during that time (trial*time interaction, $t_{(1,1354)} = 2.31$, P = 0.02; Figure 7.5).

Accuracy

Simple: There was no difference in accuracy between trials (main effect of trial, P = 0.99) and accuracy did not differ across time (main effect of time, P = 0.37). This resulted in a similar pattern of change between trials (trial*time interaction, P = 0.92).

Complex: Complex accuracy followed a similar pattern to that of the simple level, with no effect of trial (main effect of trial, P = 0.38), time (main effect of time, P = 0.84) and as a result a similar pattern of change between trials (trial*time interaction, P = 0.44).

7.3.1.3. Corsi Blocks

No effect on the number of correctly remembered sequences on the working memory task was seen between trials (main effect of trial, P = 0.21) and the sequence length did not change across time (main effect of time, P = 0.44). There was no difference in the pattern of change for sequence length (trial*time interaction, P = 0.89).

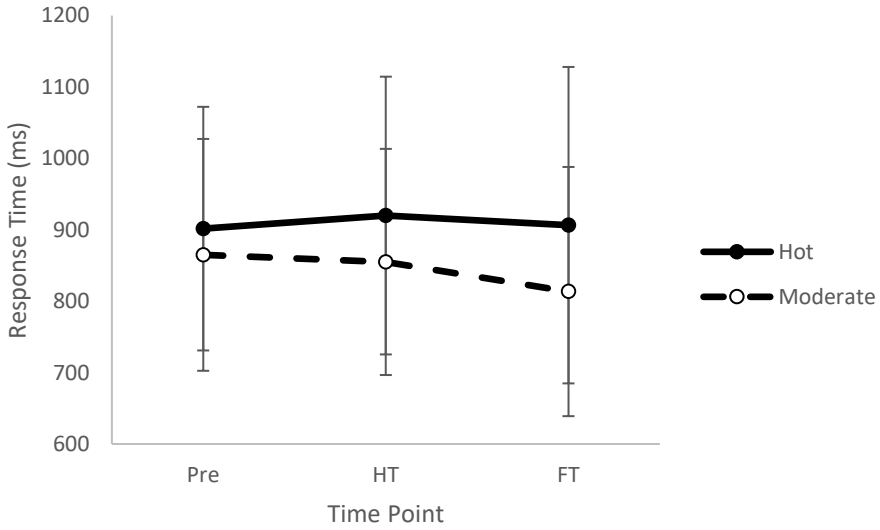


Figure 7. 5 Response times on the complex level of the Stroop test. Data are mean ± SD. Main effect of time, P<0.01. Trial*time interaction, P = 0.02.

7.3.1.4. RVIP
Response times

Response time was similar between trials (main effect of trial, P = 0.26), and were well maintained across time (main effect of time, P = 0.29). Therefore, the rate of change was not different between trials (trial*time interaction, P = 0.93).

Accuracy

Accuracy did not differ between trials (main effect of trial, P = 0.08), or across time (main effect of time, P = 0.51). This resulted in a similar pattern of change, (trial*time interaction, P = 0.22).

7.3.2. Neuromuscular Function

7.3.2.1. Cortical voluntary activation
Cortical voluntary activation was lower in the hot trial (Main effect of trial, $F_{(1,14)} = 5.26$, P = 0.04; d = 0.53, moderate effect; Figure 7.6) and decreased across time (Main effect of time, $F_{(2,28)} = 21.57$, $p<0.01$). However the pattern of change did not differ between trials (trial*time interaction, P = 0.10). For individual data, see Appendix C5

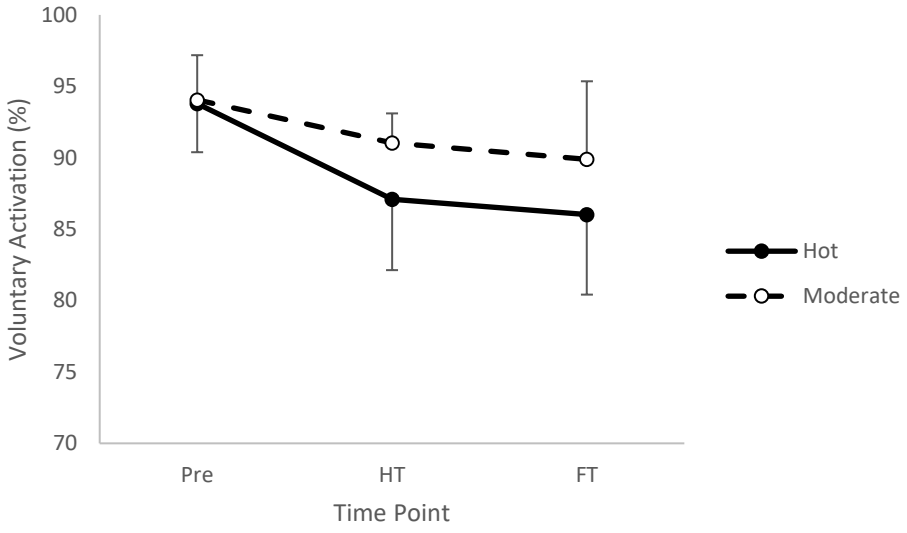


Figure 7. 6 Cortical voluntary activation values for the hot and moderate trials at baseline (Pre), half-time (HT) and full-time (FT). Main effect of trial, P = 0.04. Main effect of time, P<0.01.

7.3.2.2. Peripheral voluntary activation
Peripheral voluntary activation was lower in the hot trial (Main effect of trial, $F_{(1, 14)} = 12.36$, P<0.01; d = 0.62, moderate effect) and decreased across time (Main effect of time, $F_{(2,28)} = 23.00$, P<0.01). The pattern of change differed between trials (trial*time interaction, $F_{(2, 28)} =$

6.83, P < 0.01), with voluntary activation being worse in the hot trial at half-time (*post hoc*, P<0.01) and full-time (*post hoc*, P<0.01; Figure 7.7), compared to the corresponding time points in the moderate trial. For individual data, see Appendix C5.

7.3.2.3. Electromyography
M-wave

There was no effect of trial (P = 0.18), or time (P = 0.86) and no significant trial*time interaction (P = 0.21) for M-wave amplitude.

There was no main effect of trial (P = 0.82) and no trial*time interaction for M-wave area. However, M-wave area decreased across time ($F_{(1, 28)}$ = 5.89, P < 0.01).

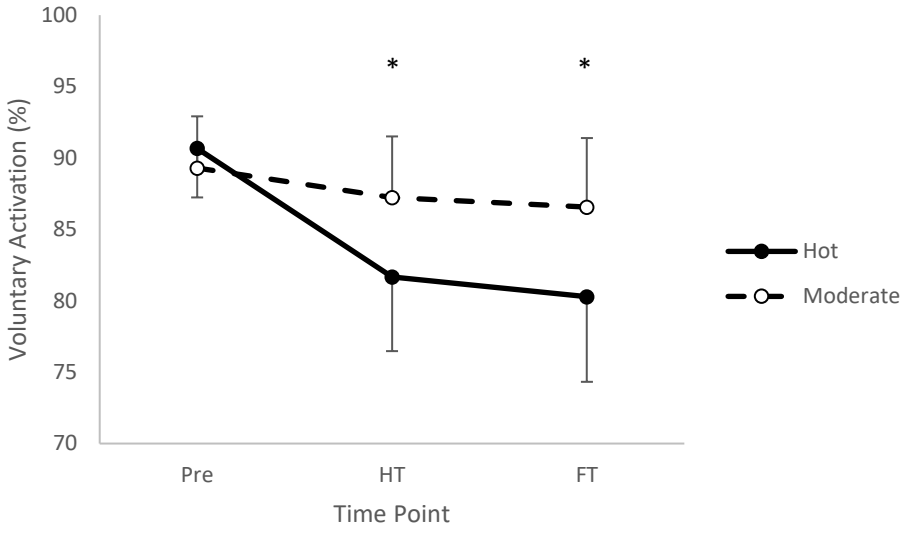


Figure 7. 7 Peripheral voluntary activation values for the hot and moderate trials at baseline (Pre), half-time (HT) and full-time (FT). * = time points where hot < moderate. Main effect of trial, P<0.01. Main effect of time, P<0.01. Trial*time interaction, P<0.01.

MEP

There was no effect of trial (P = 0.10), time (P = 0.59) and no significant trial*time interaction (P = 0.787) for MEP amplitude.

There was no effect of trial (P = 0.20) and no trial*time interaction (P = 0.60) for MEP area, however MEP area decreased over time (main effect of time, $F_{(1,28)}$ = 3.52, P = 0.04).

7.3.2.4. Potentiated twitch

Changes in potentiated twitch force did not differ between trials (main effect of trial, P = 0.68; d = 0.04, trivial effect; Figure 7.8), however decreased across time in both conditions (main effect of time, $F_{(1,14)} = 9.06$, P<0.01). The change in potentiated twitch from baseline in the hot trial was 11% at half-time and 7% at full-time. The change in potentiated twitch from baseline in the moderate trial was 3 % at half-time and 1% at full-time. However, there was no difference in the rate of change (trial*time interaction, P = 0.13).

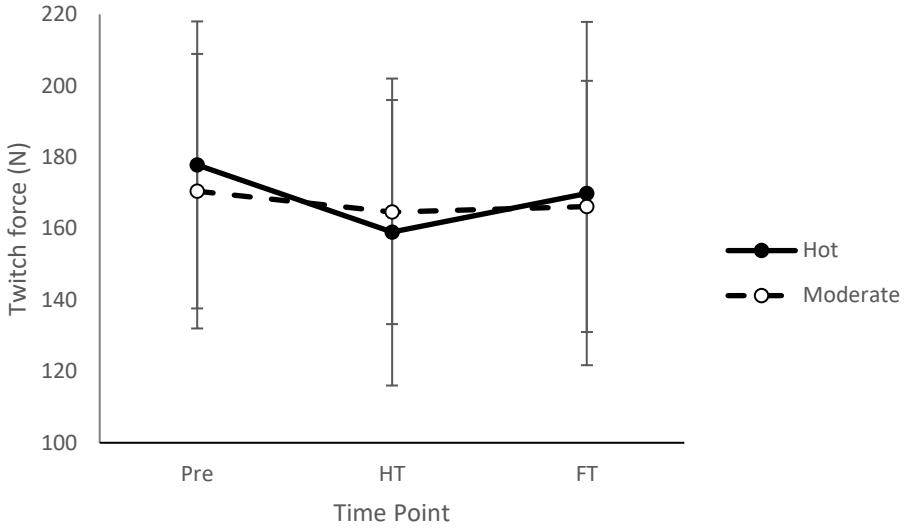


Figure 7. 8 Potentiated twitch force during the hot and moderate trials. Main effect of trial, P = 0.68. Main effect of time P<0.01. Trial*time interaction, P = 0.13.

7.3.3. Heart rate

Heart rate was greater in the hot trial (main effect of trial, $F_{(1,16)} = 59.92$, P<0.01; d = 0.40, small effect). Heart rate changed across time (main effect of time, $F_{(1, 13)} = 465.86$, P<0.01). The pattern of change in heart rate differed in the heat (trial*time interaction, $F_{(1,13)} = 6.69$, P<0.01; Figure 7.9), where heart rate was greater in the heat from 20 min onwards (P<0.01) compared to the corresponding time points in the moderate condition.

7.3.4. Core temperature

Core temperature was greater in the hot trial (main effect of trial, $F_{(1,16)} = 9.74$, P < 0.01; d = 0.67, moderate effect). Core temperature changed across time (main effect of time, $F_{(1, 13)} = 154.09$, P<0.01). The pattern of change in core temperature differed in the heat (trial*time interaction, $F_{(1,13)} = 10.06$, P<0.01; Figure 7.10), where core temperature was greater at 30, 40,

45 and 115 min in the hot trial (*post hoc*, P<0.05) compared to the corresponding time points in the moderate condition.

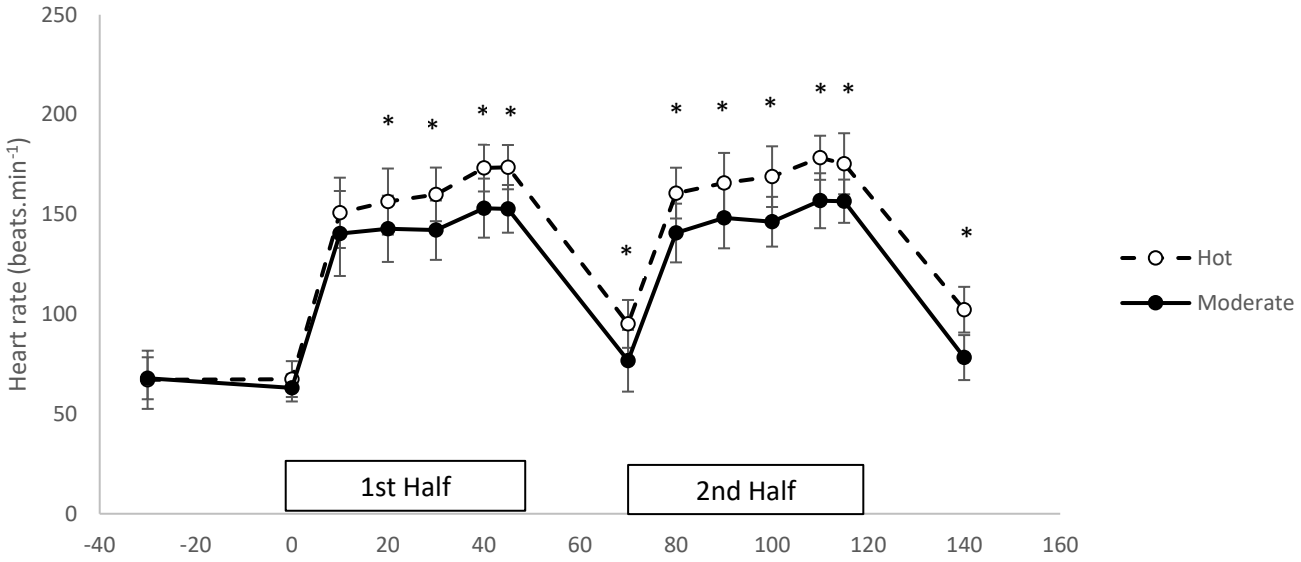


Figure 7. 9 Heart rate values during the hot and moderate trials. Main effect of trial, P <0.01; Main effect of time, P , 0.01; Trial*time interaction, P <0.01).

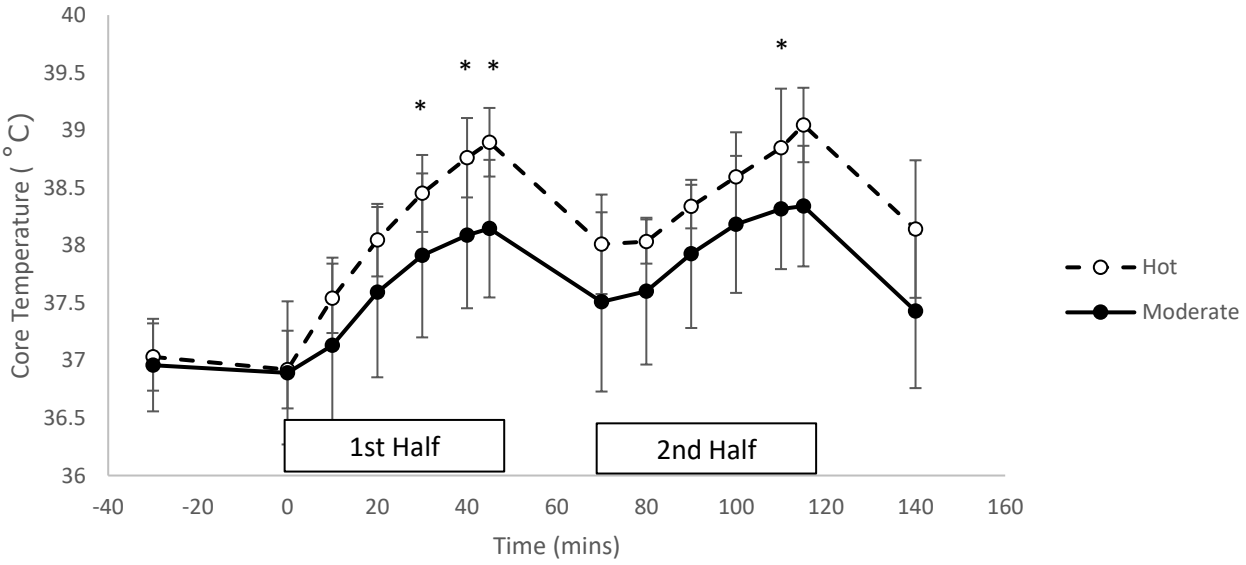


Figure 7. 10 Core temperature on the hot and moderate trials. Main effect of trial, P < 0.01; Main effect of time, P < 0.01; Trial*time interaction, P < 0.01.

7.3.5. Thigh skin temperature

Skin temperature was greater in the hot trial (main effect of trial, $F_{(1,16)} = 73.52$, $P < 0.01$; d = 2.19, large effect) and increased across time (main effect of time, $F_{(1, 13)} = 32.20$, $P<0.01$). The pattern of change in skin temperature differed in the heat (trial*time interaction, $F_{(1,13)} = 11.50$, $P<0.01$; Figure 7.11), where thigh skin temperature was greater following all time points after baseline (-30 min) (*post hoc*, $P<0.01$) compared to the corresponding time points in the moderate trial.

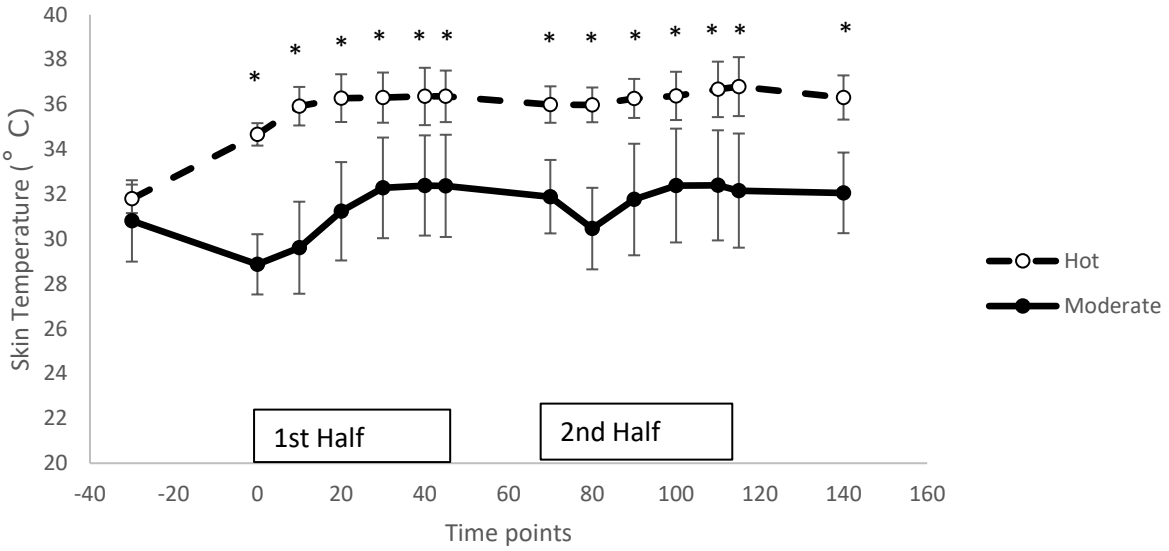


Figure 7. 11 Skin temperature values on the hot and moderate trials. Main effect of trial, $P < 0.01$; Main effect of time, $P < 0.01$; Trial*time interaction, $P < 0.01$.

7.3.6. Hydration status

Participants were hydrated (<800 mosmol.kg^{-1}) at the beginning (Hot: 561 ± 221 mosmol.kg^{-1}, Moderate: 693 ± 283 mosmol.kg^{-1}) and end (Hot: 605 ± 332 mosmol.kg^{-1}, Moderate: 559 ± 249 mosmol.kg^{-1}) of each trial. Ad libitum water intake was 1875 ± 756 ml in the hot and 699 ± 557 ml in the moderate condition. Urine osmolality did not differ between trials at baseline ($P = 0.34$) or full-time ($P = 0.61$).

Sweat rate was greater in the heat (hot: 1.7 ± 0.4 l.h^{-1}, mod: 0.8 ± 0.4 l.h^{-1}; $P < 0.01$). Body mass as a percentage of resting body mass, was well maintained in both the hot and moderate trials (hot vs moderate: -0.98 ± 1.17 *vs* -0.92 ± 0.80%), with similar changes between trials ($P = 0.71$).

7.3.7. Mood Questionnaire

Mean data for the mood questionnaires can be found in table 7.3. Anger and depression were not influenced by trial, time or trial*time interaction. Confusion was greater in the hot trial (main effect of trial, $F_{(1,14)} = 8.26$, $P = 0.01$; $d = 0.40$, small effect). Fatigue was greater in the heat (main effect of trial, $F_{(1, 13)} = 26.24$, $P<0.01$; $d = 0.95$, large effect), increased across time (main effect of time, $F_{(1,13)} = 17.28$, $P<<0.01$) and the pattern of change across trials differed (trial*time interaction, $F_{(1,13)} = 12.42$, $P < 0.01$), where it increases to a greater extent across time in the heat (*post hoc*, $P<0.01$). Tension was greater in the hot trial (main effect of trial, $F_{(1,13)} = 6.28$, $P = 0.03$; $d = 0.63$, moderate effect) and decreased across time (main effect of time, $F_{(1,13)} = 9.43$, $P < 0.01$). Vigour decreased across time (main effect of time, $F_{(1,13)} = 12.39$, $P <0.01$) and the pattern of changed differed between trials (trial*time interaction, $F_{(1,13)} = 4.67$, $P = 0.05$), with a greater drop off in vigour in the hot trial.

Table 7. 3 Mood questionnaire data. Data is Mean ± SD. * P<0.05.

	Moderate		**Hot**	
	Pre	**Full-time**	**Pre**	**Full-time**
Anger	4.0 ± 0.0	4.0 ± 2.0	4.0 ± 1.0	5.0 ± 1.0
Confusion	4.0 ± 1.0	4.0 ± 1.0	5.0 ± 1.0	5.0 ± 3.0
Depression	4.0 ± 1.0	5.0 ± 2.0	5.0 ± 1.0	5.0 ± 2.0
Fatigue	7.0 ± 3.0	9.0 ± 4.0	10.0 ± 4.0	14.0 ± 4.0*
Tension	5.0 ± 1.0	4.0 ± 0.0	6.0 ± 2.0	4.0 ± 1.0
Vigour	11.0 ± 3.0	9.0 ± 4.0	10.0 ± 2.0	8.0 ± 3.0*

7.3.8. Blood analysis

Mean values for blood parameters can be found in table 7.4.

Plasma volume changes did not differ between trials (main effect of trial, $P = 0.10$), or across time (main effect of time, $P = 0.92$). This resulted in a similar rate of change in plasma volume between trials (trial*time interaction, $P = 0.80$). The change in plasma volume was -0.2 ± 7.5 % and 6.4 ± 8.1 % in the moderate and hot trials, respectively.

Adrenaline

Plasma adrenaline did not differ between trials ($P =0.64$; $d = 0.07$, trivial effect) or across time ($P = 0.33$). This resulted in no difference in the pattern of change between trials ($P = 0.07$).

BDNF

Plasma BDNF had no main effect of trial (P = 0.79; d = 0.02, trivial effect) or time (P = 0.58). However the pattern of change across trials differed (trial*time interaction, $F_{(1,3)}$ = 3.88, P = 0.02; Figure 7.12). In the hot condition serum BDNF increased across the first half, however a similar increase was not seen in the moderate condition until full-time, despite no significant difference at each time point.

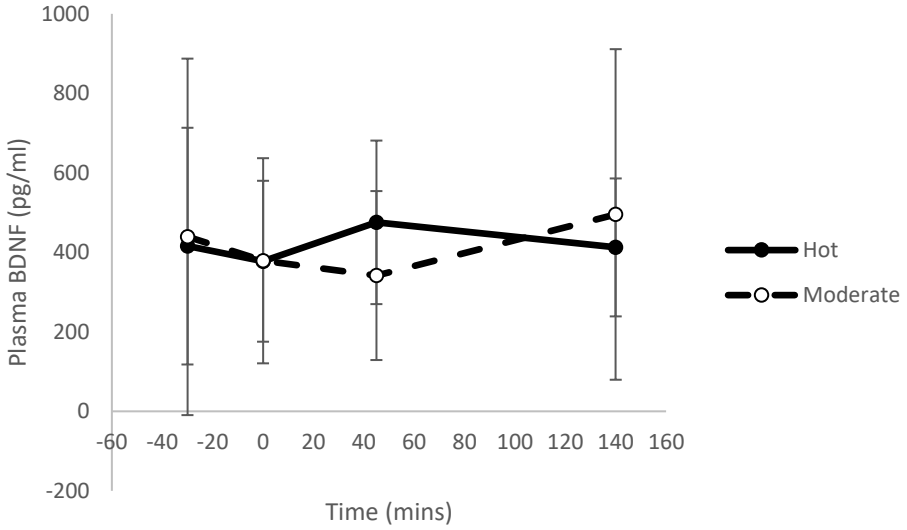


Figure 7. 12 Plasma BDNF concentrations across the hot and moderate trials. Data are mean ± SD. Trial*time interaction, P = 0.02.

Serum BDNF was greater in the hot trial (main effect of trial, $F_{(1,3)}$ = 17.19, P < 0.01; d = 0.39, small effect) and increased across time (main effect of time $F_{(1,3)}$ = 8.81, P<0.01). The pattern of change differed across trials (trial*time interaction $F_{(1,3)}$ = 6.81, P < 0.01; Figure 7.13), with greater increases seen in the heat at the half-time (*post hoc* P<0.01) and full-time (*post hoc* P<0.05) compared to the moderate trial.

Cortisol

No main effect of trial (P = 0.77; d = 0.03, trivial effect), time (P = 0.58) or interaction (P = 0.08) was seen for cortisol.

Cathepsin B

There was no effect of trial (P = 0.07; d = 0.65, moderate effect). However, cathepsin B concentration increased across time (main effect of time, $F_{(3, 39)}$ = 20.78, P <0.01) and the pattern of change differed between trials (trial*time interaction, $F_{(3,39)}$ = 4.82, P<0.01). In the moderate trial cathepsin B increased gradually across time, where a relatively stable concentration of cathepsin B was seen on the hot trial.

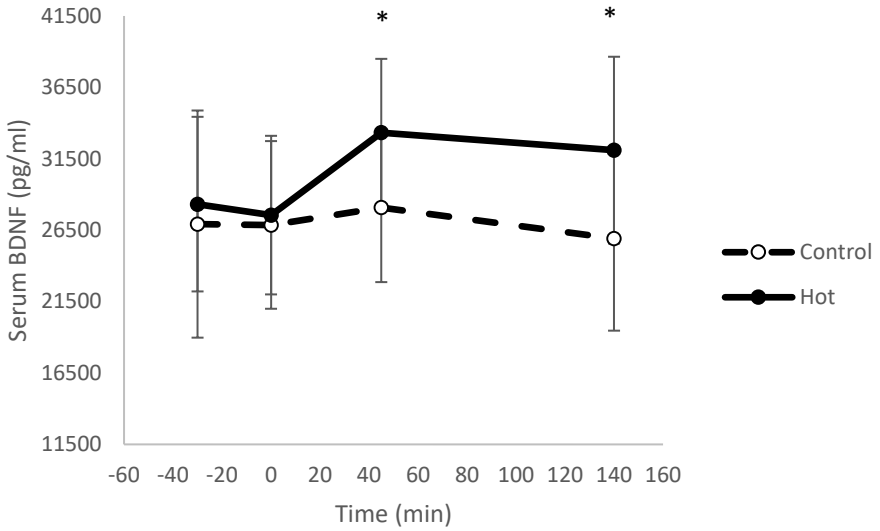


Figure 7. 13 Serum BDNF values across the hot and moderate trials. Data are mean ± SD. Main effect of trial, P < 0.01; Main effect of time, P < 0.01; Trial*time interaction, P < 0.01.

Noradrenaline

Noradrenaline concentration did not differ between trials (P = 0.42; d = 0.14, trivial effect), however increased across time ($F_{(3,39)}$ = 19.72, P<0.01), showing a significant change from baseline at pre-match (P < 0.01), half-time (P <0.01) and full-time (P<0.01). The pattern of change differed between trials (trial*time interaction, $F_{(3,39)}$ = 14.17, P<0.01; Figure 7.14), with differences in concentration at pre-match (P < 0.01) and full-time (P = 0.03), whereby noradrenaline increases during exercise in the heat and gradually decreases in the moderate condition.

Glucose

Blood glucose concentration was not different between trials (P = 0.62) and did not differ across time (P = 0.41), however the pattern of change was different between trials (trial*time interaction, $F_{(3,42)}$ = 3.11, P = 0.04), whereby blood glucose increased more following first half of the FSINT protocol in the hot trial.

Lactate

Blood lactate concentration was not different between trials (P = 0.73), however changed across time (main effect of time, $F_{(3,42)}$ = 11.02, P<0.01) and the pattern of change

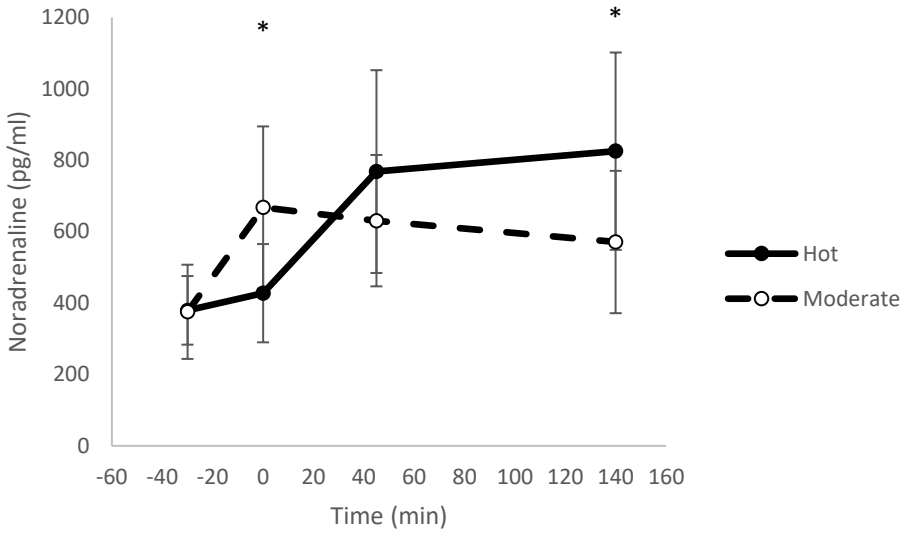


Figure 7. 14 Noradrenaline concentrations across the hot and moderate trials. Data are mean ± SD. Main effect of time, P < 0.01; Trial*time interaction, P < 0.01.

differed across trials (trial*time interaction, $F_{(3,42)}$, P = 0.02). Lactate was higher in the hot at full-time (P<0.01).

7.3.9. Perceptual measures

Rating of perceived exertion

Rating of perceived exertion was higher in the hot trial (main effect of trial, $F_{(1,16)}$= 63.30, P<0.01, d = 0.60, moderate effect; Tables 7.5 and 7.6), increased across time (main effect of time, $F_{(1,13)}$ = 90.50, P<0.01) and the pattern of change differed between trials (trial*time interaction, $F_{(1,13)}$= 7.164, P<0.01). RPE was greater in the heat at 10, 20, 30, 40, 45, 80, 90, 100, 110 and 115 min (*post hoc* P<0.05) compared to the corresponding time points on the moderate trial.

Feeling scale

Feeling was worse in the hot trial (main effect of trial, $F_{(1,15)}$ = 7.96, P = 0.01; d = 0.51, moderate effect; Tables 7.5 and 7.6), got worse across time (main effect of time, $F_{(13,195)}$ = 16.89, P<0.01), with all time points being significantly different from baseline from time point 20 min onwards. The pattern of change was different between trials (trial*time interaction, $F_{(13, 195)}$ = 4.36, P<0.01), where feeling was worse on the hot trial compared to the moderate trial at 40 and 45 min (i.e. immediately before HT) (P<0.05).

Table 7. 4 Blood parameter values (mean +/- SD) and effect sizes.

Blood Parameter	**Moderate**				**Hot**				**Trial effect**	**Time effect**	**Interaction effect**	**Effect Size**
Time (min)	**-30**	**0**	**45**	**140**	**-30**	**0**	**45**	**140**				
Adrenaline (pg/ml)	192 ± 129	174 ± 83	231 ± 136	192 ± 92	144 ± 103	241 ± 156	213 ± 90	228 ± 79	P = 0.64	P = 0.33	P = 0.07	0.07 (trivial)
BDNF (plasma) (pg/ml)	439 ± 448	379 ± 258	342 ± 213	495 ± 416	416 ± 298*	378 ± 202	475 ± 206*	412 ± 174	P = 0.79	P = 0.58	P = 0.02*	0.02 (trivial)
BDNF (serum) (pg/ml)	26928 ± 7953	26868 ± 5870	28081 ± 5218	25917 ± 6453	28325 ±6113*	27556 ± 5553	33328 ±5170*	32105 ±6541*	P<0.01*	P<0.01*	P<0.01*	0.39 (small)
Cathepsin B (ng/ml)	46 ± 12	52 ± 7	57 ± 8	59 ± 10	61 ± 14	60 ± 11	62 ± 10	61 ± 9	P = 0.07	P<0.01*	P<0.01*	0.65 (moderate)
Cortisol (ng/ml)	42 ± 11	44 ± 17	42 ± 12	41 ± 14	42 ± 14	40 ± 12	41 ± 12	48 ± 15	P=0.77	P=0.58	P=0.08	0.03 (trivial)
Noradrenaline (pg/ml)	375 ± 132	668 ± 227	631 ± 184	571 ± 199	379 ± 96*	428 ± 138*	768 ± 284	825 ± 276*	P=0.42	P<0.01*	P<0.01*	0.14 (trivial)

Table 7. 5 Perceptual measures for the first half of the FSINT protocol.

Trial	Time (min)							Trial Effect	Time Effect	Interaction	Effect size
	-30	0	10	20	30	40	45				
Rating of perceived exertion (RPE)											
Moderate	6.0 ± 1.0	6.0 ± 1.0	9.0 ± 2.0	11.0 ± 2.0	11.0 ± 2.0	12.0 ± 2.0	12.0 ± 2.0	P<0.01	P<0.01	P<0.01	0.60 (Moderate)
Hot	6.0 ± 1.0	7.0 ± 2.0	11.0±2.0*	13.0±2.0*	14.0±2.0*	15.0±2.0*	16.0 ± 2.0*				
Thermal Sensation (TS)											
Moderate	4.0 ± 1.0	2.0 ± 1.0	3.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	P<0.01	P<0.01	P<0.01	1.44 (Large)
Hot	4.0± 1.0*	5.0± 1.0*	5.0 ± 1.0*	6.0 ± 1.0*	6.0 ± 1.0*	7.0 ± 1.0*	7.0 ± 1.0*				
Feeling scale (FS)											
Moderate	2.0 ± 2.0	2.0 ± 2.0	2.0 ± 2.0	2.0 ± 2.0	2.0 ± 2.0	2.0 ± 2.0	2.0 ± 2.0	P =0.01	P<0.01	P<0.01	0.51 (Moderate)
Hot	3.0 ± 2.0	2.0 ± 2.0	2.0 ± 2.0	1.0 ± 2.0	1.0 ± 2.0	0.0±2.3*	-1.0 ± 2.0*				
Felt Arousal Scale (FAS)											
Moderate	3.0 ± 1.0	3.0 ± 2.0	3.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	P =0.58	P<0.01	P = 0.10	0.10 (Trivial)
Hot	3.0 ± 1.0	3.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	3.0 ± 2.0	3.0 ± 2.0	3.0 ± 2.0				

Table 7. 6 Perceptual measures for the second half of the FSINT protocol.

Trial	Time (min)							Trial Effect	Time Effect	Interaction	Effect size
	70	80	90	100	110	115	140				
Rating of perceived exertion (RPE)											
Moderate	7.0 ± 2.0	11.0 ± 2.0	12.0 ± 2.0	13.0 ± 3.0	13.0 ± 3.0	13.0 ± 3.0	7.0 ± 2.0	P<0.01	P<0.01	P<0.01	0.6 (Moderate)
Hot	8.0 ± 3.0	13.0±2.0*	14.0±2.0*	16.0±2.0*	17.0±2.0*	16.0±3.0*	7.0 ± 2.0				
Thermal Sensation (TS)											
Moderate	3.0 ± 1.0	3.0 ± 1.0	4.0 ± 1.0	5.0 ± 1.0	5.0 ± 1.0	5.0 ± 1.0	3.0 ± 1.0	P<0.01	P<0.01	P<0.01	1.44 (Large)
Hot	5.0 ± 1.0*	5.0 ± 1.0*	6.0 ± 1.0*	6.0 ± 1.0*	7.0 ± 1.0*	7.0 ± 1.0*	5.0 ± 1.0*				
Feeling scale (FS)											
Moderate	2.0 ± 2.0	2.0 ± 2.0	1.0 ± 2.0	1.0 ± 2.0	1.0 ± 2.0	1.0 ± 2.0	2.0 ± 2.0	P = 0.01	P<0.01	P<0.01	0.51 (Moderate)
Hot	1.0 ± 2.0	1.0 ± 2.0	0.0 ± 2.0	-1.0 ± 2.0	-1.0 ± 3.0	-1.0 ± 3.0	1.0 ± 1.0				
Felt Arousal Scale (FAS)											
Moderate	3.0 ± 2.0	3.0 ± 1.0	3.0 ± 1.0	3.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	3.0 ± 1.0	P = 0.58	P<0.01	P = 0.10	0.10 (Trivial)
Hot	3.0 ± 1.0	3.0 ± 2.0	3.0 ± 1.0	3.0 ± 2.0	3.0 ± 2.0	3.0 ± 2.0	2.0 ± 1.0				

Thermal Sensation

Thermal sensation was greater on the hot trial (main effect of trial, $F_{(1,16)} = 234.68$, $P<0.01$; d = 1.44, large effect; Tables 7.5 and 7.6) and increased across time (main effect of time, $F_{(13,208)} = 64.45$, $P<0.01$), dropping down close to baseline at half-time. The pattern of change differed between trials (trial*time interaction, $F_{(13,208)} = 7.84$, $P<0.01$), with thermal sensation greater in the hot trial at all time points following baseline (-30 min) (*post hoc* $P<0.05$).

Felt Arousal Scale

Felt arousal did not differ between trials (P = 0.58; d = 0.10, trivial effect; Tables 7.5 and 7.6) and the pattern of change did not differ between trials (P = 0.10). Felt arousal increased across both the halves of exercise (main effect of time, $F_{(13,208)} = 3.02$, $P<0.01$).

7.3.10. Blood Flow

Blood flow (thigh)

There was no difference in blood flow to the thigh between the hot and moderate trial (P = 0.56), and the pattern of change was not different between trials (trial*time interaction, P = 0.05). Blood flow increases across both FSINT halves (main effect of time, F(13,182) =6.70, P <0.01). Blood flow to the thigh was greater in the heat immediately following the cognitive testing at 0 min ($P<0.01$) and half-time ($P<0.05$).

Blood flow (brain)

Blood flow to the brain was greater in the hot trial (main effect of trial, $F_{(1,12)} = 7.01$, P = 0.02) and increased across both FSINT halves (main effect of time, $F_{(13, 156)} = 5.56$, $P<0.01$). However, the pattern of change across both trials was similar (trial*time interaction, P = 0.91).

7.3.11. Haematocrit and Haemoglobin

Haematocrit and haemoglobin were not influenced by trial (P = 0.548 and P = 0.14, respectively) and the pattern of change did not differ between trials (P = 0.88 and P = 0.14, respectively), however there is a significant effect of time for haematocrit (main effect of time, $F_{(3, 33)} = 3.735$, P = 0.02), where haematocrit increased from pre-match to half-time then decreased from half-time to full-time. There was no effect of time on haemoglobin (main effect of time, P = 0.91).

7.4. Discussion

The main findings of this study are that there was a detrimental combined influence of intermittent exercise and heat stress on both cognitive and neuromuscular function. Both peripheral and cortical voluntary activation were reduced in the hot trial, when compared to the moderate, with peripheral voluntary activation decreasing at a greater rate in the first half in the heat. Cognitive responses in the moderate trial showed either maintenance or facilitation for perception and executive function, however an opposite response was seen in these domains in the hot trial. Serum BDNF was greater in the heat, whilst both serum BDNF and plasma BDNF increased at a greater rate in the hot trial. However, this did not correlate with an improvement in cognition, suggesting that the optimal concentration of BDNF may have been exceeded. Similarly, noradrenaline demonstrated a greater rate of increase in the hot trial, which suggests the deterioration in cognition may be a result of over arousal.

Cognitive Function

Perception

Visual search response time was only affected on the simple level, where response time was well maintained in the moderate trial and deteriorated in the heat, with a greater drop off from baseline to full-time. Response times on the complex level followed a similar pattern to the simple level, however only showed a tendency (small effect, $d = 0.25$) for significance at full-time. These findings coincide with peaks in core temperature at the completion of exercise, which agrees with Bandelow et al (2010) who found a relationship between high core temperatures and a slowing in response time for visual perception. Simple level accuracy showed an improvement from baseline to half time on the moderate trial, however deteriorated in the heat. Wohlwend et al (2017) and McMorris et al (2009) both found that higher intensity exercise resulted in acutely worse accuracy. The higher physiological strain in the hot trial, demonstrated by increased heart rate and core temperatures, suggests performance could not be maintained within this domain as the cognitive load was too great for the available neural resources (Patel et al., 2014). Further, in line with the findings of chapter 4, the negative perceptual feelings and mood responses likely contributed to detriment to performance via an alliesthial effect.

Executive Function

Response time on the complex Stroop test improved in the moderate condition, whilst showing a gradual deterioration in the heat from baseline to full-time. The findings of chapter 4 highlight heat stress results in overall worse response times on both simple and complex executive function tasks. Whereas chapter 5 demonstrates an improvement in response time at half-time during a hockey match, agreeing with our improvement seen on the moderate trial, similar to previous findings (Byun et al., 2014; Lemmink & Visscher, 2005). Therefore, similar to perception, any improvement in skill performance using higher level functioning during intermittent exercise, is likely to be reversed during (football) competitions at high ambient temperatures. Hence, these findings highlight an aspect of performance which is at risk.

The prefrontal cortex is fundamental in performance of response inhibition and high-level functioning, associated with Stroop performance (Arnsten, 1998). Norepinephrine has previously been found to increase to a greater extent during exercise in the heat over moderate conditions (Morris et al., 2005; Powers, Howley & Cox, 1982), and was also much greater than passive heating alone. Hence, the combination of heat and high intensity intermittent exercise in the current study has caused a similar sympathetic nervous system response, likely detrimentally influencing the prefrontal cortex.

Cortisol has been implicated in negative cognitive responses (Lieberman et al., 2016), which is relevant due to the well documented increase in cortisol in response to both heat (Wang et al., 2015) and exercise stress (Kraemer & Ratamess, 2005; Liebermann et al., 2016). In the current study Cortisol only increased in the hot trial, showing a trend towards significance at full-time, suggesting this stress response in the heat potentially caused dysregulation in the prefrontal cortex causing an increase in response time. The beneficial effect shown in the moderate trial for response time could be related to the increase in cathepsin B concentration across time in that condition to a greater extent than the hot condition.

Working memory

Working memory was unaffected in the present study. Previous literature has demonstrated bot decrements in complex working memory tasks during heat stress (Gaoua et al., 2018), and improvements (MacLeod et al., 2018), however simple cognitive tasks are often improved or unaffected (Gaoua et al., 2018). Hence the working memory task in the current study may be unaffected due to its simplicity, not incurring a large enough cognitive load

when combined with the heat-exercise stress. Brain blood flow was increased in the hot trial on the present study, which may indicate increased resource utilisation and distribution in this domain, and therefore increased effort to maintain performance. Previous literature has demonstrated an increase in oxygen delivery to the prefrontal lobe, the brain region responsible for working memory, in response to heat stress (Wijayanto et al., 2013). Unfortunately, without electroencephalographic activity being measured in the present study, the cognitive load cannot be determined, and only speculated. However, the lack of change in this domain of cognitive function in the present study, and the findings of the aforementioned study, suggests that neural resources can be redistributed to maintain performance in this domain.

Attention

Similar to the findings of chapter 4 and chapter 5, minimal effect was seen on sustained attention, with no effect on either response time or accuracy being demonstrated. This domain appears unlikely to be at risk during team sports exercise in the heat. Similar to working memory, the brain region associated with this domain may be able to redistribute resources in response to heat stress to provide greater activation in attention networks within the brain (Liu et al., 2013). This finding, along with the increased brain blood flow seen in the current study, may have enabled maintenance of performance across all the different stressors explored within this thesis. RVIP performance has previously been shown to be unaffected by core temperature changes (Racinais, Gaoua & Grantham, 2008). This response may represent an improvement in nerve conduction velocity seen in the heat (Aird et al., 1983), counteracting any increase in the premotor reaction time caused by increases in core temperature.

Neuromuscular Function

The neuromuscular changes in response to intermittent football specific exercise in moderate conditions have been previously investigated (Goodall et al., 2017; Girard et al., 2015), however this is the first study to determine the time course of these changes in the knee extensors, during intermittent exercise in the heat. The current study found that both peripheral and cortical voluntary activation were worse in the hot condition when compared to a moderate control, with peripheral voluntary activation decreasing at a greater rate following the first half of the FSINT. Our results demonstrate the biphasic response previously shown in team sport exercise whereby the higher threshold, fast fatiguing motor

units are preferentially recruited at the onset of high intensity exercise, where the more fatigue resistant lower threshold motor units are recruited in the second half (Goodall et al., 2017; Thomas et al., 2016). The current results further demonstrate that this response is greater in the heat, showing only a 2.4% change from baseline in the moderate condition, compared to a 9.9% change in the heat for peripheral voluntary activation.

Research has shown that changes in VA and MVC have been dictated by changes in core temperature (Minett et al., 2012; Skein et al., 2012), with performance returning to baseline with the dissipation of endogenous heat gain (Minett & Duffield, 2014), allowing restoration of central coordination of function. Core temperature was greater in the hot trial and showed a greater rate of increase, in both the first and second half of exercise, on the hot trial. This highlights one of the key physiological factors that must be managed in order to optimise performance in hot environments. It is possible that these changes, combined with the skin temperature and perceptual changes influence motivation and result in a limited capacity to recruit adequate motoneurons to reach maximum force during a brief contraction (Periard et al., 2014a).

Our findings demonstrate a lesser response both in peripheral and cortical voluntary activation than the study by Goodall et al (2017), performed in moderate conditions. Goodall et al (2017) used an ecologically valid simulation of a football match (e.g. including dribbling a ball and maximal sprints), which likely exacerbated both the central and peripheral fatigue, which was evident in the higher perceived exertion (RPE = 16 vs 13 in the moderate trial in present study). However, this was completed in moderate conditions, including a hot trial in the present study meant the football simulation had to be completed in an environmental chamber, hence due to space constraints a more ecologically valid method of assessment could not be used.

Despite not reaching significance, the change in potentiated twitch from baseline mimicked the response of peripheral VA. In the heat a 10.6% decrease from baseline in the first half, compared to only a 3.4% decrease in the moderate trial. In agreement with the moderate condition used by Goodall et al (2017), M_{max} was unchanged in both conditions, hence suggesting the sarcolemmal excitability was also unchanged. This provides evidence for peripheral fatigue in the heat being exacerbated by changes in excitation-contraction coupling (Gandevia, 2001), as described in section 2.3.

Previous literature has suggested when exhaustive exercise is combined with heat stress, decrements in force producing capacity originate in the inability of the motor cortex to maximally stimulate the muscle (Periard, 2016). Cortical voluntary activation followed a similar pattern of change to peripheral and was overall worse on the hot trial. Therefore, the results of the current study are the first to highlight the increased risk of supraspinal fatigue following team sports exercise in the heat. The decrements have coincided with increased core temperature, which has previously been implicated as a moderator in cortical output following passive heat exposure (Ross et al., 2011), therefore providing strategies to manage core temperature may minimise this response. Also, physiological stress and perceptual feelings are also greater in the heat, shown via RPE, feeling scale and mood responses, which may also alter feedback to the brain and output to muscle afferents.

Implications and future research

The current study highlights that it is unlikely athletes can achieve the same level of performance, both in terms of cognition and neuromuscular function, when intermittent exercise is performed in hot climates.

The long-term effects of this type of exercise in the heat are not known, however neuromuscular decrements in moderate conditions have been demonstrated 72 h following a football simulation protocol (Thomas et al., 2017). Hence future research must investigate the rate of recovery of both neuromuscular and cognitive measures in order to understand how performance may be influenced in a multi-match tournament in the heat, for example the forthcoming Tokyo 2020 Olympics. From a physical capacity point of view, acclimation has proved beneficial (Castle et al., 2011; Sunderland, Morris & Nevill, 2008), however it is not understood how acclimation may affect both cognitive and neuromuscular function. Hence further investigations as to how these decrements can be prevented or minimised are necessary in order to enable optimal performance in extreme conditions during high level sporting competitions.

7.5. Conclusions

Confirming the findings of chapter 5, the findings of the current study suggest that intermittent exercise in the absence of heat stress enables either facilitation or maintenance of cognitive function in the domains of perception and executive function. However, the findings highlight the risk this aspect of function is under when this type of exercise is combined with heat stress, reversing the response. In addition, the peripheral and cortical

voluntary activation decrement is greater in the heat and likely contributes to the decrease in physical output seen when athletes compete in the heat. Hence, these findings provide a mechanistic explanation for decreases in skill performance and physical output when team sports athletes compete in the heat. The changes in cognition and neuromuscular function are likely mediated by the large difference in core temperature, skin temperature and perceptual feeling resulting in afferent feedback limiting output from the brain, whilst also providing a distracting stimulus for cognition. Additionally, the changes in concentration for serum BDNF, plasma BDNF and noradrenaline all likely mediate the differing cognitive responses between the moderate and hot conditions. This study provides rationale for the investigation of protective strategies to enable the maintenance of these aspects of function for athletes exposed to heat stress in order to optimise performance.

CHAPTER 8: GENERAL DISCUSSION

8.1. Introduction and Key Findings

The studies within this thesis have assessed the cognitive and neuromuscular responses to heat and intermittent exercise, with the aim of understanding the potential mechanisms responsible for physical and skill performance alterations when team sports athletes compete in the heat. The main findings of this thesis are summarised below: -

1. During heat exposure, both passively and combined with exercise, response times in the domains of perception and executive function were negatively influenced.
2. Intermittent exercise had a positive effect on response times for complex executive function tasks at half-time. Response time was better maintained during this type of exercise in moderate conditions.
3. Working memory was negatively affected by intermittent exercise alone. However, this response was not seen when intermittent exercise and heat stress are combined.
4. Attention was unaffected during passive heat stress. However intermittent exercise alone results in improved maintenance of accuracy over time and a tendency for quicker response times. Whereas attention was unaffected during intermittent exercise in the heat.
5. Large physiologically significant changes in core temperature are not necessary to influence cognition, with changes being seen during passive heating in response to large increases in skin temperature and the resultant alliesthesial effect.
6. Cortical voluntary activation can be established reliably in the knee extensors between day and within day, using transcranial magnetic stimulation.
7. The decrement in peripheral and cortical voluntary activation in response to high intensity intermittent exercise was exacerbated in the heat.
8. Peripheral voluntary activation shows a greater rate of decrease in the first half of a football match in the heat, demonstrating a preferential recruitment of high threshold, fast fatiguing motor units.
9. The lack of change in electromyography responses suggests the peripheral changes during intermittent exercise in the heat area a result of alterations in excitation-contraction coupling, rather than sarcolemmal excitability.

8.2. Heat, exercise and cognitive function

The results of chapters 4, 5 and 7 clearly demonstrate the vulnerability of cognitive function to heat, exercise and the two in combination. Broadly, the findings are in agreement with

previous literature, however what this thesis adds is the first insight into the area of team sport performance and exercise in the heat, eradicating any influence of hydration.

In order to provide greater insight into the potential mechanisms moderating cognitive alterations, we deemed it necessary to establish the cognitive response to intermittent exercise (as discussed above) and heat stress in isolation. Chapter 4 highlighted the importance of perceptual feelings on cognitive function. Previously Gaoua et al (2012) has found that changes in skin temperature and the resulting discomfort felt by participants is adequate to negatively influence cognition on a planning task. In light of these findings, we aimed to establish if this response was consistent across a range of cognitive domains. Chapter 4's findings demonstrated a negative effect on response times for perception and executive function tasks, whilst a trade-off appeared to occur for perception, whereby accuracy improved but response time slowed. In a sport specific setting, athletes who have become hot prior to exercise e.g. substitutes, may have slower perceptual responses, hence miss the appearance of an opponent or ball in their visual field. In terms of executive function, if a participant cannot deal with conflicting stimuli effectively and make a decision more slowly in the heat as a result, then their opponent may intercept a pass if they are either too slow at executing the pass or choose the wrong option. Mood state and perceptual feelings were negatively affected by the heat stress, which coincided with increases in skin temperature. This study suggested that the effect of heat stress on cognition is not solely mediated by internal core temperature changes, but an alliesthesial effect can also hamper cognition via perceptual feelings and skin temperature. Hence, when providing practical application, these aspects must be protected, as well as core temperature, in order to optimise cognition in the heat. This study provided application for vocations who are exposed to the heat (Færevik & Reinersten, 2003), and provided the rationale to explore the cognitive responses of athletes competing in the heat.

For the first time we have been able to show the cognitive responses of team sports athletes in a real-world competitive context at moderate ambient temperatures, which could provide a mechanism for altered skill performance throughout a match. Previously, Bandelow et al (2010) demonstrated the negative cognitive responses during a competitive football match, showing largely negative effects on the various cognitive domains assessed. However, all trials within this study were completed at high ambient temperatures, coupled with a number of other confounding factors. Hence, we believe chapter 5 provides the most ecologically valid assessment of how, and to an extent why, cognitive function is influenced by

intermittent, competitive team sports exercise, in this case field hockey. This study showed the positive influence a hockey match has on response time for executive function, perception and to some degree attention tasks. This would suggest that a player's ability to pick up perceptual cues such as their team mates, the ball or their opponents is improved due to the stimulus of competitive team sports exercise. Similarly, their ability to make a decision when confronted with conflicting stimuli, such as which pass to make, is also improved. Working memory was the only domain to show a negative response to this type of exercise stress. This might suggest that as a match goes on, an athlete's ability to recall tactical information may get worse. Although research has previously demonstrated the positive effects of different modes, intensities and durations of exercise (Brisselwalter et al., 1995; Chang et al., 2015; McMorris & Graydon, 1997), ours was the first study to provide insight for team sports athletes and provide an indication of the potential mechanisms involved. The increase in serum BDNF concentration on the match trial, which is known for its positive influence on cognition (Erickson et al., 2009; Rex et al., 2006; Egan et al., 2003; Grassi-Oliveira et al., 2008), has likely mediated the largely positive response for cognition on this study. Similarly, an increase in noradrenaline was seen from pre- to post-match. This demonstrated an increased sympathetic nervous system activity, which coupled with the cognitive data suggests an optimum level of arousal has been achieved for athletes to perform within the domains of perception and executive function.

The positive influence of team-based exercise was further reinforced in chapter 7, where football specific exercise was utilised as the exercise stressor in either moderate or hot conditions. The moderate condition highlighted a number of beneficial responses, such as better maintained or improved response times on perception and executive function tasks, respectively, alongside improvements in accuracy for both simple and complex perception tasks. Hence, the findings across chapter 5 and 7 suggest that performance within the domains of perception and executive function, improve across both a hockey match and football specific protocol. This response agrees with the majority of previous literature assessing exercise and cognitive function (Hogervorst et al., 1996; McMorris & Graydon, 1997; Yanagisawa et al., 2010). The findings of this thesis, combined with those of the literature allow us to speculate as to the mechanisms involved in this relationship, such as increased arousal shown via increases in noradrenaline, and improved neural function via the action of BDNF.

In the final study included in this thesis, football specific intermittent exercise stress was combined with heat. This relationship had not been previously investigated and presented a complex phenomenon due to the contrasting findings of chapters 4 and 5, in relation to heat and exercise, respectively. Core temperature has previously been highlighted as a key determinant of cognitive performance in the heat (Bandelow et al., 2010), alongside arousal (Hancock, 1989). Hence the overall greater core temperature, faster rate of increase in core temperature and greater noradrenaline response are likely responsible for the decrements in response time for perception and executive function. This is particularly true due to the greatest effect on cognition being seen at full-time, when core temperature peaked. Noradrenaline was also seen to peak at the completion of the exercise in the hot trial, indicating over arousal may have contributed to negative cognitive performance. These responses, according to the model described by Hancock (1989), are likely to drain available attentional resources, hence explaining the opposing response seen in moderate and hot conditions for this type of exercise. However, in line with the findings of chapter 4, the negative mood response and perceptual feelings, seen on the hot trial, likely contribute to the draining of neural resources and resulting negative effect on cognition. Similar to chapter 4, these findings suggest that athletes competing at high external temperatures would be less able to utilise the information presented to them efficiently. This may negatively influence their performance, for example either causing them to be tackled by their opposition or having their pass intercepted.

Therefore, the findings of the present thesis, in relation to cognitive function, have suggested that cognitive function can be improved across a match in both hockey and football. However, this effect is reversed when exercise is completed in the heat as a result of the draining of attentional resources. The mechanisms involved in this change are likely related to core temperature changes and negative perceptual feelings, and not always in combination, agreeing with previous literature (Bandelow et al., 2010; Gaoua et al., 2011). However, our findings suggest hormonal alterations also mediate the cognitive responses seen. Hence, protective strategies must now aim to protect and maintain homeostasis across all of these aspects.

8.3. Heat, exercise and neuromuscular function

Unlike cognitive function, the neuromuscular responses to intermittent exercise have previously been assessed (Goodall et al., 2015a; Goodall et al., 2017). Hence, the main aim

for this thesis was to establish how these responses were influenced when this type of exercise was performed in the heat and speculate as to the mechanisms involved.

Prior to investigating the neuromuscular responses to intermittent exercise in the heat (Chapter 7), it was deemed necessary to establish the reliability of the neuromuscular function techniques planned for this study. Previously, the reliability of these techniques (primarily peripheral nerve stimulation and transcranial magnetic stimulation) had been established (Goodall et al., 2009; Sidhu et al., 2009). However, the number of participants used in previous studies was not deemed adequate according to previous recommendations (Atkinson & Nevill, 1998), as well as minimal familiarisation being used in these studies. Although the findings of the study described in chapter 6 have provided similar reliability results to the aforementioned studies, the findings enhanced understanding by providing an extensive evaluation of necessary familiarisation techniques, whilst using a participant number in accordance with statistical guidelines (Atkinson & Nevill, 1998). Therefore, the results of the study described in Chapter 6 allowed us to design a study to establish the neuromuscular function responses to intermittent exercise in the heat, with the knowledge we were using both reliable techniques alongside an adequate familiarisation protocol to provide accurate results. Due to the computer simulation completed on this data, accurate participant numbers are now provided to guide researchers using these techniques in the future.

In comparison to cognitive function research, a greater body of literature has previously assessed the neuromuscular responses to both heat (Morrison et al., 2004; Racinais et al., 2008; Thomas et al., 2006; Todd et al., 2005) and intermittent exercise (Goodall et al., 2015a; Goodall et al., 2017). The study described in chapter 7 elaborated on these findings by combining intermittent exercise and the heat and established that both peripheral and cortical voluntary activation were worse in the hot trial. Hence providing a mechanistic explanation for any decrease in physical capacity during this type of exercise in the heat, when compared to moderate temperatures.

The findings of this thesis agree with those of previous literature, demonstrating a biphasic response for peripheral fatigue (Goodall et al, 2017), whereby a greater drop off in activation is seen in the first half when higher threshold, faster fatiguing motor units are being preferentially recruited. Hence activation was better maintained in the second half when the lower threshold and slower fatiguing motor units take preference. A similar pattern of response was shown in cortical activation, despite not reaching statistical significance. This

study was the first to provide an indication of the peripheral and cortical contributions to fatigue in the heat, which have previously been demonstrated in moderate conditions (Goodall et al., 2017). Hence, these findings now provide rational and a basis for developing protection strategies for athletes likely to be exposed to these stressors.

One of the likely mediators of neuromuscular changes was a greater increase in core temperature in the hot trial. This has previously been associated with decreased peripheral and cortical voluntary activation (Minett, 2012; Skein, 2012). The afferent feedback from negative perceptual responses, greater physiological strain and core and skin temperature, causes a protective decrease in output from the brain to the muscle, in order to limit heat production (Tucker, Rauch, Harley, & Noakes, 2004). Further, no change was seen in electromyography responses in the hot condition. This suggest that peripheral voluntary activation is negatively affected due to alterations in excitation-contraction coupling, rather than sarcolemmal excitability (Gandevia, 2001). Hence this thesis was the first to detail the time course of neuromuscular function response in the knee extensors in the heat, providing a mechanistic explanation of the changes seen.

8.4. Limitations

Despite attempts to minimise as many limitations as possible, a number of issues remain within the current thesis which may have influenced results. These are detailed below: -

1. In both chapter 4 and chapter 5 we utilised a computer-based battery of cognitive tests, at baseline, half-time and full-time. There are two issues with this strategy, the first being the lack of ecological validity due to completing the tests after exercise rather than during. As the time course of cognitive recovery for each of the tests is not known, this short delay combined with completing them in a rested state, may have negated some of the effects of both exercise and exercise-heat stress.
2. The second issue with the computer based cognitive tests are that it is still not possible to give a true estimation of the extent to which exercise, heat or exercise-heat stress will influence skill performance. Hence the development of more sport specific, skill-based tasks with cognitive elements would enable this. However, due to the constraints of where our testing was completed, this was not possible and is something we endeavour to address in the future.
3. The use of a solely treadmill-based protocol in chapter 7 to mimic team-based performance did not provide a physiological or mental strain which truly represents performance in team sport. With the exclusion of a number of essential movements

used in football, the exertion felt by participants and the physiological strain, may have under-represented the demands of football. However, due to the space constraints of the environmental chamber this was not possible.

Ecological validity remains the biggest issue when assessing performance in team sport. Due to the nature of team sport it is difficult to assess performance in a context which truly replicates the demands of the sport. However, this thesis has provided an insight, using as ecologically valid methods as possible, into the cognitive and neuromuscular responses during this type of exercise in the heat, in order to provide rationale for further investigation and ultimately protective strategies to be established.

8.5. Directions for future research

In order to provide greater practical application and real world meaning to our data, it is necessary to utilise sport specific tests and exercise protocols. In the first part this may be a computer-based test with sport specific scenarios, similar to those used by McMorris & Graydon (1997). However, ultimately to give an indication of how cognitive changes (across domains) influence skill performance, a sport specific skill test is required in conjunction with computer-based tests, similar to that of Sunderland & Nevill (2005), for hockey players.

This thesis has highlighted the risk which heat presents for both cognitive and neuromuscular function, both in isolation and in conjunction with intermittent exercise. Therefore, future research must investigate the means by which we can counteract this effect. As previously mentioned, the benefits of acclimation on physical output in intermittent exercise are known (Castle et al., 2011; Sunderland et al., 2008), however future research must now assess how we can best use similar techniques to benefit neuromuscular and cognitive performance.

A number of cooling methods have also been investigated to assess whether performance can be better maintained in the heat (Castle et al., 2006; Duffield et al., 2010; Skein et al., 2012), however again research has largely focused towards improving physical capacity and output. Hence, further investigation is required to assess if cooling techniques can be used to influence both cognition and neuromuscular function in team sports athletes, and further how this can then be implemented effectively in a competitive environment. The influence of precooling on both peripheral voluntary activation and time trial performance in the heat has previously been assessed (Randall et al., 2015). This study found that precooling of the thighs had the ability to improve exercise intensity and resulting time trial performance, however not as a result of any improvement in voluntary activation. This study provides an effective

starting point for research in the area of protective strategies for neuromuscular function. Further investigation is required to assess if cooling would have a different effect on neuromuscular function following intermittent exercise in the heat, which would be both a higher intensity and longer duration than the exercise protocol used by Randall et al (2015). It is also necessary to enhance this initial understanding by investigating the neuromuscular responses to transcranial magnetic stimulation, hence understanding the supraspinal fatigue responses.

8.6. Implications and practical advice

This thesis was solely interested in the effect of heat, intermittent exercise and the two in combination on cognition and neuromuscular function. The aim was to be able to assess whether performance of these functions was hindered and hence speculate as to the mechanisms involved. Without further investigation, we cannot say with certainty how protective strategies may counteract or improve any of the effects we have seen, however by isolating each of the stressors in chapters 4 and 5, along with measuring an extensive range of physiological and perceptual measures throughout the thesis, we can speculate as to the mechanisms influencing performance and thus how we may be able to influence these.

Our findings highlight the need to address athletes' perceptual feelings when exercising in the heat, according to our study described in chapter 4. This may be improved as a result of reducing skin temperature via cooling as this is known to relate closely to perceptual feelings and discomfort in the heat. Depending on the sport, this may be incorporated either pre-match, at half-time or during rolling substitutions in order to have the best effect on cognition. Core temperature is a known moderator of cognitive performance (Lieberman et al., 2005; Morley et al., 2012; Schlader et al., 2015), and neuromuscular function (Morrison et al., 2004; Nybo & Nielsen, 2001a; Racinais et al., 2008; Thomas et al., 2006), hence although not researched in relation to this type of exercise, providing means to alter core temperature are likely to positively influence both aspects of function. The most likely method to enable this is again through cooling, however understanding the rates of increase in core temperature could enable a more effective use of rolling substitutes in sports such as hockey, with the aim of preventing core temperature reaching levels which may put performance at risk.

A final practical recommendation to take from the current research is that attention must be paid to the time course of recovery for both cognition and neuromuscular function following intermittent exercise in the heat. Hence, during a multi-match tournament this may have to be considered. Some degree of neuromuscular fatigue has been shown in the days following a

football match in the plantar flexors (Girard et al., 2015), whereas minimal reduction was shown 24 h following a tennis match in both the knee extensors and plantar flexors (Periard et al., 2014a). However, a more extensive understanding is required in this area incorporating both peripheral nerve stimulation and transcranial magnetic stimulation in order to understand the time course of recovery. Once this has been adequately established, recovery strategies can be investigated to further optimise cognitive and neuromuscular performance to ensure restoration prior to subsequent matches.

8.7 Conclusion

The findings of this thesis have highlighted the detrimental influence which heat exposure combined with intermittent exercise can have on both cognitive and neuromuscular performance. The domains of perception and executive function appear to be the most vulnerable to the effects of heat stress, with our data suggesting this response is related to not only core temperature, but also negative perceptual feelings and hormonal responses. The findings also suggest that physical fatigue in the heat is a result of a decrease in both peripheral and cortical voluntary activation. It is likely that these alterations in function will result in poorer performance for team sports athletes competing in the heat. Therefore, it is necessary to establish protective strategies, relating to these specific aspects of function, to optimise performance for athletes competing in the heat.

References

Ackerman, P. L., & Kanfer, R. (2009). Test length and cognitive fatigue: An empirical examination of effects on performance and test-taker reactions. *Journal of Experimental Psychology: Applied, 15*(2), 163.

Aird, J., Webb, R., & Hoare, J. (1983). Heat exposure-induced changes in motor outflow component of reaction time. *Perceptual and Motor Skills, 56*(3), 699-706.

Aks, D. J. (1998). Influence of exercise on visual search: implications for mediating cognitive mechanisms. *Perceptual and Motor Skills, 87(3),* 771-783.

Allard, F. (1989). The effects of exercise on visual attention performance. *Human Performance,* 2, 131-145.

Allard, F., & Burnett, N. (1985). Skill in sport. *Canadian Journal of Psychology/Revue Canadienne De Psychologie, 39*(2), 294.

Amann, M., Goodall, S., Twomey, R., Subudhi, AW., Lovering, AT., Roach, RC. (2013). AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans. *J Appl Physiol* 115:634-642 doi:10.1152/japplphysiol.00606.2013.

Arnsten, A. F. (1998). Catecholamine modulation of prefrontal cortical cognitive function. *Trends in Cognitive Sciences, 2*(11), 436-447.

Asmussen, E., & Bøje, O. (1945). Body temperature and capacity for work. *Acta Physiologica Scandinavica*, 10(1), 1-22.

Åstrand, P., Rodahl, K., Dahl, H. A., & Strømme, S. B. (2003). *Textbook of Work Physiology: Physiological bases of exercise* Human Kinetics.

Atkinson, G., & Nevill, A. M. (1998). Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Medicine, 26*(4), 217-238.

Atkinson, G., Nevill, A. M. (2001). Selected issues in the design and analysis of sport performance research. *J Sports Sci* 19:811-827.

Audiffren M. (2009). Acute Exercise and Psychological Functions: A Cognitive-Energetic Approach. *Exercise and Cognitive Function*, 1-39.

Bain, A. R., Nybo, L., & Ainslie, P. N. (2015). Cerebral vascular control and metabolism in heat stress. *Comprehensive Physiology*,

Baker, A. J., Kostov, K. G., Miller, R. G., & Weiner, M. W. (1993). Slow force recovery after long-duration exercise: Metabolic and activation factors in muscle fatigue. *Journal of Applied Physiology (Bethesda, Md.: 1985), 74*(5), 2294-2300. doi:10.1152/jappl.1993.74.5.2294 [doi]

Ball, D., Burrows, C., & Sargeant, A. J. (1999). Human power output during repeated sprint cycle exercise: the influence of thermal stress. *European Journal of Applied Physiology and Occupational Physiology*, 79(4), 360-366.

Bandelow, S., Maughan, R., Shirreffs, S., Ozgünen, K., Kurdak, S., Ersöz, G., . . . Dvorak, J. (2010). The effects of exercise, heat, cooling and rehydration strategies on cognitive function in football players. *Scandinavian Journal of Medicine & Science in Sports, 20*(s3), 148-160.

Bangsbo, J., Mohr, M., & Krustrup, P. (2006). Physical and metabolic demands of training and match-play in the elite football player. *Journal of Sports Sciences, 24*(07), 665-674.

Banich, M. T. (2004). Cognitive neuroscience and neuropsychology. *Houghton Mifflin College Division*.

Behm, DG., St-Pierre, DM., Perez, D. (1996). Muscle inactivation: assessment of interpolated twitch technique. J Appl Physiol 81:2267-2273.

Bendiksen, M., Bischoff, R., Randers, M. B., Mohr, M., Rollo, I., Suetta, C., . . . Krustrup, P. (2012). The copenhagen soccer test: Physiological response and fatigue development. *Medicine and Science in Sports and Exercise, 44*(8), 1595-1603. doi:10.1249/MSS.0b013e31824cc23b [doi]

Bentley, D. J., Smith, P. A., Davie, A. J., & Zhou, S. (2000). Muscle activation of the knee extensors following high intensity endurance exercise in cyclists. *European Journal of Applied Physiology, 81*(4), 297-302.

Berchtold, M. W., Brinkmeier, H., & Muntener, M. (2000). Calcium ion in skeletal muscle: Its crucial role for muscle function, plasticity, and disease. *Physiological Reviews, 80*(3), 1215-1265.

Bishop, D. J., & Girard, O. (2013). Determinants of team-sport performance: Implications for altitude training by team-sport athletes. *British Journal of Sports Medicine, 47 Suppl 1*, i17-21. doi:10.1136/bjsports-2013-092950 [doi] .

Bland, JM., Altman, DG. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1:307-310.

Bohnen, N., Houx, P., Nicolson, N., & Jolles, J. (1990). Cortisol reactivity and cognitive performance in a continuous mental task paradigm. *Biological Psychology, 31*(2), 107-116.

Borg, G. A. (1962). *Physical performance and perceived exertion* Gleerup Lund.

Brisswalter J, Arcelin R, Audiffren M, & Delignieres D. (1997). Influence of Physical Exercise on Simple Reaction Time: Effect of Physical Fitness. *Perceptual and Motor Skills*, 85(3), 1019-1027.

Brisswalter, J., Durand, M., Delignieres, D., & Legros, P. (1995). Optimal and non-optimal demand in a dual task of pedalling and simple reaction time: Effects on energy expenditure and cognitive performance. *Journal of Human Movement Studies, 29*(1), 15-34.

Brisswalter, J., Collardeau, M., & René, A. (2002). Effects of acute physical exercise characteristics on cognitive performance. *Sports Medicine, 32*(9), 555-566.

Brück, K., & Olschewski, H. (1987). Body temperature related factors diminishing the drive to exercise. *Canadian Journal of Physiology and Pharmacology*, *65*(6), 1274-1280.

Buckthorpe, MW., Hannah, R., Pain, TG., Folland, JP. (2012). Reliability of neuromuscular measurements during explosive isometric contractions, with special reference to electromyography normalization techniques. *Muscle Nerve* 46:566-576 doi:10.1002/mus.23322.

Burke, L. (1997). Fluid balance during team sports. *Journal of Sports Sciences, 15*(3), 287-295.

Byun, K., Hyodo, K., Suwabe, K., Ochi, G., Sakairi, Y., Kato, M., . . . Soya, H. (2014). Positive effect of acute mild exercise on executive function via arousal-related prefrontal activations: An fNIRS study. *NeuroImage, 98*, 336-345.

Cabanac, M. (1971). Physiological role of pleasure. *Science (New York, N.Y.), 173*(4002), 1103-1107.

Cady, E., Jones, D., Lynn, J., & Newham, D. (1989). Changes in force and intracellular metabolites during fatigue of human skeletal muscle. *The Journal of Physiology, 418*(1), 311-325.

Caldwell J N, Engelen L, Van der Henst C, Patterson M J, Taylor N A. (2011). The Interaction of Body Armour, Low-Intensity Exercise, and Hot-Humid Conditions on Physiological Strain and Cognitive Function. *Mil Med*, 176 (5), 488-93.

Callard, D., Davenne, D., Gauthier, A., Lagarde, D., Van Hoecke, J. (2000). Circadian rhythms in human muscular efficiency: continuous physical exercise versus continuous rest. A crossover study. *Chronobiology International* 17:693-704.

Castle, P. C., Macdonald, A. L., Philp, A., Webborn, A., Watt, P. W., & Maxwell, N. S. (2006). Precooling leg muscle improves intermittent sprint exercise performance in hot, humid conditions. *Journal of Applied Physiology, 100*(4), 1377-1384.

Castle, P., Mackenzie, R. W., Maxwell, N., Webborn, A. D., & Watt, P. W. (2011). Heat acclimation improves intermittent sprinting in the heat but additional pre-cooling offers no further ergogenic effect. *Journal of Sports Sciences*, 29(11), 1125-1134.

Castle, P. C., Maxwell, N., Allchorn, A., Mauger, A. R., & White, D. K. (2012). Deception of ambient and body core temperature improves self paced cycling in hot, humid conditions. *European Journal of Applied Physiology, 112*(1), 377-385.

Chang, C., Bernard, T. E., & Logan, J. (2017). Effects of heat stress on risk perceptions and risk taking. *Applied Ergonomics, 62*, 150-157.

Chang, Y., Chu, C., Wang, C., Song, T., & Wei, G. (2015). Effect of acute exercise and cardiovascular fitness on cognitive function: An event-related cortical desynchronization study. *Psychophysiology, 52*(3), 342-351.

Chang, Y., Labban, J., Gapin, J., & Etnier, J. L. (2012a). The effects of acute exercise on cognitive performance: A meta-analysis. *Brain Research, 1453*, 87-101.

Chase B, Karwowski W, Benedict M E, & Queseda P M. (2005). Effects of Thermal Stress on Dual Task Performance and Attention Allocation. *Journal of Human Performance in Extreme Environments*, 8(1), 1.

Cheung, K., Hume, P. A., & Maxwell, L. (2003). Delayed onset muscle soreness. *Sports Medicine, 33*(2), 145-164.

Cian, C., Barraud, P., Melin, B., & Raphel, C. (2001). Effects of fluid ingestion on cognitive function after heat stress or exercise-induced dehydration. *International Journal of Psychophysiology, 42*(3), 243-251.

Cipolla, M. J. (2009). The cerebral circulation. *Integrated Systems Physiology: From Molecule to Function, 1*(1), 1-59.

Cohen, J. (1992). A power primer. *Psychological Bulletin, 112*(1), 155.

Cooper, S. B., Bandelow, S., Nute, M. L., Morris, J. G., & Nevill, M. E. (2015). Breakfast glycaemic index and exercise: Combined effects on adolescents' cognition. *Physiology & Behavior, 139*, 104-111.

Corsi, P. (1972). Memory and the medial temporal region of the brain. *Unpublished Doctoral Dissertation), McGill University, Montreal, QB,*

D'Esposito, M., Postle, B. R., & Rypma, B. (2000). Prefrontal cortical contributions to working memory: Evidence from event-related fMRI studies. *Executive control and the frontal lobe: Current issues* (pp. 3-11) Springer.

Dawson, B., Elliott, B., Pyke, F., & Rogers, R. (1985). Physiological and performance responses to playing tennis in a cool environment and similar intervalized treadmill running in a hot climate. *Journal of Human Movement Studies, 11*, 21-34.

De Quervain, D. J., Henke, K., Aerni, A., Treyer, V., McGaugh, J. L., Berthold, T., . . . Hock, C. (2003). Glucocorticoid-induced impairment of declarative memory retrieval is associated with reduced blood flow in the medial temporal lobe. *European Journal of Neuroscience, 17*(6), 1296-1302.

De Ruiter, C. J., & De Haan, A. (2000). Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. *Pflügers Archiv*, 440(1), 163-170.

Deboer, T. (1998). Brain temperature dependent changes in the electroencephalogram power spectrum of humans and animals. *Journal of Sleep Research, 7*(4), 254-262.

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology, 37*(2), 247-248. doi:10.1152/jappl.1974.37.2.247 [doi]

Dominique, J., Roozendaal, B., Nitsch, R. M., McGaugh, J. L., & Hock, C. (2000). Acute cortisone administration impairs retrieval of long-term declarative memory in humans. *Nature Neuroscience, 3*(4), 313.

Drust, B., Rasmussen, P., Mohr, M., Nielsen, B., & Nybo, L. (2005). Elevations in core and muscle temperature impairs repeated sprint performance. *Acta Physiologica, 183*(2), 181-190.

Duffield, R., Green, R., Castle, P., & Maxwell, N. (2010). Precooling can prevent the reduction of self-paced exercise intensity in the heat. *Medicine & Science in Sports & Exercise, 42*(3), 577-584.

Easterbrook, J. A. (1959). The effect of emotion on cue utilization and the organization of behavior. *Psychological Review, 66*(3), 183.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., . . . Dean, M. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*(2), 257-269.

Enoka, R. M. (2002). Activation order of motor axons in electrically evoked contractions. *Muscle & Nerve, 25*(6), 763-764.

Enoka, R. M., & Duchateau, J. (2008). Muscle fatigue: What, why and how it influences muscle function. *The Journal of Physiology, 586*(1), 11-23.

Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Hu, L., Morris, K. S., . . . Kramer, A. F. (2009). Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus, 19*(10), 1030-1039.

Etnier, J. L., Salazar, W., Landers, D. M., Petruzzello, S. J., Han, M., & Nowell, P. (1997). The influence of physical fitness and exercise upon cognitive functioning: A meta-analysis. *Journal of Sport and Exercise Psychology, 19*(3), 249-277.

Færevik, H., & Eidsmo Reinertsen, R. (2003). Effects of wearing aircrew protective clothing on physiological and cognitive responses under various ambient conditions. *Ergonomics, 46*(8), 780-799.

Farah, M. J. (2003). Disorders of visual-spatial perception and cognition. *Clinical Neuropsychology*, 4.

Fernandez-del-Olmo, M., Rodriguez, F., Marquez, G., Iglesias, X., Marina, M., Benitez, A., . . . Acero, R. (2013). Isometric knee extensor fatigue following a wingate test: Peripheral and central mechanisms. *Scandinavian Journal of Medicine & Science in Sports, 23*(1), 57-65.

Ferris, L. T., Williams, J. S., & Shen, C. L. (2007). The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. *Medicine and Science in Sports and Exercise, 39*(4), 728-734. doi:10.1249/mss.0b013e31802f04c7 [doi]

Fitts, R. H. (1994). Cellular mechanisms of muscle fatigue. *Physiological reviews,* 74(1), 49-94.

Fleury, M., Bard, C., Jobin, J., & Carrière, L. (1981). Influence of different types of physical fatigue on a visual detection task. *Perceptual and Motor Skills, 53*(3), 723-730.

Gabbett, T. J. (2010). GPS analysis of elite women's field hockey training and competition. *Journal of Strength and Conditioning Research, 24*(5), 1321-1324. doi:10.1519/JSC.0b013e3181ceebbb [doi]

Gandevia, S., Allen, G. M., Butler, J. E., & Taylor, J. L. (1996). Supraspinal factors in human muscle fatigue: Evidence for suboptimal output from the motor cortex. *The Journal of Physiology, 490*(2), 529-536.

Gandevia, S. (1992). Some central and peripheral factors affecting human motoneuronal output in neuromuscular fatigue. *Sports Med* 13:93-98.

Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiological Reviews, 81*(4), 1725-1789.

Ganio, M. S., Armstrong, L. E., Casa, D. J., McDermott, B. P., Lee, E. C., Yamamoto, L. M., . . . Le Bellego, L. (2011). Mild dehydration impairs cognitive performance and mood of men. *British Journal of Nutrition, 106*(10), 1535-1543.

Gaoua, N. (2010). Cognitive function in hot environments: A question of methodology. *Scandinavian Journal of Medicine & Science in Sports, 20*(s3), 60-70.

Gaoua, N., Grantham, J., Racinais, S., & El Massioui, F. (2012). Sensory displeasure reduces complex cognitive performance in the heat. *Journal of Environmental Psychology, 32*(2), 158-163.

Gaoua, N., Herrera, C. P., Périard, J. D., El Massioui, F., & Racinais, S. (2018). Effect of passive hyperthermia on working memory resources during simple and complex cognitive tasks. *Frontiers in Psychology*, 8, 2290.

Gaoua, N., Racinais, S., Grantham, J., & El Massioui, F. (2011). Alterations in cognitive performance during passive hyperthermia are task dependent. *International Journal of Hyperthermia, 27*(1), 1-9.

Gibson, A. S. C., Lambert, M. I., & Noakes, T. D. (2001). Neural control of force output during maximal and submaximal exercise. *Sports Medicine, 31*(9), 637-650.

Girard, O., Nybo, L., Mohr, M., & Racinais, S. (2015). Plantar flexor neuromuscular adjustments following match-play football in hot and cool conditions. *Scandinavian Journal of Medicine & Science in Sports, 25*(S1), 154-163.

Girard, O., Lattier, G., Maffiuletti, N. A., Micallef, J., & Millet, G. P. (2008). Neuromuscular fatigue during a prolonged intermittent exercise: Application to tennis. *Journal of Electromyography and Kinesiology, 18*(6), 1038-1046.

Gonzalez-Alonso, J., Teller, C., Andersen, S. L., Jensen, F. B., Hyldig, T., & Nielsen, B. (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *Journal of Applied Physiology (Bethesda, Md.: 1985), 86*(3), 1032-1039.

Goodall, S., Charlton, K., Howatson, G., & Thomas, K. (2015a). Neuromuscular fatigability during repeated-sprint exercise in male athletes. *Medicine & Science in Sports & Exercise, 47*(3), 528-536.

Goodall, S., Charlton, K., Hignett, C., Prichard, J., Barwood, M., Howatson, G., & Thomas, K. (2015b). Augmented supraspinal fatigue following constant-load cycling in the heat. *Scandinavian Journal of Medicine & Science in Sports, 25,* 164-172.

Goodall, S., González-Alonso, J., Ali, L., Ross, E. Z., & Romer, L. M. (2012). Supraspinal fatigue after normoxic and hypoxic exercise in humans. *The Journal of Physiology, 590*(11), 2767-2782.

Goodall, S., Howatson, G., Romer, L., & Ross, E. (2014). Transcranial magnetic stimulation in sport science: A commentary. *European Journal of Sport Science, 14*(sup1), S332-S340.

Goodall, S., Romer, L., & Ross, E. (2009). Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. *Experimental Physiology, 94*(9), 995-1004.

Goodall, S., Thomas, K., Harper, L. D., Hunter, R., Parker, P., Stevenson, E., . . . Howatson, G. (2017). The assessment of neuromuscular fatigue during 120 min of simulated soccer exercise. *European Journal of Applied Physiology, 117*(4), 687-697.

Gopinathan, P., Pichan, G., & Sharma, V. (1988). Role of dehydration in heat stress-induced variations in mental performance. *Archives of Environmental Health: An International Journal, 43*(1), 15-17.

Grandjean, A. C., & Grandjean, N. R. (2007). Dehydration and cognitive performance. *Journal of the American College of Nutrition, 26*(sup5), 549S-554S.

Grassi-Oliveira, R., Stein, L. M., Lopes, R. P., Teixeira, A. L., & Bauer, M. E. (2008). Low plasma brain-derived neurotrophic factor and childhood physical neglect are associated with verbal memory impairment in major depression—a preliminary report. *Biological Psychiatry, 64*(4), 281-285.

Grego, F., Vallier, J., Collardeau, M., Rousseu, C., Cremieux, J., & Brisswalter, J. (2005). Influence of exercise duration and hydration status on cognitive function during prolonged cycling exercise. *International Journal of Sports Medicine, 26*(01), 27-33.

Greig, M. P., Mc Naughton, L. R., & Lovell, R. J. (2006). Physiological and mechanical response to soccer-specific intermittent activity and steady-state activity. *Research in Sports Medicine, 14*(1), 29-52.

Greiwe, J. S., Hickner, R. C., Hansen, P. A., Racette, S. B., Chen, M. M., & Holloszy, J. O. (1999). Effects of endurance exercise training on muscle glycogen accumulation in humans. *Journal of Applied Physiology, 87*(1), 222-226.

Griffin, É W., Mullally, S., Foley, C., Warmington, S. A., O'Mara, S. M., & Kelly, Á M. (2011). Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males. *Physiology & Behavior, 104*(5), 934-941.

Gruet, M., Temesi, J., Rupp, T., Levy, P., Millet, G., & Verges, S. (2013). Stimulation of the motor cortex and corticospinal tract to assess human muscle fatigue. *Neuroscience, 231*, 384-399.

Hancock, P., & Dirkin, G. (1982). Central and peripheral visual choice-reaction time under conditions of induced cortical hyperthermia. *Perceptual and Motor Skills, 54*(2), 395-402.

Hancock P A. (1983). The Effect of an Induced Selective Increase in Head Temperature Upon Performance of a Simple Mental Task. *Human Factors: The Journal of the Human Factors and Ergonomics Society*, 25(4), 441-448.

Hancock, P., & Vasmatzidis, I. (2003). Effects of heat stress on cognitive performance: The current state of knowledge. *International Journal of Hyperthermia, 19*(3), 355-372.

Hancock, P. A. (1989). A dynamic model of stress and sustained attention. *Human Factors, 31*(5), 519-537.

Hancock, P. A., Ross, J. M., & Szalma, J. L. (2007). A meta-analysis of performance response under thermal stressors. *Human Factors, 49*(5), 851-877.

Hardy, C. J., & Rejeski, W. J. (1989). Not what, but how one feels: The measurement of affect during exercise. *Journal of Sport and Exercise Psychology, 11*(3), 304-317.

Harridge, S. D., Kryger, A., & Stensgaard, A. (1999). Knee extensor strength, activation, and size in very elderly people following strength training. *Muscle & Nerve,* 22(7), 831-839.

Harrison, M. H. (1985). Effects on thermal stress and exercise on blood volume in humans. *Physiological Reviews, 65*(1), 149-209. doi:10.1152/physrev.1985.65.1.149 [doi]

Helsen, W. F., & Starkes, J. L. (1999). A multidimensional approach to skilled perception and performance in sport. *Applied Cognitive Psychology, 13*(1), 1-27.

Hemmatjo, R., Motamedzade, M., Aliabadi, M., Kalatpour, O., & Farhadian, M. (2017). The effect of practical cooling strategies on physiological response and cognitive function during simulated firefighting tasks. *Health Promotion Perspectives, 7*(2), 66.

Héroux, M. E., Taylor, J. L., & Gandevia, S. C. (2016). Correction: The use and abuse of transcranial magnetic stimulation to modulate corticospinal excitability in humans. *PloS One, 11*(1), e0147890.

Hill, C. A., Thompson, M. W., Ruell, P. A., Thom, J. M., & White, M. J. (2001). Sarcoplasmic reticulum function and muscle contractile character following fatiguing exercise in humans. *The Journal of Physiology*, *531*(3), 871-878.

Hocking, C., Silberstein, R. B., Lau, W. M., Stough, C., & Roberts, W. (2001). Evaluation of cognitive performance in the heat by functional brain imaging and psychometric testing. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *128*(4), 719-734.

Hogervorst, E., Bandelow, S., Schmitt, J. A., Jentjens, R., Oliveira, M., Allgrove, J. E., . . . Gleeson, M. (2008). Caffeine improves physical and cognitive performance during exhaustive exercise.

Hogervorst, E., Riedel, W., Jeukendrup, A., & Jolles, J. (1996). Cognitive performance after strenuous physical exercise. *Perceptual and Motor Skills*, *83*(2), 479-488.

Jiménez-Pavón, D., Romeo, J., Cervantes-Borunda, M., Ortega, F. B., Ruiz, J. R., España-Romero, V., . . . Castillo, M. J. (2011). Effects of a running bout in the heat on cognitive performance. *Journal of Exercise Science & Fitness*, *9*(1), 58-64.

Johnson, MA., Sharpe, GR., Williams, NC., Hannah, R. (2015). Locomotor muscle fatigue is not critically regulated after prior upper body exercise. *J Appl Physiol* (1985) 119:840-850 doi:10.1152/japplphysiol.00072.2015

Kamijo, K., Nishihira, Y., Hatta, A., Kaneda, T., Wasaka, T., Kida, T., & Kuroiwa, K. (2004). Differential influences of exercise intensity on information processing in the central nervous system. *European Journal of Applied Physiology*, *92*(3), 305-311.

Kamijo K, Nishihira Y, Higashiura T, & Kuroiwa K. (2007). The Interactive Effect of Exercise Intensity and Task Difficulty on Human Cognitive Processing. *International Journal of Psychophysiology,* 65(2), 114-121.

Kent-Braun, J. A. (1999). Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. *European Journal of Applied Physiology and Occupational Physiology, 80*(1), 57-63.

Kirschbaum, C., Wolf, O., May, M., Wippich, W., & Hellhammer, D. (1996). Stress-and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. *Life Sciences, 58*(17), 1475-1483.

Kobayashi, M., Pascual-Leone, A. (2003). Transcranial magnetic stimulation in neurology. *Lancet Neurol* 2:145-156

Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Medicine, 35*(4), 339-361.

Labelle V, Bosquet L, Mekary S, & Bherer L. (2013). Decline in Executive Control During Acute Bouts of Exercise as a Function of Exercise Intensity and Fitness Level. *Brain and Cognition,* 81(1), 10-17.

Laitano, O., Runco, J. L., & Baker, L. (2014). Hydration science and strategies in football. *Sports Sci Exch, 27*(128), 1-7.

Lambourne, K., & Tomporowski, P. (2010). The effect of exercise-induced arousal on cognitive task performance: A meta-regression analysis. *Brain Research, 1341*, 12-24.

Lee, M., Gandevia, SC., Carroll, TJ. (2008). Cortical voluntary activation can be reliably measured in human wrist extensors using transcranial magnetic stimulation. *Clin Neurophysiol* 119:1130-1138 doi:10.1016/j.clinph.2007.12.018.

Lee, J. K., Koh, A. C., Koh, S. X., Liu, G. J., Nio, A. Q., & Fan, P. W. (2014). Neck cooling and cognitive performance following exercise-induced hyperthermia. *European Journal of Applied Physiology, 114*(2), 375-384.

Lemmink, K. A., & Visscher, C. (2005). Effect of intermittent exercise on multiple-choice reaction times of soccer players. *Perceptual and Motor Skills, 100*(1), 85-95.

Lepers, R., Hausswirth, C., Maffiuletti, N., Brisswalter, J., & Van Hoecke, J. (2000). Evidence of neuromuscular fatigue after prolonged cycling exercise. *Medicine & Science in Sports & Exercise, 32*(11), 1880-1886.

Lepers, R., Millet, G. Y., & Maffiuletti, N. A. (2001). Effect of cycling cadence on contractile and neural properties of knee extensors. *Medicine & Science in Sports & Exercise, 33*(11), 1882-1888.

Lepers, R., Maffiuletti, N. A., Rochette, L., Brugniaux, J., & Millet, G. Y. (2002). Neuromuscular fatigue during a long-duration cycling exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 92*(4), 1487-1493. doi:10.1152/japplphysiol.00880.2001 [doi]

Lieberman, H. R., Bathalon, G. P., Falco, C. M., Kramer, F. M., Morgan, C. A., & Niro, P. (2005). Severe decrements in cognition function and mood induced by sleep loss, heat, dehydration, and undernutrition during simulated combat. *Biological Psychiatry, 57*(4), 422-429.

Lieberman, H. R., Farina, E. K., Caldwell, J., Williams, K. W., Thompson, L. A., Niro, P. J., . . . McClung, J. P. (2016). Cognitive function, stress hormones, heart rate and nutritional status during simulated captivity in military survival training. *Physiology & Behavior, 165*, 86-97.

Liu, K., Sun, G., Li, B., Jiang, Q., Yang, X., Li, M., . . . Zhou, Z. (2013). The impact of passive hyperthermia on human attention networks: An fMRI study. *Behavioural Brain Research, 243*, 220-230.

Lorenzo, S., Halliwill, J. R., Sawka, M. N., & Minson, C. T. (2010). Heat acclimation improves exercise performance. *Journal of Applied Physiology (Bethesda, Md.: 1985), 109*(4), 1140-1147. doi:10.1152/japplphysiol.00495.2010 [doi]

Lothian, F. (1994). A time-motion analysis of women's hockey. *Journal of Human Movement Studies, 26*(6), 255-266.

Lothian, F., & Farrally, M. (1992). Estimating the energy cost of women's hockey using heart rate and video analysis. *Journal of Human Movement Studies, 23*, 215-215.

Lythe, J., & Kilding, A. (2011). Physical demands and physiological responses during elite field hockey. *International Journal of Sports Medicine, 32*(07), 523-528.

MacLeod, H., Bussell, C., & Sunderland, C. (2007). Time-motion analysis of elite women's field hockey, with particular reference to maximum intensity movement patterns. *International Journal of Performance Analysis in Sport, 7*(2), 1-12.

MacLeod, H., Cooper, S., Bandelow, S., Malcolm, R., & Sunderland, C. (2018). Effects of heat stress and dehydration on cognitive function in elite female field hockey players. *BMC Sports Science, Medicine and Rehabilitation, 10*(1), 12.

MacLeod, H., Morris, J., Nevill, A., & Sunderland, C. (2009). The validity of a non-differential global positioning system for assessing player movement patterns in field hockey. *Journal of Sports Sciences, 27*(2), 121-128.

Magistris, MR., Rosler, KM., Truffert, A., Myers, JP. (1998). Transcranial stimulation excites virtually all motor neurons supplying the target muscle. A demonstration and a method improving the study of motor evoked potentials. *Brain,* 121 (Pt 3):437-450

Martins A, Kavussanu M, Willoughby A, & Ring C. (2013). Moderate Intensity Exercise Facilitates Working Memory. *Psychology of Sport and Exercise*, 14(3), 323-328.

Mathur, S., Eng, JJ., MacIntyre, DL. (2005). Reliability of surface EMG during sustained contractions of the quadriceps. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology,* 15:102-110 doi:10.1016/j.jelekin.2004.06.003

McGregor, S., Nicholas, C., Lakomy, H., & Williams, C. (1999). The influence of intermittent high-intensity shuttle running and fluid ingestion on the performance of a soccer skill. *Journal of Sports Sciences, 17*(11), 895-903.

McMorris T, Collard K, Corbett J, Dicks M, & Swain J P. (2008). A Test of the Catecholamines Hypothesis for an Acute Exercise–Cognition Interaction. *Pharmacology Biochemistry and Behavior*, 89(1), 106-115.

McMorris, T., Davranche, K., Jones, G., Hall, B., Corbett, J., & Minter, C. (2009). Acute incremental exercise, performance of a central executive task, and sympathoadrenal system and hypothalamic-pituitary-adrenal axis activity. *International Journal of Psychophysiology, 73*(3), 334-340.

McMorris, T., & Graydon, J. (1996). The effect of exercise on the decision-making performance of experienced and inexperienced soccer players. *Research Quarterly for Exercise and Sport, 67*(1), 109-114.

McMorris, T., & Graydon, J. (1997). The effect of exercise on cognitive performance in soccer-specific tests. *Journal of Sports Sciences, 15*(5), 459-468.

McMorris, T., Harris, R. C., Swain, J., Corbett, J., Collard, K., Dyson, R. J., . . . Draper, N. (2006a). Effect of creatine supplementation and sleep deprivation, with mild exercise, on cognitive and psychomotor performance, mood state, and plasma concentrations of catecholamines and cortisol. *Psychopharmacology, 185*(1), 93-103.

McMorris, T., Sproule, J., Turner, A., & Hale, B. J. (2011). Acute, intermediate intensity exercise, and speed and accuracy in working memory tasks: A meta-analytical comparison of effects. *Physiology & Behavior, 102*(3-4), 421-428.

McMorris, T., Swain, J., Smith, M., Corbett, J., Delves, S., Sale, C., . . . Potter, J. (2006b). Heat stress, plasma concentrations of adrenaline, noradrenaline, 5-hydroxytryptamine and cortisol, mood state and cognitive performance. *International Journal of Psychophysiology, 61*(2), 204-215.

Medbø, J., & Sejersted, O. (1990). Plasma potassium changes with high intensity exercise. *The Journal of Physiology, 421*(1), 105-122.

Merton, P. (1954). Voluntary strength and fatigue. *The Journal of Physiology, 123*(3), 553-564.

Miller, R., Boska, M. D., Moussavi, R., Carson, P., & Weiner, M. (1988). 31P nuclear magnetic resonance studies of high energy phosphates and pH in human muscle fatigue.

comparison of aerobic and anaerobic exercise. *The Journal of Clinical Investigation, 81*(4), 1190-1196.

Minett, G. M., & Duffield, R. (2014). Is recovery driven by central or peripheral factors? A role for the brain in recovery following intermittent-sprint exercise. *Frontiers in Physiology, 5*, 24.

Minett, G. M., Duffield, R., Marino, F. E., & Portus, M. (2012). Duration-dependant response of mixed-method pre-cooling for intermittent-sprint exercise in the heat. *European Journal of Applied Physiology, 112*(10), 3655-3666.

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., & Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks: A latent variable analysis. *Cognitive Psychology, 41*(1), 49-100.

Moon, H. Y., Becke, A., Berron, D., Becker, B., Sah, N., Benoni, G., . . . Mattison, J. A. (2016). Running-induced systemic cathepsin B secretion is associated with memory function. *Cell Metabolism, 24*(2), 332-340.

Morgan, R., Patterson, M., & Nimmo, M. (2004). Acute effects of dehydration on sweat composition in men during prolonged exercise in the heat. *Acta Physiologica, 182*(1), 37-43.

Morley, J., Beauchamp, G., Suyama, J., Guyette, F. X., Reis, S. E., Callaway, C. W., & Hostler, D. (2012). Cognitive function following treadmill exercise in thermal protective clothing. *European Journal of Applied Physiology, 112*(5), 1733-1740.

Morris, J., Nevill, M., Boobis, L., Macdonald, I., & Williams, C. (2005). Muscle metabolism, temperature, and function during prolonged, intermittent, high-intensity running in air temperatures of 33 and 17 C. *International Journal of Sports Medicine, 26*(10), 805-814.

Morrison, S., Sleivert, G. G., & Cheung, S. S. (2004). Passive hyperthermia reduces voluntary activation and isometric force production. *European Journal of Applied Physiology, 91*(5-6), 729-736.

Näätänen, R. (1973). The inverted-U relationship between activation and performance: A critical review. *Attention and Performance,* 4, 4-155.

Nakamura, Y., & Okamura, K. (2001). Seasonal variation of sweating responses under identical heat stress. *Applied Human Science, 17*(5), 167-172.

Neave, N., Emmett, J., Moss, M., Ayton, R., Scholey, A., & Wesnes, K. (2004). The effects of protective helmet use on physiology and cognition in young cricketers. *Applied Cognitive Psychology, 18*(9), 1181-1193.

Newcomer, J. W., Craft, S., Hershey, T., Askins, K., & Bardgett, M. E. (1994). Glucocorticoid-induced impairment in declarative memory performance in adult humans. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 14*(4), 2047-2053.

Nielsen, B., Hales, J., Strange, S., Christensen, N. J., Warberg, J., & Saltin, B. (1993). Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *The Journal of Physiology, 460*(1), 467-485.

Nielsen, B., & Nybo, L. (2003). Cerebral changes during exercise in the heat. *Sports Medicine, 33*(1), 1-11.

Noakes, T. D., St Clair Gibson, A., & Lambert, E. V. (2005). From catastrophe to complexity: A novel model of integrative central neural regulation of effort and fatigue during exercise in humans: Summary and conclusions. *British Journal of Sports Medicine, 39*(2), 120-124. doi:39/2/120 [pii]

Nybo, L., Girard, O., Mohr, M., Knez, W., Voss, S., & Racinais, S. (2013). Markers of muscle damage and performance recovery after exercise in the heat. *Med Sci Sports Exerc, 45*(5), 860-868.

Nybo, L., & Nielsen, B. (2001a). Hyperthermia and central fatigue during prolonged exercise in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985), 91*(3), 1055-1060.

Nybo, L., & Nielsen, B. (2001b). Perceived exertion is associated with an altered brain activity during exercise with progressive hyperthermia. *Journal of Applied Physiology (Bethesda, Md.: 1985), 91*(5), 2017-2023.

Oei, N. Y., Everaerd, W. T., Elzinga, B. M., van Well, S., & Bermond, B. (2006). Psychosocial stress impairs working memory at high loads: An association with cortisol levels and memory retrieval. *Stress, 9*(3), 133-141.

Ogoh, S., Tsukamoto, H., Hirasawa, A., Hasegawa, H., Hirose, N., & Hashimoto, T. (2014). The effect of changes in cerebral blood flow on cognitive function during exercise. *Physiological Reports, 2*(9)

Parker, S. M., Erin, J. R., Pryor, R. R., Khorana, P., Suyama, J., Guyette, F. X., . . . Hostler, D. (2013). The effect of prolonged light intensity exercise in the heat on executive function. *Wilderness & Environmental Medicine, 24*(3), 203-210.

Patel, P., Lamar, M., & Bhatt, T. (2014). Effect of type of cognitive task and walking speed on cognitive-motor interference during dual-task walking. *Neuroscience, 260*, 140-148.

Périard, J. D. (2016). Hyperthermia and supraspinal fatigue. *Experimental Physiology, 101(11)*, 1323-1324.

Periard, J. D., Racinais, S., Knez, W. L., Herrera, C. P., Christian, R. J., & Girard, O. (2014). Coping with heat stress during match-play tennis: Does an individualised hydration regimen enhance performance and recovery? *British Journal of Sports Medicine, 48 Suppl 1*, i64-70. doi:10.1136/bjsports-2013-093242 [doi]

Périard, J. D., Christian, R. J., Knez, W. L., & Racinais, S. (2014b). Voluntary muscle and motor cortical activation during progressive exercise and passively induced hyperthermia. *Experimental Physiology*, 99(1), 136-148.

Périard, J. D., Thompson, M. W., Caillaud, C., & Quaresima, V. (2013). Influence of heat stress and exercise intensity on vastus lateralis muscle and prefrontal cortex oxygenation. *Euroéean Journal of Applied Physiology*, 113(1), 211-222.

Péronnet, F., & Szabo, A. (1993). Sympathetic response to acute psychosocial stressors in humans: Linkage to physical exercise and training. *Exercise Psychology: The Influence of Physical Exercise on Psychological Processes.New York: John Wiley & Sons,*

Perrey, S., Racinais, S., Saimouaa, K., & Girard, O. (2010). Neural and muscular adjustments following repeated running sprints. *European Journal of Applied Physiology, 109*(6), 1027-1036.

Perrier, E. T., Buendia-Jimenez, I., Vecchio, M., Armstrong, L. E., Tack, I., & Klein, A. (2015). Twenty-four-hour urine osmolality as a physiological index of adequate water intake. *Disease Markers, 2015*

Pesce, C., Capranica, L., Tessitore, A., & Figura, F. (2003). Focusing of visual attention under submaximal physical load. *International Journal of Sport and Exercise Psychology, 1*(3), 275-292.

Pesce, C., Tessitore, A., Casella, R., Pirritano, M., & Capranica, L. (2007). Focusing of visual attention at rest and during physical exercise in soccer players. *Journal of Sports Sciences, 25*(11), 1259-1270.

Pontifex, M. B., Hillman, C. H., Fernhall, B., Thompson, K. M., & Valentini, T. A. (2009). The effect of acute aerobic and resistance exercise on working memory. *Medicine and Science in Sports and Exercise, 41*(4), 927-934. doi:10.1249/MSS.0b013e3181907d69 [doi]

Powers, S. K., Howley, E. T., & Cox, R. (1982). A differential catecholamine response during prolonged exercise and passive heating. *Medicine and Science in Sports and Exercise*, 14(6), 435-439.

Racinais, S., Gaoua, N., & Grantham, J. (2008a). Hyperthermia impairs short-term memory and peripheral motor drive transmission. *The Journal of Physiology, 586*(19), 4751-4762.

Racinais, S., & Girard, O. (2012). Neuromuscular failure is unlikely to explain the early exercise cessation in hot ambient conditions. *Psychophysiology, 49*(6), 853-865.

Racinais, S., & Oksa, J. (2010). Temperature and neuromuscular function. *Scandinavian Journal of Medicine & Science in Sports, 20*, 1-18.

Radakovic, S. S., Maric, J., Surbatovic, M., Radjen, S., Stefanova, E., Stankovic, N., & Filipovic, N. (2007). Effects of acclimation on cognitive performance in soldiers during exertional heat stress. *Military Medicine, 172*(2), 133-136.

Rampinini, E., Bosio, A., Ferraresi, I., Petruolo, A., Morelli, A., & Sassi, A. (2011). Match-related fatigue in soccer players. *Medicine and Science in Sports and Exercise, 43*(11), 2161-2170. doi:10.1249/MSS.0b013e31821e9c5c [doi]

Ramsey, J. D., & Kwon, Y. G. (1992). Recommended alert limits for perceptual motor loss in hot environments. *International Journal of Industrial Ergonomics, 9*(3), 245-257.

Randall, C. A., Ross, E. Z., & Maxwell, N. S. (2015). Effect of practical precooling on neuromuscular function and 5-km time-trial performance in hot, humid conditions among well-trained male runners. *Journal of Strength and Conditioning Research, 29*(7), 1925-1936. doi:10.1519/JSC.0000000000000840 [doi]

Reilly, T., & Borrie, A. (1992). Physiology applied to field hockey. *Sports Medicine, 14*(1), 10-26.

Rex, C. S., Lauterborn, J. C., Lin, C. Y., Kramar, E. A., Rogers, G. A., Gall, C. M., & Lynch, G. (2006). Restoration of long-term potentiation in middle-aged hippocampus after induction of brain-derived neurotrophic factor. *Journal of Neurophysiology, 96*(2), 677-685. doi:00336.2006 [pii]

Robertson, C. V., & Marino, F. E. (2017). Cerebral responses to exercise and the influence of heat stress in human fatigue. *Journal of Thermal Biology, 63*, 10-15.

Ross, E. Z., Cotter, J. D., Wilson, L., Fan, J., Lucas, S. J., & Ainslie, P. N. (2011). Cerebrovascular and corticomotor function during progressive passive hyperthermia in humans. *Journal of Applied Physiology, 112*(5), 748-758.

Ross, E. Z., Middleton, N., Shave, R., George, K., & Nowicky, A. (2007). Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man. *Experimental Physiology, 92*(2), 417-426.

Ross, E. Z., Cotter, J. D., Wilson, L., Fan, J. L., Lucas, S. J., & Ainslie, P. N. (2012). Cerebrovascular and corticomotor function during progressive passive hyperthermia in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985), 112*(5), 748-758. doi:10.1152/japplphysiol.00988.2011 [doi]

Ross, E. Z., Gregson, W., Williams, K., Robertson, C., & George, K. (2010). Muscle contractile function and neural control after repetitive endurance cycling. *Medicine and Science in Sports and Exercise, 42*(1), 206-212. doi:10.1249/MSS.0b013e3181b07a18 [doi]

Rossini, PM et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol* 91:79-92.

Saboisky, J., Marino, F. E., Kay, D., & Cannon, J. (2003). Exercise heat stress does not reduce central activation to non-exercised human skeletal muscle. *Experimental Physiology, 88*(6), 783-790.

Sawka, M. N., & Wenger, C. B. (1988). *Physiological Responses to Acute Exercise-Heat Stress,*

Schlader, Z. J., Gagnon, D., Adams, A., Rivas, E., Cullum, C. M., & Crandall, C. G. (2015). Cognitive and perceptual responses during passive heat stress in younger and older adults. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology, 308*(10), R847-54. doi:10.1152/ajpregu.00010.2015 [doi]

Schmit, C., Hausswirth, C., Le Meur, Y., & Duffield, R. (2016). Cognitive functioning and heat strain: Performance responses and protective strategies. *Sports Medicine,* , 1-14.

Schmitt, J. A., Benton, D., & Kallus, K. W. (2005). General methodological considerations for the assessment of nutritional influences on human cognitive functions. *European Journal of Nutrition, 44*(8), 459-464.

Serwah, N., & Marino, F. E. (2006). The combined effects of hydration and exercise heat stress on choice reaction time. *Journal of Science and Medicine in Sport, 9*(1-2), 157-164. doi:S1440-2440(06)00030-2 [pii]

Sidhu, S. K., Bentley, D. J., & Carroll, T. J. (2009). Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. *Muscle & Nerve, 39*(2), 186-196.

Simmons, S. E., Saxby, B. K., McGlone, F. P., & Jones, D. A. (2008). The effect of passive heating and head cooling on perception, cardiovascular function and cognitive performance in the heat. *European Journal of Applied Physiology, 104*(2), 271-280.

Skein, M., Duffield, R., Cannon, J., & Marino, F. E. (2012). Self-paced intermittent-sprint performance and pacing strategies following respective pre-cooling and heating. *European Journal of Applied Physiology, 112*(1), 253-266.

Spencer, M., Lawrence, S., Rechichi, C., Bishop, D., Dawson, B., & Goodman, C. (2004). Time–motion analysis of elite field hockey, with special reference to repeated-sprint activity. *Journal of Sports Sciences, 22*(9), 843-850.

Starkes, J. L. (1987). Skill in field hockey: The nature of the cognitive advantage. *Journal of Sport Psychology, 9*(2), 146-160.

Strauss, E., Sherman, E. M., & Spreen, O. (2006). A compendium of neuropsychological tests. New York, NY; Oxford University Press.

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology, 18*(6), 643.

Sun, G., Qian, S., Jiang, Q., Liu, K., Li, B., Li, M., . . . Liu, Y. (2013). Hyperthermia-induced disruption of functional connectivity in the human brain network. *PloS One, 8*(4), e61157.

Sunderland, C., & Nevill, M. E. (2005). High-intensity intermittent running and field hockey skill performance in the heat. *Journal of Sports Sciences, 23*(5), 531-540.

Sunderland, C., Morris, J. G., & Nevill, M. E. (2008). A heat acclimation protocol for team sports. *British Journal of Sports Medicine, 42*(5), 327-333. doi:10.1136/bjsm.2007.034207 [doi]

Svebak, S., & Murgatroyd, S. (1985). Metamotivational dominance: A multimethod validation of reversal theory constructs. *Journal of Personality and Social Psychology, 48*(1), 107.

Tatterson, A. J., Hahn, A. G., Martin, D. T., & Febbraio, M. A. (2000). Effects of heat stress on physiological responses and exercise performance in elite cyclists. *Journal of Science and Medicine in Sport, 3*(2), 186-193. doi:S1440-2440(00)80080-8 [pii]

Taylor, J. L., Todd, G., & Gandevia, S. C. (2006). Evidence for a supraspinal contribution to human muscle fatigue. *Clinical and Experimental Pharmacology and Physiology, 33*(4), 400-405.

Taylor, J. L., & Gandevia, S. C. (2008). A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *Journal of Applied Physiology (Bethesda, Md.: 1985), 104*(2), 542-550. doi:01053.2007 [pii]

Temesi, J., Maturana, F. M., Peyrard, A., Piucco, T., Murias, J. M., & Millet, G. Y. (2017). The relationship between oxygen uptake kinetics and neuromuscular fatigue in high-intensity cycling exercise. *European Journal of Applied Physiology*, *117*(5), 969-978.

Temesi, J., Rupp, T., Martin, V., Arnal, P. J., Féasson, L., Verges, S., & Millet, G. Y. (2014). Central fatigue assessed by transcranial magnetic stimulation in ultratrail running. *Med Sci Sports Exerc, 46*(6), 1166-1175.

Terry, P. C., Lane, A. M., Lane, H. J., & Keohane, L. (1999). Development and validation of a mood measure for adolescents. *Journal of Sports Sciences, 17*(11), 861-872.

Thomas, K., Elmeua, M., Howatson, G., & Goodall, S. (2016). Intensity-dependent contribution of neuromuscular fatigue after constant-load cycling. *Medicine and Science in Sports and Exercise, 48*(9), 1751-1760.

Thomas, K., Goodall, S., Stone, M., Howatson, G., Gibson, A. S. C., & Ansley, L. (2015). Central and peripheral fatigue in male cyclists after 4-, 20-, and 40-km time trials. *Medicine & Science in Sports & Exercise, 47*(3), 537-546.

Thomas, K., Dent, J., Howatson, G., & Goodall, S. (2017). Etiology and recovery of neuromuscular fatigue following simulated soccer match-play. *Medicine & Science in Sports & Exercise*, 49(5), 955-964.

Thomas, M. M., Cheung, S. S., Elder, G. C., & Sleivert, G. G. (2006). Voluntary muscle activation is impaired by core temperature rather than local muscle temperature. *Journal of Applied Physiology (Bethesda, Md.: 1985), 100*(4), 1361-1369. doi:00945.2005 [pii]

Todd, G., Butler, J. E., Taylor, J. L., & Gandevia, S. (2005). Hyperthermia: A failure of the motor cortex and the muscle. *The Journal of Physiology, 563*(2), 621-631.

Todd, G., Taylor, J. L., & Gandevia, S. (2003). Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *The Journal of Physiology, 551*(2), 661-671.

Todd, G., Taylor, J. L., & Gandevia, S. C. (2004). Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation. *Journal of Applied Physiology (Bethesda, Md.: 1985), 97*(1), 236-242. doi:10.1152/japplphysiol.01336.2003 [doi]

Todd, G., Taylor, JL., Gandevia, SC. (2016). Measurement of voluntary activation based on transcranial magnetic stimulation over the motor cortex. *J Appl Physiol* 121:678-686 doi:10.1152/japplphysiol.00293.2016.

Tomporowski, P. D. (2003). Effects of acute bouts of exercise on cognition. *Acta Psychologica, 112*(3), 297-324.

Tucker, R., Marle, T., Lambert, E. V., & Noakes, T. D. (2006). The rate of heat storage mediates an anticipatory reduction in exercise intensity during cycling at a fixed rating of perceived exertion. *The Journal of Physiology, 574*(3), 905-915.

Tucker, R., Rauch, L., Harley, Y. X., & Noakes, T. D. (2004). Impaired exercise performance in the heat is associated with an anticipatory reduction in skeletal muscle recruitment. *Pflügers Archiv, 448*(4), 422-430.

Urbach, D., Nebelung, W., Weiler, H. T., & Awiszus, F. (1999). Bilateral deficit of voluntary quadriceps muscle activation after unilateral ACL tear. *Medicine and Science in Sports and Exercise*, 31(12), 1691-1696.

van den Heuvel, Anne MJ, Haberley, B. J., Hoyle, D. J., Taylor, N. A., & Croft, R. J. (2017). The independent influences of heat strain and dehydration upon cognition. *European Journal of Applied Physiology, 117*(5), 1025-1037.

Van Dongen, H. P., & Dinges, D. F. (2005). Sleep, circadian rhythms, and psychomotor vigilance. *Clin Sports Med, 24*, 237-249.

Vega, S. R., Strüder, H. K., Wahrmann, B. V., Schmidt, A., Bloch, W., & Hollmann, W. (2006). Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. *Brain Research, 1121*(1), 59-65.

Verin, E., Ross, E., Demoule, A., Hopkinson, N., Nickol, A., Fauroux, B., . . . Polkey, M. I. (2004). Effects of exhaustive incremental treadmill exercise on diaphragm and quadriceps motor potentials evoked by transcranial magnetic stimulation. *Journal of*

Applied Physiology (Bethesda, Md.: 1985), 96(1), 253-259. doi:10.1152/japplphysiol.00325.2003 [doi]

Wang, L. I., Liu, F., Luo, Y., Zhu, L., & Li, G. (2015). Effect of acute heat stress on adrenocorticotropic hormone, cortisol, interleukin-2, interleukin-12 and apoptosis gene expression in rats. *Biomedical Reports*, *3*(3), 425-429.

Weiner, M. W., Moussavi, R. S., Baker, A. J., Boska, M. D., & Miller, R. G. (1990). Constant relationships between force, phosphate concentration, and pH in muscles with differential fatigability. *Neurology*, *40*(12), 1888-1893.

Wendt, D., Van Loon, L. J., & Lichtenbelt, W. D. M. (2007). Thermoregulation during exercise in the heat. *Sports Medicine, 37*(8), 669-682.

Westerblad, H., Allen, D. G., & Lannergren, J. (2002). Muscle fatigue: Lactic acid or inorganic phosphate the major cause? *Physiology, 17*(1), 17-21.

Williams, A. M., Hodges, N. J., North, J. S., & Barton, G. (2006). Perceiving patterns of play in dynamic sport tasks: Investigating the essential information underlying skilled performance. *Perception, 35*(3), 317-332.

Wijayanto, T., Toramoto, S., & Tochihara, Y. (2013). Passive heat exposure induced by hot water leg immersion increased oxyhemoglobin in pre-frontal cortex to preserve oxygenation and did not contribute to impaired cognitive functioning. *International Journal of Biometeorology*, *57*(4), 557-567.

Wohlwend, M., Olsen, A., Håberg, A. K., & Palmer, H. S. (2017). Exercise intensity-dependent effects on cognitive control function during and after acute treadmill running in young healthy adults. *Frontiers in Psychology*, *8*, 406.

Yanagisawa, H., Dan, I., Tsuzuki, D., Kato, M., Okamoto, M., Kyutoku, Y., & Soya, H. (2010). Acute moderate exercise elicits increased dorsolateral prefrontal activation and improves cognitive performance with stroop test. *NeuroImage, 50*(4), 1702-1710.

Yerkes, R. M., & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology, 18*(5), 459-482.

Young, A. J., Sawka, M. N., Epstein, Y., Decristofano, B., & Pandolf, K. B. (1987). Cooling different body surfaces during upper and lower body exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 63*(3), 1218-1223.

Appendices

Appendix A

- A1 Example Participant Information Sheet
- A2 Example Participant Consent Form
- A3 Example Health Screen Form

Appendix B

- B2 Example ethical application

Appendix C

- C1 Feeling scale
- C2 Thermal Sensation Scale
- C3 Rating of Perceived Exertion Scale
- C4 Felt Arousal Scale

Appendix A1

Participant Information Sheet

"The influence of a hockey match on cognitive function in male and female hockey players"

- Brief Introduction:
  Many team games, including hockey, are intermittent and high intensity in character requiring a large cognitive component in order to perform well. The ability to maintain cognitive function and skill performance whilst performing maximal exercise is crucial in order to maintain performance in competitive match play. It is thought that exercise can actually have a beneficial influence on cognitive performance, however it is yet to be established what influence exercise which is intermittent in nature has on cognitive function. Therefore the aim of the current study is to establish how cognitive function is influenced by the physical and mental demands of a hockey match in both male and female hockey players.

- Study Requirements:
  Participants will be:
  - hockey players who play for Loughborough University hockey club.
  - All participants will be healthy and accustomed to high intensity exercise.

  Study demands:
  If you volunteer the study will require you to attend the hockey pitch/pavilion on 3 occasions. The first session will be a familiarisation session consisting of only cognitive function tests. This session will last approximately 1 hour. You will then be expected to attend the pitch for a control trial and a main trial, each of which will last approximately 2.5 hours. In the main trial, you will be expected to exercise strenuously. This entails putting in a considerable amount of effort and energy, which will result in a significant elevation in heart rate and potentially cause discomfort, particularly in the lower limbs. If discomfort becomes too great, you will be advised to stop participating in the activity. 2 blood samples will be taken on each main trial. A total of 60ml will be taken on each day i.e. maximum of 2 x 30ml samples. The blood samples will be analysed for various markers including adrenaline, cortisol, lactate, noradrenaline and glucose.

- Location:
  The waterbase hockey pitch and pavilion at Loughborough University, Epinal Way, Loughborough, LE11 3TT

- Restrictions During Testing:
  No alcohol to be consumed during the 24 hours prior to testing and during testing. No caffeine to be consumed on the day of testing. Avoid strenuous exercise in the 24 hours prior to the main experimental trial.

- Testing Protocol:
  The testing protocol consists of a baseline test (full cognitive function test battery & blood sample) followed by a 70 minute hockey match, with a 10 minute half-time. At half-time a shortened version of the cognitive function tests will be completed. Immediately following completion of the hockey match the full cognitive function test battery will be completed as well

as a final blood sample being taken. GPS and heart rate data will be taken throughout the hockey match.
Video footage of the hockey match will be taken which will include all individuals involved in the match, who will all have been asked to provide written consent. Video footage will only be taken during the 70 minutes of match play, and only those involved in match play will be in shot. No video footage will be taken during any other aspects of the protocol. This will be collected using an automatic overhead camera at the hockey pitch. In terms of anonymising the data, as with all data on all studies conducted, the data will be anonymised and kept in accordance with the Data Protection Act. Clearly, individuals will be identifiable on the video but this is unavoidable. Only study staff will have access to the video and any subsequent data to be stored will be done so anonymously, using the same coding system as all other data collected.

On the control trial all timings and measurements will be the same however the tests will separated by seated rest instead of a hockey match.

- Potential Benefits to You:
  We will be able to provide you with heart rate and GPS data from the hockey match. We will also be able to provide you with information regarding your individual cognitive function response during and following a hockey match.

- Potential Risks to You:

  Although it is extremely unlikely, high intensity exercise has been known to reveal unsuspected heart or circulation problems and very rarely these have had serious or fatal consequences. The exercise tests (as indicated above) could cause injury and illness. However, several steps will be taken to minimise this risk, including the fact an experienced experimenter will oversee all testing and you will be asked to complete a warm-up prior to completing the match. You may experience some slight delayed onset muscle soreness in the 48 hours following the testing session but this can be alleviated with some gentle stretching.

  Blood sampling through venepuncture sampling has the potential to result in bruising. Samples will be taken only by appropriately trained phlebotomists (someone who collects blood samples) and all biological material will be stored and disposed of according to Health and Safety procedures.

  You are free to withdraw from the study at any time, without providing a reason. If at any point you decide to withdraw from the study your data will be destroyed.

- Contacts:
  Dr Caroline Sunderland: Caroline. sunderland@ntu.ac.uk or 01158486379
  Dr Simon Cooper: Simon.Cooper@ntu.ac.uk or 01158488059
  Rachel Malcolm: Rachel.malcolm@ntu.ac.uk

Appendix A2

Participant Statement of Consent to Participate in the Investigation Entitled:

"The influence of a hockey match on cognitive function in male and female hockey players"

1) I, _____*[name of participant]* agree to partake as a participant in the above study.

2) I understand from the participant information sheet, which I have read in full, and from my discussion(s) with *Rachel Malcolm/ Dr Simon Cooper/ Dr Caroline Sunderland* that this will involve me *performing cognitive function tests pre, during and post a competitive hockey match. I also understand that I will be required to provide a baseline and post-exercise blood sample.*

3) It has also been explained to me by *Rachel Malcolm/ Dr Simon Cooper/ Dr Caroline Sunderland* that the risks and side effects which may result from my participation are as follows: *In individuals who have a medical problem maximal exercise can be a risk to health and in extreme cases can be a cause of sudden death. However, in active individuals the risks are minimal and all individuals who wish to undertake the field test battery will complete a health history questionnaire before they participate in any testing. If there are any reasons why maximal exercise may pose a risk to your health, you will be told not to take part in the testing procedures. You are free to withdraw from the tests at any time without penalty or prejudice.*

4) I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction.

5) I undertake to abide by University regulations and the advice of researchers regarding safety.

6) I am aware that I can withdraw my consent to participate in the procedure at any time and for any reason, without having to explain my withdrawal and that my personal data will be destroyed.

7) I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous.

8) I understand that this study requires video analysis of a hockey match I will be partaking in. I confirm that I agree to comply with this as the video will be anonymised and kept in accordance with the Data Protection Act.

9) I confirm that I have had the University's policy relating to the storage and subsequent destruction of sensitive information explained to me. I understand that sensitive information I have provided through my participation in this study, in the form of questionnaires, blood samples, heart rate data and GPS data will be handled in accordance with this policy.

10) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent me from partaking in this research.

Participant signature: Date:
Independent witness signature: Date:

Primary Researcher signature: Date:

Appendix A3

Health screen

Name or Number .....

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present,** do you have any health problem for which you are:

(a) on medication, prescribed or otherwise Yes ☐ No ☐

(b) attending your general practitioner Yes ☐ No ☐

(c) on a hospital waiting list Yes ☐ No ☐

2. **In the past two years,** have you had any illness which require you to:

(a) consult your GP Yes ☐ No ☐

(b) attend a hospital outpatient department Yes ☐ No ☐

(c) be admitted to hospital Yes ☐ No ☐

3. **Have you ever** had any of the following?

(a) Convulsions/epilepsy Yes ☐ No ☐

(b) Asthma Yes ☐ No ☐

(c) Eczema Yes ☐ No ☐

(d) Diabetes Yes ☐ No ☐

(e) A blood disorder Yes ☐ No ☐

(f) Head injury Yes ☐ No ☐

(g) Digestive problems Yes ☐ No ☐

(h) Heart problems Yes ☐ No ☐

(i) Problems with bones or joints Yes ☐ No ☐

(j) Disturbance of balance / coordination Yes ☐ No ☐

(k) Numbness in hands or feet Yes ☐ No ☐

(l) Disturbance of vision Yes ☐ No ☐

(m) Ear / hearing problems Yes ☐ No ☐

(n) Thyroid problems Yes ☐ No ☐

(o) Kidney or liver problems Yes ☐ No ☐

(p) Allergy to nuts, alcohol etc. Yes ☐ No ☐

(q) Any problems affecting your nose e.g. recurrent nose bleeds Yes ☐ No ☐

(r) Any nasal fracture or deviated nasal septum Yes ☐ No ☐

4. **Has any,** otherwise healthy, member of your family under the age of 50 died suddenly during or soon after exercise? Yes ☐ No ☐

5. Are there any reasons why blood sampling may be difficult? Yes ☐ No ☐

6. Have you had a blood sample taken previously? Yes ☐ No ☐

7. Have you had a cold, flu or any flu like symptoms in the last Month? Yes ☐ No ☐

Appendix B1

Application form

(1) Applicants (please note: supervisor's name/s only for undergraduate student work, supervisor's name/s and student's name for postgraduate student work):

Dr Caroline Sunderland, School of Science and Technology, Nottingham Trent University
Rachel Malcolm, School of Science and Technology, Nottingham Trent University
Dr Simon Cooper, School of Science and Technology, Nottingham Trent University

(2) Project Title:

The influence of a hockey match on cognitive function in male and female hockey players.

(3) Lay Summary:

Many team games, such as football, field hockey and rugby union are intermittent in character requiring performance of variable, but submaximal, speed running, interspersed with brief periods of maximal sprinting. Specifically, field hockey demands an extensive requirement for high levels of mental processing to successfully perform in a dynamic environment (Sunderland & Nevill, 2007). In addition, successful skilled performance can have a substantial cognitive requirement (Starkes, 1987), which has the potential to be susceptible to a number of physiological and environmental factors. It is known that exercise can beneficially influence cognitive performance (Chang et al, 2012), however this has never been looked at in isolation for intermittent exercise in a field setting. Therefore, the aim of the present study is to assess how a 70 minute hockey match influences cognitive function in hockey players.

(4) Background **and** aims of the project:

Background:

Team sports, which are characterized by intermittent high-intensity exercise bouts, require a contribution of motor skill performance and cognitive functioning as well as the necessary physiological contributions in order to perform optimally (Burke, 1997). Hockey is a sport which can be included in this category. A considerable cognitive demand is placed on individuals, hence any changes in cognitive functioning during and after a game may be a key determinant in overall performance, particularly skill performance. While the maintenance of physiological function is clearly important in team sports such as field hockey, soccer and rugby, during such activities the completion of the required skills is at least as important in determining success. However, very few researchers have investigated how fatiguing exercise may influence motor skill performance and cognitive functioning.

It has been suggested that the increase in metabolic load associated with exercise , as a result of an increased heart rate and core temperature, improves cognitive performance as a result of increased levels of arousal (Grego et al, 2005). High-intensity bouts of exercise may facilitate a narrowing of attention to block out any irrelevant cues and improve focus on task-relevant information (Hogervorst et al, 1993). However, when the exercise intensity or duration is sufficient to bring about symptoms of central and/or peripheral fatigue, and the concomitant hormonal changes, cognitive performance is likely to decline (Grego et al, 2005). There have been several suggested mechanisms to identify this causal link. Changes in cerebral blood flow, increases in attentional resource allocation (McMorris et al, 1999) or changes in concentrations of cortisol, noradrenaline and adrenaline (Grego et al, 2005; McMorris et al, 1999) could be related to cognitive performance, however it is clear that this relationship is complex.

To date no one has examined the influence of intermittent exercise in a temperate environment, in a field setting on cognitive function.

Aims:

Therefore the aim of the current study is to assess whether the intermittent high-intensity nature of a hockey game will influence cognitive functioning. We also aim to assess whether any changes in cognitive functioning correlate with changes in stress markers in the blood.

We aim to compare the differential influence of this type of exercise on males and females and assess whether any differences correlate with differences in stress markers in the blood.

(5) Name **and** personal experience of **each** investigator (include experience of proposed procedures and, if appropriate, qualifications):

Name: Dr Caroline Sunderland
Experience: 15 years' experience of completing high intensity intermittent exercise testing in hot environmental conditions and a trained phlebotomist.
Fully familiar with all appropriate policies eg Health and Safety, Blood Sampling.

Name: Dr Simon Cooper
Experience: 9 years' experience in exercise physiology testing, in particular working with young people in a variety of settings (schools, sports clubs etc.). Dr. Cooper has also been involved extensively in the physiological testing of elite young athletes. Completion of First Aid at Work Course. Extensive experience administering cognitive function tests.

Name: Rachel Malcolm
Experience: 2nd year PhD student, experiences gained during undergraduate and postgraduate exercise physiology laboratories. Rachel also has two years' experience of teaching in the area of Exercise Physiology. Completion of First Aid at Work course. Rachel has also gained experience in an applied setting assisting with physiological testing at Glasgow Warriors rugby club and Nottingham Forest football club.

Name: Jonathan Folland
Experience: 20 years' experience of completing neuromuscular function testing.

Name: Chris Tyler
Experience: 9 years' experience completing high intensity exercise testing in the heat. Also has extensive experience completing neuromuscular testing using electrical stimulation.

(6) Details of participants (e.g. age [units], health status, gender):

30 Males and 30 females, aged 18- 25 years and hockey players for Loughborough students hockey club. No participants under the age of 18 will be included.
Volunteers will be made aware of their right to withdraw from the study at any time. Participants will also be made aware of all requirements for taking part in the study, and will complete informed consent and health screening.
Participants will supervised by exercise physiologists from the Nottingham Trent University Sport Science department. We have at least 6 members of staff present and supervising at all times. Therefore, there will be at least 1 member of staff for every 3 participants.

(7) Location (e.g. room/building, campus, off-site, any special facilities to be used):

All testing will take part at the waterbase hockey pitch at Lougborough University, Epinal Way, Loughbrough, LE11 3TT.

(8) Duration of study (e.g. dates, timescale of study, demands on participant's time):

Participants will be required to complete a familiarisation session, one control session and one match day session. 4 different matches will be analysed (2 with the men's teams and 2 with women's teams). The familiarisation trial will last no longer than one hour. The main match day trial and control trial will last~2.5hrs. However on the control day and match day trials only 30 minutes will be spent partaking in cognitive function activities and the remainder will be spent resting(control day) between measurements or playing hockey (match day). Total time demand of 6 hours per participant.

(9) Reasons for undertaking the study (e.g. research, teaching, consultancy or contract) **and** intended dissemination of data (e.g. publication, conference presentation, undergraduate dissertation):

Reasons: PhD research project

Intended dissemination: The data will be used to write up the PhD thesis and potentially for conference presentation/publication by staff members and PhD student.

(10) Research design:

Familiarisation

Participants will be required to complete an informed consent form and health screen questionnaires prior to participating in the study (see appendices).

During familiarisation, participants will be given the opportunity to practice all of the cognitive function testing involved in the main trials.

Anthropometric measurements will also be taken during the familiarisation session (height & weight).

Experimental trial

During the experimental trial participants will complete a battery of cognitive tests lasting 10 minutes at 3 time points (prior to a hockey match, at half time and at full time). During the control trial the tests will be implemented at the same time points, however seated rest will fill the time between tests.

(11) Procedures and measurements (e.g. materials and methods):

GPS

Participants will be fitted with GPS trackers. This involves participants wearing a small transmitter device on their back. This is placed within a holder to prevent displacement. The participant can wear this under or over the clothes. If the participant wears it underneath their clothing then they will be provided a private room in order to put this on in extreme privacy. The GPS device will allow us to monitor the total distance covered and distances covered at various speeds in order to associate the amount of work done by an individual participant to the changes seen in their cognitive function.

Video analysis

Participants will be recorded during each of the match day trials in order to assess how skill performance is influenced throughout a hockey match. Participants will all provide formal consent to being filmed during match play. All individuals who are participating in the match will be asked for their consent to being videoed. In terms of anonymising the data, as with all data on all studies conducted, the data will be anonymised and kept in accordance with the Data Protection Act.

Clearly, individuals will be identifiable on the video but this is unavoidable. Only study staff will have access to the video and any subsequent data to be stored will be done so anonymously, using the same coding system as all other data collected. A separate consent form for the opposition who will also be filmed can be found in appendix 2a.

Cognitive function

The cognitive function tests will be administered via a battery of tests on a laptop computer, where the subject is asked to respond in a simple manner, often pressing a pre-determined key. Each testing session will last 15-20 minutes. The battery consists of tests of sustained attention (the Rapid Visual Information Processing test), visual search, working memory (the Sternberg paradigm) and a Stroop test.

RVIP: The RVIP (Rapid Visual Information Processing) test consists of the numbers 0-9 appearing on a screen in quick succession. The subject is asked to respond by pressing the enter key if they detect three consecutive odd or even numbers. During piloting, should this be too difficult for the younger age group, the test can be modified to identifying two consecutive odd or even numbers.

Visual Search: This test has two parts, the first of which involves responding when a triangle appears on the screen, again by pressing the enter key. The second part of the test consists of a number of dots on the screen: when these form a triangle, the subject responds by pressing the enter key.

Sternberg Paradigm: The Sternberg paradigm is a test of working memory and involves testing at three different levels. The first level has one target digit (a 3), each time this appears on the screen the subject is to press the right arrow key, whereas when any letter appears the subject presses the left arrow key. The second level has three target letters (e.g. A, G and P) and the third level five target letters (e.g. N, F, H, T and W). The subjects respond in the same manner as the first level. Again, during piloting, if this is deemed too difficult for the younger children, the top level can be removed from the test.

Stroop Test: The Stroop test involves two levels. On the first level, a word (always a colour) appears in the centre of the screen and the subject must choose either the word on the left or right of the screen that matches the central word, using the arrow keys. On the next level, the subject must select the colour the word is written in, rather than the word itself, again by choosing from the words on the left and right of the screen, using the arrow keys.

Bloods

Venepuncture blood samples will be taken prior to the first cognitive test and the final cognitive test to assess changes in markers of stress including cortisol, adrenaline, noradrenaline, lactate and glucose. Total amount of blood will be <60ml per trial. Participants will be laid down on a bed whilst the blood sample is taken. Sterile procedures and protective equipment (*e.g.* gloves) will be used at all times. Staff members will have protective inoculations against Hepatitis B. Participants are very unlikely to be in contact with any blood other than their own, making participants' risk minimal.

Additional data

Heart rate will be measured continuously using polar heart rate monitors. This will involve participants wearing a heart rate monitor belt. This will be fastened around the chest of each participant. Participants will apply their heart rate monitor belts themselves in a private room in extreme privacy.

(12) **Standard phrasing**; please delete/edit as appropriate:

BLOOD SAMPLING:

Blood sampling will be carried out by one of various qualified phlebotomists within the Sport Academic Team, all of whom are on the School's list of trained phlebotomists.

FIRST AID COVER:

This will be provided by **Rachel Malcolm, Simon Cooper & Caroline Sunderland** who are on the School's list of First Aiders as shown in the School Safety Handbook (see additional information). They will be present at the field testing site and immediately contactable to be on-site within 30 seconds.

INFORMED CONSENT AND DATA PROTECTION:

- All participants over age 18 years will be asked to give informed consent to the participation and processing/use of personal information.
- Participants will have a minimum of 24 hours between receiving the information and signing the informed consent
- All participants will have the right to withdraw at any stage without detriment. Any personal information involving any participant gained through participation in the study will be treated as confidential and only handled by individuals relevant to the performance of the study.
- All personal data (such as names, addresses, telephone numbers and email addresses) and sensitive personal data, (such as information about racial ethnic origin, physical or mental health or sex life), will only be processed with the participants informed consent. The data will not be stored for any longer than is necessary.

(13) Justification of sample size (**and** power calculations, if appropriate):

Power calculations are not appropriate given that a convenience sample of Loughborough students hockey club players will be used in the present study.

The sample used in the present study comprises of the members of the Loughborough students hockey club 1st and 2nd teams for both men and women. Approximate numbers are as follows:

Women's 1st team = 15
Women's 2nd team = 15
Men's 1st team = 15
Women's 2nd team = 15

(14) Possible risks, discomforts and distress:

Maximal exercise

Exercise carries a potential risk of muscle soreness and injury, although this will be somewhat mediated by the performance of a warm-up. However, using hockey players as our participants

means that no additional strain that they are unaccustomed to will be placed upon them.
There is also the possibility of an adverse cardiovascular event, although this is unlikely in young, healthy participants who are used to performing high intensity exercise. Participants will be instructed that they should cease exercise if they are feeling any pain or unwell.

Blood sampling

Blood sampling through venepuncture sampling has the potential to result in bruising and there are also risks as the work involves biological material. Sampling will be carried out by appropriately trained phlebotomists (Caroline Sunderland, Simon Cooper, Rachel Malcolm, Rebecca Stannard, Rebecca Townsend, Karah Dring, Daniel Martin, John Morris, Ian Varley & Neil Williams) only and all biological material will be stored and disposed of according to health and safety procedures.

(15) Procedures for taking measurements **and** for chaperoning and supervision of participants during investigations:

Participants will initially be given a verbal and written explanation of the study requirements. They will also be provided with written informed consent prior to participating in the study and be free to withdraw from the study at any time. All participants will receive information sheets on how to prepare for the testing to make the investigation valid and will complete a medical and health questionnaire prior to all testing.
Participants will be supervised at all times, from arriving for the start of their session until the end of the trial. All measurements will be taken by an experienced sport and exercise physiologist. At least 6 exercise physiologists from Nottingham Trent University's sport science department will be at each testing session. This ensures that their will be a least 1 member of staff supervising every 3 participants at all times during all periods of testing and will be closely monitored following all tests. Participants will be allowed to drink water ad libitum, and can leave the testing session once they feel comfortable.

(16) Details of any payments to be made to the participants:

None.

(17) Do any investigators stand to gain from a particular conclusion of the research project, other than publication of the research findings?

No

(18) Please provide details of funding bodies supporting this research and their commercial interest, **if none please state**:

None.

(19) Storage and destruction of sensitive materials (refer to Storage and destruction of sensitive materials policy for guidance; Appendix 8).

All sensitive materials will be stored for 5 years and destroyed by June 2020 in accordance with the

University's Storage and Destruction of Sensitive Materials Policy.
**Please edit Appendix 8 in accordance with the information stated here.*

(20) ***(Committee use only)*** Has the University's Insurers indicated that they are content for the University's Public Liability Policy to apply to the proposed investigation?

Yes ___

No ___
If No, has additional insurance cover been arranged by the investigator?

(21) In the case of studies involving new drugs or radioisotopes, written approval for the study must be obtained from the appropriate national body and submitted with the protocol. **State if applicable:**

N/A

(22) Declaration

By checking *(insert an X)* the box below, I am confirming that I have read the University's ethical constitution and protocol document and that I have completed this application. I understand that this application and approval is specific to this procedure and these participants only. X

Appendix C1

Feeling Scale

+5	Very good
+4	
+3	Good
+2	
+1	Fairly good
0	Neutral
-1	Fairly bad
-2	
-3	Bad
-4	
-5	Very bad

Appendix C2

Thermal Sensations

Rating	Sensation
0.0	Unbearably Cold
0.5	
1.0	Very Cold
1.5	
2.0	Cold
2.5	
3.0	Cool
3.5	
4.0	Comfortable
4.5	
5.0	Warm
5.5	
6.0	Hot
6.5	
7.0	Very Hot
7.5	
8.0	Unbearably Hot

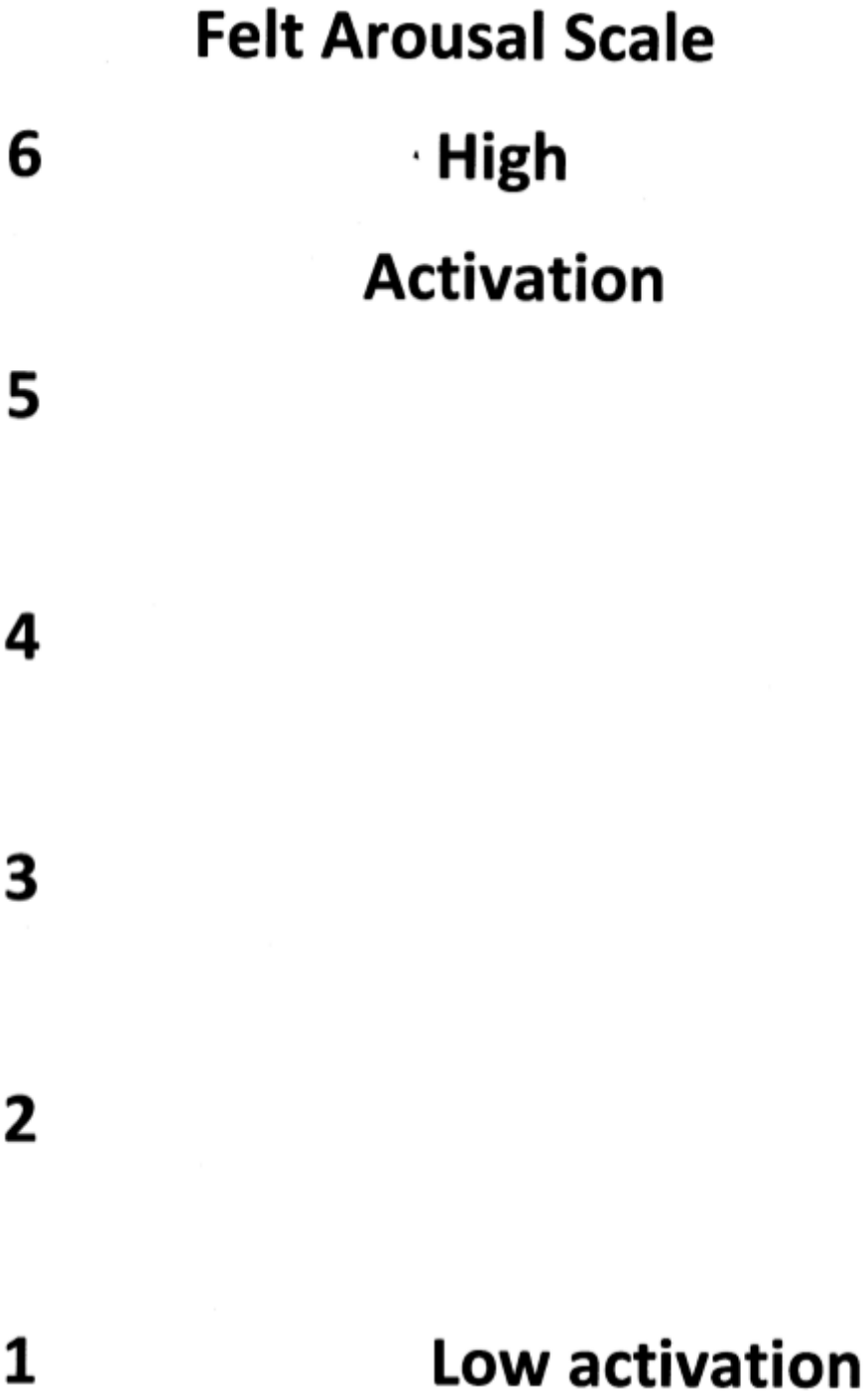
Appendix C3

RATING OF PERCEIVED EXERTION

6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

Morgan W. & Borg G. Perception of effort in the prescription of physical activity. In: Nelson T., ed. Mental Health and emotional aspects of sports. Chicago: American Medical Association, 1976:126-129.

Appendix C4



Appendix C5

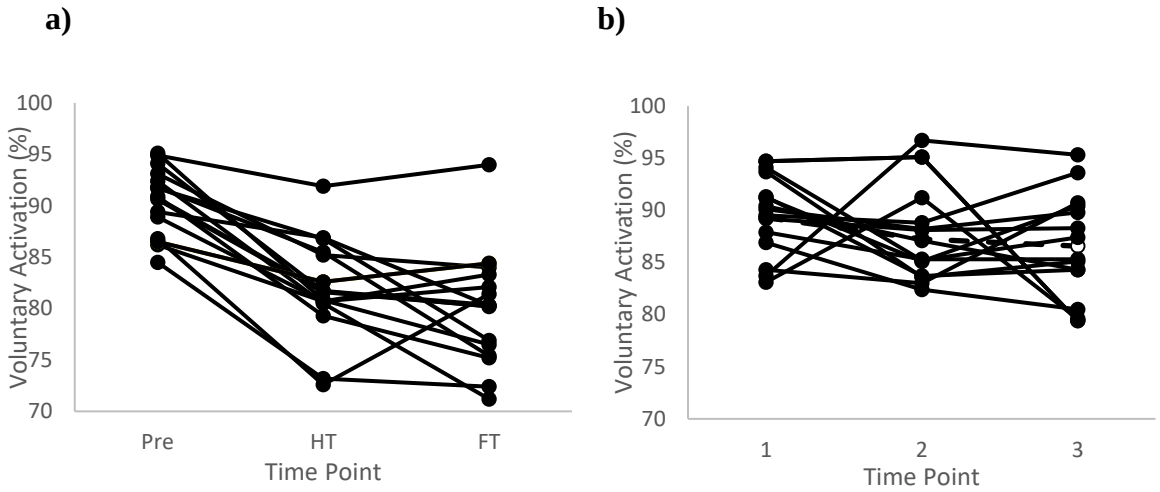


Figure 8. 1 Individual data for peripheral voluntary activation changes across time in the a) hot and b) moderate trials.

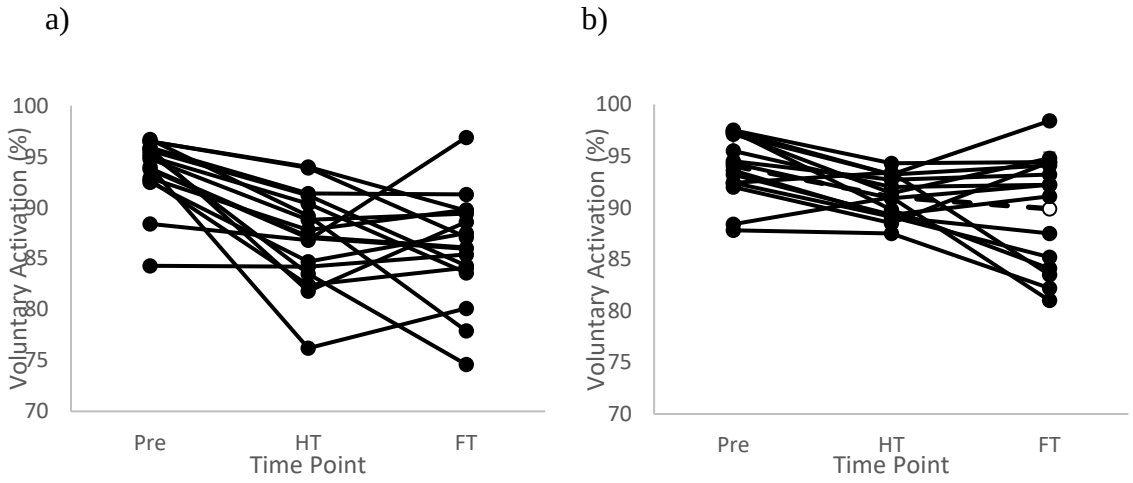


Figure 8. 2. Individual data for cortical voluntary activation changes across time in the a) hot and b) moderate trials.