THE EFFICACY OF TRANSCRANIAL DIRECT CURRENT STIMULATION TO ENHANCE ENDURANCE EXERCISE PERFORMANCE

This thesis is presented for the Degree of Doctor of Philosophy at the University of Kent.

by

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Abstract

In the last two decades, non-invasive brain stimulation techniques such as transcranial direct current stimulation (tDCS) have been proposed to increase endurance capacity within exhaustive exercise trials. Consequently, interest in the use of tDCS has spread throughout research, commercial and public communities. Despite the promising findings of an ergogenic effect detailed within exhaustive exercise, the mechanisms of action underlying tDCS are uncertain and the efficacy of tDCS to enhance self-paced performance is inconclusive. Therefore, this thesis explored the efficacy of tDCS to enhance endurance exercise performance.

The first experimental chapter (chapter 2) demonstrated that anodal tDCS applied to the dorsolateral prefrontal cortex (DLPFC) in a bilateral montage had no significant effect on 15 minute cycling TT performance. Study two explored the effect of tDCS delivered to the DLPFC in an extracephalic montage on cycling TT performance. An extracephalic montage is suggested to avoid the complications associated with the cathodal electrode seen in cephalic and bilateral montages. This study also demonstrated that tDCS had no significant effect on TT performance. Study three explored the effect of anodal tDCS applied through the Halo Sport Neurostimulation system on physiological adaptation to endurance training. This study demonstrated that tDCS applied to the motor cortex (M1) during 6 weeks of high intensity interval training did not augment the training response to a greater extent than the sham group. Finally, study four investigated the capability of the Halo Sport Neurostimulation System to induce changes in corticospinal excitability. The results demonstrated that this device had no effect on the corticospinal excitability of the M1 when delivered at rest and during submaximal exercise.

The present thesis demonstrates that the acute and chronic applications of conventional tDCS are not viable methods of enhancing endurance performance or increasing the physiological adaptations to training. Therefore, the use of tDCS to enhance performance of recreationally active participants cannot be recommended.

Declaration

No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other University or Institution of learning.

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Abbreviations

ACC- anterior cingulate cortex

ADM- adductor digiti minimi muscle

AMPAR- α-amino-3-hydroxy-5-methyl-4-isoxazolepropoionic acid receptors

ANOVA- analysis of variance

BBB- blood-brain barrier

BCAA- branched chain amino acids

BCM theory- Bienenstock-Cooper-Munro theory of synaptic modification

BDNF- brain derived neurotrophic factor

BOLD MRI- blood oxygenation level dependent magnetic resonance imaging

Ca^{2+} - calcium

CaMKII- Ca^{2+}/ calmodulin-dependent protein kinase II

CAMP- cyclic adenosine 3', 5' monophosphate

CBZ- carbamazepine

CMEP- cervicomedullary motor evoked potential

CPT- cold pressor test

CREB- CAMP-response element binding protein

CSF- cerebrospinal fluid

CSP- corticospinal silent period

CV- coefficient of variation

CVAL- cortical voluntary activation level

DLPFC- dorsolateral prefrontal cortex

DMO- dextromethorphan

E-LTP- early long-term depression

E-LTP- early long-term potentiation

ECT- electroconvulsive therapy

EEG- electroencephalography

EIP- exercise induced pain

EMG-electromyography

ERT- estimated resting twitch

FDI- first dorsal interosseous muscle

FEM- finite element modelling

FI- fixed intensity cycling trial

FLU- flunarizine

fNIRS- functional near infrared spectroscopy

FP2- supraorbital area

GABA- gamma aminobutyric acid

HIIT- high intensity interval training

H-Reflex- Hoffman's reflex

HR- heart rate

HR_{peak}- peak heart rate

IC- insular cortex

K^+ - potassium

L-LTP- late long-term depression

L-LTP – late long-term potentiation

LTP- long-term depression

LTP- long-term potentiation

M-wave – muscle action potential response

$M\text{-}wave_{p\text{-}p}$ – peak to peak amplitude of the M-wave

M1- primary motor cortex

MEP-motor evoked potential

$MEP_{p\text{-}p}$ – peak to peak amplitude of motor evoked potential

$MEP_{p\text{-}p}/M\text{-}wave_{p\text{-}p}$ ratio- the ratio of $MEP_{p\text{-}p}$ to $M\text{-}wave_{p\text{-}p}$ used for normalisation.

MIE- maximal incremental test to exhaustion

MRCP- movement related cortical potential

MRI- magnetic resonance imaging

mRNA- messenger ribonucleic acid

MRS- magnetic resonance spectroscopy

MVC- maximal voluntary contraction

Na^{2+} - sodium

NIBS- non-invasive brain stimulation

NMDA- N-methyl-D-aspartate

NMDAR- N-methyl-D-aspartate receptors

O_2 - oxygen

PAG- periaqueductal grey

PAS- paired associative stimulation

PET- positron electron tomography

PFC- prefrontal cortex

P_i – inorganic phosphate

PKA- CAMP-dependent protein kinase A

PKC- protein kinase C

PO- power output

$Power_{4mM}$- power output at which 4mmoL of lactate is produced

PPO- peak power output

QST- quantitative sensory testing

RM- repeated measures

RPE- rating of perceived exertion

rTMS- repetitive transcranial magnetic stimulation

SD- standard deviation

SICI- short interval intracortical inhibition

SIT- super-imposed twitch

SNP- single nucleotide polymorphism

sRPE- session RPE

SSES REAG- School of Sport and Exercise Science research ethics advisory group

SWC- smallest worthwhile change

T3- temporal cortex

tDCS- transcranial direct current stimulation

TMEP- thoracic motor evoked potential

TMS- transcranial magnetic stimulation

TRK-B- tropomyosin receptor kinase B

TT- time trial

TTE- time to exhaustion

VAL- voluntary activation level

VO_{2max}- maximal volume of oxygen consumed

VO_{2peak}- peak volume of oxygen consumed

Publications and Communications

Research reported in this thesis has directly contributed to the following publications and conference presentations.

Peer-reviewed publications:

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Conference Presentations:

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1. Introduction and Literature Review

Following the discovery of the electrical properties of the brain and central nervous system, researchers have used brain stimulation techniques to understand the fundamental roles and functions of individual brain cortices, in addition to the development of treatments for various pathologies relating to the central nervous system (George & Aston-Jones., 2010; Priori., 2003). The techniques used included invasive measures such as individual cortex stimulation or deep brain stimulation, requiring a surgical procedure to implant electrodes (Priori., 2003). In addition, non-invasive brain stimulation (NIBS) techniques have been used which involve the placement of an electrode over the scalp to deliver the electrical current (George & Aston-Jones., 2010; Priori., 2003). These include electroconvulsive therapy (ECT), transcranial magnetic stimulation (TMS) and the primary focus of this thesis, transcranial direct current stimulation (tDCS) (Priori., 2003).

Transcranial direct current stimulation involves the delivery of a weak direct current from a 9 V battery to anodal and cathodal electrodes placed on the scalp. Unlike ECT, the polarizing current delivered by tDCS is stated to induce subthreshold physiological changes to the resting membrane potential, influencing the spontaneous activity of the underlying neurones (Priori et al., 1998; Nitsche & Paulus., 2000). As such, tDCS avoids the disadvantages of ECT such as memory loss or the loss of consciousness (Priori., 2003). Furthermore, the use of tDCS does not require sedation or the administration of muscle relaxers (Priori., 2003). Consequently, tDCS has had extensive use for the treatment for a multitude of neurological and mental disorders (Benninger et al., 2010; Lefaucheur et al., 2008; Antal et al., 2010; Fregni et al., 2006; Nitsche et al., 2006) and to aid in the recovery of motor function following from stroke (Fridriksson et al., 2018; Hesse et al., 2007; Datta et al., 2011; Mahmoudi et al., 2011). Transcranial direct current stimulation has also allowed for the investigation of cognitive functions in both healthy and unhealthy populations (Loftus et al., 2015; Antal et al., 2007; Miniussi et al., 2008).

The clinical findings of reduced fatigue, decreased pain and improved motor function have led researchers to speculate that tDCS may confer an ergogenic effect for endurance performance (Angius et al., 2017; Angius et al., 2018; Cogiamanian et al., 2007). However, emerging literature has highlighted the inconsistencies in the modulation of corticospinal excitability (Angius et al., 2018; Abdelmoula et al., 2016; Wrightson et al., 2019), performance within sustained isometric contractions (Cogiamanian et al., 2007; Angius et al., 2016; Williams et al., 2013; Muthalib et al., 2013; Kan et al., 2013), and cycling performance (Angius et al., 2015; Angius et al., 2018; Angius et al., 2019; Okano et al 2013; Barwood et al., 2016; Vitor Costa et al., 2015; Lattari et al., 2018; Holgado et al., 2019). These differences in reported outcomes are likely due to the difference in tDCS parameters selected (Jamil et al., 2017; Li et al., 2015; Opitz et al., 2015; Horvath et al., 2014; Vöröslakos et al., 2018) in addition to intra-and-inter-individual differences to tDCS itself (Li et al., 2015; Horvath et al., 2014), all of which have cast doubts over tDCS' ability to reliably exert this ergogenic effect.

1.1 The history of brain stimulation

The earliest reported therapeutic use of electricity dates back to 43 AD, where Roman physicians treated headaches and gout through the application of a strong electrical current delivered by a live torpedo fish. Some years later, these findings were also replicated by Greek (131-401 AD) and Muslim physicians (11th century) who also used the strong electrical current from the torpedo fish and electric catfish to treat headaches and

epilepsy (Utz et al., 2010; Priori., 2003; Wagner et al., 2007). Centuries later, Luigi Galvani discovered the electrical potential of the muscle and nerve cells of frogs, which lead to the development of the galvanic cell by Alessandro Volta (1792) (Utz et al., 2010; Priori., 2003). These developments gave impetus to the possibility of electrically stimulating the human brain. In their laboratories, Giovanni Aldini (1804) and Charles Le Roy (1755) performed preliminary experiments, applying electricity to the scalp of guillotined human heads and cadavers, observing blinking, and opening of the eyes (Wagner et al., 2007; Priori., 2003). Despite having a limited understanding of the functions of individual cortices, Aldini believed that the application of electricity to the scalp would exert a beneficial effect for those suffering from severe depression (Priori., 2003). The medicinal use of galvanic currents progressively declined during the 1930's due to the advent of ECT as well as the reports of variations in response to the polarizing galvanic current's (Utz et al., 2010; Priori., 2003).

In the scientific community, galvanic currents were used to investigate the function of the central nervous system and brain. Decades after the initial observation that electricity elicited movements in cadavers, pioneering research from Eduard Hitzig and Gustav Fritsch (1870) discovered that the application of galvanic currents to the anterior half of the canine cerebral cortex, but not posterior to the central sulcus, elicited limb movements from the opposite side of the body (Uetmatsu et al., 1992). Conversely, David Ferrier (1876) observed limb movements elicited by stimulation posterior to the central sulcus of monkeys, which allowed the production of the first brain map in primates (Uematsu et al., 1992). Later Victor Horsley (1887) and Charles. S. Sherrington (1902) produced very similar maps of the brain for use on humans, based on their own work on primates (Uematsu et al., 1992; Vilensky & Gilman., 2002). Guided by the maps produced by Sherrington, Harvey Cushing (1908) stimulated the brain of two awake human patients eliciting both sensory and motor functions (Uematsu et al., 1992; Di Lazzaro et al., 2008). More advanced brain mapping studies were conducted on humans, where Wilder Penfield and Edwin Boldery (1937) discovered the integration of the sensorimotor area with other cortical areas (Penfield & Boldrey, 1937; Uematsu et al., 1992).

After three decades, the use of direct currents reappeared in the scientific community, with the discovery that very weak direct currents (10-20 $\mu A/mm^2$) were able to induce paroxysmal changes in spontaneous neural activity in animal preparations (Bindman et al., 1964; Purpura & McMurtry, 1965). Following this, researchers investigated the effects of weak anodal and cathodal transcranial direct current stimulation (tDCS) on mood, motor activity and attentiveness, finding that 50-500 μA anodal tDCS enhanced these measures, whilst cathodal tDCS induced quietness and apathy (Lippold & Redfearn., 1964). However, a replication study with the addition of a control condition, failed to corroborate these initial findings (Sheffield & Mowbray., 1986). Perhaps the disparity in findings between the two studies were due to observer bias, poor sensitivity of the psychometric tests used. Alternatively, the behavioural changes induced by the low intensity tDCS were too subtle when compared to a control condition (Sheffield & Mowbray., 1968; Priori., 2003). It is, however, intriguing to see some of the same criticisms levelled at similar research in this area today. Following these studies, the therapeutic use of tDCS was once again largely abandoned in favour of the development of psychoactive drugs (Priori., 2003; Utz et al., 2010).

Two decades later, following the advent of TMS devices, researchers sought to directly investigate the effect of weak tDCS on corticospinal excitability, reporting that 0.5 mA anodal tDCS alternated with cathodal tDCS significantly depressed the amplitude of the motor evoked potentials (MEP) elicited by TMS (Priori et al., 1998; Priori., 2003). The findings of this initial study allowed for the development of the common dose of tDCS seen in research and clinical use today (Nitsche & Paulus., 2000; Nitsche & Paulus., 2001).

1.2 Mechanisms of Action

The application of tDCS via the placement of anodal and cathodal electrodes on the scalp are reported to alter both corticospinal excitability and behaviour through the modulating the activity status of underlying neurons (Nitsche & Paulus., 2000; Nitsche & Paulus., 2001; Liebetanz et al., 2002). Due to the subthreshold nature of tDCS the current intensities associated with conventional tDCS (1-2 mA) are too weak to elicit an action potential. Instead, polarization of the resting membrane potential is thought to be the primary mechanism underpinning the observed alterations in corticospinal excitability (Nitsche & Paulus., 2000; Nitsche & Paulus., 2001; Liebetanz et al., 2002). The effects of tDCS are reported to extend for several hours post stimulation which is reflective of the induction of synaptic plasticity, the activity-dependent alteration of structure, function, and neural connectivity (Agboda et al., 2020; Nitsche & Paulus., 2000). Early views of tDCS suggested that the excitability changes induced by tDCS were polar-dependent (Nitsche & Paulus., 2000), where the anodal electrode exerts an excitatory effect, depolarising the underlying neurons (Nitsche & Paulus., 2000). This is often demonstrated as an increase in MEP amplitude elicited by TMS (Nitsche & Paulus., 2000; Angius et al., 2016; Angius et al., 2018; Tremblay et al., 2013; Lang et al., 2004) as well as alterations in regional blood flow characterised by increased oxygenation measured by functional near infrared spectroscopy (fNIRS) (Merzagor et al., 2010). On the other hand, cathodal tDCS was purported to hyperpolarise the membrane in which reductions in MEP amplitude from baseline and reductions in active pixels identified by blood oxygenation dependent magnetic resonance imaging (BOLD MRI) are observed (Lang et al., 2004; Nitsche & Paulus., 2000; Baudewig et al., 2001). However, it is now apparent that such expositions fail to consider the complexity of the tDCS-dose response which appears to be dependent upon the intricate relationship between the non-linear dose response and a multitude of inter-and intra-individual differences (Agboda et al., 2020; Wiethoff et al., 2014; Salehinejad & Ghanavati., 2020; Batsikadze et al., 2013).

To date, the exact cellular targets of tDCS are uncertain. However, it is apparent that both the initial membrane changes and inductions of neuroplasticity are dictated by neurotrophin, and neurotransmitter mediated signalling cascades which allow for the modification of voltage- gated ion channels in addition to intracellular and extracellular ionic changes (Pelletier & Cicchetti., 2015; Fritsch et al., 2010; Purpura & McMurtry., 1965; Stagg et al., 2018). In the following sections, the tDCS mediated polarization of resting membrane potential and induction of neuroplasticity will be explored in greater detail.

1.2.1 Polarization of the membrane potential.

In neuroscience, the membrane potential refers to the difference in electric potential (voltage) across the membrane at any given moment. This is purported to vary from -90 mV to + 60 mV. Changes in membrane potential are induced by modifications of intracellular and extracellular ion concentrations. As such, excitation of the underlying neuron following tDCS is induced by depolarisation of the membrane potential (Pellitier & Cicchetti., 2015; Purpura & McMurtry., 1965; Nitsche & Paulus., 2000). Through the induction of neuron depolarisation, the membrane potential undergoes tonic increases, towards the spike threshold (~ -55 mV) and therefore increases the probability of action potential elicitation (Pelletier & Cicchetti., 2015; Purpura & McMurtry., 1965) (Figure 1). The inverse is true for neural hyperpolarisation, which induces negative changes in the membrane potential (figure 1).

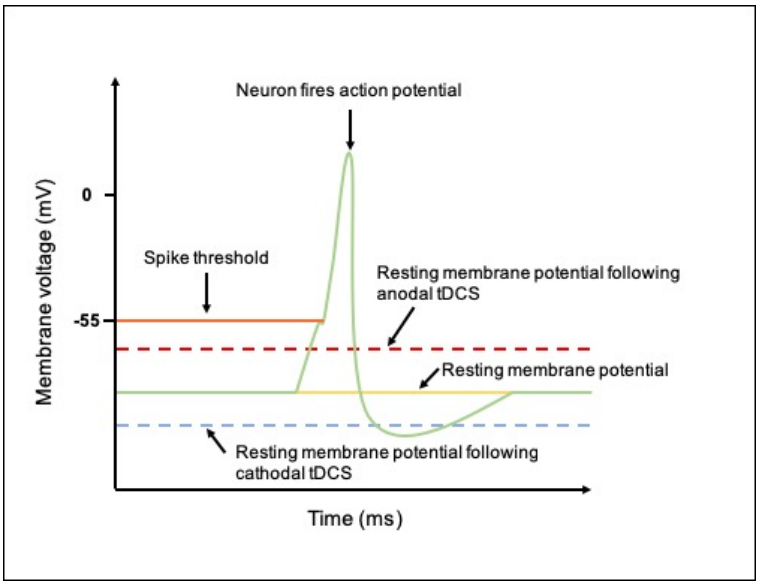

Figure 1 The application of tDCS changes the membrane potential of the neural cell. At rest the membrane potential (yellow line) varies between -60 and +80 mV. Following anodal tDCS (red dotted line), the membrane potential increases closer to the spike threshold (orange line). Whereas cathodal tDCS reduces the membrane potential (blue dotted line).

Transcranial direct current stimulation is thought to influence the activity of the voltage gated ion channels, where tDCS induced depolarisation elicits an influx of Ca^{2+} and Na^{+} into the intracellular space (Pelletier & Cicchetti., 2015; Islam et al., 1995; Nitsche et al., 2003; Purpura & McMurtry., 1965; Rahman et al., 2017). Pharmacologically blocking both Ca^{2+} and Na^{+} via central nervous system (CNS) using the CNS active drugs flunarizine (FLU) and carbamazepine (CBZ) have shown to completely suppress the enhancement of corticospinal excitability associated with anodal tDCS (Nitsche et al., 2003; Liebetanz et al., 2002). However, there were no significant changes in MEP amplitude following cathodal tDCS when both FLU and CBZ were administered, likely due to the voltage dependency of both drugs. As both FLU and CBZ prevent against the lowering of the spike threshold, they are unable to influence hyperpolarisation induced by cathodal tDCS (Nitsche et al., 2003; Liebetanz et al., 2002).

The influx of Ca^{2+} and Na^{+} into the intracellular space is thought to be mediated by the release of excitatory or inhibitory neurotransmitters, glutamate, or gamma aminobutyric acid (GABA) (Pelletier & Cicchetti., 2015; Stagg et al., 2009; Stagg et al., 2011). Magnetic resonance spectroscopy (MRS) has previously shown an increase in glutaminergic activity and a decrease in GABA release following anodal tDCS (Clark et al., 2011; Stagg et al., 2009; Stagg et al., 2011). Whereas cathodal tDCS was categorised by an increased GABA release (Stagg et al., 2009; Stagg et al., 2011). Increased glutamatergic activity following anodal tDCS is thought to mediate the influx of ions into the intracellular space through the removal of the Mg^{2+} ion blocking the N-methyl-D-aspartate receptors (NMDAR) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropoionic acid receptors (AMPAR) (Pelletier & Cicchetti., 2015; Kabakov et al., 2012); Minichiello., 2009). The mediation of the NMDAR is an important mechanism underlying the effect of tDCS as these receptors have previously been shown to be important for excitatory synaptic transmissions as well as the induction of plasticity (Nitsche et al., 2003; Liebetanz et al., 2002) (an important factor cited in the physiological response to exercise training; Mellow et al., 2020).

1.2.2 Induction of plasticity.

Alongside the immediate alterations in corticospinal excitability, prolonged applications of tDCS (10-30 mins) are supposedly able to influence corticospinal excitability for hours post cessation of stimulation (Nitsche & Paulus., 2001). These long-lasting changes in excitability are thought to be reflective of the induction of plasticity via long-term potentiation (LTP) or long-term depression (LTD), a persistent increase or decrease in synaptic strength induced by electrical stimulation (Minichiello., 2009; Baudewig et al., 2001). LTP or LTD can be split into early and late phases, which are discernible by the duration of excitability changes (Michiello., 2009; Monte-Silva et al., 2013). Early LTP (E-LTP) or LTD (E-LTD) are known to induce changes in corticospinal excitability lasting for more than 3 hours, and therefore the singular application of tDCS provides a unique opportunity to potentially alter behavioural outcomes. Alterations in corticospinal excitability persisting for greater than 24 hours are thought to be reflective of the induction of late LTP (L-LTP) or LTD (L-LTD) (Agboda et al., 2020; Monte-Silva et al., 2013). L-LTP and L-LTD are thought to be induced by repeated high frequency stimulation separated by several minutes of rest, which is thought to allow for the occurrence of protein synthesis and gene transcription to alter the synaptic strength (Agboda et al., 2020; Minichiello., 2009; Monte-Silva et al., 2010; Monte-Silva et al., 2013; Sajikumar et al., 2005; Goldsworthy et al., 2015; Reymann & Frey., 2007). Therefore, researchers have sought to use multiple applications of tDCS to induce lasting changes as a therapeutic technique in clinical populations where synaptic strength is impaired (Ridding & Ziemann., 2010).

Early LTP

The induction of E-LTP or E-LTD is reliant upon the activation of NMDAR following depolarisation or hyperpolarisation of the cell membrane (Pelletier & Cicchetti., 2015). The involvements of the NMDAR in the induction of LTP and LTD has previously been examined through the ingestion of dextromethorphan (DMO) (Liebetanz et al., 2002; Nitsche & Paulus., 2000). Whilst the application of DMO had no effect on the changes in corticospinal excitability induced immediately, DMO was shown to completely abolish the aftereffects of both anodal and cathodal stimulation (Liebetanz et al., 2002; Nitsche &

Paulus., 2000). Thus, activation of the NMDAR is thought to initiated by the release of various neurotransmitters (Minichiello., 2009; Liebetanz et al., 2002; Nitsche et al., 2003; Nitsche et al., 2006; Nitsche et al., 2009; Stagg et al., 2009; Stagg & Nitsche., 2011; Wang et al., 2012; Pelletier & Cicchetti., 2015). The excitatory and inhibitory neurotransmitters glutamate and GABA are implicated in the induction of synaptic plasticity (Stagg et al., 2009; Stagg et al., 2011; Clark et al., 2011). Imaging via MRS has shown that following anodal tDCS, localised GABA concentrations are reduced, whilst cathodal tDCS sees a localised reduction in glutamate activity alongside a reduction in GABA (Stagg et al., 2009; Stagg et al., 2011; Clark et al., 2011). It is therefore suggested that the increased glutamate release following anodal tDCS preferentially mediates the opening of NMDAR gated Ca^{2+} ion channels (Pelletier & Cicchetti., 2015; Clark et al., 2011; Stagg & Nitsche., 2011; Sale et al., 2008). Whereas the enhanced concentration of GABA following cathodal tDCS preferentially opens Cl^- channels to induce LTD (Nitsche et al., 2004; Sale et al., 2008; Sale et al., 2010; Inghilleri et al., 2004). However, there seems to be a complex interaction between neurotransmitter release and other physiological systems which may mitigate the effect of tDCS (Sale et al., 2008; Sale et al., 2010; Inghilleri et al., 2004). For example, the neuromodulator cortisol enhances the effect of GABA (Sale et al., 2008; Sale et al., 2010). To date the influence of diurnal variations on tDCS response has not been investigated, however the excitatory effect of paired associative stimulation (PAS) was reduced in the morning when cortisol is at its peak concentration and following the administration of oral hydrocortisone (Sale et al., 2008). Menstrual cycle hormones are also reported to influence neurotransmitter activity (Inghilleri et al., 2004). Both oestrogen and progesterone are presumed to cross the blood-brain barrier to influence NMDAR activity through the upregulation of glutamate or GABA respectively (Inghilleri et al., 2004). As such, researchers have previously demonstrated that corticospinal excitability is reduced following repetitive TMS (rTMS) during the luteal phase of the menstrual cycle, due to greater levels of progesterone inducing GABAergic activity (Inghilleri et al., 2004). As such oral contraceptives used may also serve as a source of variability to tDCS (Li et al., 2015; Inghilleri et al., 2004).

The activation of NMDAR to initiate LTP or LTD is also dependent on the release of neurotransmitters dopamine and serotonin (Medeiros et al., 2012; Nitsche et al., 2006; Nitsche et al., 2009). Dopamine is suggested to play a stabilising role during the induction of plasticity, where both D1 and D2 receptors are purported to be important for the facilitation and consolidation of both LTP and LDP (Medeiros et al., 2012; Nitsche et al., 2006; Otmakhova & Lisman., 1996). Following the pharmacological blockade of the D2 receptors, the after-effects of both anodal and cathodal tDCS were almost completely abolished (Nitsche et al., 2006). Previous research has also made it apparent that the administration of serotonin enhancers are able to enhance memory formation and motor function in healthy and clinical populations (Nitsche et al., 2009; Meeusen et al., 2006; Leite et al., 2010). Serotonin is reputed to enhance the K^+ conductance to facilitate the intracellular influx of Ca^{2+}, therefore the enhancement of motor function observed following the increased uptake of serotonin is believed to be caused by a serotonin dependent improvement in plasticity (Nitsche et al., 2009). Increasing the circulating concentration of serotonin prior to the application of tDCS has been shown to enhance the faciliatory response to anodal tDCS but also to reverse LTD induced by cathodal tDCS to LTP (Nitsche et al., 2009). In neurological disorders such as Parkinson's disease and depression, the regulation of neurotransmitter concentration is vital. Therefore, as tDCS

and other NIBS techniques are reported to alter neurotransmitter concentration, they are often promoted as an efficacious adjunctive therapy (Hadoush et al., 2018; Nitsche et al., 2006; Nitsche et al., 2006; Nitsche et al., 2009; Brunoni et al., 2013; Fregni et al., 2006). Alterations of neurotransmitter concentration is also purported to occur within prolonged exercise. Indeed, according to the serotonin hypothesis, the increased availability of tryptophan and subsequent increases in serotonin concentration increases lethargy whilst simultaneously reducing central motor drive and motivation (Meeusen et al., 2006). As tDCS is purported to enhance the secretion of dopamine (Fontenau et al., 2018), perhaps the reputed ergogenic effect of tDCS occurs as a result of maintaining the dopamine-serotonin ratio within exercise. However, this is likely to only benefit prolonged low-intensity exercise or exercise in hot conditions (this will be discussed further in Section 1.7.1 Fatigue).

The neurotransmitter mediated activation of NMDAR channels and increased intracellular Ca^{2+} concentrations within the post-synaptic cell are thought to trigger the transient activation of protein kinases such as Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) (Minichiello., 2009; Agboda et al., 2010; Lagemann et al., 2009; Nguygen & Woo., 2003). The activation of CaMKII and PKC ultimately results in the phosphorylation of AMPAR (Agboda et al., 2020; Lagemann et al., 2008). This allows for an increase in AMPAR availability and activity and therefore strengthens synaptic plasticity through increased postsynaptic depolarization (Agboda et al., 2020). The effects of E-LTP are thought to be limited to durations of 1-2 hours due to the rapid depletion of the CaMKII and PKC pathways, therefore leaving the neural membrane to undergo depotentiation if further excitation does not occur (Woo & Nguyen., 2003; Langemann et al., 2008; Nguygen & Woo., 2003; Agboda et al., 2020; Monte-Silva et al., 2010).

Through transiently modulating CaMKII, PKC and NMDAR activity, the induction of E-LTP via tDCS has previously shown to significantly increase corticospinal excitability (Nitsche & Paulus., 2001). Reduced corticospinal excitability of the primary motor cortex (M1) has previously been demonstrated to occur in supraspinal fatigue associated with exhaustive exercise (Ross et al., 2007; Ross et al., 2010). It is therefore purported that the induction of E-LTP via tDCS may confer an ergogenic effect on exercise performance through delaying the onset of supraspinal fatigue (Cogiamanian et al., 2007; Angius et al., 2016; Angius et al., 2018) (this literature will be discussed further in section 1.7.1).

Late LTP

The induction of L-LTP is actuated through repeated high-frequency stimulation, interspersed with rest intervals of several minutes (Agboda et al., 2020). The persistent activation of the CaMKII and PKC pathway through repeated stimulation protocols allows for the activation of Ca/CaM-sensitive adenylyl cyclase which synthesises the intracellular messenger molecule, cyclic adenosine 3' ,5' monophosphate (cAMP) (Nguyen & Woo., 2003). The synthesis of cAMP leads to the activation of cAMP-dependent protein kinase A (PKA), cAMP-response element binding protein (CREB), messenger ribonucleic acid (mRNA) and protein synthesis allowing for the production and insertion of NMDAR and AMPAR into the subsynaptic membrane, which furthermore enhances the synaptic efficacy for longer durations (Agboda et al., 2020; Nguyen & Woo., 2003; Pelletier & Cicchetti., 2015).

Tropomyosin receptor kinase B (TRK-B) and its ligand molecule brain-derived neurotrophic factor (BDNF) are also suggested to play a fundamental role in the induction and maintenance of L-LTP (Minichiello., 2009). Previous studies have found that the magnitude of synaptic response is reduced when the binding of TRK-B and BDNF is inhibited (Fritsch et al., 2010). Whereas the application of anodal tDCS has been shown to increase BDNF concentration in addition to the facilitation of cognitive functions (Podda et al., 2016). Following membrane depolarisation, the activity dependent secretion of BDNF occurs alongside the secretion of glutamate (Langemann et al., 2008; Minichiello., 2009). It is thought that the secretion of BDNF is responsible for the coding of positive and negative signals through the concentration and frequency (Langemann et al., 2008). Positive signals and the long-lasting cAMP activation elicit the fast activation of TRK-B receptors for the binding of BDNF, which activates protein and gene synthesis (Minichiello., 2009; Langemann et al., 2008; Pelletier & Cicchetti., 2015; Nguyen & Woo., 2003). Additionally, the prolonged activation of cAMP and binding of TRK-B and BDNF promotes the activation of the three main signalling pathways; neuronal differentiation, neural cell growth (through the production of new dendritic spines and/or the enlargement of pre-existing spines) and neural cell survival (Minichiello., 2009; Langemann et al., 2008; Pelletier & Cicchetti., 2015; Nguyen & Woo., 2003). In the case of L-LTD induction following cathodal stimulation and neural membrane hyperpolarisation, a negative signal is coded by BDNF and therefore initiates the binding of BDNF to pan-neurotrophin receptor $P75^{NTR}$, a low-affinity nerve growth factor which instigates the reduction in protein synthesis (Minichiello., 2009; Langemann et al., 2008).

Whilst the benefits of regular aerobic exercise on cardiorespiratory and musculoskeletal health are well-established, it is now apparent that aerobic exercise also confers a neuroprotective effect on brain and CNS health and bolsters cognitive function (Ferris et al., 2007; Vaynman et al., 2004; Gòmez-Pinilla et al., 2002; Berchtold et al., 2005; Cotman et al., 2007; Gold et al., 2003; Mellow et al., 2020). As part of the physiological adaptation, it is presumed that regular aerobic exercise induces L-LTP like plastic changes (Mellow et al., 2020). Indeed, following daily or intermittent high intensity interval training (HIIT) and moderate intensity continuous training, hippocampal CREB mRNA levels and BDNF expression are shown to increase (Berchtold et al., 2005; Vaynman et al., 2004; Gòmez-Pinilla et al., 2002; Ferris et al., 2007; Reycraft et al., 2020; Tang et al., 2008; Saucedo Marquez et al., 2015; Zoladz et al., 2008; Seifert et al., 2010). These increases in CREB mRNA and BDNF expression are thought to be associated with improved learning and the formation of long-term memory (Gòmez-Pinilla et al., 2002; Vaynman et al., 2004; Ferris et al., 2007). Furthermore, the exercise-induced increase in BDNF expression improves the neuronal survival and resistance to brain insult, increased neurogenesis and increases the resistance to the development of Alzheimer's disease, dementia, and depression (Cotman et al., 2007; Berchtold et al., 2005). These neuroprotective and cognitive benefits of regular aerobic exercise are more clearly demonstrated in older adults, where increased concentration of these plasticity related molecules are associated with improved executive function, learning and long-term memory (Cotman et al., 2007). Furthermore, Colcombe et al (2003) reported that the participation in regular physical activity reduces the age-related decline in cortical tissue density of the frontal, temporal, and parietal cortices, which is consistent with the neurogenic actions of BDNF (Ferris et al., 2007).

However, there appears to be a genetic influence of a common polymorphism of BDNF on the induction of LTP (Pelletier & Cicchetti., 2015; Antal et al., 2010; Teo et al., 2014; Kleim et al., 2006). Thirty five percent of the Caucasian population exhibit a valine (val) to methionine (Met) substitution in the 5'-pro regions of the 66 codon (Cheeran et al., 2008). In comparison to the val66val polymorphism, carriers of the met polymorphism (either met66met or val66met) exhibit a reduced activity-dependent secretion of BDNF and disturbed cell trafficking, in addition to the impairment of the induction of LTP and synaptic transmissions (Brunoni et al., 2013; Kleim et al., 2006; Egan et al., 2003; Pezawas et al., 2004; Erickson et al., 2013; Cheeran et al., 2008). This is perhaps due to the reduced rate of cleavage of mature BDNF from its pro-BDNF precursor (Cheeran et al., 2008). As such, polymorphisms with the Met substitution have previously been linked to structural (Cheeran et al., 2008; Pezawas et al., 2004) and functional deficits including reduced motor skill acquisition (Fritsch et al., 2010; Kleim et al., 2006), reduced cognitive benefits from regular aerobic exercise (Erickson et al., 2013) and episodic memory (Egan et al., 2003; Pezawas et al., 2004; Cheeran et al., 2008). As this polymorphism has a greater propensity for the induction of LTD, it also serves as a source of variability in the response to tDCS and other NIBS techniques (Li et al., 2015; Kleim et al., 2006). When used alone, the faciliatory effect of anodal tDCS appears to become delayed in met carriers (Antal et al., 2010; Teo et al., 2014). In contrast, when tDCS is used as a priming technique, the excitatory effects are inhibited (Cheeran et al., 2008). Similarly, met carriers displayed no faciliatory effects following intermittent theta burst stimulation (Kleim et al., 2006; Antal et al., 2010).

1.2.3 Metaplasticity

It is presumed that through modifying the excitability of the target cortical area via the application of tDCS, secondary alterations in behaviours will occur. However, it is now apparent that the likelihood of LTP or LTD induction is dependent upon the prior synaptic activity (Thirugnanasambandam et al., 2011; Abraham & Bear., 1996; Bienestock et al., 1982; Müller-Dahlhaus et al., 2015). Metaplasticity confers a higher-order form of plasticity, acting to regulate the duration and magnitude of LTP or LTD (Müller-Dahlhaus et al., 2015; Abraham & Bear., 1996). To date, two primary mechanisms of metaplasticity have been identified; gating and homeostatic plasticity (Ziemann & Siebner., 2008; Bienestock et al., 1982; Thirugnanasambandam et al., 2011).

Transcranial direct current stimulation is often applied to induce the 'gating' concept of metaplasticity. According to this concept, an excitatory task (such as an endurance cycling trial or motor learning task) administered alone may not exert any overt changes in corticospinal excitability (Ziemann & Siebner., 2008; Kuo et al., 2008; Sriraman et al., 2014). However, if the task is preceded by an excitatory protocol, such as anodal tDCS, both corticospinal excitability and task performance are supposedly bolstered (Ziemann & Siebner., 2008; Kuo et al., 2008) (Figure 2, Panel A). To date, much of the reported use of tDCS in sport and exercise science literature has been predicated upon this concept of metaplasticity. Indeed, the application of tDCS prior to or during an exercise task has shown to improve M1 excitability (Angius et al., 2018), and enhance isometric (Cogiamanian et al., 2007; Angius et al., 2016., Williams et al., 2013) and cycling (Angius et al., 2018; Angius et al., 2019; Vitor Costa et al., 2015) time to exhaustion (TTE).

However, in some cases the presumed task enhancement does not occur with the prior application of anodal tDCS (Filmer et al., 2013; Filmer et al., 2014; Ferrucci et al., 2008; Sandrini et al., 2012; Baltar et al., 2018; Angius et al., 2015; Barwood et al., 2016; Holgado et al., 2019; Antal et al., 2007; Thirugnanasambandam et al., 2011). Indeed, it is suggested that the application of tDCS prior to another stimulation or excitatory task initiates a homeostatic response (Antal et al., 2007; Baltar et al., 2019; Bienestock et al., 1982). According to the Bienestock-Munroe-Cooper theory (1982) of homeostatic plasticity, stabilization of the neuronal activity is ensured by a dynamically sliding threshold, where prior low post-synaptic activity favours the induction of LTP. Whereas prior high post-synaptic activity increases the threshold for LTP to occur in a subsequent activity, and therefore a reversal in excitability is often observed when another NIBS technique or excitatory protocol is applied (Bienestock et al., 1982; Baltar et al., 2018; Abraham & Bear., 1996; Abbott & Nelson., 2000; Turrigano & Nelson., 2004) (Figure 2, Panel B).

The effects of homeostatic plasticity have previously been observed in trials utilizing multiple NIBS techniques (Siebner., 2004; Lang et al., 2004). Indeed, suppression of the faciliatory effect of rTMS has been observed due to the prior application of anodal tDCS (Siebner., 2004; Lang et al., 2004). However, when rTMS was superseded by the application of cathodal tDCS, lasting enhancements of corticospinal excitability occurred (Siebner., 2004; Lang et al., 2004). This is not only limited to NIBS techniques, as the application of anodal tDCS immediately before cognitive tasks, motor learning (Antal et al., 2007), isometric contractions (Thirugnanasambandam et al., 2011) and high or moderate intensity prolonged running trials (Baltar et al., 2018) have resulted in decrements in corticospinal excitability. However, it should be noted that these studies did not report behavioural performance (i.e., endurance performance or retention of motor learning). Indeed, several studies to date have reported a beneficial effect of tDCS on endurance (Abelmoula et al., 2016; Angius et al) and motor learning (Kuo et al., 2008) irrespective of changes in corticospinal excitability. Therefore, it is still uncertain to what extent homeostatic plasticity will influence the effects of tDCS (Kuo et al., 2008).

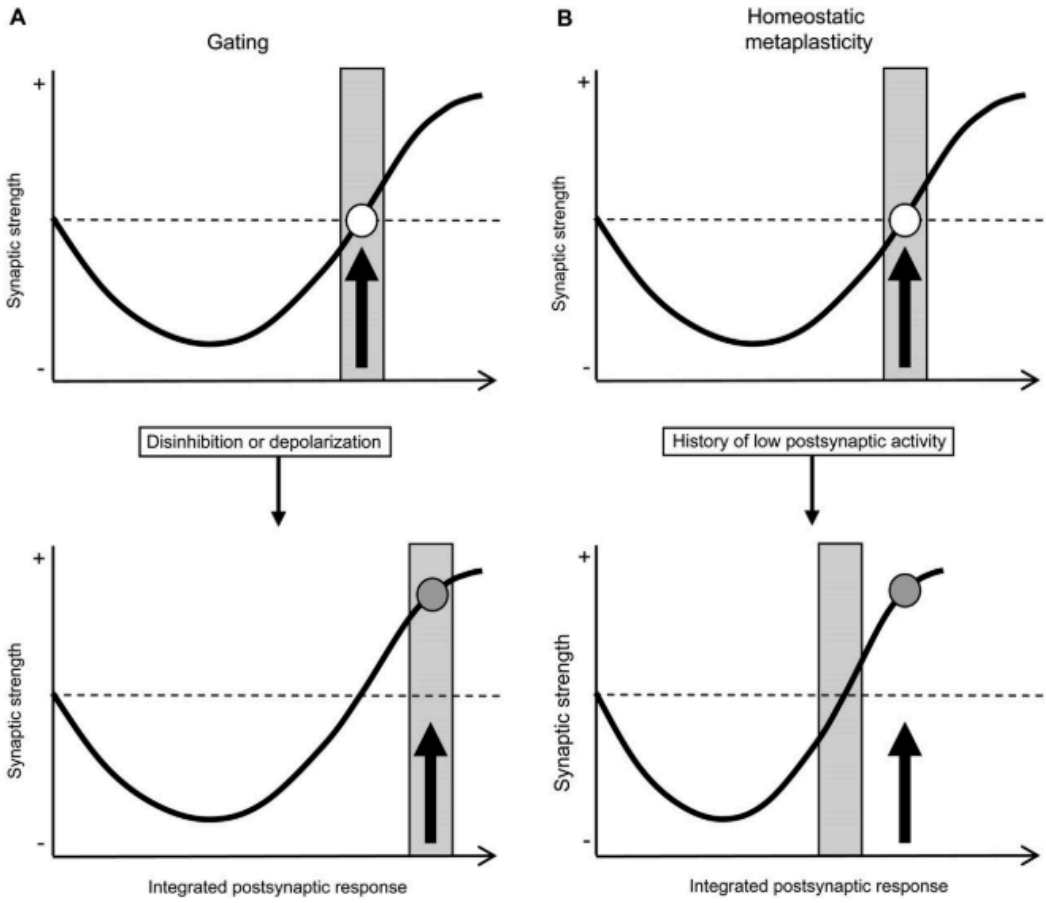

Figure 2 Changes in synaptic strength induced by gating (panel A) and homeostatic plasticity (Panel B). The upper diagram depicts that a stimulatory or learning protocol (arrow) may not induce any overt changes in synaptic strength (indicated by the white circle on the dashed line), when task is administered when post-synaptic activity is under normal conditions (depicted by the rectangle). However, if the stimulatory or learning protocol is administered at a point of increased post-synaptic activity (depicted by a rightward move of the rectangle) induced by depolarisation of neural cells via anodal tDCS, then the same stimulatory or learning protocol (arrow) will induce an increase in synaptic strength (grey circle). Similarly, according to homeostatic plasticity, a stimulatory or learning protocol alone may not induce overt changes in synaptic strength (white circle) under normal post-synaptic conditions (rectangle). However, the lower diagram shows, that if the learning or stimulatory protocol (arrow) is delivered after a period of low post-synaptic activity (depicted by a leftward shift of the rectangle) then increases in synaptic strength such as LTP are likely to occur (depicted by grey circle). Taken from Ziemann and Siebner (2008).

1.3 The tDCS current dose: The effects of montage, duration of stimulation and intensity

The biological and behavioural outcomes associated with NIBS techniques such as tDCS, TMS and rTMS occur through the modification of electromagnetic fields within the body. As such, the current dose associated with NIBS techniques are defined by the parameters of the NIBS device which can modify the electromagnetic field (Peterchev et al., 2012;

Bikson et al., 2008). The current dose elicited by tDCS is thought to be contingent upon the size and montage of electrodes, current intensity, and stimulation duration (Peterchev et al., 2012; Bikson et al., 2008; Datta et al., 2012). Despite the resurgence in use of tDCS earlier this century, the optimal current dose produced by tDCS is still uncertain, largely due to the ease of customization which has produced a vast diversity in current doses across research groups preventing a consensus from being drawn.

1.3.1 *Electrode Montage and size*

Like water, the current injected into the brain via tDCS follows a path of least resistance (Reinhart et al., 2017). Conventional tDCS usually involves the passage of current through the placement of one or more electrodes on the scalp. Electrodes are placed under the premise of the occurrence of excitation under the anodal electrode and inhibition under the cathodal. However, computational modelling studies have also identified a significant amount of current flow within the intermediary regions (Datta et al., 2009; Datta et al., 2012), where the direction of current flow is thought to be determined by the montage of electrodes (Bikson et al., 2010; Moliadze et al., 2010). Therefore, the array of electrodes used play a pivotal role in the excitability and behavioural outcomes.

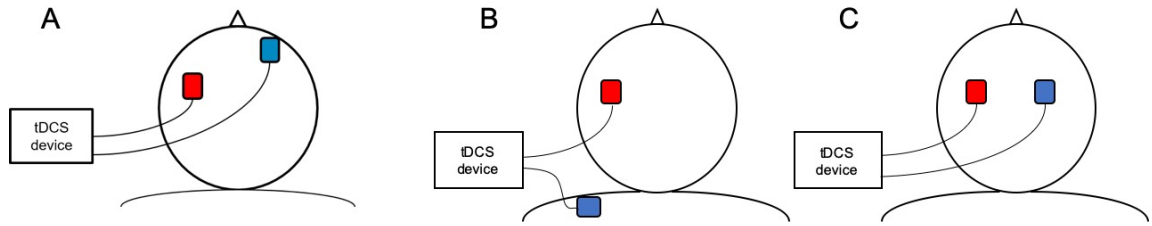

Figure 3. Common electrode montages used within the endurance performance literature. Panel A depicts the 'traditional' M1 cephalic montage originally used by Nitsche & Paulus (2000) to alter corticospinal excitability. Panel B depicts the extracephalic montage described by Cogiamanian et al (2007) to enhance isometric time to exhaustion. Lesser used is the bilateral M1 montage where the active (red electrode) is placed over the left M1 and the return electrode (blue electrode) is placed over the right M1

Traditionally, tDCS involves the placement of one electrode over the brain region of interest, whilst the other is placed over another brain area (intracephalic) or a non-cephalic body area (extracephalic) commonly the deltoid muscle (Nasseri et al., 2015). Researchers have previously referred to these electrodes as the ‘active’ and ‘reference’ electrodes (Nasseri et al., 2015; Brunoni et al., 2013). However, due to the contribution of the ‘reference’ electrode in the determination of the electric field and physiological outcome, it is recommended that this electrode should be referred to as a ‘return’ electrode instead (Nasseri et al., 2015; Brunoni et al., 2013). Due to the inherent flexibility of tDCS, a variety of montages have been introduced which can be broadly categorized into four classifications based upon their physical characteristics (Nasseri et al., 2015). These classifications include unilateral montages which target a brain area in one hemisphere only; bilateral montages which involve the placement of electrodes across both hemispheres; midline montages which are categorized by the placement of the target or active electrodes across the midline (Cz, Fz, Pz according to the 10-20 international electroencephalography (EEG) system); and dual channel montages which involve the use of two independent tDCS devices and therefore two pairs of electrodes (Nasseri et al., 2015). Each of these broad montage classifications can be further divided into multiple

subsections. However, for the purpose of this literature review, the montages most prevalent within the sports performance literature will be discussed.

Cephalic montages (Figure 3, panel A) are an example of asymmetrical bilateral montage, requiring the placement of the active electrode over the brain region of interest, with the return electrode placed over another brain region on the opposite hemisphere (Nasseri et al., 2015). Nitsche and Paulus (2000) compared a number of cephalic and bilateral montages to establish the optimal electrode montage to modulate corticospinal excitability. From this, they discovered that MEP amplitudes were significantly enhanced or diminished from baseline levels only when the active electrode was placed over the right M1, and the return electrode over the contralateral forehead (Nitsche & Paulus., 2000). Since this discovery, much of the current literature reports the use of a cephalic montage to enhance endurance performance, either by replicating the 'traditional' M1 montage (Angius et al., 2015; Angius et al., 2016; Bastani & Jaberzadeh., 2013; Hendy & Kidgell., 2013; Hendy & Kidgell., 2014; Hendy et al., 2019; Jeffery et al., 2007; Oki et al., 2016; Tanaka et al., 2009; Williams et al., 2013) used by Nitsche and Paulus (2000) or by placing the active electrode over the DLPFC (Lattari et al., 2016; Lattari et al., 2018; Angius et al., 2019) or the temporal cortex (Barwood et al., 2016; Okano et al., 2013; Okano et al., 2017).

Across the research groups, there are numerous inconsistencies in reported outcomes, which may be partially explained by the montage used (Angius et al., 2015; Angius et al., 2016). Cephalic montages are thought to induce unintended diminutions in excitability due to the placement of the return electrode over another brain area (Bikson et al., 2010; Yanamadala et al., 2014; Moliadze et al., 2010; Angius et al., 2016; Nitsche et al., 2007). For example, the 'traditional' M1 cephalic montage may induce decrements in excitability of the prefrontal areas such as the DLPFC, which are key areas in the regulation of cognitive functions (Loftus et al., 2015; Angius et al., 2019) and emotional valences (Brunoni et al., 2014). As the DLPFC is proposed to provide a supportive role during fatigue, decreasing the excitability of this brain area via the placement of the return electrode may act as a hinderance to endurance performance (Lattari et al., 2016).

In support of this, computational modelling studies have identified that the placement of the return electrode influences the direction of the current flow. In the 'traditional' M1 montage, a significant proportion of the electric field density occurs within the anterior brain rather than the M1, suggesting that this montage induces a posteroanterior direction of current flow (Datta et al., 2009; Datta et al., 2011; Wagner et al., 2007; Miranda et al., 2009; Im et al., 2012; Yanamadala et al., 2014). It is likely that temporal cortex-supraorbital area (Fp2) montages will also induce posteroanterior current flow, however this montage has not been computationally modelled to date. Comparatively, the F3- FP2 montages targeting the left DLPFC, are shown to maintain much of the electric field density within the targeted brain area (Bai et al., 2014). However, due to the close proximity of electrodes, a significant amount of current shunting across the scalp occurs (Bai et al., 2014; Miranda et al., 2006).

Extracephalic montages are an example of unilateral monopolar electrode arrays (Nasseri et al., 2015). These montages are proposed to circumvent the reputed negative consequence of the cephalic montage through the placement of the return electrode over a non-brain area such as the mastoid process or the shoulder (Nasseri et al., 2015) (figure

3, panel B). The reported use of extracephalic montages occurs less within the tDCS literature due to early fears of modulation of the autonomic nervous system and cardio-respiratory centres through unintended stimulation of the brain stem (pons, medulla, and spinal cord) induced when current flows towards the return electrode (Lippold & Redfearn., 1964; Im et al., 2012). Computational models and behavioural studies using extracephalic montages have failed to corroborate this argument finding no significant changes in blood pressure, respiratory frequency, heart rate or body temperature (Im et al., 2012; Vandermeeren et al., 2010; Accornero et al., 2007; Cogiamanian et al., 2007) as well as no significant differences in the maximal electric field and current density located in the brain stem when compared to a cephalic montage (Im et al., 2012).

In comparison to cephalic montages, extracephalic montages are suggested to have a greater propensity for localised stimulation, as demonstrated by increased current density and electric field in the brain region of interest, as measured by computational models (Im et al., 2012; Bai et al., 2014). This is due to a reduction in shunting of current across the scalp induced by the increased inter-electrode distance (Bai et al., 2014; Miranda et al., 2009). As such, researchers have used an extracephalic montage to enhance endurance performance through the placement of the active electrode on the M1 (Cogiamanian et al., 2007; Angius et al., 2016; Abdelmoula et al., 2016; Liu et al., 2019; Lampropoulou & Nowicky., 2013; Muthalib et al., 2013; Kan et al., 2013) or DLPFC (Holgado et al., 2019) and the return electrode over the ipsilateral (Cogiamanian et al., 2007; Muthalib et al., 2013; Kan et al., 2013) or contralateral shoulder (Abdelmoula et al., 2016; Angius et al., 2016; Liu et al., 2019; Holgado et al., 2019; Lampropoulou & Nowicky., 2013). Whilst the results of the computational modelling studies imply that an extracephalic montage will allow for greater changes in excitability of a targeted area, contrasting results have been garnered by Moliadze et al (2010), who observed significantly greater MEP amplitudes using a cephalic M1-FP2 montage and no significant differences between the ipsilateral and contralateral shoulder placements in the extracephalic montage. The authors also noted the requirement of increased voltage to maintain a current intensity of 1mA or 2mA in the extracephalic montage (Moliadze et al., 2010). Therefore, it has been suggested that intensities of 1-2mA are too low for use in an extracephalic montage and therefore should be adapted for the montage selected (Bikson et al., 2010; Moliadze et al., 2010).

Bilateral montages are an example of bilateral bipolar-balanced electrode arrays (Nasseri et al., 2015). Due to the placement of the active electrode a targeted region and the return over the contralateral counterpart, bilateral montages are reported to deliver current in a medio-lateral direction (Nasseri et al., 2015; Naros et al., 2016). To date, this montage has been extensively used in the motor learning paradigm in both healthy and clinical populations (Vines et al., 2008; Naros et al., 2016; Khan., 2013; Goodwill et al., 2013). However, there are no studies to date that have investigated the effect of a typical bilateral montage on endurance performance. Sports such as running or cycling rely on the co-activation of both left and right hemispheres, as such the placement of the return electrode in this montage may induce an imbalance in cortical activity which may negatively impact performance (Angius et al., 2016; Angius et al., 2018). This, however, requires further exploration.

Instead, variants of a bilateral montage have been used. For example, Angius et al (2018) used a dual-processing paradigm to apply anodal tDCS to both the left and right hemisphere in a bilateral extracephalic montage, observing enhanced corticospinal

excitability, a greater time to exhaustion and a reduction in rating of perceived exertion (RPE). A commercial tDCS device has also been used to deliver current to both hemispheres. Under the Halo Sport Neurostimulation System, the active electrode is applied to the Cz whilst two cathodal electrodes are placed over C5 an C6 according to the 10-20 international system for EEG placement. This device has been reported to enhance cycling sprint performance (Huang et al., 2019) and running time to exhaustion (Park et al., 2019) however due to participant awareness and a lack of a non-stimulation control it is possible that participants may have performed in a manner that supported the study hypothesis. Further research is warranted for the validation of the Halo device as well as the effect of a typical bilateral montage on endurance performance.

The direction of current flow is also influenced by the size of electrodes used to stimulate. Conventional tDCS involves the use of large sponge-based rectangular electrodes to deliver the current to the scalp (Miranda., 2013; Nitsche et al., 2007; Datta et al., 2009; Datta et al., 2012). As tDCS has previously been suggested to have the potential to burn the skin underneath the electrode, researchers have promoted the use of large with an area of 35 cm^2 (Fregni et al., 2005) or 25 cm^2 (Iyer et al., 2005). The use of large electrodes is thought to reduce the electrical density applied to the skin and therefore ensures the safety of tDCS (Brunoni et al., 2013; Miranda., 2013).

However, one drawback of conventional tDCS is the low spatial focality (Nitsche et al., 2007; Miranda et al., 2009; Cano et al., 2013; Datta et al., 2009). Due to the large size of the electrodes, cortical areas adjacent to the brain region of interest are likely to also be stimulated, consequently creating ambiguity in the interpretation of experimental findings (Nitsche et al., 2007). In addition to this, computational modelling studies and measurements of cerebral blood flow have also reported the widespread dispersal of current throughout the brain (Lang et al., 2005; Im et al., 2012; Datta et al., 2009; Datta et al., 2012). This is thought to occur due to the shunting of current through the cerebrospinal fluid (CSF), where the peaks and troughs of the gyri and sulci which protrude into the CSF receive comparatively greater current densities than the walls of the sulci (Datta et al., 2012; Uylings et al., 2005; Faria et al., 2011). This results in clusters of electric fields in un-associated cortical areas (Datta et al., 2011; Datta et al., 2012; Bikson et al., 2012).

To rectify the focality issue associated with conventional tDCS researchers have investigated the effect of altering the sizes of the conventional tDCS electrodes, discovering that the use of different sized electrodes allowed for asymmetries in current distribution and greater focality underneath a smaller active electrode (Nitsche et al., 2007; Farria et al., 2011). Nitsche et al (2007) explored the effects of reducing and increasing the active and return electrodes respectively on corticospinal excitability and a cognitive task. In comparison to a conventional 35 cm^2 tDCS electrode, using an electrode 10% of the conventional size allowed for a significantly greater focality of stimulation (Nitsche et al., 2007). This was categorized by significant changes in corticospinal excitability of the adductor digiti minimi (ADM) representation of the M1, with no significant changes in the first dorsal interosseous (FDI) representation in the M1 (Nitsche et al., 2007). Whereas 35 cm^2 electrodes induced significant changes in excitability of both the ADM and FDI motor cortical representations (Nitsche et al., 2007). Increasing the area of the return electrode to 100 cm^2 was also found to render the return electrode functionally inept, as measured by the performance accuracy in a cognitive trial (Nitsche et al, 2007).

The change in functionality occurs as the current density emitted by the electrode decreases as the area of the electrode increases. These findings have been corroborated through the use of finite element modelling (FEM) (Faria et al., 2011). This technique identified that focality of stimulation rapid improved when the electrode size decreased (Faria et al., 2011).

However, focality of stimulation appears to come at an expense of depth of stimulation (Nitsche et al., 2007; Faria et al., 2011). The use of FEM has identified that the use of small electrodes (3.5 cm^2 – 12 cm^2) restricts the depth of stimulation whilst increasing the amount of current shunting across the scalp (Faria et al., 2011). Indeed, Nitsche et al (2007) noted that in comparison to conventional 35cm^2 electrodes, smaller electrodes with an area of 3.5cm^2 induced greater variability in the MEP response, which could be explained by the increase in shunting of current. Increased current density and shunting of current through the scalp can also reduce tolerability of tDCS, therefore it is recommended that the current density is reduced via lowering the intensity (Faria et al., 2011). It is also apparent that the peak current density is concentrated near to the edge of the electrodes for both the conventional, and small area electrodes which may increase the risk of hazards and reduce the tolerability of stimulation if current densities are uncontrolled (Faria et al., 2011; Wagner et al., 2007). It is therefore implored that researchers use the correct conductive gels and appropriate electrode material to improve the tolerability of stimulation (Faria et al., 2011; Minhas et al., 2010).

Techniques such as high definition tDCS (HD-tDCS) are also demonstrated to elicit a comparatively greater focality of stimulation when compared to conventional tDCS (Datta et al., 2009). This technique involves the use of multiple small diameter (<12 mm) gel-based electrodes, often deployed in a 4 x 1 ring configuration (Datta et al., 2009; Datta et al., 2012; Kuo et al., 2013). The small design of the electrodes allows for precise control of the stimulation geometry, with computational models based on MRI scans demonstrating that the placement of the active electrode in the middle of a ring of return electrodes results in the maximal electric field strength to be produced over the target of interest (Datta et al., 2009; Datta et al., 2012). The ring of return electrodes also restricts the current spread to unrelated cortical areas (Datta et al., 2009; Datta et al., 2012).

Whilst HD-tDCS has been promoted to facilitate the focalized enhancement of corticospinal excitability, these alterations have not yet been shown to manifest in improvements in endurance performance (Radel et al., 2017; Flood et al., 2017; Machado et al., 2019). Indeed, whilst the application of HD-tDCS has been shown to improve excitability of the M1 and prefrontal cortex (PFC), these results were accompanied by no significant differences in time to exhaustion in a sustained isometric contraction of the elbow flexors, fatigue indices and RPE (Radel et al., 2017). Likewise, Flood et al (2017) also observed that improvements in experimental pain threshold were not accompanied by improvements in maximal force or time to exhaustion of the knee extensors, following the application of HD-tDCS to the M1. In both studies the HD-tDCS electrodes were placed according to the 10-20 and the 10-10 international system for EEG placement, which predicts the location of a desired cortical area based off of the correlation of prominent external skull features (Herwig et al., 2003; De Witte et al., 2018). Whilst this system considers head size, it fails to consider individual differences in neocortical morphology caused by differences in head shape, brain position or placement of gyri and sulci (Uylings et al., 2005; De Witte et al., 2018). Consequently, the net null effects

reported in both studies may be due to the inaccurate location of the brain region of interest. Perhaps the use of prediction-based location systems such as the 10-20 EEG system would be more effective with the use of conventional 25 cm^2 or 35 cm^2 where the large area is likely to span across the desired brain area (Herwig et al., 2003). However, it should also be highlighted, that robust responses to tDCS are more likely to occur if tDCS is optimised for the individual, which will likely require the use of HD-tDCS alongside various neuroimaging techniques to ensure the precise electrode placement (Bikson et al., 2012; Datta et al., 2011).

1.3.2 Current intensity

Throughout the years of reported tDCS use, wide varieties of current intensities have been used in the search of the optimal current dose. Through the mid 1960's researchers reported that current intensities of 500 μA were efficacious for the treatment of depression (Lippold & Redfearn., 1965). As the technique developed, researchers investigated the effects of greater current intensities, recommending the use of current intensities of at least 0.6 mA to induce long-lasting changes in corticospinal excitability (Priori et al.,1998; Nitsche & Paulus., 2000). From investigating current intensities from 0.2 mA to 1 mA, Nitsche and Paulus (Nitsche & Paulus., 2000) noted that increasing the current intensity produced larger and more prolonged changes in corticospinal excitability. As such, clinical trials involving tDCS often reported use of a greater stimulation intensity (1.5 mA – 2 mA) to ensure the efficacy of the treatment (Brunoni et al., 2013). Similarly, current intensities ranging between 1.5 mA -2 mA have also been reported within the sport and exercise science literature (Machado et al., 2019).

However, in healthy populations increasing the current intensity of stimulation does not uniformly enhance the response to tDCS (Jamil et al., 2017; Mosayebi Samani et al, 2019; Agboda et al., 2020; Salehinejad & Ghanavati., 2020; Batsikadze et al., 2013; Bastani & Jaberzadeh., 2013). To date, several studies have systematically evaluated the influence of different current intensities on measures of corticospinal excitability (Jamil et al., 2017; Mosayebi Samani et al., 2019; Agboda et al., 2020; Salehinejad & Ghanavati., 2020; Batiskadze et al., 2013; Bastani & Jaberzadeh., 2013; Kidgell et al., 2013). Bastani and Jaberzadeh (2013) sought to compare the aftereffects of corticospinal excitability induced by a range of current densities induced by anodal tDCS (0.3 mA, current density = 0.013 mA/cm^2; 0.7 mA, current density = 0.029 mA/cm^2; 1.4 mA, current density = 0.058 mA/cm^2; 2mA, current density = 0.083 mA/ cm^2), observing a non-linear increase in corticospinal excitability as stimulation intensity increased. The lowest intensity (0.013 mA/cm^2, 0.3 mA) used was shown to induce greater aftereffects on corticospinal excitability than the second (0.029mA/cm^2, 0.7 mA) and third (0.058 mA/cm^2, 1.4 mA). However, 0.013 mA/cm^2 is thought to be too low to initiate the opening of AMPAR or NMDAR (Bastani & Jaberzadeh, 2013; Jamil et al., 2017). It is therefore thought that the significant changes in corticospinal excitability initiated by the lowest current density is produced by the opening of voltage gated Ca^{2+} channels which have a comparatively lower threshold than AMPAR and NMDAR channels (Bastani & Jaberzadeh., 2013; Jamil et al., 2017). The activation of these gated Ca^{2+} channels is thought to allow for the increase in intracellular Ca^{2+} and depolarisation of the membrane (Bastani & Jaberzadeh., 2013; Jamil et al., 2017). Additional investigations corroborated the non-linear effects of current intensity, showing no significant differences in the aftereffects of corticospinal excitability (Jamil et al., 2017; Kidgell et al., 2013) and short interval intracortical inhibition

(SICI) (Kidgell et al., 2013) over a wide range of current densities (0.014-0.057 mA/cm^2; Jamil et al., 2017). Furthermore, recent research has also demonstrated that intensified tDCS protocols (3 mA, current density = 0.086 mA/cm^2) increases corticospinal excitability to a similar magnitude of conventional protocols (1mA, current density = 0.029 mA/cm^2) (Agboda et al., 2020).

Indeed, variability in the physiological response to cathodal tDCS is also observed over a range of current intensities (Batsikadze et al., 2013; Jamil et al., 2017; Mosayebi Samani et al., 2019). Whilst lower current intensities (< 1.0 mA, current densities ≤ 0.04 mA/cm2) appear to reliably induce diminutions in corticospinal excitability (Jamil et al., 2017; Mosayebi Samani et al., 2019), higher current intensities (1.5 mA or 2 mA) induce variations in physiological responses are observed, where participants either exhibit no consistent responses to tDCS (Wiethoff et al., 2014; Jamil et al., 2017) or alternatively facilitation is seen to occur (Batsikadze et al., 2013; Mosayebi Samani et al., 2019). However, increasing the stimulation intensity to 3 mA resulted in diminutions of corticospinal excitability (Mosayebi Samani et al., 2019; Salehinejad & Ghanavati., 2020). It is speculated that these bi-directional alterations in corticospinal excitability are related to NMDAR related glutaminergic plasticity (Mosayebi Samani et al., 2019; Jamil et al., 2017). It is thought that different current intensities will lead to different activation levels of the NMDAR, resulting in different concentrations of intracellular Ca^{2+} (Lisman., 2001; Cho et al., 2001; Mosayebi Samani et al., 2019; Jamil et al., 2017). It is therefore postulated that cathodal tDCS delivered at intensities of 1 mA and 3mA result in LTD due to a low post-synaptic membrane Ca^{2+} concentration and Ca^{2+} overflow respectively (Mosayebi Samani et al., 2019; Jamil et al., 2017; Cho et al., 2012; Lisman., 2001). Whereas cathodal tDCS delivered a 2 mA may allow for a sufficient Ca^{2+} concentration to induce LTP (Mosayebi Samani et al., 2019; Jamil et al., 2017; Cho et al., 2012; Lisman., 2001).

Questions have been raised about the efficacy of traditional current intensities used by NIBS techniques (Vöröslakos et al., 2018). Whilst it is generally assumed that current density leaving the electrode equates to the density of current received by the underlying cortex, the actual current density received by the brain has been shown to be muted due to the shunting of current and non-conductive layers (Miranda et al., 2006). For example, FEM has predicted that if electrodes were to be placed approximately 20 cm apart, 60% of the current injected would be received by the cortical area (Faria et al., 2011). However, when placed 8 cm apart, the proportion of current density received by the cortical area is reduced to 35% (Faria et al., 2011), which can be further influenced by individual differences in anatomy. Using rat models and human cadavers, Vöröslakos et al (2018) suggested that an electrical gradient of 1 mV/mm is required to influence neuronal firing rate and brain rhythms, which would require current intensities of 4mA to 6mA to elicit this electrical gradient. Intensities greater than 2mA have been previously avoided due to potential reductions in tolerability of stimulation in addition to the possibility of compromising skin integrity and induction of more adverse side effects (Russo et al., 2013; Keller et al., 2018). However, it has recently been reported that it is feasible to employ current densities of 4mA without breaching the integrity of the skin barrier function or inducing injury (Chhatbar et al., 2017; Nitsche & Bikson., 2017).

Blinding of stimulation condition is dependent upon the current intensity. During a sham or placebo stimulation protocol, current is delivered for the scalp for a short period of time,

usually up to 30 seconds, to induce a similar sensation as the active condition without inducing any neurophysiological changes (Davis et al., 2013; Fontenau et al., 2019; Palm et al., 2013). The use of higher intensity currents (≥ 2.5 mA) may compromise the blinding of participants to the sham condition due to the increased likelihood of irritation and redness occurring underneath the electrodes (Davis et al., 2013; Fontenau et al., 2019; Palm et al., 2013; Bastani & Jaberzadeh., 2013). Moreover, greater stimulation intensities may increase the ease of discerning the ramp down period when compared to the active condition (Davis et al., 2013; Fotenau et al., 2019; Palm et al., 2013; Bastani & Jaberzadeh., 2013). In the sport science literature, the use of an adequate sham or ramping procedures has not been consistently adopted. Indeed, the use of discrepant protocols may mean that the blinding procedures used may be effective in some studies than others and may partially explain the inconsistencies in findings within the isometric (Cogiamanian et al., 2007; Angius et al.,2016; Kan et al., 2013; Muthalib et al., 2013) and cycling (Angius et al., 2018; Vitor Costa et al., 2015; Barwood et al., 2016) exercise trials. Indeed, in cases where the sham condition is easily distinguished from the experimental trial, it is possible that participants might perform according to the studies aims.

Direct comparison of the studies is also limited by the inconsistent use of electrode montage, electrode size and the variability induced by individual covariates such as anatomy, sensitivity to stimulation and genetics (Strube et al., 2015; Labruna et al., 2016; Jamil et al., 2017; Wiethoff et al., 2014; Ridding & Ziemann., 2010). Recent investigations have highlighted that the efficacy of anodal tDCS is correlated to the sensitivity to other NIBS techniques, particularly TMS (Labruna et al., 2016; Jamil et al., 2017). It appears that those with a high baseline sensitivity to TMS (requiring a lower TMS output to fulfil a particular MEP amplitude) often exhibit a greater change in corticospinal excitability following the application of tDCS, in comparison to those with a low TMS sensitivity (Labruna et al., 2016; Jamil et al., 2017). Whilst TMS and tDCS influence cortical activity under different physiological mechanisms, it is suggested that individual differences in anatomy and physiology may influence the efficacy of these NIBS techniques in a similar manner. It should be noted that this relationship between TMS sensitivity and tDCS efficacy has not been observed in cathodal tDCS (Labruna et al., 2016; Jamil et al., 2017). This may be associated with the restricted range of current intensities required for diminutions in corticospinal excitability to occur (Labruna et al., 2016; Jamil et al., 2017).

Previous studies have intimated the relationship between TMS sensitivity and the coil-to-cortex distance, which modelling studies have revealed to be nuanced by the thickness of the cranium, subcutaneous fat, and CSF (Jamil et al., 2017; Opitz et al., 2015; Laakso et al., 2015; Truong et al., 2013; Kozel et al., 2000). Cranial tissue is the least conductive medium in the human head, and therefore is considered to strongly determine the proportion of current that can be passed into the cortical area (Opitz et al., 2015). This has been characterised by the observation of increased electric field strength within areas of low cranial density (Opitz et al., 2015). Intra-individual differences in cranial density may also mean that current intensities used may have a greater efficacy in regions of thinner density than others (Opitz et a l., 2015; Wagner et al., 2007). Computational modelling of spherical head models have demonstrated that a greater electric field strength occurs under an area of less curvature, such as the M1 (Wagner et al., 2007). Whereas areas with increased curvature such as the DLPFC have greater resistive matrixes thus limiting the current density reaching the underlying cortical area (Wagner et al., 2007). The CSF is

also implicated as a significant source of variability in electric field strength and the distance between the TMS coil and cortex (Laakso et al., 2015; Opitz et al., 2015). Although the CSF is a highly conductive medium, its ability to shunt current influences the proportion of current reaching the cortical area (Laakso et al., 2015; Datta et al., 2012; Opitz et al., 2015). Therefore, individuals with a thin layer of CSF have been shown to have a greater current density in the targeted area (Laakso et al., 2015; Datta et al., 2012; Opitz et al., 2015). Whilst those individuals with a thicker layer of CSF are shown to experience comparatively lower current density, due to a greater proportion of current being shunted away from the cortical area (Laakso et al., 2015; Datta et al., 2012; Opitz et al., 2015). These individual differences in anatomy emphasise the need for optimising the current intensity for the individual.

1.3.3 Duration and repetition of stimulation

In a similar principle to current intensity, researchers have also sought to enhance the longevity of the tDCS induced after-effects through altering the duration of stimulation or through the inclusion of multiple repetitions. Durations of tDCS application greater than 5 minutes have previously been shown to be capable of altering corticospinal excitability for several hours post stimulation, synonymous to the induction of E-LTP. The early studies conducted by Nitsche and Paulus (2001) also established a near linear relationship between the duration of tDCS application (from 1 to 13 minutes) and the duration of the elicited after effects. As such, to reap greater neurophysiological and behavioural outcomes, researchers have sought to induce late-LTP through the prolonged application of tDCS with common durations of up to 30 minutes (Agboda et al., 2020; Tremblay et al., 2016; Vignaud et al., 2018; Hassanzahraee et al., 2020; Mosayebi Samani et al., 2019; Monte-Silva et al., 2010; Monte-Silva et al., 2013). Indeed, within the sport and exercise literature the duration of stimulation commonly employed ranged between 10 to 30 minutes of application (See Table 1 and 2 in section 1.6).

However, due to the inherent complexities of the dose response relationship associated with tDCS, a growing body of literature has reported null effects of increasing duration on corticospinal excitability (Tremblay et al., 2016; Vignaud et al., 2018; Hassanzahree et al., 2020; Monte-Silva et al., 2010; Monte-Silva et al., 2013; Agboda et al., 2020; Bastani & Jaberzahed., 2013; Mosayebi Samani et al., 2019). To investigate the influence of stimulation duration on tDCS induced after effects, Monte-Silva et al (2013) compared the difference in corticospinal excitability following 13 minutes of stimulation and 26 minutes of stimulation. Whilst 13 minutes of 1 mA anodal-tDCS significantly enhanced corticospinal excitability for an hour post stimulation, the application of anodal-tDCS for 26 minutes surprisingly diminished corticospinal excitability for 2 hours post stimulation (Monte-Silva et al., 2013). Indeed, through titrating the duration of tDCS application from 22 to 30 minutes, Hassanzahraee et al (2020) determined the existence of a restricted window of linear effects of anodal tDCS duration, where durations under 26 minutes facilitate the increase in corticospinal excitability. Non-linearities in the corticospinal response occur when tDCS duration exceeds this window, as demonstrated by diminutions in corticospinal excitability when durations upwards of 26 minutes were implemented (Hassanzahraee et al., 2020; Monte-Silva et al., 2013). The reduction in MEP amplitude observed when duration of anodal-tDCS exceeded 26 minutes were also accompanied by reductions of intracortical facilitation (ICF) and increases in SICI (Hassanzahraee et al., 2020). As SICI is dependent upon the activation of GABAergic interneurons

(Hassanzahraee et al., 2020; Stagg & Nitsche., 2011), the enhancement of SICI during the prolonged tDCS protocol indicates the role of inhibitory mechanisms in the reversal of stimulation polarity (Hassanzahraee et al., 2020).

Prior synaptic plasticity is known to influence the induction of LTP and LTD in a subsequent neuroplastic protocols through the mechanisms of homeostatic plasticity (Ridding & Ziemann., 2010; Bienestock et al., 1982; Abraham & Bear., 1996; Turrigiano & Nelson., 2004., Siebner., 2004; Monte-Silva et al., 2013; Hassanzahraee et al., 2020). Researchers have implied that the mechanisms underlying the reversal of polarity observed when the duration of 1 mA anodal-tDCS exceeds 26 minutes can be explained by the BCM theorem of homeostatic plasticity (Hassanzahraee et al., 2020; Monte-Silva et al., 2013). It is speculated that the initial application of tDCS (durations $\leq$ 24 minutes) drive the threshold for LTD induction lower (Hassanzahraee et al., 2020; Monte-Silva et al., 2013). Therefore, when stimulation is prolonged ($\geq$ 26 minutes) homeostatic mechanisms activate the K^+ channels to mitigate the overflow of intracellular Ca^{2+} and thus converts the effects of anodal-tDCS to diminution (Hassanzahraee et al., 2020; Lisman., 2001; Monte-Silva et al., 2013). Indeed, the changes in ICF and SICI observed in conjunction with reductions of corticospinal excitability occur in accordance with the sliding scale of plasticity proposed by the BCM theorem, and therefore indicates that the strength of synaptic plasticity is reduced under high levels of neuronal activity due to the enhancement of the intracortical inhibitory interneurons (Hassanzahraee et al., 2020; Swanick et al., 2006).

Although the corticospinal response appears to be impinged by homeostatic counter-regulatory mechanisms during prolonged applications of tDCS, this does not necessarily guarantee the occurrence of unexpected alterations in the behavioural response to tDCS. For example, Angius et al (2019) reported an enhancement in cognitive and cycling performance following the 30-minute application of anodal tDCS to the DLPFC. An earlier study by Martin et al (2014) also demonstrated that the 30-minute application of anodal tDCS significantly enhanced skill acquisition during a cognitive task. This further highlights the inherent complexities associated with the application of tDCS. Nevertheless, researchers have since explored the viability of multiple repetitions of tDCS application to enhance the behavioural or neurophysiological outcomes. It is common practice for clinical trials to administer daily or twice daily applications of tDCS over several consecutive days on the assumption that repeated stimulation will induce cumulative and long-lasting changes in cortical function (Ferrucci et al., 2009; Benninger et al., 2010; Boggio et al., 2007; Loo et al., 2010; Loo et al., 2012). Outcomes thus far have supported this theory with the use of tDCS for treatment of depression showing the persistent alleviation of depressive symptoms which have lasted up to 4 weeks post treatment (Loo et al., 2010; Loo et al., 2012; Ferruci et al., 2009). Consolidation of hand motor function has also been observed up to two weeks post treatment in stroke patients (Boggio et al., 2007), whilst the improvements in bradykinesia and gait have lasted for up to three months post treatment in Parkinson's disease patients (Benninger et al., 2010). Indeed, the application of anodal tDCS over consecutive days has also been shown to induce cumulative increases in corticospinal excitability in healthy participants (Gálvez et al., 2013; Alonzo et al., 2012).

Despite the findings of cumulative increases in corticospinal excitability, debate still ensues regarding the optimal rest interval between successive tDCS applications. Converse to the findings of Galvez et al (2013) and Alonzo et al (2012) who reported the cumulative increases in corticospinal excitability from daily tDCS application, other research suggested that smaller intervals between successive applications of tDCS may be more effective at bolstering the effects of tDCS through elicitation of late-LTP (Agboda et al., 2020; Monte-Silva et al., 2010; Monte-Silva et al., 2013; Fricke et al., 2011). Short interludes of 3 minutes and 20 minutes have been shown to induce significant increases in MEP amplitude lasting for longer than 24 hours. Whilst long interludes of 3 hours or 24 hours have been shown to induce no effects or abolish the tDCS-induced after effects (Agboda et al., 2020; Monte-Silva et al., 2013). Greater effects of short intervals between multiple applications of tDCS has also been observed in cathodal tDCS (Monte-Silva et al., 2010). In comparison to a singular 9 minute application of 1mA cathodal tDCS, two 9 minute applications of tDCS interspersed by either 3 minutes or 20 minutes of rest has been shown to induce greater and longer-lasting reductions in corticospinal excitability (Monte-Silva et al., 2010). Longer rest durations of 3 hours and 24 hours induced a great amount of variability in the response to cathodal tDCS, initially showing attenuation or abolishment of after-effects with a delayed reduction in corticospinal excitability occurring at 2 hours post stimulation when there was 24 hours rest between tDCS application (Monte-Silva et al., 2010). Likewise, the use of short inter stimulus intervals of a similar duration have also been found to be more efficacious than long intervals at inducing late-LTP-like plasticity in animal slice studies (Reymann & Frey., 2007; Goldsworthy et al., 2015; Woo & Nguyen., 2003) and other NIBS techniques such as PAS (Müller-Dahlhaus et al., 2015) and intermittent theta burst stimulation (Tse et al., 2018).

However, the induction of late-LTP through multiple repetitions of tDCS appears to be dependent upon other stimulation parameters including the intensity and the duration of tDCS (Fricke et al., 2011; Agboda et al., 2020). Similar, to Monte-Silva et al (2013), Agboda et al (2020) also demonstrated that greater increases in corticospinal excitability lasting for over 24 hours occurred when two sessions of 15-minute 1 mA anodal tDCS were separated by short intervals of 20 minutes, whereas corticospinal excitability was minorly elevated for 30 minutes when the applications of tDCS were separated by 3 hours. However, when using an intensified tDCS protocol (20 minutes of 3mA anodal tDCS), enhancements in corticospinal excitability were restricted to 2 hours post-stimulation, and long rest intervals induced no changes (Agboda et al., 2020). The greater efficacy of the 1mA stimulation condition in comparison to the intensified 3 mA condition may be due to homeostatic plasticity's regulation of neural activity, where the intensified protocol may have induced a greater influx of Ca^{2+} and therefore may have experienced greater counter-regulation (Agboda et al., 2020). A reduction or reversal of stimulation polarity was observed when 5 minutes of 1 mA anodal or cathodal tDCS was interspersed by 3 minutes and 10 minutes of rest, whereas short intervals of 1 minute, 20 minutes or 30 minutes rest failed to induce a significant effect on corticospinal excitability (Fricke et al., 2011). Previous studies have shown that corticospinal excitability returns to baseline levels within minutes following short duration stimulations (< 6 minutes) and therefore are unlikely to induce E-LTP, the precursor for the occurrence of L-LTP (Nitsche & Paulus., 2000; Nitsche & Paulus., 2001). Additionally, results from this study may have resulted from individual differences in baseline MEP amplitude and responsiveness to tDCS as

several different participant groups were used to compare the efficacy of different rest intervals (Fricke et al., 2011).

Whilst it is still unclear why short inter stimulus intervals confer greater and longer lasting L-LTP or L-LTD-like changes in corticospinal excitability, researchers have speculated that the mechanisms underlying this process may be in part related to the time course of E-LTP or E-LTD (Agboda et al., 2020; Monte-Silva et al., 2010; Monte-Silva et al., 2013). It is well established that E-LTP and E-LDP are reliant upon alterations in NMDAR and CaMKII/ PKC pathway activity (Nguyen & Woo., 2003; Langemann et al., 2008; Monte-Silva et al., 2010). It is therefore suggested that a restricted time window exists for the induction of L-LTP or L-LTD due to the rapid depletion of the CaMKII/ PKC pathway and fast depotentiation of NMDAR's (Langemann et al., 2008; Monte-Silva et al., 2010; Agboda et al., 2020). As such, the repeated stimulations delivered in close succession may have allowed for stabilisation of NMDAR, CaMKII and PKC activity allowing for the induction of L-LTP or L-LTD (Langemann et al., 2008; Monte-Silva et al., 2010).

The 'synaptic tagging' or 'capture' hypothesis may also explain the differences observed between long and short rest intervals (Agboda et al., 2020; Sajikumar et al., 2005; Reymann & Frey., 2007; Woo & Nguyen., 2003; Nguyen & Woo., 2003). According to this hypothesis plasticity-related proteins and mRNA complexes likely synthesized within the nucleus are transported and distributed at multiple synapses (Woo & Nguyen., 2003; Nguyen & Woo., 2003; Sajikumar et al., 2005). It is thought that these proteins can be utilised for the induction of L-LTP at recently activated or 'tagged' synapses (Agboda et al., 2020; Reymann & Frey., 2007; Nguyen & Woo., 2003). However, these plasticity-related proteins and tagged synapses are thought to have a half-life of less than 2 hours, meaning that protocols employing short rest intervals between stimulations will provide a more efficient means of capturing these plasticity-related proteins and furthermore inducing L-LTP or L-LTD (Agboda et al., 2020; Nguyen & Woo., 2003; Woo & Nguyen., 2003).

Whilst both hypotheses highlight why short inter stimulation intervals may be more efficacious for inducing L-LTP and L-LDP, they fail to consider the mechanisms restricting the induction of E-LTP and E-LTD following the second stimulation, as often evidenced by null or abolished changes in corticospinal excitability (Monte-Silva et al., 2010; Monte-Silva et al., 2013; Agboda et al., 2020). The suppressive effect of long inter stimulation intervals may be caused by homeostatic mechanisms of metaplasticity in which alter the efficacy of neural networks to prevent destabilisation (Agboda et al., 2020; Abbott & Nelson., 2000; Turrigiano & Nelson., 2004; Monte-Silva et al., 2010; Monte-Silva et al., 2013; Siebner., 2004). It is argued that the effects of tDCS may outlast the overt changes in corticospinal excitability and presumably incorporate separate intracortical plasticity mechanisms (Monte-Silva et al., 2010). As the stimuli provided from the second stimulation following 3 to 24 hours rest falls outside the time window for the induction of L-LTP or L-LTD these homeostatic mechanisms are thought to raise the threshold and thus make it difficult for further applications of tDCS to induce E-LTP or E-LTD (Monte-Silva et al., 2010; Agboda et al., 2020; Turrigiano & Nelson., 2004; Abbott & Nelson., 2000). As alluded to in a previous section of this literature review (1.2.3 Metaplasticity) when tDCS is applied in short succession with another neuroplastic protocol such as rTMS (Siebner., 2004), PAS (Antal et al., 2007) or voluntary muscular contractions (Thirugnanasambandam et al., 2011; Baltar et al., 2018) homeostatic counter-regulatory

mechanisms are elicited, which contrasts the findings of enhanced efficacy of tDCS when applied in close succession. However, these neuroplastic interventions will differ in magnitude and duration of after effects, time dependency, focality of effect, nature of stimulation (subthreshold versus suprathreshold) to tDCS and therefore may be the basis of the homeostatic plasticity induced reductions in corticospinal excitability (Monte-Silva et al., 2010; Monte-Silva et al., 2013). Further research is warranted to clarify whether tDCS induces alterations in intra-cortical excitability, occurring between 90 minutes and 3 hours post stimulation, that are independent to changes in corticospinal excitability.

1.3.4 Summary

The use of conventional tDCS has been deemed advantageous in comparison to alternative NIBS techniques due to the ease of customisation. This allows the current dose to be adapted for the therapeutic use in clinical populations, and for the modification or cognitive and motor performance in healthy individuals. However, through customisation the intricate nature of tDCS has been unearthed. For example, increasing the current dose induces non-linear responses in corticospinal excitability (Bastani & Jaberzadeh., 2013; Batsikadze et al., 2013; Jamil et al., 2013; Agboda et al., 2020; Mosayebi Samani et al., 2019), manipulation of electrode montage changes the direction of current through the brain and can induce unintended effects underneath the return electrode (Bikson et al., 2010; Moliadze et al., 2010; Yanamadala et al., 2014) and finally, the traditional large (25 cm^2 – 35 cm^2) rectangular electrodes delivers unfocalized stimulation and therefore alters the excitability of multiple areas simultaneously (Nitsche et al., 2007; Datta et al., 2011). Furthermore, inter-, and intra-individual differences make the response to tDCS more unpredictable (Li et al., 2015). Due to these inherent complexities, researchers thus far have failed to draw a consensus for the optimal current dose.

Currently opinions are mixed on whether tDCS can exert an ergogenic on endurance performance, this literature will be discussed in Section 1.6. These contrasting findings are likely due to the vast inter-individual differences which are not taken into consideration when a standard current dose is applied to all participants. To mitigate the effects of the intra- and inter-individual differences tDCS should be optimised to the individual (Li et al., 2015). As a first port of call, researchers have sought to increase the focality of stimulation with HD-tDCS, which is predicted to restrict the current spread (Datta et al., 2012) and enhance corticospinal excitability (Radel et al., 2017). However, the enhancements in corticospinal excitability have yet to manifest in changes of motor performance (Flood et al., 2017; Radel et al., 2017). It is likely that for HD-tDCS to exert an ergogenic effect on performance, the placement of electrodes will need to be guided by prior neuroimaging and computational modelling (Datta et al., 2012). The continual monitoring and adjustment of tDCS parameters such as current intensity may also be necessary to reap the greatest effects of tDCS (Sood et al., 2016; Muthalib et al., 2018). The application of fNIRS and EEG in conjunction with HD-tDCS has recently been proposed to provide real-time evaluations of the current dose, through observing changes in brain metabolism and activation (Sood et al., 2016). However, the use of this technique may be constrained within a sports environment due to movement artefacts lowering the signal-to-noise ratio (Perrey & Besson., 2018). Whilst these techniques are likely to be fundamental for the optimization of tDCS for an individual athlete, it is potentially not feasible for both recreational and elite athletes. Firstly, the use of HD-tDCS in conjunction with various neuroimaging techniques are highly specialised and therefore are likely to be used within

a hospital or university setting, of which may not be accessible for the athlete. Due to the sensitive nature of these techniques, additional time may be required for application to ensure accurate results. This may impose time constraints and therefore may not be easily incorporated into an everyday training session or as a pre-competition intervention.

1.4 Sources of specificity

Transcranial direct current stimulation has been proposed for a plethora of uses. For example, stimulating the M1 is purported to reduce pain associated with spinal cord injury (Lefaucheur et al., 2008; Yoon et al., 2014; Fregni et al., 2006), fibromyalgia (Antal et al., 2010) and experimental pain (Flood et al., 2017; Angius et al., 2015; Boggio et al., 2008), improve gait and reduce bradykinesia in Parkinson's disease (Benninger et al., 2010; Hadoush et al., 2018), restores motor function following a stroke (Mahmoudi et al., 2011; Hesse et al., 2007; Boggio et al., 2007) and enhances endurance performance through the reduction in supraspinal fatigue and the rating of RPE (Cogiamanian et al., 2007; Angius et al., 2016; Angius et al., 2018; Vitor-Costa et al., 2015; Sasada et al., 2017; Park et al., 2019), to name a few. Due to the simplistic and low-intensity nature of tDCS, it is currently uncertain how tDCS can produce desired behavioural outcomes in complex cognitive functions, how it can manipulate multiple neural pathways, and how it can achieve specific effects (Bikson & Rahman., 2013).

The origins of specificity associated with tDCS have previously been grouped together based upon functional (task-specificity or input-selectivity) and anatomical factors (Bikson & Rahman., 2013). These sources of specificity are not thought to occur exclusively and therefore may be used mutually in a tDCS protocol. Understanding these sources of specificity is therefore deemed important for the advancement of tDCS due to the complexity associated with brain function and the supposed simplicity of tDCS (Unal & Bikson., 2018).

1.4.1 Anatomical specificity

By knowing the function of a cortical area or the location of pathology associated with a neurological disorder, researchers can theoretically apply tDCS to preferentially modulate a targeted area (Bikson & Rahman., 2013). For example, researchers have reported improvements in hand motor function when anodal tDCS was placed over the affected hemisphere in ischemic stroke patients (Boggio et al., 2007; Mahmoudi et al., 2011). In healthy populations, brain regions such as the insular cortex (IC), the dorsolateral prefrontal cortex (DLPFC) and the M1 are often targeted when investigating endurance exercise performance (Okano et al., 2013; Lattari et al., 2018; Angius et al., 2016; Angius et al., 2018; Angius et al., 2019; Vitor-Costa et al., 2015), due to their role in the control of motor functions and fatigue related perceptions (discussed further in section 1.7).

Anatomical specificity of tDCS is primarily determined by the current dose, and therefore largely influenced by the size of the electrode and the montage selected (Bikson & Rahman., 2013). The accuracy of locating desired cortical area is also thought to influence anatomical specificity (Bikson & Rahman., 2013; De Witte et al., 2018). Whilst the gold standard method for accurately locating the targeted cortical area is through magnetic resonance imaging (MRI) derived computational models (Bikson et al., 2012), a vast number of researchers have located the targeted area through locating TMS hotspots (Opitz et al., 2015; Ho et al., 2016; Jeffery et al., 2007; Williams et al., 2013), or through

the 10-20 international EEG system (Baltar et al., 2018; Angius et al., 2015; Angius et al., 2019). The 10-20 international EEG system is commonly used for the EEG electrode and TMS coil placement through the correlation of anatomical landmarks on the external skull to underlying cortical areas (De Witte et al., 2018; Herwig et al., 2003). Whilst the 10-20 EEG system accounts for head size in its calculations, it fails to consider the effects of head position, shape, or morphology of the underlying cortices (Uylings et al., 2005; De Witte et al., 2018; Hannah et al., 2019). Therefore, accuracy of cortical location is limited due to individual differences in neocortical morphology (De Witte et al., 2018; Datta et al., 2012; Hannah et al., 2019; Uylings et al., 2005).

Anatomical differences are a significant source of variance in the response to tDCS (Berker et al., 2013; Datta et al., 2012; Li et al., 2015). To allow for greater cortical space, the brain has a folded nature which yields a highly individualised pattern of gyri and sulci (Moliadze et al., 2010). As mentioned previously, computational modelling studies have demonstrated that individual differences in gyral patterns exacerbates the diffuse nature of conventional tDCS delivered through the large pad electrodes, inducing inter-individual variability in the areas influenced by tDCS (Bikson et al., 2012; Data et al., 2011; Datta et al., 2012). For example, a study modelled on three different participants demonstrated that conventional tDCS using large pad electrodes (35 cm^2) in a M1 cephalic montage (anode over the left M1, cathode over the right FP2) produces current clusters is several cortical areas, including deep brain structures and the prefrontal cortex (Datta et al., 2012). Current clusters in unintended cortical areas are thought to be produced by shunting of current through CSF (Li et al., 2015; Datta et al., 2012; Nathan et al., 1993). Therefore, grey matter (such as the peaks and troughs of gyri and sulci) that protrude into the CSF receive a greater current density, whilst the current densities of the sulci walls are much lower (Li et al., 2015).

The individualised folded nature of the brain will also influence the orientation of neurons within a cortex (Li et al., 2015; Kronberg et al., 2017; Kronberg et al., 2020; Kabakov et al., 2012; Bindman et al., 1964; Das et al., 2016). Whilst many neurons will exist in the same electric field, their orientation will not necessarily be the same and therefore will produce a different response to tDCS (Bikson et al., 2004; Kabakov et al., 2012; Das et al., 2016; Bindman et al., 1964). Hippocampal slice studies have indicated that polarity of effects on a neuron are dependent upon axonal orientation (Kabakov et al., 2012). An axon orientated towards the cathodal electrode will reduce the probability of glutamate release into the presynaptic terminals, whereas when the axon of a neuron is orientated towards the anodal electrode exhibiting the opposite effect (Kabakov et al., 2012; Das et al., 2016). It is also speculated that polarization of the neuronal dendrites decides the magnitude of effects induced by tDCS (Kabakov et al., 2012; Das et al., 2016).

To ensure anatomical specificity it is recommended that individual MRI derived computational models with tractography are implemented to understand cortical and neuronal morphology (Datta et al., 2012; Kabakov et al., 2012; Bikson & Rahman et al., 2013). Additionally, to ensure focality of stimulation, the use of HD-tDCS is recommended (Datta et al., 2012; Kabakov et al., 2012; Bikson & Rahman et al., 2013). However, these techniques are expensive, time consuming and potentially unattainable for athletes to use within training. Additionally, whilst individualisation of stimulation via computational modelling accounts for anatomical differences, many other neurophysiological and genetic precursors to variability will remain uncontrolled (Li et al., 2015; Horvath et al., 2015).

1.4.2 Task specific modulation

The application of tDCS concurrent to a task is suggested to enhance the focality and anatomical specificity of conventional tDCS, through the preferential modulation of active networks (Bikson & Rahman., 2013; Lapenta et al., 2013; Kronberg et al., 2017; Reato et al., 2013). The diffuse and subthreshold nature of tDCS is thought to be too weak and unspecific to modulate synaptic efficacy alone. Therefore, it is suggested that tDCS endogenously facilitates pre-existing exogenous task-induced plasticity through polarization of the post-synaptic membrane and removal of the Mg^{2+} NMDAR block (Stagg & Nitsche., 2011; Kronberg et al., 2017; Kronberg et al., 2020; Reato et al., 2013; Bikson & Rahman., 2013).

In clinical populations, the deliverance of tDCS at rest has previously shown to be functionally specific, with many studies reporting long-lasting enhancements in motor and cognitive performance (Bikson & Rahman., 2013; Olma et al., 2013). It is proposed that these enhancements occur due to the increased sensitivity within the pre-existing neural network associated with the pathology (Olma et al., 2013; Bikson & Rahman., 2013). However, to increase the efficacy of the rehabilitative programme, clinical studies have also demonstrated the improvement both motor and cognitive impairment when tDCS was administered in conjunction with a task (Hummel et al., 2005; Hesse et al., 2007; Bikson & Rahman., 2013; Ochi et al., 2013; Leśniak et al., 2014). In chronic stroke patients, the application of tDCS combined with motor training improved motor function (Hummel et al., 2004; Hesse et al., 2007; Ochi et al., 2013). These improvements in motor function also occurred in parallel to enhancements of motor cortical recruitment curves and reduced SICI (Hummel et al., 2005). In comparison to a sham condition, anodal tDCS targeting both the left temporal lobe and the left DLPFC enhanced a component of recognition memory in Alzheimer's disease patients (Boggio et al., 2008), similar effects of DLPFC stimulation on working memory have also been observed in Parkinson's disease patients (Boggio et al., 2006).

Task-specific modulation has also been explored in healthy populations. Fregni et al (2005) first demonstrated that tDCS delivered in conjunction with a cognitive task guided the neuromodulatory effects to become more functionally specific. By applying 5 minutes of anodal tDCS to the DLPFC during a cognitive task, significantly enhanced aspects of verbal working memory in comparison to the sham condition, cathodal DLPFC tDCS and anodal tDCS of the M1. Similarly, pain thresholds to experimental pain were shown to increase when tDCS was applied to either the DLPFC or the M1 during both peripheral electrical stimulation (PES) (Boggio et al., 2008) and thermal pain (Mylius et al., 2012). Only recently have researchers attempted to make the application of tDCS functionally specific for exercise performance (Park et al., 2019; Codella et al., 2020; Frazer et al., 2016; Hendy & Kidgell., 2014; Radel et al., 2017). Indeed, several studies demonstrated that the application of tDCS during a sport specific warm up or exercise trial enhanced wrist flexor one repetition max (Hendy & Kidgell., 2014), running (Park et al., 2019) and isometric TTE (Frazer et al., 2016), and performance within a battery of field tests (Codella et al., 2020).

It appears that the behavioural outcomes of tDCS are dependent upon the task completed and timing of stimulation. The combination of tDCS applied during performance of a task seems to bolster the learning rate during cognitive and motor tasks, as well as pain

thresholds (Fregni et al., 2005; Boggio et al., 2008; Mylius et al, 2012; Reis et al., 2009; Park et al., 2019). However, when tDCS is applied prior to a task, a number of studies have reported that learning (Kuo et al., 2008), pain thresholds and tolerance (Brasil-Neto et al., 2020) and motor performance (Kan et al., 2013; Muthalib et al., 2013; Barwood et al., 2016; Holgado et al., 2019) are left unchanged. Indeed, studies have demonstrated that the 'online' application of tDCS (applied during the task) leads to greater skill acquisition during both cognitive (Martin et al., 2014; Andrews et al., 2011) and motor learning (Stagg et al., 2011). Whereas 'offline' tDCS (prior application of tDCS) lead to slower learning times (Martin et al., 2014; Stagg et al., 2011). It therefore could be suggested that the 'online' tDCS manipulates cortical excitability in a similar manner to a motor learning task and therefore takes advantage of the gating mechanisms of metaplasticity (Ziemann & Siebner., 2008). Whereas tDCS delivered 'offline' may be influenced by homeostatic mechanisms of metaplasticity leading to diminished corticospinal excitability elicited by tDCS and slower learning (Stagg et al., 2011; Martin et al., 2014).

To date, research investigating the effect of tDCS on endurance performance has primarily adopted the 'offline' approach of tDCS application, applying tDCS following a prior exhaustive bout (Cogiamanian et al., 2007; Kan et al., 2013; Williams et al., 2013) or in a rested state (Abdelmoula et al., 2016; Angius et al., 2015; Angius et al., 2016; Angius et al., 2018; Angius et al., 2019; Vitor-Costa et al., 2015). Four studies to date have implemented an 'online' tDCS protocol, delivering tDCS within a sport specific protocol or during the exercise trial, demonstrating improvements in performance in on in a battery of field tests (Codella et al., 2013) and running (Park et al., 2019) and isometric TTE (Frazer et al., 2016). Interestingly, Radel et al (2017) reported no significant differences between the tDCS and sham condition when anodal HD-tDCS was delivered during an isometric TTE trial. To date have directly compared the effects of 'online' and 'offline' applications of tDCS on endurance performance. Therefore, it is still uncertain whether the 'online' application of tDCS will exert a beneficial effect during an endurance task.

1.4.3 Net-zero sum model of neuroenhancement

The human brain is thought to be constrained by limited processing power and energy. Therefore, functional reallocation is thought to occur to manage demands (Luber., 2014; Brem et al., 2014). The Net-zero sum model of neuroenhancement is based upon the mathematical framework constructed by game theorists which describes a situation where the sum of all payments won or lost by all players is zero by the end of the game (Brem et al., 2014). Therefore, the improvement in one player or party is always associated with a decrement to another player or party (Brem et al., 2014). According to the Net-zero sum model of neuroenhancement (Brem et al., 2014), to conserve the finite amount of energy available to the brain, tDCS induced enhancements in cognitive or motor ability must be accompanied by a cost (Luber., 2014; Brem et al., 2014). Brem et al (2014) postulated the existence of a central processor in which distributes the power and energy sources according to the need and demand, suggesting this would occur through top-down modulation processes in which brain areas or neurons higher in the 'hierarchy' increase or suppress task-relevant networks. Alternatively, when neural pathways work independently to top-down modulation, trade-offs are stated to occur due to competition between sub-processes which leads to a negative impact on one or more process (Brem et al., 2014; Luber., 2014).

Neuroenhancement through the application of tDCS is proposed to enhance the processing power for a specific function through a direct increase in processing power in either the brain region of interest or supplementary areas or through the reduction in background noise or interference induced by other cortical areas (Brem et al., 2014). Iuculano and Kadosh (2014) draws our attention to the existence of cognitive side effects that occur in parallel to enhancements following tDCS, describing that anodal stimulation of the posterior parietal cortex enhanced learning whilst automaticity (the effortless or speed of performance) was compromised. The authors also reported that the opposite effects on learning and automaticity were observed when anodal tDCS was applied to the DLPFC (Iuculano & Kadosh., 2014). This supports the notion that enhancements in performance elicited by tDCS may also be accompanied by a deficit in another (Iuculano & Kadosh., 2013; Brem et al., 2014). However, it should be noted that different stimuli were presented to the participants in the learning and test phases (the test phase included non-adjacent symbols whilst only adjacent symbols were presented to the participants within the learning phase) which therefore may have influenced automaticity rather than the tDCS induced redistribution favouring a specific cognitive task. Similar 'trade-offs' in accuracy or detection speed have also been observed in alternative NIBS techniques (Brem et al., 2014; Luber., 2014; Walsh et al., 1998; Hilgetag et al., 2001). The application of 1 Hz rTMS to lower excitability within the parietal cortex improved the accuracy of detection of visual stimuli in the ipsilateral field of vision to the stimulation site, at the cost of visual accuracy in the contralateral field (Hilgetag et al., 2001). TMS applied over the visual cortex has also been shown to induce detrimental effects to reaction time performance (Walsh et al., 1998). For example, when motion was irrelevant to the task, or when participants were required to pay attention to either the colour or form of the presented stimuli, reaction time was quicker (Walsh et al., 1998). However, when required to pay attention to motion, reaction time was significantly lengthened (Walsh et al., 1998). It should be noted that the decrements in cognitive functions observed in these studies are likely to originate from similar cortical areas and neural pathways, and therefore do not necessarily represent the redistribution of all neural functions as suggested by the net-zero sum model and therefore could be better explained by input-selectivity and bias.

The application of the net-zero sum model to performance within endurance tasks is also limited. In addition to motor function, endurance performance depends upon the prior experience, the cumulation of various stimuli (distance covered, terrain, energy stores, weather, competitor location) and cognitive functions such as attention, working memory and inhibitory control to continually refine the pacing strategy and supress irrelevant cues (Boya et al., 2017; Martin et al., 2016; Renfree et al., 2014). According to the net-zero sum proposition, the application of tDCS would enhance endurance performance through improving motor function at the expense of all other functions, including the cognitive functions required for accurate pacing. Arguably if this was the case, endurance performance would be disrupted due to suboptimal pacing. Thus far there has been no evidence of the net-zero sum model within the sport science literature. Indeed, contrasting the net-zero sum model Angius et al (2019) recently demonstrated that the application of anodal tDCS to the DLPFC induced significant improvements in cycling TTE performance and inhibitory control.

By all accounts, the net-zero sum model raises important issues concerning the underreporting of side effects in the NIBS literature, including decrements in irrelevant

cognitive functions (Davis et al., 2013; Luber., 2014). However, this framework fails to account for circumstances where enhancements in one function does not entail a cost. For example, the enhancements in endurance cycling performance and cognitive functions following the application of anodal tDCS (Angius et al., 2019). This framework also considers the brain and nervous system to be a closed-loop thermodynamic system, where energy can undergo change, but matter cannot (Luber., 2014; Brem et al., 2014). This therefore undermines the process of synaptic plasticity, through which the ever-evolving nature of the nervous system allows for the automaticity of various functions and therefore frees the limited energy available for novel interactions (Luber., 2014). The net-zero sum model of neuroenhancement therefore may not be the most appropriate model to explain task specificity associated with tDCS (Luber., 2014).

1.4.4 Input selectivity and bias

Several similarities exist between the net-zero sum model of neuroenhancement and specificity originating from input-selectivity. Input-selectivity assumes that a neuronal network or pathway is predisposed to operate in two states and at least two functions (Bikson & Rahman., 2013). Like the net-zero sum model, input-selectivity assumes that a neuronal network or pathway cannot operate in more than one function or state at any given point, and therefore the application of tDCS is postulated to activate gating systems where the tDCS current switches the network or pathway from operating in one state to another (Bikson & Rahman., 2013). It is therefore implied, that an enhancement in one function may occur at a cost of another (Bikson & Rahman., 2013; Iuculano & Kadosh., 2013; Walsh et al., 1998). In contrast to the Net-zero sum model, the effects of input-selectivity are thought to be confined to be within the region of stimulation, therefore input-selectivity is not thought to encompass the same detrimental effects as described by the net-zero sum model (Bikson & Rahman., 2013).

Findings from animal studies have postulated that the cellular basis of input selectivity could be determined by neuronal orientation (Bikson & Rahman., 2013). Hippocampal slice studies have demonstrated that due to the orientation of a neuron, tDCS induced changes in synaptic efficacy will differentially modulate neurons located within the same region (Bikson & Rahman., 2013; Rahman et al., 2013; Kabakov et al., 2012; Bikson et al., 2004). As such it has been suggested that the orientation of the axon may influence pathway specific alterations (Kabakov et al., 2012). Alternatively, it has also been suggested that the polarising current delivered by tDCS may be unevenly distributed across a neuron. For example, polarisation may occur across one dendritic branch, but not another. This is thought to create weighting or imbalances across the cell which may induce the input-bias within a network (Rahman et al., 2013; Fritsch et al., 2010; Bikson & Rahman., 2013).

Brain areas such as the DLPFC provide a good example of input-selectivity. The DLPFC has been implicated as a primary structure for the control of executive functions (Loftus et al., 2014; Miller., 2005), emotional valences (Rêgo et al., 2015; Brounoni et al., 2013), working memory (Gill et al., 2015), attention (Mariano et al., 2016) and the control of the pain response (Ong et al., 2019). Therefore, according to the concept of input-selectivity, tDCS applied to the DLPFC, one of these functions will be enhanced whilst others inhibited. Angius et al (2019) recently demonstrated that DLPFC stimulation using a cephalic montage (anode: left DLPFC, cathode: right supraorbital area) significantly

enhanced inhibitory control but had no effect on pain naturally induced by intense exercise. Inhibitory control was assessed via the Stroop test before and immediately after the application of tDCS, therefore inputs may have been biased towards enhancing this executive function instead of the pain pathway (Angius et al., 2019; Bikson & Rahman., 2013).

Input-selectivity supposedly occurs independently to both anatomical-specificity and functional-specificity via task-specific modulation, as it does not require an anatomical target or the co-activation of brain areas (Bikson and Rahman 2013). Due to the inherent difficulties in distinguishing between the different concepts of specificity, it is uncertain whether the functional specificity of tDCS induced by task-specific modulation is influenced by anatomical-specificity guiding the current to specific cortical structures; whether active-networks are preferentially modulated by tDCS or whether the administration of tDCS during a cognitive or motor task biases the inputs towards the activity-specific pathways (Bikson & Rahman., 2013). However, it is likely that a combination of the concepts of specificity are used during effective tDCS protocols. Future research should aim to explore the sources of specificity associated with tDCS through the use of multi-modal imaging, to enable the establishment of changes in functional brain states and cortical pathways elicited by tDCS. It is however likely that a combination of concepts of specificity.

1.5 Safety

With the increasing popularity of tDCS, the development of commercial devices and new protocols, the development of stringent safety criteria is required. Resultantly to date conventional tDCS ($\leq$ 40 min duration, $\leq$ 4 mA) has been reported to be used in over 33,200 human trials, with the occurrence of a singular serious adverse event (Sierawska et al., 2020; Bikson et al., 2016). A serious adverse event is defined by the Regulation of the European Parliament and The Council on Medical Devices (2017) as any untoward event that leads to a death, hospitalisation, permanent illness or injury or the requirement of medical intervention in order to prevent permanent illness, injury or irreversible damage to a body structure or function in either a participant or a researcher. In the case of the singular serious adverse event reported, an incident of juvenile myoclonus epilepsy occurred 5 days following a second application of anodal DLPFC tDCS in a clinical trial of healthy adolescents and children (Sierawska et al., 2020). According to the authors following the incident the participant had reported previous cases of trembling which could be classified as minor seizures (Sierawska et al., 2020). Moreover, the participant and consenting guardian were reported to pay little attention during the pre-screening and informed consent procedure. Whilst it is uncertain whether tDCS triggered this epileptic incident, this case study highlights the necessity of thorough screening of medical history, adherence to strict exclusion criteria and participant awareness of potential side effects (Sierawska et al., 2020).

The reporting of mild side effects is much more prevalent within the tDCS literature. During tDCS application, cutaneous sensations such as tingling, itching or a mild burning are commonly reported (Bikson et al., 2016; Brunoni et al., 2013; Poreisz et al., 2007; Nikolin et al., 2017; Antal et al., 2017). As tDCS is thought to induce negligible changes to skin temperature, brain injury via heating is improbable (Bikson et al., 2016). Furthermore, imaging via MRI has demonstrated that single applications of tDCS does not cause

changes to cerebral tissue, the blood-brain barrier (BBB) or brain edema (Nitsche et al., 2004). These sensations are therefore believed to be due to the stimulation of cranial nerve afferents (Paneri et al., 2016). In rare incidences atypical irritations to the skin can occur, which are thought be associated with poor skin preparation (Palm et al., 2008; Paneri et al., 2016; Bikson et al., 2016; Poreisz et al., 2007; Nikolin et al., 2017; Antal et al., 2017). The daily application of adhesive electrodes has previously shown to induce skin irritation, due to the adhesive glue (Paneri et al., 2016). Skin lesions have also been reported following the use of sponge electrodes soaked in water (Palm et al., 2008). The use of water is suggested to increase the impedance and therefore causing more pronounced thermal effects (Palm et al., 2008). Tolerability and skin integrity have previously been shown to improve through the use of sponge electrodes soaked in a sodium chloride solution (Minhas et al., 2010; Paneri et al., 2016). Within the tDCS literature mild headaches, nausea, fatigue, insomnia, and dizziness are commonly reported to occur after the tDCS application, these however are thought to resolve within a few hours reported (Bikson et al., 2016; Poreisz et al., 2007; Nikolin et al., 2017; Antal et al., 2017).

To date there are no known reports of serious adverse effects associated with self-directed home use, still the cautious use of devices is recommended (Bikson et al., 2016). With the increase in prevalence of commercial and homemade devices, there are several potential risks which may not be accounted for, such as the reliability of device's construction and design, implementation of safety meters to prevent overuse and quality of skin contact from the electrodes (Bikson et al., 2016; Fitz & Reiner., 2015; Wexler., 2016). Whilst tDCS is lauded for its supposedly transient influence on corticospinal excitability, the presence of long-term consequences on cognitive or motor functions are still unknown (Fitz & Reiner., 2015; Wexler., 2016). Therefore, researchers are concerned that the lack of regulations associated with home-use devices will promote misuse and therefore heightens the risk of serious adverse events (Fitz & Reiner., 2015; Wexler., 2016; Davis & van Koningsbruggen., 2013).

1.6 The use of tDCS to enhance endurance performance.

Endurance performance can be defined as whole-body dynamic exercise, involving a continuous effort lasting upwards of 75 seconds (McCormick et al., 2015). As such sports such as rowing, running, swimming, cycling or a combination such as triathlon are commonly associated. Endurance events are not only for the elite athlete but are popular within a recreational community, with major events such as the London Triathlon reporting over 11,000 participants every year (British Triathlon, 2019). The regulation of pace is deemed to be the crux of endurance sports. For which, pace is known to be determined by a coalescence of physical (terrain, altitude, temperature, wind, humidity, aerodynamics and athlete positioning) (De Koning et al., 2011; Jeukendrup et al., 2011; Kay et al., 2001), physiological (development of peripheral and central fatigue, VO_{2max}, Lactate thresholds, running economy or cycling efficiency) (McKenzie et al., 1992; Amann et al., 2008; Place et al., 2007; Joyner & Coyle et al., 2008) and psychological (self-efficacy, internal and external motivation, emotions, mental fatigue, the perception of pain) (Mauger et al., 2010; Mauger et al., 2013; Pageaux et al., 2013; Marcora., 2008; McCormick et al., 2015) factors. Throughout time, clubs and coaches have sought to develop the fittest, fastest, and strongest athlete, as such it is not uncommon for athletes to turn to illegal

substances or methods to enhance performance. Indeed, a recent study estimated the prevalence of blood doping to be between 15-18% of endurance athletes at the 2011 and 2013 World Athletics World Championships (Faiss et al., 2020). With a growing acceptance that the brain may regulate the development of fatigue, pace and therefore endurance performance focus has been shifted towards the role of centrally acting performance modifiers such as tDCS to enhance endurance performance (Machado et al., 2019).

From the observations of enhanced corticospinal excitability in healthy populations and the improvement in motor function, fatigue, and pain in clinical patients (Nitsche & Paulus., 2000; Cunningham., 2007; Antal et al., 2010; Benninger et al., 2010); tDCS is intimated as a potential tool to enhance endurance performance (Cogiamanian et al., 2007; Angius et al., 2017). Indeed, the early studies conducted within this field noted that through altering the excitability of the M1 or the IC induced improvements in isometric TTE of the elbow flexors and knee extensors, cycling TTE and improved peak power output (PPO) within a maximal incremental test to exhaustion (MIE) (Cogiamanian et al., 2007; Angius et al., 2016; Okano et al., 2013; Vitor-Costa et al., 2015). However, with the proliferation of studies conducted within the last two decades, a vast number of researchers have reported null effects of anodal tDCS on cycling time trial (TT) performance in addition to isometric and cycling TTE trials (Muthalib et al., 2013; Kan et al., 2013; Angius et al., 2015; Holgado et al., 2019; Barwood et al., 2016). As summarised in table 1 and table 2, the diversity in presented outcomes may be instigated by the differences in tDCS current dose (see section 1.3) and exercise paradigm evaluated. Or it may also be indicative of the numerous individual differences which influence the response to tDCS (see section 1.4.1).

Cogiamanian et al (2007) were the first to investigate the effect of tDCS on elbow flexor muscular endurance. In two experiments, the authors explored whether the application of anodal or cathodal tDCS (10 mins, 0.043 mA/cm^2) to the right M1 in an extracephalic montage would influence the development of fatigue during an isometric TTE of the elbow flexors when compared to a separate non-stimulation control group (Cogiamanian et al., 2007). In this experiment, both the polarization (anodal and cathodal tDCS) and non-stimulation control group completed two isometric TTE at 35% maximal voluntary contraction (MVC) interspersed with 60 minutes of rest. The second experiment sought to investigate the effect of tDCS on fatigue through monitoring changes in excitability. For this, TMS was applied to six participants during a submaximal isometric contraction (5% MVC) before and after the application of anodal tDCS. Despite reporting no significant differences in MVC, the authors observed a significantly longer endurance time following anodal tDCS and increased MEP amplitude within the second experiment. To date the mechanisms underlying the supposed ergogenic effect are uncertain, however the authors postulated that anodal tDCS may influence activity upstream of the M1 to protect the M1 from inhibitory feedback from the muscles limiting the motor cortical output, facilitation of the supraspinal drive, manipulation of fatigue-related perceptions such as pain or RPE, increase motivation or enhance the coherence of synergistic muscles (Cogiamanian et al., 2007).

Concurring with the findings of Cogiamanian et al (2007), a later study conducted by Williams et al (2013) observed that anodal tDCS applied to the M1 in a cephalic montage (20 min, 0.043 mA/cm2) enhanced endurance time during an isometric TTE trial of the

elbow flexors at 20% MVC. In a crossover repeated measures design, participants performed the exhaustive isometric contraction during both anodal tDCS and a sham control trial, during which RPE and corticospinal excitability (through the application of TMS) were monitored throughout. During analysis, participants were split into two groups; full-time (n = 8, where TTE occurred prior to tDCS cessation) and part time (n = 10, where TTE was extended after the termination of tDCS). In comparison to sham, anodal tDCS increased endurance time by 31% in the full-time group. This was accompanied by greater fatigue and RPE at task failure, but no significant changes in MEP amplitude. Additionally, there were no significant differences in TTE between the anodal tDCS and sham condition of the part-time group. Interestingly, a later study conducted by Angius et al (2016) explored the influence of electrode montage on corticospinal excitability and subsequent TTE of the knee extensors. Nine participants completed an isometric TTE trial at 20% MVC following a sham, non-stimulation control and two anodal M1 tDCS trials utilizing a cephalic and extracephalic montage (See table 1). Interestingly, in contrast to Williams et al (2013) and corroborating the findings of Cogiamanian et al (2007), the authors (Angius et al., 2016) found a significant improvement in TTE and a reduction in RPE during the extracephalic montage alone. Like Williams et al (2013), these findings occurred independently to changes in corticospinal excitability.

Six to nine years later several studies have investigated the influence of tDCS on isometric TTE, highlighting contrasting outcomes (Table 1). Most notably, two studies conducted in 2013, failed to corroborate the findings of enhanced endurance time or changes in corticospinal excitability (Muthalib et al., 2013; Kan et al., 2013). Replicating the tDCS montage and exercise paradigm, Kan et al (2013) explored whether an increased current dose (0.083 mA/cm^2) would produce greater changes in TTE. The authors reported no significant difference between the anodal tDCS and sham conditions in MVC strength of the elbow flexors or TTE. In a second, separate investigation, the authors investigated the influence of the intensified protocol (0.083 mA/cm^2) on the corticospinal excitability of a separate 10 male participants to the original investigation. As a group, there were no evident changes in corticospinal excitability, however the authors noted a large variance in response where the change in MEP amplitude ranged from 0% to an 135% increase from baseline (Kan et al., 2013). However, as the participants used in this second experiment are different from the original 15, it is still uncertain whether individual differences contributed to the null findings on MVC strength and TTE (Kan et al., 2013). Muthalib et al (2013) also sought to investigate the effects of M1 anodal tDCS on TTE of the elbow flexors, and PFC oxygenation with fNIRS. Like Kan et al (2013) the authors replicated the electrode montage and exercise paradigm from Cogiamanian et al (2007), finding no significant difference in endurance time between the sham and anodal tDCS condition. The authors also failed to detect any difference in PFC oxygenation between the sham and anodal tDCS condition. Both research groups suggested the existence of a ceiling effect where lower intensities of tDCS induce greater changes in corticospinal excitability and endurance performance of the elbow flexors (Muthalib et al., 2013; Kan et al., 2013). However, this explanation is unlikely as recent research has demonstrated no significant differences in corticospinal excitability and TTE following both 1 mA (0.029 mA/cm^2) and 2 mA (0.058 mA/cm^2) tDCS (Wrightson et al., 2019). Whilst others have reported ergogenic effects using a much greater current density (Angius et al., 2016).

The first study to investigate the influence of tDCS on dynamic, whole-body exercise was conducted by Okano et al (2013). Within this study, ten national level male road cyclists completed a cycling MIE trial following anodal tDCS or sham stimulation applied to the temporal cortex (TC). The TC was targeted within this study to influence the activity of the IC, an area thought to regulate the perceived exertion during exercise as well as cardiac autonomic function (Okano et al., 2013). As a result, the authors reported a 4% improvement in PPO which was accompanied by a significantly lower submaximal HR and RPE in comparison to the sham condition. The authors concluded that the increased excitability of the IC induced by anodal tDCS may have made the MIE trial feel easier, and consequently allowed for improvements in performance.

To explore the impact of tDCS on endurance capacity, most studies have employed a cycling, or in one instance running, TTE test (Table 2). The first cycling TTE trial was conducted by Angius et al (2015). In this study, the researchers investigated whether the application of anodal tDCS in a cephalic montage could induce analgesia to exercise-induced pain. In part A of the investigation, nine participants received 10 minutes of anodal tDCS, sham or control condition prior to completing a cycling TTE trial at 70% of PPO, finding no significant differences in endurance time, RPE or the rating of pain between the three conditions. However, part B of the investigation saw significant reductions in the pain perception during an experimental cold pressor test (CPT) (Angius et al., 2015). Consequently, the authors concluded that the use of the cephalic montage may have reduced the excitability of the right DLPFC which may have had detrimental effects on cognitive functions, the processing of exercise-induced pain and emotions, which are thought to be important for the regulation of exercise (Angius et al., 2015). Alternatively, the cephalic montage used is thought to influence excitability of the brain related to the contralateral limb and therefore may not be beneficial for whole-body dynamic exercise. Indeed, employing an electrode montage which spans both left and right M1 appears to enhance whole-body performance (Angius et al., 2018; Vitor-Costa et al., 2015; Park et al., 2019; Huang et al., 2019). Through applying the active electrode to the Cz, spanning both right and left M1, Vitor-Costa et al (2015) demonstrated an improvement in cycling performance within a TTE. Interestingly, this change in endurance performance occurred without any significant changes in RPE, HR and EMG activity. A later study conducted by Angius et al (2018) also found that a bilateral extracephalic montage applied to the M1 was also capable of significantly enhancing performance during a TTE trial, reducing RPE and increasing corticospinal excitability.

Whilst TTE trials allow for important observations in endurance capacity, performance in endurance events are known to have fluctuations in pace, and rarely involve an athlete exercising to the point of exhaustion. Therefore, limiting the application of TTE models within a sporting context (Marino., 2010). Therefore, it could be argued that the findings from TT's may provide greater information on the efficacy of tDCS to enhance endurance performance. As shown in table 2, to date TT's have been employed in a total of four studies, all of which displayed no significant effects of tDCS (Andre et al., 2019; Barwood et al., 2016; Holgado et al., 2019; Valenzuela et al., 2018). It is still uncertain why these null effects were found. As none of these studies employed measures to investigate the physiological change following tDCS (such as the use of TMS or fNIRS) it is unknown whether the net null effect was due to individual differences to tDCS or whether these findings are attributable to the individualisation in pacing strategy adopted.

With the increase in availability of commercial devices, the chronic application of tDCS has been reported for the use during training. Thus far, only one study has investigated the influence of repetitive tDCS application during a three-week strength training programme (Hendy & Kidgell., 2013). This programme required thirty participants to complete nine training sessions in total consisting of four sets of 6-8 reps of wrist extension exercises completed at 70% of one-repetition maximum. Twenty minutes of anodal M1 tDCS (active left M1, return Fp2; 0.083 mA/cm^2) or sham stimulation was applied throughout the strength training session. Interestingly whilst the authors reported that tDCS induced changes in markers of cortical plasticity such as increases in MEP amplitude and decreased SICI, tDCS did not exert any beneficial effects on dynamic strength adaptations. Consequently, the authors suggested that this effect may be due to the muscle group selected. The wrist extensor muscles are important for the control of fine motor movements, therefore heavy strength training may have induced excessive fatigue or muscle soreness, as such strength gain at the end of the training period may not have been evident (Hendy & Kidgell., 2013).

To date the effects of repeated applications of tDCS during endurance training has not yet been systematically evaluated. Nevertheless, it is now apparent that the self-directed use of conventional tDCS has now outpaced research. Indeed, tDCS is thought to be commonly used to enhance physiological adaptation to endurance training or prior to competition in a naturalistic setting (Hornyak., 2017; Davis., 2013; Edwards et al., 2017; Machado et al., 2019; Lefaucher., 2019). In a 2015 interview by The Guardian, Sir Dave Brailsford, the general manager of Team Ineos and the former performance director of British Cycling, referenced the use of tDCS in training with Team Sky cyclists to ‘over-ride’ the brain during fatigue (Ingle., 2015). Since, several American athletes within the American cycling, skiing and snowboard teams have been reported to use a commercial tDCS to boost their training ahead of the 2021 Tokyo Olympics, 2017 Tour De France and 2018 PyeongChang Winter Olympics respectively (Halo Neuroscience., 2018; McMahon., 2017; Reardon., 2016). Therefore, given the recent media attention surrounding the use of tDCS to enhance training, this area of research requires desperate attention to address the efficacy and validity of these devices to influence training adaptations. Moreover, the commercialisation of these devices is thought to pose issues, given that there is incomplete evidence that tDCS provides an ergogenic effect, the exact mechanisms underlying this supposed effect are still unknown, and the lack of longitudinal studies limits the understanding of potential maladaptation and safety issues (Davis & van Koningsbruggen., 2013; Fitz & Reiner., 2015; Wexler., 2016). Arguably, if tDCS was found to provide an ergogenic effect for training and competition, it is fair to consider such device as a form of ‘brain doping’ and therefore would breach the integrity of sportsmanship (Davis., 2013; Park., 2017).

Table 1. Summary of studies that have investigated the effect of conventional tDCS on isometric contractions

Reference	Target area	Montage, participants (n)	Duration (mins)	Current Density (mA/cm^2)	Control	Exercise Paradigm	Result
Cogiamanian et al., 2007	Right M1	Active R M1 return ipsilateral shoulder, Experiment 1 group 1 *n* = 9, group 2 *n* = 15. Experiment 2 *n* = 6	10	0.043	Control	35% MVC TTE of left elbow flexors	tDCS enhanced endurance time by 15%
Kan et al., 2013	Right M1	Active R M1 return ipsilateral shoulder, *n* = 15	10	0.083	Sham	30% MVC TTE of left elbow flexor	No significant difference
Muthalib et al.,2013	Right M1	Active R M1 return ipsilateral shoulder, *n* = 15	10	0.083	Sham	30% MVC TTE of left elbow flexor	No significant difference
Williams et al., 2013	Right M1	Active R M1 return contralateral Fp2, *n* = 18	20	0.043	Sham	20% MVC TTE of elbow flexors	tDCS enhanced endurance time by 31%
Abdelmoula et al., 2016	Left M1	Active L M1 return contralateral shoulder, *n* = 11	10	0.043	Sham	35% MVC TTE of elbow flexors	tDCS enhanced endurance time by 17 %
Angius et al., 2016	Left M1	Cephalic montage active L M1 return contralateral DLPFC. Extracephalic montage active L M1 Return ipsilateral shoulder, *n* = 9	10	0.167	Sham & control	20% MVC TTE of knee extensors	tDCS with extracephalic montage enhanced endurance time by 27%. Cephalic no

							significant differences
Oki et al., 2016	Right M1	Cephalic montage active R M1 return contralateral Fp2, *n* = 13	20	0.043	Sham	20% MVC TTE of elbow flexors	tDCS enhanced endurance time by 15%
Wrightson et., al (2019)	Left M1	Active L M1 return ipsilateral shoulder, *n* = 22	10	0.029 (1 mA condition), 0.058 (2mA condition)	Sham	20% MVC TTE of right knee extensors	No significant differences

Primary motor cortex (M1); Supraorbital area (Fp2); Dorsolateral prefrontal cortex (DLPFC); Maximal voluntary contraction (MVC); Time to exhaustion (TTE).

Table 2. Summary of studies investigating the influence of conventional tDCS on endurance performance (dynamic exercise).

Reference	Target area	Montage, participants (n)	Duration (mins)	Current Density (mA/cm^2)	Control	Exercise Paradigm	Result
Okano et al., 2013	Left TC & IC	Active L TC return Fp2, *n* = 10	20	0.058	Sham	Cycling MIE	4% improvement PPO
Angius et al., 2015	Left M1	Active L M1 return contralateral DLPFC, *n* = 9	10	0.167	Sham & control	Cycling TTE at 60% PPO	No significant differences
Vitor Costa et al., 2015	M1	Active L & R M1 (Cz) return occipital protuberance, *n* = 15	13	0.058	Sham & control	Cycling TTE at 80% PPO	Enhanced endurance time by 20%
Barwood et al., 2016	Left TC & IC	Active L TC return Fp2, study 1 *n* = 6, study 2 *n* = 8	20	Study 1 0.430, study 2 0.440	Sham	Study 1 20 km cycling TT. Study 2 cycling TTE at 75% PPO	No significant differences
Okano et al., 2017	Left TC & IC	Active L TC return FP2, *n* = 13	20	0.058	Sham	30 min cycle at 120% HR_{max}	No significant differences
Angius et al., 2018	M1	Bilateral extracephalic montage active L & R M1 return ipsilateral shoulders, *n* = 12	10	0.058	Sham & Control	Cycling TTE at 70% PPO	Enhanced endurance time by 23%
Lattari et al., 2018	Left DLPFC	Active L DLPFC return Fp2, *n* = 11	20	0.058	Sham	Cycling TTE at 100% PPO	Enhanced endurance time by 46%

Valenzuela et al., 2018	Left M1	Active L M1 return Fp2, *n* = 8	20	0.080	Sham	800 m swimming TT	No significant difference
Angius et al., 2019	Left DLPFC	Active L DLPFC return contralateral Fp2, *n* = 12	30	0.058	Sham	Cycling TTE at 70% PPO	Enhanced endurance time by 13%
Holgado et al., 2019	Left DLPFC	Active L DLPFC return contralateral shoulder, *n* = 36	20	0.080	Sham	20 min cycling TT	No significant differences
Park et al., 2019	M1	Bihemispheric montage active M1 (Cz), return C5 & C6 (10-20 EEG system), *n* = 10	20	0.825	Sham	Running TTE at 80% VO_{2max}	Enhanced endurance time by 15%
Andre et al 2019	M1, DLPFC, V1	M1 tDCS active Cz, return Fp2, DLPFC active L DLPFC, return Fp2, V1 tDCS active Oz, return Fp2, *n* = 9	20	0.06	V1 stimulation	16.1 km cycling TT	No significant differences

Temporal cortex (TC); Insular cortex (IC); Primary motor cortex (M1); Dorsolateral prefrontal cortex (DLPFC); Supraorbital area (Fp2); Maximal incremental test to exhaustion (MIE); Time to exhaustion (TTE); Peak power output (PPO); Time trial (TT); Maximal heart rate (HR_{max}); Maximal volume of oxygen consumed (VO_{2max}).

1.7 The putative mechanisms of action underpinning the influence of tDCS upon endurance performance

The contradictory evidence presented for an ergogenic effect of tDCS upon endurance performance is also compounded by the uncertainty surrounding the underlying mechanisms (Wrightson et al., 2019; Davis et al., 2019; Angius et al., 2017). This dearth in understanding is thought to be constrained by the inability to combine robust neuroimaging methods with sporting activities, due to immobility and the sensitivity of these methods to movement artefacts (Perrey & Besson., 2018). However, based on the assumption that tDCS induces depolarization or hyperpolarization, it is presumed that the ergogenic effect exerted by tDCS occurs through facilitation of M1 activity and therefore delaying supraspinal fatigue (Angius et al., 2017; Cogiamanian et al., 2007). Alternatively, researchers have also suggested that tDCS enables exercise to feel easier (by reducing RPE or pain) and therefore may influence the mechanisms related to the generation of fatigue related perceptions (Okano et al., 2013; Cogiamanian et al., 2007; Angius et al., 2018). The following sections aim to fully address the speculative mechanisms underlying the ergogenic effect of tDCS.

1.7.1 Fatigue

For many decades' researchers have noted the progressive decline in performance of intensely exercised muscles, a phenomenon now termed neuromuscular fatigue (from here termed fatigue). To date many definitions of fatigue have been proposed, however the standard definition provided by Gandevia et al (2001) states that fatigue is an exercise-induced reduction in maximal force. However, many have argued that this definition is incomplete, as it fails to elucidate the origins of performance decrement and fails to reflect the progressive but transient nature of fatigue, likening it to an event or breakpoint (Mauger., 2014; Boyas & Guével., 2011; Søgaard et al., 2006; Taylor & Gandevia., 2008). Instead, a more complete definition of fatigue has been proposed, defining fatigue as "an exercise-induced decline in the muscle's ability to exert force or power, regardless of whether or not the task can be maintained" (Bigland-Ritchie & Woods., 1984). It is important to recognise that the occurrence of a voluntary muscular contraction is underpinned by a series of complex events, leading from high brain areas via the spinal cord and motoneurons, to the muscle fibres and the occurrence a cross-bridge cycle to generate force (Gandevia., 2001; Allen et al., 2008). As such, fatigue is thought to occur at any point within these processes (Figure 4) and consequently, fatigue is often categorized into peripheral and central origins. As the primary mechanisms of

tDCS supposedly act upon mechanisms of central fatigue, a review of literature surrounding peripheral fatigue is outside of the scope of this literature review.

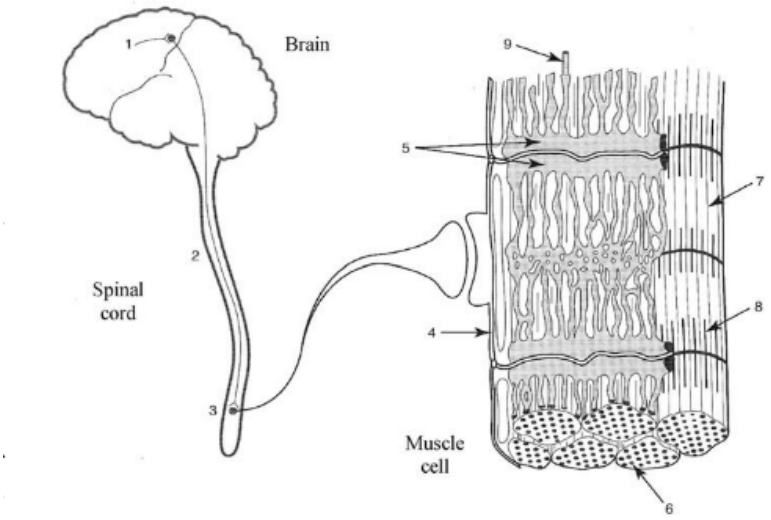

Figure 4 Schematic representation of sites contributing to fatigue. Fatigue may occur due to alterations in (1) activation of M1; (2) propagation of the motor command from the CNS to motoneurons (the pyramidal tract); (3) activation of motor units and muscle fibres; (4) neuromuscular propagation (this is inclusive of propagation at the neuromuscular junction); (5) excitation-contraction coupling; (6) the different availability of metabolic substrates; (7) state of the intracellular system; (8) contractile capacity; (9) blood flow to the muscle. From Boyas & Guével (2011).

Central Fatigue

Central fatigue is defined as the exercise-induced gradual attenuation in voluntary activation level (VAL) of the muscle, and therefore refers to the alteration of processes occurring proximally to the neuromuscular junction (Boyas & Guével., 2011; Ament & Verkerke., 2009; Taylor et al., 2000; Gandevia., 2001). Central fatigue is easily investigated through the use of the twitch interpolation technique administered during an MVC (Gandevia., 2001). This technique allows for the estimation of VAL through the comparison of a superimposed evoked contraction (peripheral nerve stimulation delivered within the MVC). The stimulation delivered utilises supramaximal intensities and therefore is thought to recruit the entire motoneuron pool (Gandevia., 2001). Central fatigue is demarcated by an increase in force elicited by the application of percutaneous nerve stimulation during an MVC (Gandevia., 2001). This increase in force evoked is thought to implicate the suboptimal recruitment of motor units (Taylor & Gandevia., 2008; McKenzie et al., 1992).

Through interspersing MVCs and the twitch interpolation method at regular intervals, researchers have noted the presence of central fatigue during maximal and submaximal contractions (Taylor & Gandevia., 2008; Søgaard et al., 2006; McKenzie et al., 1992). For which, central fatigue has been shown to account for up to 40% of the force lost when contractions were completed at low percentages (< 30%) of MVC (Taylor & Gandevia., 2008; Søgaard et al., 2006). Søgaard et al (2006) observed a profound reduction in voluntary activation following a 43-minute sustained contraction at 15% of the elbow flexors MVC force. The authors also reported that the voluntary EMG amplitude, MEP

amplitude and RPE progression rose throughout the submaximal contraction (Søgaard et al., 2006). This is thought to reflect the increase in M1 output and motor unit firing rate in order to maintain the required force throughout the contraction (Søgaard et al., 2006; Gandevia., 2001). The authors also reported a gradual increase in corticospinal silent period (CSP) duration throughout both the MVC's and the sustained submaximal contraction (Søgaard et al., 2006). The CSP, observed as a period of EMG silence following the elicitation of a MEP, is thought to represent the degree of which disynaptic inhibition at the spinal cord (occurring within the initial ~ 50 to 80 ms) and intracortical inhibition (the latter portion of the CSP) occur (Søgaard et al., 2006; Škarabot et al., 2019). Søgaard et al (2006) noted that the CSP elicited were over 100 ms and therefore can be attributed to an exercise-induced increase in intracortical inhibition.

During maximal contractions the majority of fatigue occurs due to the development of peripheral fatigue. However due to the recruitment of the entire motoneuron pool, central fatigue develops from the onset of maximal exercise as demarcated as an early decline in VAL (Taylor & Gandevia., 2008; Bilodeau., 2006; Gandevia., 1996; Søgaard., 2006). During a sustained 2-minute contraction, Gandevia et al (1996) observed the immediate decline in VAL accompanied by an increased amplitude of a superimposed twitch evoked by TMS applied to the M1. This signifies the reduced ability of the CNS to recruit all motor units and therefore the early induction of central fatigue. A later study has indicated that the severity of central fatigue developed within maximal contractions appears to be task dependent (Bilodeau., 2006). Bilodeau et al (2006) reported that in comparison to an intermittent MVC protocol, a sustained 3-minute MVC induced an earlier and greater reduction in VAL.

To date the mechanisms underlying the decline in VAL are poorly understood. However, it has been suggested that the slowing of the motoneuron firing rate occurs as a result of (1) increased inhibitory input; (2) a reduction in excitatory input and (3) a reduction in responsiveness of the motoneuron (Taylor & Gandevia., 2008). This indicates that fatigue occurs within any point of the CNS, resultantly researchers have sought to distinguish between spinal and supraspinal sources of central fatigue. The application of anodal tDCS is thought to modulate the supraspinal sources of fatigue (Angius et al., 2017; Angius et al., 2018; Wrightson et al., 2019). Indeed, following the application of anodal tDCS to the M1, VAL has been shown to increase (Frazer et al., 2016) as a result of improved M1 excitability (demonstrated through increases in MEP amplitude elicited via TMS; Hendy& Kidgell., 2013; Angius et al., 2018) and reduced inhibitory input (demonstrated via reductions in SICI and CSP duration; Hendy & Kidgell., 2013). Therefore, the application of anodal tDCS is thought to enhance performance through delaying the onset of supraspinal fatigue and providing a greater compensation for the decline in motoneuron responsiveness associated with spinal fatigue.

Spinal Mechanisms

The spinal mechanisms of fatigue refer to the inhibition of central motor drive induced by alterations of the excitability of the motoneuron pool. Stimulating the corticospinal tract has provided means of observing muscle responses (cervicomedullary motor evoked potentials (CMEP) and thoracic motor evoked potentials (TMEP)) which reflect alterations at a spinal level and therefore provide an indication of motoneuron excitability (Petersen et al., 2002; Kennedy et al., 2016). Indeed, both Gandevia et al (1999) and Butler et al

(2003) observed a decline in CMEP amplitude size during a 2 minute sustained MVC, indicating that the responsiveness of the motoneurons to synaptic inputs had decreased. The decline in responsiveness of motoneurons has also been demonstrated through the recording of individual motor units via fine-wire and tungsten EMG microelectrodes (Peters & Fuglevand., 1999). During a sustained MVC, Peters and Fuglevand (1999) observed that six out of the thirteen motor units tracked had stopped discharging prior to task failure. Consequently, it was suggested that repeated excitation of motoneurons may lead to the decrease in motoneuron responsiveness and therefore may require increased excitatory input (as demonstrated by increases in EMG) to maintain the firing frequency and furthermore the required force (Johnson et al., 2004; Taylor & Gandevia., 2008).

It is well recognised that the spinal projections of muscle afferents exert a diminishing effect on motoneuron excitability. Muscle spindles (type Ia and II muscle afferents) are predominantly responsible for the detection of changes in muscle length (Macefield et al., 1991; Hagbarth & Macefield., 1995). However, Hagbarth and Macefield (1995) hypothesized that the type Ia and II afferents may contribute to the development of central fatigue through the progressive withdrawal of fusimotor support provided by the type Ia and II muscle afferents inducing disfacilitation of the α-motoneuron pool. In support of this Macefield et al (1991) reported an inverse relationship between the activity of these afferents and central motor drive during a sustained contraction at 30% MVC, where the muscle spindle firing rate declined and the surface EMG increased. The authors concluded that the reduction in spindle firing rate induced the progressive disfacilitation of the α-motoneuron and therefore central motor drive was required to increase in order to sustain the contraction (Macefield et al., 1991; Hagbarth & Macefield., 1995).

Type III and IV muscle afferents are also reported to induce a reflex inhibitory response to the α-motoneuron pool (Gandevia et al., 1996; Kaufman et al., 2002; Kaufman., 2012). These small diameter muscle afferents are sensitive to the accumulation of metabolites (K^+, H^+, P_i) and mechanical changes associated with muscle fatigue (Gandevia et al., 1996; Kaufman et al., 2002). Indeed, a number of investigations have investigated the associated inhibitory reflex response via inducing post-exercise muscle ischemia, observing the prolonged decline in motoneuron firing rate, which returned to baseline values following cessation of occlusion (Garland., 1991; Bigland-Ritchie et al., 1986; Duchateau & Hainaut., 1993. In addition to their action upon the α-motoneuron pool, the type III and IV muscle afferents are also thought to diminish M1 excitability and the voluntary descending drive via top-down modulation (Gandevia., 1998; Amann et al., 2009; Amann & Dempsey., 2008; Sidhu et al., 2014; Sidhu et al., 2017). Indeed, compelling evidence from afferent blockade studies have reported that afferent feedback promotes the development of central fatigue (Gandevia., 1998; Amann & Dempsey., 2008; Amann et al., 2009; Sidhu et al., 2014; Sidhu et al., 2017). During exhaustive exercise, type III and IV afferents have been demonstrated to exert an inhibitory effect upon the M1 whilst inducing no significant effect upon the motoneurons, whilst the inverse was reported to be true during intense but non-fatiguing exercise (Sidhu et al., 2017).

Transcranial direct current stimulation influences VAL primarily through modulating supraspinal mechanisms of fatigue. Indeed, the observed change in VAL is thought to occur through enhancement of the M1 excitatory input whilst the inhibitory input is reduced (Frazer et al.,2016). Anodal tDCS is commonly applied to the M1, which has

shown to induce increases in MEP amplitude elicited by TMS (Nitsche & Paulus; Angius et al., 2018; Cogiamanian et al., 2007; Abdelmoula et al., 2016). This increase in MEP amplitude is purported to delay the onset of supraspinal fatigue (described in greater depth in Supraspinal Mechanisms) and compensates for the decline in motoneuron responsiveness through increasing the central neural drive (Williams et al., 2013; Angius et al., 2018; Abdelmoula et al., 2016). tDCS has also been applied to combat the inhibitory influence of the type III and IV muscle afferents, through targeting afferent projection sites including the IC and the DLPFC. Notably, Okano et al (2013) indirectly targeted the IC observing an improvement in PPO which was accompanied by reductions in submaximal HR and RPE. The IC is proposed to be a fundamental area for the establishment of the sentient self, and furthermore the generation of RPE, due to its role in the collation of afferent feedback from the periphery and central factors from the parietal and premotor cortices (Okano et al., 2013; Craig., 2009; Craig., 2010). The reduction in submaximal RPE following anodal tDCS could be reflective of centrally altered processing of afferent information (Okano et al., 2013), however no neuroimaging modalities have been employed to investigate this. Using similar tDCS parameters, Barwood et al (2016) examined the influence of anodal tDCS indirectly targeting the IC on TTE and TT cycling performance in hot conditions, reporting no significant differences between the anodal tDCS and sham conditions. The discrepancies between these two studies could be attributed to the different blinding procedures. Whilst the participants and experimenters in both studies were blinded to the stimulation order, the participants in Okano et al's (2013) study were aware of the sham condition and therefore may have behaved accordingly. Recent studies have highlighted that effective experimenter and participant blinding has been hard to achieve and therefore may serve as another source of variability (Fontenau et al., 2019; Horvath et al., 2015).

The DLPFC is another frequently targeted cortical area in the tDCS community. This area is proposed to collate afferent signals from the ACC and the orbitofrontal cortex in which are thought to be related to the emotional processing and motivation (Robertson & Marino., 2016; Holgado et al., 2019). Therefore, Holgado et al (2019) hypothesized that targeting this area may improve self-paced TT performance. As shown in table 2, the authors reported no significant effects on TT performance, in which was accompanied by no significant differences in EEG amplitude or session RPE (Holgado et al., 2019. Overall, this suggests, that tDCS is likely to be ineffective at modulating afferent signals.

Supraspinal Mechanisms

The supraspinal mechanisms of fatigue refer to the decline in central motor drive produced by a reduction in excitability of the M1 (Boyas & Guevel., 2011; Gandevia., 1998). The progressive decline in M1 excitability can be monitored through the use of TMS, measuring indices of corticospinal excitability including cortical VAL (cVAL), MEP amplitude and CSP length (Goodall et al., 2014). Although the twitch interpolation technique using peripheral nerve stimulation is traditionally used to measure central fatigue, it is limited in its inability to discern where in the CNS fatigue has occurred (Goodall et al., 2014). Instead, it is believed that estimating cVAL via the use of TMS to deliver a superimposed twitch can provide greater insight to the location of neural impairment (Dekerle et al., 2019; Goodall et al., 2014; Todd et al., 2003). Like the traditional twitch interpolation technique, TMS is applied during an MVC to find the

superimposed twitch (Goodall et al., 2014; Todd et al., 2003). However, due to the reduced corticospinal excitability at rest, the TMS stimuli applied will activate few motoneurons. Therefore, the resting twitch value is required to be estimated through the extrapolation of the negative linear relationship of voluntary force between 50%-100% MVC (Todd et al., 2003; Goodall et al., 2014, Figure 5).

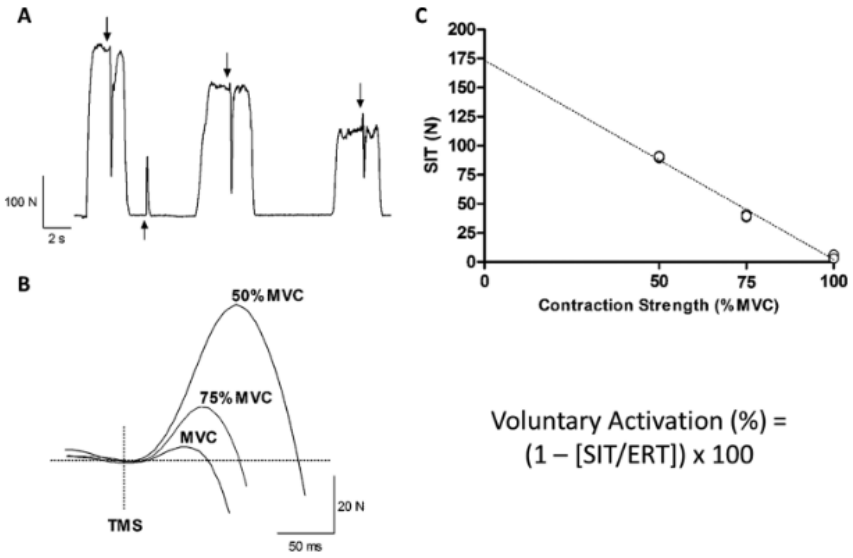

Figure 5 An illustration of a force trace demonstrating three levels of voluntary knee extension during a typical measurement of cortical voluntary activation (cVAL). The timing of TMS stimuli is denoted by downwards arrows, with the upwards arrow indicating the deliverance of electrical nerve stimulation applied to the femoral nerve. The negative linear relationship between the superimposed twitch force produced in the three voluntary contractions are extrapolated to estimate the resting twitch value to calculate cVAL (c). Taken from Goodall et al (2014).

The application of TMS during submaximal isometric contractions also provides insight into the integrity of the corticospinal tract (Badawy et al., 2012; Goodall et al., 2014). The amplitude of the MEP and the duration of the CSP elicited by TMS are thought to reflect the magnitude of corticospinal excitability and intracortical inhibition respectively (Badawy et al., 2012; Goodall et al., 2014; Gandevia., 2001). Whereas the proportion of the pool of motor units recruited is determined by the examination of the MEP area normalized to the maximal response of the M-wave elicited by peripheral electrical nerve stimulation (Goodall et al., 2014; Gandevia., 2001).

Supraspinal fatigue has been demonstrated following fatiguing dynamic and isometric exercise, as shown by a reduction in cVAL, MEP amplitude and an increase in CSP duration (Goodall et al., 2014; Ross et al., 2007; Weavil & Amann., 2018). Brasil-Neto et al (1993) were amongst the first to report a significant depression in MEP amplitude following the intermittent contractions of the wrist flexors to exhaustion. The authors attributed the reduction in MEP amplitude to changes in M1 cell excitability, as there were no significant changes in the Hoffman reflex (H-reflex) or M-wave (Brasil-Neto et al., 1993). Similar findings have also been reported following sustained isometric MVC of the dorsiflexors (McKay et al., 1995), anaerobic exercises including isometric dumbbell hold,

400 m sprinting and press-ups to exhaustion (Höllge et al., 1997) and running MIE tests (Verin et al., 2004). Later studies have also demonstrated that supraspinal fatigue contributes to fatigue following endurance events such as running a marathon (Ross et al., 2007) or stage cycling events such as the Tour de France (Ross et al., 2010). Ross et al (2007) reported that following the completion of a treadmill marathon, the most prominent source of fatigue originates within the CNS, finding a 67% and 14% reduction in MEP amplitude and cVAL respectively. The authors also noted a significant increase in silent period duration (Ross et al., 2007). As there were no changes in MEP response following peroneal nerve magnetic stimulation, the authors attributed fatigue of the dorsiflexors to be due to suboptimal M1 output (Ross et al., 2007). Sidhu et al (2009) also investigated the force generating capacity of the knee extensors following dynamic exercise. A significant reduction of cVAL was observed following the 8 x 5-minute cycling intervals, which was sustained for 45 minutes post cessation (Sidhu et al., 2009). Interestingly, the authors found that high intensity cycling intervals had no significant effect upon the M1 or motoneuron excitability as demonstrated by a lack of significant differences in the MEP amplitude or CSP duration. This indicates that fatigue may also occur upstream of the M1 (Goodall et al., 2014; Sidhu et al., 2009; Søgaard et al., 2006).

In their seminal papers, Nitsche and Paulus (2000; 2001) noted the significant augmentation of MEP amplitude following the application of anodal tDCS to the M1. Therefore, many have assumed that tDCS mitigates the development of supraspinal fatigue (Cogiamanian et al., 2007; Angius et al., 2016; Angius et al., 2017; Angius et al., 2018; Abdelmoula et al., 2016). To date, numerous studies have employed neuromuscular assessments to validate the performance effects induced by tDCS (Cogimanian et al, 2007; Kan et al., 2013; Williams et al., 2013; Angius et al., 2016; Angius et al., 2018; Lampropoulou & Nowicky., 2013; Hendy & Kidgell., 2014). Whilst a few studies have confirmed that performance improvements are associated with changes in MEP amplitude (Frazer et al., 2016; Hendy & Kidgell., 2014; Angius et al., 2018; Cogiamanian et al., 2007), several studies have also demonstrated that performance enhancements occur following tDCS in the absence of alterations in MEP amplitudes (Angius et al., 2016; Williams et al., 2013; Abdelmoula et al., 2016). Researchers have recently acknowledged that changes of MEP amplitude may not be the optimal measure to assess the validity of tDCS due to the inherent variability and dependence upon technical factors (Horvath et al., 2014; Rotenberg et al., 2014). MEP amplitudes are subject to natural variability; thus, alterations may occur irrespective of the presence of an intervention (Horvath et al., 2014; Rotenberg et al., 2014). Therefore, the original assumption is problematic due to the paucity of reliable evidence of tDCS altering other markers of corticospinal excitability (Wrightson et al., 2019; Machado et al., 2019).

Although much research has focused upon the involvement of the motor pathways in supraspinal fatigue, it is not the only purported mechanism. Indeed, researchers have advocated the role of impaired brain oxygenation, alterations in brain neurotransmitter concentrations and impaired brain glycogen content in the decline of VAL. Whole-body exercise has previously been shown to be impaired by hypoxic conditions (Goodall et al., 2010; Goodall et al., 2012; Amann et al., 2006; Amann et al., 2007). Whilst many studies have attributed the performance decrements to the increased type III and IV afferent firing rate elicited by the increased metabolic demands (Amann et al., 2006; Amann et al., 2007; Goodall et al., 2010), Goodall et al (2012) identified that performance decrements in

hypoxic conditions were attributed to reduced cerebral oxygenation (demonstrated via parallel reductions in cerebral O_2 delivery and cVAL). Decrements in cerebral oxygenation has also been shown to occur in normoxic conditions (Billaut et al., 2010; Bhambhani et al., 2007; Subudhi et al., 2009). During high-intensity exercise, deoxygenation is shown to occur in multiple brain areas including the prefrontal cortex, the premotor cortex and the M1 (Subudhi et al., 2009; Bhambhani et al., 2007). During a self-paced 5 km TT, Billaut et al (2010) highlighted that oxygenation of the prefrontal lobe remained constant from 2.5km to 4.5 km, but declined within the last 0.5 km. This reduction in oxygenation was shown to coincide with the end sprint and the increased recruitment of skeletal muscle (Billaut et al., 2010). Overall, this suggests that cerebral oxygenation contributes to supraspinal fatigue through inducing a reduction in neuronal activation (Goodall et al., 2012; Bhambhani et al., 2007). It is also apparent that deoxygenation of the prefrontal, premotor and motor cortices may contribute to the integrative decisions made within a pacing strategy (Billaut et al., 2010; Subudhi et al., 2009).

The application of tDCS may also enhance endurance performance through improving the cerebral O_2 delivery. Previous studies have demonstrated that tDCS is capable of altering the regional cerebral blood flow and cerebral oxygenation (Baudewig et al., 2001; Khan., 2013; Merzagora et al., 2010). In resting states, the application of anodal tDCS to the PFC has been shown to induces significant increases in oxyhaemoglobin within the PFC, whilst cathodal tDCS had a negligible effect (Merzagora et al., 2010). However, these effects have not yet been shown in exercise. Muthalib and colleagues (2013) were the first to explore the influence of conventional tDCS applied to the M1on PFC oxygenation throughout a TTE of the elbow flexors at 30% MVC, reporting no significant differences between anodal tDCS and the sham condition for TTE performance or PFC oxygenation. Analogous findings were also reported by Angius et al (2016) who reported no significant effects of M1 stimulation in a cephalic and extracephalic montage on PFC oxygenation, despite reporting a significant increase in TTE performance. The lack of effect of anodal tDCS upon cerebral oxygenation was suggested to be due to the exercise-induced cerebral response disabling the differences induced by tDCS. This may have been also due to the distance between the tDCS target locations and the NIRS monitoring sites (Angius et al., 2016; Muthalib et al., 2013). Interestingly, during a TTE trial of the elbow flexors, Radel et al (2017) observed a significant reduction in PFC oxyhaemoglobin content following the application of PFC HD-tDCS. This was suggested to reflect an increase in neuronal efficacy. However, the same study reported negligible changes in M1 activation following HD-tDCS applied to the M1, nor were there any improvements in performance associated with either PFC or M1 HD-tDCS. The authors hypothesized that the lack of change in M1 oxygenation observed may have been due reduced current dispersal induced by increased distances between the tDCS electrode and the cortex (Radel et al., 2017). However, this assumption negates the findings of previous computational modelling studies which have identified a smaller electrode-to-cortex distance over the M1, whilst curved regions such as the PFC have a greater electrode-to-cortex distance (Wagner et al., 2007). Further research using several neuroimaging modalities is required to fully elucidate the effects of tDCS on cerebral activation during both isometric and dynamic exercise (Machado et al., 2019).

Lastly, researchers have also proposed a role for the alterations in neurotransmitter concentration in the development of supraspinal fatigue. The serotonin hypothesis is

predicated on the supposition that prolonged exercise influences the synthesis and metabolism of central monoamines, specifically serotonin and dopamine (Nybo et al., 2003; Nybo & Secher., 2004; Meeusen & Roelands., 2018; Meeusen et al., 2006; Blomstrand et al., 1988). As serotonin is unable to cross the BBB, the cerebral neurons are required to synthesize it themselves (Meeusen et al., 2006). During exercise, the plasma concentration of the serotonin precursor molecule, tryptophan has been shown to increase (Blomstrand et al., 1988). This occurs as a result of the adenosine mobilisation of free fatty acids induces a greater displacement of tryptophan from its binding molecule albumin (Blomstrand et al., 1988). The observed rise in free tryptophan, alongside the marked reduction in plasma branched chain amino acids (BCAA) produces an increase in the tryptophan/BCAA ratio and therefore increases the transportation of tryptophan across the BBB (Blomstrand et al., 1988; Nybo et al., 2003; Nybo & Secher., 2004; Meeusen et al., 2006). The successive increase in serotonin concentration is thought induce augmentations in lethargy whilst reducing motivation and central motor drive (Meeusen et al., 2006).

As brain function is known to be reliant upon the interplay of multiple systems, it is unlikely that serotonin is singularly responsible for the development of supraspinal fatigue (Nybo et al., 2003; Nybo & Secher., 2004). Thus, it is now thought that the development of supraspinal fatigue is dependent upon the interaction of serotonin and dopamine (Nybo et al., 2003; Nybo & Secher., 2004). Accordingly, a high ratio of serotonin to dopamine is thought to initiate the development of fatigue through the augmentation of lethargy and reduced motivation (Nybo et al., 2003; Nybo & Secher., 2004; Meeusen et al., 2006; Meeusen & Roelands., 2018). Whereas a low serotonin to dopamine ratio favours performance enhancement through the maintenance of motivation and arousal (Meeusen et al., 2006; Meeusen & Roelands., 2018; Nybo & Secher., 2004). However, support for this hypothesis is equivocal. Increasing the serotonin activity through the selective serotonin reuptake inhibitor Citalopram, Roelands et al (2009) reported no significant effect on TT performance in comparison to a placebo condition in both normal and hyperthermic conditions. Moreover, the administration of L-Dopa had no significant effect on performance within a TTE trial (Meeusen et al., 1997). Roelands et al (2008) however observed a significant enhancement of time trial performance in the heat through the administration of the dopamine reuptake inhibitor Ritalin. However, no significant effects on performance were seen following the administration of Ritalin at normal temperatures (18 ^{o} c) (Roelands et al., 2008). Whilst this suggests that dopamine may have a role in the prevention of supraspinal fatigue in hyperthermic conditions, the role of serotonin in exercise should not be completely discounted. Nybo et al (2003) suggested that the plasma concentration of tryptophan and free fatty acids increase with exercise duration, and therefore exercise durations of under 2 hours, as used in these studies mentioned, may not be long enough to induce a net uptake of tryptophan.

The induction of E-LTP following the application of anodal tDCS is reliant upon the glutaminergic, GABAergic, dopaminergic, and serotonergic activity (Medeiros et al., 2012). Dopamine is thought to facilitate and stabilize the tDCS induced alterations in excitability via modulation of the cAMP mechanisms and furthermore, the induction of NMDAR-dependent LTP or LTD (Medeiros et al., 2012; Otmakhova & Lisman., 1996). Using positron electron tomography (PET), Fontenau et al (2018) observed a significant increase in the extracellular release of dopamine from the striatum, suggesting that

increased dopamine transmission may be associated with the underlying effects of tDCS. Further support for the role of dopamine in the tDCS induced after-effects comes from pharmacologically blocking the D2 receptors, which demonstrated a complete abolishment of after-effects following both anodal and cathodal tDCS (Nitsche et al., 2006). It could be argued that the application of tDCS may allow for the maintenance of the dopamine-serotonin ratio within exercise. Given that enhancing the effect of dopamine has only resulted in improved performance in the heat, it would be likely that this effect from tDCS would also be observable in the heat, which wasn't the case in the study by Barwood et al (2016). No studies to date have investigated the effect of tDCS upon prolonged exercise. As the alterations in corticospinal excitability induced following a singular application of tDCS are proposed to last for only a few hours, it is unlikely that tDCS would exert a modulatory effect upon the serotonergic system during the development of supraspinal fatigue.

1.7.2 Perception of effort

The perception of effort (also referred to as exertion) is often considered as the sensory manifestation of how hard, heavy, or strenuous a physical task is (Abbiss et al., 2015; Pageaux., 2016). The perception of effort is commonly used to monitor and prescribe exercise intensity through the use of psychophysical scales such as the Borg 6-20 scale (Borg., 1998), the category ratio (CR) 10 scale (Borg., 2007) and the CR100 scale (de Morree & Marcora., 2015). Using these scales, researchers have reported the exacerbation of RPE in the presence of physical (de Morree et al., 2012; Christian et al., 2014; Crewe et al., 2008) and mental fatigue (Pageaux et al., 2015), resulting in the supposition that the perception of effort limits endurance performance (Marcora & Staiano., 2010; Pageaux., 2016). Despite its popularity, the aetiology underlying this perception is hotly contested (Pageaux., 2016). So far, three models (Figure 6) have suggested that the RPE reflects the neural processing of (1) afferent feedback from the working skeletal and respiratory muscles (Pageaux., 2016); (2) the corollary discharge associated with central motor command (Marcora., 2009; Pageaux., 2016); or (3) the combination of afferent feedback and corollary discharge (Amann et al., 2010).

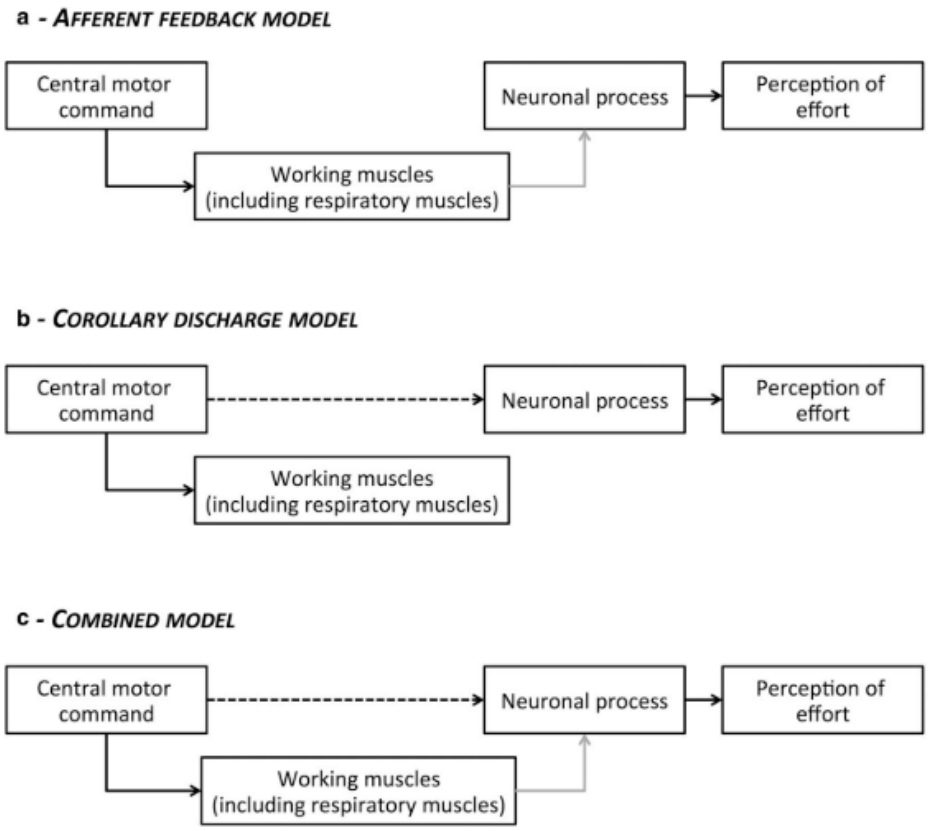

Figure 6 The Afferent feedback model (A). Corollary discharge model (B), and the combined model (C) of the perception of effort. The grey lines represent afferent feedback, and the dashed lines denote the corollary discharge associated with central motor command. Pageaux et al (2016).

According to the afferent feedback model of the perception of effort, during exercise the perception of effort is generated through the convergence of peripheral type III and IV afferent signals within the brain (Amann et al., 2011; Amann et al., 2010; St Clair Gibson et al., 2006). Therefore, it is assumed that as the intensity of exercise increases, the RPE will also rise due to the increased accumulation of metabolites (St Clair Gibson et al., 2006). Using intrathecal fentanyl injections to selectively block the transmission of type III and IV afferent signals, Amann et al (2010) observed a significant reduction in RPE in comparison to a placebo trial. However, other studies using the same intervention failed to report similar findings (Amann et al., 2011; Amann et al., 2008; Amann et al., 2009), nor has there been any evidence of improved performance following the administration of fentanyl (Gallagher et al., 2001; Amann et al., 2001; Amann et al., 2008; Amann et al., 2009).

The role of afferent feedback in development of the perception of effort has been strongly contested by Marcora (2009), who instead proposes that the perception of effort is centrally derived from the neural processing of corollary discharges associated with central motor commands (the activity within the motor and premotor areas in relation to voluntary muscle contractions, de Morree et al., 2012; Pageaux., 2016). Therefore, this model assumes that any increase in central motor drive will induce subsequent increases in the RPE. Indeed, de Morree et al (2012) observed a significant correlation between the RPE and movement-related cortical potentials (MRCP) measured via EEG during

dynamic elbow flexion tasks of fatigued and non-fatigued arms. However, it should be noted that the increase in MRCP in relation to RPE does not necessarily substantiate that afferent feedback has no role in the determination of endurance performance (Bishop et al., 2010). Additionally, the argument that corollary discharge is the sole determinant of RPE fails to explain how RPE rises in situations where PO and muscle EMG decline during repeated sprint exercises (Mendez-Villanueva et al., 2007; Bishop et al., 2010), or when the force and cVAL declines during MVC's (Sidhu et al., 2009).

Instead, the combined model (Figure 6) postulates that the perception of effort occurs as a result of the integration of both afferent feedback and corollary discharge (Amann et al., 2010; Christian et al., 2014; Bergstrom et al., 2015; Pageaux., 2016). However, despite several studies proposing the validity, there have been no studies to date that have specifically investigated this model (Pageaux., 2016).

In addition to its supposed influence on the development of supraspinal fatigue, modulation of corticospinal excitability via tDCS has also been suggested to influence sporting performance through reducing fatigue-related perceptions including RPE (Cogiamanian et al., 2007). Indeed, several investigations have shown improved performance accompanied with significant reductions in RPE within resistance training exercises (Lattari et al., 2016), isometric TTE (Angius et al., 2016; Oki et al., 2016), cycling TTE (Angius et al., 2018; Angius et al., 2019) and MIE trials (Okano et al., 2013) following the application of anodal tDCS to the DLPFC (Angius et al., 2019), M1 (Angius et al., 2016; Angius et al., 2018; Oki et al., 2016) and IC (Okano et al., 2013). In light of the corollary discharge model of the perception of effort, and the psychobiological model of endurance performance, Angius et al (2016) proposed that the performance enhancements and reduction in RPE following M1 anodal tDCS occurs due to a reduction in premotor cortex activity resulting from the tDCS induced facilitation of the descending motor drive. Angius et al (2019) also suggested that the application of anodal tDCS to the DLPFC enhances motivation and inhibitory control and therefore reduces the effort associated with the endurance cycling bout. On the other hand, Okano et al (2013) suggested that the reduction in submaximal RPE following anodal tDCS applied to the IC could be due to the altered processing of afferent signals. Overall, several studies have also demonstrated inconsistencies in the influence of tDCS upon RPE where enhancement of performance has been found in the absence of changes in RPE (Park et al., 2019; Huang et al., 2019; Codella et al., 2020; Vitor-Costa et al., 2015; Abdelmoula et al., 2016; Lattari et al., 2018; Williams et al., 2013; Holgado et al., 2019), it is therefore unlikely that the manipulation of RPE is the sole cause of performance enhancement following tDCS.

1.7.3 Exercise-induced pain

Intense and prolonged muscular contractions are known to induce acute pain which is proportional to the intensity and duration of the associated exercise (Cook et al., 1997). Therefore, it is not uncommon to hear axioms such as 'no pain no gain' or 'pain is temporary but quitting lasts forever' in competitive sports environments. Despite the occurrence of EIP being widely acknowledged by athletes, coaches, and commentators alike, EIP has received peculiarly little attention within sport and exercise science. Consequently, the exact aetiology of EIP is unclear. However, it is believed that EIP arises as a consequence of the sensitization and stimulation of the group III and IV nociceptive

afferents in response to the increased intramuscular pressure and accumulation of endogenous algesics (e.g., prostaglandin, bradykinin, substance P) within the contracting musculature (O'Connor & Cook., 1999; Angius et al., 2015; Almeida et al., 2004; Smith et al., 2020).

As EIP is closely bound to the intensity and duration of exercise, it has been advocated that EIP may play a role in the regulation of pace and the metabolic reserve (Mauger., 2014; Astokorki & Mauger., 2017a). In support of this notion, tolerance to EIP has been demonstrated to influence endurance performance, with superior performance occurring in those who have a greater tolerance to this sensation (Astokorki & Mauger., 2017a). The means by which EIP influences endurance performance is likely to involve both physiological and psychological mechanisms (Astokorki & Mauger., 2017a). Indeed, the firing of the nociceptive muscle afferents have previously demonstrated to induce the subjective sensation of pain and fatigue, impede central motor drive, and elicit a reduction in VA (Pollak et al., 2014; Graven-Nielsen et al., 2002; Khan et al., 2011; Kennedy et al., 2013). Therefore, it is suggested that the occurrence of EIP exacerbates fatigue through the afferent induced impedance of central motor drive and recruitment of motor units (Amann et al., 2011; Hureau et al., 2019). Pain is also known to induce a negative affective state, and therefore provides a powerful stimulus encouraging the disengagement from a behaviour or action (Astokorki & Mauger., 2017a; Astokorki & Mauger., 2017b). It is therefore feasible that EIP may influence the decision to reduce the intensity of exercise or disengage completely (Astokorki & Mauger., 2017a).

Thus far, several interventions that manipulate the sensation of pain peripherally have been employed to examine the pain-performance relationship. The hypertonic saline model is a well-established method of investigating the relationship between pain and motor function (Khan et al., 2011; Smith et al., 2020). The injection of hypertonic saline solutions is reported to activate the non-myelinated muscle afferents, inducing muscle pain described as aching or cramping (Khan et al., 2011). Through the use of this model to induce muscle algesia, previous studies have reported a reduction in pain pressure thresholds (Graven-Nielsen et al., 2003), torque during MVC's of the knee extensors (Graven-Nielsen et al., 2002) and VA of the elbow flexors (Khan et al., 2011). However, recently Smith et al (2020) demonstrated that the descriptive characteristics of pain naturally occurring within exercise when the hypertonic saline model was combined with low-intensity exercise. In this study the authors reported a significant reduction in TTE of the knee extensors when hypertonic saline was injected immediately prior to the commencement of 10% sustained contraction, when compared to injections of isotonic saline and a non-injection control trial (Smith et al., 2020). Furthermore, in the hypertonic saline trial, the rating of pain intensity was significantly elevated within the first 20% of the TTE trial, which continued to increase in a linear fashion until task failure (Smith et al.,2020). As there were no significant differences in the rating of fatigue or RPE, the authors concluded that the injection of hypertonic saline impeded performance through reaching the sensory limit sooner than the isotonic and control trials (Smith et al., 2020). Additionally, the increased firing of nociceptive muscle afferents induced by hypertonic saline, may have reduced central motor drive and VA of the knee extensors (Smith et al., 2020; Amann et al., 2011; Sidhu et al., 2018). Psychological interventions have also shown to impair endurance performance and increase the perceived pain intensity (Astokorki et al., 2020). Indeed, eliciting compassional hyperalgesia through showing

painful images has been shown to increase pain sensitivity to experimental pain measures and induce a negative affective state (Godinho et al., 2006; Astokorki et al., 2020). During exercise, Astokorki et al (2020) noted that compassional hyperalgesia increased the rating of EIP during a fixed intensity cycling trial and impaired TT performance in comparison to neutral and pleasant images. This confirms that the occurrence of EIP is a psychophysical phenomenon, which may alter the pacing strategy through manipulating the motivational state (Astokorki et al., 2020).

Inversely, lowering pain during exercise has shown to result in improved performance (Mauger et al., 2010; Foster et al., 2014; Astokorki & Mauger., 2017b; Motl et al., 2003). Mauger et al (2010) reported a significant improvement in 16.1 km cycling TT performance following the ingestion of acetaminophen. In this study, the participants were able to sustain a greater PO in comparison to the placebo condition, for a given rating of pain (Mauger et al., 2010). The ingestion of acetaminophen has also shown to improve repeated sprint performance (Foster et al., 2014). Surprisingly, the ingestion of aspirin (Ray & Carter., 2007; Hudson et al., 2008). and other non-steroidal anti-inflammatories (Howatson & Van Someren., 2008) have described opposing findings to the ones previously presented (Mauger et al., 2010; Foster et al., 2014). Consumption of the CNS stimulant caffeine has also shown to reduce the rating of EIP during a fixed intensity cycling trial (Motl et al., 2003). However, investigating the relationship between EIP and endurance performance through peripheral manipulations is challenging due to secondary mechanisms of action, and therefore changes in performance cannot be solely attributed to EIP (Angius et al., 2015). For example, whilst the primary property of acetaminophen is analgesia, it is also known to be an antipyretic agent and manipulates corticospinal excitability, therefore it could be argued that acetaminophen may influence performance through mechanisms other than pain (Foster et al., 2014; Mauger et al., 2014; Mauger & Hopker., 2013). Methods of increasing the rating of EIP and furthermore the firing of the nociceptive muscle afferents may also induce performance decrements through altering cardiorespiratory regulation (Angius et al., 2015; Amann et al., 2011; Kaufman., 2012). Thus, it may be beneficial to use interventions in which manipulate the central processing of pain, rather than modifying the strength of the nociceptive signal peripherally (Angius et al., 2015).

Transcranial direct current stimulation has previously been described as an efficacious adjunctive therapy for the treatment of chronic pain disorders which are characterised by maladaptive central sensitization (e.g., fibromyalgia, refractory pain elicited by traumatic spinal cord injury or stroke, and chronic migraine disorders) (Fregni et al., 2007; Valle et al., 2009; Yoon et al., 2014; Antal et al., 2010; Vaseghi et al., 2014; Vaseghi et al., 2015; Lefaucheur et al., 2008). Indeed, the daily application (5-10 days) of anodal tDCS either to the M1 or the DLPFC has been reported to induce a marked reduction in chronic pain (up to 58% reductions, in 63% of enrolled participants; Fregni et al., 2007) (Valle et al., 2009; Yoon et al., 2014; Lefaucheur et al., 2008; Vaseghi et al., 2014). The significant reduction in pain following anodal M1 tDCS has also been reported to last for 2 months following the end of stimulation in fibromyalgia patients (Valle et al., 2009). It is postulated that application of M1 anodal tDCS reduces somatosensory pain through upregulating the activity of the M1 and associated cortical and subcortical areas within the pain network (e.g., ACC, periaqueductal grey, and the thalamus), and therefore reduces the intracortical facilitation induced by the maladaptive plastic alterations associated with

central sensitization (Antal et al., 2010; Yoon et al., 2014; Meeker et al., 2019; Fregni et al., 2007; Lefaucheur et al., 2008).

The DLPFC is implicated as a primary structure for nociceptive control, modulation of emotional valences, executive functions, and the control of attention and working memory functions (Lorenz et al., 2002; Lorenz et al., 2003; Graff-Guerrero et al., 2005; Fierro et al., 2010; Brighina et al., 2004). It is therefore suggested that tDCS applied to the DLPFC modulates the affective-emotional networks in which regulates the unpleasantness associated with pain (Valle et al., 2009; Boggio et al., 2008). However, this brain site has also been shown to influence the activity of the M1 in the presence of pain (Fierro et al., 2010; Farina et al., 2001). When tonic pain was induced by capsaicin application, excitability of the M1 has been shown to be notably reduced (Farina et al., 2001), this however was reversed when rTMS was applied to the DLPFC (Fierro et al., 2010). Therefore, stimulation of the DLPFC may also mediate pain through top-down modulation of M1 activity (Lorenz et al., 2003).

In healthy individuals, tDCS is also purported to improve the pain threshold and tolerance to acute pain (Boggio et al., 2008; Boggio et al., 2009; Mylius et al., 2012; Angius et al., 2015; Zandieh et al., 2013; Vaseghi et al., 2014; Flood et al., 2017; Lefaucheur et al., 2008; Mariano et al., 2016). However, like much of the tDCS literature, the efficacy of tDCS to exert an analgesia is dependent upon intra-individual differences, the type of pain experienced, the timing of stimulation, polarity, intensity, and the montage of electrodes (Borckardt et al., 2012; Mordillo-Mateos et al., 2017; Brasil-Neto et al., 2020). The majority of current tDCS research has employed classical experimental pain measures to investigate the supposed analgesic effect, including tests such as the CPT, PES, and quantitative sensory testing (QST). Indeed, the online (during task) application of anodal tDCS to the M1 in a cephalic montage (anodal M1, cathodal contralateral forehead) has proven to be the most efficacious tDCS parameters for the enhancement of pain perception and pain thresholds to PES and cold pain elicited within the CPT and QST (Angius et al., 2015; Mordillo-Mateos et al., 2017; Boggio et al., 2008; Zandieh et al., 2013; Borckardt et al., 2012; Lefaucheur et al., 2008). It is purported that tDCS applied to the M1 indirectly reduces the somatosensory component through the modulation of the downstream inhibitory M1-thalamic projections (Angius et al., 2015; Zandieh et al., 2013; Boggio et al., 2008). In support of this concept, neuroimaging studies have identified wide-spread activations of many cortical and subcortical areas following M1 tDCS, including the thalamic nuclei (Lang et al., 2005; García-Larrea et al., 1999). Anodal tDCS applied to the DLPFC has also shown to improve the pain thresholds to experimental pain measures (Boggio et al., 2008; Mariano et al., 2016; Mylius et al., 2012). Boggio et al (2008) demonstrated that the application of anodal tDCS in a cephalic montage (anodal left DLPFC, cathodal contralateral forehead) enhanced pain threshold to PES, but had no significant influence upon the pain perception thresholds. Furthermore, Mylius et al (2012) and Mariano et al (2016) observed an analgesic effect of anodal DLPFC stimulation in response to heat and cold pain. The DLPFC is proposed to alleviate pain through distinct mechanisms to the M1, where upregulation of the DLPFC via tDCS is proposed to alleviate pain through modulation of the affective emotional networks (Boggio et al., 2008; Mylius et al., 2012; Mariano et al., 2016; Angius et al., 2015). Supporting this notion, the application of anodal tDCS has been reliably shown to increase pain empathy and reduce

self-unpleasantness when viewing painful or aversive images (Boggio et al., 2009; Maeoka et al., 2012; Wang et al., 2014).

To date, no studies have attempted to map the particular brain areas involved in the processing of EIP (Angius et al., 2015). However, O'Connor and Cook (1999) theorised that the primary and secondary somatosensory cortices, the ACC, IC, and the thalamus were likely to be involved. Furthermore, neuroimaging studies observed activations of the thalamus and the basal ganglia during muscle pain (Wardman et al., 2014; Peyron et al., 2000), therefore demonstrating an overlap in the central processing of experimental and muscle pain (Wardman et al., 2014; Angius et al., 2015). As such, several research groups to date have attempted to investigate the effects of tDCS on EIP and endurance performance through stimulating the M1 (Angius et al., 2015; Angius et al., 2016; Angius et al., 2018; Flood et al., 2017). Using conventional tDCS, Angius et al (2015) applied anodal tDCS to the M1 in a cephalic montage (cathodal electrode placed over the right DLPFC). In this study, the authors reported that tDCS was efficacious at inducing an analgesic effect during the CPT, but no significant difference in endurance performance or the rating of EIP during the TTE cycling trial were discovered. Similarly, Flood et al (2017) reported an enhancement in the pain response during a conditioned pain modulation protocol following the application of HD-tDCS to the M1 but reported no significant differences in MVC force or isometric TTE. Despite reporting a positive effect of anodal tDCS on TTE performance and RPE, stimulating the M1 in an extracephalic montage and a bilateral extracephalic montage did not elicit any changes in the pain perception (Angius et al., 2016; Angius et al., 2018). Although the application of anodal tDCS to the M1 has been proven to be efficacious in the amelioration of acute experimentally induced pain, these measures are known to also induce perceptions of cutaneous pain and therefore are qualitatively different to EIP (Smith et al., 2020). Experimental pain measures such as PES and CPT are known to induce sensations of "stabbing" or "aching" whilst EIP or muscle pain are often described as "aching" "cramping" or "burning" (Smith et al., 2002; Olesen et al., 2012). It is therefore likely that EIP is processed within different brain regions than experimental pain measures, and therefore tDCS applied to the M1 is unable to confer an analgesic effect to EIP.

It is widely acknowledged that EIP elicits a strong emotional response, which likely contributes to the decision to reduce the intensity or cease exercising (Angius et al., 2015; Astokorki & Mauger., 2017a). Therefore, altering the central processing of EIP within the responsible brain regions may confer an analgesic effect. As alluded to previously, PET scanning has previously identified that the activity of the left DLPFC is negatively correlated with pain and is therefore thought to interact with the ACC, thalamus, midbrain, amygdala, striatal and limbic structures manipulating the behavioural dominance of pain dependent upon motivational and emotional context (Lorenz et al., 2003). Therefore, the DLPFC may be a suitable target region to apply tDCS to in the aim of alleviating EIP (Angius et al., 2015). Thus far, five studies to date have examined the influence of anodal tDCS stimulation applied to the DLPFC on exercise performance (Lattari et al., 2018; Lattari et al., 2016; Holgado et al., 2019; Angius et al., 2019; Andre et al., 2019). Three of which reported a significant improvement in cycling TTE (Lattari et al., 2018; Angius et al., 2019) or resistance training exercise (Lattari et al., 2016). Whilst Andre (2019) and Holgado (2019) both demonstrated no significant influence of DLPFC tDCS on TT performance. In addition to the improvement in resistance exercise performance, anodal

DLPFC stimulation significantly reduced the rating of RPE which integrated pain into its definition, and therefore agrees that DLPFC stimulation may be an appropriate site to alleviate EIP (Lattari et al., 2016). To date, only Angius et al (2019) has specifically measured the intensity of EIP within their protocol. Indeed, through applying anodal tDCS to the left DLPFC in a cephalic montage (cathodal contralateral FP2), the authors reported that tDCS significantly enhanced cycling TTE performance, RPE and inhibitory control, but no significant effect of tDCS on the rating of EIP. Within this study, the Stroop test to measure inhibitory control was administered immediately before and after the application of tDCS. It therefore plausible that the baseline measure of inhibitory control led to task specific modulation or input-selectivity bias towards improving inhibitory control rather than pain. Further research is warranted to elucidate whether the application of anodal tDCS to the DLPFC is an effective means of ameliorating EIP and furthermore, improving endurance performance.

1.8 Summary and aims of the thesis

In summary, tDCS is a NIBS technique known to induce long lasting but reversible alterations in the excitability of a targeted brain region. Through applying a low intensity, direct current via electrodes on the scalp, tDCS is proposed to influence the resting membrane potential and induce E-LTP or E-LTD (Nitsche & Paulus., 2000; Nitsche & Paulus., 2001; Liebetanz et al., 2002; Pelletier & Cicchetti., 2015). Since the turn of the century, tDCS has had extensive use as a tool to investigate various cognitive functions and as an adjunctive therapy for multiple neurological disorders (DaSilva et al., 2012; Valle et al., 2009; Boggio et al., 2007; Benninger et al., 2010; Marshall et al., 2004; Loftus et al., 2015). With the agreement that the brain is involved in the regulation of endurance performance, tDCS has also been ascribed as an ergogenic aid (Cogiamanian et al., 2007; Angius et al., 2016; Angius et al. 2017; Angius et al., 2019; Williams et al., 2013). To date, much of the tDCS literature has employed isometric and dynamic TTE trials in a multifaceted approach to evaluate the efficacy of the device to enhance endurance capacity and to gain a mechanistic understanding of how tDCS induces the supposed ergogenic effect. It is postulated that increased excitability of a brain region of interest delays the onset of supraspinal fatigue and/or lowers the exercise generated perceptions including EIP and RPE (Angius et al., 2018; Angius et al., 2016; Cogiamanian et al., 2007). However, due to contrasting results, the exact mechanisms underlying this supposed ergogenic effect remain uncertain (Wrightson et al., 2019; Vitor-Costa et al., 2015; Angius et al., 2015).

Thus far, the small number of studies which have implemented TT's to simulate endurance performance have failed to corroborate the supposed ergogenic effect (Holgado et al., 2019; Andre et al., 2019; Barwood et al., 2016; Valenzuela et al., 2019). Currently it is uncertain whether these findings are because of the varied tDCS parameters selected (i.e., montage of electrodes, current intensity, duration of stimulation, blinding procedure) or whether tDCS is insufficient to influence endurance performance. These studies may also be subject to the intricacies associated with the non-linearity of the tDCS dose response and the profuse differences in anatomy, genetics, baseline neurochemical concentrations or sensitivity to brain stimulation (Agboda et al., 2020; Monte-Silva et al., 2010; Monte-Silva et al., 2013; Li et al., 2015). Regardless of the disparities in findings, there are now numerous tDCS devices on the commercial market

promoted to enhance athlete performance in competition and training, many of which are endorsed by elite endurance athletes. However, the efficacy of these devices to alter the corticospinal response or enhance the response to training have not yet been explored. Therefore, it is argued that further understanding is required to elucidate whether tDCS can confer an ergogenic effect on endurance performance.

1.8.1 Aims of thesis

The overall aim of this thesis was to assess the efficacy of anodal tDCS to enhance endurance exercise performance. Accordingly, the following chapters present a series of studies which contribute to the overall aim of this thesis. The aims and hypotheses of the four experimental chapters are as follows:

1. Previous research has predominantly focused on the effects of tDCS applied to the M1 on endurance performance and the rating of EIP. However, recent research has suggested that the DLPFC may provide a more suitable target to ameliorate EIP.
    - Aim: To investigate whether the application of anodal tDCS to the DLPFC would reduce the rating of EIP within a fixed intensity cycling trial and improve performance in a cycling time trial.
    - H1 $_0$: The rating of EIP within the fixed-intensity trial will not be significantly different between the experimental conditions.
    - H1 $_1$: The rating of EIP within the fixed-intensity trial will be significantly different between the experimental conditions.
    - $H2_0$: Time trial performance will not be significantly different between the experimental conditions.
    - $H2_1$: Time trial performance will be significantly different between the experimental conditions.

2. Traditional bilateral montages are thought to cause unexpected diminutions in excitability resulting from the placement of the cathodal electrode. Extracephalic montages are reported to mitigate this issue, therefore this study explored the effect of this montage on the rating of EIP and TT performance
    - Aim: To investigate whether tDCS applied in an extracephalic montage would decrease the rating of EIP within a fixed intensity cycling trial and improve performance within a cycling TT.
    - $H3_{0:}$ The rating of EIP within the fixed-intensity trial will not be significantly different between the experimental conditions.
    - $H3_{1:}$ The rating of EIP within the fixed-intensity trial will be significantly different between the experimental conditions.
    - $H4_0$: Time trial performance will not be significantly different between the experimental conditions.
    - $H4_1$: Time trial performance will be significantly different between the experimental conditions.

3. Recently tDCS has been suggested as method of deriving the benefit of overload within training, yet no research has investigated whether tDCS can enhance the physiological adaptations to endurance training.

- Aim: To determine whether tDCS applied throughout a 6 week HIIT intervention can increase the physiological adaptation to a greater magnitude than a no-stimulation sham group.
- To investigate whether the chronic application of tDCS can be detected through changes in serum, platelet-poor plasma, and saliva BDNF samples.
- $H5_0$: The physiological adaptations will not be significantly different between the tDCS and sham group.
- $H5_1$: The physiological adaptation will be significantly different between the tDCS and sham group.
- $H6_0$: The chronic use of tDCS will not change peripheral BDNF samples.
- $H6_1$: The chronic use of tDCS will change peripheral BDNF samples.

4. Although the Halo Sport Neurostimulation System has been shown to improve sprint and TTE performance, no studies have investigated whether this device can alter the corticospinal excitability.
    - Aim: To evaluate whether the Halo Sport Neurostimulation System can alter the corticospinal excitability.
    - To determine whether the 'online' application of tDCS will confer greater changes in corticospinal excitability.
    - $H7_0$: The Halo Sport Neurostimulation System will have no significant effect on corticospinal excitability.
    - $H7_1$: The Halo Sport Neurostimulation System will have a significant effect on corticospinal excitability.
    - $H8_0$: The corticospinal excitability will not be significantly different between 'online' and 'offline' applications of tDCS.
    - $H8_1$: The corticospinal excitability will be significantly different between 'online' and 'offline' applications of tDCS.

1.8.2 Thesis structure

The experimental chapters contained within this thesis are not presented in the order that they were conducted. Study two (chapter 3) was the first to be explored. However, as study one (chapter 2) used a conventional electrode montage (vs. the non-traditional extracephalic montage used in study two) it was placed ahead of study two in the thesis. Part A of study four (chapter 5) was conducted simultaneously with study three. Part B of study 4 was the last to be conducted. As the first two studies explored the effect of tDCS applied 'offline' during quiet rest and study 3 used the 'online' application during a sport-specific warm up, Part B was designed to explore whether the timing of stimulation impacted corticospinal excitability.

Chapter 2. Effect of transcranial direct current stimulation of the Dorsolateral Prefrontal Cortex on the modulation of exercise-induced pain and cycling performance

2.1 Abstract

Introduction: Non-invasive brain stimulation techniques such as tDCS have been purported to enhance endurance performance through reducing fatigue-related perceptions, such as EIP. Therefore, this study aimed to investigate whether tDCS applied to the DLPFC would induce analgesia to a fixed intensity cycling trial (FI) and furthermore enhance time trial (TT) performance. **Methods**: Eleven participants completed a 10 min FI at 75% of their peak power output and a 15 min TT following the administration of either DLPFC tDCS, sham stimulation or control. tDCS was delivered for 10 minutes at 2mA in a bilateral montage. Pain and heart rate (HR) were recorded in both FI and TT, with power output (PO) monitored throughout the TT. **Results**: There were no significant differences in the rating of pain (tDCS: 4.3 ± 2.0, sham: 4.0 ± 1.8, control: 3.8 ± 1.4, $P \geq 0.098$) during the FI trial. Furthermore, there were no significant differences in the distance covered (tDCS: 8.00 ± 0.56 km, sham: 7.98 ± 0.57 km, control: 7.93 ± 0.59 km, $P = 0.478$), the rating of pain ($P = 0.332$), HR ($P = 0.575$) or PO ($P = 0.419$) during the TT. **Conclusion**: These results demonstrate that tDCS delivered in a bilateral montage was insufficient to induce analgesia to EIP and provided no ergogenic effect for endurance performance. This may be due to the montage selected, where activity of the right DLPFC is supressed by the cathodal electrode.

2.2 Introduction

In the last decade, neuromodulatory techniques such as tDCS have become accepted tools in the treatment of numerous neuropsychiatric disorders (Brunoni et al., 2013). In healthy populations, tDCS is also used as an investigative tool for cognitive functions (Loftus et al., 2015) and to enhance physical performance (Machado et al., 2019). Briefly, tDCS involves the passage of a weak electrical direct current to a targeted brain region through anodal and cathodal electrodes placed on the scalp. Originally it was presumed that tDCS induces polar dependent shifts in the neuronal resting membrane potential (Nitsche & Paulus., 2000; Stagg & Nitsche., 2011). However, the response to tDCS is more complex than this, due to the intricate relationship between the non-linearity of the dose response and multifarious inter-and intra-individual differences (Agboda et al., 2020; Salehinejad & Ghanavati., 2020; Batsikadze et al., 2013; Wiethoff et al., 2014). Therefore, changes in neuronal excitability are thought to produce an ergogenic effect through enhancing the synergist muscle coupling, and attenuation of the decline in M1 excitability (Cogiamanian et al., 2007). However, due to inconsistencies within the research designs and reported outcomes, the exact mechanisms remain uncertain (Machado et al., 2019).

Intense and prolonged muscular contractions are known to induce acute pain which is proportional to the intensity of the associated exercise (Cook et al., 1997). It's thought that this EIP arises as a consequence of the accumulation of noxious metabolites (e.g., prostaglandin, bradykinin) combined with increased intramuscular pressure which sensitize or stimulate the type III and IV peripheral nociceptors for the interpretation of pain (Almeida et al., 2004; O'Connor & Cook., 1999; Angius et al., 2015). Tolerance to EIP has previously been shown to influence TT performance (Astokorki & Mauger., 2017a), with improvements in performance shown when pain is reduced with acetaminophen (Astokorki & Mauger., 2017a; Foster et al., 2014). As tDCS applied to the M1 and the DLPFC has been successfully used to treat acute and chronic pain in clinical groups (Antal et al., 2010; Valle et al., 2009), it is reasonable to assume that tDCS induced analgesia could confer and analgesic effect to EIP in healthy individuals. Transcranial direct current stimulation has already been demonstrated to enhance tolerance to endurance performance through the reduction in RPE (Cogiamanian et al., 2007; Lampropolou & Nowicky., 2013; Angius et al., 2016; Angius et al., 2018). However, it has not yet been demonstrated to be effective to reduce EIP. Indeed, analgesia during a cold pressor test (Angius et al., 2015) and enhanced conditioned pain modulation (Flood et al., 2017) has been observed following conventional (Angius et al., 2015) and HD-tDCS (Flood et al., 2017) applied to the M1, despite having no effect on EIP (Angius et al., 2015; Flood et al., 2017). However, EIP and experimental pain are different (Smith et al., 2020), therefore the M1 may be more effective at modulating pain of a type III origin (Fierro et al., 2010). Exercise-induced pain is commonly described as an aching or burning sensation (akin to type IV afferent stimulation), so the M1 may be less effective at reducing this type of pain (Angius et al., 2015). Stimulating the DLPFC has previously been shown to induce analgesia (Boggio et al., 2008), and has been used to treat chronic pain disorders (Seminowicz & Moayedi., 2017), increase pain tolerance to experimental pain measures (Boggio et al., 2008; Mylius et al., 2012) and increase pain empathy to aversive images (Boggio et al., 2009). The DLPFC is a key structure for nociceptive control, modulation of attention, emotional valences and working memory (Fierro et al., 2010, Lorenz et al. 2002; Lorenz et al., 2003; Graff-Guerrero et al., 2005) and contains

reciprocal connections to the M1 (Fierro et al., 2010). Transcranial direct current stimulation applied to the left DLPFC has been shown to enhance resistance exercises with reductions in RPE which integrated pain into the definition (Lattari et al., 2016). Therefore, anodal tDCS applied to the DLPFC may serve as an effective means to reduce the sensation of EIP and thus improve endurance performance.

The present study aimed to investigate whether tDCS can reduce the perception of EIP during a fixed intensity cycling trial. This study also investigated whether the analgesic effect would improve cycling time trial performance.

2.3 Methods

Participants

Eleven healthy volunteers (7 males, 4 females, age: 26 ± 6 yr, height: 1.77 ±0.09 m, body mass: 72 ± 13 kg; Peak Power output (PPO): 273 ± 49 W) were recruited to take part in this study. A sample size of eleven participants was chosen based upon the sample sizes of similar studies. All participants were active, completing a minimum of 180 minutes of aerobic exercise per week. Based upon these descriptors, the participants meet the criteria of 'untrained' described by De Pauw et al (2013). The exclusion criteria included any reports of mental health (i.e., depression or schizophrenia) or brain disorders (i.e., epilepsy, brain lesions), implants from surgery or were taking any medication at the time of the study. Prior to providing written informed consent, participants were provided with an overview of experimental procedures, but not the aims or hypothesis to limit subject-expectancy bias. Ethical approval was obtained from the School of Sport and Exercise Science Research Ethics Advisory Group (SSES REAG) (approval number: Prop_92_2015_2016)

Experimental Protocol

Participants visited the laboratory on four occasions, one preliminary and three experimental visits, separated by a minimum of 48 hours. In the three experimental visits (visits 2-4) participants were assigned in a single-blind randomised order to a tDCS, sham and control condition (for more detail see transcranial direct current stimulation procedures). Trials were randomised using randomised permutations generated by randomisation.com. Visits were conducted at a similar time of day in a temperature-controlled room (20 ^{o}C, relative humidity between 40 and 50%). Participants were asked to abstain from consumption of caffeine, and analgesic substances for a minimum of 6 hours preceding each visit and avoid completing strenuous physical activity and consuming alcohol for 24 hours before each visit.

Visit 1 familiarised participants with the laboratory equipment and experimental procedures. This was preceded by the completion of a maximal incremental (2 minutes at 100 W with 30 W increases every 2 minutes) test to exhaustion (operationally defined as a pedal frequency less than 60 revolutions/ minute (RPM) for more than 5 seconds despite strong verbal encouragement) on an electromagnetically braked cycle ergometer (SRM ergometer, Welldorf, Germany). This ergometer is reported to have a workload accuracy of 0.5%. Prior to completing the incremental test and familiarisation procedures, the ergometers were adjusted to fit the participant. All measurements were recorded and

repeated across subsequent visits. During the MIE, a fan was positioned at the front right of the ergometer, approximately 2m away from the participant. The fan was switched on upon request and airflow was determined by the participant.

In visits 2 to 4, following the completion of 10 minutes tDCS, sham or quiet rest (control), pain threshold and tolerance were evaluated through peripheral electrical stimulation (PES) and ischemic pain test (see *Evaluations of pain threshold and tolerance*). After this and following a 5-minute self-paced cycling warm up, participants completed a 10-minute constant load cycling trial (see *Fixed intensity cycling*), followed by a 15-minute time trial separated by 10 minutes of rest (see *Time Trial*). Mood was assessed through the administration of questionnaires before tDCS application and at the end of each visit (See Brunel Mood Scale).

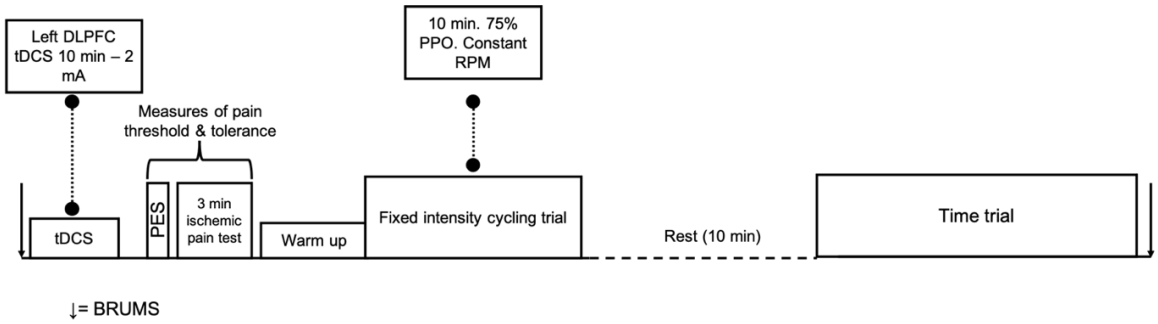

Figure 7 Schematic of study protocol.

Transcranial Direct Current Stimulation Procedures

Transcranial direct current stimulation was administered using a battery-driven stimulator (TCT research limited, Hong Kong) through a pair of rubber electrodes (size: 5cm x 7cm, 35cm^2) encased in saline-soaked sponges (9% NaCl). This tDCS has a precision of ± 0.004 mA. The anodal and cathodal electrodes were placed over the F3 and F4 to stimulate the left and right DLPFC respectively. These were secured in place by elastic straps. A bilateral montage of electrodes was adopted based upon previous findings of enhanced current density within the targeted region (Neuling et al., 2012).

In the experimental condition, participants received 2 mA tDCS (current density 0.057 mA/cm^2) for a duration of 10 minutes (Lang et al., 2005; Angius et al., 2015). Whereas in the sham condition, stimulation lasted for 30 s and subsequently ramping down to nothing. In both conditions, the current intensity was ramped up and down over a 10s period. The electrical resistance was continuously monitored on the stimulators display and maintained between 4- 6 kΩ. In the control condition participants rested quietly for 10 minutes.

Evaluations of Pain Threshold and Pain Tolerance

Pain threshold and tolerance to experimental pain were measured as a manipulation check to ascertain whether changes in performance were due an analgesic effect. Pain thresholds were evaluated through the application of PES (Pulse duration 100 μs) to the right index finger using an electrical stimulator (Digitimer DS7AH, Whelwyn Garden City, UK). An Ag/Cl electrode coated (2 x 2 cm; Nessler, Medizintechnik, Innsbruck, Austria) in

conductive gel acting as the cathode was applied to the fingertip of the right index finger, while the anode, a carbon rubber electrode (100 mm x 50 mm; Phoenix Healthcare Products ltd, Nottingham, UK) was applied to the back of the hand proximal to the knuckles. Intensity of the current started at 0.0 mA, increasing in steps of 0.1 mA until the participant reported sensation (perception threshold) and then pain (pain threshold) (Boggio et al., 2008).

Pain tolerance was assessed through the administration of an ischemic pain test (Scott & Gijsbers., 1981). The participant's right arm was flexed to 90^{o}, with the hand elevated and elbow supported by a table. A manual sphygmomanometer (Accoson, Dekamet, UK) was fitted to the upper arm, where the cuff was inflated and held at the pressure corresponding to 100 mmHg above the resting systolic pressure, as measured prior to the trial by a digital sphygmomanometer (Carescape- V100, Dinamap, GE technology, USA). Participants contracted their hand at a rate of one contraction per second to induce ischemia, a metronome set at 60 bpm was used to establish a regular rhythm. Pain intensity was verbally rated every 15 seconds on a 10-point scale (Cook et al., 1997). Pain tolerance was defined as the maximum number of times the participants were willing to contract their hand under the ischemic conditions. If the participants had not already disengaged from the trial, the experimenter ended the trial at 3 minutes to avoid complications such as tourniquet pain, participants were however blinded to this cut off point. In total, this cut-off point was reached by 6 participants.

Fixed Intensity Cycling Trial

Participants completed a fixed intensity cycling trial to measure changes in tolerance to EIP following tDCS. For this, participants were instructed to cycle at 75% of their PPO for 10 minutes on a cycle ergometer (Lode Excalibur sport, Lode, Groningen, Netherlands). The intensity of 75% PPO was selected as an intensity great enough to elicit a sensation of strong pain (5 out of 10 on Cook's pain intensity scale; Cook et al., 1997). The ergometer used for this study has a reported workload accuracy of 2 W in loads less than 100 W, from 100 to 1500 W of 2% and over 1500 W of 5%. Earnest et al (2005) reported that this ergometer had a coefficient of variation (CV) of 6.3%. During each visit, participants maintained a constant cadence which was established during the familiarisation trial. Pain intensity (1-10, Cook et al., 1997) and RPE (6-20 scale, Borg., 1982) were recorded at the end of every minute. Heart rate was also recorded at the end of every minute through a telemetric device (Polar FT1, Polar Electro Oy, Kempele, Finland). A fan positioned to the side of the participants was provided on all trials and turned on upon request.

Time Trial

As the premise of this study was to investigate the influence of tDCS on the rating of EIP, a time-based time trial was adopted. Previous research has identified that time-based protocols induce a greater sense of effort or discomfort as a change in intensity does not change the overall duration of exercise, unlike distance-based protocols (Abbiss et al., 2016). Therefore, on a cycle ergometer (SRM ergometer, Welldorf, Germany) participants cycled as far as they could for a period of 15 minutes. This ergometer is reported to have a measurement error of 0.5% and a CV of 2.2% in untrained populations (Hopker et al., 2010). Participant's regulated intensity through RPM alone, keeping the same gear

selected from the familiarisation trial. Participants were provided with feedback on time elapsed throughout the trial, but were blinded to PO, distance or work completed. At the end of every minute participants were asked to provide their perception of pain and RPE, whilst HR and PO were recorded continuously. Thus far no studies have examined the test-retest reliability of a 15-minute TT completed on the SRM ergometer. However, 20-minute TTs completed by trained cyclists on a Velotron ergometer have been shown to have a CV of 4.6%, an intraclass correlation of 0.99 and typical error of the mean of 4.6 W (MacInnis et al., 2018). Due to the use of untrained participants, the reliability of the TT's in the present study are likely to be lower. Additionally, whilst the Velotron and SRM ergometers have been reported to measure similar PO during constant load cycling trials, the Velotron has been shown to report higher PO during a self-paced time trial (Abbiss et al., 2008) and therefore the reliability between the two ergometers cannot be directly compared.

Brunel Mood Scale

Mood was assessed at the start and end of each visit through the Brunel Mood Scale. This questionnaire is based upon the profile of mood states containing a total of 24 items, which are divided into 6 subsets: depression, anger, tension, fatigue, vigour, and confusion. These items are scored on a 5-point likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a lot, 4 = extremely). Each subset has four relevant items and can achieve a raw score between 0-16.

Statistical Analysis

Assumptions of statistical tests such as the normal distribution, equality of variance and sphericity of data were checked using the Shapiro-Wilk, Levene's and Mauchly's tests respectively. Where the assumption of sphericity was violated the Greenhouse-Geiser adjustment was applied to the degrees of freedom. Where appropriate, post-hoc tests using the Bonferroni correction were applied.

A one-way analysis of variance (ANOVA) with repeated measures (RM) was used to analyse pain thresholds as well as the work completed, and distance covered in the time trial. In this study, participants only completed one familiarisation session, a one-way ANOVA with RM was also used to assess for any order effects. As the assumption of normality was violated, Friedmans test was used to analyse pain tolerance. In the event of a statistically significant difference a Wilcoxon-signed rank test was performed. To analyse differences in pain reported in the ischemic pain test a 3×5 RM ANOVA was performed. To account for difference in time to pain tolerance, a combination of intra-individual iso-times and percentage of time to task disengagement (TD) was used. For this, the individuals shortest time to TD was identified as 100% isotime and compared to the equivalent time of the two other conditions. This 100% isotime was further divided by 5 and rounded up to ascertain values corresponding to 20- 80% isotime, providing 5 data points for statistical comparison (20-100 %) for each condition.

For time-based analysis of PO, HR, RPE and pain during the time trial (TT) and analysis of HR, RPE and pain in the fixed intensity cycling trial (FI), the first two minutes were excluded from the ANOVA to accommodate the time delay in achieving adequate feedback (Ulmer., 1996). For analysis of the TT, a 3×13 RM ANOVA was performed on

PO, HR, RPE and pain data. A 3x8 RM ANOVA was used to analyse HR, RPE and pain data in the FI trial. A 3 $\times$ 2 ANOVA + RM was performed on mood subsets reported in the BRUMS questionnaires. All data are presented as means $\pm$ standard deviation. Effect sizes were calculated to establish the size of the difference between the three conditions, this was reported as partial eta-squared (η_p^2) (small = 0.01, medium = 0.06, large = 0.14). The α level was set to $P = 0.05$. All statistics were performed using SPSS (Version 24, SPSS, IBM Corp, Armonik, NY, USA).

2.4 Results

No adverse effects of tDCS occurred in this study. Participants reported a mild itching sensation underneath the electrodes during both the tDCS and sham conditions. No other side effects were reported during or after tDCS administration. Four participants correctly identified the order they completed the trials in. Of these four participants, only one participant was confident that they had identified the trials correctly.

Pain thresholds & tolerance

Between-group analysis revealed no differences between the experimental conditions for pain thresholds to PES ($F_{2, 20} = 0.0736$, $P = 0.492$, $\eta_p^2 = 0.069$, Figure 8, panel A). No differences between the tDCS, sham and control conditions were detected for tolerance to ischemic pain (χ^2 (2) = 3.6, $P = 0.163$, Figure 8, panel B). Analysis of the iso-time data revealed no significant interactions between experimental condition and pain rating over time ($F_{4.0, 39.6} = 0.8$, $P = 0.558$, $\eta_p^2 = 0.01$). There was no significant difference in the rating of pain intensity between the three experimental groups ($F_{2,20} = 0.2$, $P = 0.824$, $\eta_p^2 = 0.02$). Where pain intensity increased over time, a significant main effect of time was observed ($F_{1.7, 16.6} = 125.1$ $P < 0.001$, $\eta_p^2 = 0.93$).

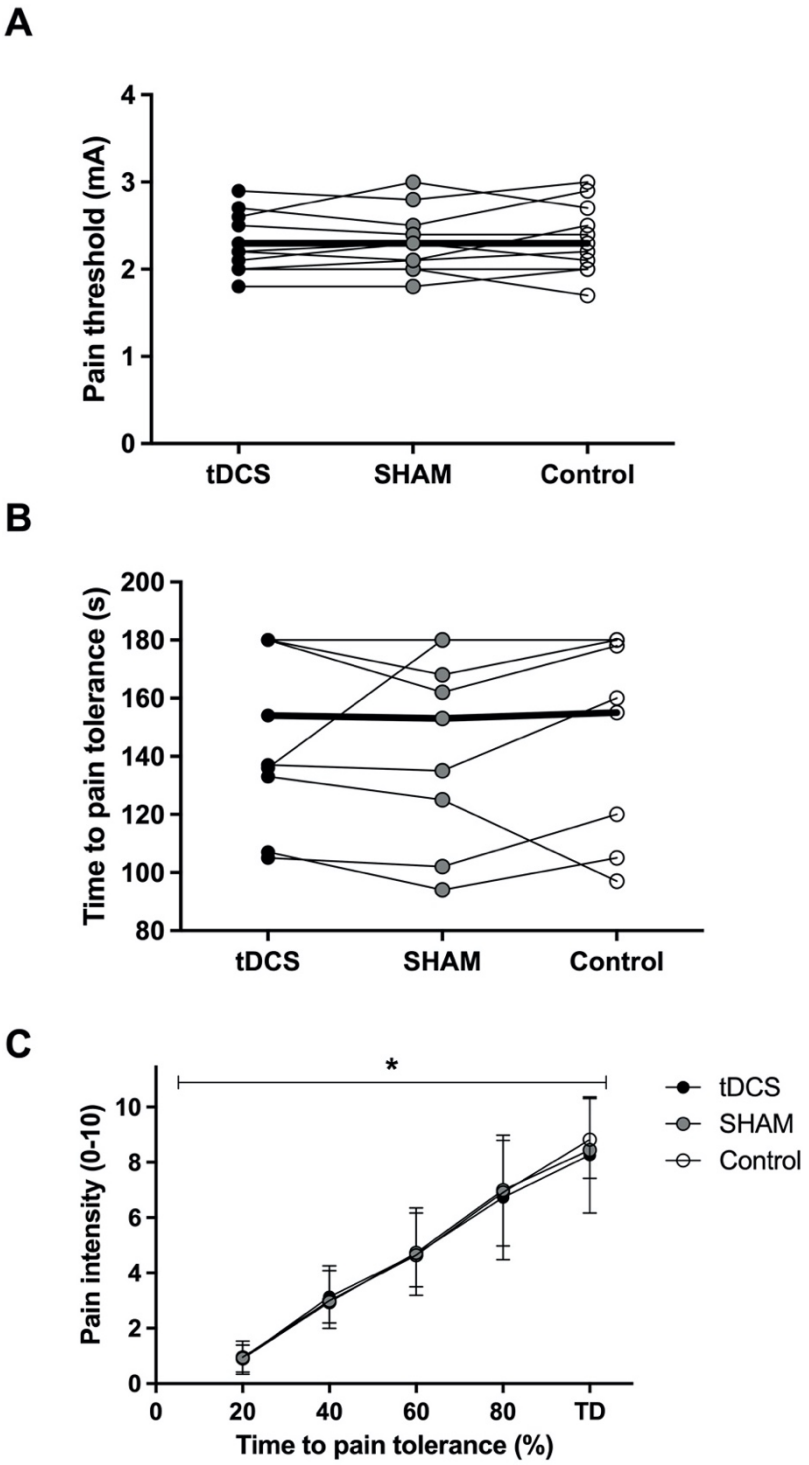

*Figure 8. The effects of tDCS on experimental pain thresholds and tolerance. Panel A shows pain threshold to peripheral electrical stimulation (mA); Panel B shows time to pain tolerance during the ischemic pain test (s); Panel C shows the time course of pain reported during the ischemic pain test. All data is presented as mean ± SD, bold line on panels A and B signifies the mean. * Denotes a significant main effect of time.*

Fixed Intensity Cycling Trial

In the FI trial, a condition × time interaction was detected for the rating of pain intensity ($F_{4.1, 41.1} = 3.4$, $P = 0.016$, $\eta_p^2 = 0.25$), however post-hoc analysis did not show any significant between group differences (all P's ≥ 0.098). Additionally, there was no main effect of condition observed for pain intensity ($F_{2, 20} = 0.9$, $P = 0.417$, $\eta_p^2 = 0.08$). A main effect of time was observed for the rating of pain intensity ($F_{1.4, 13.7} = 46.3$, $P < 0.001$, $\eta_p^2 = 0.82$) with all conditions displaying a greater rating of pain at minute 10 than minute 1 (Figure 9, panel A). No significant condition × time interactions found for RPE ($F_{5.3, 52.5} = 1.7$, $P = 0.141$, $\eta_p^2 = 0.15$) and HR ($F_{2.9, 29.2} = 0.3$, $P = 0.847$, $\eta_p^2 = 0.03$), nor was there any significant main effects of condition for either of these variables ((RPE: $F_{2, 20} = 0.05$, $P = 0.956$, $\eta_p^2 = 0.01$) (HR: $F_{2, 20} = 3.\ 4$, $P = 0.054$, $\eta_p^2 = 0.25$)). Like pain, RPE and HR rose gradually throughout the trial therefore a significant main effect of time was observed ((RPE $F_{1.4, 14.1} = 50.0$, $P < 0.001$, $\eta_p^2 = 0.83$, Figure 9, panel B) (HR $F_{1.7, 17.5 =} 54.5$, $P < 0.001$, $\eta_p^2 = 0.85$, Figure 9, panel C)).

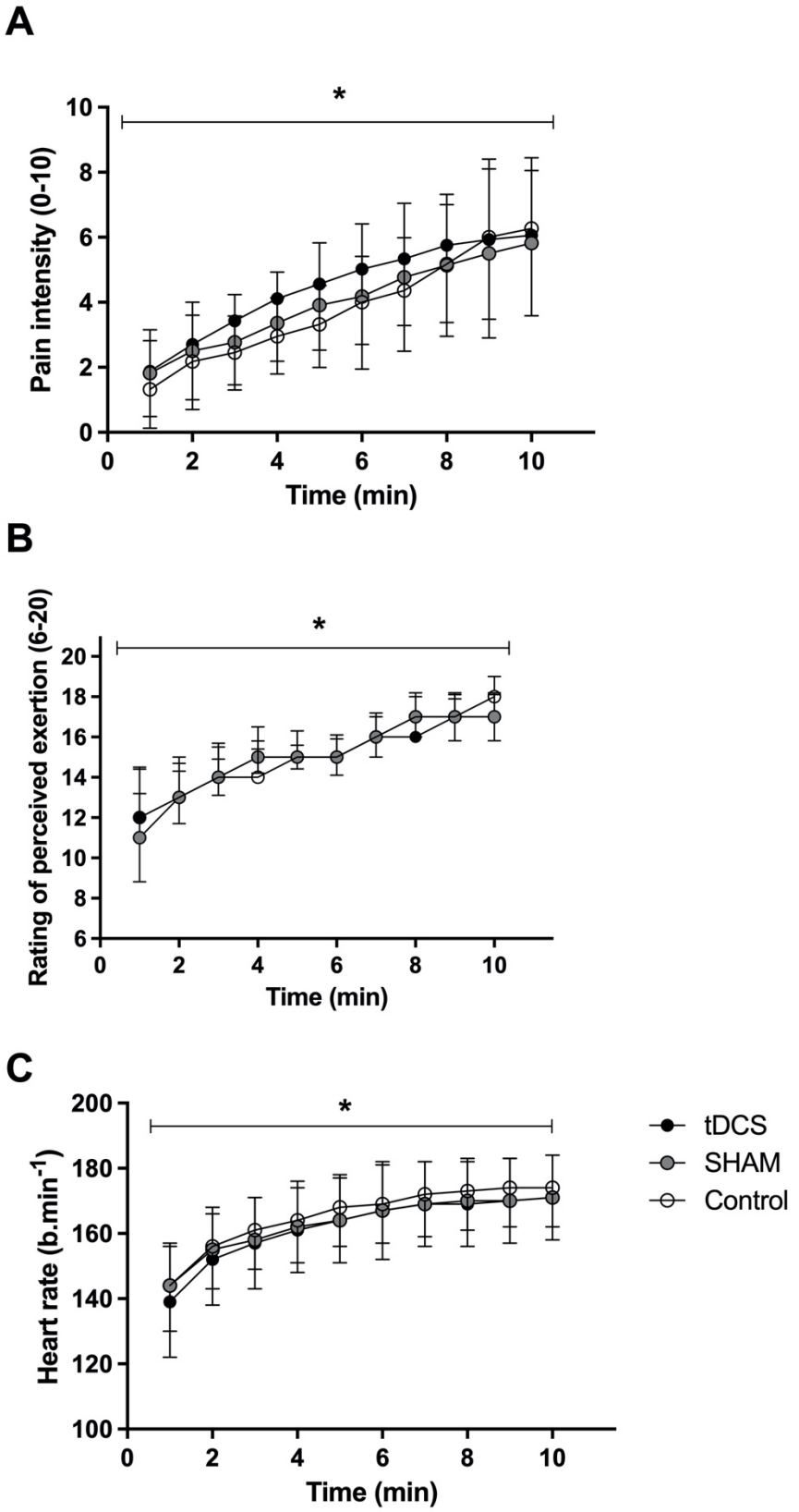

*Figure 9 The physiological and perceptual responses recorded during the fixed intensity cycling trial. Panels A-C show the time course of pain intensity, RPE and HR during the FI trial respectively. Data presented as mean ± SD. * denotes a significant main effect of time.*

Time trial

Distance covered in the TT was 8.00 ± 0.56 km in tDCS condition, 7.98 ± 0.57 km in the sham condition and 7.93 ± 0.59 km in the control conditions. The distance covered was not found to be significantly different between the experimental conditions ($F_{2, 20}$ = 0.8, P = 0.478, η_p^2 = 0.07). There were also no significant order effects detected ($F_{2,20}$ = 0.4, P = 0.694, η_p^2 = 0.04). Despite the large effect size, there was no difference detected between the experimental groups for work completed in the TT ($F_{2, 20}$ = 2.2, P = 0.133, η_p^2 = 0.18, Figure 10, Panel A). No condition × time interactions were detected for the rating of pain ($F_{5.3,53.3}$ = 1.2, P = 0.332, η_p^2 = 0.11), RPE ($F_{3.2, 31.6}$ = 2.0, P = 0.129, η_p^2 = 0.17), HR ($F_{4.8, 48.1}$ = 0.9, P = 0.575, η_p^2 = 0.29) and PO ($F_{4.5, 45.4}$ = 1.0, P = 0.419, η_p^2 = 0.09), nor were there any significant main effects of condition for any of these measures ((Pain: $F_{1.2, 12.4}$ = 0.4, P = 0.603, η_p^2 = 0.04) (RPE: $F_{2, 20}$ = 0.03, P = 0.967, η_p^2 = 0.003) (HR: $F_{2, 20}$ = 0.3, P = 0.745, η_p^2 = 0.03) (PO: $F_{2,20}$ = 1.7, P = 0.204, η_p^2 = 0.15). A significant main effect of time was observed for pain ($F_{1.7, 17.3}$ = 127.2, P < 0.001, η_p^2 = 0.93), RPE ($F_{2.5, 25.4}$ = 73.896, P < 0.001, η_p^2 = 0.88), HR ($F_{1.8, 17.6}$ = 22.9, P < 0.001, η_p^2 = 0.70) and PO ($F_{2.5, 25.3}$ = 17.2, P < 0.001, η_p^2 = 0.63).

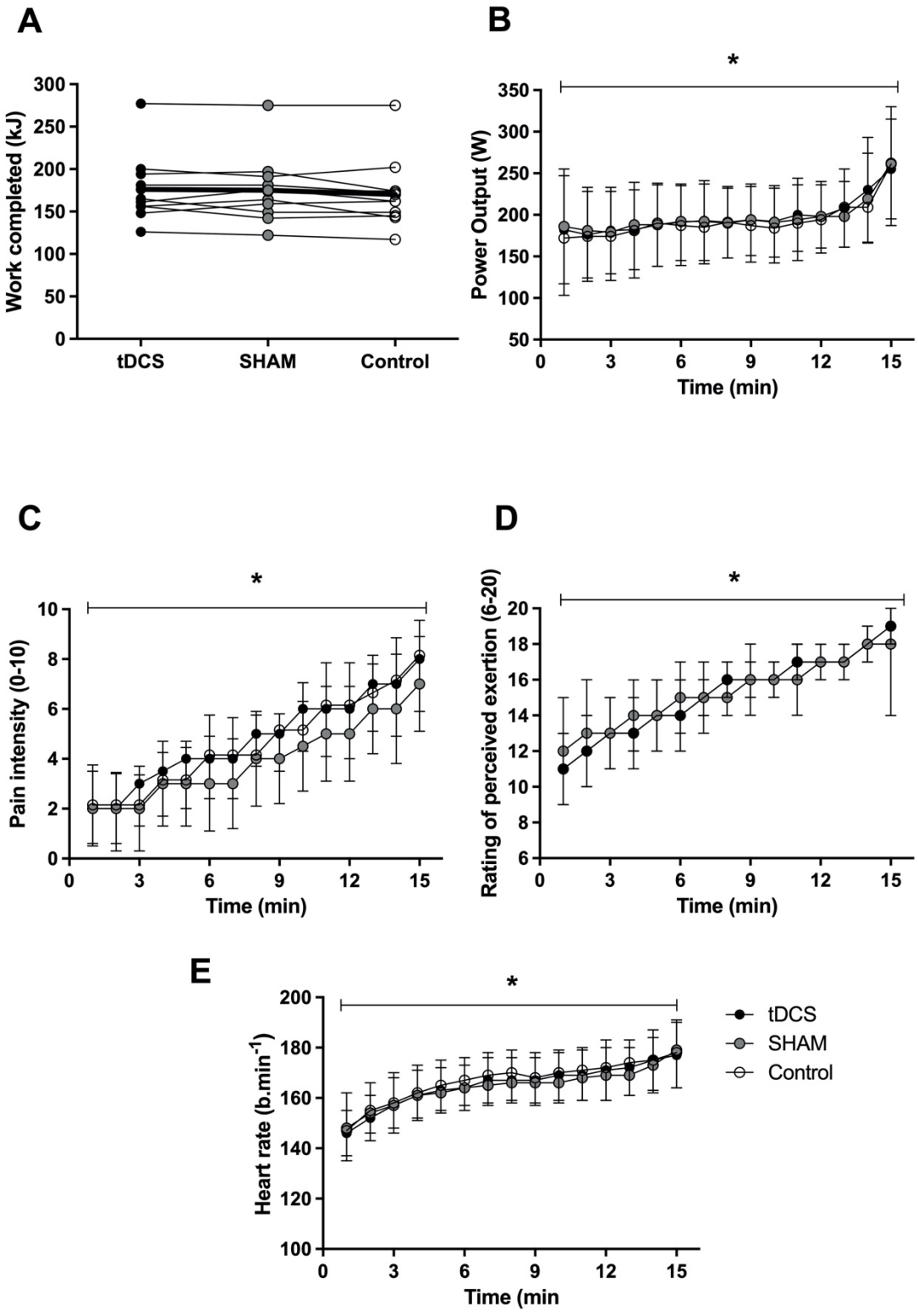

*Figure 10 Perceptual and performance responses during the 15 min TT. Panel A shows the work completed (kJ). Panels B-E show the time course of power output (W), pain intensity, RPE and HR respectively. Data presented as mean ± SD, bold line on panel A signifies the mean. * Denotes a significant main effect of time.*

Mood Scales

In the fatigue subset, a significant condition × time interaction was detected ($F_{2, 20}$ = 5.6, P = 0.012, η_p^2 = 0.547). However post-hoc analysis using the Bonferroni correction revealed no significant between group differences (all P's ≥ 0.05). No main effects of condition were detected for the fatigue subset ($F_{2, 20}$ = 1.1, P = 0.363, η_p^2 = 0.1). A main effect of time was detected for fatigue, where participants reported that they felt more fatigued at the end of the visit ($F_{1,10}$ = 121, P = 0.006, η_p^2 = 0.55). For the depression, anger and vigour subsets there was no condition × time interactions detected (Depression ($F_{2,20}$ = 2.3, P = 0.127, η_p^2 = 0.19) Anger ($F_{2,20}$ = 0.8, P = 0.473, η_p^2 = 0.07) Vigour ($F_{2,20}$ = 0.7, P = 0.504, η_p^2 = 0.07)), main effect of condition (Depression ($F_{2,20}$ = 0.2, P = 0.786, η_p^2 = 0.02) Anger ($F_{2,20}$ = 0.9, P = 0.420, η_p^2 = 0.08) Vigour ($F_{2,20}$ = 0.3, P = 0.709, η_p^2 = 0.349)) or time observed (Depression ($F_{2,20}$ = 2.3, P = 0.127, η_p^2 = 0.19) Anger ($F_{2,20}$ = 0.8, P = 0.473, η_p^2 = 0.07) Vigour ($F_{2,20}$ = 0.7, P = 0.504, η_p^2= 0.07)). For the tension and confusion subsets there were no significant condition x time interactions detected (Tension ($F_{2,20}$ = 1.3, P = 0.306, η_p^2 = 0.11) Confusion ($F_{2,20}$ = 0.2, P = 0.789, η_p^2 = 0.46). A significant main effect of condition was found for both tension and confusion subsets (Tension ($F_{2, 20}$ = 3.5, P = 0.049, η_p^2 = 0.26) Confusion ($F_{2,20}$ = 3.6, P = 0.046, η_p^2 = 0.27)), follow up post-hoc analysis revealed that there was no significant between group differences for both subsets (Tension (all P's ≥ 0.088) Confusion (all P's ≥ 0.128). Furthermore, there was no significant main effects of time detected (Tension ($F_{1,10}$ = 3.5, P = 0.092, η_p^2 = 0.26) Confusion ($F_{1,10}$ = 0.5, P = 0.482, η_p^2 = 0.05).

2.5 Discussion

This study's primary objective was to investigate the effect of anodal tDCS applied to the DLPFC on the modulation of EIP and how this impacted TT performance. The principal finding of the present investigation was that the application of anodal tDCS to the DLPFC in a bilateral montage was incapable of inducing analgesia. Consequently, no significant changes in TT performance occurred.

The effect of tDCS on the perception of pain

In the present investigation, the response to experimental pain was measured as a manipulation check to ascertain whether changes in performance were due to analgesic effect. With no significant differences in experimental pain threshold or tolerance detected, our results demonstrate that tDCS delivered in a bilateral montage was insufficient to induce an analgesic effect. These findings are surprising given that the use of tDCS to treat chronic pain and augment acute pain thresholds or tolerance has been well documented. Indeed, studies that have specifically targeted the left DLPFC have reported significant improvements in experimental pain thresholds (Boggio et al., 2008; Mariano et al., 2016) and reductions in perceived unpleasantness or discomfort associated with painful images (Boggio et al., 2009). The discrepant findings could be due to the timing of stimulation procedures. Our study, like many other studies investigating the effect of tDCS on endurance performance (Angius et al., 2015; Angius et al., 2016; Angius et al., 2018; Angius et al., 2019; Barwood et al., 2016), applied tDCS at rest, and assessed pain tolerance and threshold to experimental pain once stimulation was complete. However, it is a common practice for researchers to assess the effect of tDCS on experimental pain by inducing pain whilst tDCS is active, often allowing 3-5 minutes of stimulation prior to

inducing experimental pain (Boggio et al., 2008; Mylius et al., 2012; Mariano et al., 2016). Previous studies have suggested that the effectiveness of tDCS is enhanced through task-specific modulation (Bikson & Rahman., 2013). This assumes that the subthreshold nature of tDCS is unable to induce changes in resting networks (such as the pain networks were in this study), instead tDCS induces an 'online' effect preferentially stimulating the more sensitive active networks (Bikson & Rahman., 2013).

A bilateral montage of electrodes was adopted for this study based upon the finding of increased current density within the targeted brain region (Neuling et al., 2012). However, the lack of analgesia observed in the present study may be due to placement of the cathodal electrode over the right DLPFC. Indeed, Mylius et al (2012) identified that anodal tDCS applied to the right DLPFC in a cephalic montage significantly enhanced tolerance to heat pain. As stated in 1.3.1 Electrode montage and size, the placement of the cathodal electrode over a brain area can induce unintended diminutions in excitability (Moliadze et al., 2010; Bikson et al., 2010; Nitsche et al., 2007). Therefore, the bilateral montage used in the present investigation may have induced decrements in right DLPFC excitability, counteracting the analgesic effect induced by the placement of the anodal electrode over the left DLPFC.

This study also observed no analgesic effects of tDCS during the FI cycling trial or the TT. Resultantly it was not surprising that no significant changes in work completed, or distance covered within the TT were observed. Like the present investigation, several studies have also reported that anodal tDCS applied to both the M1 and the DLPFC was insufficient at modulating EIP (Angius et al., 2015; Angius et al., 2016; Angius et al., 2018; Angius et al., 2019; Flood et al., 2017). It is well established that physical activity activates the body's inherent analgesic system, allowing for the release of endorphins, catecholamines, growth factors and stimulates the suprapsinal nociceptive inhibitory systems (Nijs et al., 2012; Angius et al., 2015). Therefore, the lack of change in the perception of EIP seen in the present study may be reflective of the inability of tDCS to exert an additive effect to the body's inherent system. However, these previous trials were able to demonstrate a significant enhancement in experimental pain tolerance (Angius et al., 2015; Flood et al., 217) or a reduction in RPE during the cycling or isometric TTE trials. As the present study found no significant effects of tDCS on experimental pain, EIP and the profile of RPE during both the FI and the TT, it is arguable that the tDCS parameters adopted were insufficient for manipulating the perceptual measures recorded.

Attention paid to the nociceptive stimuli is a prerequisite for the interpretation of pain and the formation of coping strategies (Linton & Shaw., 2001). Therefore, distraction from the nociceptive stimuli may decrease the perceived pain intensity (Linton & Shaw., 2001). During the FI trial and the TT, participants are required to focus on the exercise task (for example, maintaining the required cadence in the fixed intensity trial), which may distract from the nociceptive stimuli, reducing the pain intensity perceived. In this study, the participants may have been more focused on the exercise task itself in the FI cycling trial and the TT. Additionally, within the ischemic pain test, participants were required to pay attention to completing the contractions in time with a metronome, which may have also detracted from the painful stimuli.

The effect of tDCS on endurance performance

Due to inconsistent use of tDCS parameters and experimental methodology, the efficacy of anodal tDCS to enhance endurance performance is unclear. To date, only four studies have investigated the effect of anodal tDCS applied to the DLPFC on cycling performance. Lattari et al (2018) reported that anodal tDCS of the DLPFC significantly increased the time to exhaustion (TTE) when cycling at 100% PPO. Similarly, Angius et al (2019) also found an improvement in TTE from stimulating the left DLFPC. In this study the authors reported that tDCS significantly reduced RPE and HR in comparison to sham but found no significant difference in the rating of pain. The authors concluded that tDCS applied to the DLPFC enhanced exercise regulation due to the improvement in inhibitory control (as assessed via the Stroop task) and RPE. In contrast, the results of the present study indicate that stimulating the DLPFC was unable to produce any changes in TT performance. In support of the present study, both Andre et al (2019) and Holgado et al (2019) reported no significant effects of DLPFC tDCS upon TT performance. Although less ecologically valid, perhaps the open loop design of a TTE test provides a more sensitive measure of tDCS' ergogenic effect as participants are more aware of feedback from metabolic processes such as EIP or their RPE.

2.6 Conclusions

In summary, anodal tDCS of the DLPFC delivered in a bilateral montage was inadequate to induce an analgesic effect to both experimentally provoke pain and EIP. As a result, there were no changes in TT performance. This could be reflective of the montage selected, downregulating the activity of the DLPFC in the right hemisphere. Further research is required to establish the optimal electrode positioning, perhaps using an extracephalic montage which is suggested to avoid the unwarranted effects of the cathodal electrode.

Chapter 3. The effect of tDCS applied to the Dorsolateral Prefrontal Cortex via an extracephalic montage on the modulation of exercise-induced pain and cycling performance

3.1 Abstract

Introduction: The placement of the cathodal electrode over another brain area has previously been suggested to induce unintended alterations in excitability, and therefore creates ambiguity in the interpretation of experimental outcomes. Instead, the use of an extracephalic montage has been proposed to offset the unwanted effects observed in a cephalic or bilateral montage. **Aim:** This study investigated the effect of tDCS applied to the left DLPFC using an extracephalic montage on cycling TT performance and the modulation of EIP during a FI cycling trial. **Methods:** On separate days, 20 recreationally active participants completed a 10-minute FI cycling trial at 75% of their peak power output and a 15-minute cycling TT preceded by 10 minutes of 2 mA tDCS, sham or the control condition. For the tDCS and sham condition, electrodes were arranged in an extracephalic montage where the anodal and cathodal electrodes were placed over the left DLPFC and the ipsilateral shoulder respectively. During the cycling trials, Pain, RPE and HR were recorded every minute. PO was also monitored throughout the TT. **Results:** No significant changes in pain occurred within the FI cycling trial (tDCS 3.7 ± 1.9; sham 3.8 ± 1.6; control 3.7 ± 1.8; $P = 0.217$). No significant differences in distance completed within the TT were also observed ($P = 0.239$) **Conclusion:** tDCS delivered in an extracephalic montage did not induce analgesia and provided no ergogenic effect for TT performance.

3.2 Introduction

Chapter 2 examined the effects of a bilateral montage used to stimulate the DLPFC on cycling performance and the rating of exercise-induced pain (EIP). The principal finding of this study was that there was no significant difference in time trial performance between the tDCS, sham and the control conditions. Furthermore, there was no effect of tDCS condition on the rating of EIP reported within the fixed intensity cycling trial. As there was no significant difference observed between the three experimental groups for pain threshold and pain tolerance to experimental pain methods (PES and ischemic muscle pain), it was concluded that tDCS applied within this study was insufficient to induce an analgesic effect. Previous studies have reported analgesic effects from targeting both the left and right DLPFC (Boggio et al., 2008; Mylius et al., 2016; Mariano et al., 2016). Therefore, it is possible that the placement of the cathodal electrode over the right DLPFC may have induced unintended diminutions in excitability, counteracting the analgesia induced by the placement of the anodal electrode over the F3 (left DLPFC).

Transcranial direct current stimulation has been proposed as a tool to treat numerous neuropsychological disorders or induce changes in a variety of behavioural responses (Benninger et al., 2010; Loo et al., 2010; Fregni et al., 2008; Fregni et al., 2006; Marshall., 2004; Machado et al., 2019). It is thought that the specificity of stimulation is achieved through functional and anatomical factors in addition to the montage of electrodes selected (Bikson & Rahman., 2013; DaSilva et al., 2011). Within the sport and exercise science literature, tDCS is often applied to achieve anatomical specificity, targeting brain areas such as the M1, IC and DLPFC to delay the onset of supraspinal fatigue, and reduce fatigue related perceptions such as RPE and EIP (Angius et al., 2017; Machado et al., 2019). The findings to date have been mixed (As shown in tables 1 and 2), which may be due to disparities between tDCS current dose, exercise paradigm and electrode montage selected (Machado et al, 2019).

The montage of electrodes is known to determine the direction of current flow throughout the brain. Indeed, FEM studies have demonstrated that the traditional M1 cephalic and bilateral montages induce greater current densities within the prefrontal cortex (Yanamadala et al., 2014). As such the bilateral montage adopted within chapter 2, and the cephalic montage selected by Angius et al (2015) could have induced unintended reductions in excitability of the right DLPFC. An extracephalic montage has been suggested as an alternative approach (Cogiamanian et al., 2007; Holgado et al., 2019; Moliadze et al., 2010; Yanamadala et al., 2014; Accornero et al., 2007). This refers to the placement of the cathodal electrode on a non-cephalic area such as the shoulder (Cogiamanian et al., 2007; DaSilva et al., 2011). Finite element modelling studies have identified that extracephalic montages allow for greater and deeper current densities within the brain region of interest when compared to a cephalic montage (Miranda et al., 2006; Yanamadala et al., 2014). The increased inter-electrode distance associated with the extracephalic montage is thought to reduce the resistance, allowing a greater proportion of current to be injected into the brain region of interest whilst simultaneously reducing the shunting of current across the scalp and current spread to adjacent brain areas (Miranda et al., 2006; Yanamadala et al., 2014).

To date, a small number of studies have investigated the effect of tDCS using an extracephalic montage on endurance performance. Cogiamanian et al (2007) first

demonstrated that stimulating the M1 with the cathodal electrode on the ipsilateral shoulder enhanced the time to exhaustion (TTE) of elbow extensors, without any changes in the maximal voluntary contraction or electromyography measures. The placement of the cathodal electrode on the contralateral shoulder has also shown to be efficacious for improving TTE of the knee extensors (Angius et al., 2016; Abdelmoula et al., 2016). Thus far, only Holgado et al (2019) has investigated the influence of tDCS delivered in an extracephalic montage on cycling performance. In this study, the authors placed the anodal electrode over the left DLPFC and the cathodal over the contralateral shoulder, reporting no significant effects of tDCS on cycling TT performance (Holgado et al., 2019). These null findings may be due to the contralateral placement of the cathodal electrode.

The primary aim of this study was to investigate the effect of tDCS applied to the DLPFC using an extracephalic montage with the cathodal electrode placed on the ipsilateral shoulder, on the rating of exercise induced pain measured during a fixed intensity cycling trial, and time trial performance.

3.3 Methods

Participants

Twenty healthy volunteers (14 males, 6 females, age: 25 ± 5 yr, height: 1.75 ± 0.08 m, body mass: 70 ± 12 kg, PPO: 274 ± 69 W) participated in this study. The sample size was chosen to equal or exceed the sample size of similar studies. Participants were eligible to take part in the investigation if they were aged 18- 44 years and habitually performed a minimum of 180 minutes of aerobic exercise per week. Based upon these descriptors the participants were deemed as ‘untrained’ as described by De Pauw et a (2013). Participants were excluded from the study if they reported any mental health (i.e., depression or schizophrenia) or brain disorders (i.e., epilepsy, brain lesions), implants from surgery or were taking any medication at the time of the study. Prior to providing written informed consent, participants were provided with an overview of experimental procedures, but not the aims or hypothesis to limit subject-expectancy bias. Ethical approval for this study was obtained from the SSES REAG (approval number: Prop_92_2015_2016).

Experimental Protocol

This study adopted the same methods discussed in chapter 2 [1]. Therefore, the following sections provide an overview of where the methodologies differ between the present study and chapter 2.

[1] For full details of the methods used in this study please see the sections titled ‘Evaluations of pain threshold and tolerance’, ‘Time trial’, ‘Brunel Mood Scale’ and ‘Statistical Analysis’ reported in Chapter 2.

Transcranial Direct Current Stimulation Procedures

Transcranial direct current stimulation was administered using a battery-drive stimulator (First 9 participants completed the study receiving tDCS from Neuroconn Eldith DC stimulator, Magstim, Carmarthenshire. The final 11 participants completed the study receiving tDCS from TCT research limited, Hong Kong stimulator) through a pair of rubber electrodes (size: 5 cm x 7 cm, 35 cm^2) encased in a saline soaked sponge (9% NaCl). According to the manufacturers, the Neuroconn Eldith Stimulator has an error rate of 0.02%. The electrodes were arranged in an extracephalic montage, with the anodal electrode placed over the left DLPFC (F3 according to the 10-20 EEG system), whilst the cathodal electrode was placed on the ipsilateral shoulder and held in place by elastic straps. This montage was selected to minimise undesired effects of the cathodal electrode on other cortical areas (Cogiamanian et al., 2007; Yanamadala et al., 2014). In the experimental condition, participants received 2 mA tDCS (current density 0.057 mA/cm^2) for a duration of 10-minutes (Lang et al., 2005; Angius et al., 2015). Whereas in the sham condition, stimulation lasted for 30 s and was subsequently ramped down to no sensation. In both conditions, the current intensity was ramped up and down over a 10 s period. The electrical resistance was continuously monitored on the stimulators display and maintained between 4- 6 kΩ. In the control condition participants rested quietly for 10 minutes

Fixed Intensity Cycling Trial

Participants were instructed to cycle at 75% of their PPO for 10 minutes on a cycle ergometer. Eleven participants completed the trial on the Lode Excalibur Sport ergometer (Lode, Groningen, Netherlands). Whilst the final 9 participants completed the study on a road bike (Merida Scultura 400, 2017 model) affixed to a Cylcus 2 ergometer (RBM elekronik-automotion, GmbH, Leipzig, Germany). The cyclus 2 has a reported 2% error (± 2 W for PO < 100 W). Furthermore, Rodgers et al (2016) reported that the cyclus 2 is a reliable ergometer for the measurements of power in tests with a duration greater than one minute and PO less than 500 W. All participants completed all three trials on the same ergometer. Workloads have shown to be transferrable across these two ergometers, with no difference in heart rate (HR) or volume of oxygen reported across a range of PO (Reiser et al., 2000). Like study 1, participants were required to maintain a constant cadence throughout the trial, which was replicated across the conditions. At the end of every minute, participants were asked to report their pain intensity and RPE. Heart rate was also collected at the end of the minute via a telemetric device (Polar FT1, Polar Electro Oy, Kempele, Finland).

3.4 Results

No adverse effects to tDCS occurred in this study. Participants reported a mild itching sensation underneath the electrodes during all tDCS conditions. No other side effects were reported during or after tDCS administration. Seven participants correctly identified the order they had completed the trials in. Of these seven participants, three were confident that identified the trials correctly whilst four were uncertain but guessed correctly.

Pain thresholds & tolerance

The influence of tDCS on the pain threshold and tolerance to PES and ischemic pain are demonstrated in Figure 11. The difference in pain threshold was detected between the three experimental conditions ($F_{2,38}$ = 3.3, P = 0.047, η_p^2 = 0.15). However, post-hoc analysis with the Bonferroni correction did not show significant between group differences (all P's $\geq$ 0.05). There were no differences in tolerance to ischemic pain between the tDCS, sham and control conditions (χ^2 (2) = 0.3, P = 0.876). Analysis of iso-time data showed no condition $\times$ time interaction for the rating of pain intensity throughout the ischemic trial ($F_{4.6, 87.5}$ = 0.7, P = 0.587, η_p^2 = 0.04). No main effect of condition was detected for the rating of pain ($F_{2, 38}$ = 2.9, P = 0.7, η_p^2 = 0.13). As pain rose throughout the ischemic trial, a main effect of time was observed ($F_{1.6, 30.50}$ = 148.5, P < 0.001, η_p^2 = 0.89). In the ischemic pain trial, a total of 8 participants reached the 3-minute cut- off point.

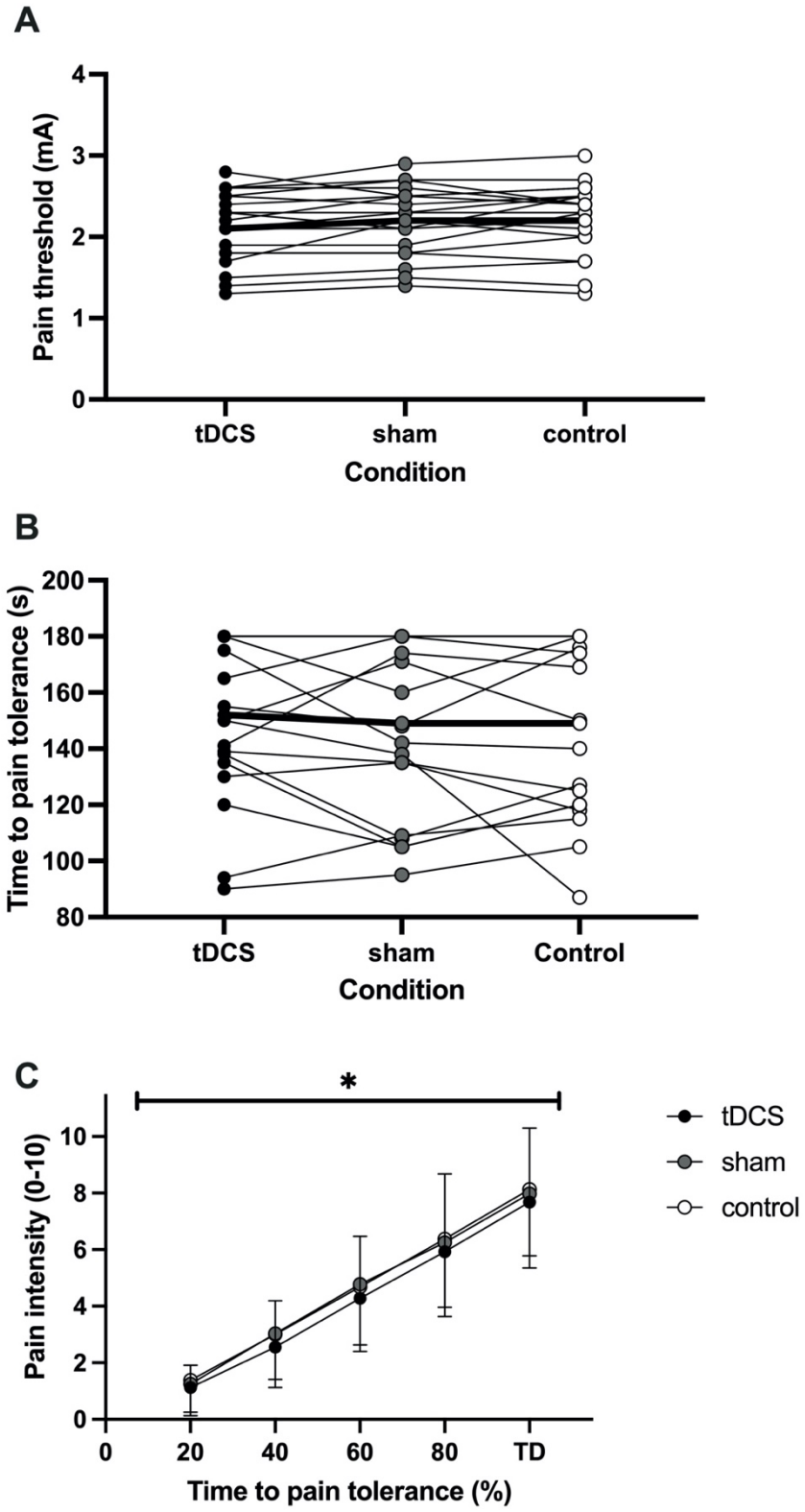

*Figure 11. the effect of tDCS applied to the DLPFC via an extracephalic montage on experimental pain threshold and tolerance. Panels A-C demonstrate the pain threshold to peripheral electrical stimulation (mA), time to pain tolerance during the ischemic pain test (s), and the time course of pain perceived during the ischemic pain test respectively. All data is presented as mean ± SD. Bold lines on panels A and B signify the mean. * Denotes a significant main effect of time.*

Fixed Intensity Trial

The effects of tDCS on the physiological and perceptual responses during the FI cycling trial are detailed in Figure 12. No condition × time interactions were detected for the rating of pain intensity throughout the FI trial ($F_{4.8, 91.6}$ =1.4. P = 0.217, η_p^2 = 0.07), nor was there a significant main effect of condition ($F_{2,38}$ = 0.6, P = 0.559, η_p^2 = 0.03). A main effect of time was detected ($F_{1.3, 25.1}$ = 51.2, P < 0.001, η_p^2 = 0.73) where participants reported a greater pain intensity at minute 10 in all experimental conditions. Similarly, there was no condition × time interactions for the rating of RPE throughout the FI trial ($F_{5.6, 106.3}$ = 0.9, P = 0.461, η_p^2 = 0.05). There were also no main effects of condition on the RPE ($F_{2, 38}$ = 1.1, P = 0.347, η_p^2 = 0.05). All the experimental groups reported a greater RPE at minute 10 than minute one, therefore a significant main effect of time was detected ($F_{1.4, 27.1}$ = 68.8, P < 0.001, η_p^2 = 0.78). For HR measured throughout the FI trial, no condition × time interaction effect was detected ($F_{5.5, 104.5}$ = 0.5, P = 0.763, η_p^2 = 0.03), nor was there any main effects of condition ($F_{5.5, 104.5}$ = 0.5, P = 0.763, η_p^2 = 0.03). A main effect of time was detected ($F_{2.2, 41.2}$ = 169.0, P < 0.001, η_p^2 = 0.90) where HR was greater at minute 10 for all the experimental conditions.

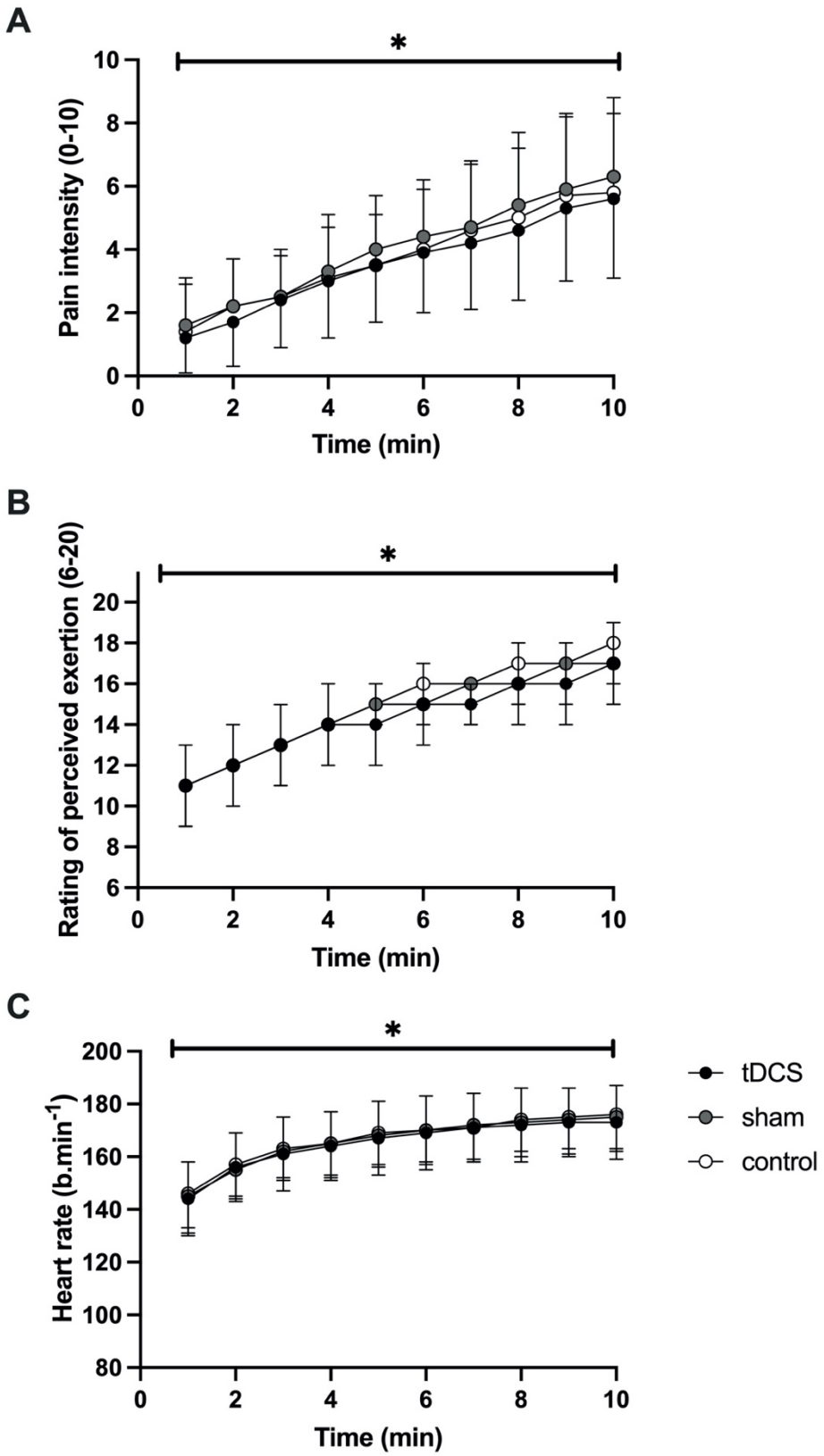

*Figure 12. The perceptual and physiological responses observed during the 10 min fixed intensity cycling trial. Panels A-C display the time course of the perceived pain intensity, RPE and HR respectively. Data presented as mean ± SD. * denotes a significant main effect of time.*

Time trial

The distance covered in the 15-minute TT was 8.02 ± 0.56 km in the tDCS condition, 7.89 ± 1.08 km in the sham condition and 7.95 ± 1.03 in the control condition. No significant differences were detected in the distance completed between the experimental conditions ($F_{2, 38}$ = 1.5, P = 0.239, η_p^2 = 0.07). There was also no significant order effect detected on the distance completed in the TT ($F_{2,38}$ = 0.1, P = 0.912, η_p^2 = 0.005). Whilst not a strict measure of test-retest reliability the CV calculated between the distance covered in the sham and the control condition was 2.44%. Work completed within the 15-minute TT varied considerably around the mean, as shown in Figure 13, Panel A. Indeed, the CV calculated between the sham and control conditions was 8.01%. This variation is likely due to the use of untrained participants who are less familiar with completing TT. There were also no significant differences between the experimental conditions detected for work completed ($F_{2, 38}$ = 1.9, P = 0.16, η_p^2 = 0.09). The pacing profile of PO is shown in Figure 13 panel B. No significant condition × time interactions were detected for PO ($F_{5.0, 94.4}$ = 0.971, P = 0.439, η_p^2 = 0.05). There were also no main effects of condition detected ($F_{2,38}$ = 1.3, P = 0.272, η_p^2 = 0.07). A main effect of time was detected ($F_{1,7, 32.2}$ = 26.5, P < 0.001, η_p^2 = 0.58) where the PO in the final two minutes were greater than the preceding 13 minutes in all three experimental conditions ($F_{1.7, 32.2}$ = 26.5, P < 0.001, η_p^2 = 0.58). No interactions between condition × time were detected for the rating of pain intensity throughout the 15-minute TT ($F_{6.1, 116.0}$ = 1.1, P = 0.361, η_p^2 = 0.06). There were also no significant main effects of condition detected ($F_{2, 38}$ = 1.0, P = 0.393, η_p^2 = 0.05). For all three experimental conditions, the perception of pain intensity gradually increased throughout the trial ($F_{1.5, 30.3}$ = 79.1, P < 0.001, η_p^2 = 0.81), with pain intensity being the greatest within the final minute of the TT. No condition × time interactions were detected for RPE during the 15-minute TT ($F_{5.0, 94.5}$ = 1.088, P = 0.372, η_p^2 = 0.05), nor was there a main effect of condition ($F_{2,38}$ = 0.3, P = 0.776, η_p^2 = 0.01). Where the RPE gradually rose throughout the TT, with the greatest RPE being reported in the final minute, a significant main effect of time was detected ($F_{1.8, 34.5}$ = 90.3, P < 0.001, η_p^2 = 0.83). No significant condition × time interaction was detected for HR during the TT ($F_{1.2, 22.2}$ = 0.8, P = 0.393, η_p^2 = 0.04), nor was there a main effect of condition ($F_{1.5, 27.6}$ = 0.5, P = 0.565, η_p^2 = 0.03). The participants HR was highest in the final minute of the time trial across all experimental conditions; therefore, a main effect of time was observed ($F_{1.7, 32.9}$ = 12.8, P < 0.001, η_p^2 = 0.4).

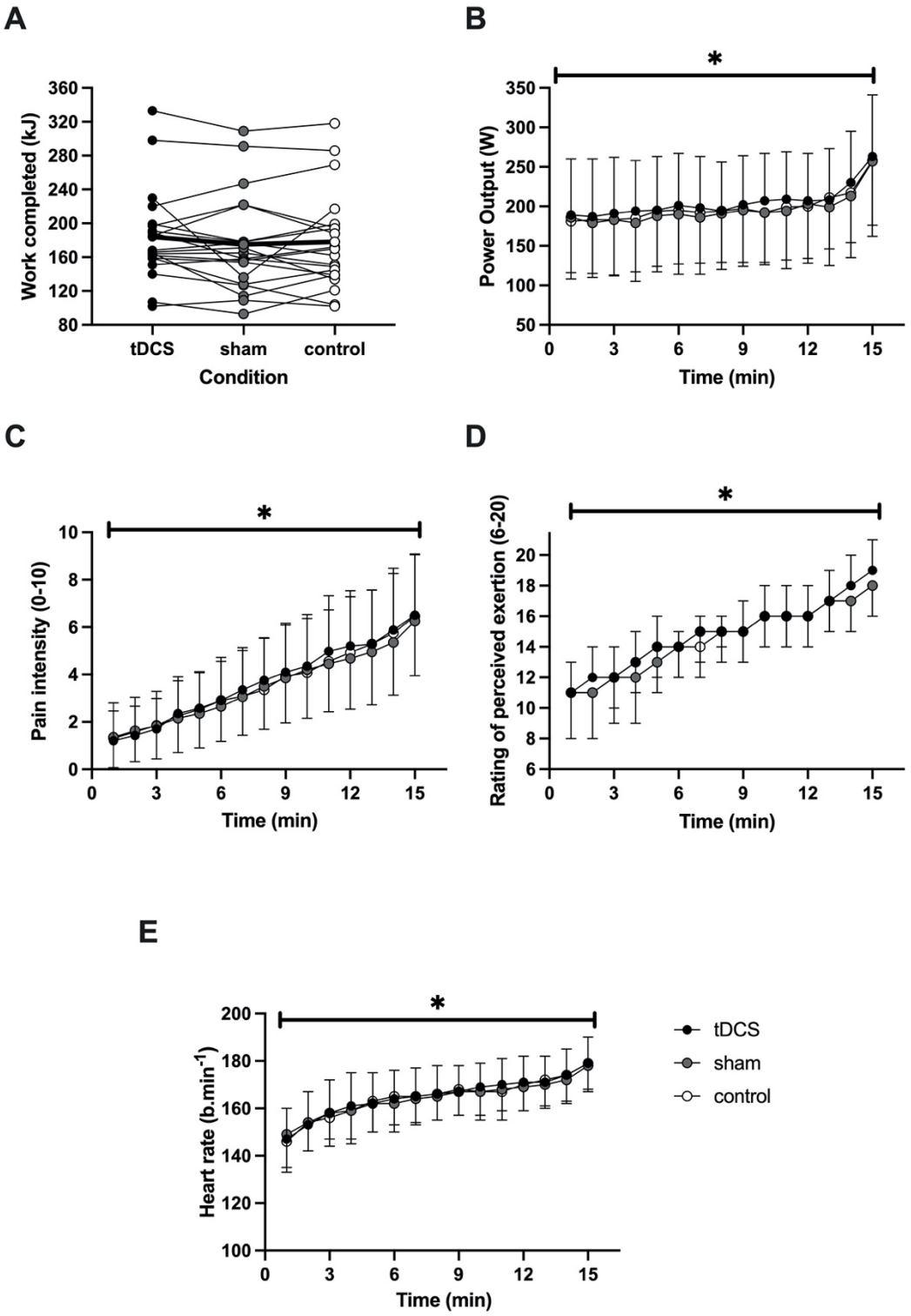

*Figure 13. The performance and perceptual responses observed during the 15 min. TT. Panel A demonstrates the work completed (kJ). Panels B-E display the time course of power output (W), pain intensity, RPE and HR respectively. Data are presented as mean ± SD, bold line on panel A signifies the mean. * Denotes a significant main effect of time.*

Mood Scales

No condition × time interactions were demonstrated for the depression ($F_{2, 38}$ = 1.4, P = 0.264, η_p^2 = 0.07), tension ($F_{1.4, 26.3}$ = 2.1, P = 0.156, η_p^2 = 0.10), anger ($F_{2, 38}$ = 2.0, P = 0.145, η_p^2 = 0.10), vigour($F_{2,38}$ = 0.2, P = 0.828, η_p^2 = 0.01) , fatigue ($F_{2,38}$ = 0.7, P = 0.48, η_p^2 = 0.04) and confusion ($F_{2,38}$ = 2.6, P = 0.089, η_p^2 = 0.12) subsets. No main effects of condition were detected for depression ($F_{2,38}$ = 0.4, P = 0.697, η_p^2 = 0.02), anger ($F_{2,38}$ = 0.1, P = 0.868, η_p^2 = 0.01), vigour ($F_{1.5, 28.4}$ = 2.4, P = 0.121, η_p^2 = 0.001), fatigue ($F_{2,38}$ = 0.04, P = 0.962, η_p^2= 0.002) or confusion ($F_{2, 38}$ = 0.3, P = 0.717, η_p^2 = 0.02). A significant main effect of time was found for depression ($F_{1\ 19}$ = 7.4, P = 0.014, η_p^2= 0.28), tension ($F_{1, 19}$ = 19.0, P < 0.001, η_p^2 = 0.50) and anger ($F_{1,19}$ = 7.8, P = 0.011, η_p^2 = 0.29) subsets where the rating of each of these three subsets was reduced at the end of the trial in comparison to the beginning. Likewise, there was also a main effect of time on the rating of fatigue ($F_{1, 19}$ = 11.4, P = 0.003, η_p^2 = 0.38) observed, where the ratings of fatigue increased at the end of the session in comparison to the start. No significant main effects of time were found for the vigour ($F_{2, 38}$ = 2.0, P = 0.145, η_p^2 = 0.10) and confusion ($F_{2,38}$ = 2.6, P= 0.089, η_p^2 = 0.12).

3.5 Discussion

The present study aimed to examine whether tDCS applied in an extracephalic montage to the left DLPFC could induce analgesia to EIP. Furthermore, this study examined if an analgesic effect was induced, whether time trial performance would have been enhanced. As in chapter 2, experimental pain threshold and tolerance were examined as a manipulation check to ascertain whether performance changes were due to analgesia. Like chapter 2, the major findings of this study were that tDCS delivered in an extracephalic montage was unable to (1) modulate experimental pain thresholds and tolerance, (2) induce an analgesic effect to EIP and (3) improve cycling TT performance.

The use of an extracephalic montage is proposed to circumvent the unwarranted effects of the cathodal electrode associated with bilateral and cephalic electrode montages (Cogiamanian et al., 2007; Yanamadala et al., 2014). Thus far, several studies have investigated the impact of an extracephalic montage on endurance performance. Indeed, the use of M1 extracephalic montages (anode M1, cathodal ipsilateral or contralateral shoulders) has been shown to increase the TTE of the elbow flexors (Cogiamanian et al., 2007; Abdelmoula et al., 2016) and the knee extensors (Angius et al., 2016). In contrast, the findings indicate that tDCS delivered in an extracephalic montage did not modulate performance within a self-paced TT or induce analgesia to EIP. Similar findings have also been reported by Holgado et al (2019) who also applied tDCS to the DLPFC in an extracephalic montage (anode left DLPFC, cathode contralateral shoulder). Indeed, the authors of this study reported that tDCS was insufficient to induce changes in RPE, TT performance, cognitive performance and brain activation measured by EEG. To date, several studies have failed to observe an ergogenic effect of tDCS applied to the M1 (Valenzuela et al., 2018; Andre et al., 2019), the IC (Barwood et al., 2016) and the DLPFC (Holgado et al., 2019; Andre et al., 2019) in self-paced TT performance. This is perhaps indicative of an activity-dependent effect of tDCS, where tDCS preferentially enhances brain regions in a state of reduced excitability such as within exhaustive exercise (Andre et al., 2019).

The effect of Electrode Montage

Within the last decade, research has suggested that the complex interaction between inter-individual differences and the flow of current between the stimulation electrodes will influence the efficacy of tDCS (Bikson et al., 2010; Data et al., 2011; Wagner et al., 2007). In a cephalic or bilateral montage, the placement of electrodes over multiple brain areas reduces the focality of stimulation due to widespread alterations in cortical excitability (Lang et al., 2005). Additionally, clustering of current density in the intermediate regions has also been observed due to differences in tissue architecture and conductivity (Bikson et al., 2010; Datta et al., 2009). This creates ambiguity in the experimental outcome as it becomes uncertain whether the behavioural outcome occurred as a result of targeting a specific brain area or due to activation of intermediate cortical regions (Nitsche et al., 2007).

Extracephalic montages are proposed to enhance the magnitude of current injected into a brain region of interest, through reducing the shunting of current across the scalp (Cogiamanian et al., 2007; Yanamadala et al., 2014; Miranda et al., 2006; Bai et al., 2014; Datta et al., 2008). However, studies have also suggested that increased current intensities may be required for extracephalic montages to be effective (Bikson et al., 2010; Moliadze et al., 2010). Indeed, Moliadze et al (2010) reported that extracephalic montages with the contralateral placement of the cathodal electrode demonstrated the lowest change in MEP amplitude, in comparison to the extracephalic montage with the ipsilateral placement of the cathodal electrode and cephalic montages. Due to the placement of the anodal electrode over the DLPFC, the inter-electrode distance would be comparatively greater than the M1 montages presented by Moliadze et al (2010). Therefore, the current intensity (2 mA) selected may have been too low to exert an analgesic or ergogenic effect.

Computational modelling has also identified that maximal cortical current density is dependent upon the curvature of superficial structures such as the cranium (Wagner et al., 2007). Due to the resistive matrix located superficially to the targeted cortical area, current paths appear to be shunted across the superficial layers and skin when applied to a curvilinear surface (Wanger et al., 2007). This could explain the discrepant findings between those of the present study, and the previous uses of the M1 extracephalic montage (Cogiamanian et al., 2007; Angius et al., 2016), where the more planar surface of the M1 allowed for greater current density in comparison to the more curvilinear area of the DLPFC (Wagner et al., 2007).

Individual Variations in Response to tDCS

It is now recognised that responses to tDCS do not always follow the polar-dependent fashion where anodal excites and cathodal inhibits (Wiethoff et al., 2014). In their seminal paper, Wiethoff et al (2014) noted that the response to 2 mA M1 tDCS varied considerably. Indeed, the authors highlighted that the corticospinal response to tDCS was poor or absent in 50% of participants tested. Furthermore, in those who demonstrated a response to tDCS, 21% exhibited an inverted classical response where cathodal stimulation resulted in increased MEP amplitude, whilst anodal tDCS induced inhibition (Wiethoff et al., 2014). Variance in results was also observed in the present study, where performance in the tDCS condition was greater than the smallest worthwhile change (SWC) (calculated from the standard deviation of the control condition) in 35% (7

participants) of the participants. In the remaining participants, 23% (3 participants) showed a change in performance greater than the SWC, however the performance in the sham group was similar or greater than the tDCS and therefore was considered as a placebo response. Another 23% of participants demonstrated a decline in performance below the SWC threshold in the tDCS condition. Whereas a change in performance was absent in the remaining 54% of participants (7 participants). However, as the CV calculated from the sham and control trials exceeds the SWC, the use of a more conservative measure of variability is recommended, such as doubling the CV (Hopkins et al., 2004). In this instance, work completed within the tDCS trial exceeded this threshold by two participants, whilst performance declined below this threshold in only one participant. It is possible that the variation observed in the present study is due to individual responses to tDCS which would highlight the need for an individualised approach to tDCS. However, it is more likely that this variation is due to the use of untrained participants who would be less familiar on how to optimally pace a TT in comparison to their trained counterparts.

Other studies have also reported non-linear correlations between tDCS intensity and changes in excitability (Jamil et al., 2017; Opitz et al., 2015). Resultantly, Vöröslakos et al (2018) recently suggested that conventional tDCS utilised are insufficient to induce long-lasting changes in cortical excitability. Indeed, using rat models and human cadavers, the authors (Vöröslakos et al., 2018) reported that an electrical gradient of 1 mV/mm (4 – 6 mA) would be required to influence neuronal firing rate, and activity in brain regions. However, it should be noted that Vöröslakos et al (2018) used tACS within their investigation and therefore restricts the direct comparison between NIBS techniques. It is likely that the variability in response to tDCS are due to an array of individual differences which influence the conductivity of the underlying tissues or the susceptibility of the targeted areas, these include; anatomical differences (e.g. cranial and corticospinal fluid layer thickness, cortical folding and neural circuitry organisation) (Opitz et al., 2015; Hannah et al., 2019); neurochemistry and baseline neurophysiological states (e.g. circadian rhythms, TMS sensitivity, BDNF polymorphism) (Labruna et al., 2016; Cheeran et al., 2008; López-Alonso et al., 2015); Physiological states, gender and genetics (Li et al., 2015). A limitation of the present study is that the menstrual cycle phase and use of oral contraceptives were not controlled for in the female participants. The concentration of female sex hormones has been previously shown to impact the pain response (Sherman et al., 2006; Màximo et al., 2015) and is also thought to impact sporting performance (Ansdell et al., 2019; Ansdell et al., 2020; Elliott-Sale et al., 2020; McNulty et al., 2020). The lack of control of the phase of the menstrual cycle and hormonal contraceptive use could explain some of the variability seen in the TT. However, it should be noted that removing the female participants from the statistical analysis did not change the overall outcome of the results. Therefore, it is likely that a combination of the aforementioned factors influences the response to tDCS.

Indeed, given the great deal of individual variability in the responses to tDCS it is not surprising that a growing number of studies published are reporting null effects of conventional tDCS. The experimental findings of this study provide agreement with the growing literature that conventional tDCS does not appear to be a viable method of enhancing athletic performance in both untrained and highly trained populations (Holgado et al., 2018; Holgado et al., 2019; Machado et al., 2019; Barwood et al., 2016). Furthermore, a vast amount of research is required to fully elucidate the optimal

stimulation parameters needed to elicit a reliable and repeatable response to tDCS. It is likely for this to occur individualisation of stimulation parameters would be required.

3.6 Conclusion

In conclusion, the novel application of tDCS to the left DLPFC using an ipsilateral extracephalic montage was insufficient to induce an analgesic effect to exercise-induced pain. Furthermore, as a result of the lack of analgesic effect there was no difference in TT performance between the tDCS, sham and control conditions.

Chapter 4. The application of transcranial direct current stimulation to enhance the physiological adaptation from endurance training; is it effective and can it be detected?

5.1 Abstract

Background: Acute applications of transcranial direct current stimulation (tDCS) have previously been used to enhance endurance performance. Based on this, commercial devices have been released on the market for athletes to use during training. However, it is not yet known whether the repeated use of tDCS during training will enhance the physiological adaptations. **Aim:** This study investigated whether the application of tDCS during a 6-week high intensity interval training (HIIT) programme would augment physiological adaptations to a greater extent than no stimulation. This study also investigated whether the chronic use of tDCS could be detected through changes in BDNF concentration. **Method:** Twenty recreationally active participants were randomised into two training groups (tDCS & sham) and instructed to complete a 6-week training programme consisting of 10 HIIT sessions and performance testing on a cycle-ergometer. Both experimental groups completed 2 interval sessions per week with the Halo Sport neurostimulation system (or sham) applied during the warm-up. **Results:** Peak O_2 uptake, PPO, 5 km TT performance and PO at 4 $mmoL^{-1}$ of blood lactate concentration was improved at post-intervention by 4-14% (P's ≤ 0.009), however there were no significant differences between groups (P's ≥ 0.103). There were also no changes in BDNF concentration in serum (P =0.499) or platelet-poor plasma samples (P = 0.305). **Conclusion:** The application of tDCS prior to interval training does not improve the physiological or perceptual responses during training, nor does it provide a viable means of augmenting adaptation to training. These results challenge the validity of this commercially available device.

5.2 Introduction

The physiological determinants of endurance sports are well documented, with the maximal oxygen consumption (VO_{2max}), lactate thresholds and work efficiency being considered as important variables explaining the heterogeneity of performance (Joyner & Coyle., 2008; Ronnestad et al., 2012; Midgley et al., 2007). Physiological make-up and performance aptitude are partially explained by genetics; however, endurance training elicits physiological adaptations which produce profound enhancements to performance (Laursen & Jenkins., 2002; Midgley et al., 2007). To ensure the continuation of these physiological adaptations, athletes must progressively overload training through the manipulation of intensity, duration, or frequency (Sandbakk et al., 2013; Seiler., 2010). Consequently, this has led to the creation of various training programmes including high intensity interval training (HIIT), threshold and high-volume continuous training. Conceptually, it appears that athletes are likely to match the duration or intensity to the overall perceived effort and the perceived accumulation of fatigue rather than to total energy expenditure (Seiler et al., 2013; Seiler., 2010). Therefore, minimising the RPE during training may allow an athlete to realise greater performance and physiological adaptations.

In the last decade, NIBS techniques such as tDCS have proposed to exert an ergogenic effect within endurance performance (Angius et al., 2017). Indeed, early studies employing tDCS have demonstrated an enhancement of endurance capacity within isometric (Cogiamanian et al., 2007; Williams et al., 2013; Angius et al., 2016; Abdelmoula et al., 2016) and dynamic exercise trials (Huang et al., 2019; Angius et al., 2018; Angius et al., 2019; Park et al., 2019; Vitor-Costa et al., 2015; Okano et al., 2013), which are often accompanied by parallel reductions in RPE (Angius et al., 2016; Angius et al., 2018; Angius et al., 2019; Okano et al.,2013; Cogiamanian et al. 2007). At present, the efficacy of tDCS to enhance endurance performance has come into question resulting from discrepant research outcomes, which are likely due to differing tDCS parameters and exercise paradigm (Machado et al., 2019). Nevertheless, researchers have suggested that tDCS may provide a viable means of deriving the benefits of overload, if applied during training (Williams et al., 2013). If true, tDCS may also allow for greater augmentation of physiological adaptations (Williams et al., 2013). Currently a single study has used tDCS to enhance adaptations to training (Hendy & Kidgell., 2013), finding that after 3 weeks of strength training with tDCS significant increases in markers of cortical plasticity, but no significant difference in dynamic strength of the wrist extensors. The authors concluded that the heavy training regime used may have induced excessive fatigue and muscle damage as the wrist extensors are suggested to be more accustomed to fine motor skills (Hendy & Kidgell., 2013).

Singular applications of tDCS are known to induce long lasting alterations in corticospinal excitability, extending for several hours post stimulation in both animal and human data (Nitsche & Paulus., 2001; Lang et al., 2004; Lang et al., 2005). The cellular mechanisms underpinning this effect are thought to be synonymous to the induction of E-LTP or E-LTD (Nitsche & Paulus., 2000; Hendy & Kidgell., 2013; Liebetanz et al., 2002). In support of this, BDNF (an important mediator of LTP, neural cell survival and proliferation) has been shown to increase in human and animal serum samples following the application of anodal tDCS (Frtische et al., 2010; Hadoush et al., 2018). Thus, confirming the existence

of LTP-like plasticity in the anodal tDCS induced aftereffects (Minichiello., 2019; Cocco et al., 2018; Podda et al., 2016). Multiple applications of anodal tDCS are reported to enhance the efficacy of tDCS through inducing L-LTP (Monte-Silva et al., 2013; Agboda et al., 2020; Boggio et al., 2007). Indeed, there has been some evidence that the application of tDCS over several consecutive days induces cumulative increases in corticospinal excitability (Alonso et al., 2014; Gálvez et al., 2013; Reis et al., 2009; Martin et al., 2014; Fricke et al., 2011; Hendy & Kidgell., 2013). Furthermore, clinical studies have reported that the repeated application of tDCS confers a greater and longer lasting improvement in symptoms (Boggio et al., 2007; Hadoush et al., 2018; Fridriksson et al., 2018). Therefore, tDCS could potentially be applied to optimise performance during training.

To date no studies have investigated the effects of repeated applications of tDCS during endurance training, yet tDCS is reportedly used by a number of elite endurance athletes. The use of tDCS in endurance performance is still considered to be in its experimental phase as the efficacy of tDCS to improve endurance performance, the underlying mechanisms and the safety of long-term use is still uncertain (Park., 2017; Davis., 2013). Therefore, the commercialisation and wide use of tDCS poses inherent issues. Moreover, it could be argued that the use of tDCS to enhance training and performance is a method of 'brain doping' and therefore violates the integrity of sportsmanship, and therefore requires a method of detection (Park., 2017; Davis., 2013).

Therefore, this study aimed to identify the degree to which tDCS augments physiological adaptations to endurance training. Secondly, this study also examined whether the repeated application of tDCS during a training intervention can be detected through the sampling of BDNF from human serum, platelet-poor plasma, and saliva sampling.

5.3 Methods

Participants

Twenty recreationally active participants were recruited to take part in this study. The Inclusion criteria required participants to; 1) habitually complete a minimum of 180 minutes of moderate intensity exercise per week and meet the criteria of 'recreationally active' as outlined by De Pauw et al (2013), 2) be healthy with the absence of mental health (i.e., depression, schizophrenia) or brain disorders (i.e., brain lesions, epilepsy), 3) free from surgical implants (i.e., pacemakers, cochlear hearing implants, intracranial metal implants). The study was approved by the SSES REAG (approval number: Prop 59_2017_18). All participants provided written and verbal consent before study participation.

Table 3 Baseline participant characteristics. Data presented as mean ± SD.

	tDCS (*n =10)*	**sham** (*n =10)*
Sex (M, F)	6, 4	5, 5
Age (yr)	24 ± 5	24 ± 5
Body mass (kg)	70.4 ± 10.5	67.1 ± 14.5
VO_{2peak} ($ml.kg^{-1}.min^{-1}$)	45 ± 8	46 ± 8

Pre-Post Testing Procedures

Participants completed the baseline and post-training procedures one week before and after the training intervention respectively. For both testing periods, participants completed several tests over two days, separated by a minimum of 48 hours. Participants were instructed to avoid strenuous exercise 24 hours preceding each test, as well as consuming a similar meal no later than 2 hours before each visit. Participants were also asked to abstain from the consumption of caffeine 2 hours before each test. From completing the pretesting procedures, participants were randomly and systematically (based upon baseline peak volume of oxygen consumed (VO_{2peak})) allocated to either the tDCS (6 males & 4 females) or sham (5 males and 5 females) group in a single-blind fashion.

Day One

To examine for physiological adaptations following the training intervention, participants completed a lactate profile test to identify the minimum workload eliciting 4 mmol. L^{-1} blood lactate concentration ($Power_{4mM}$) in addition to an incremental test to exhaustion to identify the VO_{2peak}, PPO and heart rate peak (HR_{peak}).

Participants completed all tests on a road bike (Merida Scultura 400, 2017 model) mounted to an electromagnetically braked ergometer (Computrainer Pro, Racer Mate Inc., Seattle WA, USA) with the rear tyre inflated to 6 Bar. Previous research has shown these ergometers to provide an accurate (± 2.5%) and reliable measure of PO (Mauger et al., 2010; Davidson et al., 2006; Sparks et al., 2016). Sparks et al (2016) recently noted that the CV for this ergometer ranged between 0.7 to 3.2% in trained cyclists. However, this ergometer is also reported to provide reliable measurements in untrained participants (Williams et al., 2012). Prior to completing any tests, participants were fitted for the correct frame size, and saddle height was adjusted as desired. The same ergometer and bike set-up were used across the entirety of the study. All tests were completed at a similar time of day in a temperature-controlled room (20 ^{o}C, relative humidity 40-50%).

Before commencing the lactate profile test, participants completed a 10-minute warm-up to habituate themselves and warm-up the ergometer's rear tyre. Immediately following

this, the ergometers were calibrated according to the manufacturer's specifications. For this, participants were required to increase their speed to 40 $km.h^{-1}$ 3-6 times to adjust the resistance on the tyre of the flywheel to an appropriate level. After a short recovery period, participants were connected to the online gas analysis system (Cortex Metalyzer 3B, Cortex Biophysik, Leipzig, Germany). This gas analyser is reported to have the following technical specifications:

- Flow: Range 0.05 – 20 L/s, Accuracy ± 2%
- O_2: Range ≤ 25% vol., Accuracy ± 0.1% vol.
- CO_2: Range ≤ 13% vol. Accuracy ± 0.1% vol.

Before every test, the gas analyser was calibrated according to the manufacturer's specifications. The gas sensors were calibrated using a two-point procedure which consisted of measurements of ambient air, and measurement of a standard compressed gas 17% O_2 and 5% CO_2. A 3-litre syringe (Hans Rudolph Inc, Kansas, USA) was used to calibrate the flow sensor and turbine.

The lactate profile test began with 5 minutes cycling at 60 W, after which power was increased by 25 W/5 min until the blood lactate concentration reached ≥ 4 mmol. L^{-1}. Capillary blood lactate was sampled from the fingertip at the 4-minute point during each increment and subsequently analysed (Biosen C-Line, EKF Diagnostic, Barbleben, Germany). According to the manufacturers, the Biosen C-Line has a CV of 1.5% (12 mmoL). The perceived pain intensity and rating of perceived exertion (RPE) was collected at the end of every stage using Cook's pain 0-10 scale, and Borg's 6-20 RPE scale (Borg., 1982) respectively. The power eliciting $Power_{4mM}$ was determined after plotting the power-lactate curve for each participant, by fitting a polynomial regression model.

After 10 minutes of recovery, participants completed a maximal incremental test to exhaustion. The test started with cycling for 1 minute at 2 $W.kg^{-1}$ (rounded down to the nearest 50 W) and subsequently increased at a rate of 20 W/min until volitional exhaustion or the inability to maintain a cadence of ≥ 60 rpm. A capillary blood lactate sample was collected 1-min post exhaustion to measure the peak lactate concentration. The highest 30 second and 5 second average of VO_2 and HR was used to define VO_{2peak} and H_{Rpeak} respectively.

Day Two

Day two consisted of a 10-minute fixed intensity cycling trial (FI) and a 5km time trial (TT) to measure for changes in performance and perceptual responses. In FI trial, participants cycled for 10 minutes at the power output equating to $Power_{4mM.}$ Throughout the trial, participants maintained a constant cadence which was replicated when this trial was repeated on weeks two, four and at post training. Pain intensity and RPE were recorded at the end of every minute. A telemetric device (Polar FT1, Polar Electro Oy, Kempele, Finland) was also used to record the HR at the end of every minute. The FI trial was used to examine the change in tolerance to the perceptual sensations generated in exercise, such as EIP and RPE. Therefore, as Power4mM was set as a minimum load throughout the interval training, we expected to see a greater reduction in the perceptual responses within the tDCS condition.

Following 10 minutes rest, participants completed the 5km TT. Although a 5km cycling TT is not a conventional distance, this distance was selected based upon previous findings of its validity to assess changes in aerobic endurance (Dantas et al., 2015). Participants were instructed to complete the 5km TT in the quickest time possible, manipulating workload through pedalling frequency and gearing in the same manner as they would on a flat road. The computrainer ergometers were connected to a central PC running dedicated software (PerfPRO studio, Hartware Technologies, Rockford, MI), allowing the continuous recording of PO and HR. Participants were given feedback on the distance that they had covered during the trial but were blinded to time elapsed, and power output. Pain intensity and RPE were recorded for every km completed.

Intervention Period

Training Organisation

Over 6 weeks participants were prescribed 10 supervised HIIT sessions in addition to two supervised mid-intervention testing points and ad libitum self-organized moderate to low intensity endurance or strength training. The intervention period was split into three mesocycles, each lasting two weeks which aimed to progressively overload participants. Participants attended the laboratory twice a week over the 6-week intervention period to complete the supervised sessions. Mesocycle 1 consisted of three HIIT sessions comprised of 4x8 minute intervals with 4 minutes of active recovery and 1 mid-intervention testing session. Mesocycle 2 consisted of three HIIT sessions comprised of 4x8 minute intervals with 3 minutes of active recovery, and 1 mid-intervention testing session. The third mesocycle consisted of 4 HIIT sessions comprised of 4x8 minute intervals interspersed with 2 minutes of active recovery (figure 14). The two mid-intervention testing points completed in the first and second mesocycles consisted of the 10-minute FI cycle and the 5km TT (see pre-post testing, day two).

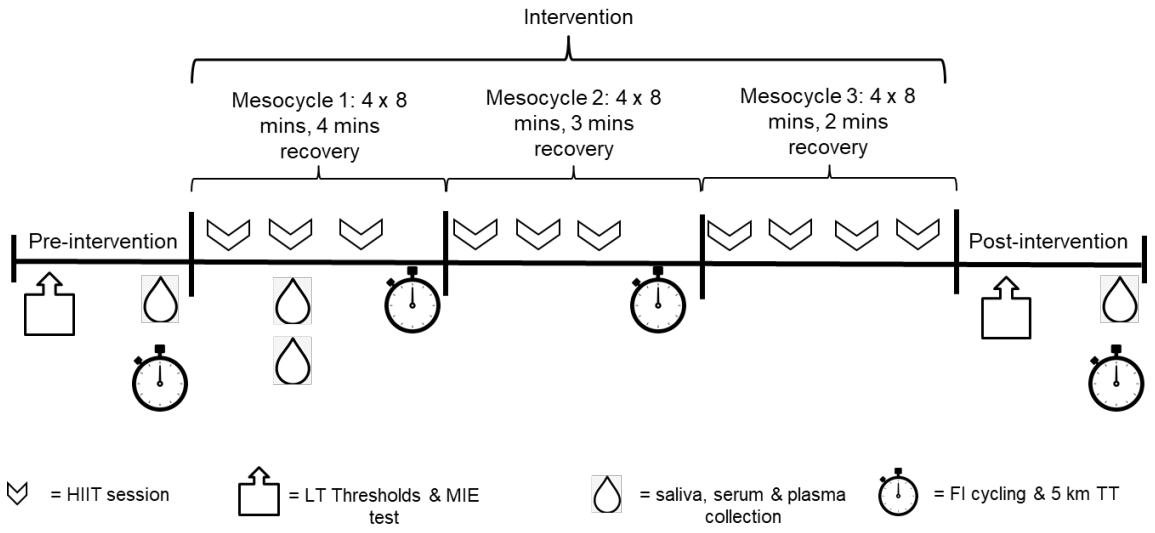

Figure 14. Schematic of study protocol.

Interval Sessions

Participants completed all HIIT sessions indoors in a temperature-controlled room (18-20 ° C, relative humidity 50-60%) as a supervised group interval session. Each session included 20 minutes of either tDCS or sham stimulation (see transcranial direct current stimulation procedures), a 10-minute standardized warm up followed by 4 interval bouts of 8 minutes in duration followed by a period of active recovery and concluded with up to a 10-minute low intensity cool down (Figure 15).

Throughout the four interval bouts, participants were instructed to cycle at their maximal sustainable intensity (isoeffort), where they were able to complete the described session structure and with either an even or progressive pacing strategy from the first to the fourth interval bout. Participants individually selected their cadence for each interval bout and were able to manipulate cycling load electronically by adjusting the ergometer with ± 3W precision. Participants were provided continuous feedback on time elapsed, absolute, and average power, HR, and cadence on a large video screen. Participants reported their RPE and pain intensity at the end of each interval bout. Session RPE (sRPE) was recorded 30 minutes after the end of each interval session.

The physiological responses to the HIIT sessions were indexed according to the physiological values for HR_{peak}, PPO and $Power_{4mM}$ measured at baseline.

Training Monitoring

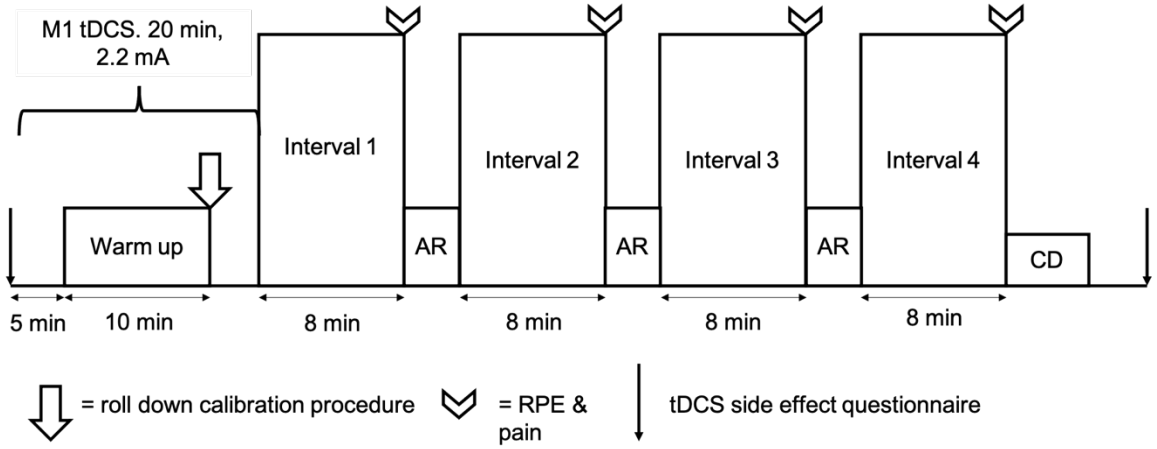

Figure 15. Schematic of the 4 x 8 min HIIT sessions.

All participants were provided with a training diary to record their training throughout the baseline testing week and the following 6 week-intervention period. The following variables were registered for each training session: 1) description of activity. Training activity was later categorised to endurance, strength, or other training (team sports, stretching, yoga) to calculate the volume of training completed in each type. 2) total duration of activity and 3) sRPE (1-10) (Foster et al., 2001) 30 minutes post-exercise cessation. Training load was calculated using the session RPE method where session RPE was multiplied by duration accumulated in the activity. Participants were also asked to maintain a similar diet and to abstain from the introduction of new supplements

throughout the intervention period, adherence to this was confirmed after the completion of the study.

Transcranial Direct Current Stimulation Procedures

Transcranial direct current stimulation was delivered during the supervised interval sessions. Before the intervals commenced, participants received 20 minutes of tDCS via a commercial device, the Halo Sport (Halo Neuroscience, San Francisco, USA). This tDCS device has an output precision of ± 10%. Three studded electrodes (24 cm^2) dampened with water were connected to the tDCS device and placed over the vertex to deliver bihemispheric stimulation of the M1. The anodal electrode was placed horizontally over the Cz, whilst the cathodal electrodes were placed over the C5 and C6 according to the 10-20 EEG system. In the tDCS condition, participants received 2.20 mA tDCS (level 10 amperage on device; current density 0.0916 mA/cm^2) for a duration of 20 minutes. At the start and end of the stimulation periods, the current intensity was ramped up and down over a period of 30 seconds. In the sham condition, the tDCS device ramps up over a 30 second period to 1.8 mA (level 5 amperage on tDCS device; current density 0.075 mA/cm^2), after which the intensity ramped down over 30 seconds to 0 mA.

Participants rested quietly for the first 5 minutes of tDCS, which was followed by the completion of the 10 minute standardised warm up and calibration procedure (Figure 15). This was conducted to allow participants to acclimate fully to the stimulation (Mariano et al., 2016; Fecteau et al., 2007). After completion of the standardized warm-up and calibration procedures, participants lightly stretched until the stimulation duration had finished.

Participants were required to complete a self-reported ‘adverse effects questionnaire’ at the start and end of each training session to classify the within-session and between-session occurrence of side effects. The questionnaire included a list of twelve side effects indexed from commonly reported adverse effects (Brunoni et al., 2011; Kessler et al., 2012; Poreisz et al., 2007) including tingling, itching, burning sensation, nausea, headaches, neck pain, scalp pain, blurred vision, mood change, difficulty concentrating, and dizziness. Participants reported the incidence of each side effect using a binary code

system (no= 0, yes= 1), as well as evaluating the severity of the side effect (minimal = 1, mild= 2, moderate=3, severe= 4).

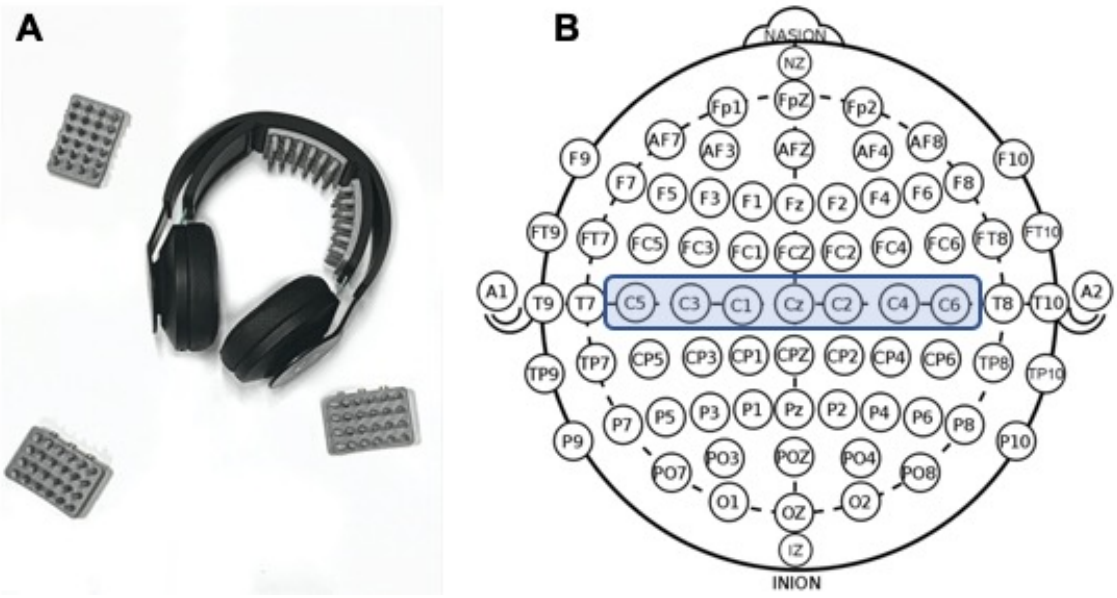

Figure 16. The Halo Sport Neurostimulation System (A) and the placement of the device (horizontal bar) on the 10-20 EEG system (B)

Methods of Blood and Saliva Sampling

To assess for chronic changes in BDNF concentration, blood (serum and platelet-poor plasma) and saliva samples were collected on day 2 of the pre-intervention and post-intervention week. All samples were collected at the same time of day to prevent any diurnal variations, in a 10-minute window before exercise commenced. In the last 10 participants (n= 5 tDCS group, n = 5 sham group), blood samples were taken to assess for acute changes in the peripheral BDNF concentration. Therefore, in these participants venous blood samples were collected 10 minutes before the application of tDCS and immediately following the completion of the HIIT session.

Saliva Samples

The passive expectoration technique was used to collect 1 mL of unstimulated whole resting saliva in a 30 mL sterile specimen tube from the participants. Ten minutes prior to providing a saliva sample, participants were asked to thoroughly rinse their mouth to remove debris from the oral cavity. Immediately prior to collection participants were also asked to empty their oral cavity by swallowing, after which the stopwatch was started, and collection began. Participants were asked to passively drool into the collection tubes whilst remaining seated, head titled, chin to chest making minimal orofacial movements. Saliva was collected for a minimum duration of 2 minutes or until 1 mL of saliva was deposited into the collection tubes. After collection, tubes were weighed to the closest mg to calculate the volume of saliva. Samples were then centrifuged for 5 minutes at 17,000 x g to remove debris (Accuspin Microspin 17R, Fisher Scientific, Hampton, New Hampton, USA). Ahead of analysis, the supernatant was aliquoted into 1.5 mL microcentrifuge tubes and stored at -80 ^{o} C.

Blood Samples

Standard venepuncture techniques were used to sample blood from the antecubital vein into 6 mL vacutainer (Becton-Dickinson, Oxford, UK) tubes (containing K_3EDTA for separation of plasma and silicone coated for the separation of serum). Plasma samples were centrifuged within 20 minutes of sampling at 1500 x g at 4 ° C, after which the samples were aliquoted into three 1.5 mL microcentrifuge tubes. Two of these tubes were frozen as platelet-rich plasma, whilst the third tube was centrifuged for a further 10 min at 10,000 x g at 4 ° C to obtain platelet-poor plasma. Serum samples were left at room temperature for an hour to clot before being centrifuged. Both serum and plasma samples were stored at -80 ° C as aliquots until analysis.

Enzyme-linked immunosorbent Assay

The serum, platelet-poor plasma and saliva samples were analysed for BDNF concentration via enzyme linked immunosorbent assay (ELISA), using a commercially available kit (DBNT00 Total BDNF Quantikine Kit, R&D systems, Minneapolis, MN, USA). According to the manufacturers, this assay has an intra-assay CV of 2.87% ± 0.42%. All samples were analysed according to the standard protocol provided by the manufacturer, validated for human serum and platelet poor plasma samples, but not saliva. All samples and standards were added in duplicate to a flat-bottom 96 well microplate coated in monoclonal antibody specific for BDNF. Prior to sample addition, 50 μL of a buffered protein solution was added to each well. For each kit, standards were created fresh, and samples were diluted prior to addition to the well. Serum samples were diluted 100-fold with assay diluent, whilst platelet poor plasma and saliva underwent a 5-fold and 2-fold dilution respectively. 50 μL of samples and standards were added to the wells and incubated at room temperature for 2 hours on a horizontal orbital shaker, followed by four wash/aspiration cycles with the appropriate wash buffer. Plates underwent another 1-hour incubation under shaking conditions after the addition of 200 μL monoclonal antibody conjugated to horseradish peroxide. After another wash/aspiration cycle, the plates were then incubated for 30 minutes in a substrate solution of hydrogen peroxide and tetramethylbenzidine to induce a colour reaction. The reaction was stopped with 50 μL of 2 N sulfuric acid. A microplate reader (FLUOstar OPTIMA, BMG Labtech, Durham, NC, USA) set at 450 / 530 nm was used to read the optical density of the colour reaction.

Analysis of BDNF Genotype

Participants were genotyped for BDNF polymorphism post-hoc via quantitative real time polymerase chain reaction using a Roche Light cycler 96 device (Roche, Basel, Switzerland). Prior to analysis, genomic DNA was isolated from serum samples using the QIAamp DNA blood mini kit (QIAgen GmbH, Hilden, Germany), and were subsequently frozen at -80 ° C for later analysis. Genotyping of the rs6265 SNP of the BDNF polymorphism was conducted using the rhAMP assay (Integrated DNA Technologies, Coralville, Iowa, USA) according to the manufacturer's specifications. Participants were categorized as either G: G homozygotes (val66val carriers), or carriers of the recessive A allele (A: A homozygotes (met66met carriers) or G: A (val66met carriers).

Statistical Analysis

Normality and equality of variances of the data were assessed via the Shapiro Wilk test and Levene's test respectively. Sphericity of the data was assessed by Mauchly's test, which if violated was corrected by the Greenhouse-Geiser adjustment to the degrees of freedom. Where appropriate, post-hoc tests were conducted with the Bonferroni correction applied.

An independent samples T-Test was used to determine the existence of baseline differences in VO_{2peak}, PPO, and 5km TT performance between the tDCS and sham condition. Due to the violation of normality, baseline differences in $Power_{4mM}$ between the tDCS and sham condition were assessed by the Mann-Whitney U test.

To assess for changes in VO_{2peak}, PPO, $Power_{4mM,}$ the fractional use of VO_{2peak} at VO_2 corresponding to $Power_{4mM}$ (%VO_{2peak}@4 mM) and 5km TT performance from baseline to post-intervention measurements for both the tDCS and sham condition, a 2 x 2 factorial analysis of variance (ANOVA) was used. Changes in concentration of BDNF in serum, saliva, and platelet poor-plasma from baseline measurement to post-intervention as well as pre and post a single training session were also analysed using a 2 x 2 factorial ANOVA. To assess the influence of BDNF polymorphism on the changes in physiological and performance measures, a two-way analysis of covariance (ANCOVA) was performed on the TT performance and $Power_{4mM}$ data, controlling for BDNF polymorphism as a covariate. To assess the influence of BDNF polymorphism on baseline serum BDNF concentration and the chronic change in serum BDNF concentration across the intervention, a one-way ANCOVA was performed.

Differences in perceptual (RPE and pain) responses recorded during the FI cycling trial collected at baseline, week 2, week 4 and post-intervention were analysed by a 2 x 4 factorial ANOVA. A 2 x 4 factorial ANOVA was also used to assess differences in TT performance, average HR, RPE and Pain for TT performed at baseline, week 2, week 4, and post-intervention. For each variable, data recorded from km 1-5 were averaged to analyse the average differences in performance over the course of the study. To assess for differences in PO during the time trial, the percent change from pre-training to post-training, pre-training to week 2, pre-training to week 4, week 2 to post-training and week 4 to post-change were calculated. A 2 x 3 factorial ANOVA was performed on the percent change from kilometres 1, 3 and 5.

A 2 x 3 factorial ANOVA was used to assess for within training differences between the tDCS and sham group. For this, a single session from each mesocycle were selected for comparison (session 1, session 5 and session 10). For each of the selected sessions, absolute PO, PO relative to body mass, HR, pain, and RPE were averaged over the four interval bouts. A 2 x 3 factorial ANOVA was also used to analyse sRPE reported in the participants training diaries for these training sessions.

To assess for the stability of ad libitum additional training completed, a 2 x 7 factorial ANOVA analysed weekly training volume and load from the pre-intervention period to the end of the intervention period. Additionally, independent samples T-Test were employed to assess for differences in overall training volume, load, and percentage of sessions completed as strength training. For analysis of the percentage of sessions completed as endurance or other, Mann-Whitney U tests were completed.

All data were analysed using SPSS 27.0 (SPSS, IBM Corp, Armonik, NY, USA) and the significance level was set at $p = 0.05$. Data is presented as mean ± standard deviations. Effect sizes were calculated to estimate the size of the differences between the tDCS and sham group. In the ANOVA and ANCOVA, effect sizes are reported according to partial eta-squared (η_p^2) (small = 0.01, medium = 0.06, large = 0.14). For the results of the independent samples T-Test and Mann-Whitney U test are reported according to Cohen's *d* (small = 0.2, medium = 0.5, large = 0.8).

5.4 Results

A total of 19 participants completed the study, one participant withdrew after completing week 4 of the intervention. As such their results have been removed from statistical analysis of baseline and post-intervention measures but have been retained for analysis of the HIIT training prescription. Participants in both the tDCS and sham group reported minimal tingling itching and burning sensations on the scalp during the stimulation period, however these side effects did not persist once the tDCS device was removed or after the session.

Physiological Responses

At baseline there was no differences between the tDCS and sham groups for VO_{2peak} (t (18) = -0.1, $P = 0.891$, $d = 0.12$) or $Power_{4mM}$ (tDCS 133 ± 31 W, sham 134 ± 19 W, t (18) = -0.1, P =0.880, $d = 0.16$). After the 6-week intervention, VO_{2peak} significantly increased ($F_{1, 17} = 8.8$, $P = 0.009$, $\eta_p^2 = 0.34$, figure 17A) for both the tDCS (45 ± 8 ml.kg.min^{-1} to 48 ± 8 ml.kg.min^{-1}) and sham groups (46 ± 7 ml.kg.min^{-1} to 49 ± 6 ml.kg.min^{-1}). There was however no significant group × time interaction ($F_{1, 17} = 0$, $P = 0.835$, $\eta_p^2 = 0.001$) or main effect of groups ($F_{1, 17} = 0.3$, $P = 0.608$, $\eta_p^2 = 0.02$) detected. Likewise, after the intervention $Power_{4mM}$ significantly increased from baseline for both groups ($F_{1, 17} = 11.8$, $P = 0.003$, $\eta_p^2 = 0.41$, Figure 17C), but there was no significant group × time interactions ($F_{1, 17} = 0.2$, $P = 0.649$, $\eta_p^2 = 0.01$) or main effects of group ($F_{1, 17} = 0.2$, $P = 0.635$, $\eta_p^2 = 0.01$) were detected.

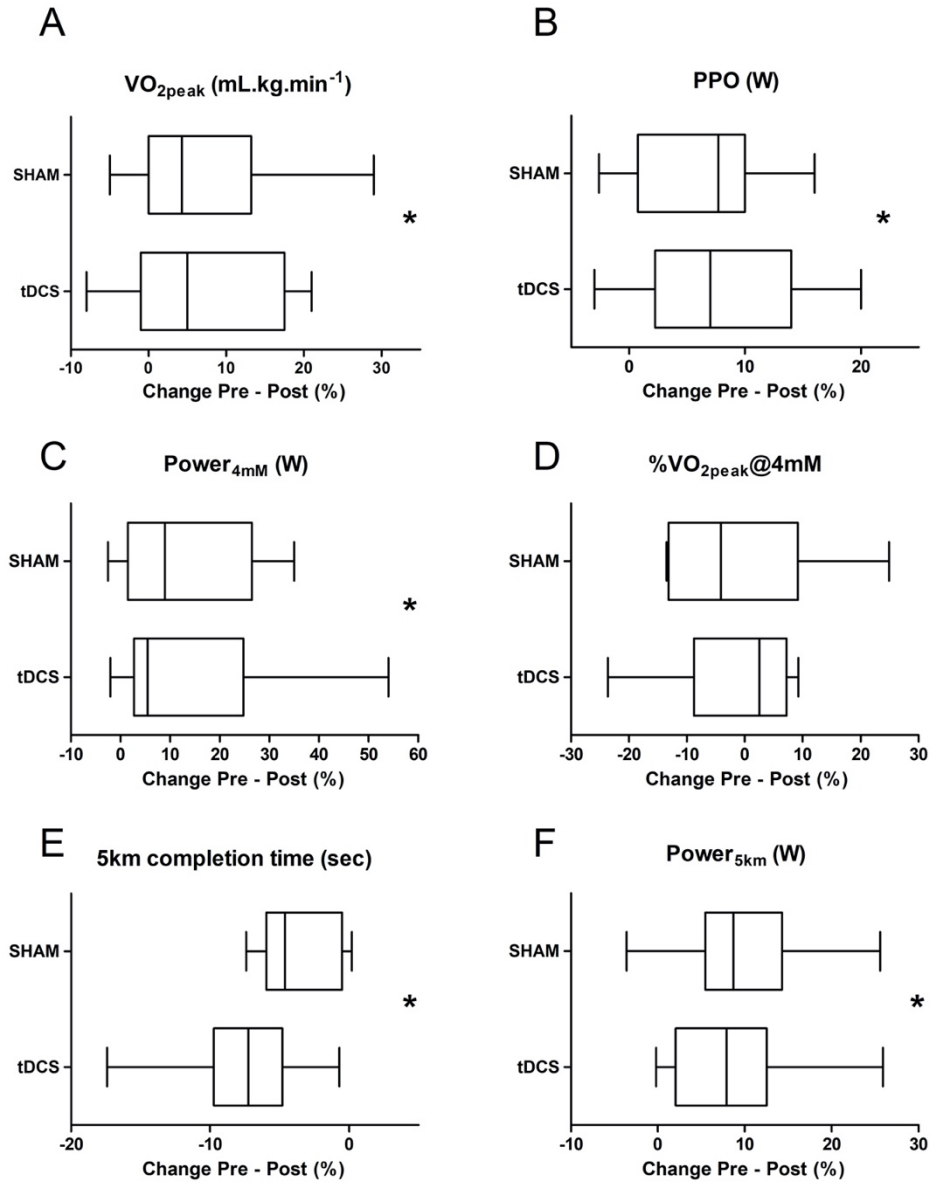

*Figure 17. 95% confidence intervals for relative change after the 6-week HIIT intervention with tDCS in VO_{2peak} (A), PPO (B), $Power_{4mM}$ (C), $\%VO_{2peak}$@4mM (D), 5km completion time (E) and $Power_{5km}$ (F) in the tDCS (n = 10) and sham (n = 9) groups. $Power_{4mM}$, power output eliciting 4mmoL of lactate; $\%VO_{2peak}$@4mM, the percentage of VO_{2peak} uptake at 4mmoL of lactate; $Power_{5km}$, average PO during the TT. * Denotes a significant main effect of time.*

Performance Responses

At baseline there were no differences between the tDCS and sham group for PPO (tDCS 239 ± 50 W, sham 236 ± 41 W, t (18) = 0.1, P = 0.924, d = 0.17) and 5 km TT performance (tDCS 576 ± 119 s, sham 587 ± 37 s, t (18) = -0.2, P = 0.873, d = 0.12). Peak power output improved from baseline in both the tDCS and sham groups ($F_{1, 17}$ = 20.4, $P \leq 0.001$, η_p^2 = 0.55, Figure 17B). However, no significant group × time interactions ($F_{1, 17}$ = 0.2, P = 0.630, η_p^2 = 0.01) or main effect of group ($F_{1, 17}$ = 0.2, P = 0.672, η_p^2 = 0.01) were detected. In comparison to baseline, participants in both the tDCS and sham

condition completed the 5 km TT quicker at post-intervention ($F_{1,17}$ = 23.2, $P \leq 0.001$, η_p^2 = 0.58, Figure 17E), however no group × time interactions ($F_{1, 17}$ = 0.2, P = 0.103, η_p^2 = 0.15) or main effect of condition ($F_{1, 17}$ = 0.4, P = 0.528, η_p^2 = 0.02) were detected. The average PO cycled at during the 5km TT also increased from baseline for both the tDCS and sham groups ($F_{1,17}$ = 27.1, $P \leq 0.001$, η_p^2 = 0.61, Figure 17F), however there were no group × time interactions ($F_{1, 17}$ = 0.4, P = 0.681, η_p^2 = 0.01) or main effect of condition ($F_{1,17}$ = 0.03, P = 0.517, η_p^2 = 0.03) detected.

Time trials were completed on four occasions throughout the study; baseline and post-intervention in addition to trials completed on week 2 and week 4 during the HIIT intervention period. A significant improvement in the time taken to complete the 5km TT across the study occurred for both tDCS and sham conditions ($F_{1.5, 18.4}$ = 10.3, P = 0.002, η_p^2 = 0.41), but no group × time interactions ($F_{1,12}$ = 1.5, P = 0.242, η_p^2 = 0.11) or significant main effects of group ($F_{1,12}$ = 0.0, P = 0.965, η_p^2 = 0.0) occurred.

The power profile performed during the TT's were compared across the study through calculation of the percent change (Figure 18). No group x distance interaction existed for the percent change between pre- and post-training ($F_{1,.5, 25}$ = 2.5, P = 0.112, η_p^2 = 0.13), nor was there a main effect of group ($F_{1,17}$ = 0.3, P = 0.569, η_p^2 = 0.02). A main effect of distance was detected, where the change in power output at 1 km (tDCS 22 ± 23%; sham 20 ± 13%) and 3km (tDCS 18 ± 6%, sham 10 ± 12%) was greater than 5 km (tDCS -11 ± 23%; sham 6 ± 19%) in both the tDCS and sham group (P = 0.017, CI = 3.7 – 43.2%). However, post-hoc comparisons revealed no differences between the change in PO at 1km and 3 km (P = 0.44, CI = -5.1 – 18.7%). When the change in PO was compared between pre-training and week two, no group x distance interaction ($F_{1.4, 25}$ = 0.6, P = 0.521, η_p^2 = 0.03) or main effects of group were detected $F_{1,17}$ = 2.4, P = 0. 140, η_p^2 = 0.12). A main effect of distance was detected ($F_{1.4, 25}$ = 8.0, P = 0.005, η_p^2 = 0.32) where the change in PO was greater at 1 km and 3 km than 5km (P = 0.024, CI = 1.4 – 22.2 %). No difference was detected in the change in PO between 1 km and 3 km (P = 1.000, CI = -4.3 – 9.0%). No group x distance interaction was detected between pre-training and week four ($F_{2,32}$ = 1.5, P = 0.229). There was also no significant main effect of group ($F_{1,16}$ = 3.7, P = 0.072, η_p^2 = 0.19). A main effect of distance was detected ($F_{2,32}$ = 7.0, P = 0.003, η_p^2 = 0.31). Indeed, the change in PO was greater at 1km when compared to 5 km (P = 0.019, CI = 3.3 – 42.1%). However, there were no significant differences between the change in PO at 1 km and 3 km (P = 1.000, CI = -7.5 – 16.2%). Nor were there any differences detected between 3km and 5km (P = 0.064, CI = -0.9 – 37.7%). Despite the large effect size, there was no group x distance interaction was detected for the change in PO between week two and post-training ($F_{2,34}$ = 2.8, P = 0.075, η_p^2 = 0.14). This result is possibly due to the large variation seen at 5km (Figure 18D). No main effects of group were detected for the percent change in PO when post-training was compared to week two ($F_{1,17}$ = 1.0, P = 0.320, η_p^2 = 0.06). There was also no main effect of distance ($F_{2,34}$ = 1.4, P = 0.253, η_p^2 = 0.08). When the percent change in PO was compared between post-training and week four, no group x distance interaction was observed ($F_{2,32}$ = 0.2, P = 0.838, η_p^2 = 0.01). Nor was there a main effect of group ($F_{1,16}$ = 3.0, P = 0.103, η_p^2 = 0.16) or a main effect of distance ($F_{2,32}$ = 0.1, P = 0.947, η_p^2 = 0.00, Figure 18E).

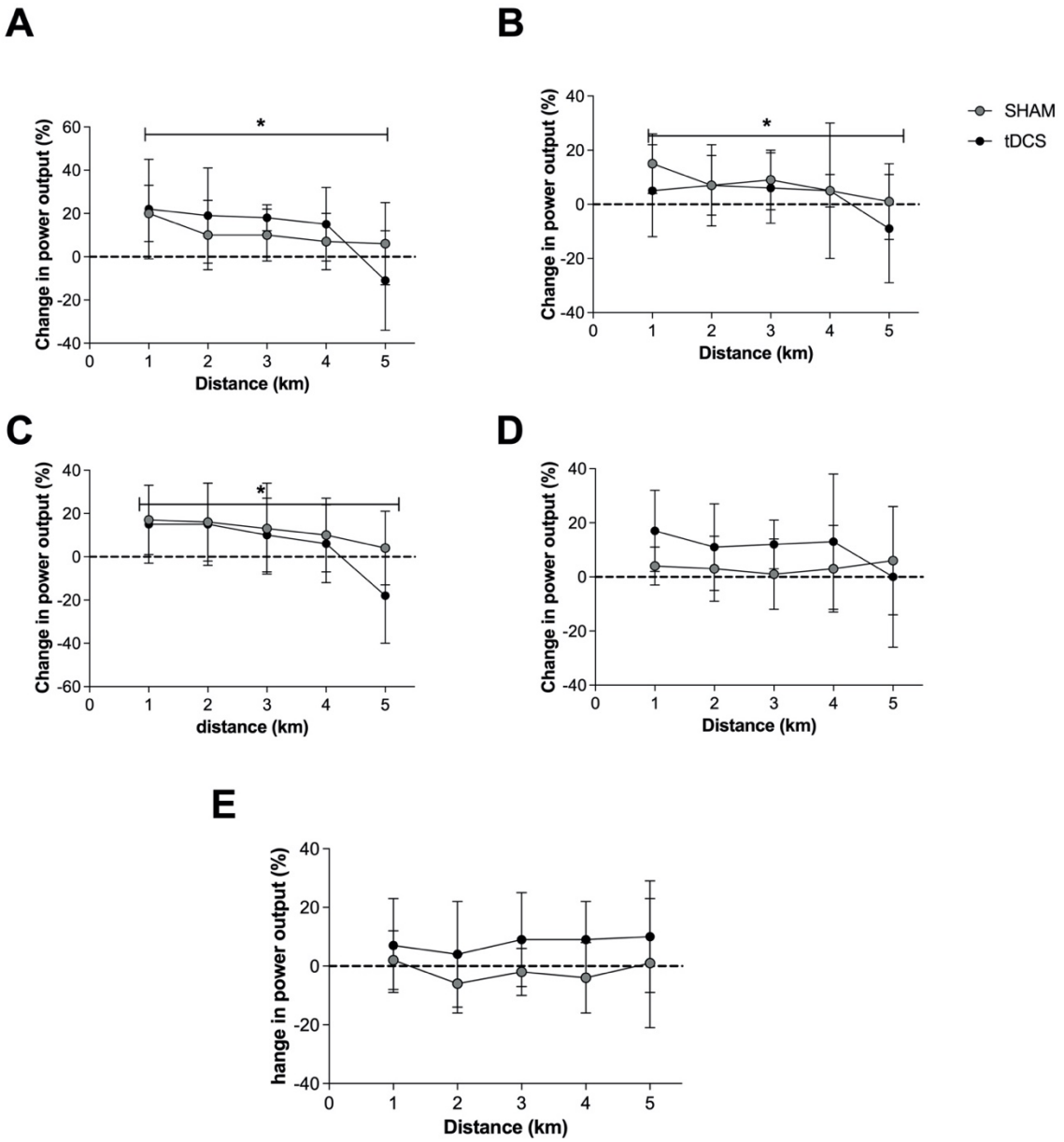

*Figure 18. Percent change in power output recorded within the 5 km TT. Panel A compares the change in power output between pre- and post- training. Panel B compares the change between pre-training and week two. Panel C displays the change in power between pre-training and week four. Panel D shows the percent change in power output between week two and post-training. Panel E presents the percent change in power output between week four and post-training. * Denotes a significant main effect of distance.*

Perceptual Responses

A main effect of time was detected for the average RPE reported during the 5 km TT ($F_{3,36}$ = 11.7, $P \leq 0.001$, $\eta_p^2 = 0.50$). Post-hoc analysis revealed that in comparison to the average RPE at baseline, RPE's reported at week 2 ($P = 0.025$), week 4 ($P = 0.046$) and post-intervention ($P = 0.003$) were significantly greater. However, no group × time interaction ($F_{3,36} = 0.6$, $P = 0.645$, $\eta_p^2 = 0.04$) or main effect of group occurred ($F_{1,12} = 0.8$, $P = 0.381$, $\eta_p^2 = 0.07$). There were no significant group-time interactions ($F_{1.6, 19.4} = 2.2$, $P = 0.141$, $\eta_p^2 = 0.16$), main effect of group ($F_{1, 12} = 0.5$, $P = 0.481$, $\eta_p^2 = 0.04$) or time ($F_{1.6, 19.4} = 0.6$, $P = 0.539$, $\eta_p^2 = 0.05$) found for the average rating of pain during the TT.

A main effect of time ($F_{3, 36} = 7.2$, $P = 0.001$, $\eta_p^2 = 0.38$) was also observed for the average RPE reported in the FI cycling trial. Post-hoc analysis revealed a significant increase in RPE at week 2 in comparison to the post-intervention trial ($P = 0.048$), however no group-time interactions ($F_{3, 36} = 0.3$, $P = 0.796$, $\eta_p^2 = 0.03$), or main effect of group ($F_{1, 12,} = 0.0$, $P = 0.943$, $\eta_p^2 = 0.0$) were observed. For the average pain reported during the FI trial, there were no significant group-time interactions ($F_{3, 36} = 1.4$, $P = 0.246$, $\eta_p^2 = 0.11$) or main effect of group ($F_{1, 12} = 0.8$, $P = 0.390$, $\eta_p^2 = 0.06$). A significant main effect of time for average pain during the FI trial was detected ($F_{3, 36} = 0.9$, $P = 0.035$, $\eta_p^2 = 0.21$), however post-hoc analysis failed to detect a difference (P's ≥ 0.416).

BDNF concentration and genotyping

Serum, platelet-poor plasma, and saliva samples were collected at baseline and post-intervention to analyse for changes in BDNF concentration. The BDNF concentration in thirty-three out of 38 saliva samples fell below the calibrated standard curve, leaving the remaining five samples without a comparator. All detectable samples were from samples collected at the pre-testing point across both the tDCS ($n = 3$) and sham ($n = 2$) groups. As such, saliva samples were omitted from statistical analysis. Saliva samples have previously been shown to have a low concentration, therefore the 2-fold dilution used in this study may have reduced the detectability. Furthermore, the commercial ELISA kit used is not validated to detect BDNF from saliva, indicating that saliva is not a valid means of BDNF detection.

In total 19 serum samples (tDCS $n = 10$, sham $n = 9$) were analysed for difference in BDNF concentration after the training intervention, one participant's baseline measurement was removed from analysis due to study withdrawal. There were no group × time interactions ($F_{1, 17} = 0.5$, $P = 0.499$, $\eta_p^2 = 0.03$), main effect of group ($F_{1, 17} = 0.4$, $P = 0.557$, $\eta_p^2 = 0.02$) or time ($F_{1, 17} = 0.1$, $P = 0.707$, $\eta_p^2 = 0.01$) for BDNF concentration detected in serum samples. Platelet poor plasma samples from 17 (tDCS n = 9, sham n = 8) participants were analysed for differences in BDNF concentration, finding no group x time interactions ($F_{1, 15} = 1.1$, $P = 0.305$, $\eta_p^2 = 0.07$), main effects of group ($F_{1, 15} = 0.631$, $P = 0.439$, $\eta_p^2 = 0.04$) or time ($F_{1, 15} = 1.1$, $P = 0.305$, $\eta_p^2 = 0.07$) (Figure 19B).

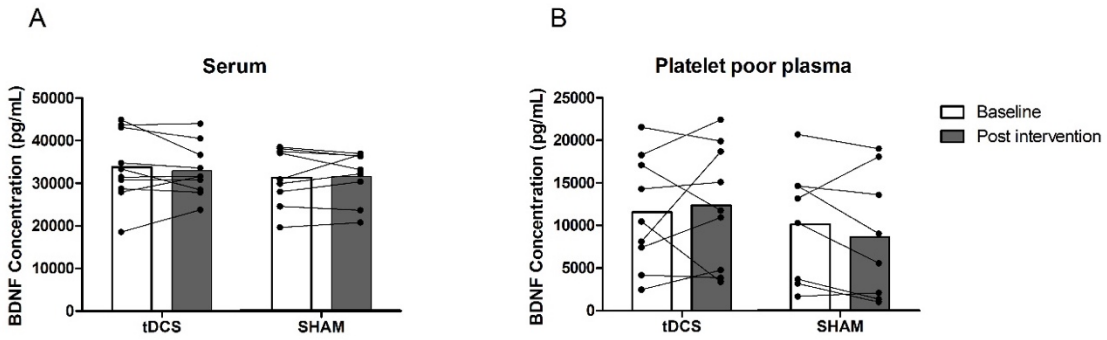

Figure 19. The concentration of BDNF collected within serum (A) and platelet-poor plasma (B) samples at baseline and after the 6-week intervention period. Bars signify the mean response; individual lines represent the response by each participant.

In the last 10 participants (tDCS *n* = 5, sham *n* = 5) serum and platelet-poor plasma samples were collected before and after a single training session to analyse the acute effects of tDCS on BDNF concentration (Figure 20). Serum samples of nine participants (tDCS *n* = 5, sham *n* = 5) were analysed for the acute effects of tDCS on serum BDNF. Samples from one participant was removed as an outlier as the concentration was more than three SD away from the mean. There was no group × time interaction ($F_{1,7}$ = 4.2, P = 0.079, η_p^2 = 0.38), main effect of group ($F_{1,7}$ = 0.3, P = 0.643, η_p^2 = 0.03) or time ($F_{1,7}$ = 0.4, P = 0.543, η_p^2 = 0.06) detected for acute changes in BDNF concentration of serum samples. Likewise, nine participants platelet-poor plasma samples (tDCS *n* = 4, sham *n* = 5) were analysed, revealing no significant group x time interactions ($F_{1,7}$ = 0.1, P = 0.717, η_p^2 = 0.02), main effects of group ($F_{1,7}$ = 1.8, P = 0.220, η_p^2 = 0.21) or time ($F_{1,7}$ = 0.1, P = 0.993, η_p^2 = 0.01).

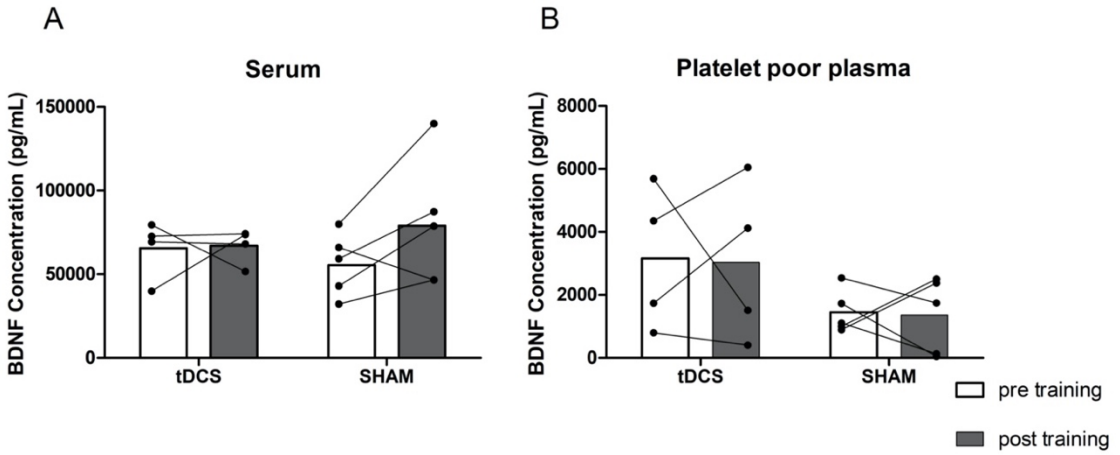

Figure 20. BDNF concentration measured within serum (A) and platelet-poor plasma before and after as single training session, sampled from the final 10 participants. Bars signify the mean response; individual lines represent the response from each participant.

For the post-hoc analysis of BDNF polymorphism, genotyping of the rs6265 SNP revealed that nine participants were categorized as homozygous for the G allele (G: G, i.e., val66val), and one participant was homozygous for the A allele (A: A, i.e., met66met) in the tDCS condition. In the sham condition, two participants were homozygous for the G

allele (val66val), two participants were homozygous for the A allele (met66met), and five participants were categorized as heterozygous (G: A, val66met). When assessing for the effects of BDNF polymorphism and tDCS condition on $Power_{4mM}$, no BDNF polymorphism × condition interaction was detected ($F_{1,13} = 3.2$, $P = 0.096$, $\eta_p^2 = 0.20$). No main effect of condition were detected either ($F_{1,13} = 2.4$, $P = 0.143$, $\eta_p^2 = 0.16$). A significant influence of BDNF polymorphism on post-training Power4mM was detected ($F_{1,13} = 4.6$, $P = 0.031$, $\eta_p^2 = 0.41$), however pairwise comparison with the Bonferroni correction failed to detect a between group difference (P's ≥ 0.052). When the influence of BDNF polymorphism and tDCS condition on 5 km TT performance was assessed, no polymorphism × condition interactions ($F_{1,13} = 0.001$, $P = 0.975$, $\eta_p^2 \leq 0.001$), main effects of condition ($F_{1,13} = 4.0$, $P = 0.067$, $\eta_p^2 = 0.24$) or main effects of polymorphism ($F_{1,13} = 3.0$, $P = 0.072$, $\eta_p^2 = 0.33$) were detected. The polymorphism of BDNF did not influence the BDNF concentration measured within serum samples at baseline ($F_{1,15} = 0.3$, $P = 0.763$, $\eta_p^2 = 0.04$). The BDNF polymorphism also had no influence on the percent change in serum BDNF concentration from baseline to post-intervention ($F_{1,15} = 0.5$, $P = 0.598$, $\eta_p^2 = 0.07$).

Physiological and perceptual responses to HIIT training

The physiological and perceptual responses of both the tDCS and sham group within the three training mesocycles are presented in table 4. Participants in both the tDCS and sham groups completed each session at a relatively constant PO, with no significant group × time interaction ($F_{1.4, 21.2} = 0.2$, $P = 0.748$, $\eta_p^2 = 0.01$), main effect of group ($F_{1, 15} = 0.1$, $P = 0.733$, $\eta_p^2 = 0.01$) or time ($F_{1.4, 21.2} = 1.6$, $P = 0.219$, $\eta_p^2 = 0.10$) being detected. There was also no significant group × time interaction effect ($F_{2, 30} = 0.6$, $P = 0.563$, $\eta_p^2 = 0.04$), main effect of group ($F_{1, 15} = 2.9$, $P = 0.109$, $\eta_p^2 = 0.16$) or time ($F_{2, 30} = 0.4$, $P = 0.672$, $\eta_p^2 = 0.03$) detected for average HR. Despite the prescription of cycling at the maximal sustainable intensity, there was a significant main effect of session found for the average RPE ($F_{1.4, 21.7} = 4$, $P = 0.045$, $\eta_p^2 = 0.21$). However post-hoc analysis of the session difference in RPE failed to find any between session differences ($P \geq 0.063$). No group × time interactions ($F_{1.4, 21.7} = 2.5$, $P = 0.116$, $\eta_p^2 = 0.14$) or main effect of group ($F_{1, 15} = 1.7$, $P = 0.206$, $\eta_p^2 = 0.10$) were detected for the RPE within the HIIT sessions. No differences were detected in the average pain reported throughout the HIIT sessions (group × time interaction $F_{2, 30} = 0.1$, $P = 0.896$, $\eta_p^2 = 0.01$; main effect of group $F_{1, 15} = 0.3$, $P = 0.579$, $\eta_p^2 = 0.02$; main effect of time $F_{2, 30} = 0.1$, $P = 0.867$, $\eta_p^2 = 0.01$).

Table 4 the physiological and perceptual responses during the 4x8 min intervals. Recovery periods decreased with each mesocycle and therefore were executed as mesocycle 1- 4 min recovery; mesocycle 2- 3min recovery; mesocycle 3, 2 min recovery. Data presented as mean ± SD

	tDCS			**sham**			***P***
	MC 1	MC 2	MC 3	MC 1	MC2	M3	
Power (W)	145 ± 32	147 ± 28	149 ± 21	142 ± 27	142 ± 23	144 ± 21	0.748
Power (W.kg)	2.0 ± 0.3	2.0 ± 0.3	2.1 ± 0.3	2.3 ± 0.5	2.3 ± 0.4	2.3 ± 0.5	0.786
% PPO	61 ± 6	63 ± 9	63 ± 5	62 ± 9	63 ± 7	64 ±	0.580
% Power $_{4mM}$	108 ± 14	110 ± 13	111 ± 13	103 ± 11	103 ± 7	104 ± 6	0.689
HR	163 ± 15	166 ± 10	163 ± 13	153 ± 15	154 ± 12	157 ± 13	0.479
% HRpeak	88 ± 6	89 ± 6	88 ± 9	87 ± 5	87 ± 4	89 ± 7	0.563
Pain	4.2 ± 2.2	4.2 ± 1.8	4.5 ± 2.1	4.6 ± 1.9	4.9 ± 2.2	4.8 ± 1.8	0.116
RPE	16 ± 2	16 ± 1	16 ± 1	16 ± 1	17± 1	17 ± 1	0.896
sRPE	8 ± 1	8 ± 1	8 ± 1	9 ± 0.5	8 ± 1	8 ± 1	0.563

MC = mesocycle

Additional ad libitum training volume and load were analysed from 16 training diaries. Four diaries were removed from statistical analysis due to incompletion (n = 3) or drop out (n = 1). There were no differences in the average training volume (t = -1.1, P = 0.274, d = 0.58) or load (t = -0.8, P = 0.414, d = 0.43) between the tDCS (volume 156 ± 82 minutes; sRPE load 949 ± 629) and sham (volume 197 ± 56 minutes; sRPE load 1182 ± 424) groups. Additionally, for both the tDCS and sham groups, training load (group × week interaction $F_{3.3, 46}$ = 0.6, P = 0.658, η_p^2 = 0.04; main effect of group $F_{1, 14}$ = 1.3, P = 0.274, η_p^2 = 0.09; main effect of week $F_{3.3, 46}$ = 0.8, P = 0.490, η_p^2 = 0.06) and volume (group × week interaction $F_{3.3, 46}$ = 0.5, P = 0.733, η_p^2 = 0.03; main effect of group $F_{1, 14}$ = 0.7, P = 0.413, η_p^2 = 0.05; main effect of week $F_{3.3, 46}$ = 0.9, P = 0.480, η_p^2 = 0.06) remained stable from the baseline testing week to week 6 of training. Over the course of the study, the sham condition completed more endurance type training than the tDCS group (tDCS 16 ± 24%;

sham 54 ± 32%; U = 8, *P* = 0.013, *d* = 1.34). However, there were no significant differences between the tDCS and sham groups for amount of strength (tDCS 37 ± 43%; sham 36 ± 28%; t = 0.06, *P* = 0.953, *d* = 0.03) and other (tDCS 47 ± 39%; sham 10 ± 20%; U = 14.5, *P* = 0.055, *d* = 1.19) training completed.

5.5 Discussion

This is the first study to investigate the efficacy of tDCS to enhance physiological adaptations to endurance training. Therefore, it provides important implications regarding the repeated use of tDCS to enhance cycling performance. This study aimed to elucidate to what extent the application of a commercial tDCS device could augment physiological adaptations to training. Secondly, this study aimed to see whether repeated use of tDCS could be identified by the concentration of BDNF in serum, platelet-poor plasma, and saliva samples. As anodal tDCS has previously demonstrated the ability to reduce fatigue related perceptions such as RPE (Okano et al., 2013; Angius et al., 2016; Angius et al., 2018; Angius et al., 2019) and extend endurance time (Cogiamanian et al., 2007; Vitor-Costa et al., 2015; Angius et al., 2018; Angius et al., 2019), it was hypothesized that the application of tDCS during training would allow for the adoption of greater intensities, providing a greater stimulus for adaptation than the sham group. The principal findings of this study demonstrate that in comparison to baseline, there were significant improvements in VO_{2peak}, $Power_{4Mm}$, PPO and TT performance. However contrary to the hypothesis, the tDCS and sham groups improved at a similar magnitude, and crucially, tDCS did not facilitate training at a higher intensity. Furthermore, there were no significant differences between the tDCS and sham groups for training variables or BDNF concentration detected within serum and platelet poor plasma samples.

High intensity exercise is known to induce central and peripheral fatigue (Sidhu et al., 2018; Ament & Verkerke., 2009). Consequently, this results in the reduction of the force generating capacity of the muscles, increases the perception of effort for a given task and impairs endurance performance (Marcora et al., 2008; Angius et al., 2018). The Halo Sport neurostimulation device is promoted to bolster endurance training. The founders state that the application of this device during a warm-up period induces a state of 'hyperplasticity' delaying the onset of central fatigue in addition to the modulation of fatigue related perceptions which is purported to allow athletes to work harder, for longer. Therefore, as isoeffort intervals were used as the training prescription, it was hypothesized that the tDCS group would opt to cycle at a greater percentage of their lactate threshold and PPO than the sham group, if the Halo Sport did induce an ergogenic effect. Contrary to the hypothesis, our results demonstrate that training with anodal tDCS did not produce any significant differences in training intensity or perceptual responses to the HIIT prescription in comparison to the sham group. Although no studies to date have investigated the effect of tDCS on HIIT endurance training, our results contrast with several studies that have demonstrated a beneficial effect of anodal tDCS on TTE performance (Cogiamanian et al., 2007; Angius et al., 2016; Angius et al., 2018; Angius et al., 2019; Vitor-Costa et al., 2015; Williams et al., 2013) and RPE (Angius et al., 2016; Angius et al., 2018; Angius et al., 2019). However direct comparisons are limited due to differences in exercise paradigm, tDCS device used and current dose applied.

The bihemispheric electrode montage may in part explain the null findings presented within this study. The Halo Sport Neurostimulation System places the anodal electrode

horizontally over the Cz, with the cathodal electrodes are placed over the C5 and C6. This leaves a relatively small distance between the anodal and cathodal electrodes. Finite element modelling of tDCS induced effects has previously identified that the magnitude of current density within a target area is determined in part by the distance between electrodes. Indeed, placing the anodal and cathodal electrodes within a close proximity increases the resistance for current to penetrate through the superficial layers, reducing the current density within the brain region of interest, whilst also increasing the proportion of current that is shunted across the scalp (Miranda et al., 2006; Miranda et al., 2009; Faria et al., 2011). Therefore, the portion of current reaching the M1 in the present study may be insufficient to modulate M1 excitability hence the lack of difference between the tDCS and sham groups for performance within the interval sessions. Furthermore, as there was no significant effect of tDCS on training, the physiological characteristics of both the tDCS and sham group improved to a similar magnitude.

Like tDCS, high intensity exercise is suggested to enhance synaptic plasticity (O'Leary et al., 2017; Baltar et al., 2018; Andrews et al., 2019; Ferris et al., 2007; Seifert et al., 2010). Indeed, both acute exercise bouts and endurance training programmes have shown to improve cognitive functions (Ferris et al., 2007; Berchtold et al., 2005; Gold et al., 2003; Mellow et al., 2020) and increases the activity-dependent secretion of BDNF (Berchtold et al., 2005; Reycraft et al., 2019; Zoladz et al., 2008; Seifert et al., 2010; Ferris et al., 2007; Vaynmann et al., 2004; Gómez-Pinilla et al., 2002). It is therefore interesting that the present study observed no significant differences between the tDCS and sham groups for changes in physiological characteristics and BDNF concentration. Within the present study, the application of tDCS and the HIIT sessions potentially acted as competing sources of neuroplasticity. As such the lack of significant difference in training performance may be indicative of homeostatic mechanisms of metaplasticity.

The BCM theorem of homeostatic plasticity (1982) postulates that physiological limits exist to prevent unrestricted neuronal excitability and the destabilisation of neurons (Baltar et al., 2018; Abraham & Bear., 1996; Siebner., 2004; Fricke et al., 2011). Therefore, when two excitatory protocols are conducted simultaneously (for example preconditioning with anodal tDCS followed by high-intensity exercise) the neuroplastic threshold is increased for the second excitatory protocol, resulting in reductions in excitatory or the reversal of facilitation (Bienestock et al., 1982; Baltar et al., 2018; Abraham & Bear., 1996; Fricke et al., 2011; Siebner., 2004; Thirugnanasambandam et al., 2011). In humans, preconditioning rTMS with the application of anodal tDCS has previously shown to induce sustained reversals in corticospinal excitability, reducing the excitability of the M1 to below baseline levels (Siebner., 2004). Whilst the opposite effects have been noted for cathodal tDCS (Siebner., 2004). Indeed, preconditioning rTMS with cathodal tDCS resulted in significant enhancement of corticospinal excitability (Siebner., 2004). The effects of homeostatic plasticity have also been observed when anodal tDCS is applied prior to exercise (Antal et al., 2007; Thirugnanasambandam et al., 2011; Baltar et al., 2018). Indeed, Baltar et al (2018) observed a significant decline in M1 excitability when anodal tDCS was applied prior to low and high intensity running trials. Anodal tDCS was used to prime the M1 in the present study, based upon the recommended use by the manufacturers of the Halo Sport device. Furthermore, the Halo Sport Neurostimulation does not currently allow for the application of cathodal tDCS. Therefore, according to the BCM theorem, the Halo Sport Neurostimulation system may not be an effective tool to

derive the benefit of overload. It is also recommended that future studies establish whether priming with cathodal tDCS can augment the responses to training.

The induction of homeostatic plasticity may also explain the non-significant difference in serum and platelet-poor plasma BDNF concentrations between the experimental groups. In the last 10 participants, BDNF concentration was measured in serum and platelet-poor plasma samples collected before and immediately after a single training session. The serum BDNF concentration in four out of five participants within the sham group, increased from baseline. This change however was not significant. Whereas no changes in BDNF concentration were observed in the tDCS group. The concentration of BDNF has previously been shown to increase following acute exercise bouts (Ferris et al., 2007; Tang et al., 2008) and acute applications of anodal tDCS (Fritsche et al., 2010). However, no studies conducted on healthy participants have reported an increase in the BDNF concentration from combining anodal tDCS with exercise. Indeed, supporting the findings of the present study, Hendy et al (2019) demonstrated that the application of anodal tDCS immediately after the completion of a cycling HIIT trial had no influence upon the concentration of BDNF or cognitive functions. Perhaps to ensure neural activity is maintained within the physiological range, homeostatic plasticity mechanisms reduce the rate at which mature-BDNF is cleaved from its proBDNF precursor molecule. This therefore may reflect the lack of change observed in tDCS group for serum BDNF concentration. However, it should be noted that this trend was not observed for platelet-poor plasma. Although studies have reported a positive correlation between serum and cortical BDNF samples in rodents (Karege et al., 2005), the platelet degranulation produces a 100-fold greater concentration of BDNF and therefore platelet-poor plasma may provide a better comparison for synapse changes at a cortical level (Piccinni et al., 2008; Lommatzch et al., 2005).

Caution should also be taken when comparing the findings of the present study given that the acute measurement of BDNF was conducted on the final 10 participants in the study (tDCS n = 5, sham n= 5), and therefore are likely to be underpowered. However, inferences can be made from the effect size calculated. In the present study, the effect sizes calculated indicate that there was a small and large effect size was observed for platelet-poor plasma ($\eta_p^2 = 0.02$) and serum ($\eta_p^2 = 0.38$) BDNF concentration respectively. It is therefore plausible that a greater difference in the BDNF concentration in sham and tDCS group may have been observed had there been a greater number of participants. This is unlikely to exert a beneficial effect. It is now recognised that in the field of tDCS, larger effect sizes are often shown in studies with small sample sizes and large standard error (Holgado et al., 2018). Therefore, due to the small samples sizes and publication bias the efficacy of tDCS has previously been overestimated.

The present study is the first known study to investigate the viability of using peripherally sampled BDNF (within saliva, serum, and platelet-poor plasma) to detect the chronic use of tDCS in healthy physically active participants. Therefore, it was uncertain whether any tDCS induced elevations of BDNF would remain until the post-training sampling point. Whilst no studies have investigated the longevity of the tDCS induced increase in BDNF, several studies have investigated the influence of training on BDNF concentration in healthy and clinical populations, disclosing varied findings. Consistent with the results of the present study, Schiffer et al (2009) reported no significant changes in resting plasma BDNF concentration following 12 weeks of strength or endurance training, despite the

improvement in isometric and dynamic strength and endurance performance. Similarly, Schulz et al (2004) reported a significant increase in aerobic capacity in multiple sclerosis following an 8-week training programme but failed to detect a significant change in resting serum BDNF concentrations. Contrasting this, Zoladz et al (2008) reported a significant increase in resting plasma BDNF following 5 weeks of endurance training. It is thought that the peripheral sampling of BDNF may lower the brain derived contribution within the systemic circulation, and therefore could potentially explain the discrepancies between findings (Seifert et al., 2010). In support of this, Seifert et al (2010) compared the BDNF concentration within samples collected via catheter from the jugular vein and the brachial artery following 8 weeks of endurance training completed by over-weight sedentary participants. Within this study, the authors reported a significant increase in resting serum BDNF when sampled from the jugular vein, but no significant effect of training upon BDNF sampled peripherally in the brachial artery (Seifert et al., 2010). Overall, these finding insinuate that peripheral BDNF sampling may not be a viable method of detecting increases in brain derived BDNF induced by tDCS. Furthermore, whilst sampling BDNF from the jugular vein may provide a more accurate representation of alterations in BDNF concentration occurring within the brain, this method of sampling is likely to be too invasive to be employed as a routine tDCS detection method had tDCS been demonstrated as an ergogenic aid.

The lack of significant differences presented within the current study may also be attributed to the time interval between HIIT sessions and tDCS applications. Indeed, previous studies that have reported tDCS to increase motor function, cortical excitability and BDNF concentration in blood samples have often applied tDCS over consecutive days, whilst this study applied tDCS twice a week on non-consecutive days. The effect of consecutive applications on BDNF samples are unknown. However, it appears that the consecutive application of tDCS produces a cumulative effect, gradually increasing the baseline corticospinal excitability (Gálvez et al., 2013; Alonzo et al., 2012). Therefore, the significant improvement in motor function increased BDNF concentration observed by Hadoush et al (2018) could have been due to the cumulative effect of tDCS applied over consecutive days. However, it should be noted that within that study there were no control or sham conditions. Moreover, tDCS was applied 1 hour after the consumption of Levodopa, therefore it is uncertain whether the increases in BDNF were due to the application of tDCS or Levodopa. It remains a possibility that for enhanced physiological adaptation and greater detectability of tDCS use via BDNF sampling, regular applications on consecutive days may be required.

Transcranial direct current stimulation is associated with large inter-individual differences, resulting from differences in anatomy, genetics, and neurophysiology (Li et al., 2015). These differences have previously manifested as a variance in the corticospinal response (Whiethoff et al., 2014). In the present study, these variations may manifest as a variance in the physiological adaptation to training. By using the statistical analysis framework described by Swinton et al (2018), it was estimated that 29% of the participants had a meaningful change in $Power_{4mM}$ within the tDCS group. Whereas, the tDCS group had no meaningful change in VO_{2peak}. Surprisingly despite the lack of significant differences between the tDCS and sham group, 79% of participants in the tDCS were considered to have a meaningful change in performance in the 5 km TT. However, this finding may be a

result of the use of recreationally active participants and the lack of familiarisation of the TT procedures prior to the start of the intervention.

This study also explored the influence of a common SNP of BDNF, val66met, which has previously been implicated as a source of variability to NIBS (Teo et al., 2014; Cheeran et al., 2008; Puri et al., 2015; Antal et al., 2010). The val66met polymorphism has been found to reduce the amount of BDNF that is cleaved from its precursor molecule, proBDNF (Cheeran et al., 2008; Puri et al., 2015; Teo et al., 2014). As a result, researchers have reported abolishment of cortical excitability following transcutaneous spinal direct current stimulation (Lamy & Boakye., 2013), intermittent theta burst stimulation (Antal et al., 2008), and median nerve paired associative stimulation (Cheeran et al., 2008). Depending on the experimental protocol, met carriers have been reported to experience a greater increase in corticospinal excitability than val carriers following tDCS application (Antal et al., 2010; Teo et al., 2014; Puri et al., 2015). However, Cheeran et al (2009) reported that corticospinal excitability was diminished in met carriers when cathodal tDCS was used to precondition rTMS; a protocol which has previously shown to overcome homeostatic regulation to enhance corticospinal excitability (Lang et al., 2004; Siebner., 2004). Furthermore, corticospinal excitability in met carriers has also shown to reduce following both motor (Kleim et al., 2006) and endurance training (Lemos et al., 2016). Surprisingly, the results of the present study are in contrast with the aforementioned literature. Indeed, the BDNF polymorphism had no significant influence on the null findings of tDCS induced enhancement of endurance performance and physiological adaptations. This is likely due to the higher proportion of val66val carriers being within the tDCS condition. Instead, the null findings presented within this study could be attributed to other sources of variances such as individual anatomical differences (Li et al., 2015). Alternatively, the results of the present study may indicate that tDCS delivered by the Halo Sport Neurostimulation System is incapable of altering the corticospinal excitability of the M1, and therefore provides no benefit to performance and training.

5.6 Limitations

Whilst the present study provides a unique insight into the efficacy of the repeated use of a commercial tDCS to enhance physiological adaptation following endurance training, it is not without limitations. Due to the matched group study design comparisons drawn between groups become limited. Firstly, due to differences in the interpretation of the ordinal scales presented for the measurement of pain during exercise, RPE and sRPE as well as the subjective nature of the perception-intensity relationship, perceptual responses reported during training are not directly comparable between participants (Seiler & Sylta., 2017; Sylta et al., 2016).

Secondly, whilst participants within the tDCS and sham group were matched for age, body mass and baseline physiological variables that determine endurance performance, they were not matched according to BDNF polymorphism or responsiveness to tDCS. Due to the many intra-and inter-individual differences to tDCS, it is likely that tDCS would need to be optimised for the individual. Indeed, this is likely to require the use of MRI scans, computational modelling of the brain, the use of HD-tDCS as well as extensive testing for correct polarity (Wagner et al., 2007), current dose (Hassanzahraee et al., 2020; Bastani & Jaberzadeh., 2013; Jamil et al., 2017) and the optimal timing of application due to

potential circadian effects (Li et al., 2015; Sale et al., 2008; Sale et al., 2010). All of which may not be accessible or logistical for use outside of a clinical or research environment, or unavailable if relying on commercial tDCS devices such as the Halo Sport.

Like other traditional forms of tDCS, the Halo Sport device also uses large electrodes which are likely to span across multiple brain areas (Bikson et al., 2010). Previous research has indicated that large electrodes lack focality, inducing widespread distribution of electrical current (Miranda et al., 2006). Moreover, due to the size of the electrodes and the bihemispheric montage adopted, the cortical target of the Halo Sport device is uncertain. Considering this, mathematical modelling of the bihemispheric montage used by the Halo Sport should be conducted to evaluate how current density is distributed throughout the brain. Moreover, this will ascertain whether the Halo Sport will allow for current to be injected into the brain or whether current is shunted across the scalp caused by the proximity of the cathodal electrodes (Miranda et al., 2006).

Due to the length of the study, the menstrual cycle phase and hormonal contraceptive use in the female participants was not controlled for. The female sex hormones oestrogen and progesterone are known to exhibit neurosteroidal properties, where oestrogen exerts an excitatory effect whilst progesterone exerts an inhibitory effect. Therefore, the sensitivity of the female participants to tDCS may differ across the menstrual cycle. It has also been recently established that the concentration of oestrogen and progesterone may impact the response to exercise (Ansdell et al., 2020; Elliott-Sale et al., 2020; McNulty et al., 2020). Indeed, in exercise the fluctuation in hormone concentration manifests as an alteration to the recruitment of skeletal muscle, fatiguability, substrate metabolism and the ventilation rate (Ansdell et al., 2019; Ansdell et al., 2020; McNulty et al., 2020). Due to the variation in the integrative response to exercise, it is currently unclear whether female participants adapt to training in a similar manner to male participants (Ansdell et al., 2020). Overall, by not controlling for the menstrual cycle phase and hormonal contraceptive use, the fluctuation in hormones may have contributed to some of the variance seen in the results. However, it should be noted that removing female participants from the analysis had no effect on the overall outcome, indicating that several factors cause the variation in the response to tDCS.

5.7 Conclusions

Although tDCS has previously been proposed as a potential tool to help derive the benefit of overload, the results of the present study demonstrate that the six-week application of bihemispheric tDCS during a HIIT intervention had little to no effect on the performance or perceptual response during training, nor did it exert any effect on the physiological adaptations to training. These findings may be due to the electrode montage, where the proximity of the electrodes limits the amount of current which is able to penetrate the M1. Therefore, the Halo Sport Neurostimulation system may be insufficient to induce noticeable changes in corticospinal excitability which is reflected as the lack of difference in performance between the tDCS and sham group. This is further evidenced by no differences in BDNF concentration of serum and platelet-poor plasma samples immediately after a single HIIT session, in addition to differences in BDNF concentration at the post-intervention point when compared to baseline. Future research should investigate the impact of the Halo Sport Neurostimulation system on corticospinal excitability of the M1. It is also recommended that researchers identify whether the use of

inhibitory or cathodal tDCS prior to a HIIT programme would augment performance or the adaptation to training. The impact of individualised applications of tDCS should also be investigated.

Chapter 5. Evaluating the influence of the Halo Sport Neurostimulation System on the Corticospinal Excitability of the Motor Cortex

4.1 Abstract

Introduction: Transcranial direct current stimulation (tDCS) of the M1 has previously been shown to enhance corticospinal excitability. Therefore, this study investigated the efficacy of a commercial tDCS device to modulate the corticospinal response. This study also compared the influence of inducing anatomical-specificity and function-specificity on the corticospinal response. **Methods:** This study was comprised of two investigations (part A and part B). In part A, 14 participants completed neuromuscular assessments (MEP_{p-p} and SP elicited by TMS) before and immediately after the application of 20 min 2.20 mA bihemispheric M1 tDCS delivered at rest. In Part B, 12 participants completed the same neuromuscular assessments before and immediately following 20 min 2.20 mA bihemispheric M1 tDCS delivered during a series of submaximal contractions. **Results:** In part A, baseline CSP duration was significantly shorter in the sham condition ($P = 0.009$), with no significant differences at post-intervention. There were no differences in MEP_{p-p} ($P = 0.982$). Part B saw no differences in MEP_{p-p} ($P = 0.579$) or CSP duration ($P = 0.887$). **Conclusion:** This is the first study to examine the efficacy of the Halo Sport device to induce changes in the corticospinal response. As there were no significant changes in corticospinal parameters in both part A and B this questions the validity of this device.

4.2 Introduction

In addition to its purported effects on exercise-generated perceptions, transcranial direct current stimulation (tDCS) is also suggested to elicit sustained but transient alterations in corticospinal excitability (Nitsche & Paulus., 2000; Williams et al., 2013). Since this discovery, tDCS has become an eminent technique for the investigation of the cortical mechanisms associated with neuroplasticity, motor learning and supraspinal fatigue (Williams et al., 2013; Cogiamanian et al., 2007; Angius et al., 2016; Angius et al., 2018). Indeed, according to the manufacturers, the Halo Sport Neurostimulation System enhances performance through increasing the excitability of the M1. In turn, this is thought to ameliorate the induction of supraspinal fatigue

Transcranial magnetic stimulation (TMS) provides a non-invasive method of evaluating changes in corticospinal excitability in vivo (Badawy et al., 2012; Fatemi-Ardekani., 2008; Rossini & Rossi., 2007). When applied over the M1, the discharge elicits a twitch in the corresponding muscle referred to as a motor evoked potential (MEP). This TMS stimuli is thought to be reflective of the global excitability of interneurons, fast corticospinal pathways and spinal motoneurons (Badawy et al., 2013). Therefore, the amplitude or size of the corresponding MEP is used as an indicator of corticospinal excitability (Badawy et al., 2012). The use of TMS to examine changes in corticospinal excitability of the M1 has allowed for the observation of significant and repeatable changes in corticospinal excitability following the use of tDCS (Nitsche & Paulus., 2000; Nitsche & Paulus., 2001; Williams et al., 2013; Angius et al., 2018; Cogiamanian et al., 2007). However, it should be noted that not all studies have been able to detect changes in the MEP amplitude or the CSP duration following tDCS but have nevertheless observed an enhancement of performance (Angius et al., 2016; Abdelmoula et al., 2016).

As the Halo Sport Neurostimulation system is already available on the consumer market, an evaluation of the efficacy of this device is imperative. Therefore, the present study sought to evaluate the capability of this device to alter the corticospinal excitability, using TMS and PES to quantify changes in several neuromuscular parameters. Transcranial direct current stimulation is often applied at both rest and during exercise (Codella et al., 2020; Park et al., 2019; Angius et al., 2016). Indeed, several studies have suggested that the ‘online’ application induces greater specificity of tDCS aftereffects due to directly targeting active networks (Andrews et al., 2011; Bikson & Rahman., 2013; Martin et al., 2014). However, the BCM theorem of homeostatic plasticity (1982) postulates that the concurrent application of anodal tDCS and a task induces a reversal in polarity (Abraham & Bear., 1996; Baltar et al., 2018; Siebner., 2004; Thirugnanasambandam et al., 2011). Therefore, this study also sought to investigate the influence of both ‘online’ and ‘offline’ applications on corticospinal excitability.

4.3 Methods

Participants

The current investigation comprised of two parts (Part A and Part B). In part A, fourteen recreationally active volunteers (10 males and 4 females: age; 22 ± 5 yrs, height; 175 ± 8 cm, body mass; 69± 10 kg) volunteered as participants. In part B, twelve recreationally active volunteers (7 males and 5 females; age 25 ± 5 yrs, height; 171.4 ± 8.8 cm, body

mass 68.9 ± 11.5 kg) were recruited to participate. Exclusion criteria included a history of cardiorespiratory disease, mental health or brain disorders, intracranial implants, or the use of medication at the time of the study. Participants provided written informed consent and were briefed on the experimental procedures. Written informed consent was obtained from the participants after receiving an overview of the experimental procedures. Ethical approval for this study was obtained from the SSES REAG (approval number: Prop 94_2018_19).

Experimental Protocol

In both part A and part B, participants attended the laboratory on three separate occasions. Visit one familiarized the participants with the experimental protocol. On the two subsequent visits, maximal voluntary isometric force (MVC), cortical voluntary activation (cVAL), corticospinal excitability and peripheral fatigue (as measured by the peak torque of resting evoked contractions) were assessed before and after the administration of either tDCS or sham stimulation delivered in a double-blind counterbalanced design (figure 21). All visits were conducted at the same time of day within a two-week period. Before each visit, participants were asked to refrain from completing strenuous exercise and consuming alcohol for 24 hours, avoid the consumption of caffeine and painkillers for 6 hours and eating within 2 hours of the visit.

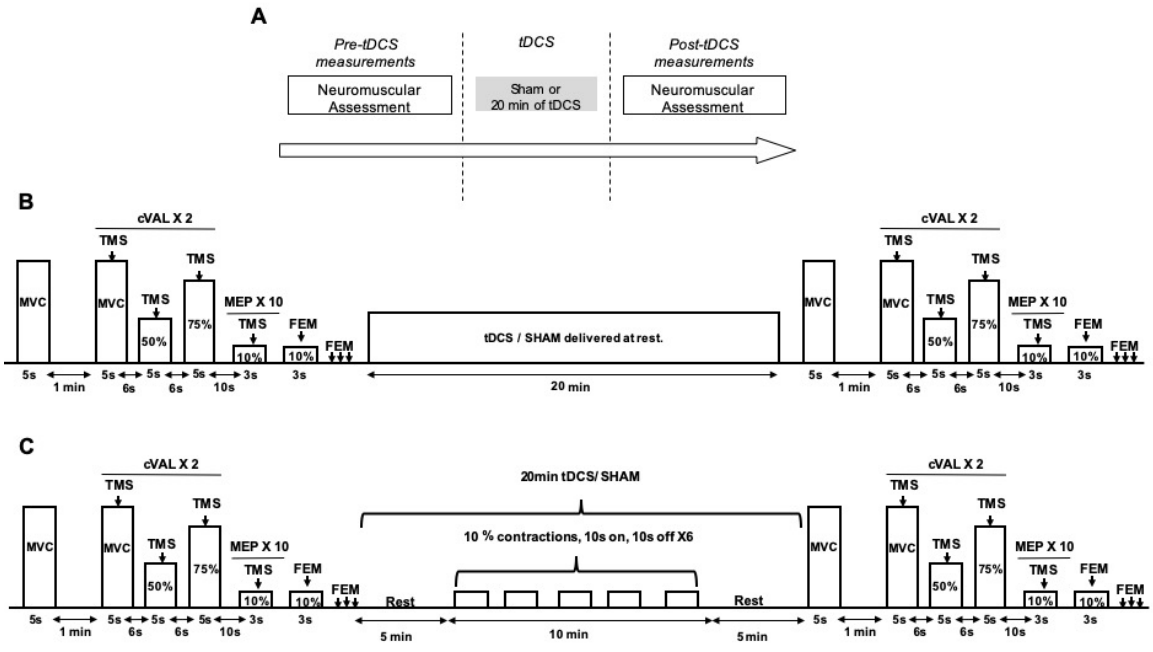

Figure 21. Overview of the experimental protocol, where neuromuscular assessments were conducted before and after the application of tDCS or sham (Panel A). Panel B displays the experimental protocol for Part A where tDCS was delivered at rest. Panel C displays the experimental protocol for part B, where participants completed 10 minutes of low intensity contractions throughout the application of tDCS. Maximal voluntary contraction (MVC); Cortical voluntary activation level (cVAL); Transcranial magnetic stimulation (TMS); Motor evoked potential (MEP); Femoral stimulation (FEM); Transcranial direct current stimulation (tDCS).

Transcranial Direct Current Stimulation Procedures

A commercial tDCS device, the Halo Sport Neurostimulation System (Halo Neuroscience, San Francisco, USA) was used in this study. Current was delivered through three water-soaked studded electrodes connected to the headband of what looks like audio headphones (Figure 16). The device was placed directly over the vertex to stimulate the M1 in both hemispheres. The anodal electrode was placed horizontally over the Cz, whilst the cathodal electrodes was placed over the C5 and C6 according to the 10-20 EEG system. In the tDCS condition, participants received 2.20 mA (equivalent to a level 10 amperage on the Team Halo app; current density 0.0916 mA/cm^2) for a duration of 20 minutes, with an additional 30 second ramp up and down at the start and end of stimulation respectively. In the sham condition, stimulation ramped up to 1.8 mA (equivalent to a level 5 amperage according to the Team Halo app; current density 0.075 mA/cm^2) over a 30 second period, where it subsequently ramped down over a 30 second period to 0 mA.

Part A

Following the completion of the baseline corticospinal excitability and neuromuscular assessments, the 20 min of anodal or sham tDCS was applied at rest.

Part B

Part B aimed to investigate how task-specific modulation would influence the corticospinal response. Therefore, participants completed submaximal contractions during the application of tDCS. tDCS was applied for 5 minutes prior to starting the contractions to acclimate participants fully to the stimulation (Fecteau et al., 2007; Maraiano et al., 2016). After this, participants completed 30 submaximal contractions over a 10-minute period. For this, participants completed 10 s contractions interspersed with 10 s of rest at 10% MVC.

Neuromuscular Assessments

Following a brief standardised warm up, participants completed a single 5 second MVC to assess the force generating capacity of the knee extensor muscles. Following a minute of rest, participants completed 2 sets of 3, 5- second contractions at 100%, 50% and 75% MVC with superimposed TMS to measure the cVAL (Figure 21). Each contraction was separated by 6 seconds with 10 seconds separating the first and second set of contractions. For the measurement of corticospinal excitability and assessment of the cortical silent period, participants completed 10 submaximal contractions (3 s) at 10% MVC with superimposed TMS. This was followed by a single 3 s contraction at 10% MVC with a superimposed femoral nerve stimulation, and 3 femoral nerve stimulations delivered at rest (Figure 21).

Transcranial magnetic stimulation procedures

Transcranial magnetic stimulation was used to examine the cortical voluntary activation and corticospinal excitability of the M1. A TMS stimulator (Magstim 2002, The Magstim Company Ltd, Whitland, UK) with a 110 mm diameter concave coil was used to stimulate the M1. At the beginning of each visit the precise of site of stimulation was determined by the largest MEP response of the right vastus lateralis (VL) muscle with a small MEP

response (<10%) of the biceps femoris. Once determined, this site was marked on tight swimming hat with a marker pen. Once the stimulation site was obtained, the optimal stimulation intensity was set according to the highest MEP elicited during a 3 second contraction at 10% MVC. Stimulation intensity commenced at 45% of stimulator output and increased by 5% until a plateau in MEP response was observed. In part A, the stimulation intensity across participants and visits was 62 ± 6% of the maximum stimulator output. In part B, the average stimulation intensity used across participants and visits was 58 ± 6% of the stimulator output.

Femoral Nerve Stimulation

Transcutaneous electrical stimulation of the right femoral nerve was delivered by a high-voltage constant current stimulator (stimulus duration 200 ms, 100 Hz frequency: Digitimer D7AH, Digitimer, Hertfordshire, UK). The femoral nerve was located within the femoral triangle and stimulated with by a cathode electrode (2 x2 cm, Swaromed, Nessler Medizintechnik, Innsbruck, Austria) with the anodal electrode (10 x 5 cm; Phoenix Healthcare Products ltd., Nottingham, UK) placed in the right gluteal fold. Stimulation intensity commenced at 100 mA, increasing by 20 mA until a plateau in the electrical compound action potential response (M-wave) occurred at rest and during a submaximal contraction at 10% MVC. The optimal intensity of the stimulation was set at 130% of the intensity required to elicit the highest M-wave (Part A: 219 ± 61 mA Part B: 219 ± 32 mA). Stimulation intensity was determined during each experimental visit before commencing the neuromuscular assessment.

Mechanical recordings

Part A

All experimental procedures were performed on a Cybex isokinetic dynamometer (HUMAC NORM, CMsi, Computer Sports Medicine inc., Stoughton, USA), initialised and calibrated according to the manufacturer's specifications. This dynamometer has previously been shown to have a good relative reliability for knee extension exercises (ICC = 0.840; Habets et al., 2018). Participants were seated in the dynamometer chair with the seated position adjusted to ensure that the axis of rotation of the lever arm was in line with the lateral epicondyle of the right femur. The experimental procedures were completed with a relative hip and knee angle of 90 degrees, with full extension being 0 degrees. Padded Velcro straps placed above the malleoli were used to secure the lower leg to the lever arm. Participants shoulders and waist were also firmly secured with straps to prevent extraneous movements or hip extension during contractions. Seating position was adjusted and recorded on the familiarisation visit and replicated for the subsequent experimental visits. Mechanical signals were digitized on-line at a sampling frequency of 100 kHz and stored on a computer for subsequent analysis (Acqknowledge 4.2, Biopac Systems inc., Goleta, USA).

Part B

For all experimental procedures, participants were seated in a custom-made dynamometer chair, with the seated position adjusted to ensure a relative knee angle of 90 degrees. Seating position was adjusted and recorded on the familiarisation visit and

were replicated across experimental sessions. Participants were secured to the dynamometer via straps around the torso to prevent any extraneous movements. A non-compliant Velcro strap was also secured 2 cm above the malleoli. This ankle strap was connected to a transducer to measure the isometric force of the right knee extensors. The transducer was connected to a signal amplifier (DA100c, Biopac Systems Inc, California, USA) and a data acquisition module (MP150, Biopac Systems Inc, California, USA). Mechanical signals were digitized on-line at a sampling frequency of 100 kHz and stored on a computer for subsequent analysis (Acqknowledge 5.0, Biopac Systems Inc., Goleta, USA)

Figure 22. Image of the custom-built dynamometer used in Part B.

Electromyographical recordings

Surface electromyography (EMG) of the VL was acquired via two square surface electrodes with a circular recording site in the centre of the electrode (10 mm diameter, 20 mm centre-to-centre distance; Swaromed, Nessler Medizintechnik, Innsbruck, Austria). Electrodes were placed according to SENIAM guidelines, specifically electrodes for the VL were placed on the muscle belly at 2/3 of the line from the anterior spina iliac superior and the lateral side of the patella. Prior to electrode placement, skin was prepared by shaving and cleaning with alcohol swabs. Using a commercially available software (Acqknowledge 5.0, Biopac Systems Inc., Goleta, USA) the electrical signal was amplified with a bandwidth frequency ranging from 10 Hz to 500 Hz (gain = 500).

Data Analysis

Peak torque produced within the MVC was used as a measure of the force-generating capacity of the knee extensor muscles. Cortical voluntary activation was used to assess changes in voluntary activation of the knee extensors. This method has previously been shown as a valid and reliable (CV < 3%; Goodall et al., 2009; Goodall et al., 2017; Thomas et al., 2015; Thomas et al., 2016) technique to directly estimate the change in the cortical activation of the target muscle group rather than including the spinal components.

Cortical voluntary activation was quantified by the measurement of the torque responses to single-pulse TMS applied to the M1. For each participant, the estimated resting twitch (ER) was calculated by the extrapolation of the linear regression between the super-imposed twitch (SIT) and voluntary torque over 50-100% mean maximal torque. The y-intercept was taken as the estimated amplitude of the ERT; therefore, each set of contraction yielded an ERT. The level of cVAL was quantified using the following equation:

$$\boldsymbol{voluntary\ activation}\ (\%) = \left(\mathbf{1} - \frac{\boldsymbol{SIT}}{\boldsymbol{ERT}}\right)\boldsymbol{x100}$$

Once calculated, cVAL was averaged for the two sets of contractions. Peak-to-peak amplitude (including both positive and negative phases of the EMG signal) of the EMG signal following TMS (MEP) and femoral nerve stimulation during the brief (3s) 10% MVC contractions were used to obtain MEP peak-to-peak amplitude ($MEP_{p\text{-}p}$) and the M-wave ($M\text{-}wave_{p\text{-}p}$). The $MEP_{p\text{-}p}$ was normalized for the $M\text{-}wave_{p\text{-}p}$ ($MEP_{p\text{-}p}/M\text{-}wave_{p\text{-}p}$ ratio). The $MEP_{p\text{-}p}/M\text{-}wave_{p\text{-}p}$ ratio was calculated and averaged for the 10 stimulations. The SP was measured from the stimulation artefact to the return of the EMG signal. It's suggested that as the mechanisms prompting the silent period in the EMG signal are elicited after the stimulus, therefore the stimulation artefact provides the most standardized reference point to measure the SP (Škarabot et al., 2019). For the three evoked contractions produced by femoral stimulations delivered at rest, the peak torque of all three contractions were analysed and then averaged.

Statistical Analysis

All data are presented as mean ± standard deviation. The assumptions of statistical tests such as normal distribution, equality of variance and sphericity were assessed through the Shapiro-Wilk, Levene's and Mauchley's tests respectively. When violations to the assumption of sphericity were present, the Greenhouse-Geisser correction to the degrees of freedom were applied. As the SP duration from Part A violated the assumption of normality, the data were log transformed to ensure a normal distribution. A 2 x 2 RM ANOVA was performed to assess the effect of tDCS condition on MVC, cVAL, $MEP_{p\text{-}p}/M\text{-}wave_{p\text{-}p}$ ratio, CSP duration and the resting evoked contractions. When a significant main effect of condition or time was observed, follow-up analysis using the Bonferroni correction was performed. Statistical significance was set at an α level of 0.05. Effect sizes were used to establish the size of the differences between the tDCS and sham conditions. This was reported as partial eta squared (η_p^2) (small = 0.01, medium = 0.06, large = 0.14). All statistical analysis was performed using SPSS (Version 25, SPSS, IBM Corp, Armonik, NY, USA)

4.4 Results

Part A

The corticospinal responses before and after tDCS and sham stimulation are demonstrated in figure 23. No condition × time interaction was found for the MEP amplitude ($F_{1,13}$ = 0.1, P = 0.730, η_p^2 = 0.01), nor was there a main effect of condition ($F_{1,13}$ = 0.02, P = 0.892, η_p^2 = 0.001) or time ($F_{1,13}$ = 0.03, P = 0.864, η_p^2 = 0.002). The small effect size highlights that there were no meaningful differences detected in the MEP response between the tDCS and sham condition. When the MEP amplitude was normalised to the amplitude of the M-wave, no differences were detected. Indeed, there was no interaction between condition × time ($F_{1,13}$ = 0.01, P = 0.922, η_p^2 = 0.001), main effects of condition ($F_{1,13}$ = 0.02, P = 0.905, η_p^2 = 0.001) or main effect of time ($F_{1,13}$ = 0.6, P = 0.436, η_p^2 = 0.047) observed (Figure 23B). An interaction effect was observed for CSP duration ($F_{1,13}$ = 9.5, P = 0.009, η_p^2 = 0.42, figure 23C), where the tDCS and sham condition were significantly different at baseline (tDCS 214 ± 39 ms, sham 193 ± 44 ms; $F_{1,13}$ = 6.9, P = 0.021, η_p^2 = 0.35), but not significantly different following the application of the Halo Sport Device (tDCS 206 ± 38 ms, sham 242 ± 108 ms; $F_{1,13}$ = 1.7, P = 0.213, η_p^2 = 0.12). However, no main effect of condition $F_{1,13}$ = 0.0, P = 0.957, $\eta_p^2 \leq 0.001$) or time ($F_{1,13}$ = 3.0, P = 0.109, η_p^2 = 0.19) were observed.

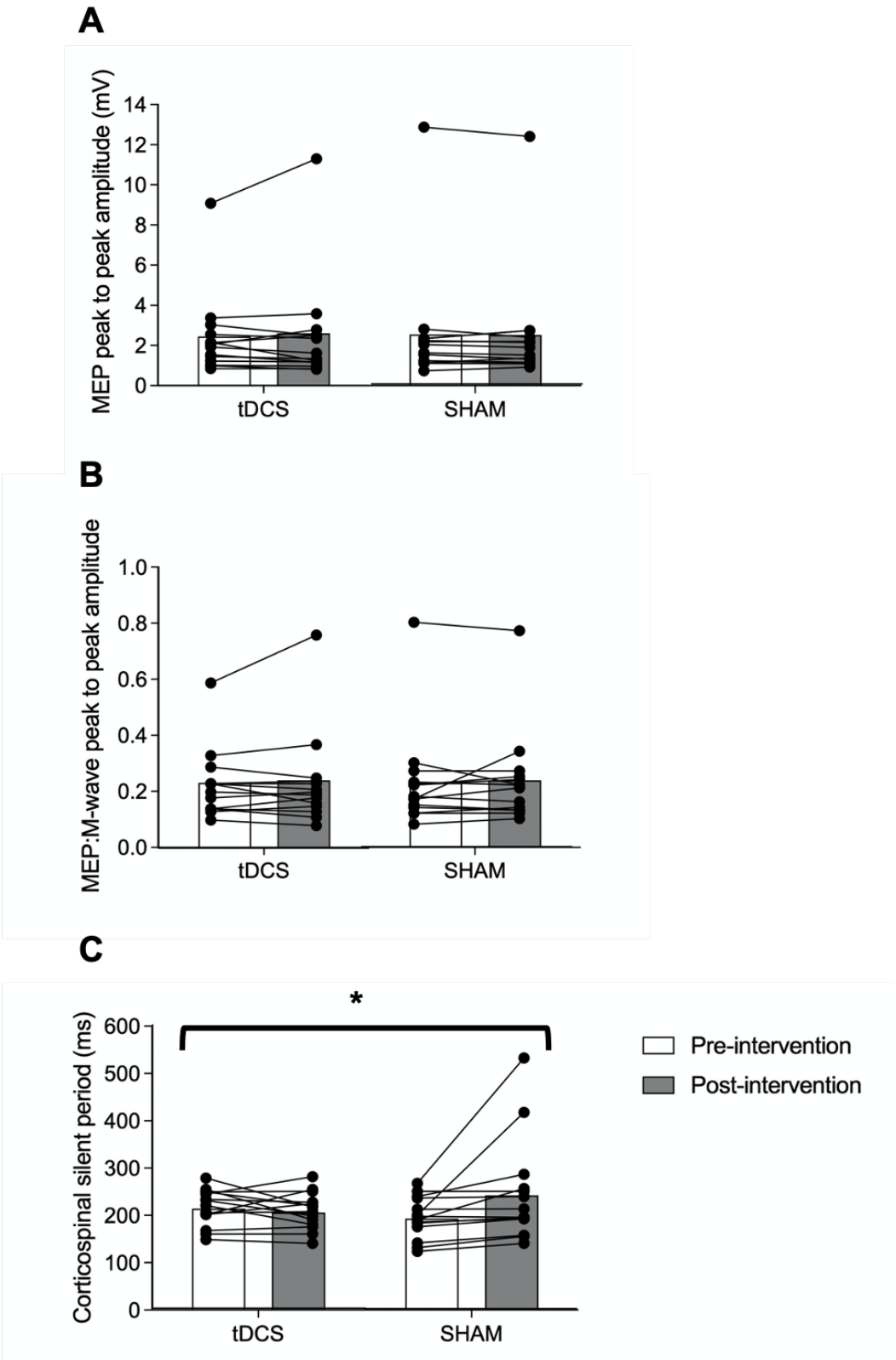

*Figure 23. The corticospinal responses to bihemispheric M1 tDCS delivered by the Halo Sport Neurostimulation system in Part A. Panels A-C compare the MEPp-p amplitude, MEPp-p/M-wavep-p ratio and CSP at baseline and following the application of tDCS at rest. Bars signify the mean response; individual lines represent the response of each participant. * Denotes a significant interaction effect.*

There was no difference in the MVC force between the tDCS and sham condition before and after the application of tDCS at rest. Indeed, there were no condition × time interaction effects ($F_{1,13}$ = 0.1, P = 0.751, η_p^2 = 0.01, Figure 24A), main effect of condition ($F_{1,13}$ = 0.1, P = 0.727, η_p^2 = 0.01) and time ($F_{1,13}$ = 0.1, P = 0.776, η_p^2 = 0.01). For cVAL, there was no condition × time interactions ($F_{1,13}$ = 0.2, P = 0.677, η_p^2 = 0.01), nor was there any main effects of condition ($F_{1,13}$ = 0.3, P = 0.571, η_p^2 = 0.03) or time ($F_{1,13}$ = 0.8, P = 0.777, η_p^2 = 0.01). There was no evidence that peripheral fatigue had occurred as no condition × time interactions ($F_{1,13}$ = 0.9, P = 0.352, η_p^2 = 0.07), main effect of condition ($F_{1,13}$ = 0.1, P = 0.741, η_p^2 = 0.01) or main effect of time ($F_{1,13}$ = 0.1, P = 0.761, η_p^2 = 0.01) for the force produced within the resting evoked contractions (Figure 24C).

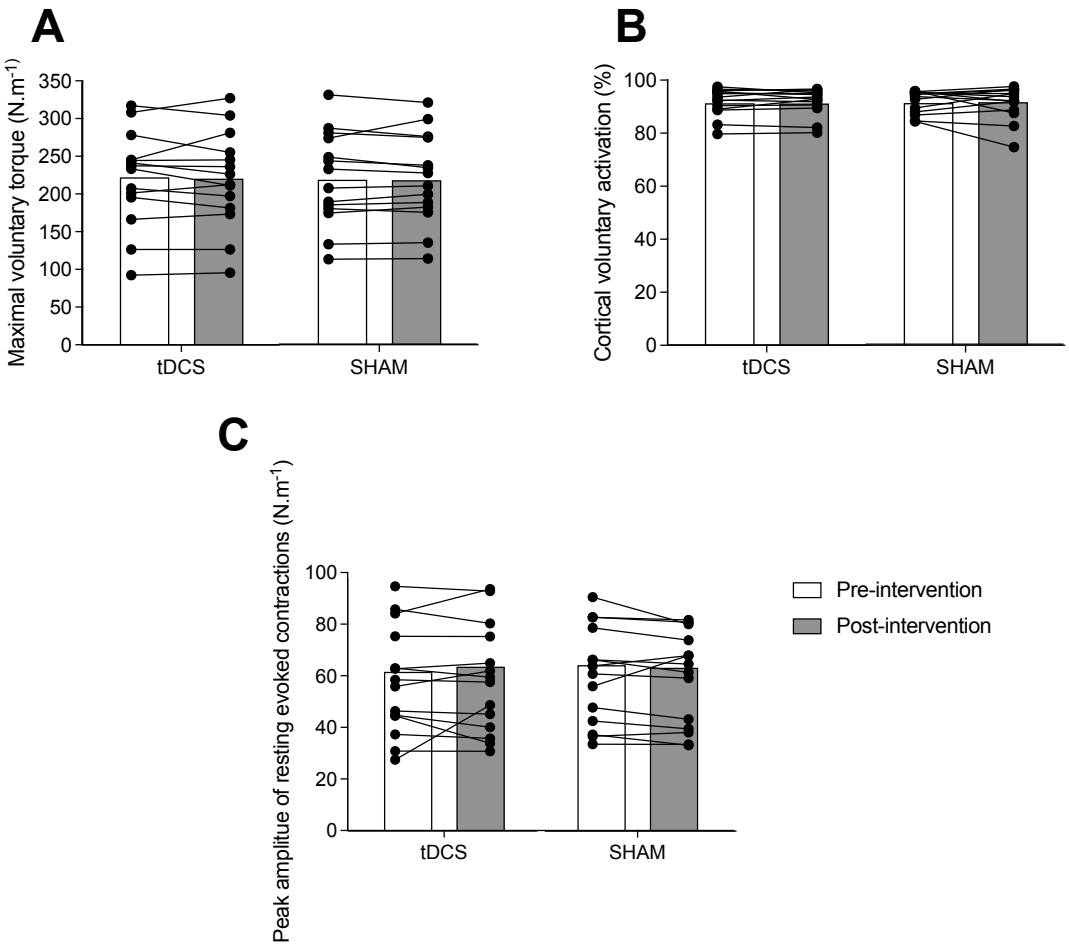

Figure 24. The neuromuscular responses to 20 minutes of bihemispheric M1 tDCS delivered by the Halo Sport Neurostimulation System at rest. Panels A-C compare the MVC, cVAL and resting evoked contractions at pre-intervention and post-intervention. Bars signify the mean response; individual lines represent the response from each participant.

Part B

There was no evidence that anodal M1 tDCS applied during submaximal contractions had any effect on corticospinal excitability (Figure 25). Indeed, no condition × time interactions ($F_{1,11}$ = 0.3, P = 0.579, η_p^2 = 0.03), main effect of condition ($F_{1,11}$ = 0.1, P = 0.754, η_p^2 = 0.01) or main effects of time ($F_{1,11}$ = 2.0, P = 0.186, η_p^2 = 0.153) were detected for the MEPp-p amplitude. The small effect sizes that emphasize that no meaningful differences existed between the tDCS and sham groups. When the $MEP_{p\text{-}p}$ amplitude was normalised to the amplitude of the M-wave, no condition × time interaction ($F_{1,11}$ = 1.1, P = 0.310, η_p^2 = 0.09), main effect of condition ($F_{1,11}$ = 2.3, P = 0.159, η_p^2 = 0.17) and time ($F_{1,11}$ = 4.2, P = 0.065, η_p^2 = 0.276) were observed. Similarly, there were no condition × time interactions ($F_{1,11}$ = 0.2, P = 0.887, η_p^2 = 0.002), main effect of condition ($F_{1,11}$ = 0.146, P = 0.710, η_p^2 = 0.013), or main effect of time ($F_{1,11}$ = 0.3, P = 0.622, η_p^2 = 0.02) for CSP duration.

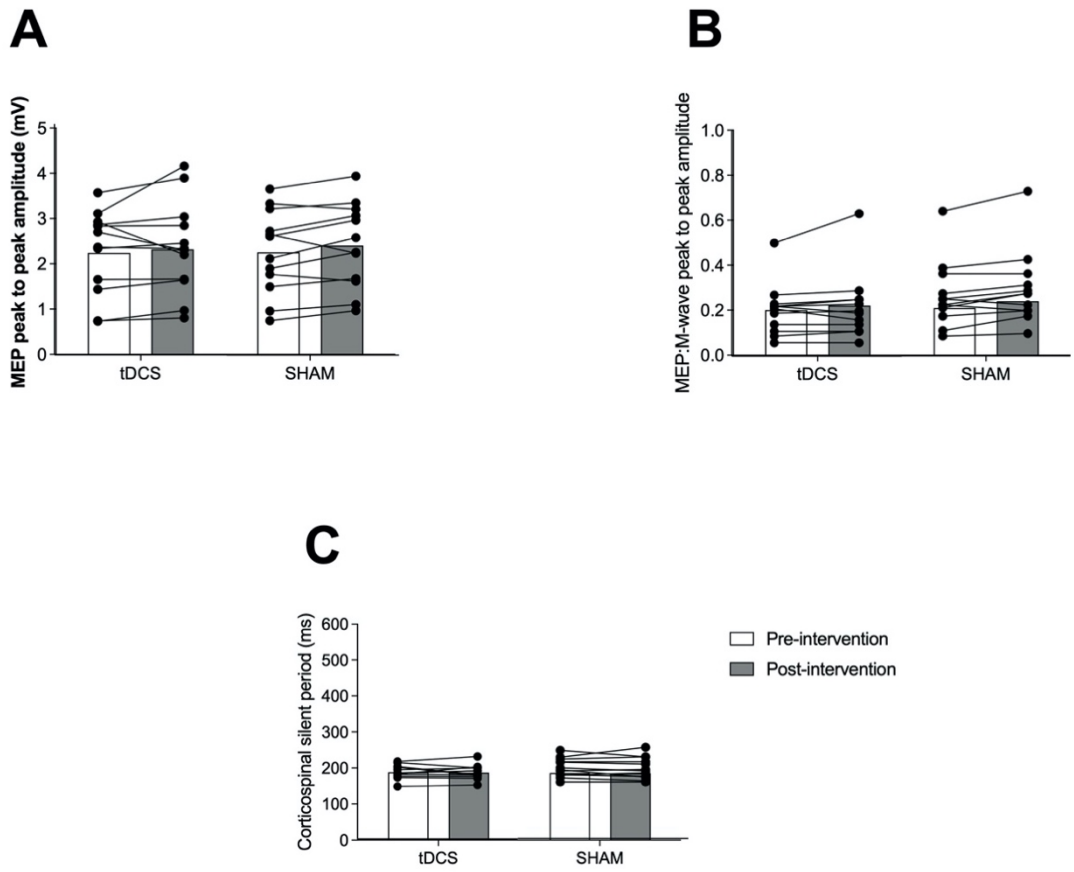

Figure 25. The corticospinal responses to bihemispheric M1 tDCS delivered during submaximal contractions. Panels A-C compare the $MEP_{p\text{-}p}$ amplitude, MEPp-p amplitude/ M-$wave_{p\text{-}p}$ ratio and the CSP at pre- and post-intervention. Bars signify the mean; individual lines represent the response of each participant.

The completion of neuromuscular assessments alongside submaximal contractions during the application of tDCS decreased the MVC force, as a result a main effect of time was detected ($F_{1,11}$ = 9.4, P = 0.011, η_p^2 = 0.460). This occurred in both stimulation conditions therefore no condition × time interactions ($F_{1,11}$ = 0.03, P = 0.860, η_p^2 = 0.003), or main

effects of condition ($F_{1,11}$ = 0.1, P = 0.732, η_p^2 = 0.01) were observed. For the estimation of cVAL, no significant condition × time interaction ($F_{1,11}$ = 0.005, P = 0.944, η_p^2 = ≤ 0.001) was detected. A significant main effect of condition was detected ($F_{1,11}$ = 6.5, P = 0.027, η_p^2 = 0.371, Figure 26B). However, there was no main effect of time observed ($F_{1,11}$ = 0.005, P = 0.944, η_p^2 = ≤ 0.001). No condition × time interaction ($F_{1,11}$ = 0.4, P = 0.526, η_p^2 = 0.04) or main effect of condition ($F_{1,11}$ = 0.6, P = 0.459, η_p^2 = 0.05) were observed in the resting evoked contractions, however there was a main effect of time ($F_{1,11}$ = 42.9, P = ≤ 0.001, η_p^2 =0.80), where the peak torque reduced after the application of tDCS was lower (tDCS 150 ± 37 N.m^{-1}; sham 147 ± 40 N.m^{-1}) in comparison to baseline (tDCS 162 ± 33 N.m^{-1}, sham 162 ± 36 N.m^{-1}).

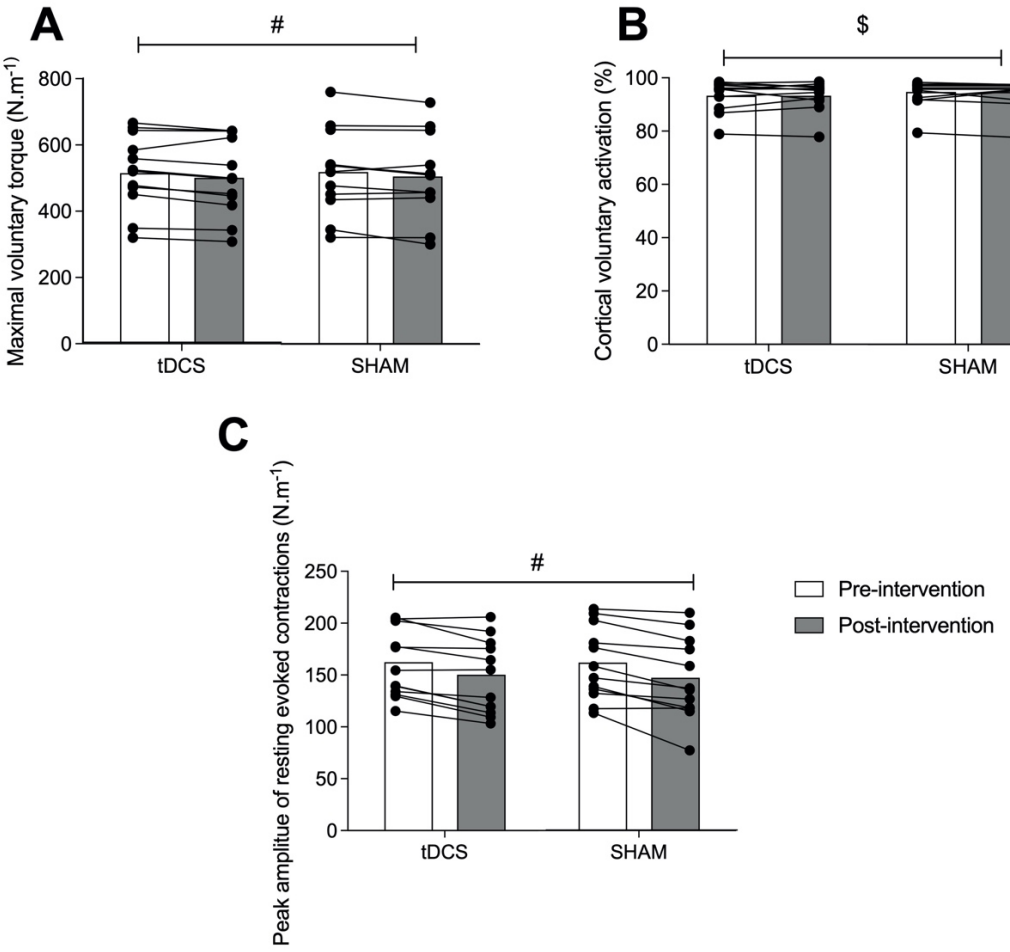

Figure 26. The neuromuscular responses to bihemispheric M1 tDCS delivered during submaximal contractions. Panels A-C compare the MVC force, estimated cVAL and force elicited during resting evoked contractions before and after the application of tDCS. # Denotes a significant main effect of time. $ Denotes a significant main effect of condition.

4.5 Discussion

The primary aim of this study was to validate the efficacy of a commercial tDCS device to modulate the corticospinal response. As the commercial device used within the present study is recommended for the use within a sport-specific warm up, this study also sought to compare the change in corticospinal excitability when tDCS was delivered at rest (part A) or during submaximal contractions (part B). The main findings of this study were that tDCS delivered in a bihemispheric montage by the Halo Sport device was unable to alter measures of corticospinal excitability (MEP_{p-p} and MEP_{p-p}/M-$wave_{p-p}$ ratio). These findings were consistent across both parts of the investigation indicating that (1) the Halo Sport Neurostimulation System was insufficient to induce a physiological change in cortical excitability, and (2) timing of stimulation (delivery at rest or during submaximal contractions) had no influence on the corticospinal response.

The present study is the first to try to externally evaluate the efficacy of the Halo Sport Neurostimulation system to modulate the corticospinal excitability. The results of part A and B indicate that this commercial tDCS device did not elicit any significant effect on measures of the excitability transmission; MEP_{p-p} amplitude or the MEP_{p-p}/M-$wave_{p-p}$ ratio. Interestingly a significant interaction effect was observed for CSP duration in Part A. This finding may indicate that tDCS induced a reduction in inhibitory transmission induced by a γ-amino-butyric-acid B ($GABA_B$) receptor mediated decline in glutamate concentration (Škarabot et al., 2019; Tremblay et al., 2013; Stagg & Nitsche., 2011). However, CSP duration is dictated by both cortical and spinal mechanisms (Škarabot et al., 2019). Therefore, it is difficult to distinguish whether tDCS influenced the cortical mechanisms without measuring the H-reflex, especially given that the pairwise comparison revealed that a significant difference occurred between the tDCS and sham condition at baseline only. This interaction effect observed in the CSP duration is unlikely to be meaningful as whilst there is a slight reduction in the CSP duration within the tDCS group after the application of tDCS, the interaction primarily appears to be determined by changes in the sham condition. Therefore, instead of a change in neurotransmitter concentration, this interaction effect detected is likely to be a consequence of variation in the measurement of CSP. Low intensity contractions such as the 10% contraction used within the present study is known to increase the variation CSP measurement, whereas greater contraction intensities (40-60% MVC) have previously shown to produce a lower coefficient of variation (Säisänen et al., 2008; Škarabot et al., 2019). This is perhaps due to the increased background EMG levels allowing for better distinction of the CSP offset (Säisänen et al., 2008; Škarabot et al., 2019).

Unlike the results of the present study, previous studies have reported tDCS to be effective in altering the corticospinal response (Priori et al., 2003; Nitsche & Paulus., 2000; Nitsche & Paulus., 2001; Angius et al., 2018; Frazer et al., 2018; Hendy & Kidgell., 2013; Hendy & Kidgell., 2014). However, modulation of the corticospinal response may be montage dependent (Nitsche & Paulus., 2000). Notably, Nitsche & Paulus (2000) reported that the improvement in M1 excitability following anodal tDCS was only obtained through the use of a cephalic montage (anodal M1, cathode FP2). Therefore, the contrasting results of the present study could be attributed to the bihemispheric montage delivered by the Halo Sport device. Indeed, the bihemispheric montage of the Halo Sport requires both an anodal and cathodal electrode to be placed over both hemispheres, leaving a relatively

small distance between the electrodes. Due to the non-invasive application of electrodes on the scalp, a great proportion of the current delivered by tDCS is shunted across the scalp, with a small portion entering the targeted brain region (Miranda et al., 2006; Miranda et al., 2013; Faria et al., 2011; Nathan et al., 1993; Bikson et al., 2010). Finite element modelling studies have previously identified that montages with a greater electrode reduce the resistance for current to penetrate the cortex, allowing increased current density within the brain region of interest (Miranda et al., 2006; Faria et al., 2011; Miranda et al., 2009; Miranda., 2013). In contrast, small inter-electrode distances increase the resistance, resulting in a large proportion of current being shunted across the scalp interest (Miranda et al., 2006; Faria et al., 2011; Miranda et al., 2009; Miranda., 2013). Therefore, it is likely that the bihemispheric montage resulted in increased current shunting across the scalp, and the small portion of current injected into the M1 was insufficient to alter the corticospinal excitability.

It is apparent that individual anatomical, genetic, and physiological factors predispose the response to both tDCS and TMS (Laakso et al., 2015; Li et al., 2015; Horvath et al., 2014; Labruna et al., 2016). Consequently, not all participants are likely to respond uniformly to anodal or cathodal tDCS (Wiethoff et al., 2014). In the present study, participants appeared to have markedly different responses to anodal tDCS which are likely to arise from inter-individual differences. Using the statistical framework outlined by Swinton et al (2018), it was determined the 53% of participants (n = 8) had a meaningful change in the MEPp-p amplitude following the application of anodal tDCS at rest (part A). Whereas, in part B, 47% of participants (n = 5) were classed to have a meaningful change in the MEPp-p response following tDCS delivered during submaximal contractions (Part B). This variability in response explains the overall null finding of tDCS. In addition to anatomical & genetic differences, hormonal fluctuations and diurnal variations are also thought to alter the response to NIBS techniques (Sale et al., 2008; Sale et al., 2009; Inghilleri et al., 2004; Horvath et al., 2014). Whilst this study controlled for circadian rhythm by ensuring all visits were conducted at the same time of day, the type of hormonal contraceptives used, or menstrual cycle phase of the female participants was not controlled for. To overcome the issues associated with intra and inter-individual differences, it is likely that the use of tDCS will need to be optimised for the individual and therefore commercial devices such as the Halo Sport may not provide a viable method of optimizing performance for many users.

The application of tDCS is usually applied to elicit the gating mechanism of metaplasticity. This mechanism presumes that the depolarization-induced by anodal tDCS will allow for transient changes in cortical excitability, and therefore bolster cognitive or motor functions (Ziemann and Siebner., 2008). However, the findings of the present study may be better explained by the homeostatic regulation of metaplasticity instead (Bienestock et al., 1982; Ziemann & Siebner., 2008).

According to the BCM theorem of homeostatic plasticity (1982), neuronal excitability is stabilized through alterations to the neuroplastic threshold to prevent unrestricted alterations in excitability. In the present study the use of TMS and submaximal contractions preceding the application of anodal tDCS may have instigated the homeostatic mechanisms of metaplasticity. Indeed, priming the M1 with rTMS immediately before the application of anodal tDCS has previously been demonstrated to induce a reversal in corticospinal excitability (Siebner., 2004; Lang et al., 2004). Whilst it is known

that the application of rTMS induces greater changes in neuronal excitability and synaptic plasticity than the single-pulse TMS used within the present study, it is presumed that the repeated application of single-pulse TMS may still be capable of instigating the secretion of neuromodulators (such as dopamine, glutamate, and serotonin), BDNF and the transcription of plasticity-related genes (Huerta & Volpe., 2009). The completion of voluntary muscle contractions prior to the applications of anodal tDCS are also thought to raise the neuroplastic threshold (Thirugnanasambandam et al., 2011; Baltar et al., 2018). Indeed, the completion of both isometric isolated muscle and whole-body dynamic exercise have shown to reduce or reverse the effects of anodal tDCS.

In line with previous studies, part A saw no changes in the force generating capabilities of the muscles, observing no significant differences in MVC, or the central (cVAL) and peripheral (resting evoked contractions) parameters of fatigue (Angius et al., 2016; Angius et al., 2018; Abdelmoula et al., 2016). However, part B saw significant reductions in torque produced during the MVC and the resting evoked contractions. This likely indicates that peripheral fatigue was induced through completing the 10% contractions during the online application of tDCS. cVAL was also significantly greater in the sham condition in comparison to the tDCS. The cVAL protocol requires the completion of a single MVC superimposed with TMS to estimate the ERT via linear regression. Consequently, the performance within the MVC is thought to critically influence the estimation of both the ERT and cVAL (Dekerle et al., 2019). Both the SIT and ERT are reputed to share similar mechanisms, and therefore can be influenced by similar variables (e.g., peripheral fatigue and poor MVC performance; Dekerle et al., 2019). As both the MVC and peak torque of the resting evoked contractions were influenced by the delivery of tDCS during submaximal contractions, it is likely that these factors contributed to the observed decline in cVAL.

4.6 Conclusions

This study evaluated the influence of the Halo Sport Neurostimulation system on its efficacy to induce changes in the corticospinal response. Considering the findings of both the present study and those detailed in study three, the lack of change in corticospinal excitability and physiological adaptations to training suggest that the Halo Sport Neurostimulation system is incapable of altering performance through reducing the magnitude of supraspinal fatigue. The results of this study also highlight that the application of tDCS during submaximal contractions to derive the benefit of task-specific modulation. These findings are likely due to the inter- and intra-individual responses to tDCS. Therefore, future use of tDCS should be optimised to the individual using HD-tDCS guided via MRI scans and computational models.

Chapter 6. General Discussion

6.1 Summary of findings

The present thesis sought to comprehensively assess the efficacy of anodal tDCS to enhance endurance exercise performance. In the last decade, tDCS has been reported as a potential ergogenic tool for use within endurance performance. According to the results within TTE trials, the application of anodal tDCS increases the activity in a brain region of interest, producing enhancements to corticospinal excitability and reducing the perceptual responses to fatiguing exercise (Cogiamanian et al., 2007; Williams et al., 2013; Angius et al., 2016; Angius et al., 2017; Angius et al., 2018; Angius et al., 2019). However, the ergogenic effects of anodal tDCS reported within the TTE trials are yet to be replicated within self-paced exercise (Andre et al., 2019; Holgado et al., 2019; Valenzuela et al., 2019; Barwood et al., 2016). Excluding the studies reported within the present thesis, the influence of tDCS on performance within a TT has been investigated a total of four times. All of which have reported no significant differences between tDCS and sham stimulation (Andre et al., 2019; Holgado et al., 2019; Valenzuela et al., 2019; Barwood et al., 2016).

Despite the lack of convincing evidence of an ergogenic influence of tDCS, there are numerous commercial tDCS devices currently sold on the consumer market which are specifically endorsed to bolster athletic performance. To date, several researchers have argued that tDCS (*if found to be ergogenic*) could be considered as a method of 'brain doping' (Davis., 2013; Reardon., 2016; Park., 2017). Although the present thesis focused upon the potential benefits of tDCS to endurance performance through the delaying the onset of supraspinal fatigue, the potential benefits extend much further due to the ease of customisation. For example, tDCS is touted to enhance cognitive functions, therefore neuro-doped athletes such as sprinters or swimmers may respond faster to the starter's pistol due to enhancement of reaction time (Davis., 2013; Huang et al., 2019). Performance in tennis is largely determined by the probability of getting the first service (Magnus & Klaasen., 1999). This is a learned skill and therefore potentially susceptible to neuro-enhancement (Davis., 2013). Transcranial direct current stimulation could also be used to reduce tremor, which would enhance the performance in precision sports such as pistol shooting (Kamali et al., 2018). It is therefore important to determine whether tDCS exerts an ergogenic effect and find a potential method of detection. By doing so, this may allow governing bodies to decide whether neuro-doping via tDCS poses a violation to sporting ethos (Davis., 2013; Park., 2017).

Chapter 2 aimed to determine the effects of tDCS applied to the DLPFC on the modulation of EIP and performance in a 15-minute cycling TT. Whilst the use of tDCS to reduce the rating of pain is well renowned, this is the first study to investigate the influence of tDCS applied to the DLPFC on the rating of EIP and TT performance. Moreover, this study is also the first to employ a bilateral montage of electrodes to influence endurance performance, despite its previous use in clinical populations (Marshall et al., 2005; Kidgell et al., 2013; Chrysikou et al., 2020). The results of this study revealed no significant effects of tDCS upon rating of EIP during a FI cycling trial. Furthermore, tDCS had no influence upon distance covered, PO or the rating of EIP during the TT. In contrast to previous findings in a clinical setting, tDCS applied in a bilateral montage to the DLPFC was unable to exert an analgesic effect to experimental pain measures. Therefore, it is thought that the bilateral electrode montage selected for this study could have contributed

to this outcome through inducing a down-regulation of the right DLPFC activity (Bikson et al., 2010).

Based upon the findings of the previous chapter, chapter 3 investigated the effect of acute applications of tDCS applied in an extracephalic montage on tolerance to EIP and TT performance. An extracephalic montage was selected for this study as the placement of the cathodal electrode on the shoulder supposedly removes the unintended down-regulatory influence that is observed in a cephalic or bilateral montage (Bikson et al., 2010; Bikson et al., 2011l Cogiamanian et al., 2007; Angius et al., 2016; Yanamadala et al., 2014). In agreement with Holgado et al (2019), the application of anodal tDCS in an extracephalic montage to the left DLPFC was insufficient to alter performance within a 15 minute cycling TT. Transcranial direct current stimulation delivered in such a montage was also incapable of exerting an analgesic effect to EIP and experimental pain measures. These null findings confirm that the acute application of anodal tDCS to the DLPFC is ineffective at enhancing endurance performance in untrained participants.

Chapters 4 and 5 investigated the effects of applying tDCS to the M1 using a commercial tDCS device. The Halo Sport Neurostimulation System is currently endorsed by several elite teams including World Champion triathletes, Paralympic swimmers, Team USA cycling and Team USA ski and snowboard (Reardon., 2016). Therefore, external validation of this device is paramount. Chapter 4 is the first study to empirically investigate the influence of chronic tDCS application, applied through the Halo Sport Neurostimulation System, on physiological adaptation to training. It therefore provides important perspectives to athletes and team coaches who are contemplating the use of tDCS to seek marginal gains. Whilst the 6-week HIIT programme significantly enhanced the VO_{2max}, PPO, $Power_{4mM}$ and TT performance in all participants, this study demonstrates that the chronic application of tDCS was unable to induce greater augmentations in comparison to the sham condition. This study also demonstrated no significant differences in the peripheral BDNF concentration following the chronic use of tDCS.

The study performed in chapter 5 is the first to evaluate whether the Halo Sport Neurostimulation System could alter the corticospinal response. To test the concept of task-specific modulation, this study also compared the effect of bihemispheric M1 tDCS on corticospinal excitability when delivered at rest and during a series of submaximal contractions. No significant differences in measures of corticospinal excitability (MEPp-p, MEPp-p/M-wavep-p) between the tDCS and sham conditions were observed for part A and part B. Therefore, it is reasonable to conclude that there was no evidence of task-specific modulation. Like previous research, tDCS failed to improve MVC torque and cVAL. The results disclosed within the present study highlight that tDCS delivered in a bihemispheric montage is unable to influence corticospinal excitability, and therefore is contrary to the manufacturers claims. Due to the proximity of the anodal and cathodal electrodes within the bihemispheric montage, it is likely that current flow to the brain region of interest is reduced because of increased shunting, this however warrants further investigation through computational modelling.

A recurring theme throughout this thesis has been the discrepancies in the ergogenic effects of tDCS reported in exhaustive and self-paced exercise. The majority of studies that have employed an exhaustive exercise protocol (such as an isometric TTE or the upper and lower limbs, dynamic TTE at a set percentage of VO_{2max} or PPO, or MIE tests)

have reported a beneficial effect of tDCS upon performance (Cogiamanian et al., 2007; Williams et al., 2013; Angius et al., 2016; Oki et al., 2016; Okano et al., 2013; Vitor-Costa et al., 2015; Angius et al., 2018; Lattari et al., 2018; Angius et al., 2019; Park et al., 2019), notwithstanding a number of differences in the tDCS current dose, measures of performance and brain area targeted (Machado et al., 2019). Despite the use of similar electrode montages, albeit in different participant groups, the ergogenic effects disclosed in exhaustive exercise paradigms have failed to be translated to self-paced exercise (Andre et al., 2019; Valenzuela et al., 2019; Holgado et al., 2019; Barwood et al., 2016; Okano et al., 2017). Potentially, this is indicative of an activity-dependent effect where tDCS preferentially enhances brain regions in a state of reduced excitability (Andre et al., 2019).

Whilst many studies have primarily focused upon the implications of supraspinal fatigue upon M1 activity due to its role in driving the muscles, recent neuroimaging studies have also demonstrated that fatigue from exhaustive exercise also induces down-regulations in distinct subcortical areas including the supplementary motor area, cerebellum, prefrontal cortex and to some extent the visual cortex (Sidhu et al., 2017; Thomas & Stephane., 2008; Liu et al., 2003; Fontes et al., 2015; Benwell et al., 2006). Whilst there are no known studies which have recorded the brain activity during endurance trials such as a TT, through the use of TMS Sidhu et al (2017) recently demonstrated that exhaustive and intense but non-exhaustive exercise influences central fatigue differently. In Sidhu et al's (2017) study, afferent feedback was demonstrated to significantly diminish M1 excitability in exhaustive exercise only, whilst intense but non-exhaustive exercise diminished the force producing capacity through impairing the activity of the motoneurons (Sidhu et al., 2017). Due to the pacing of energy expenditure throughout self-paced exercise, exhaustion rarely occurs. Therefore, if the effects of afferent feedback are also true for self-paced exercise such as a TT, perhaps tDCS is unable to exert an additive effect (Andre et al., 2019; Holgado et al., 2019).

Despite the increased interest in the use of tDCS the underlying mechanisms of action are still inconclusive. Therefore, in addition to investigating the effects on performance this thesis also sought to gain a clearer understanding of the mechanisms. It is proposed that tDCS exerts an ergogenic effect through delaying the onset of supraspinal fatigue and reduces the severity of fatigue related perceptions (Cogiamanian et al., 2007; Angius et al., 2016; Angius et al., 2017; Angius et al., 2018; Angius et al., 2019). Indeed, Angius et al (2016) demonstrated that tDCS applied in an extracephalic montage to the M1 significantly enhanced the TTE of the knee extensors, whilst reducing the RPE. Later, Angius et al (2018) demonstrated that M1 tDCS delivered in a bilateral extracephalic montage enhanced $MEP_{p\text{-}p}$ amplitude, reduced RPE and improved cycling TTE. By stimulating the DLPFC in a cephalic montage, Angius et al (2019) also demonstrated an improvement in cycling TTE and reduced RPE. Interestingly, despite the proposed analgesic effect of tDCS upon clinical pain, no studies (including the ones contained herein this thesis) have demonstrated any influence tDCS upon EIP (Angius et al., 2015; Flood et al., 2017; Angius et al., 2018; Angius et al., 2019). As it has previously been demonstrated that athletes will exercise to a given perception of RPE and EIP during self-paced exercise (Mauger et al., 2010), chapters 2 and 3 of the present thesis examined the tolerance to these perceptions using a FI cycling trial. The results of these chapters highlight that stimulating the DLPFC in a bilateral and an extracephalic montage was not

able to significantly modulate the rating of these perceptions. Whilst it appears that tDCS in these two chapters was unable to influence the cortical excitability of the DLPFC, as there were no physiological measures of brain activity collected (i.e., through fMRI, fNIRS or TMS) it cannot be categorically stated that tDCS was insufficient. An alternative explanation for the lack of change in perceptual measures could be due to the preferential modulation of a distinct neural pathway. The DLPFC is known to regulate multiple functions in addition to the emotional control of pain, including executive functions, working memory and attention (Lorenz et al., 2002; Lorenz et al., 2003; Fierro et al., 2010; Graff-Guerrero et al., 2005). The delivery of tDCS at rest may therefore lead to the upregulation of one of these distinct functions due to the inactivity of the intended pain pathway (Bikson & Rahman., 2013).

Chapter 4 also sought to use tDCS to manipulate the fatigue related perceptions generated during the HIIT training programme. In a similar manner to Seiler and Sylta (2017), chapter 4 prescribed isoeffort intervals which required participants to work at their maximal sustainable intensity. As such, it was expected if tDCS was able to influence the rating of EIP or RPE, those in the tDCS condition would cycle at a greater PO or percentage of their VO_{2peak} in comparison to the sham condition. However, the results of this study demonstrated that tDCS delivered by the Halo Sport Neurostimulation system was unable to influence the rating of RPE and EIP and furthermore did not serve a beneficial effect on training performance, as demonstrated by the non-significant differences in PO, HR, pain and RPE. This was also reflected in the lack of significant difference in the physiological adaptations to training between the tDCS and sham condition. Considering the combined findings of chapter 4 and 5, it is reasonable to assume that the discrepant findings between those reported in previous trials targeting the M1 could be in part due to the electrode design and montage. The proximity of the anodal and cathodal electrodes within the bihemispheric montage may increase the amount of current shunted across the scalp. Therefore, tDCS delivered by the Halo Sport Neurostimulation system was unable to manipulate M1 excitability and the rating of RPE and Pain. Unlike the traditional flat plastic electrode design commonly used, the Halo Sport Neurostimulation system electrodes or ‘primers’ contains 24 foam nibs, pre-soaked in an electrolyte solution. No studies to date have investigated the effects of the nib electrode design on current delivery, resistance induced or focality. Therefore, further research is warranted to investigate how this design impacts the efficacy of the Halo Sport to alter M1 excitability. Future research should also compare the tolerability of stimulation using this electrode design.

Overall, the results of this thesis have developed a greater understanding of the influence of acute and chronic tDCS applications upon endurance performance, highlighting that the use of conventional tDCS is not a valid method of exerting an ergogenic effect. A point that has been consistently stressed throughout this thesis is the necessity to implement an individualised approach to tDCS. As discussed within the literature review (sections 1.3 and 1.4) numerous methodological considerations such as the timing of stimulation and chosen current dose are known to impact the efficacy of conventional tDCS (Jamil et al., 2017; Bastani & Jaberzadeh., 2013; Hassanzahraee et al., 2020). Through using three different electrode montages, the studies within the present thesis attempted to elucidate the optimal montage to exert an influential effect on endurance performance. Furthermore, chapter 4 also sought to compare the influence of stimulation timing on changes in the

corticospinal response. Instead, the null findings reported in each of these studies confirms the influence of inconsistencies which the use of conventional tDCS cannot consider. It is now apparent that individual factors such as anatomical differences, sensitivity to TMS, chronotype and genetic differences significantly impact the response to tDCS (Li et al., 2015; Wagner et al., 2007). In the present thesis, the only potential source of variation which was explored was the influence of BDNF polymorphism in which was shown to have no influence on the findings of chapter 4. The present thesis and much of the previous literature have failed to consider the influence of anatomical differences or other sources of variation. These results highlight the intricacies underlying the influence of tDCS and solidifies the notion that the use of tDCS is not as simple as the 'plug in and play' vision of tDCS promoted by commercial tDCS companies.

6.2 Limitations

This thesis contains several limitations which may impact the final interpretation of the results. The main limitation of the present thesis is the sample size. This limitation is a common occurrence within the sport and exercise neurophysiology research, with the majority of previous tDCS literature employing sample sizes of 6-15 participants. Whilst all studies in the present thesis recruited an equivalent or greater number of participants, all four experimental studies within this thesis may be underpowered (see table 5) and therefore have an increased likelihood to commit a type II error (Barwood et al., 2016). However, the effect sizes reported within the experimental chapters highlight that tDCS had no effect on the outcome measures. In a recent review and meta-analysis, Holgado et al (2018) highlighted a positive effect of tDCS upon sporting performance, the effect size of this influence however was shown to be small (Hedges' *g* = 0.27). It was suggested that this positive effect was largely driven by studies employing small sample sizes with large standard errors and a 'file drawer' effect where reporting and publication is biased towards selecting significant results. The results of the present thesis agree with this statement, and further highlight that the current results available do not provide a strong conclusion that conventional tDCS is a viable means of enhancing endurance performance.

*Table 5. Post-hoc power analyses (calculated in G*power version 3.1)*

Chapter	Number of participants	Effect size ($\eta_p^{2)}$)	Achieved power (1-β)
2	11	0.07	0.44
3	20	0.07	0.74
4	20	0.01	0.15
5 (Part A)	14	0.01	0.11
(Part B)	12	0.03	0.20

Whilst the results of this thesis are presented considering the potential use of by elite athletes, the participants recruited within this thesis were of an untrained or recreationally trained standard according to De Pauw et al (2013). Initially, it was intended to recruit trained cyclists to the studies within this thesis, however due to difficulties in recruiting and retaining trained cyclists, a convenience sample of predominantly sport science students were used instead. These difficulties included the inability to organise visits to fit alongside a busy training and competition schedule and participant concerns over the safety of tDCS. The use of recreationally trained and untrained participants may limit the generalisation of results to elite athletes. However, it should be noted that like the studies presented within this thesis, Holgado et al (2019) and Andre et al (2019) reported no-significant differences in TT performance in highly trained cyclists. The sporting background of the participants was also not controlled for and therefore differences in pacing strategies adopted and baseline physiological characteristics may serve as an additional source of variance in the performance measures of chapters 2, 3 and 5. Indeed, previous studies have demonstrated that a pacing strategy becomes more robust with experience (Albertus et al., 2005; Mauger et al., 2009). In chapters two and three, participants completed one familiarisation trial prior to the experimental trials and therefore may not have gathered enough experience to develop a robust pacing strategy. However, when the trials of both study one and two were analysed in order of completion, there were no significant differences detected in the distance completed, indicating that no learning effects occurred.

Failing to control for the menstrual cycle phase and oral contraceptive use in the female participants is another limitation of this thesis. At the point of designing the studies, there was a dearth of literature which explored the impact of the menstrual cycle on endurance performance or the effects of tDCS. However, it has since come to light that the female sex hormones act as ‘neurosteroids’ which modulates neuronal excitability (Ansdell et al., 2019). Inghilleri et al (2004) reported that oestrogen and progesterone enhance and diminish neuronal excitability respectively. Therefore, in periods of high oestrogen concentration (such as the follicular phase) have demonstrated an improvement in corticospinal excitability following rTMS when compared periods of low oestrogen such as the menstrual phase (Inghilleri et al., 2004). The fluctuation of female sex hormones also manifests in exercise as changes in the voluntary activation, substrate metabolism and ventilatory rate (Ansdell et al., 2020; McNulty et al., 2020). These alterations are thought to indicate that the integrative response to acute exercise may differ across the menstrual cycle (Ansdell et al., 2019; Ansdell et al., 2020; Sheel et al., 2016). It is also become apparent that menstrual cycle hormones also alter the perception of pain. Whilst the impact of the menstrual cycle on the perception of EIP has not yet been explored, measures of pain quality, pain thresholds and pain tolerance to experimental pain measures has been shown to vary across the menstrual cycle and in users of oral contraceptives (Sherman et al., 2006; Màximo et al., 2015). Indeed, during the late follicular phase (days 6-14), ischemic, cold, heat and pressure pain thresholds have been shown to increase (Sherman et al., 2006). Interestingly pain threshold to electrical stimulation has been shown to improve in the luteal phase (days 14-28) (Sherman et al., 2006). Variance in the pain response has also been observed when comparing eumenorrheic females to hormonal contraceptive users (Sherman et al., 2006; Màximo et al., 2015). In comparison to eumenorrheic women and combined contraceptive users, those using progestin-only contraception demonstrated a greater pressure pain threshold

(Màximo et al., 2015). Menstrual cycle hormones also influence the circulating BDNF concentration (Pluchino et al., 2009). Indeed, during the follicular phase of the menstrual cycle and in hormonal contraceptive users, the BDNF concentration demonstrated a diurnal rhythm in a similar pattern that has been shown in male participants. Plasma BDNF concentrations were shown to increase significantly in the luteal phase of the eumenorrheic menstrual cycle, where BDNF concentration has a positive correlation to progesterone levels (Pluchino et al., 2009). Therefore, the lack of control for hormonal contraceptive use and menstrual cycle phase may have provided a source of variability in the TT performances, pain response, measurements of corticospinal excitability, BDNF concentrations and tDCS responses measured within this thesis. However, removing female participants from the statistical analysis of the studies in this thesis did not change the overall findings which indicates that the null findings reported are likely a consequence of several different factors. Nevertheless, researchers should strive to identify how menstrual cycle hormones influence the response to tDCS.

Finally, the lack of control for individual anatomical variations could also be considered as a limitation of this thesis, and a potential source of variance. However, it should be noted that the optimal measures to control for individualization have not yet been fully established. Furthermore, the use of neuroimaging techniques such as PET scans or fMRI were outside the reach of this present thesis. However, it is arguable that the methods presented within the current thesis are reflective of how conventional tDCS would presently be used by athletes to enhance performance.

6.3 Practical applications and future considerations

This thesis presents novel information concerning the efficacy of conventional tDCS to improve performance following acute and chronic applications. From the findings that targeting both the M1 and the DLPFC in multiple arrays of electrodes are unable to influence TT performance and physiological adaptations to training, it is clear that the use of tDCS is not a viable method of enhancing performance. As highlighted throughout within section 1.3, the unfocalized nature of conventional tDCS also creates ambiguity in the results, as it is uncertain if the results occurred as a result of targeting a specific brain region or through manipulating a number of brain regions due to current dispersal (Nitsche et al., 2007). Therefore, conventional tDCS has a limited application within the research community. Due to the increased use of tDCS by the public it is recommended that greater number of longitudinal studies are conducted which fully explore the influence of tDCS on brain morphology and cognitive functions. Indeed, to abide by health and safety regulations, many institutions require the use of a minimal interval between applications of tDCS is required to prevent the accumulation of deleterious effects of repeated tDCS application on the desired and distant brain areas (Davis & van Koningsbruggen., 2013). However, with the increase in DIY tutorials and commercial devices the unregulated use of tDCS increases the risk of cognitive or morphological maladaptation (Davis & van Koningsbruggen., 2013; Fitz & Reiner., 2015; Wexler., 2016). Therefore, further longitudinal data is required to elucidate whether changes in brain morphology can occur resulting from chronic tDCS use, for more stringent regulations to be created.

The use of HD-tDCS is recommended to solve the issue of focality underlying conventional tDCS, and therefore may provide an interesting insight into the role of

different cortical areas within endurance performance (Radel et al., 2017; Datta et al., 2011; Datta et al., 2012). However, as demonstrated by Flood et al (2017) and Radel et al (2017), the use of HD-tDCS alone will not necessarily lead to performance enhancements, especially if applied without consideration for individual differences in anatomy. To optimise the electrode placement for the individual, it is advised that researchers place electrodes according to MRI-derived computational models to accurately locate the brain region of interest (Bikson et al., 2012; Datta et al., 2011). The orientation of neurons is also proposed to influence the outcome of tDCS (Hannah et al., 2019; Kronberg et al., 2017; Kabakov et al., 2012). Therefore, it is likely that researchers will need to use tractograms produced via diffusion-weighted MRI to gain the desired outcome from HD-tDCS (Datta et al., 2011; Bikson et al., 2012; Kabakov et al., 2012).

Due to the individual differences in anatomy, the current dose applied should also be individualised. Indeed, previous studies have demonstrated that thickness of the cranium, CSF and subcutaneous fat determine the sensitivity to TMS and the electric field strength following tDCS (Opitz et al., 2015; Laakso et al., 2015; Truong et al., 2013; Labruna et al., 2016; Jamil et al., 2017; Kozel et al., 2000; Strube et al., 2015; Li et al., 2015). Reductions in electric field strength have previously been demonstrated when CSF thickness is increased (Opitz et al., 2015). Consequently, the intensity of tDCS should be altered according to an individual's anatomy. Participant chronotype is demonstrated to influence the diurnal variation of M1 excitability (Tamm et al., 2009), therefore it is also reasonable to assume that chronotype may also influence the efficacy of tDCS throughout the day. As such researchers may need to implement neuroimaging methods through the stimulation period to monitor the tDCS induced changes in activation to ensure the intended effects of tDCS occur (Siebner et al., 2009; Sood et al., 2016; Muthalib et al., 2018). Indeed, the coupling of neuroimaging techniques such as fNIRS and EEG has recently been proposed as a viable method of monitoring alterations in resting brain activation during the application of anodal HD-tDCS and therefore may also provide a means of individualising the application of HD-tDCS (Sood et al., 2016). If this is required, commercial tDCS devices such as the Halo Sport Neurostimulation System may be rendered useless as the bulky electrodes used do not allow for the concurrent application of other neuroimaging techniques. Furthermore, users of this device are unable to alter the duration of stimulation and therefore would not be able to achieve a personalised application of tDCS.

It is also likely that researchers will need to screen participants for genetic composition. Whilst the results of the present study demonstrate the BDNF polymorphism had no influence on training performance and physiological adaptations following HIIT training combined with tDCS, this result was due to the greater proportion of val66val carriers (n = 9) within the tDCS group. Therefore, the results of the present study cannot be taken as conclusive evidence that BDNF polymorphism does not impact the influence of tDCS on endurance performance. Further research with significantly greater sample sizes is warranted to fully elucidate the links between BDNF polymorphism and tDCS efficacy in healthy individuals.

Whilst much of these recommendations may be accessible for research and clinical applications, it is unlikely that this will be a viable option for athletes to incorporate into their training and competition schedule. In comparison to conventional tDCS, the use of the proposed techniques will be more time consuming, costly and require the constant presence of a trained individual to maintain the precision of application. Furthermore,

these techniques are also likely to only be conducted within a university or hospital. All of which may not be logistically convenient or accessible for athletes to implement during training.

6.4 Conclusion

The overarching aim of this thesis was to investigate the efficacy of conventional tDCS to enhance endurance exercise performance. The studies contained within this thesis were unable to detect an ergogenic effect of anodal tDCS for self-paced TT performance, HIIT cycling sessions and physiological adaptation to training. Furthermore, due to the lack of change in both physiological and perceptual measures observed, the mechanisms underlying tDCS' supposed ergogenic effect remain unclear. As such the use of tDCS by the public to seek marginal gains cannot be recommended.

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Appendices

Appendix A. Example of Participant Information Sheet (Chapter 5, Version 2)

PARTICIPANT INFORMATION SHEET

School of Sport and Exercise Sciences

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The application of transcranial direct current stimulation to enhance physiological adaptation from aerobic interval training; is it effective, and can it be detected?

Name of Researchers

Megan Judge

Dr Lex Mauger

Dr James Hopker

You are being invited to take part in a research study, which has been ethically approved by the SSES Research Ethics Committee. Before deciding whether to participate, please carefully read this information sheet before deciding whether to participate, it is important that you understand why this information is being collected and what the study involves. Please ask if anything is not clear, or you require further information.

What is this study about?

Transcranial direct current stimulation (tDCS) is a safe non-invasive brain stimulation technique, used to alter the excitability of a targeted brain area. This technique was made popular from its use in research studies aiming to study and improve the brain function of healthy and clinical populations. Recently, tDCS has attracted attention for its potential to enhance endurance performance. This has led to the manufacture of many commercial tDCS devices targeted for the use by athletes during training.

In this study we want to investigate whether tDCS application over a 6-week training programme will lead to greater physiological adaptations and improve performance, and to see whether chronic use of tDCS can be detected.

Inclusion Criteria

We are looking for healthy, recreationally active (minimum of 3 hours of moderate intensity exercise per week) males and females aged between 18 and 55 years old to take part in this study. You must be free from cardio-respiratory disease, a history of brain/ mental disorders (e.g., lesions, epilepsy, depression and schizophrenia), any implants from surgery (e.g., cochlear implants, cardiac pacemakers or intracranial metal implants) or current skeletal muscle injuries. Prior to testing you will be asked to complete a health questionnaire and an exclusion criteria checklist to ensure your readiness to participate in physical activity.

It should also be advised that participants consider the appropriateness of taking part in this study if they take part in sport competitively. Although there is no current implications of the use of tDCS in doping regulation of sport, this method has been suggested as a potential method to enhance performance and adaptations to training.

Do I have to take part?

No, but if you do decide to take part you will be given this information sheet to keep and will be asked to sign an informed consent form. You will be free to withdraw from this study at any time, without having to provide a reason and you can ask to have your data removed from the data analysis.

What will I have to do?

You will be asked to visit the Sport and Exercise Science laboratory at Medway Park Leisure Centre on 16 separate occasions in total. You will be required to attend the laboratory twice a week for 6 weeks to complete a training programme of high-intensity cycling. As well as this you will be required to visit the laboratory for two baseline visits, and two post-training visits to assess your VO_{2max}, cycling efficiency and cycling performance. Each visit will be separated by a minimum of 48 hours. All visits will take no more than 1 hour in total and all visits will be completed within 8 to 10 weeks.

Baseline & Post-training testing

Day 1- Upon arrival, a general health screening will be conducted to ensure your readiness to participate in this study. You will then complete a threshold cycling test, combined with a maximal intensity cycling test, to obtain values for your cycling efficiency, blood lactate thresholds, VO_{2max} and maximum minute power output (W_{max}). You will start off the test at a light intensity, cycling at stages of 5 minute duration. The intensity will continue to increase by 25 W until your blood lactate reaches 4 mmol.L. After this you will immediately complete the maximal intensity part of the test, whereby the intensity will increase by 1 W every 2 seconds until volitional exhaustion.

Day 2- When you arrive at the lab you will be asked to provide a blood sample and a saliva sample. After these two tests you will be required to complete two cycling tests. The first test assesses your perceptual response to cycling at a constant power output. For this you will be cycling for 10-minutes at a power output determined by when your blood lactate reached 4 mmol.L in the previous visit. After 5 minutes of recovery, you will then complete a 5 km self-paced time trial, aiming to complete this distance as quickly as possible.

Training Programme

Over a 6-week period you will be asked to attend the laboratory twice a week to complete the aerobic intervals training programme. For each training session, you may have up to 3 other participants training with you like a group exercise class. Each session will consist of 4x8 minute intervals, separated by 2 minutes of active recovery. You will be instructed to complete each interval at the maximal intensity that you can sustain. Throughout each interval the researchers will record your power output, heart rate, and perception of effort. On weeks 2 and 4 one of the training sessions will be replaced with a 10-minute fixed power trial and a 5 km time trial. On one of the trials on weeks 1, 3 and 6 fingertip capillary and saliva sample will be taken before and after the training session.

Figure 1: Halo neuro-stimulation device

Before you start exercising, we will stimulate your motor cortex for 20 minutes using the Halo Sport tDCS device (fig. 1). The tDCS procedure does not produce any pain, however there is an unusual itching sensation produced underneath of the electrodes, this usually disappears after a couple of minutes.

Are there any risks or disadvantages of taking part in this study?

During exercise

There are foreseeable discomforts during the exercise sessions, similar to what you may feel during normal training and competition. However, there exists the possibility of changes occurring during each visit. These include fainting, abnormal blood pressure; irregular, fast or slow heart rhythms; and in rare instances heart attack, stroke, and death. Every effort will be made to minimize these risks through evaluation of preliminary information relating to your health and fitness as well as careful observation throughout the tests. Emergency equipment and trained personnel are available to deal with unusual situations that may arise. It is also important that you understand that you are able to stop when you wish because of feelings of fatigue or any other discomfort.

tDCS

During tDCS you will feel a mild tingling sensation that might be accompanied by a slight itching sensation under the stimulation electrodes. After tDCS, occasionally a few people might feel a mild headache or nausea, although this occurs rarely. In case you feel any of the side effects, we suggest you to not schedule any activity such as important meetings or test in the following hours.

An infrequent, harmless, but uncomfortable effect is a mild headache, which is probably caused by the activation of scalp and neck muscles. The headache may persist after the end of the stimulation session. In some cases, you may experience feelings of elevated mood as a consequence of tDCS. In case you get a headache, we won't proceed with the experiment, and we can reschedule the session. If you continue to get a headache in the following sessions, we will withdraw you from the experiment. The headache can be treated with standard pain relievers (e.g., paracetamol and ibuprofen). Please consult your doctor if the headache persists for longer than 24 hours. There is also a small chance of increased skin irritation (reddening) following tDCS application, to ensure that any injury to the skin is prevented, the researchers will check the area prior to tDCS application. Additionally, you will be required to fill in an adverse effect's questionnaire before and after each training session. If the researcher deems that any injury or atypical responses have/ or are likely to occur, you will be withdrawn from the study.

What are the advantages of taking part in this experiment?

Through participating in this present study, you will be instructed through a 6 week structured training programme, allowing you to improve your overall fitness. You will also be able to train using the Halo Sport Neuro-stimulation device, this cutting edge piece of technology has been tried and tested by various NFL, NBA, Tour de France and Ironman Triathlon athletes. In addition to this, you will also be provided with free performance tests, before and after completing the training programme. This will provide you with an accurate measure of your cardiovascular fitness including your VO_{2max} (the maximal volume of oxygen you can utilise during exercise), as well as your cycling efficiency and blood lactate thresholds. This study will allow you to assess the effectiveness of your previous training was, as well as ideas of how to structure your training in the future. Also, as part of your participation in this study, you will receive up to £50 for your time and expenses, which will be provided pro-rate across the 16 testing sessions.

Will I get to know the results of the study?

If you are interested in getting the results of the study, please leave your contact information with the researcher and we will send you a summary of the findings and a full PDF copy of the study, should the study be published.

Will the information be confidential?

All the data collected and analysed during the study and any personal information will be kept in accordance with the Data Protection Act 1998 and the University of Kent's own data protection requirements. No data will be passed on to any third party. This study will be written up as an experimental Chapter of a PhD thesis and may be further published in scientific journals or conference papers, however no references will be made that could reveal an individual participants identity.

Other considerations

Please eat a similar meal before each visit (throughout your time in the study you will be asked to fill out a training and food diary), and report to the lab in appropriate sports kit. You are also asked to adhere to the following:

- Whilst in the study please do not take up any new types of training (e.g., do not start a new resistance training programme)
- Do not complete any additional interval training outside of what is completed in the laboratory.
- Please complete the training and food diaries provided
- No strenuous exercise 24 hours before each visit
- No Alcohol 24 hours before each visit
- No Caffeine 2 hours before each visit
- No heavy meals within the 2 hours before each visit
- No consumption of pain killers 6 hours before each visit
- Free of illness or infection 2 weeks prior to testing.

Who can I contact about this study?

If you are interested in participating, would like more information or have any questions please do not hesitate to contact me

Megan Judge Mj361@kent.ac.uk

In the unlikely event that you wish to make a complaint about the conduct of this study please contact Dr Lex Mauger l.mauger@kent.ac.uk

Thank you for taking the time to read this information sheet. If you are happy to participate in this study, please indicate your consent by signing the informed consent form.

Appendix B. Example of Participant Informed Consent (Chapter 5)

Title of project: The application of tDCS to enhance physiological adaptation from aerobic interval training; is it effective, and can it be detected?

Name of investigator: Megan Judge (mj361@kent.ac.uk)

Name:…… D.O.B:…….

Please initial box

1. I confirm I have read and understand the information sheet dated 1/11/2017 (Version 1.) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. (If you wish to withdraw, please contact me via email: mj361@kent.ac.uk) ☐

3. I understand that my data will be anonymised before analysis. I give permission for members of the research team to have access to my anonymised data, and for my blood samples to be used for research purposes. ☐

4. I agree to take part in the above research project. ☐

Name of participant	Date	Signature

ADDITIONAL DECLARATION in relation to attached Health Questionnaire

Please read and sign the declaration below: ***I, the undersigned, have read, understood and completed the attached questionnaire (PAR-Q) to the best of my knowledge.***

NAME: ……SIGNATURE: ……DATE: ……

Name of person taking consent	Date	Signature

(If different from lead researcher) To be signed in the presence of the participant.

Lead researcher	Date	Signature

5. If you wish to be contacted after the study has been completed to receive a summary of the findings please initial the box and leave contact details below. ☐

Contact details:……

Appendix C. Example of tDCS Exclusion Criteria Questionnaire (Chapter 5).

RESEARCH PARTICIPANT EXCLUSION CRITERIA

Title of Study: The application of tDCS to enhance physiological adaptation from aerobic interval training; is it effective, and can it be detected?

Dear Participant,

Thank you for showing an interest in participating in the study. Please read the questions below carefully and answer honestly. Undertaking a test with any of the below conditions can be dangerous – your safety is our priority

YOU SHOULD NOT PERFORM THIS TEST IF YOU ANSWER 'YES' TO ANY OF THE CRITERIA BELOW

	YES	NO	I'M NOT SURE
• Do you have a history of cardiovascular disorders (e.g., Angina, high blood pressure, heart attack)			
• Do you have a history of seizures?			
• Do you have a history of mental health issues (e.g., depression, anxiety, bipolar disorder? schizophrenia)			
• Do you have a history of brain disorders (e.g., epilepsy, lesions, Parkinson's, multiple sclerosis)?			
• Do you have any implants from surgery (e.g., cochlear hearing implant, cardiac pacemakers, or intracranial metal implants)?			
• Do you have (or have you had in the last 12 months) a bone, soft tissue (ligament, tendon, cartilage) or joint injury?			
• Do you have any blood borne viruses (e.g., hepatitis B, HIV)			
• Do you have any anxiety/ phobias towards needles?			
• Are you taking any medication? If yes, please state what medication you're taking. ……			
• Do you have any other conditions that you think may be a danger to you participating?			

IF YOU HAVE ANY QUERIES PLEASE CONTACT **MEGAN; mj361@kent.ac.uk**