THE EFFECT OF TRANSCRANIAL DIRECT CURRENT STIMULATION ON EXERCISE PERFORMANCE

Thesis submitted at the University of Kent
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Doctor of Philosophy

by

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"La professione del ricercatore deve tornare alla sua tradizione di ricerca per l'amore di scoprire nuove verità. Poiché in tutte le direzioni siamo circondati dall'ignoto e la vocazione dell'uomo di scienza è di spostare in avanti le frontiere della nostra conoscenza in tutte le direzioni, non solo in quelle che promettono più immediati compensi o applausi".

Enrico Fermi (E. Fermi, dal discorso tenuto presso lo Union College nel Commencement Day dell'anno 1947).

"The role of the researcher must return to its tradition of pursuit of love to discover new truths. As in all directions we are surrounded by the unknown and the vocation of the scientist is to move forward the frontiers of our knowledge in all directions, not just those that promise more immediate rewards or applause".

Enrico Fermi (E. Fermi, from the speech at the Union College Commencement Day in the year 1947).

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ACC	anterior cingulate cortex
ANOVA	analysis of variance
ATP	adenosine triphosphate
B[La^-]	blood lactate concentration
BF	biceps femoris
CAR	central activation ratio
CATHODAL	cathodal tDCS stimulation
CG	central governor
CGM	central governor model
CMEP	cervical motor evoked potential
CNS	central nervous system
CO	cardiac output
CON	control condition
CPT	cold pressor test
CSP	cortical silent period
CV	coefficient of variation
cTBS	continuous theta burst stimulation
DAP	diastolic arterial pressure
DLPFC	dorsolateral prefrontal cortex
EEG	electroencephalogram
EIP	exercise-induced pain
EMG	electromyography
EXE	exercise
EXP	experimental condition
fMRI	functional magnetic resonance imaging
GABA-B	aminobutyric acid type B
HEAD	tDCS cephalic montage
HR	heart rate
HRV	heart rate variability

IASP	International Association for the Study of Pain
IC	insular cortex
iEMG	integrated electromyography
LDLPC	left dorso lateral prefrontal cortex
M1	motor cortex
M_{amp}	M_{wave} amplitude
MAP	mean arterial pressure
M_{area}	M_{wave} area
MBP	mean blood pressure
MEP	motor evoked potential
MEP_{amp}	motor evoked potential amplitude
MEP_{dur}	motor evoked potential duration
MVC	maximal voluntary contraction
MVCs	Maximal voluntary contractions
M_{wave}	muscular wave
NIRS	near-infrared spectroscopy
O_2Hb	oxyhaemoglobin
PAR-Q	physical activity readiness questionnaire
PEMI	post exercise muscle ischemia
Pt	peak twitch
rTMS	repetitive transcranial direct current stimulation
RER	respiratory exchange ratio
RMS	root mean square
RPE	rating of perceived exertion
rpm	revolutions per minute
SAP	systolic arterial pressure
SHOULDER	tDCS extracephalic montage
SMA	somatory-sensory cortex area
SV	stroke volume
SVR	systemic vascular resistance
TC	temporal cortex
tDCS	transcranial Direct Current Stimulation
TES	transcranial electrical stimulation
tHb	total haemoglobin
TMS	transcranial magnetic stimulation

TSI	tissue saturation index
TTE	time to exhaustion
Tw	twitch
SHAM	tDCS sham or placebo condition
SV/VET	stroke volume ventricular ejection time ratio
VA	voluntary activation
VAL	voluntary activation level
VET	ventricular ejection time
VL	vastus lateralis
WMA	World Medical Association
W_{max}	maximal peak power output
ΔO_2Hb	delta oxyhaemoglobin
ΔtHb	delta total haemoglobin
ΔHHb	delta deoxyhaemoglobin
ΔHbdiff	delta haemoglobin difference

Publications arising from the thesis

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General abstract

This thesis was supervised by Dr. Lex Mauger (University of Kent, UK), co-supervised by Dr. James Hopker (University of Kent, UK) and Prof. Samuele Marcora (University of Kent UK).

The physical limits of the human being have been the object of study for a considerable time. Human and exercise physiology, in combination with multiple other related disciplines, studied the function of the organs and their relationship during exercise. When studying the mechanisms causing the limits of the human body, most of the research has focused on the locomotor muscles, lungs and heart. Therefore, it is not surprising that the limit of the performance has predominantly been explained at a "peripheral" level. Many studies have successfully demonstrated how performance can be improved (or not) by manipulating a "peripheral" parameter. However, in most cases, it is the brain that regulates and integrates these physiological functions, and much of the contemporary literature has ignored its potential role in exercise performance.

This may be because moderating brain function is fraught with difficulty, and challenging to measure. However, with the recent introduction and development of new non-invasive devices, the knowledge regarding the behaviour of the central nervous system during exercise can be advanced. Transcranial direct current stimulation (tDCS) and repetitive transcranial magnetic stimulation (rTMS) are two such methods. These methods can transiently moderate the activity of a targeted brain area, potentially altering the regulation of a particular physiological (or psychological) system, and consequently eliciting a change in exercise performance.

Despite the promising theory, there is little or no experimental data regarding the potential to moderate neurophysiological mechanisms through tDCS to improve exercise performance. Consequently, the experiments performed as part of this thesis investigated the capacity for tDCS to alter physical performance. The ability of tDCS as a targeted and selective intervention at the brain level provides the unique opportunity to reduce many methodological constraints that might limit or confound understanding regarding some of the key physiological mechanisms during exercise. Therefore, the primary aim of this thesis was to investigate how tDCS may moderate both

central and peripheral neurophysiological mechanisms, and how this may effect various exercise tasks.

The first study investigated the effect of a well-documented analgesic tDCS montage on exercise-induced muscle pain. This study demonstrated for the first time, that although anodal tDCS of the motor cortex (M1) reduces pain in a cold pressor task, it does not elicit any reduction in exercise-induced muscle pain and consequently has no effect on exercise performance. As reductions in exercise-induced pain have previously been documented to improve performance, probably the lack of effect was due to either the M1 having a limited processing role in exercise-induced pain, or that the cathodal stimulation of the prefrontal cortex negated any positive impact of anodal M1 stimulation.

Given the lack of guidelines for tDCS electrode montage for exercise, the second study examined the effect of different electrode montages on isometric performance and the neuromuscular response of knee extensor muscle. Given that the anode increases excitability and the cathode decreases excitability, the placement of these has the potential to elicit significant effects on exercise performance. The results showed that exercise performance improved only when an extracephalic tDCS montage was applied to the M1, but in the absence of changes to the measured neuromuscular parameters. These results suggest that tDCS can have a positive effect on single limb submaximal exercise, but not on maximal muscle contraction. The improvement in performance was probably the consequence of the reduction in perceived exertion for a given load. This is the first experiment showing an improvement in exercise performance on single joint exercise of the lower limbs following tDCS. The results suggest that the extracephalic set-up is recommended for exercise studies in order to avoid any potential negative effect of the cathodal electrode.

Previous studies investigating tDCS have shown its potential to alter autonomic activity, and in some circumstances reduce the cardiovascular response during exercise. Considering the emerging studies and applications of tDCS on exercise and the potential benefits of tDCS in the treatment of cardiovascular diseases, the third study monitored multiple cardiovascular variables following tDCS in a group of healthy volunteers. Using more advanced techniques and methods compared to previous research, including the post exercise ischemia technique and transthoracic bioimpedance, the results suggest

that tDCS administration has no significant effect on the cardiovascular response in healthy individuals.

The final study sought to apply the findings obtained in the study 2 to whole body exercise. The same extracephalic set up was applied over both the motor cortices, with both anodal and cathodal stimulation conditions. The neuromuscular response and cycling performance was also monitored. Following anodal tDCS, time to exhaustion and motor cortex excitability of lower limbs increased. Interestingly, cathodal stimulation did not induce any change in cycling performance or neuromuscular response. This study demonstrated for the first time the ability of anodal tDCS to improve performance of a constant load cycling task, and highlights the inability of cathodal tDCS to decrease cortical activation during muscle contraction.

Taken together, the experiments performed as part of this thesis provide new insights on how brain stimulation influences exercise performance, with notable findings regarding the role of M1 excitability and perception of effort. Furthermore, considering the lack of knowledge regarding the use of tDCS on exercise, these findings will help further understanding of how to apply tDCS in exercise science. This consequently improves the knowledge base regarding the effect of tDCS on exercise and provides both a methodological and theoretical foundation on which future research can be based.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.0 Physiology of exercise induced fatigue

Fatigue is a common word used for a wide range of daily activities experienced by each person during their life. In general, the term fatigue refers to a condition of tiredness from mental or physical exertion and/or disease (Zwarts, Bleijenberg, & van Engelen, 2008). However, given the various conditions where fatigue is experienced, further and more specific definitions have been proposed. The complexity of fatigue during exercise can be easily represented by the widespread range of organs and physiological systems involved (Fig. 1), therefore it is not surprising the variety and number of models which have been proposed to explain its causes (Abbiss & Laursen, 2005).

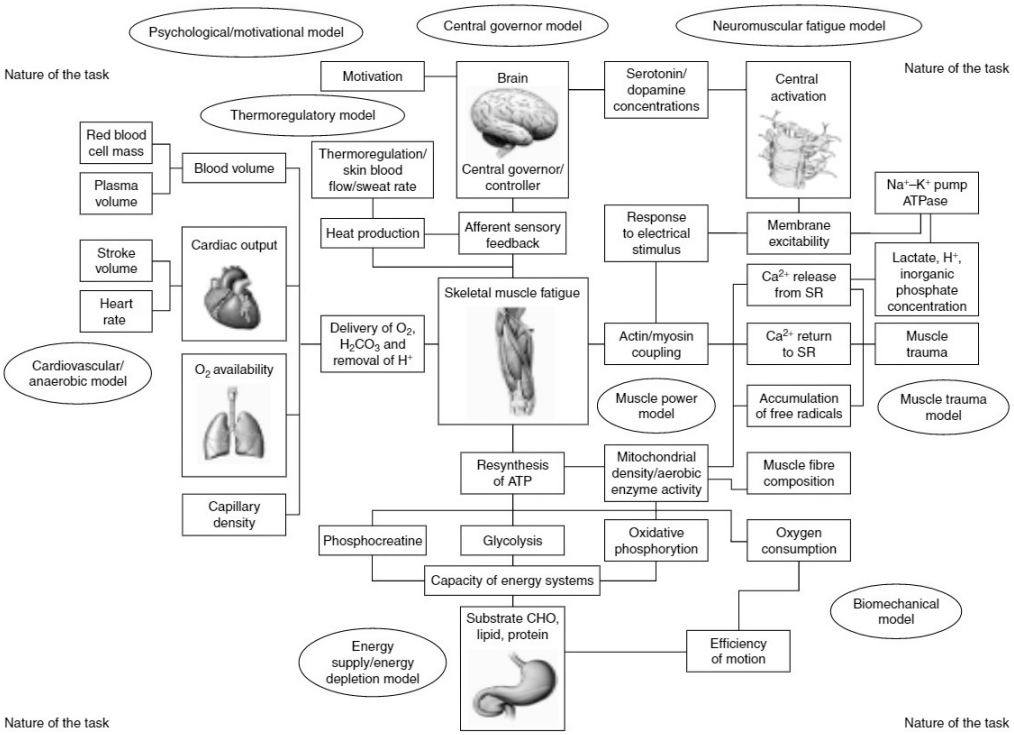


Fig 1. Diagram showing various physiological mechanisms causing fatigue. From (Abbiss & Laursen, 2005).

When exercising, the state of muscle, energy stores and the neuromuscular system change, decreasing the capacity of the muscle to produce force (Boyas & Guével, 2011; Gandevia, 2001). Fatigue has been defined as a failure to maintain the required or expected force (Edwards, 1981), which coincides with the point of voluntary exhaustion. This definition however, is still very general and does not take in account the specificity or the various origins of fatigue. Therefore, in the last few decades further classifications and definitions of fatigue have been proposed, in order to describe the neurophysiological mechanisms underlying the decrement in the maximal force capacity of the muscle and/or voluntary exhaustion.

Currently, the standard definition fatigue has been proposed by Gandevia (2001), who defined muscle fatigue as any exercise-induced decrease in the maximal force production of the muscle. Muscle fatigue is commonly measured with brief maximal isometric contraction (MVC), and so a decline in maximal force produced during an MVC indicates the development of muscle fatigue. By using this measure, many experiments have adopted the repetition of MVCs at regular intervals to monitor the development of muscle fatigue (Søgaard, Gandevia, Todd, Petersen, & Taylor, 2006; Taylor & Gandevia, 2008). In these cases, during prolonged exercise such as running, cycling or isometric tasks, muscle fatigue has been documented to start developing early and progressively, and well before the point of exhaustion (Marcora & Staiano, 2010; Søgaard et al., 2006; Taylor & Gandevia, 2008). Initially, researchers commonly believed that the reduction in maximal force capacity was mainly (or solely) caused by peripheral mechanisms. However, with the introduction of new methodologies and techniques, experimental research demonstrated that the central nervous system also plays an important role in the physiological processes leading to muscle fatigue.

Since the capacity to generate voluntary force requires a chain of events starting from the higher brain centres and ending at the muscle, fatigue can occur at any site of this neuromuscular pathway (i.e. either peripheral and/or central fatigue) (Boyas & Guével, 2011; Gandevia, 2001). This subdivision is important as it takes into account the contribution of both the central nervous system and muscle, and consequently it is possible to quantify, isolate and study the contribution of each system (Fig 2).

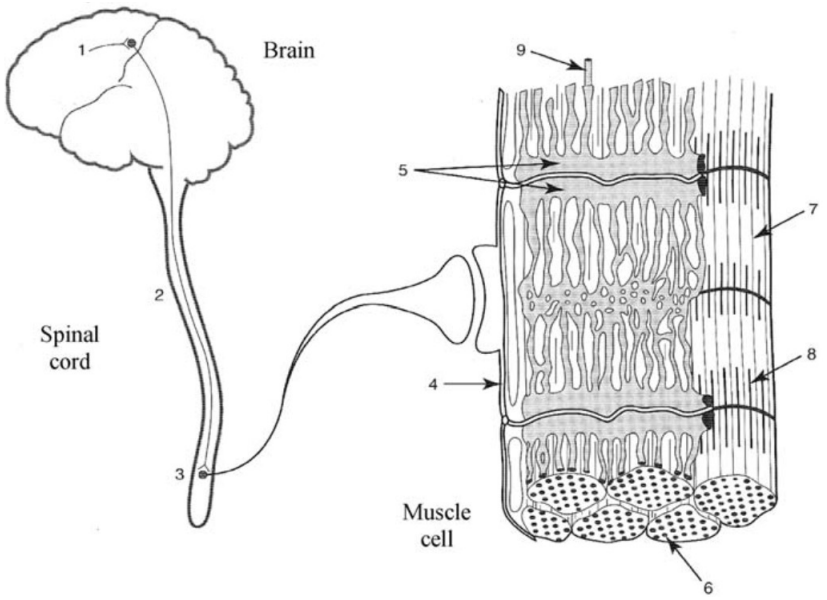


Fig 2. Schematic representation of different sites contributing to muscle fatigue. From Boyas and Guevel (2011).

Each number corresponds to a different site where muscle fatigue can occur: (1) activation of the primary motor cortex; (2) propagation of the motor command from the central nervous system to the motoneurons (the pyramidal tract); (3) activation of the motor units and muscles fibres; (4) neuromuscular propagation; (5) excitation-contraction coupling process; (6) different metabolic substrates availability; (7) state of the intracellular system; (8) contractile capacity; (9) muscle blood flow.

1.0.1 Peripheral fatigue

Peripheral fatigue refers to any change at, or distal to, the neuromuscular junction (Boyas & Guével, 2011; Gandevia, 2001), without involving any physiological process occurring at central nervous system level. Therefore, it refers to intrinsic factors in the muscle, which impair the muscle fibre's ability to produce force. Peripheral fatigue has been documented to be caused by alteration of excitation-contraction coupling (Allen, Lamb, & Westerblad, 2008; Fitts, 2008), propagation of the muscular wave (Mwave) (Boyas & Guével, 2011) and neuromuscular transmission (Ollivier-Lanvin, Lemay, Tessler, & Burns, 2009; Pagala, Namba, & Grob, 1984).

A variety of cellular mechanisms participate in the generation of peripheral fatigue, and their contribution changes according to the duration or the intensity

of muscle contraction. The balance of electrolytes inside and outside the cell is fundamental and therefore any change in the electrochemical properties of muscle cells might compromise the force generation. The observed changes in concentration of Na^+ inside the cell, with an increase of K^+ outside the cell (Sjøgaard, 1991), might in part explain the altered propagation of the action potential (Sjøgaard, 1991). Peripheral fatigue has been also associated with alteration of Ca^{++} (Allen et al., 2008). Since Ca^{++} is fundamental in the formation of cross-bridges, and any reduction of Ca^{++} availability or kinetics will reduce the force generation capacity of the muscle fibre (Allen & Westerblad, 2001).

Peripheral fatigue has been observed during both short (Amann, Proctor, Sebranek, Pegelow, & Dempsey, 2009) and prolonged exercise tasks (Lepers, Hausswirth, Maffiuletti, Brisswalter, & van Hoecke, 2000), and it changes according to the type, duration and intensity of the exercise performed (Abbiss & Laursen, 2005). A greater contribution of peripheral fatigue has been observed during short duration intense exercise (Amann, Blain, et al., 2011; Amann et al., 2009), which is also characterised by a significant contribution from anaerobic metabolism (Abbiss & Laursen, 2005). Anaerobic breakdown of glycogen is well known to increase the level of intracellular acids such as lactate and the associated H^+. The accumulation of lactate and H^+ causes a decline in pH which has been correlated with a decline in force production (Westerblad, Allen, & Lännergren, 2002). The contribution of these mechanisms is also dependent on the level of oxygen available to the exercising muscles. This is particularly evident in extreme conditions such as hypoxia or muscle contraction with blood flow restriction, where the metabolic status of the muscle is compromised and the level of peripheral fatigue is exacerbated (Amann & Calbet, 2008). Thus, peripheral fatigue may partly be effected by peripheral hemodynamic factors, which is discussed in section 1.0.1.

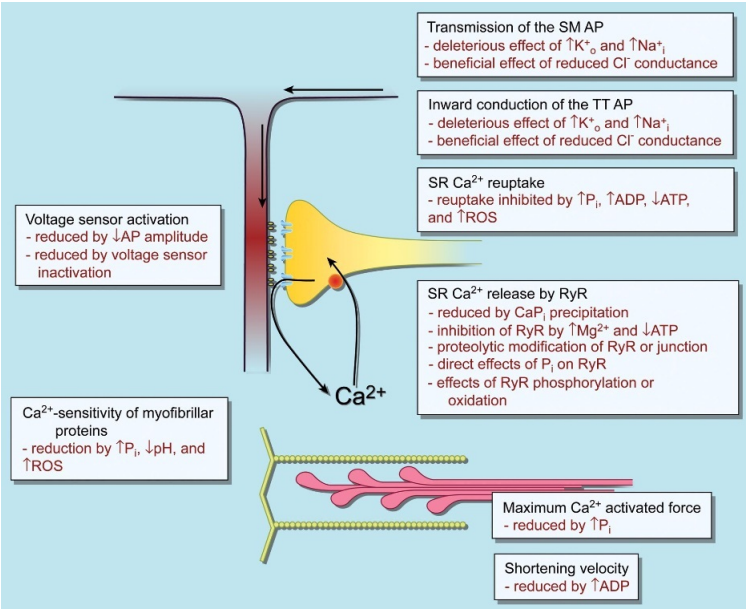


Fig 3. List of cellular and chemical mechanisms causing peripheral fatigue muscle during voluntary contraction. From Allen et al (2002).

1.0.2 Central fatigue

The voluntary production of force implies a complex series of events starting from the brain and ending at the muscle. As a consequence, the ability to generate force not only depends on peripheral mechanisms (e.g. muscle fibres) but also in the ability of the nervous system to drive and control the muscle (Boyas & Guével, 2011; Gandevia, 2001). Given the neuroanatomical and physiological link between the nervous system and muscle, the complex interplay between these two systems provides various hypotheses regarding the causes and origin of fatigue within the central nervous system (Boyas & Guével, 2011; Gandevia, 2001; Taylor & Gandevia, 2008). A decrease in voluntary activation level (VAL) of the muscle has been defined as central fatigue (Gandevia, 2001). However, because central fatigue is known to develop at any site of the central nervous system (e.g. supraspinal and spinal level) a sub-distinction is necessary. The physiological mechanisms causing fatigue within the nervous system are less clear than those involved in the developing peripheral fatigue (Gandevia, 2001; Taylor & Gandevia, 2008).

Fatigue occurring at a spinal level is defined as spinal fatigue, and mainly refers to a reduction in excitability of the motoneuronal pool (Gandevia, 2001). This may involve a bottom up process, as the discharge rate of the motoneurons is also regulated in response to both mechanical and metabolic reflexes of muscle contraction (Bigland-Ritchie, Dawson, Johansson, & Lippold, 1986). It has been hypothesized that a complex system arising from muscle reflexes projecting at spinal level, might be the major contributor to the motorneuron's inhibition (Boyas & Guével, 2011; Gandevia, 2001).

Muscle spindles (group Ia and II afferents) are well known to detect variations in the mechanical tension of muscles during exercise and their inputs at spinal level have been suggested to contribute to the spinal fatigue (Boyas & Guével, 2011; Gandevia, 1998, 2001). However, it should be taken into account that their inhibitory effect at a spinal level is still uncertain. This is likely due to the difficulty in isolating these structures and their variable and rapid discharge rates during muscle contraction (Boyas & Guével, 2011; Gandevia, 1998, 2001) (Fig 4).

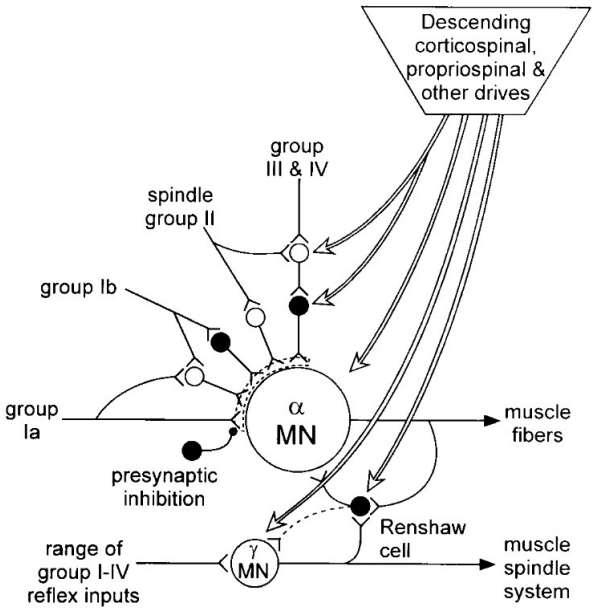


Fig 4. Summary of different inputs to ∝- and γ-motorneurons for an agonist muscle during voluntary contraction. From Gandevia, 2001.

Solid circles represent inhibitory cells. Dotted curved region at symbolises presynaptic inhibition of the afferent pathways to motor-neurons.

Another further group of muscle afferents, classified as group III/IV, likely contribute to the inhibitory effect at a spinal level due to their projection at the dorsal horn of the spinal cord (Almeida, Roizenblatt, & Tufik, 2004; Wilson, Andrew, & Craig, 2002). These afferents have been demonstrated to be sensitive to exercise induced metabolites (K^+, La^-, H^+, phosphates) and mechanical variations in the muscle (Adreani, Hill, & Kaufman, 1997; Kaufman, Longhurst, Rybicki, Wallach, & Mitchell, 1983). Indeed, several experiments have shown an alteration of the motor-neuronal pool when group III/IV afferents were activated, thus supporting the hypothesis of an inhibitory effect at spinal level (Duchateau & Hainaut, 1993; Garland & McComas, 1990) (Fig 4).

Supraspinal fatigue can be described as a suboptimal output from the motor cortex to drive the muscle (Gandevia, 2001). Defining the contribution of supraspinal sites in the development of central fatigue has been furthered by the development of the transcranial magnetic stimulation (TMS) technique in exercise sciences. However, despite the considerable amount of experiments in this area, the neurophysiological factors underlying supraspinal fatigue still remain unclear (Boyas & Guével, 2011; Gandevia, 2001; Taylor & Gandevia, 2008). The use of TMS in fatigue-based research is more widely discussed in section 2.0.10. Recent evidence suggests that a reduction in oxygen availability to the brain might in part lead to supraspinal fatigue (Amann, Romer, Subudhi, Pegelow, & Dempsey, 2007; Subudhi, Miramon, Granger, & Roach, 2009), which further increases during acute exposure to hypoxia (Goodall, González-Alonso, Ali, Ross, & Romer, 2012). Furthermore, metabolic alterations within the brain have also been demonstrated to increase supraspinal fatigue (Fernstrom & Fernstrom, 2006; Matsui et al., 2011; Meeusen, Watson, Hasegawa, Roelands, & Piacentini, 2006).

Reflexes arising from group III/IV muscle afferents have also been hypothesized to increase central fatigue. Many experiments have investigated the impact of III/IV muscle afferents on cortical response with different experimental approaches. When post exercise muscle ischemia has been used to increase activity of group III/IV muscle afferents, motoneuron discharge rates have been shown to decrease and return to baseline values when the occlusion is stopped (Bigland-Ritchie et al., 1986). However, this experiment did not measure cortical response and therefore the exact cause of the decline in motoneuron discharge rates were not clear. The cortical response of muscle afferents was later monitored both in fatigued and rested muscle in some experiments (Kennedy, McNeil, Gandevia, & Taylor, 2013, 2014). Although they observed a reduction in voluntary activation, no changes in cortical parameters were found. Another interesting experimental

approach has been used in a series of experiments through the use of saline solution injected into the muscle, which is able to stimulate muscle afferents. These experiments have shown a decrease in electrical activity of the muscle (Falla, Farina, Dahl, & Graven-Nielsen, 2007; Madeleine, Leclerc, Arendt-Nielsen, Ravier, & Farina, 2006), reduction of maximal force production (Graven-Nielsen, Arendt-Nielsen, & Mense, 2002), increase in spinal response (Martin, Weerakkody, Gandevia, & Taylor, 2008) and decrease in cortical response (Le Pera et al., 2001). Selective blockade of muscle afferents has been also performed on lower limbs but no changes in central fatigue were demonstrated (Amann et al., 2008, 2009). These series of experiments demonstrate that despite the neuroanatomical connection of group III/IV muscle afferents at spinal and cortical level (and the resultant changes in cortical excitability) (Almeida, Roizenblatt, & Tufik, 2004), whether this translates into an effect on central fatigue still remains unclear.

1.0.3 Cortical behaviour during submaximal fatiguing contractions

Prolonged isometric exercise has been demonstrated to reduce maximal force capacity (Gandevia, 2001; Pageaux, Lepers, Dietz, & Marcora, 2014; Søgaard, Gandevia, Todd, Petersen, & Taylor, 2006) and eventually exhaustion (i.e. inability to maintain the force required). The majority of the decline in force is caused by peripheral fatigue, as demonstrated by the decreased resting twitch (Gandevia, 2001; Place, Maffiuletti, Martin, & Lepers, 2007). However, given the evidence in support of a contribution of the supraspinal sites during exercise, the behaviour of cortical neurons has received particular attention when explaining fatigue in this context (Gandevia, 2001; Gandevia, Allen, Butler, & Taylor, 1996). The role of the motor cortex during fatiguing contractions has been explored with the use of TMS, which permits the study of the behaviour of the cortico-spinal tract during exercise (Gandevia, 2001; Gandevia et al., 1996; Taylor & Gandevia, 2008). Classically, these experiments are performed by matching a required force (e.g. ~20% MVC), and in some experiments additional brief MVCs are introduced to monitor the development of fatigue (Bigland-Ritchie et al., 1986; Søgaard et al., 2006; Taylor & Gandevia, 2008). At a peripheral level, this type of exercise is characterized by a decline in substrate stores and an increase in intramuscular metabolites (Allen, Lamb, & Westerblad, 2008; Jones, Turner, McIntyre, & Newham, 2009), which impair the biochemical processes necessary for muscle contraction, thus leading to peripheral fatigue (Allen et al., 2008).

In some circumstances local blood flow delivery can be impaired when the intramuscular pressure is too high, thus reducing the oxygen availability and further increasing the accumulation of muscle metabolites (Gaffney, Sjøgaard, & Saltin, 1990; Sjøgaard, Kiens, Jørgensen, & Saltin, 1986). However, when the supraspinal sites were monitored by TMS during the brief MVCs, an increase in motor cortex superimposed twitch has been documented (Søgaard et al., 2006; Taylor & Gandevia, 2008). In some experiments, this response has been observed from the early stages of the task with a progressive increase until exhaustion (Søgaard et al., 2006). This behaviour has been associated with a suboptimal output from the motor cortex (Gandevia, 2001; Gandevia et al., 1996).

The regulation of force during submaximal contractions is complex, due to the interplay between both peripheral and central mechanisms (Gandevia, 2001; Taylor & Gandevia, 2008). For example, during prolonged submaximal tasks when the activated muscle fibers become fatigued over time, in order to maintain the required force the activation of additional fresh motor units and or an increase in firing rate is required (Adam & De Luca, 2003; Bigland-Ritchie, Johansson, Lippold, Smith, & Woods, 1983; Gandevia, 2001). This is demonstrated by an increase in EMG over time during fixed intensity exercise (Bigland-Ritchie et al., 1983; Sacco, Thickbroom, Thompson, & Mastaglia, 1997). Furthermore, a simultaneous gradual increase in motor evoked potential (MEP) and cortical silent period (CSP) size has been documented (Gandevia, 2001; Gandevia et al., 1996; Taylor, Allen, Butler, & Gandevia, 2000). MEP and CSP are respectively used in physiology as index of cortical-spinal excitability and intracortical inhibition, as discussed in Chapter 2 section 2.0.10. The increase in MEP response during sustained contractions likely represents the increase in voluntary drive to the motoneuronal pool (Gandevia, 2001; Gandevia et al., 1996), which is also supported by the rise in ratings of perceived exertion (Søgaard et al., 2006). The increase in CSP has been demonstrated to reflect the level of intracortical inhibition (Gandevia, 2001; Gandevia et al., 1996).

These experiments demonstrate that supraspinal fatigue can be documented not only in maximal contractions (Gandevia, 2001; Gandevia et al., 1996), but can also be seen during prolonged submaximal tasks (Søgaard et al., 2006). The increase in MEP response further demonstrates that cortical excitability does not decline during fatiguing exercise, thus suggesting that supraspinal fatigue is likely caused by processes upstream of the motor cortex.

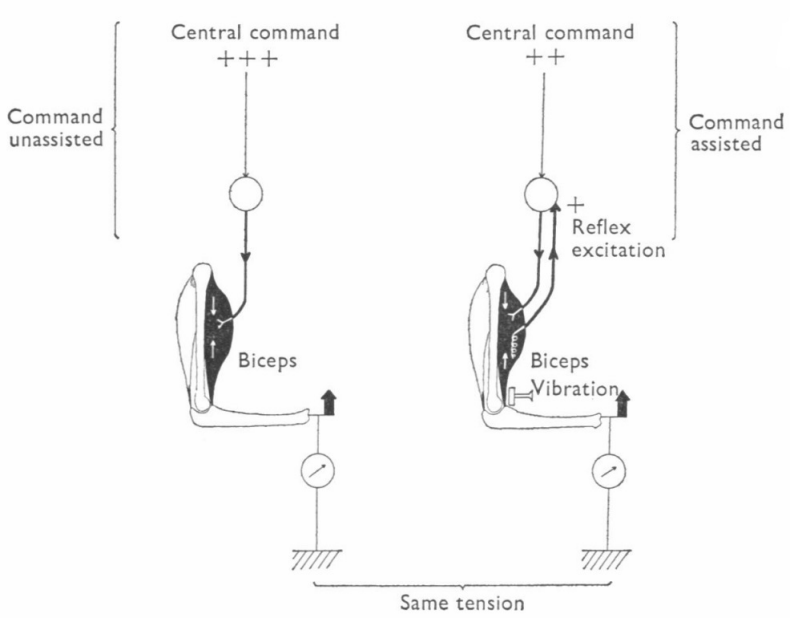


Fig 5. Illustration showing the hypothetical central command required to match the external muscle same tension required. (From Goodwin et al., 1972).

The left side shows a condition where there are no factors influencing muscle contraction, the magnitude of the central command is presumed as ++. The right side shows a condition where an inhibitory influence (inhibition reflex) negatively impact muscle contraction, the magnitude of the central command is presumed as +++.

1.0.4 Fatigue in isolated muscle

There has been considered experimental work investigating the mechanisms of central and peripheral during exercise in isolated muscles. Most of this work has examined the effect of fatigue on muscle activity during isometric tasks. These isometric tests are generally performed on dynamometers, and since the introduction of the model from Andersen (Andersen, Adams, Sjøgaard, Thorboe, & Saltin, 1985), dynamic contraction has also been studied in this manner. Unlike whole body exercise, isolated muscles or single joint exercise involves the activation of a specific muscle group and therefore the requirement of the cardiovascular and respiratory systems is reduced. The model is widely adopted to assess cortical parameters, as the delay between the end of the task performed and the assessment (i.e. moving the subject from the ergometer to the dynamometer) is removed. This is particularly important as cortical parameters quickly return

to baseline level (Gandevia, 2001; Taylor & Gandevia, 2008) and therefore any delay may underestimate their measurement. This methodological limitation is always present when performing whole body exercise, where subjects must be moved to the dynamometer used to measure neuromuscular function.

The incidence of fatigue during exercise has been generally examined by involving MVCs during exhaustive exercise, which requires subjects to maintain a constant force or power until exhaustion (defined as the inability to further sustain the task required (Edwards, 1981). Along with exhaustion, a decline in maximal force production has been often reported at the end of both dynamic and isometric prolonged exhaustive contraction (Pageaux, Angius, Hopker, Lepers, & Marcora, 2015; Søgaard et al., 2006). Much of the decline in MVC at exhaustion has been attributed to peripheral factors affecting muscle contraction capacity, and therefore peripheral fatigue has been posited to "limit" exercise performance. Therefore, numerous experiments have been performed to tease out the peripheral causes leading to exhaustion and the reduction of maximal force or power generation (Enoka & Stuart, 1992; Gandevia, 2001).

Although exhaustion is the inevitable, many experimental manipulations have been used to delay exhaustion as well as to reduce the decline in maximal force capacity, thus permitting an understanding of the contribution of specific physiological mechanisms leading to exhaustion. Time to task failure has been shown to differ between different muscle groups, even under the same relative load. For example, sustained isometric contraction of 20% MVC has been reported to be shorter in elbow flexors compared to abduction of the index finger (Maluf & Enoka, 2005; Rudroff, Barry, Stone, Barry, & Enoka, 2007; Rudroff, Poston, Shin, Bojsen-Møller, & Enoka, 2005). The authors suggested that the diverse capacity to perform exercise might be caused by the different mechanical and neurophysiological organisation of the muscle structure. Indeed, in the elbow flexors task duration has been shown to be influenced by the orientation of the shoulder independent of the relative force required (Rudroff et al., 2007). This experiment showed that task duration is greater when the upper arm is in a vertical position compared to horizontal. Similar findings have been provided by varying forearm position (Rudroff et al., 2005). The differences in time duration have been attributed to the work required from accessory muscles to stabilise the joint and therefore the likely increased demand of the CNS to augment descending drive (Le Bozec & Bouisset, 2004).

Oxygen availability has been also demonstrated to alter muscle contractile capacity in single joint exercise. Indeed, peripheral fatigue and change in motor

unit recruitment were demonstrated to be altered in hypoxia (Katayama, Amann, Pegelow, Jacques, & Dempsey, 2007). The study of Katayama and colleagues (2007) showed that after intermittent isometric contractions, the percentage reduction in quadriceps twitch force was greater in hypoxia, alongside a higher iEMG signal. This indicates a higher motor unit recruitment, probably caused by a higher fatigability of the muscle. Similar findings were also demonstrated by Fulco et al., (1996), where exhaustive knee-extension exercise was reduced, with a greater rate of decrease in MVC during hypoxia compared to normoxia. The authors associated the causes of exhaustion with both peripheral and central mechanisms. Since the presence of muscle metabolites has been demonstrated to reduce the muscle contractile capacity (Allen et al., 2008), some studies have investigated the effect of food supplementation on muscle function (Culbertson, Kreider, Greenwood, & Cooke, 2010). Craig et al., (2012) demonstrated that the improvement of exhaustive isometric knee extension was greater after 4 weeks of ß-Alanine supplementation compared to a placebo group, which was most likely caused by an improvement of intracellular buffering capacity.

The role of supraspinal sites in the development of fatigue have often been the object of study (Gandevia, 2001; Taylor & Gandevia, 2008), with particular interest in the behaviour of the motor cortex (which has been investigated by means of TMS - see section 1.0.3) during prolonged isometric submaximal contractions. One of the first pieces of evidence to demonstrate motor cortex impairment was provided by Gandevia et al., (1996), where motor cortex superimposed twitch increased during a sustained MVC. Similar findings have been also provided during prolonged isometric contraction of elbow flexors, along with a progressive decline in MVC (Søgaard et al., 2006). Evidence in favour of suboptimal output from the motor cortex has been shown to occur since the early phases of a sustained isometric contraction (Søgaard et al., 2006; Taylor & Gandevia, 2008). Interestingly, although during prolonged submaximal contraction the supraspinal twitch increases, motor cortex excitability has been shown to increase, as evidenced by the increase in MEP amplitude (Gandevia et al., 1996). This response demonstrates that supraspinal fatigue might occur upstream of the motor cortex rather than a decrease in cortical excitability (Gandevia et al., 1996, 2001, Taylor et al., 2006) (see section 1.0.3). Recently, sustained isometric exercise has been shown to be affected under different psychological states. Interestingly, when mental fatigue was induced prior to exercise, exhaustive knee extensor exercise was reduced (Pageaux, Marcora, & Lepers, 2013) without affecting MVC, VAL or any other physiological variables. The authors suggested that this was probably the

consequence of higher ratings of RPE caused by a change in the central processing of the sensory inputs generating perception of effort during exercise.

Taken together, the observation provided from the aforementioned studies suggest that fatigue in single joint exercise is a complex phenomenon which is affected by numerous physiological (and possibly psychological) mechanisms. As suggested in the famous review of Gandevia (2001), there may be some circulatory, metabolic factors or events at supraspinal sites that produce exhaustion, but no factor has yet been identified in exercise in healthy human as the cardinal "exercise stopper."

1.1 Cardiovascular regulation during exercise

This section describes the regulation of the cardiovascular system during exercise. The physiological principles described here are fundamental to the understanding of cardiovascular control during exercise, which has implications for understanding the development of fatigue and the theoretical premise of study 3 of the Thesis (see Chapter 5). The control of the cardiovascular system during exercise has been the object of study for more than a century, and is one of the most studied topics in human and exercise physiology. Consequently, this section provides an overview relevant to the themes of the thesis, but it is acknowledged that there is considerably more depth and breadth to the area.

An adequate ventilatory and circulatory response during exercise is necessary to satisfy oxygen demand of the working muscles. The resting quantity of oxygen consumption of the body in healthy and adult subject is ~250 $ml \cdot min^{-1}$, with a cardiac output of ~5 $l \cdot min^{-1}$. However, during strenuous aerobic efforts, endurance athletes can reach values of oxygen consumption of 6 $l \cdot min^{-1}$, with values of cardiac output above 35 $l \cdot min^{-1}$ (Lewis et al., 1983). Accordingly, the cardiovascular apparatus controls important adjustments to enhance blood flow delivery, such as an increase in heart rate (HR), stroke volume (SV) and cardiac output (CO). Both mechanical and neural mechanisms act in favour of an increase in CO during exercise. The rhythmic contraction of the working muscles transport an important quantity of blood towards the heart (e.g. muscle pump) thus facilitating the cardiac filling (Laughlin, 1987). The increase in cardiac activity is also the result of a complex balance between the sympathetic and parasympathetic systems. The two neural mechanisms underpinning this involve central and peripheral structures, and are respectively called central command and the exercise pressor reflex. Both of these systems are well known to participate in the shift in the sympathetic drive to the heart.

Central command has been described as a feed-forward mechanism involving descending signals from higher brain centers which cause a parallel activation of motor and cardiorespiratory areas (located in the brainstem) (Goodwin, McCloskey, & Mitchell, 1971; Krogh & Lindhard, 1913). Although the role of central command has been widely accepted, the neuroanatomical structures located in

the brain are not yet clearly defined (Williamson, 2015). According to human and animal models, it has been proposed that cortical and subcortical areas of the brain such as the insular cortex (IC), anterior cingular cortex (ACC), thalamus, hypo-thalamus, amygdala, and medial prefrontal region are involved in the central control of circulation (Benarroch, 1993; Cechetto & Shoemaker, 2009; Williamson, Fadel, & Mitchell, 2006). The anatomical location responsible for central command has also been reinforced by studies involving patients affected by lesions in specific brain areas, who experience an altered cardiovascular regulation (Critchley et al., 2003; Talman, 1985). It has been proposed that the magnitude of the central command increases with exercise intensity (Turner, 1991; Williamson et al., 2006) and therefore its contribution is hugely important during exercise performed at high intensities. Although the relevance of central command is well established, the impossibility of gaining a direct measurement of this necessitates alternative methods of assessment. Since there is a parallel activation of motor and cardiorespiratory centers (Goodwin et al., 1971; Krogh & Lindhard, 1913) when muscular movement is generated, individual ratings of perception of effort have been used to indirectly measure the level of central command (Mitchell, 1990). This assessment however has been debated, since many researchers consider other factors to exert a considerable influence on perception of effort (Williamson et al., 2006). More details on this area are discussed in section 1.2.3.

Unlike central command, the exercise pressor reflex peripherally contributes to the regulation of the cardiac activity (Kaufman et al., 1983, 2012; Mitchell, 1990; Mitchell, Kaufman, & Iwamoto, 1983). This neural mechanism involves the activation of afferent nervous fibres classified as group III/IV. Myelinated type III fibres are more sensitive to mechanical variations (i.e. mechano-receptors), while unmyelinated fibres are more sensitive to metabolic variations of the muscle milieu, such as accumulation of H^+, K^+ and La^- protons (Kaufman et al., 1983, 2012; Mitchell et al., 1983; Rotto & Kaufman, 1988). The afferent fibres then converge at the dorsal horn of the spinal cord, travelling through the spinal cord and then reaching cortical and subcortical areas (Almeida et al., 2004). Therefore, when oxygen supply is not sufficient to satisfy the muscular demand, accumulation of metabolites in the muscle stimulate the group III and IV, which subsequently activate cardiovascular control areas located in the brainstem to increase the cardiac activity. Recent work suggests that the activation of this system is dependent on muscle contraction intensity and varies according to the intensity of the exercise performed (Crisafulli et al., 2006, 2008), thus providing

more evidence that this system acts to correct a possible mismatch between oxygen supply and demand.

Signals from both central command and the exercise pressor reflex converge in the cardiorespiratory areas located in the brainstem. The specific area of the nucleus tractus solitarii is particularly important, as this is where the integration of these stimuli occur and the cardiorespiratory activity is regulated (Mitchell et al., 1983) (Fig. 6).

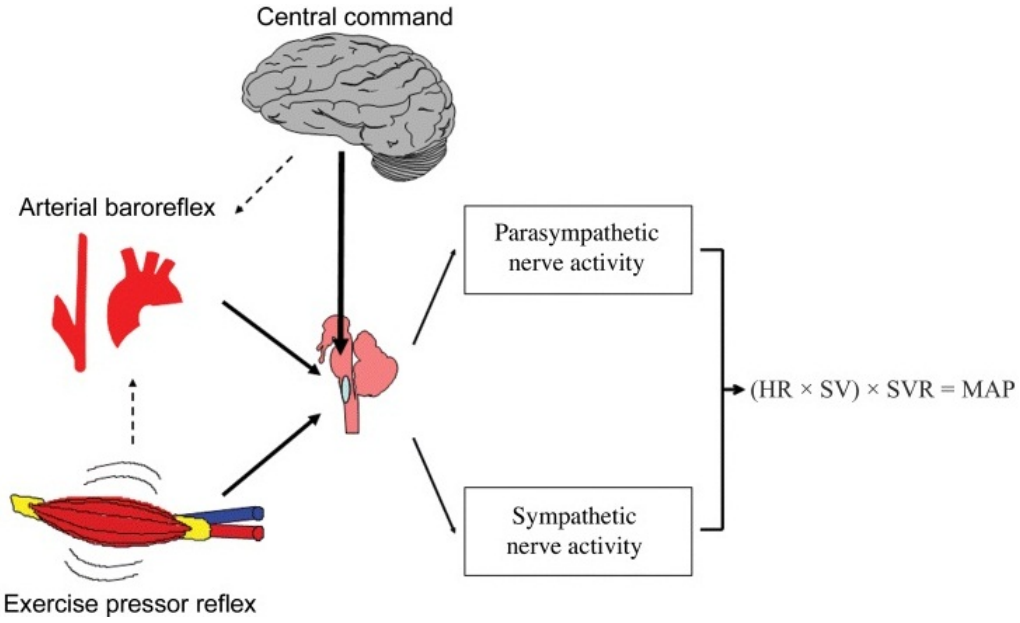


Fig 6. Schematic illustration of the peripheral and central neural mechanisms of cardiovascular control during exercise. From Williamson et al., (2006).

Heart rate (HR), stroke volume (SV), systemic vascular resistance (SVR), mean arterial pressure (MAP).

Previous studies involving spinal blockade of muscle reflexes have been documented to impair cardiovascular response and this demonstrates the importance of III/IV muscle afferents for a normal cardiovascular regulation (Hill & Kaufman, 1990; Pomeroy, Ardell, & Wurster, 1986). Even stronger evidence in support of this has been recently provided by Amann (2011; 2010; 2011). In this study, Amann and colleagues (2011) demonstrated that central and peripheral hemodynamic response were impaired during rhythmic exercise in humans along with hypoventilation and arterial hypoxemia. In terms of performance, oxygen

transport was compromised and the rate of locomotor muscle fatigue was exacerbated with a combined net effect of a reduced cycling time performance (Amann, Blain, et al., 2011). Although these experiments attempted to block a possible inhibitory effect of muscle afferents on supraspinal centres, these reflexes are essential to achieve an appropriate hemodynamic and ventilatory response during exercise which are both necessary to avoid any premature increase in fatigue.

1.2 Physiology and role of perception of effort and pain during exercise

During the experiments performed for this thesis, two main perceptual parameters were recorded throughout exercise; the exercise-induced muscle pain and perception of effort. These factors both play an important role during exercise as well as during various common activities in our daily life. Given their importance for the experiments performed for this thesis, the purpose of this section is to discuss and explain the aetiology of these sensations, and the role they both have during exercise.

1.2.1 Perception of effort

Perception of effort has been commonly defined as how hard an exercise is (Marcora, 2009; Marcora & Staiano, 2010). The neurophysiology of perception of effort has been debated for a long time and therefore many theories have been proposed to explain where and how perception of effort is generated (Marcora, 2009). These models can be generally divided in two main categories: i) peripheral generation; or ii) central generation of perception of effort.

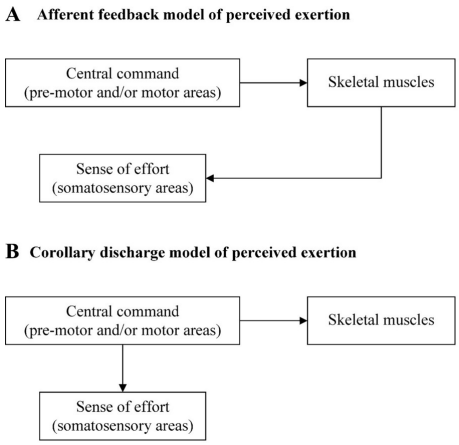


Fig 7. Schematic diagrams illustrating the two main models of the generation of perception of effort during exercise. From Marcora (2009).

Afferent feedback model (A) and corollary discharge model (B).

1.2.2 Peripheral generation of perception of effort

This model is also called the afferent feedback model of perception of effort, and states that perception of effort is mainly generated from peripheral signals from the body which ultimately converge in the brain (Amann, Blain, et al., 2011; Amann et al., 2010; Kjaer et al., 1999; St Clair Gibson et al., 2006). In particular, group III/IV muscle afferents located in muscles, heart and lungs have received attention, likely given their sensitivity to metabolic and mechanical variations during muscle contraction (Kaufman, 2012; McCord & Kaufman, 2010; Pickar, Hill, & Kaufman, 1994). When performing exercise, the accumulation of exercise-induced muscle metabolites (La^-, Na^+, K^+) stimulate these peripheral receptors (Hanna, Hayes, & Kaufman, 2002; Hill, Adreani, & Kaufman, 1996; Pickar et al., 1994). Therefore, as the exercise intensity increases, further accumulation of metabolites stimulate peripheral receptors leading to an increase in perception of effort (St Clair Gibson et al., 2006). Despite the neuroanatomical evidence showing a connection between group III/IV muscle afferents with cortical and subcortical areas (Almeida et al., 2004), this model has been strongly criticized (Marcora, 2009). In part, the debate has been caused by experiments involving selective blockade of group III/IV muscle afferents (Amann, Blain, et al., 2011; Amann et al., 2010; Kjaer et al., 1999). This experimental manipulation (i.e. intra lumbar injection of fentanyl) blocks any signal from the of group III/IV muscle afferents of exercising muscles from reaching the brain (Amann, Blain, et al., 2011; Amann et al., 2010; Kjaer et al., 1999), yet in these studies, despite the lack on afferent feedback no changes in perception of effort were found between conditions. The results of these experiments provide support for the model supporting the central generation of perception of effort.

1.2.3 Central generation of perception of effort

Unlike the afferent feedback model of perception of effort, the corollary discharge model of perception of effort proposes that perception of effort is centrally generated by the efferent neural processes of central command, termed the corollary discharge (Marcora, 2009; McCloskey, 1978; McCloskey, Gandevia, Potter, & Colebatch, 1983). Accordingly, any increase in magnitude of central command should be immediately followed by a parallel increase in perception of effort (Lafargue & Sirigu, 2006; McCloskey, 1978; McCloskey, Ebeling, & Goodwin, 1974;

McCloskey et al., 1983). This model was originally proposed several decades ago (McCloskey, 1978; McCloskey et al., 1974), and recently further studies have demonstrated a relationship between the activation of motor and premotor areas and the increase in perception of effort (de Morree, Klein, & Marcora, 2012, 2014). With the use of EEG, the studies performed by de Morree and colleagues (2012, 2014) showed a relationship between activation of the motor and premotor areas with perception of effort. This model provides a simple explanation for the increment in perception of effort during various kinds of exercise tasks. For example, when locomotor muscle weakness is induced prior to an exercise task, a compensatory increase in central command is required in order to produce the same amount of force or power (i.e. in order to overcome muscle weakness). This has been demonstrated in experiments showing a significant increase in RPE during exercise in pre-fatigued muscles (de Morree et al., 2012; Marcora, Bosio, & de Morree, 2008). Similarly, when skeletal muscle weakness was induced by curare, a significant increase in RPE during cycling exercise was observed (Gallagher et al., 2001). A gradual increase in RPE has been also observed both during prolonged isometric and dynamic exercise (Marcora, Staiano, & Manning, 2009; Pageaux, Marcora, & Lepers, 2013; Søgaard et al., 2006). During this type of exercise, the increase in RPE is likely the consequence of the increase in central motor command required to compensate the exercise-induced muscle fatigue (Marcora et al., 2008; McCloskey et al., 1983; Søgaard et al., 2006).

It has also been shown that perception of effort can be affected by psychological factors including the suggestion of a gradient (Williamson et al., 2001), the presence of an attractive female (Winchester et al., 2012) and the optic flow of the exercise (Parry, Chinnasamy, & Micklewright, 2012). These studies suggest that changes to perception of effort may be induced in the absence of changes to the collorary discharge, and so the efferent neural processes of central command may not be able to solely explain changes to perception of effort. However, there are psychological manipulations which may concurrently change motor output (for example, motivation), and thus provide a neurophysiological basis for changes to perception of effort (Blanchfield, Hardy, & Marcora, 2014; Marcora et al., 2009; Pageaux et al., 2013). These experiments demonstrate that perception of effort can be modified by neurophysiological changes, but that there may also be psychological influences in addition to this, which remain to be explained and clarified.

The corollary discharge model of perception of effort has also been criticized by Amann and colleagues (2011), as in their experiments a significant reduction in

perception of effort under the blockade of muscle afferents was found. According to the authors, this demonstrated that muscle afferents had some contribution to the generation of perception of effort. However, it should be taken into account that the same authors in other studies did not show any change in RPE after spinal blockade (Amann, Blain, et al., 2011; Sidhu et al., 2014). Moreover, other experiments involving blockade of muscle afferents do not support this hypothesis (Gallagher et al., 2001; Kjaer et al., 1999), and therefore the evidence in favour of a sole peripheral contribution to perception of effort is limited.

1.2.4 Pain perception during exercise

Pain is a common experience, perceived regularly in daily life, and can be elicited by a wide variety of factors. Pain is inherent to intense and prolonged muscular contractions and has been mooted as a contributor to fatigue. Pain perception was recorded across experiments in this thesis (and was for target manipulation in study 1, Chapter 3), and so this section will provide a background detailing factors effecting exercise-induced muscle pain.

The International Association for the Study of Pain (IASP) define pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Exercise-induced muscle pain is a common perception experienced during exercise and involves a complex interplay between central and peripheral structures of the human body as well as a considerable psychological component. Peripheral nociceptors are generally classified in type III and IV muscle afferents, and are sensitive to variations in concentration of metabolites, mechanical pressure, heat, cold, and endogenous substances producing pain (O'Connor & Cook, 1999). The aforementioned metabolites are largely the result of anaerobic metabolism during exercise (e.g. H^+, K^+, La^- and prostaglandins) and so their concentration will vary according to the duration and intensity of the exercise performed, as well as the size of the muscle mass involved.

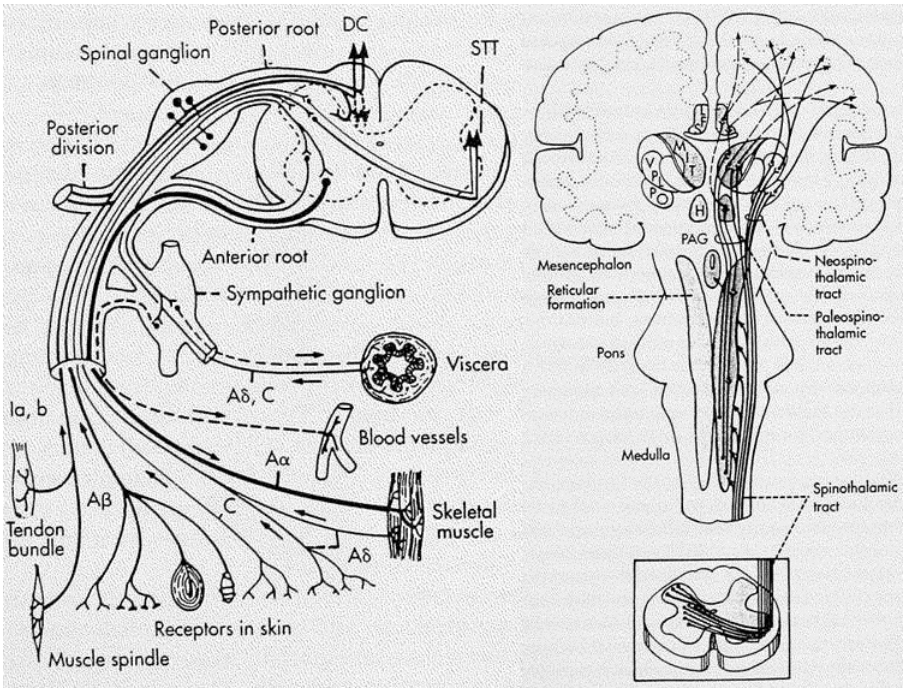


Fig 8. Peripheral and central structures involved in the processing of pain perception. From O' Connor & Cook, (1999).

Peripheral pain receptors originate in and around the muscle and/or other peripheral structures, and converge at the dorsal horn of the spinal cord. Once sensed by the nociceptor, a nociceptive signal ascends to subcortical and cortical brain regions, such as the somatosensory cortex and ventroposterior lateral nucleus of the thalamus (Almeida, Roizenblatt, & Tufik, 2004; Brodal, 1981; O'Connor & Cook, 1999), where the nociceptive stimulus becomes conscious and is perceived as pain (Fig 8). The integration of numerous other brain regions, such as the dorsolateral prefrontal cortex, in the processing on the pain response means that pain has a considerable subjective element, and so it is not always relative to the magnitude of the nociceptive signal.

Some authors have suggested that pain tolerance could be higher be in athletes than non-athletes and this might be an important requirement for athletes in specific disciplines (Ryan & Kovacic, 1966). Although this sensation is very commonly experienced, the role of the pain during exercise has received little attention and so its role on performance is still the matter of some speculation. Compounding the lack of literature is that experiments investigating the regulation and effect of pain during exercise often involve many different experimental procedures which either increase, decrease or block the peripheral signals from

the muscle. Although these procedures may provide some understanding of the role of pain during exercise, because pain is often coupled with afferent feedback (which regulates cardiovascular control), in some circumstances the experimental manipulation negatively affects the exercise performed – this often makes it difficult to interpret the experimental findings (Mauger, 2013).

Incremental tests performed on cycle a ergometer have demonstrated a relationship between pain ratings and exercise intensity (Cook, O'Connor, Eubanks, Smith, & Lee, 1997) thus supporting the premise that pain might affect and/or limit exercise performance. Some recent studies have investigated the effect of pain using simulated self-regulated cycling time trials of 5-16.1 km (Amann, Proctor, Sebranek, Pegelow, & Dempsey, 2009; Mauger, Jones, & Williams, 2010). In the study of Amann and colleagues (2009), despite spinal blockade of nociceptive signals, performance was not improved, although this was likely due to the cardiovascular response being impaired by the complete afferent blockade. Another study performed by Mauger and colleagues (2010) demonstrated an improved performance following ingestion of acetaminophen (paracetamol). In their study, subjects were able to perform at a higher power output for a given perception of pain. However, the interpretation of this experiment is difficult as ingestion of acetaminophen has also been demonstrated to increase spinal excitability and reduce body temperature (Mauger et al., 2014; Mauger & Hopker, 2013). Graven-Nielsen et al. (2002) have shown that pain induced through an intramuscular injection of hypertonic saline solution decreases MVC of the knee extensors. Additionally, a recent study provided further support for the notion that exercise may be regulated in part by the perception of pain arising from muscle contraction (Gonglach, Ade, Bemben, Larson, & Black, 2015). Collectively, these studies suggest that whilst pain may not be a sole determinant of endurance performance, it may play at least some role in the regulation of work rate during exercise. However, methodological difficulties make this notion difficult to confirm and further studies are required to explore this paradigm.

1.3 General models to explain fatigue during prolonged whole body exercise

Sections 1.1 and 1.2 discussed the more relevant physiological systems involved in the aetiology of fatigue during exercise. The operation of these systems is relatively well-known and widely accepted, however, how they are integrated into a model which explains the causes of fatigue is the topic of hot debate. This section will discuss some of the most common models proposed in the literature to explain fatigue during prolonged exercise, with particular attention on the central nervous system, as this will be particularly important in understanding the experiments performed as part of this thesis.

Endurance performance has been defined by Coyle (1999) as the prolonged maintenance of submaximal velocity or power and the ability of athletes to sustain prolonged exercise has been widely studied, reflecting scientist's interest in the mechanisms leading to fatigue and exhaustion. To satisfy energy demand during whole body exercise, the human body makes many adjustments that involve multiple physiological systems, and so one or more of these systems has the potential to effect performance. Therefore, different physiological systems may be more or less responsible for exercise depending on the task. Thus, it is not surprising that various models to explain differences in exercise performance have been proposed (Abbiss & Laursen, 2005; Ament & Verkerke, 2009). This is further compounded by the use of different tests as a measure performance and to study the physiological mechanisms leading to fatigue and exhaustion. One of the most common tests used in the laboratory is the open-loop task, whereby subjects are required to maintain a constant intensity until the point of exhaustion. This test is also commonly called a time to exhaustion task/test, with a longer time to exhaustion indicative of an improved performance. The duration of the time to exhaustion is well known to be mainly affected by the exercise intensity and whether the exercise is whole-body or single limb. The other most commonly used exercise model is closed loop exercise tests. Often called a time trial, this is where a fixed distance, time, or amount of work is completed, and exercise intensity during this can be self-paced in order to improve performance (i.e. more distance, faster time or more work done). The inherent differences between closed and open loop exercise tests have contributed to a lack of agreement between the different models of fatigue. Both open and closed loop exercise tests

have merits, but researchers often fail to recognize their shortcomings. Closed loop exercise provides a good basis for monitoring the mechanisms which may change following an intervention, whereas open loop exercise provides a better basis for assessing whether these changes integrate to produce a performance change. Because the premise of this thesis is to primarily explore how tDCS moderates different systems in the body that regulate endurance performance, the experimental chapters in this thesis have focused solely on open loop exercise (to better explain mechanisms). However, further studies using closed loop exercise may be subsequently needed to demonstrate a self-paced performance effect.

1.3.1 The oxygen transport model

The ability to sustain prolonged whole body exercise is correlated with aerobic capacity, and therefore the capacity to satisfy the oxygen required to the working muscle is fundamental to exercise performance. The level of oxygen utilization by the muscle is dependent on the oxygen delivery (e.g. central cardiac output and blood flow) and oxygen extraction (capillary diffusion and mitochondrial utilization). Accordingly, the oxygen transport model suggests that performance is limited (or at least affected) by the ability of the body's cardiovascular system to supply the level of oxygen required to sustain the energy demand of the muscle. The relationship between the rate of development of fatigue and oxygen availability has been studied in numerous experiments (Amann et al., 2006; Goodall, González-Alonso, Ali, Ross, & Romer, 2012; Goodall, Ross, & Romer, 2010). A popular methodological approach to test this relationship is the manipulation (increasing or decreasing) of the percentage of oxygen inspired (FiO_2), together with the quantification of central and peripheral neuromuscular parameters at exhaustion (Amann & Calbet, 2008). In these examples, duration of prolonged, submaximal, exhaustive exercise has been shown to be reduced in hypoxia (Goodall et al., 2010, 2012), along with a decline in MVC (Amann et al., 2006). The reduction in performance and MVC has been associated with the increased rate of accumulation of muscle metabolites, which are known to alter the excitation-contraction coupling within the muscle fibers (Allen et al., 2008) and therefore an exacerbated level of peripheral fatigue. It should be taken into account that exercise performed at the same absolute work load, but at reduced FiO_2, leads to an increase in relative exercise intensity and therefore increases the utilization of type II fibers. Taken together, these experiments demonstrate

that reducing oxygen to the muscle exacerbates the development of peripheral fatigue.

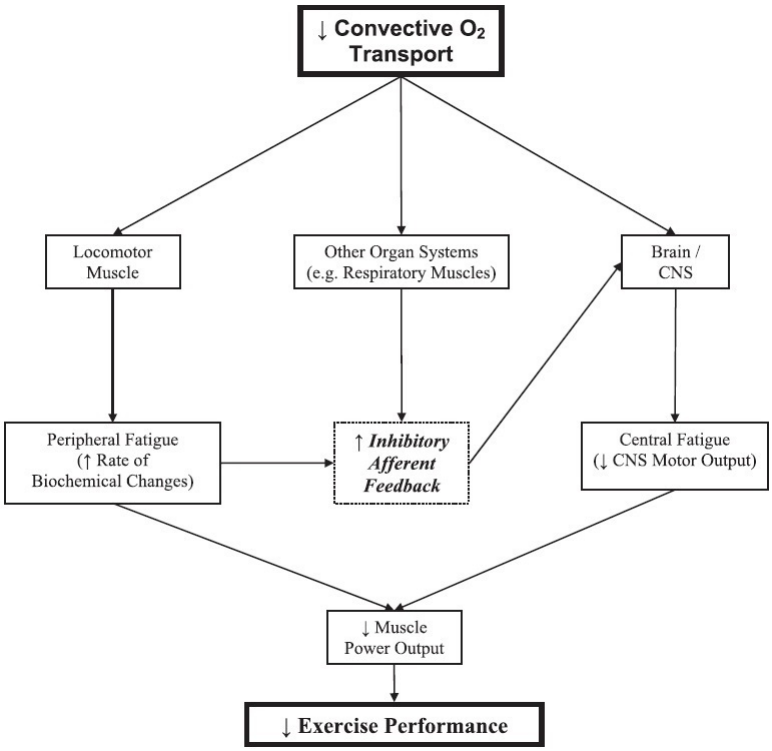


Fig 9. Diagram linking the effect of oxygen transport on exercise induced fatigue and performance. From Amann & Calbet (2008).

Recently, experiments have demonstrated that the observed reduction in performance is not only caused by alteration at peripheral level, but also by an increase in supraspinal fatigue. The study of Goodall et al (2012) provided important insight regarding the effect of systemic low oxygen at a supraspinal level during exercise. This study demonstrated for the first time that reduced content of oxygen in the brain during simulated hypoxia increases central fatigue, with an impairment at a supraspinal level. Since the reduction of oxygen in the body has been shown to increase the metabolic accumulation of muscle metabolites, some authors suggested that this might increase the development of central fatigue by a possible inhibitory effect on group III/IV afferents at a supraspinal level. This experiment suggested that during hypoxia the increased rate of accumulation of muscle metabolites, and therefore the discharge of group III/IV muscle afferents, might have in part contributed to the increase in supraspinal fatigue together

with an additional reduction of cerebral oxygenation. Collectively, these studies demonstrate that the same physiological factors occurring in normal conditions are amplified when the oxygen availability is reduced.

1.3.2 The afferent feedback model

During prolonged exercise, exhaustion occurs when the subject is not able to produce the force or power required (Edwards, 1981). Accordingly, some authors have proposed that this is caused by a deficiency of the neuromuscular system (Allen et al., 2008; Amann & Calbet, 2008; Enoka & Stuart, 1992), caused both by central and peripheral factors (Gandevia, 2001). This model states that a certain level of peripheral fatigue is never exceeded during exhaustive exercise (Amann, 2011). Observations supporting this model have been provided in experiments where despite the different experimental manipulations, the biochemical status of the muscle is very similar and never exceeds an individual critical threshold at exhaustion (Burnley, Vanhatalo, Fulford, & Jones, 2010; Hogan, Richardson, & Haseler, 1999). Accordingly, metabo-sensitive afferents act at a cortical level to inhibit the voluntary descending drive to the locomotor muscle by reducing the force produced (Amann, 2011; Amann, Runnels, et al., 2011; Amann et al., 2009). This process should increase the level of central fatigue through an inhibitory effect upstream of the motor cortex. This inhibitory mechanism has been suggested to be accelerated under hypoxic conditions (Goodall et al., 2012). To test this model, experiments involving spinal blockade of muscle afferents prior exercise have been implemented (Amann, Runnels, et al., 2011; Amann et al., 2009). By blocking the possible contribution of muscle afferents, subjects should have been able to improve exercise performance and reduce the degree of central fatigue. However, these experiments failed to find any change in central fatigue or in exercise performance. It is worth noting that blockade of muscle afferents have been shown to impair cardiovascular response (see section 1.2) and therefore negatively affect performance. Moreover, the lack of change in central fatigue might have been caused by a delay of the assessment of the neuromuscular function following exercise (~3 min). Alteration in oxygen availability can alter performance and fatigue, as working muscles and/or other organs can send inhibitory feedback, which may reduce central motor drive. Reduced oxygen supply can also alter the biochemical status of the muscle and reduce power output. Thus, both inhibitory systems and biochemical status of the muscle might decrease exercise performance (Amann & Calbet, 2008)

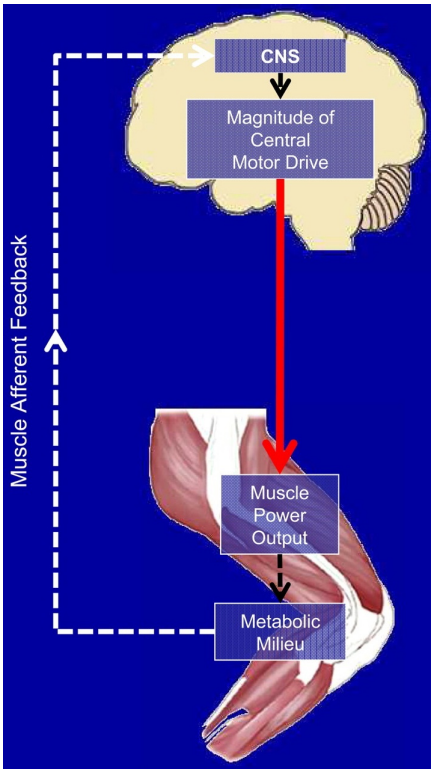


Fig 10. Schematic illustration of the afferent feedback model. From Amann (2010).

The continuous line represents the central motor drive to the exercising muscles, while the dotted lines indicates the afferent feedback signal originating from group III/IV afferent fibers. Central nervous system (CNS).

There have been challenges to the afferent feedback model that remain to be addressed. Firstly, if the peripheral perturbations of the muscle reduce central motor drive, any increase in power at the end of a time trial (end spurt) should not be possible. Secondly, experiments involving spinal blockade have demonstrated that RPE is not peripherally generated (see section 1.2.3). It should be taken into account that creating an experimental design which isolates the role of afferents is likely impossible, and experiments to date cannot rule out other explanations for the observed effects. Therefore, caution regarding interpretation of experimental findings is required with these studies. However, whilst afferents as a sole mechanism limiting exercise performance seems unlikely, their integration into a wider system has received considerable attention.

1.3.3 The Central Governor Model

The first notable model proposing the brain as a regulator of exercise performance was suggested by Ulmer (Ulmer, 1996). The processes this model described, in a mechanism called teleoanticipation, stated that a hypothetical control system in the brain acts to optimize exercise performance to maintain physiological homeostasis and avoid terminal physiological disturbance. Accordingly, optimization of performance is achieved by integrating the afferent information from muscles and other peripheral organs inside a central "black box" located in the brain, which subsequently modifies muscle power output. Based on this proposal, Noakes and colleagues (Noakes, St Clair Gibson, & Lambert, 2005) elaborated further and devised the Central Governor Model (CGM). This model suggests that a central governor (CG), located in the brain, serves as an 'intelligent' regulator of muscle recruitment with the primary role of protecting the body from a catastrophic failure of homeostasis (i.e. terminal failure of a physiological system). The integration of all physiological and environmental cues in the brain results in a conscious generation of the perception of 'fatigue' and this is regulated by the consequent perception of effort. As such, by preventing the failure of homeostasis, exercise is never voluntarily performed at to a maximal capacity. The CGM states two very important assumptions for exercise performance. The first is that during maximal exercise the brain does not recruit any additional motor units, as any additional recruitment would threaten homeostasis. The second is that the increase in perception of effort serves to ensure athletes to do not increase exercise intensity to a dangerous level. Accordingly, to optimize performance in closed-loop exercise (e.g. 5 km or marathon), comparisons between feedforward and feedback information (in response to peripheral information from different physiological systems), provides the athlete with sufficient information on whether muscle recruitment (or work rate) can be increased or must be decreased. This is manifested in the athletes pacing strategy. This suggests that the pacing strategy adopted during exercise is continuously adjusted through both unconscious and conscious control (although this point has been consistently redeveloped since the original proposition of the CGM).

Despite the CGM receiving great interest from the scientific and wider community, many criticisms have been levelled at the model. For example, the primary aim of the CG is to prevent dangerous myocardial ischemia during exercise. However, is well demonstrated that a considerable proportion of athletes and older adults can exhibit myocardial ischemia during exercise (Shephard, 2009). Furthermore, the CGM suggests that RPE is the result of afferent signals represent-

ing peripheral physiological changes of the body during exercise. However, this concept has been further disproved by some experiments, where despite spinal blockade of afferent signals from exercising muscle, RPE during exercise was not affected (Amann, 2011; Amann et al., 2009, Kjaer et al., 1999). Together with previous experiments, this evidence further demonstrates that RPE is independent of afferent feedback from muscle and heart (Marcora, 2009) (see also section 1.2.3).

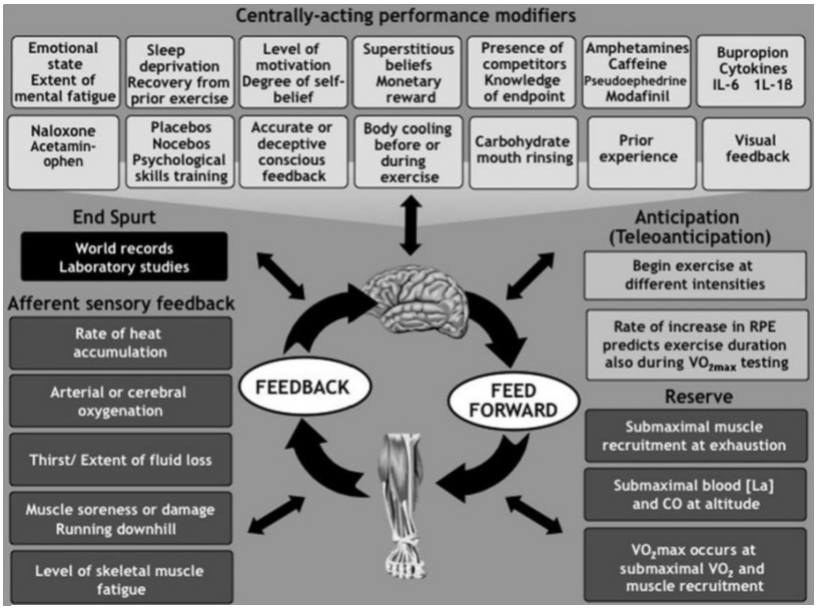


Figure 11. Updated representation of the Central Governor Model. From Noakes (2012).

1.3.4 The psychobiological model of endurance exercise

The psychobiological model of endurance exercise, proposed by Marcora (2010; 2008), is based on the Brehm's motivational intensity theory (Brehm & Self, 1989; Wright, 2008) and is described through two main concepts: potential motivation and effort. Potential motivation refers to the maximum effort the subject is disposed to achieve an objective or a task, while effort can be expressed as the amount of effort the subject exerts. According to this model, each subject will engage in a task until the level of effort exerted reaches the maximum level. In closed-loop tasks the psychobiological model provides important explanations regarding the pacing strategy adopted. During time trials, pacing strategy is

consciously regulated and mainly determined by: I) perception of effort, II) potential motivation, III) knowledge of the distance/time to cover, IV) knowledge of the distance/time remaining and V) previous experience (Pageaux, 2014). During open loop tasks, RPE increases until a maximal level that coincides with the point of exhaustion. In practical terms, the point of this can be postponed by increasing the potential motivation or decreasing the effort. This model is able to explain the causes of exhaustion during various physiological and psychological manipulations. For example, in prolonged open loop tasks, anticipated exercise termination has been demonstrated in pre-fatigued muscles (Marcora et al., 2008), mental fatigue state (Marcora et al., 2009), hypoxia (Romer & Polkey, 2008) and subconscious visual manipulations (Blanchfield et al., 2014). The shorter time to exhaustion of the tasks can be explained by the higher perception of effort perceived for the same power output and consequently reaching the maximal rating of perception of effort. From a physiological perspective, the higher perception of effort can be explained by the increased central motor command required to maintain the same amount of force (see section 1.0.3).

Contrarily to the afferent feedback model and the CGM, the point of exhaustion as a form of task disengagement is on the basis of psychological exercise intolerance rather than a subconscious/anticipatory process or physiological inability. In closed loop tasks, mental fatigue has been shown to impair self-paced running performance (Pageaux et al., 2014) and increase RPE. These experimental findings demonstrate that both physiological and psychological manipulations can alter conscious behavioral strategy (pacing) and can be explained by the psychobiological model of endurance performance. Phenomena such as the end-spurt can be explained with this model, as the strategy to maintain a constant pace during the race with a sudden increase near the end is a conscious decision adopted by the athletes rather to what is proposed by the CGM and the afferent feedback model. From a physiological perspective, it is important to specify that the psychobiological model assumes that perception of effort is generated from central processing of the corollary discharge associated with the central motor command (de Morree et al., 2012; Marcora, 2009) (see also section 1.2.3) rather than peripheral information arising from peripheral level (unlike the afferent feedback model and the CGM).

The psychobiological model is valid provided experimental evidence demonstrates a change in performance alongside a concurrent change in perception of effort. The broad agreement in the literature showing that this indeed occurs would appear to provide support for this. However, the majority of these studies

define perception of effort according to a combination of effort and peripheral signals (which according to the corollary discharge model, do not affect perception of effort). Additionally, the model is stated as superior (at least compared to the CGM) because of its relative simplicity (i.e. corollary discharge produces perception of effort, perception of effort regulates performance). However, in this case interventions such as muscle fatigue still affect performance (albeit through moderating perception of effort), so the argument of the model being less complicated is only valid in terms of systems other than RPE exerting an indirect effect (rather than a direct effect). Finally, the psychobiological number fully depicted in a single published paper, and instead many of the points are discussed across a series of separate papers (Blanchfield, Hardy, & Marcora, 2014; Marcora & Staiano, 2010; Marcora et al., 2008; Marcora, Staiano, & Manning, 2009). This has made it difficult for independent researchers to test the entirety of the model, and it is perhaps a reason for the many misunderstandings in critical papers and a lack of empirical refutation of the model. Moreover, since the exact nature of perception of effort is not well established (Marcora, 2009), many authors have argued about the ability of perception of effort to explain the cause of exhaustion or pacing strategies adopted during self-paced exercise (Abbiss et al., 2015)

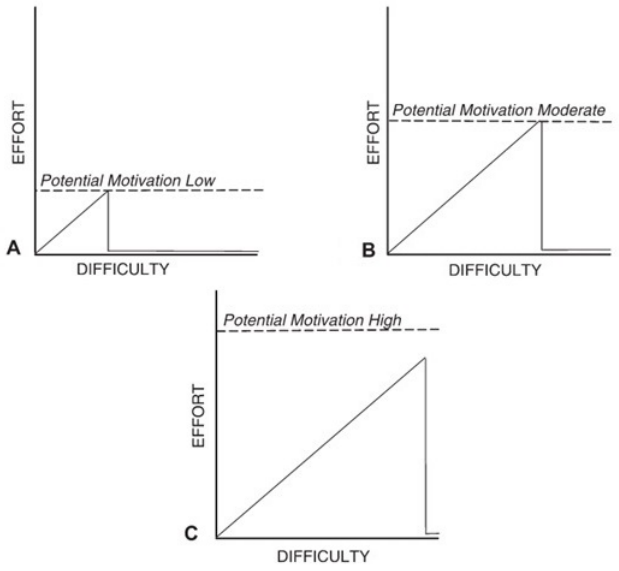


Fig 12. Graphs describing the relationship of perceived effort as function of task difficulty. From Wright, (2008).

Oblique line represent the time courses of effort at low (A), moderate (B) and high (C) intensity levels of potential motivation. Higher potential motivation (B and C) causes longer exercise duration compared to an exercise performed with a lower potential motivation (A).

1.4 Brain stimulation and exercise performance

1.4.1 A brief history of brain stimulation techniques

The brain has fascinated scientists for millennia, however its electrical properties were only discovered a few centuries ago by Luigi Galvani (1737-1798), who first discovered that the nerve and muscles in frogs were electrically excitable. Two scientists were then able to demonstrate the possibility of electrically stimulating the human brain; Charles Le Roy (1723-1789) and Giovanni Aldini (1762-1834). In their laboratories, Aldini and Le Roy applied similar techniques used by Galvani, on cadavers by evoking responses such as blinking or opening of the eyes. Subsequently, Luigi Rolando (1773-1831) performed an interesting series of experiments on the surface of the central nervous system, where he obtained limb movements. This discovery was furthered by Alexander von Humboldt (1769-1859), Carlo Matteucci (1811-1868) and Emil Heinrich du Bois-Reymond (1818-1896), who demonstrated that muscle and nerves are able to generate a type of electricity by themselves, and thus developed a more advanced technique to stimulate the central and peripheral nervous system. Decades later, experiments carried out by Eduard Hitzig (1838-1907), Gustav Fritsch (1838-1927) and David Ferrier (1843-1924), involving selective stimulation of animal brains, produced limb movements and permitted an accurate map of the motor cortex to be drawn. Their findings were further supported by Charles S. Sherrington (1852-1952) and Harvey W. Cushing (1869-1939), who later mapped the brain of great primates. More advanced brain mapping studies were performed by Wilder G. Penfield (1891-1976) on awake humans, which also explored the somatosensory cortex and its relationship with other cortical areas.

With the progression of devices able to produce electrical or magnetic impulses, non-invasive brain techniques were subsequently developed. In the 1960's, the studies of D. J. Albert demonstrated the differing effects of negative and positive stimulation on changing brain cortical excitability and function. His work provided the basis for the modern tDCS technique. Twenty years later in the 1985, Barker and colleagues introduced the first model of TMS, which permitted the non-invasive stimulation of a targeted brain.

Given the electrical properties of the central nervous system, brain stimulation techniques provide a means by which stimulation of a targeted brain area is

able to moderate behaviour. Consequently, stimulation techniques have been developed mainly for two reasons: i) understanding the role and function of a specific brain area; ii) treatment of pathologies involving the central nervous system. Brian stimulation techniques are generally classified as invasive and non-invasive. The term non-invasive refers to a technique that does not involve craniotomy or implantation of an electrode into the brain. For the purpose of this thesis, the two main non-invasive brain stimulation techniques used in sport science with be discussed (respectively called TMS and tDCS), with a particular focus on tDCS.

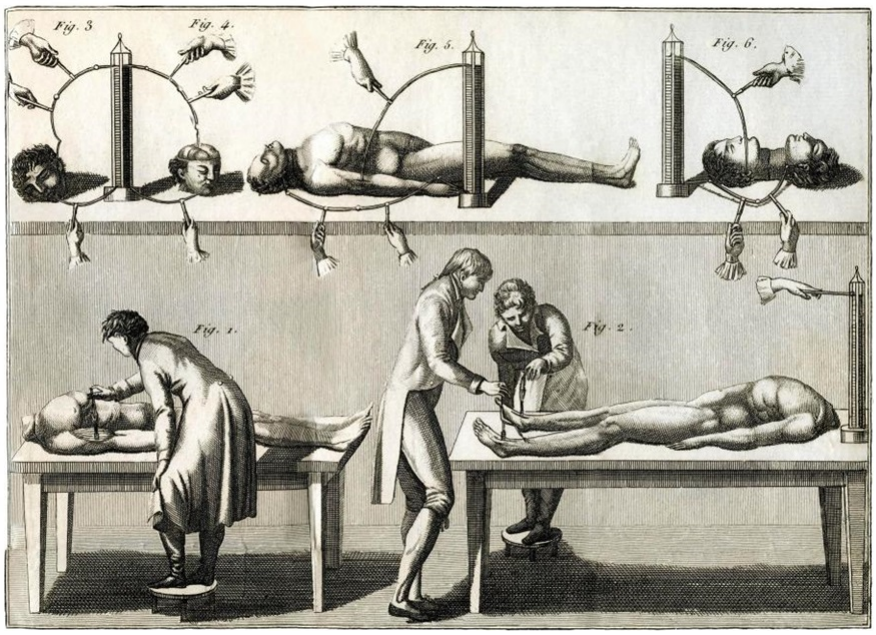


Fig 13. Illustration showing the experiments performed by Giovanni Aldini (1762-1834) on human's dead bodies.

1.4.2. Transcranial magnetic stimulation

Along with techniques to stimulate the motor nerve, other techniques have been developed to stimulate the cortical spinal tract, such as the transcranial electrical stimulation (TES) and transcranial magnetic stimulation (TMS). The main principle of TMS involves the creation of a magnetic field outside the brain which then penetrates the skull and induces an electrical stimulation of the area underneath the coil. Given the neuroanatomical connection between neurons,

TMS stimulation not only induces a change at the targeted area but also on neurons far from the site of stimulation (Klomjai, Katz, & Lackmy-Vallée, 2015; Mills, 2000; Ridding & Rothwell, 2007; Wassermann et al., 2008).

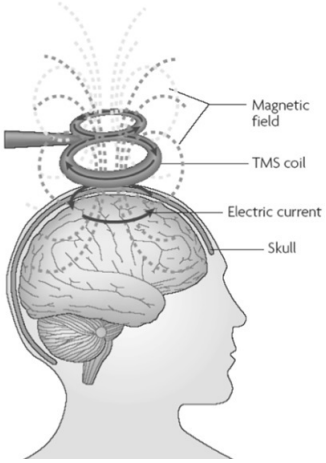


Figure 14. A circular coil showing the lines of force generated when current flows through the winding of a Transcranial Magnetic Stimulator device. From Ridding & Rothwell (2007).

When delivered to the motor cortex, the muscles of the targeted area contract. The force produced is recorded with a dynamometer while the electrical activity is monitored by surface EMG of the muscle investigated. The typical TMS electrical response is called motor evoked potential (MEP) which is used as an index to quantify the excitability of the corticospinal tract (Bestmann & Krakauer, 2015; Kobayashi & Pascual-Leone, 2003; Mills, 2000; Wassermann et al., 2008). Another parameter obtained after TMS stimulation is the cortical silent period (CSP). CSP is a momentary interruption of EMG signal immediately following the MEP, with a typical duration >200 ms (Gandevia, 2001; Orth & Rothwell, 2004). The CSP is generally measured from the point of TMS stimulation until the return of the normal EMG signal (Orth & Rothwell, 2004; Wassermann et al., 2008). The physiological mechanisms are still not clear, but CSP is believed to be influenced by the activation of GABAB receptors (McDonnell, Orekhov, & Ziemann, 2006; Wassermann et al., 2008).

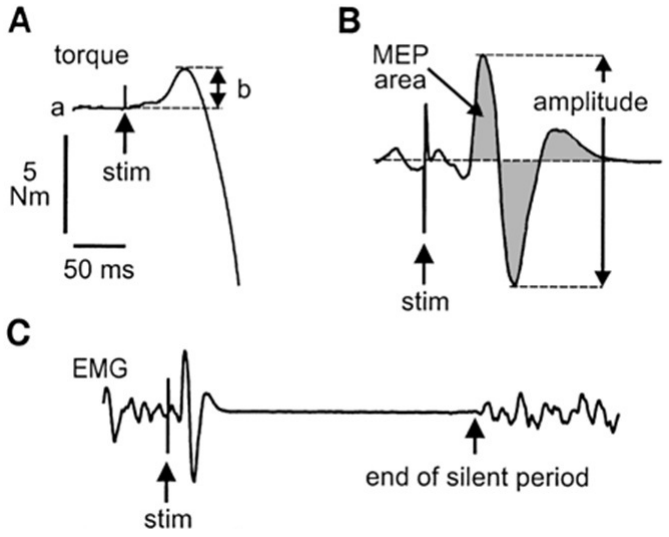


Figure 15. Representations of measurements of torque and EMG signals after a transcranial magnetic stimulation on motor cortex. From Taylor et al., (2000).

Black arrow indicates the point of stimulation. Panel A shows the force produced before stimulus (a) and the superimposed twitch from motor cortex (b). Panel B shows EMG recording of motor evoked potential (MEP). Panel C shows the cortical silent period (CSP).

Cortical excitability can be monitored both at rest (Gandevia, 2001; Rossini et al., 1994; Wassermann et al., 2008) and at various intensities of muscle contraction (Gandevia, 2001; Goodall, Howatson, Romer, & Ross, 2014; Taylor & Gandevia, 2008). MEP response is usually higher during voluntary contraction compared to resting state as the motor cortex is more activated and therefore more excitable (Gandevia, 2001; Goodall et al., 2014; Taylor & Gandevia, 2008). MEP response mainly depends on the intensity of stimulation and excitability of the brain area and motoneuronal pool (Gandevia, 2001; Goodall et al., 2014; Todd, Taylor, & Gandevia, 2003). When the intensity of the contraction increases, MEP response is larger up to an intensity corresponding to 50% MVC, where any increase in contraction force does not provide further increment in MEP (Hess, Mills, & Murray, 1987; Sidhu et al., 2009, 2009; Taylor & Gandevia, 2008; Todd et al., 2003).

Since the contribution of supraspinal sites in the development of central fatigue have been recognized, many experiments have been performed (Goodall et al., 2014; Gruet et al., 2013). TMS permits the stimulation of the targeted area during various types of exercise tasks (Gandevia, 2001; Goodall et al., 2014; Gruet et al., 2013), therefore important findings have been provided regarding

the behaviour of the cortical neurons during exercise and their role on supraspinal fatigue (Gandevia, 2001; Goodall et al., 2014; Gruet et al., 2013). TMS has been delivered at exhaustion in both isometric (Gandevia, 2001; Søgaard et al., 2006) and dynamic muscle contraction (Pageaux et al., 2015; Sidhu, Bentley, & Carroll, 2009; Sidhu, Cresswell, & Carroll, 2012) to quantity the contribution of the supraspinal sites in central fatigue. It should be noted that from a methodical point of view, the timing of the assessment of the cortical spinal tract after exercise is fundamental. Many experiments showed that cortical activity following exercise quickly recovers to baseline level (~20 s), and thus cortical assessment should be performed immediately after exhaustion (Gandevia, 2001). This is not possible in experiments involving whole body exercise such as running and cycling as specific ergometers are used, which therefore produce an inevitable delay between the termination of exercise and cortical assessment on a dynamometer (e.g. ~3 min). This has probably caused an underestimation or lack of estimation of supraspinal fatigue (Gandevia, 2001) and a consequent misinterpretation of experimental findings.

In the experiments 2 and 4, MEPs were elicited on the right VL by using transcranial magnetic stimulator (Magstim TMS 2002; Magstim, Whitland, UK). The concave double-cone coil (110 mm diameter) was placed over the contralateral M1 to deliver one single magnetic stimulation (1 ms). The optimal coil position was determined in order to elicit the largest MEP response of VL with a minimal MEP response of the antagonist muscle (biceps femoris, BF). The optimal position was then marked on the scalp, in order to more accurately stimulate the same area. Stimulation intensity was determined by starting from an intensity of 40% of the maximal stimulator intensity (100%), and then increased by 5% until the largest MEP of VL response was found with a small response of the BF. For each intensity interval, cortical stimulation was delivered during two brief voluntary contractions (3 s) interspaced by 3 s. 50% MVC was firstly chosen in study 2 as it has been demonstrated to reduce the variability of CSP (Pageaux et al., 2015; Säisänen et al., 2008). As changes in MEP have been well-established with the tDCS set-up used in study 2, using the 50% MVC allowed a measure of the previously unestablished CSP response. Contrarily in study 4, submaximal intensity was set at 10%. This decision was made as MEP amplitude does not further increase after 50% MVC and therefore any possible increase in excitability following anodal tDCS stimulation might not be detected. As the tDCS set-up in study 4 had not been used previously, it was important to document whether there was a change in MEP response, whilst still allowing a measurement of CSP

(if less reliable). Visual feedback of the force produced was constantly displayed on a screen in front of the subject.

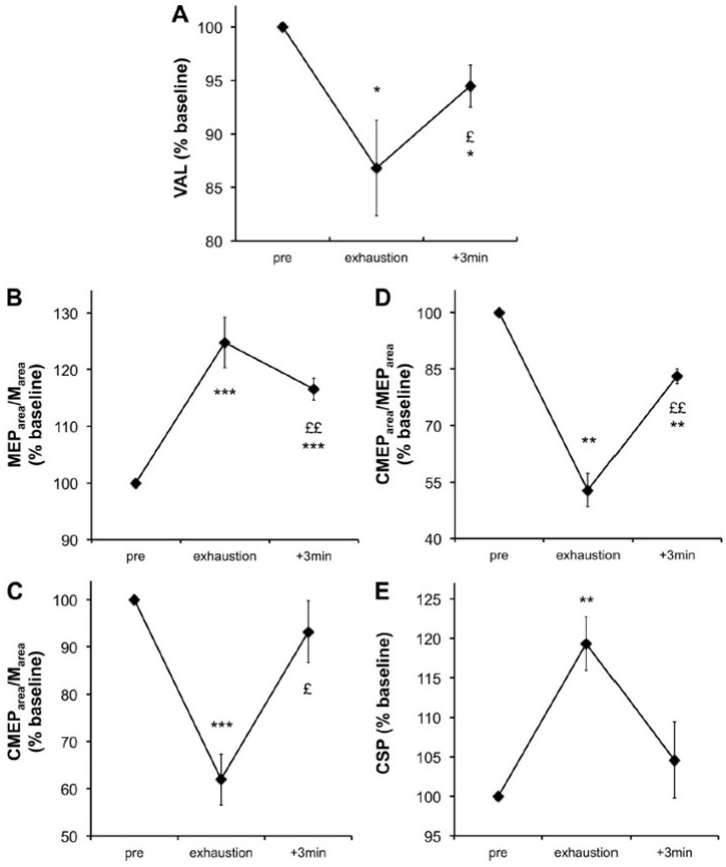


Fig 16. Effect of assessment time delay following exhaustive exercise on neurophysiological parameters. From Pageaux et al., (2015).

Parameters were recorded at baseline, immediately after exhaustion and after three min after exhaustion. Cervical motor evoked potential (CMEP), cortical silent period (CSP), voluntarily activation level (VAL), motor evoked potentials (MEP), maximal muscular wave (Mwave).

1.4.3 Repetitive Transcranial Magnetic Stimulation (rTMS)

In the previous section, the utility of TMS to monitor the behaviour of the motor cortex during exercise was discussed (Gandevia, 2001; Taylor & Gandevia, 2008). This technique, also called single pulse TMS, implies only a single TMS burst with short lasting effect in order to monitor the level of excitability of a targeted area (Gandevia, 2001; Taylor & Gandevia, 2008). TMS however can be also utilised to produce long lasting effects. Unlike single pulse TMS, repetitive

TMS (rTMS) produces long lasting changes of cortical neuronal activity (Chen, Rappelsberger, & Filz, 1998; Ridding & Rothwell, 2007). The increase or decrease of the neuronal activity depends on the type of stimulation. Low frequency stimulation (<1 Hz) has been shown to reduce neuronal activity (Chen et al., 1998; Di Lazzaro et al., 2004, 2008) while high frequency stimulation (>5 Hz) produces opposite effects (Di Lazzaro et al., 2004, 2008; Fierro et al., 2005). The duration of the effect depends on the length of stimulation (Dayan, Censor, Buch, Sandrini, & Cohen, 2013; Di Lazzaro et al., 2004, 2008). The neurophysiological response following rTMS is supported by studies monitoring the level of cortical excitability by single pulse TMS, where MEP size and CSP have been measured (Chen et al., 1998; Dayan et al., 2013; Di Lazzaro et al., 2004, 2008). The ability to alter excitability of the targeted area gives the opportunity to study various aspects of the brain, or use rTMS as co-therapy in patients with neurological and psychiatric disorders (Lefaucheur et al., 2008; Ridding & Rothwell, 2007). The use of rTMS as a therapy has rapidly increased in recent years (Ridding & Rothwell, 2007) and it is generally well accepted in patients as it does not involve any invasive procedures. However, despite the large amount of clinical trials, the way rTMS changes cortical activity and its relationship with the therapeutic benefits are still uncertain (Ridding & Rothwell, 2007).

1.4.4 Transcranial direct current stimulation (tDCS)

Transcranial direct current stimulation (tDCS) is a non-invasive technique used to stimulate a specific area of the brain. Unlike some other techniques such as TMS and transcranial electrical stimulation (TES), tDCS does not induce neuronal action potentials on the targeted area but rather acts as a neuromodulatory intervention (George & Aston-Jones, 2010; Nitsche et al., 2008). tDCS is a form of neurostimulation technique which has been widely accepted be effective for the treatment of depression (Brunoni et al., 2016), cognitive enhancement in both healthy and clinical population (Hsu, Ku, Zanto, & Gazzaley, 2015), treatment of chronic pain (Lefaucheur et al., 2008) and improving motor function of limbs in post stroke patients (Elsner, Kugler, Pohl, & Mehrholz, 2016).

The physiological principle of tDCS is based on the alteration of the membrane potential of the targeted area. The polarization of the brain tissue is obtained by the passage of a weak constant electrical flow from the anodal to the cathodal electrode. By leaving the electron pool through the cathodal electrode, an increase of negative charges occurs, which inhibits (hyperpolarization)

the area underneath the cathodal electrode and excites (depolarization) the area underneath the anodal electrode (George & Aston-Jones, 2010; Nitsche et al., 2008). As a consequence, the spontaneous firing rate increases under the anodal electrode and decreases under the cathodal. The effect of tDCS on cortical excitability mainly depends on the intensity (mA), size of electrode (cm^2), density (mA/cm^2), duration of the stimulation and position of the electrodes (Poreisz, Boros, Antal, & Paulus, 2007; Utz, Dimova, Oppenländer, & Kerkhoff, 2010). Density is calculated as the ratio between current intensity and the size of the electrode. Manipulation of these parameters has been shown to alter the magnitude and effect of tDCS stimulation on the targeted area (Stagg & Nitsche, 2011). The multiple variations of these parameters in research (particularly in the few studies in exercise science) has contributed to some divergent findings, and a difficulty in comparing studies.

The first experiment investigating different tDCS intensity dosages was performed by Nitsche and Paulus (2000), who maintained the electrode size of 35 cm^2 and monitored the cortical response following an increased intensity of stimulation from 0.2 and 1 mA. This experiment showed for the first time that cortical excitability was increased more in higher compared to lower intensities. Consequently, most studies now use the same electrode size, but with higher intensities to produce stronger effects (Nitsche et al., 2008). However, it should be noted that contradictory results were reported by Kidgell and colleagues (Kidgell et al., 2013), who reported no significant differences between an anodal intensity of 0.8 - 1.2 mA (0.032 - 0.048 mA/cm^2, 25 cm^2). By comparing different stimulation protocols, Nitsche & Paulus, (2001) showed an elevation of cortical excitability (increased MEP size) for up to 90 min following a 9-13 min stimulation protocol. However, when tDCS was applied for 5-7 min, the effects lasted for no longer than 5 min. Regarding the electrode set up, only a few experiments have investigated the effect of different electrode size, and generally findings show a similar or greater effect when using smaller electrodes (Bastani & Jaberzadeh, 2013; Ho et al., 2015; Nitsche et al., 2007).

Given the passage of electrical flow between the two electrodes and its distribution on the brain, another series of experiments investigated the effects of different electrode montages. Comparisons were facilitated by using an electroencephalogram (EEG) and computer based modelling techniques which analyzed the current distribution of different electrode montages, including cephalic and extracephalic set-ups. Greater current density has been shown when the distance between the electrodes increases (Accornero et al., 2014; Miranda, Lomarev, &

Hallett, 2006), whilst other studies have suggested that an extracephalic montage reduces the uncertain outcomes and better clarifies the tDCS effects (Angius et al., 2015; Cogiamanian, Marceglia, Ardolino, Barbieri, & Priori, 2007). The aforementioned parameters change between studies and according to the objective of the stimulation. Therefore, it is not surprising that across studies there is considerable variation in the tDCS set-up used. It is also important to note that on receiving stimulation, the brain does not just passively receive it but reacts in some way (Clemens et al., 2014; Miniussi et al., 2008). Therefore, the exact effects of tDCS on brain tissue are still not clear and yet to be defined.

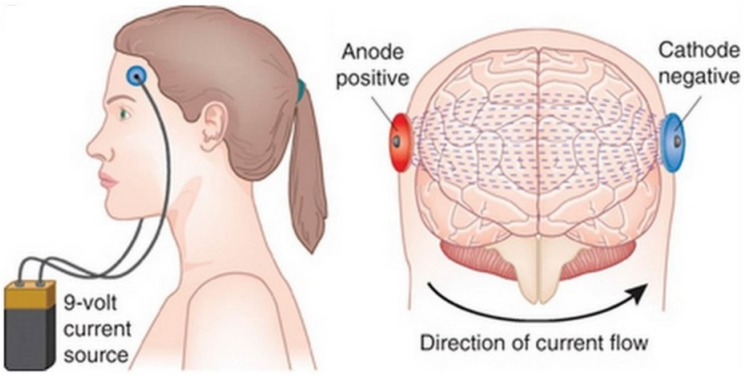


Figure 17. Description of transcranial direct current stimulation (tDCS) mechanism. From George et al., (2010).

A tDCS device uses an anode and cathode connected to a direct current source much like a 9 V battery. The direct current passes through the intervening tissue, with some shunting through the skull but much of it passes through the brain and changes resting electrical charge, particularly under the cathode.

1.4.5 Side effects and safety criteria for tDCS

Together with the increasing number of tDCS protocols, development of new devices and the use of tDCS as therapy, stricter safety criteria were required. Currently, a stimulation of 2 mA for 20 min is considered safe for humans (Iyer et al., 2005; Nitsche et al., 2003) in both a single and as a repeated session (Fregni, Boggio, Nitsche, Rigonatti, & Pascual-Leone, 2006). These parameters, in terms of intensity and duration, are frequently used in the treatment of various neurological disorders (Fregni et al., 2006). Commonly, the adverse side effects of tDCS are characterized by itching or a light burning sensation under the electrode during the stimulation (Poreisz et al., 2007; Utz et al., 2010), while only in rare

occasions has skin tissue has been damaged (Frank et al., 2010; Palm et al., 2008). Post tDCS stimulation, side effects are commonly described as a mild headache or dizziness, which usually disappears a few hours (generally less) after stimulation (Frank et al., 2010; Palm et al., 2008). Furthermore, no cognitive or motor impairments have been reported following tDCS stimulation (Fregni et al., 2006; Poreisz et al., 2007). These studies suggest tDCS to be a safe neuromodulatory brain technique, with no or only minor side effects. However, safety procedures during a subject's preparation and contraventions to participation are required in order to reduce any possible adverse effects.

1.4.6 tDCS and exercise

Numerous investigations have demonstrated that anodal tDCS increases cortical excitability of the M1 (Nitsche & Paulus, 2000, 2001) and that its effect can last up to 90 min after the cessation of the stimulation (Nitsche & Paulus, 2000, 2001). In the last decade, there has been emerging literature demonstrating the possibility to improve exercise performance following anodal tDCS stimulation. A summary of the details of the most important studies regarding tDCS stimulation and exercise performance results are shown in Table 1.

Figure 18. Commercial transcranial direct current stimulation (tDCS) stimulator device.

The first study investigating the effect of tDCS on exercise performance was performed by Cogiamanian and colleagues (2007). Their study was formed of two experiments. In the first experiment subjects were divided in two different

groups - brain polarization and control. Both groups underwent two isometric time to exhaustion tasks of left elbow flexors at 35% MVC interspaced by a 60 min recovery. In the last part of the recovery, the brain polarized group received anodal or cathodal tDCS for 10 min at 1.5 mA over the right motor cortex with the cathodal electrode over the right shoulder. The control group did not receive any tDCS treatment. The second experiment was performed to monitor the change in cortical excitability following tDCS. Six subjects were tested and TMS was delivered during an isometric contraction of the left elbow flexors at 5% MVC, before and after anodal tDCS with the same set up as experiment 1. No changes in MVC were found, but interestingly time to exhaustion was significantly longer in the anodal condition, and MEP amplitude increased in experiment 2. However, the authors were not able to provide a precise explanation for the increased endurance time. The authors suggested that tDCS could act upstream of the motor cortex by facilitating the supraspinal drive or by protecting the motor cortex from inhibitory feedback arising from working muscles.

Six years later two different experiments from the same group studied the effect of anodal tDCS on isometric exhaustive performance. Unlike the study of Cogiamanian and colleagues (2007), the study of Kan et al. (2013) performed a crossover experimental design study involving only male volunteers. Subjects performed the same protocol from experiment 1 of Cogiamanian and colleagues (2007), but the tDCS set up differed in terms of intensity of stimulation (2 mA), electrode size (24 cm^2), and the type of stimulations (anodal and sham). In the Kan et al. (2013) study, subjects performed a sustained isometric contraction corresponding to 30% MVC. Moreover, a follow up experiment to monitor MEP response following tDCS involving 10 different subjects was performed. No changes in MVC post tDCS intervention were found and unlikely to Cogiamanian and colleagues (2007) this study failed to find any improvement in performance or increase in MEP response following anodal tDCS. In the same year, a study of Muthalib and colleagues (2013) repeated the protocol of Kan and colleagues (2013) with the main aim of monitoring the prefrontal oxygenation following tDCS during exercise. However, no cortical excitability assessment was performed. Similarly to Kan et al (2013) there was no improvement in performance or MVC along with no changes in prefrontal oxygenation.

A further experiment investigating the effect of tDCS on sustained isometric contraction following anodal tDCS was performed by Williams et al., (2013). In a crossover experimental design, volunteers were asked to perform an exhaustive isometric contraction at 20% MVC. Anodal and sham tDCS were administered

for 20 min over the motor cortex. Both cortical excitability (by delivering TMS) and RPE were monitored during the fatiguing task. Initially no improvement in performance after anodal tDCS compared to sham was observed. Subsequently, the investigators divided the entire number of subjects (n=18) in two sub groups: one group included all the subjects whose performance time lasted less than the tDCS stimulation time (n=8), while the second group included subjects whose performance time exceeded the tDCS stimulation time (n=10). Accordingly, the first group showed a significant improvement in performance compared to the second. No significant changes in MEP were found between conditions or group but RPE was significantly reduced in the anodal tDCS condition. The authors associated the improvement in performance with an increase in excitability following anodal tDCS, which added a "boost" to motivation and/or descending drive to the motoneuronal pool.

The only study investigating the effect of anodal tDCS on whole body exercise was conducted by Okano et al., (2015). In a crossover, randomized design, a group of healthy volunteers performed a maximal incremental cycling test starting at 15 W, with increases in 25 $W \cdot min^{-1}$ up to volitional exhaustion. tDCS administration consisted of anodal stimulation for 20 min at 2 mA or sham tDCS. The anodal electrode was applied over the left temporal cortex and the cathodal over the contralateral prefrontal area. Maximal performance improved by ~4% with a significant reduction in RPE and HR in the anodal condition. The authors suggested that anodal stimulation could have affected the activity of the insular cortex, thus affecting the perception of effort and making the exercise feel easier, which allowed to consequent improvement in performance.

Collectively the aforementioned experiments provide interesting insights regarding the possible effects of tDCS on exercise in healthy individuals. However, the different outcomes in terms of improvement in exercise performance make the potential benefits of tDCS still uncertain. The inconsistency of the results make the experimental findings difficult to interpret and might be in part be caused by the large differences between the experiments in terms of exercise type and/or tDCS set up. The exact mechanisms which tDCS may moderate during exercise are not clear, but the research suggests it is likely to facilitate the output from the motor cortex (Cogiamanian et al., 2007; Williams et al., 2013). Indeed, many of the aforementioned studies were not designed to specifically assess the mechanism by which performance was hypothesised to improve. Therefore, more studies which systematically control the tDCS variables (e.g. montage, identity, location etc.) and allow assessment of the mechanisms are required.

Despite the differences in prior research regarding the experimental design, type of exercise performed and tDCS montage (making it difficult to interpret and conclude the effect of tDCS on exercise performance), there are some experimental findings which are similar across the various experiments. Firstly, tDCS does not seem to improve isometric maximal force capacity (Cogiamanian et al., 2007; Kan et al., 2013; Williams et al., 2013). Secondly, submaximal tasks are appear to respond preferentially (compared to maximal) to anodal tDCS intervention (Cogiamanian et al., 2007; Okano et al., 2015; Williams et al., 2013). However, given the uncertain mechanisms and the inconsistency of outcomes of tDCS prior to exercise, speculation or future application of tDCS during exercise should be treated with caution. Additionally, more experiments should be performed to explore and clarify the physiological mechanisms underlying the effect of tDCS prior to exercise.

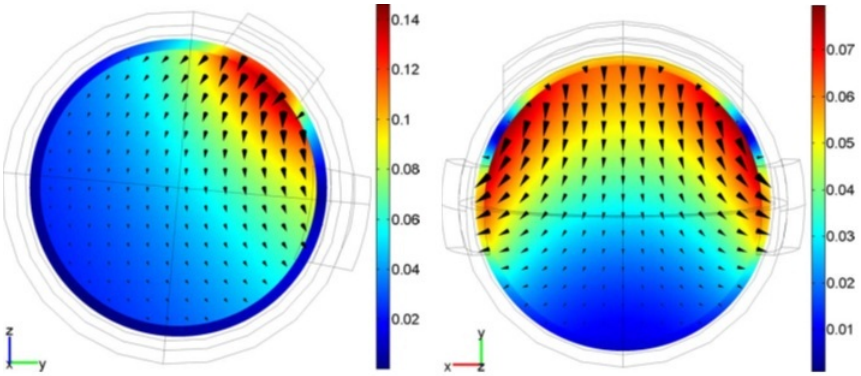


Fig 19. Top view illustration showing the magnitude and direction of the current density vectors in the brain and skull during tDCS stimulation. From Miranda et al., (2006).

Left panel: small anode electrode placed over the left dorso lateral prefrontal cortex (LDPC) and a small cathode electrode placed above the right eyebrow. Right panel: large anode placed above both eyebrows and two small cathodes placed over the mastoids. Black arrows represent vectors indicate the direction and density of the current flow while crossing the skull and the brain. In both panels, the nasion has been placed on the top of each figure.

1.4.7 Unknowns and the potential for tDCS to alter exercise performance

This literature review has demonstrated that exercise performance is regulated by a number of (psycho) physiological systems, and that the brain is at least partly involved in this integrative process (either by controlling a system, or synthesizing several inputs into an overall perception). tDCS is capable of transiently moderating the excitability of a targeted brain area, and previous research has shown that this is capable of moderating human behaviour and perception. Some exercise science researchers have used tDCS to bring about a change in performance, but the methodology used in these studies lacks any sort of systematic approach to deduce whether changes in performance are a result of the brain area stimulated, the electrode set-up, the exercise task or the stimulation duration. tDCS has conclusively been shown to increase cortical excitability of the M1 and to be capable of reducing pain. These systems have the potential to moderate endurance performance through a change in perception of effort and a reduction in pain (for a given exercise intensity), and therefore a series of studies are required to examine this potential. In the exercise literature, a change cardiovascular response alongside a performance change has been shown following tDCS (Okano et al., 2015). However, this study was not designed to examine the cardiovascular response, and so the potential for tDCS to moderate this needs to be explored more robustly. Consequently, this thesis will seek to explain whether tDCS is capable of changing endurance performance through the moderation of a brain areas responsible perception of effort, pain perception and cardiovascular regulation.

Table 1. List of all studies involving tDCS on exercise performance.

Paper of studies	Target Region(s)	Montage, subjects (n)	Duration	Intensity	Electrode size	Performance result	Muscle investigated	Control	Exercise protocol
Cogiamanian et al., (2007)	Right M1	Anodal right M1, cathodal right shoulder, Group 1, n= 9; group 2, n= 15	10 min	1.5 mA	35 cm^2	Improved	Left elbow flexors	Control	Isometric TTF 35% MVC
Muthalib et al., (2013)	Right M1	Anodal right M1, cathodal right shoulder, n=15	10 min	2 mA	24 cm^2	No diff	Left elbow flexors at 90º flexion	Sham	Isometric TTF 30% MVC
Kan et al., (2013)	Right M1	Anodal right M1, cathodal right shoulder, n=15	10 min	2 mA	24 cm^2	No diff	Elbow flexors	Sham	Isometric TTF 30% MVC
Williams et al., (2013)	Right M1	Anodal right M1, cathodal left forehead, n=18	20 min	1.5 mA	35 cm^2	Improved	Left elbow flexors	Sham	Isometric TTF 20% MVC
Okano et al., (2013)	Right scalp (T3)	Anodal over the scalp (T3), cathodal over the contralateral supraorbital area (Fp2), n=10	20 min	2 mA	35 cm^2	Improved ~4%	Lower limbs	Sham	Cycling, from 15W + 25 $Wmin^{-1}$

Primary motor cortex (M1); maximal voluntary contraction (MVC); time to task failure (TTF);

1.5 Aims and hypotheses of the thesis

As previously discussed in the previous chapters, fatigue is a complex phenomenon involving a wide variety of physiological systems of the human body as well as an important psychological component. In the previous sections, many experiments have demonstrated that the brain plays a central role in the development of fatigue. Experiments have demonstrated that tDCS stimulation can modulate excitability of the targeted brain area and this can provide an analgesic effect during various forms of experimental pain or can alter autonomic cardiovascular response. More recently, experimental research has demonstrated that tDCS can in some situations, improve exercise performance. This might be the consequence of a facilitated descending drive from the motor cortex, induced analgesic effect (thus reducing exercise induced muscle pain), or altered autonomic cardiovascular regulation (thus increasing oxygen delivery to the muscle). The exact neurophysiological and psychological mechanisms following tDCS stimulation are still unknown and therefore further experiments should be performed. In light of this deficiency, the overall aim of the thesis is to explore and further identify the key mechanisms involved following tDCS administration. Accordingly, the experiments performed for this thesis aimed to fill this gap in the literature and elucidate the aforementioned physiological and psychological mechanisms. Four experimental studies are included in this thesis; the titles, aims and hypothesis for each study are provided below:

Chapter 3 - Study 1
Title: The effect of transcranial direct current stimulation of the motor cortex on exercise-induced pain.
Aims: To investigate whether an analgesic tDCS intervention could reduce pain perception during a high intensity constant workload cycling task.
Hypothesis: It was hypothesised that pain during exercise would be reduced following tDCS stimulation and this would consequentially improve cycling time to exhaustion.

Chapter 4 - Study 2

Title: Transcranial direct current stimulation improves isometric time to exhaustion of the knee extensors.

Aims: The purpose of this study was to investigate the neurophysiological mechanisms following tDCS stimulation and identify the optimal tDCS montage to improve isometric performance of knee extensor muscle.

Hypothesis: It was hypothesised that the extracephalic montage would result in a greater improvement of endurance performance compared to cephalic montage.

Chapter 5 - Study 3

Title: The effect of anodal tDCS over left and right temporal cortex: a comparative study.

Aims: The aim of this experiment was to investigate whether tDCS stimulation applied over the left and right temporal cortex can alter the cardiovascular response.

Hypothesis: it was hypothesised that tDCS application can alter autonomic cardiovascular regulation and in turn change cardiovascular response.

Chapter 6 - Study 4

Title: Transcranial direct current stimulation improves cycling performance in healthy individuals.

Aims: The aims of this experiment were to monitor the effect of bilateral tDCS stimulation of motor cortex on perception of effort during exercise and to monitor whether any alteration of perception of effort following tDCS stimulation can alter high intensity cycling performance.

Hypothesis: It was hypothesised that anodal tDCS would decrease perception of effort and consequently improve high intensity cycling performance and cathodal tDCS would lead to an opposite effect.

CHAPTER 2

GENERAL METHODS

2.0.1 Introduction

This chapter critically describes the main methodologies used in the experimental research reported in the following chapters. The particular individual experimental protocols are also detailed in the methods section of each chapter. All data collection and all sample analyses in this thesis were conducted in the laboratories of the School of Sport and Exercise Sciences of the University of Kent.

2.0.2 Ethical approval

All experiments were approved by the School of Sport and Exercise Sciences Ethics Committee of the University of Kent, in line with the Declaration of Helsinki. The purposes and the procedures of each study were clearly documented in the participant information sheet and also verbally explained to all participants. Participants were also given the opportunity to receive more information regarding testing procedures and other details of the experiments. Before starting each study, participants completed a health questionnaire (Physical Activity Readiness Questionnaire, PAR-Q) to assess their suitability to participate each study. Once each participant satisfied all the health criteria they were permitted to sign the consent form and also permitted to withdraw from the study at any time and for any reason.

2.0.3 Participants and familiarization procedures

All participants were students or staff at the University of Kent, or residents of the local community. For all investigations, participants were recruited mainly through personal contacts and emails. All participants were aged between 18 and 35 years old and free from any particular medical condition or medication. Before participating in a study, participants attended a familiarization session that corresponded to the first visit for all the four experiments. This session served to introduce each participant to all the procedures performed in the following visits and reduce the learning effect. Individual participant details were recorded in this session, together with their anthropometric data. The Physical Activity Readiness Questionnaire (PAR-Q) was used in each study to monitor individual health status, and to identify any use of medication or pathologies that

would have precluded them to participate in the experimental procedures. During this visit, each participant fully performed all the procedures required in the following visits. This was important as they were fully aware of the experimental procedures and could prepare for the following experimental sessions.

2.0.4 Incremental test

Study 2 and 4 required the performance of a cycling time to exhaustion (TTE) task at 70% of peak power output (W_{max}). Individual W_{max} was determined during the familiarization visit by an incremental test performed on cycle ergometer (Lode, Excalibur Sport, Groningen, Netherlands). The cycle ergometer used for this thesis has been reported to have a workload accuracy below 100 W of 2 W, from 100 to 1500 W of 2% and over 1500 W of 5%. The reliability of this ergometer has been measured by Earnest et al., (2005). In details the coefficient of variation of the peak power during kindermmaximal incremental test was 6.3%.

The incremental test was preceded by a 5 min warm up at 100 W, followed by an intensity of 100 W for one minute and a subsequent increase of 5 W every 15 s until the participant was no longer able maintain the required cadence (60 rpm). $\dot{V}O_{2max}$ was considered as the attainment of at least two of the following criteria: (1) plateau of $\dot{V}O_2$ despite any increase in workload (<80 mL $\cdot$ min^{-1}), (2) respiratory exchange ratio (RER) above 1.10, and (3) heart rate (HR) within $\pm$10 bpm of predicted maximum heart rate (calculated as 220-age).

Each participant was strongly verbally encouraged during all the phases of the incremental test. RPE and pain perception were also monitored in order to familiarize them for the following visits. Familiarization for the TTE was performed in the same visits after of the completion of incremental test, following 30 min of recovery.

2.0.5 Measurement of performance

In sport science, performance testing is often used to determine the efficacy of interventions and therefore each test must be able to provide a reliable and consistent measurement of performance. Endurance performance has been measured in laboratories with two types of tests - the time trial and the time to exhaustion test. In time trials participants work at a self-selected intensity in order to complete a set distance or work as fast as possible. Time to exhaustion tests

however, are usually performed at a predetermined and constant work rate until volitional exhaustion, which corresponds to the point where the subject is not able to maintain the required power. Time to exhaustion tests have a subjective termination point, as the termination is usually determined by the subject's task disengagement (Marcora & Staiano, 2010) has been measured with the coefficient of variation (CV) which represents the error of the measurement expressed as a percentage of the mean. A smaller percentage means less variation and more accuracy. Time to exhaustions tests have been documented to have a greater variation compared to time trials and it has been demonstrated that a time to exhaustion performed at 75% of peak power has a greater CV than a similar duration time trial (26.6% vs. 3.4%).

Recently the implementation of time trials in research have increased since this type of test is a better representation of the performance. Indeed, an actual race is never based at fixed intensity, and instead the duration is dependent and characterized by periods of different intensities. Accordingly, many tests performed in the lab have been developed to simulate similar conditions occurring during the race. Time to exhaustion tests however are still often used in experimental research as the steady state intensity permits a better analysis of the physiological response during the test. This is not possible during time trials as each participant is allowed to self-regulate the power or speed (i.e. pacing) and therefore this makes it difficult to interpret the results. For the purpose of this thesis, because of the lack knowledge regarding the effect of tDCS, in an attempt to define its mechanistic effect the experimental studies utilized a time to exhaustion test. In order to reduce factors that can potentially increase the variation of TTE duration, participants were always fully familiarized with the TTE, strongly motivated and all the experimental sessions were randomized in order to avoid any learning effect for TTE. This involved both a whole body cycling exercise (study 1 and 4) and a single limb %MVC (study 2 and 3).

2.0.6 Methods to measure fatigue

One of the primary aims of this thesis was to investigate the cortical and muscular function following tDCS stimulation and exhaustive exercise of the knee-extensors. The assessment of these parameters was monitored at rest and during maximal and submaximal knee-extensor contractions. As discussed in the previous chapters, fatigue can occur at any site of the neuromuscular system, therefore various techniques and methods have been have developed in order to isolate and

quantify each component. The first step is to locate the exact site of fatigue in order to exclude any other variables that might interfere with the measurement. The following paragraph will describe the most common and validated techniques used to measure the various types of fatigue.

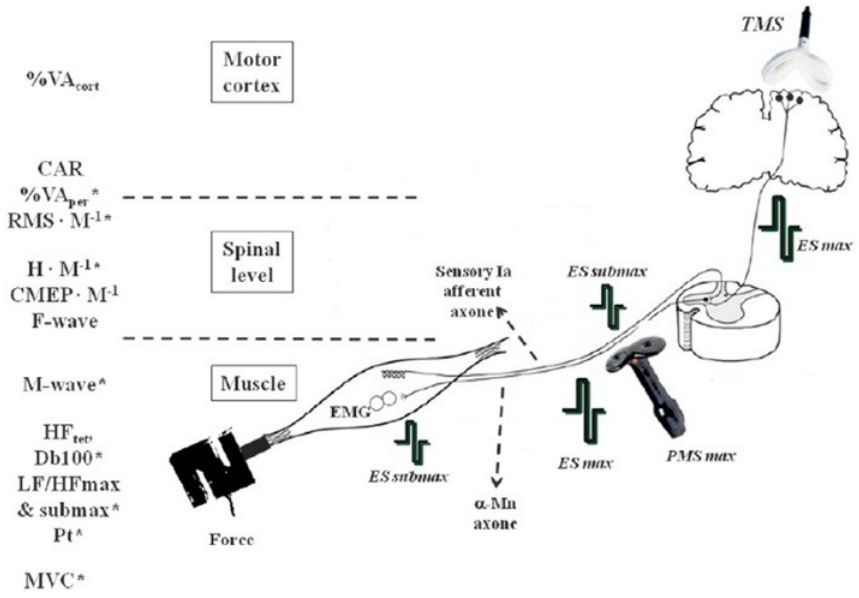


Fig 20. Schematic view of the main electrical and magnetic stimulation techniques used to measure neuromuscular fatigue at different sites of the cortical spinal tract and peripheral nerve. Adapted from Millet et al. (2012).

Transcranial magnetic stimulation (TMS); central activation ratio (CAR); maximal voluntary activation measured from motor nerve stimulation (%VAper); RMS · · · M-1: EMG (root mean square) measured during maximal voluntary contraction (MVC) normalized to Mwave amplitude; peak twitch (Pt) force evoked by a single pulse; maximal voluntary contraction (MVC).

2.0.7 Measurement of peripheral fatigue

Electrical stimulation of the motor nerve at rest is the most common technique used to measure peripheral fatigue and permits a measure of the degree of contractility and excitability of the muscle investigated (Bigland-Ritchie, Furbush, & Woods, 1986; Merton, 1954; Place, Maffiuletti, Martin, & Lepers, 2007). This is commonly performed by percutaneous electrical stimulation, with the stimulating electrode placed over the skin in correspondence to the motor nerve. The

main parameter recorded is called Twitch (Tw), which corresponds to the mechanical response of the muscle (i.e. force) immediately following the stimulation. The force is usually recorded with a dynamometer. Accordingly, any decrease in resting Tw represents an impairment of muscle contraction and thus an increase in peripheral fatigue. Peripheral femoral stimulation is usually delivered before and after the MVC. When followed by a strong muscular contraction such as an MVC, Tw is potentiated (Hodgson, Docherty, & Robbins, 2005). This response is believed to be caused by a sensitization of the actin-myosin to Ca^{++} released from the sarcoplasmic reticulum (Hodgson et al., 2005). The potentiated twitch is more sensitive to the changes occurring in fatigued muscles compared to the unpotentiated twitch (Kufel, Pineda, & Mador, 2002). For these reasons, all the experiments performed for this thesis have adopted the potentiated twitch technique to monitor peripheral fatigue. When stimulated, the electrical response of the muscle is recorded by means of the electromyography technique (EMG) of the muscle investigated. The electrical response recorded is called the muscle compound action potential (Mwave). Mwave is very useful to measure the level of excitability of the muscle investigated. Variations in Mwave are generally caused by changes in excitability of the membrane (Millet et al., 2002), while reduction in Tw without variations in Mwave might refer to limitations in excitation contraction coupling of the muscle (Allen et al., 2008).

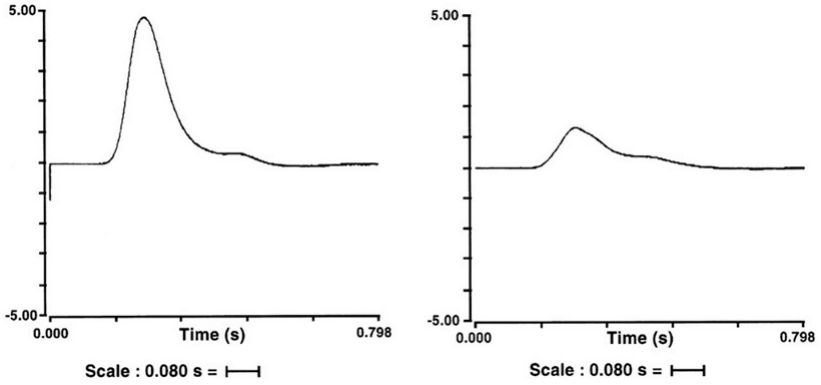


Fig 21. Effect of peripheral fatigue on potentiated resting twitch following maximal voluntary contraction. From Behm & St-Pierre, (1997).

This picture shows the effect of exercise induced muscle fatigue on potentiated resting twitch. The left panel shows mechanical response in fresh muscle while the right panel shows the mechanical response of the same muscle group in fatigued condition.

For the purpose of this thesis, the intensity of the stimulation was always detected prior to starting the experimental procedures. Stimulation of the femoral nerve was delivered by using a high-voltage constant-current stimulator (model DS7 modified, Digitimer, Hertfordshire, UK) where the cathodal and anodal electrodes (Phoenix Healthcare Products Ltd., Nottingham, UK) were respectively positioned over the femoral triangle and over the gluteal fold. Before detecting the stimulation intensity, brief low electrical stimulation was delivered in order to check signal quality. The optimal electrical intensity was detected, starting from 100 mV and increasing by 20 mV until no further increase in Mwave and Tw were found in order to recruit all the muscle fibres. Once the final intensity was detected, the last was increased by 30%.

2.0.8 Measurement of voluntary activation

Voluntary activation has been defined as the ability of the central nervous system to drive the muscle and has been extensively studied across various muscles (Bigland-Ritchie et al., 1986; Gandevia, 2001; Merton, 1954; Place et al., 2007; Shield & Zhou, 2004). Several methods involving superimposed electrical stimuli (single, double, or trains of stimuli) have been proposed to quantify the degree of voluntary activation. The most used and validated method to measure voluntary activation is the interpolated twitch technique (Bigland-Ritchie et al., 1986; Merton, 1954; Place et al., 2007). The twitch interpolation technique, introduced by Merton (1954), involves the stimulation of the nerve during an MVC. This requires a superimposed stimulation of the motor nerve during an isometric maximal voluntary contraction (MVC) of the muscle investigated (Bigland-Ritchie et al., 1986; Gandevia, 2001; Merton, 1954; Shield & Zhou, 2004). In non-fatigued conditions, superimposed stimulation evokes a small additional force of the muscle (i.e. maximal activation), conversely in fatigued state, the extra force produced following superimposed stimulation is much greater, thus indicating that not all motor units are recruited (Bigland-Ritchie et al., 1986; Gandevia, 2001; Merton, 1954; Place et al., 2007; Shield & Zhou, 2004) and therefore central fatigue is evident. In support of this, it has been demonstrated that the size of the superimposed Tw decreases when the voluntary force increases in linear fashion (Gandevia, 2001).

This technique however does not isolate the exact site of fatigue (Gandevia, 2001; Shield & Zhou, 2004). The measurement of central fatigue can be performed only during an MVC because it reflects the inability to fully drive the muscle

(Gandevia, 2001; Shield & Zhou, 2004). Conversely, if measurement is performed during submaximal contraction some other mechanism might compensate for the inability to recruit the muscle fibres (Gandevia, 2001; Taylor & Gandevia, 2008). The voluntary activation level (VAL) is measured by comparing the amplitude of the superimposed twitch evoked during the MVC with the twitch evoked at rest (potentiated doublet), according to the following formula:

$$VAL = 100 \cdot \left(1 - \frac{superimposed\ doublet\ amplitude}{potentiated\ doublet\ amplitude}\right)$$

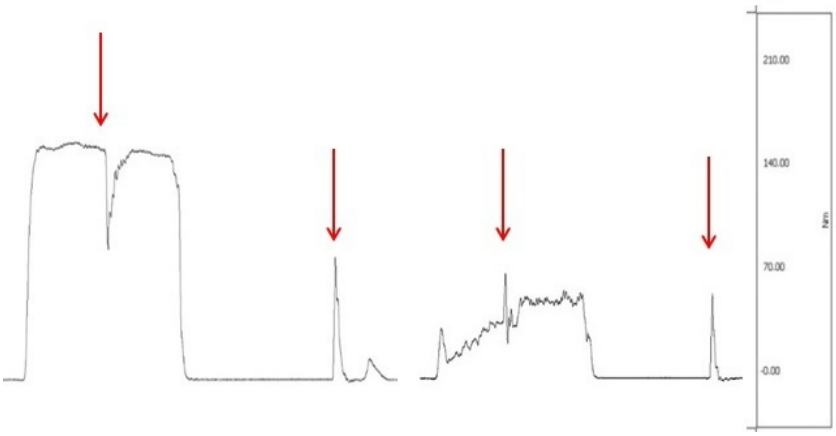


Fig 22. Effect of fatiguing exercise on maximal force, voluntary activation and peripheral fatigue.

Maximal force and potentiated twitch declined following exhaustive exercise while superimposed twitch increased. Red arrow indicates the moment of nerve stimulation.

A similar method was been later introduced by Newham and colleagues (1991), where a series of supramaximal pulses (burst) was applied to enhance the increase in force during voluntary contraction. However, there is no evidence in the literature regarding the optimal frequency of stimulation to detect central activation (Stackhouse, Dean, Lee, & Binder-MacLeod, 2000). Another method to quantify the inability of the CNS to drive the muscle has been proposed by using a burst superimposition technique called the central activation ratio (CAR) (Kent-Braun & Le Blanc, 1996).

Unlike the method proposed by Merton (1954), in the CAR method the electrical stimulus is delivered only during the MVC and it is calculated according

to the following formula:

$$CAR = \left(\frac{MVC}{MVC + stimulated\ force} \right)$$

In order not to underestimate the VAL, each subject was strongly encouraged during the execution of the MVC. By doing this, the maximal force can be expressed and so the superimposed twitch can be reduced. It also important to recommend the subject to relax once the MVC is performed as the following stimulation must be delivered at resting conditions, if not the muscle contraction invalidates the VAL measurement. In both methods an error is introduced if the stimulation is not delivered at the peak force produced. In order to reduce the error associated with the stimulus timing, a modified version of the CAR has been developed (Krishnan & Williams, 2010a), which is calculated with the following formula:

$$Modified\ CAR = \left(\frac{MVC\ prior\ stimulation}{superimposed\ Tw} \right)$$

Since the quantification of central fatigue is important in both healthy and clinical populations, the comparison between these methods have been performed (Krishnan & Williams, 2010). Comparison between methods showed a significant difference in muscle activation and so this should be taken into account when designing studies. Across the various methods proposed, the validity a reliability of the technique proposed by Merton has been well-validated in a range of muscle groups (Gandevia, 2001; Pageaux et al., 2015; Place et al., 2007) and has been described to be a valid method able to reveal any change in muscle activation.

2.0.9 Cortical voluntarily activation

Given the chain of events starting from the brain and ending at the muscle during voluntarily contraction, relatively few experiments have investigated the possibility of measuring central fatigue with superimposed motor cortex stimulation by TMS (Gandevia et al., 1996; Goodall, Romer, & Ross, 2009; Sidhu,

Bentley, & Carroll, 2009). In this technique, if extra force is produced, it means that supraspinal fatigue occurs (Gandevia, 2001; Gandevia et al., 1996; Søgaard et al., 2006; Taylor & Gandevia, 2008). Although this technique better identifies the site where central fatigue occurs (i.e. motor cortex), some limitations are present. The lack of accuracy when stimulating the motor cortex makes it difficult to target only a specific muscle group. Furthermore, only muscle groups with a strong excitatory flexor response and a small extensor response can be stimulated (Taylor, Todd, & Gandevia, 2006), therefore much of the research has involved elbow flexors muscles (Gandevia, 2001; Taylor et al., 2006). It should be noted that few experiments have validated this technique on lower limbs (Goodall et al., 2009; Sidhu et al., 2009), which is surprising given the importance of the lower limbs in common daily and competitive activities. Due to the limitations regarding the cortical voluntary activation technique and the other techniques (CAR and modified CAR), in this thesis (Study 2 and 4) voluntary activation was always measured by the superimposed twitch interpolation technique, as proposed by Merton (1954).

2.0.10 Electromyography

Skeletal muscle contraction produces force via excitation-contraction coupling, but also produces electrical activity. There are various techniques able to record the electrical of the muscle that involve both invasive and non-invasive methods. Invasive techniques require invasively monitoring the muscle by a needle or fine wire (intramuscular EMG). In the studies performed for this thesis, the electrical activity of the muscle was monitored non-invasively by means of surface EMG, which unlike the intramuscular EMG, permits a free execution of movements and does not imply any risk. During voluntary exercise, bursts of EMG are not always consistent and therefore the signal must be processed before being analysed. There are a wide number of approaches used to process the EMG signal, which generally vary according to the regime of muscle contraction or the purpose of the analysis. The EMG process used in these studies involved an automatic digital smoothing algorithm to obtain the root mean square EMG (EMG_{RMS}). In some circumstances it is also called quadratic mean and reflects the mean power of the signal, which is recommended for measuring muscle activation during muscular contractions (de Luca, 2010). EMG recording can also be used for other purposes and not only to monitor the electrical activity of the muscle. Recently integrated EMG signal (iEMG) from vastus lateralis has been

used to estimate changes in the level of central drive during cycling (Amann et al., 2008; Amann, Proctor, Sebranek, Pegelow, & Dempsey, 2009). However, it should be taken into account that many factors can influence EMG signal during exercise and so the validity of this parameter might be compromised and not reliable (Enoka & Stuart, 1992; Farina, Merletti, & Enoka, 2004).

In this thesis, electrical activity of the VL and BF muscles was recorded by means of surface EMG. A pair of electrodes (10 mm diameter, Swaromed, Ref. 1066; Nessler Medizintechnik) were placed on each muscle while the reference electrode was placed over the patella. Before placing each electrode, the skin was shaved and cleanec by using alcohol swabs. Electrode placement was marked with permanent ink in order to maintain the same placement across each experimental session. The electrical signal was recorded and digitalized with commercially available software (AcqKnowledge 4.2 for MP Systems; Biopac Systems). The signal was amplified with a bandwidth frequency ranging from 10 to 50 Hz at a sampling frequency of 2 kHz. As discussed previously, the stimulations of the cortical spinal tract or femoral nerve result in electrical responses called MEP and M-wave, respectively. Since the TMS response is recorded at a peripheral level, it is important to normalize MEP with Mwave in order to take in account peripheral influences that change the impedance during the EMG recordings.

2.0.11 Near-infrared spectroscopy

In this thesis, near-infrared spectroscopy (NIRS) was used to monitor changes in cerebral oxygenation of the left and right prefrontal cortex throughout exercise. NIRS has been shown to be one of the most used tools to non-invasively monitor continuous changes in oxygenation of the tissue investigated in vivo. The quantification of tissue oxygenation depends on the method of NIRS used. The most common equipment used in sport and exercise science have been developed to measure the relative saturation of Hb of the muscle (SmO_2), however for the purpose of this thesis a model able to monitor Hb of the brain was used (NIRS, Portamon, Artinis Medical Systems, Zetten, The Netherlands). Unlike invasive techniques involving microelectrodes on the muscle investigated, NIRS uses infrared light in the range of 700–900 nm, which penetrates the biological tissues to estimate tissue oxygenation (Hamaoka, McCully, Niwayama, & Chance, 2011). The quantification of regional oxygenation is made by the emission of wavelengths of near-infrared light that pass through the bone and brain tissues underneath the sensor (Fig 23). The wavelengths are transmitted from

the light source to cross the tissue and are then received by the sensors. The Hb molecules in the red blood cells have the highest absorption of light and therefore are able to indicate the amount of oxygen carried. According to the type and quantity of Hb absorbed from the light, an estimation of tissue saturation is calculated. The standard model used to accurately quantify oxyhaemoglobin (HbO_2) and de-oxyhaemoglobin (Hb) is based on the Beer–Lambert law (Delpy et al., 1988). The most common parameters obtained by the NIRS devices are the oxyhaemoglobin (HbO_2), deoxyhaemoglobin (Hb), total haemoglobin (tHb) difference between oxyhaemoglobin and deoxyhaemoglobin (Hbdiff) and tissue saturation index (TSI). Briefly, HbO_2 and Hb are obtained according to the different wave light absorption in relation to the content of Hb in the blood. tHB, Hbdiff and TSI are calculated as follows:

$$tHb = HbO_2 + HHb; \quad Hbdiff = HbO_2 - HHb; \quad TSI = \frac{HbO_2}{HbO_2 + Hb}$$

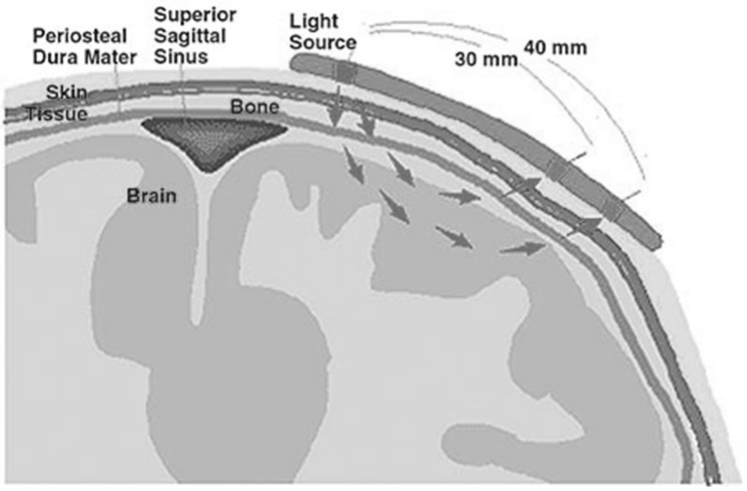


Fig 23. Illustration showing the passage of the near infrared light through the skull and brain tissue.

2.0.12 Measurement of hemodynamic parameters

The gold standard techniques for the measurement of cardiac output (CO) are the direct Fick and dye-dilution methods (Warburton, Haykowsky, Quinney,

Humen, & Teo, 1999) which are both invasive and potentially represent a risk for the volunteers during the test. In the second experiment performed for this thesis, the hemodynamic response was continuously monitored non-invasively by means of PhysioFlow (Manatec Biomedical, Paris, France), which estimates CO by applying the principles of impedance cardiometry. This technique is based on the model of electrical velocimetry, which monitors changes in the electrical signal caused by the velocity and speed of the blood flow in the aorta (Bernstein, 1986). Impedance cardiometry permits a wide variety of exercise that can be performed and thus reduces some of the methodological constraints of invasive measures. The measurement of hemodynamic parameters with PhysioFlow requires the placement of six electrodes on the thorax on the left side of the neck (Z1 and Z2), two on the chest (EKG1 and EKG2) and the last two placed on the back in at the same height of the xiphoid process (Z3 and Z4) (Fig 24).

The PhysioFlow has been previously demonstrated to be a reliable device to estimate CO in both resting condition and during high intensity whole body exercise (Charloux et al., 2000; Tordi, Mourot, Matusheski, & Hughson, 2004). In details the comparison compared to the direct Fick assessment in the study of Charloux et al., (2000) revealed a difference of 0.07 $l \cdot min^{-1}$ at rest and 0.26 $l \cdot min^{-1}$ during exercise. In this thesis, blood pressure measurements were performed using an automated blood pressure device (Tango$^{+\text{TM}}$, SunTech) specifically designed for whole body exercise. Use of the Tango involves the placement the inflatable blood pressure cuff on the left arm and two electrodes on the thorax respectively in position V2 and V6 and the reference electrode placed on RL according the international guidelines for ECG placement. Blood pressure is monitored by a microphone placed inside the cuff, able to detect the arterial pulse from the brachial artery. The Tango has been demonstrated to be a reliable device to measure arterial blood pressure and also during fatiguing exercise. (Cameron et al., 2004; Hartwich, Doreen, Dear, Waterfall, & Fisher, 2011; Pageaux et al., 2015). In details, Cameron et al., (2004) validated this device in both supine and treadmill exercise by reporting a difference of 3.68 mmHg and 6.33 mmHg respectively compared to invasive measurement of blood pressure.

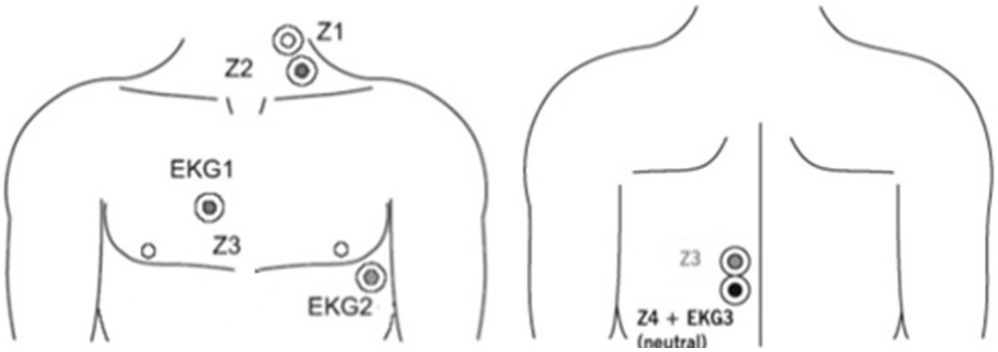


Fig 24. Placement for PhysioFlow electrodes in the thorax.

2.0.13 Measurement of afferent feedback from group III/IV muscle afferents

Cardiovascular reflex control by afferent nerves has been largely studied in research because of its importance for normal hemodynamic regulation and its implication in various chronic diseases (Piepoli & Crisafulli, 2014). In animal models, the activity of III and IV muscle afferents is monitored by means of laminectomy, where the exposition of the dorsal roots of spinal cord permits a direct measurement (Kaufman, 2012). Given the impossibility to apply this techniques in humans, the classic model proposed by Alam and Smirk (1937) involving post exercise muscle ischemia (PEMI) has been largely used to non-invasively monitor the contribution of muscle afferents in a various range of conditions and clinical populations (Boushel, 2010). PEMI arrests the circulation of the exercising limb immediately after exercise termination in order to keep the metabolites in the area and stimulate group III and IV muscle afferents. PEMI is generally maintained for 3 min while cardiovascular parameters are monitored. By maintaining the metabolites in the muscle milieu, peripheral afferents are still stimulated, but given that no exercise is performed, central command is absent and any variation of MAP is generally used as indirect index of activity of muscle afferents.

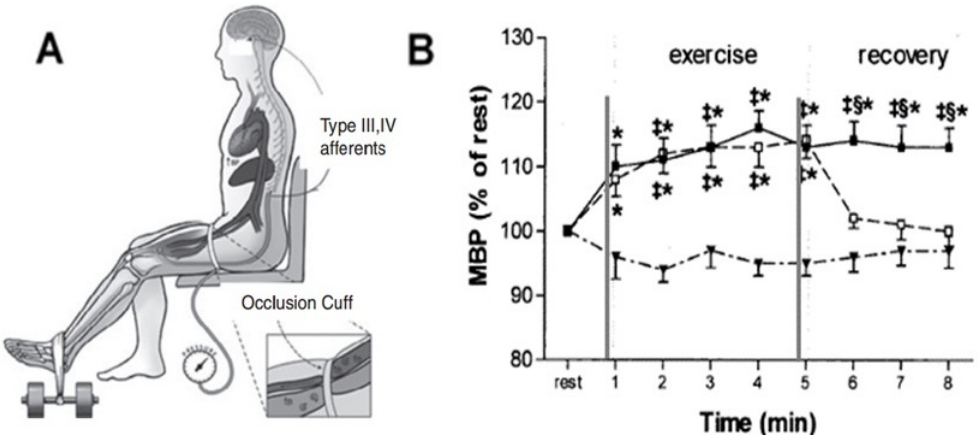


Fig 25. Illustration showing the classic method involving post exercise muscle ischemia to monitor the effect of group III and IV muscle afferents. From Alam & Smirk (1937) on panel A and Crisafulli et al., (2006) on panel B.

Panel A shows the execution of PEMI on lower limbs. Panel B shows the effect of group III & IV muscle afferents. Mean blood pressure (MBP) remained elevated during the PEMI protocol. Filled triangles refers to baseline condition, filled squares refer to PEMI condition, while empty circles refer to control condition

The typical response during PEMI is the maintained elevation of MAP despite exercise not being performed, which is immediately reduced once the circulatory occlusion is interrupted. This behaviour reflects the contribution of muscle afferents to cardiovascular regulation.

2.0.14 Metabolic measurements

In the studies reported in this thesis, gas collection was performed using an online gas analyzer (CORTEX Biophysik CPX system) and heart rate was measured via telemetry by using a commonly available commercial heart rate monitor (Polar Electro Oy, Kempele, Finland), with the transmitter placed on the chest. The reliability of this device has been tested by (Meyer, Georg, Becker, & Kindermann, 2001) by providing a reliability of 0.969 (VCO_2), 0.964 (VCO_2), and 0.953 (VE). The gas analyser used for this thesis has been reported to have the following technical specifications:

- Volume transducer: range: 0.1 – 12 l/s, resolution: 7ml, accuracy: 2%;
- O_2 analyzer: range: 0 – 35 % O_2, t90: 100 ms, accuracy: 0.1 Vol.%;
- CO_2 analyzer: range: 0 – 13 % CO_2, t90: 100 ms, accuracy: 0.1 Vol.%

- Temperature sensor: range: -55°C - +155°C, accuracy: 1° C;
- Pressure sensor Type: range: 200 – 1050 mbar, accuracy: 1.8%.

2.0.15 Measurement of perceptual parameters

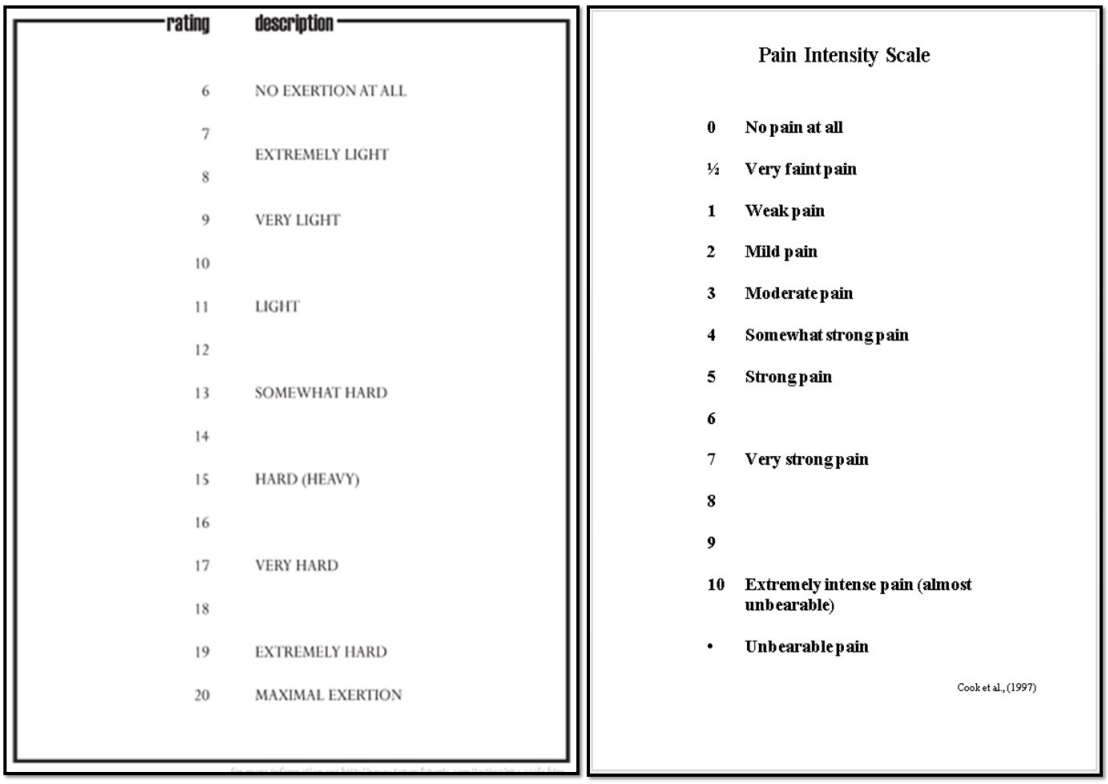
Any sensory signal must be centrally processed by the brain to become perception, and is therefore conscious (Mesulam & others, 1998). The same input might be processed differentially across each individual and is therefore highly subjective (Mesulam & others, 1998). Considering the subjective response and nature of these parameters, there is a great debate between researchers regarding how to objectively quantify perceptive parameters such as effort, emotions or pain. For the purpose of this thesis the most common perceptual parameters such as pain and RPE were monitored during exercise. Perception of effort was measured by using a common numerical scale, rating the magnitude of the effort perceived during exercise. In the experiments performed for this thesis, a fifteen point numerical scale first introduced by Borg (Borg, 1970; Gunnar Borg, 1998) was used. The 6-20 scale was originally designed according to the average heart rate response during an incremental maximal test, where 6 corresponds to 60 bpm and 20 to 200 bpm. The scale presents a list of numbers starting from 6 on the top left side up to 20 bottom left. On the right side of the scale there is list of words used to anchor the feeling perceived according to the corresponding number (see Fig 26). The RPE scale was originally developed to monitor the effort perceived during exercise, but recently it has also been used to monitor the effort perceived during the various types of training (session RPE) (Foster, 1998; Impellizzeri, Rampinini, Coutts, Sassi, & Marcora, 2004).

For the experiments performed for this thesis, participants were encouraged to use the verbal descriptors together with the numbers on the scale. When reporting their perception, participants were asked to rate 'how hard the exercise is' and therefore ignore their physiological responses or any other sensation during the test (Borg, 1998). The instructions provided were the following:

While doing physical activity, we want you to rate your perception of exertion. This feeling should reflect how heavy and strenuous the exercise feels to you, combining all sensations and feelings of physical stress, effort, and fatigue. Do not concern yourself with any one factor such as leg pain or shortness of breath, but try to focus on your total feeling of exertion.

Look at the rating scale below while you are engaging in an activity; it ranges from 6 to 20, where 6 means "no exertion at all" and 20 means "maximal exertion." Choose the number from below that best describes your level of exertion. This will give you a good idea of the intensity level of your activity, and you can use this information to speed up or slow down your movements to reach your desired range.

Try to appraise your feeling of exertion as honestly as possible, without thinking about what the actual physical load is. Your own feeling of effort and exertion is important, not how it compares to other people's. Look at the scales and the expressions and then give a number.



rating	description
6	NO EXERTION AT ALL
7	
8	EXTREMELY LIGHT
9	VERY LIGHT
10	
11	LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD (HEAVY)
16	
17	VERY HARD
18	
19	EXTREMELY HARD
20	MAXIMAL EXERTION

Figure 26. The 6-20 Borg scale on the left, from Borg (1998) and the 0-10 pain scale on the right. From (Cook et al., 1997).

In human experimental models, pain sensation can be measured both qualitatively (e.g. questionnaires), quantitatively (e.g. visual scales) and objectively by monitoring the relationship between stimulus response (e.g. evoked potentials).

There are many techniques used to monitor the level of pain (Olesen, Andresen, Staahl, & Drewes, 2012) or the pain threshold in a wide range of conditions or population type, however for the purpose of the experiments in this thesis a visual pain scale was used. Pain perception was originally measured by Borg (Borg, Ljunggren, & Ceci, 1985) using a visual 10 points numerical scale (CR10) in a group of 28 male participants. However, the authors did not provide any instructions for obtaining the ratings of pain during exercise, and moreover the values obtained in the experiments were very similar to the RPE values. A more accurate and reliable pain scale has been proposed by Cook (1997). This scale has been used in several experiments to quantify the level of exercise-induced muscle pain. Similarly to the RPE scale, this scale presents numbers corresponding to the magnitude of perceived pain, starting from 0 (no pain) on the top of the scale through to 10 (extremely intense pain) on the bottom. The description for each item is placed on the right of the number. The pain scale instructions used were the following:

The scale before you contains the numbers 0 to 10. You will use this scale to assess the perceptions of pain in your legs during the test. In this context, pain is defined as the intensity of hurt that you feel. Don't underestimate or overestimate the degree of hurt you feel, just try to estimate it as honestly and objectively as possible.

The numbers on scale represent a range of pain intensity from "very faint pain" (number ½) to "extremely intense pain-almost unbearable" (number 10). When you feel no pain in your legs, you should respond with the number zero. When the pain in your legs becomes just noticeable, you should respond with the number ½. If your legs feel extremely strong pain that is almost unbearable, you should respond with the number 10. If the pain is greater than 10 respond with the number that represents the pain intensity you feel in relation to 10. In other words, if the pain is twice as great then respond with the number 20.

Repeatedly during the test, you will be asked to rate the feelings of pain in your legs. When rating these pain sensations, be sure to attend only to the specific sensations in your legs and not report other pains you may be feeling.

It is very important that your ratings of pain intensity reflect only the degree of hurt you are feeling in your legs. Do not use your ratings as an expression of fatigue (i.e. inability of the muscle to produce force) or belief that the exercise task is completed.

In summary you'll be asked to: (a) provide pain intensity ratings in your legs only; (b) give ratings as accurately as possible; and (c) not under-or-over-

estimate the pain, but simply rate your pain honestly. You should use the verbal expressions to help rate your sensations.

In this thesis, the instructions for each scale were given at the beginning of each experimental session. Participants were fully familiarized during the first visit in order to help them to not overestimate or underestimate each parameter. This permitted a better precision of the measurement in the following experimental visits. Both perceptual parameters were regularly monitored during the execution of the task. Both scales were placed in front of the participant in order to be always available at any moment during each task.

CHAPTER 3

EXPERIMENTAL STUDY 1

The effect of transcranial direct current stimulation of the motor cortex on exercise-induced pain

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Abstract

Transcranial direct current stimulation (tDCS) provides a new exciting means to investigate the role of the brain during exercise. However, this technique is not widely used in exercise science, with little known regarding effective electrode montages. This study investigated whether tDCS of the motor cortex (M1) would elicit an analgesic response to exercise-induced pain (EIP). Nine participants completed a $\dot{V}O_{2max}$ test and three time to exhaustion (TTE) tasks on separate days following either 10 min 2 mA tDCS of the M1, a sham or a control. Additionally, seven participants completed 3 cold pressor tests (CPT) following the same experimental conditions (tDCS, SHAM, CON). Using a well-established tDCS protocol, tDCS was delivered by placing the anodal electrode above the left M1 with the cathodal electrode above dorsolateral right prefrontal cortex. Gas exchange, blood lactate, EIP and ratings of perceived exertion (RPE) were monitored during the TTE test. Perceived pain was recorded during the CPT. During the TTE, no significant differences in time to exhaustion, RPE or EIP were found between conditions. However, during the CPT, perceived pain was significantly ($P < 0.05$) reduced in the tDCS condition (7.4 ± 1.2) compared with both the CON (8.6 ± 1.0) and SHAM (8.4 ± 1.3) conditions.

Pain experienced during high intensity exercise is commonly believed to originate as a consequence of accumulation of muscle metabolites (e.g. H^+, potassium, lactate and prostaglandins), produced as a result of anaerobic resynthesis of ATP (O'Connor & Cook, 1999; Olesen et al., 2012). Peripheral muscle nociceptors that detect exercise-induced metabolites are generally classified as group III and IV muscle afferents. The contribution of exercise-induced pain to exercise performance has received little attention in experimental research (Mauger, 2013). However, the wider contribution of afferent feedback, which rises in proportion of the metabolic demand, combined with multiple psychological and physiological systems (Noakes, 2012; St Clair Gibson & Noakes, 2004), has created significant debate and complexity regarding the understanding of endurance performance. It is difficult to uncouple afferent feedback and pain, as both travel through Type III and IV afferents, which may explain the limited number of studies which focus solely on changes in pain during exercise. In an attempt to explicate the role of afferent feedback (i.e., not pain specifically) in both regulation of work rate in self-paced exercise (Amann et al., 2009) and time to exhaustion tasks (Amann, Blain, et al., 2011), a recent series of studies have used the opioid agonist fentanyl to prevent afferent feedback signals to reach cortical areas.

However, because afferent feedback plays an important role for cardiovascular regulation (Kaufman, 2012), performance in a time to exhaustion task was impaired (Amann, Blain, et al., 2011; Kaufman, 2012) and performance in time trial type tasks was no different (Amann, Blain, et al., 2011) after administration of fentanyl in these studies. Whilst the studies of Amann et al. (Amann, Blain, et al., 2011; Amann et al., 2009) demonstrate the importance of afferent feedback for cardiovascular regulation during exercise, they are not able to explain how pain contributes to performance. Concomitant with afferent feedback during intense exercise is the stimulation of muscle nociceptors and the subsequent perception of pain and discomfort. This exercise-induced pain has been suggested to play an important role in work rate selection and thus consequently affect endurance performance (Mauger, 2014; Mauger et al., 2010). However, as the sensation of pain during exercise is not only reliant on the noxious peripheral stimuli from skin and muscle nociceptors, but also the processing of this input in the primary sensorimotor cortex, secondary somatosensory cortex, anterior insular and cingulate cortex and thalamus (O'Connor & Cook, 1999; Olesen et al.,

2012), the effect of pain on endurance performance can be assessed by blocking the input or moderating the processing of it. Thus, many of the methodological difficulties associated with complete blockade of afferent feedback can be avoided or reduced. Several interventions that alter (i.e., increase or decrease) the sensation of pain at a peripheral level (moderating the pain signal before it reaches the brain) have been used to test this theory. These include: cuff occlusion of the exercising legs to increase pain (Hollander et al., 2010), administration of analgesics to reduce pain (Mauger, 2014; Mauger et al., 2010) and administration of algesic substances to increase pain (Khan, McNeil, Gandevia, & Taylor, 2011).

However, studies which investigate the role of pain by reducing/increasing feedback during exercise might still present some methodological constraints (Mauger, 2013). Therefore, methods, which solely alter the central processing of pain, would provide a useful means by which the pain performance relationship can be tested. In recent years, non-invasive modulation of cortical areas related to brain processing have been developed to relieve pain (Boggio, Zaghi, Lopes, & Fregni, 2008; Lefaucheur et al., 2008), and thus provide a targeted method of inducing analgesia during exercise. Transcranial direct current stimulation (tDCS) provides a reliable, safe, non-pharmacological and non-invasive way to alter excitability of a targeted brain area (Nitsche et al., 2008), and therefore moderate the manner in which a given area of the brain processes a stimulus. The benefits of this technique in the treatment of pain both in clinical populations and in healthy volunteers are well accepted (Boggio et al., 2008; Lefaucheur et al., 2008). However, because the processing of pain in the brain is complex, and will often depend on the type of pain experienced, the optimal tDCS electrode set-up for various types of pain is yet to be elucidated. Much of the tDCS pain research uses classical implementation of experimental pain (such as a cold pressor test) to assess analgesic efficacy, and for this type of pain, anodal tDCS of the M1 and cathodal over the contralateral prefrontal cortex proves most effective (Bachmann et al., 2010; Lefaucheur et al., 2008; Zandieh et al., 2013). In support of this M1 tDCS montage, studies which have monitored cerebral blood flow using positron emission tomography (PET) during motor cortex stimulation demonstrate that this stimulation indirectly effects pain areas such as thalamic and sub-thalamic nuclei (García-Larrea et al., 1997, 1999), and produces an overall analgesic effect. Consequently, as tDCS is only able to directly stimulate areas of the brain which are closer to the scalp, an electrode montage which stimulates the M1 may be able to indirectly moderate deeper brain areas involved in the processing of exercise-induced pain. Processing of pain arising from a CPT pri-

marily involves the thalamus, and specifically the ventral medial nucleus, which cortically projects to the insula and provides a specific network for the processing of thermal pain (Craig, Bushnell, Zhang, & Blomqvist, 1994).

However, there is also likely to be a significant level of psychological processing, involving arousal, attention, memory, emotion and evaluation in response to CPT pain (Chen et al., 1998; Craig et al., 1994), which will involve cross processing in a number of different brain areas. Although brain mapping of particular areas involved in exercise-induced pain processing is yet to be attempted, it has been suggested that the primary sensorimotor cortex, secondary somatosensory cortex, anterior insular and cingulate cortex and thalamus are all involved (O'Connor & Cook, 1999). When muscle pain has been experimentally induced, increased activation of the thalamus and basal ganglia has been reported (Peyron, Laurent, & García-Larrea, 2000; Svensson, Minoshima, Beydoun, Morrow, & Casey, 1997; Wardman, Gandevia, & Colebatch, 2014) showing an "overlap" of central processing of muscle pain and cold pain in the brain. Similarly to cold pain, because exercise also involves a multitude of other psychological processes, it is likely that mood, emotional and memory constructs also form an important part of EIP processing. Although tDCS of the M1 likely provides some analgesic effect to experimental pain, it should be recognized that moderation of a brain area may cause a number of secondary effects. As the M1 is involved in instigating muscle contraction, excitability changes in this area may elicit motor effects which may alter exercise performance. Whilst there appear to be some positive effect for tDCS stimulation of the M1 on fine movements in small muscle groups (Reis & Fritsch, 2011), its effect on exercise performance in the upper limbs remains equivocal (Cogiamanian et al., 2007; Lampropoulou & Nowicky, 2013).

There are currently no studies investigating the effect of tDCS stimulation of the M1 on exercise using the lower limbs. Therefore, the aims of the current study were (1) to monitor whether the effect of a well-established analgesic tDCS intervention could reduce pain perception during a fixed high intensity cycling task, and (2) whether tDCS induced analgesia would improve cycling time to exhaustion. As this tDCS intervention has been shown to reduce experimental pain, it was hypothesized that pain during exercise would be reduced and that this would consequentially improve cycling time to exhaustion.

Methods

Subjects. This investigation consisted of two separate studies (Part A and Part B). In the first study (Part A), 9 healthy recreationally active males (age: 23 ± 4 year, height: 179.7 ± 8.2 cm, mass: 75.4 ± 9.9 kg, $\dot{V}O_{2max}$: 48 ± 7 $mL \cdot min^{-1} \cdot kg^{-1}$) were recruited, while in the second study (Part B) 7 healthy recreationally active males (age: 23 ± 4 year, height: 179.7 ± 6.8 cm, weight: 75.11 ± 9.9 kg) were recruited. Six subjects participated in both studies. Each participant gave their written informed consent and was informed about the procedures of the study but not of the aims and hypothesis. Consent forms were approved by the School of Sport and Exercise Sciences local Ethics Committee (University of Kent). The present investigation was conducted according to the standards set by the World Medical Association (WMA) of Helsinki. None of the volunteers had any history of cardiac or respiratory disease or were taking any medication at the time of the study. Tests were conducted at the same time of the day for each volunteer in a temperature controlled room (20 °C, relative humidity 50 %). All participants refrained from intense exercise (48 h), alcohol (48 h), caffeine (6 h) and analgesic ingestion (6 h) prior to each visit.

Experimental design. Part A. Each participant visited the laboratory on 4 occasions, each separated by at least 48 h, but no more than 5 days. Visit 1. The purpose of this visit was to familiarize the participants with all the procedures performed during the experimental protocol. In the same visit, they performed an incremental test on a cycle ergometer (Lode, Excalibur Sport, Groningen, Netherlands) to establish maximal oxygen uptake ($\dot{V}O_{2max}$) and peak power output (W_{max}). Following a 30 min rest period, participants completed a familiarization of the same time to exhaustion task that would be completed in the experimental visits.

Visits 2–4. Using a double-blind and randomized according to balanced permutations design, participants underwent a control (CON), placebo (SHAM) and experimental (EXP) session. They underwent 10 min of tDCS administration in the experimental (EXP) and SHAM tDCS (SHAM) condition, respectively (see "transcranial direct current stimulation procedure"), while during the control condition, the participant was seated in a chair for 10 min. Two minutes after tDCS administration or control, participants performed a 5 min warm up at 100 W on the cycle ergometer, and then a time to exhaustion (TTE) at 70 % of W_{max} until they were unable to maintain their cadence above 60 rpm for more than

5 s. During the incremental test (visit 1) and TTE tests, respiratory variables were monitored by an automated gas analyser (Cortex Metalyser 3B, Cortex GmbH, Lepzig, Germany), and heart rate (HR) by a telemetric device (Polar, FS1, Birmingham, United Kingdom). A 20 μl capillary sample of whole blood was taken at rest and immediately at the end of the TTE by pricking the volunteers' right thumb, collected blood was subsequently analysed for lactate concentration (B[La^-]) by a laboratory lactate analyser (Super GL2, Dr. Müller Gerätebau, Germany). Rating of perceived exertion (RPE) was monitored during the TTE using Borg 6–20 scale (Borg, 1998). Exercise-induced pain perception during the TTE was assessed using the validated 10-point numerical Cook scale (Cook et al., 1997). RPE and pain were recorded at predetermined intervals (varying between 1 and 3 min) so that knowledge of elapsed time would not affect participants reporting of these values.

Part B. Each participant visited the laboratory on 4 occasions, each separated at least by 48 h, but not more than 5 days. Visit 1. The purpose of this visit was to familiarize the participants with the cold pressor test performed during the subsequent visits. Visits 2–4. Using a single-blind, randomized, counterbalanced design, participants underwent a control (CON), placebo (SHAM) and experimental (EXP) session. They underwent the same tDCS procedures performed in Part A (see "transcranial direct current stimulation procedure"). During these visits, participants underwent a cold pressor test (CPT) to investigate the effect of tDCS administration on pain perception and thus demonstrate that the tDCS set-up used in this study elicited an analgesic effect (manipulation check). Participants submerged their right hand into a container filled with iced water at a temperature between 0 and 1 °C, which was kept consistent between visits (± 0.1 °C). During the measurements, participants were required to circulate their hand around the water to prevent the development of a microclimate around the skin. After each elapsed minute, participants were asked to report their perception of pain on a 10-point numerical scale (Cook et al., 1997). They were told to withdraw their hand from the water when the pain became too much to tolerate. If the participant had not already withdrawn their hand from the water, the experimenter terminated the test after 8 min had elapsed to prevent cold-induced damage. The participants were not aware of the 8-min cut-off time. During the CPT task, the participants faced a plain wall, with the experimenter standing out of sight and offering no encouragement in order to prevent any experimenter bias.

Transcranial direct current stimulation procedure. tDCS was delivered by a direct current stimulator (TCT Research Limited, Hong Kong) using a pair of rubber electrodes in a 4 × 3 cm water-soaked synthetic sponge. One electrode (anodal) was placed over the left motor cortex (M1) whereas the other electrode (cathodal) was placed above dorsolateral right prefrontal cortex (Boggio et al., 2008; Zandieh et al., 2013). Electrode positioning was made according to the 10–20 system for EEG placement to replicate the exact position for both experiments. This electrode montage has been previously shown to elicit an analgesic effect to experimentally induced pain (Boggio et al., 2008; Zandieh et al., 2013). In the experimental session, the current was applied with an intensity of 2.0 mA for 10 min, whereas during the SHAM session stimulation lasted 30 s and subsequently ramped down to no stimulation. This induced the slight itching sensation which is commonly experienced during tDCS at the beginning of the stimulation, but has been shown to produce no cortical changes (Boggio et al., 2008; Mylius et al., 2012). Participants were blinded as to the polarity of tDCS and the SHAM and EXP conditions. Following the study, participants stated that they were unable to tell the difference between the EXP and SHAM conditions.

Statistical analysis

All data are presented as mean ± SD. An isotime of 6 min plus the final min for both the TTE and CPT were used to include all participants' data in the subsequent analyses. Furthermore, RPE and pain during TTE were analyzed by 0, 25, 50, 75 and 100 % of total time. Gas and HR were averaged for each min during the TTE. Time to exhaustion duration and B[La^-] were assessed by using one-way ANOVA with repeated measures. Analysis of gas data, HR, RPE, pain during TTE and CPT was performed by using two-way ANOVA with repeated measures, followed by Bonferroni post hoc when appropriate. Difference in pain perception during the last min between CPT and TTE was assessed by using an independent t test. The normality assumption was checked using the Kolmogorov–Smirnov test, homogeneity of variance for ANOVA was checked by Levene's test. The α level was set at $P < 0.05$. Statistics were calculated using SPSS version 20.

Results

All participants completed the experimental protocols and none of them reported any adverse effect during or after tDCS stimulation or cold pressor test. All participants at the beginning of the tDCS perceived a tingling sensation, but no participants could distinguish between the EXP and SHAM conditions.

Part A. There were no significant differences ($F_{(2,16)} = 3.26$, $P = 0.06$) in TTE time between EXP, SHAM and CON condition (16.58 ± 8.49; 14.68 ± 8.62; 18.22 ± 9.48 min, respectively). Pain and RPE increased during the TTE (main effect of time $P = 0.001$) but did not present any significant difference between the conditions at isotime ($F_{(2,12)} = 0.92$, $P = 0.47$ and $F_{(2,16)} = 0.81$, $P = 0.51$) or as percentage of total time ($F_{(2,12)} = 0.89$, $P = 0.48$ and $F_{(2,12)} = 0.27$, $P = 0.79$) (see Fig 27). Heart rate, $\dot{V}O_2$ and $\dot{V}e$ increased during the TTE (main effect of time, $P = 0.001$) but did not present any difference between conditions ($F_{(2,16)} = 0.35$, $P = 0.718$, $F_{(2,16)} = 0.81$, $P = 0.46$, $F_{(2,16)} = 1.24$, $P = 0.31$). Blood lactate collected after the TTE did not present any difference between the conditions ($F_{(2,12)} = 0.48$, $P = 0.62$.) (see Fig. 28).

Part B. Pain reported during the CPT increased over time (main effect of time $P = 0.001$) and was significantly lower in the tDCS condition compared to SHAM and CON (main effect of condition, $F_{(2,8)} = 5.68$, $P = 0.001$) while no difference in pain tolerance (time to remove hand) were found ($F_{(2,10)} = 3.18$, $P = 0.85$) (see Fig 27). The pain reported at the end of the CPT was significantly higher than the pain reported during the TTE ($P = 0.001$). In the CON condition, two participants reached the 8-min cut-off time, while in the tDCS and SHAM condition three participants reached the 8-min cut-off time.

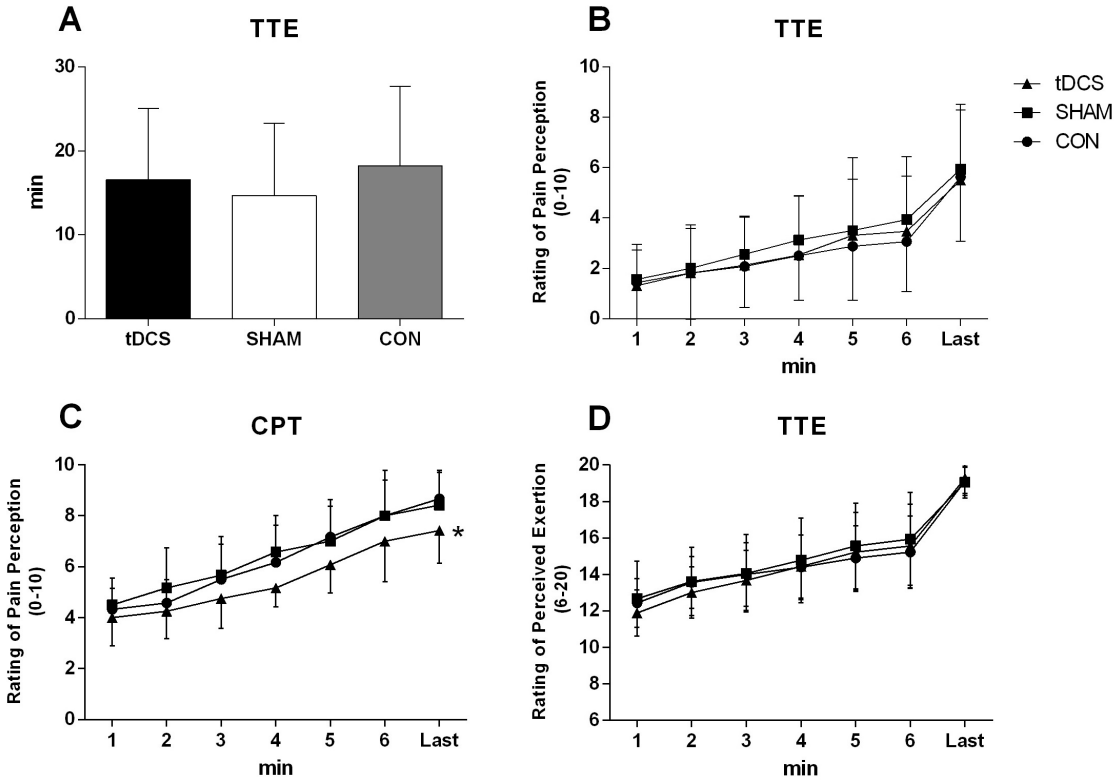

Fig 27. Performance results and perceptual response during exercise.

Panel A shows time to exhaustion (TTE) performance. Panel B shows time courses of pain perception during the time to exhaustion. Panel C shows time courses of pain perception during the cold pressor test (CPT) and rating of perceived exertion during time to exhaustion are shown in panel C. Values are presented as mean ± SD. *P < 0.05 shows a significant main effect of condition.

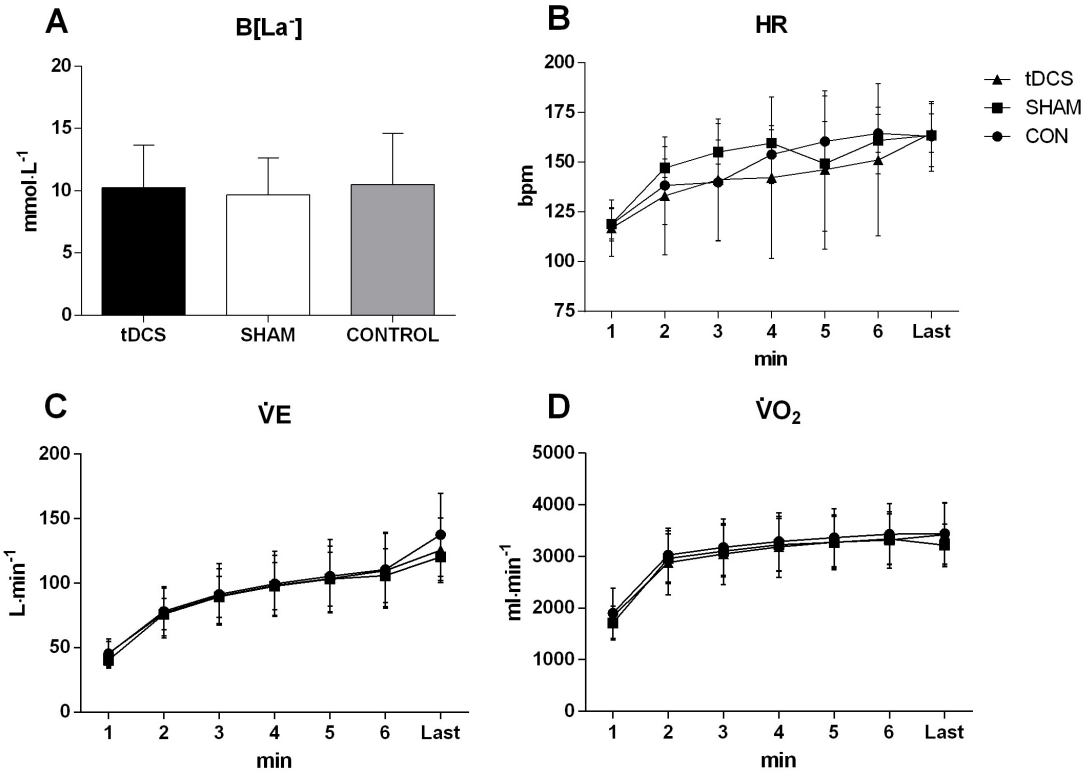

Fig 28. Overall metabolic response of all tests performed.

Panel A shows blood lactate values (B[La^-]) at exhaustion. Panel B shows time courses of heart rate (HR); Panel C shows time courses of pulmonary ventilation ($\dot{V}E$); Panel D shows time courses of pulmonary ventilation ($\dot{V}O_2$). Values are presented as mean ± SD.

Discussion

This is the first study to present data regarding tDCS M1 stimulation during whole-body exercise, and consequently provides important findings regarding the advancement for the use tDCS in exercise science. This experiment aimed to assess whether a recognized tDCS montage that has been shown to induce analgesia to experimental pain would lead to (1) a reduction in exercise-induced pain, and (2) an improvement in cycling time to exhaustion. The main findings of the current study demonstrate that anodal tDCS over the primary motor cortex reduced pain perception during a cold pressor test in healthy subjects, but did not change pain perception during a fixed high intensity cycling task. In the present study, pain perception after anodal tDCS of the M1 was lower during the

CPT compared to no stimulation (SHAM and CON conditions). This demonstrates that the tDCS intervention elicited an analgesic effect in response to the pain associated with cold thermal stimuli. These findings are in agreement with previous studies performed on healthy subjects where the tDCS intervention was able to evoke an analgesic effect during a cold pressor test (Zandieh et al., 2013) and in response to painful peripheral electrical stimulation (Boggio et al., 2008). This finding demonstrates a manipulation check for the intervention and that the established tDCS protocol used in this study did induce a central analgesic effect. However, while this form of analgesia moderated pain in the CPT, it did not affect pain perception during the exercise task. These findings demonstrate that the tDCS montage used in this study (anodal stimulation of M1, cathodal stimulation of the dorsolateral right prefrontal cortex) is not capable of producing an analgesic response to exercise-induced pain.

As the neural pathways from nociception to the brain, and the processing of the pain signal within the brain are highly complex and are related to the type of pain (e.g., thermal, pressure, metabolic, etc.) (Boggio et al., 2008; Millan, 2002), this suggests that whilst the M1 (and moderation of it) is, at least indirectly (García-Larrea et al., 1997, 1999), important in the processing of cold pain, it has a limited role in the processing of exercise-induced pain. Although not assessed in the current study, it is generally accepted that sensitivity to somatosensory inputs is reduced after cathodal tDCS administration to the motor and somatosensory cortex, probably because of the alteration of the resting membrane potential in the targeted area (Nitsche et al., 2008; Schestatsky, Simis, Freeman, Pascual-Leone, & Fregni, 2013). However, the analgesic effect observed in our study is unlikely to be caused by a reduction of activity in the somatosensory cortex, but rather through an alteration of the cold signaling pathway in the thalamus or insular cortex following anodal stimulation of the motor cortex (Zandieh et al., 2013). Indeed, investigations on animal models indicate an anatomical connection between the motor cortex with insula and thalamus (Schestatsky et al., 2013; Stepniewska, Preuss, & Kaas, 1994; Zandieh et al., 2013), and so the effect of tDCS may be extended to other brain regions distant from the targeted area (i.e., spatial effect) as previously hypothesized by Zandieh et al. (2013). Therefore, the tDCS-induced analgesia demonstrated in the current study could be due to an inhibition of the nociceptive center at the ventroposterior and medial thalamic nuclei via corticothalamic pathway, which would have a greater anti-nociceptive action for thermal pain signaling (Stepniewska et al., 1994; Zandieh et al., 2013).

With regard to the lack of analgesic effect of M1 tDCS on exercise-induced

pain, it has been shown that different populations of afferent fibers process cold and mechanical stimuli (Olesen et al., 2012). Therefore, whilst M1 tDCS stimulation reduces thermal and electrical pain, according to the results from the current study, stimulation of this brain area produces no such effect for exercise-induced pain. It has been suggested that the important areas for pain processing during exercise include the primary sensorimotor cortex, secondary somatosensory cortex, anterior insular and cingulate cortex and thalamus (O'Connor & Cook, 1999). tDCS stimulation of the M1 has been proposed to induce acute analgesia through a corticothalamic inhibition of epicritic (consistent with type III afferents) and nociceptive sensation at the VPL and VPM thalamic nuclei (Boggio et al., 2008). However, as skeletal muscle is more densely populated by type IV afferents, which are more consistent with a gradual build-up of pain which is dull, burning and aching in nature (Boggio et al., 2008; O'Connor & Cook, 1999), it may be that tDCS over the M1 elicits little analgesic effect to this type of pain. There is a strong emotional response to exercise-induced pain, which is likely important in its classification in terms of the unpleasantness. tDCS stimulation of the dorsolateral prefrontal cortex (DLPFC) has been shown to correlate negatively with the perception of pain (Lorenz, Minoshima, & Casey, 2003) and reduce the emotional response to pain (Boggio, Zaghi, & Fregni, 2009), likely through a modulation of brain structures including the anterior cingulate cortex, insula and amygdala. Consequently, future studies should use tDCS to moderate the DLPFC during exercise to assess its role in the processing of exercise-induced pain.

The pain arising from intense exercise presents a unique set of circumstances which makes its processing unique. Firstly, the pain arising from the CPT was rated as 'very strong pain' (Cook scale value of 7.4–8.6), whereas the rating for the TTE task was that of 'strong pain' (Cook scale value of 5.5–6). Therefore, it may be the case that the TTE task did not elicit levels of pain high enough for an analgesic effect to be detected. This may be in part due intense exercise stimulating the body's inherent analgesic system, including the release of endogenous opioids and growth factors, an activation of brain controlled supraspinal nociceptive inhibitory mechanisms and the release of catecholamines (Nijs, Kosek, Van Oosterwijck, & Meeus, 2012), all of which are likely to mitigate the strength of the pain signal reaching the brain, or the processing of it. Thus, the additive effect of tDCS may not supplement this already powerful natural analgesic response to exercise. Additionally, it is well known that one of the requisites of pain perception is the direct attention to the stimuli, and so distraction from

the pain sensation can reduce reporting of pain (Boggio et al., 2009; Linton & Shaw, 2011). So, it is likely that during the CPT participants focused solely on the nociceptive stimuli, while during the TTE, attention was more focused on the exercise task (Linton & Shaw, 2011). Subjective experience represents a significant portion component of pain processing (Linton & Shaw, 2011) and participants (although familiarized in this study) are not usually experienced with the unusual nociceptive stimuli which a CPT elicits. Consequently, participants may tend to report a higher rating of pain compared to experienced stimuli such as muscle pain.

In the current study, there was no improvement in TTE duration following tDCS compared to the SHAM and CON conditions. Because the tDCS intervention did not induce analgesia to exercise-induced pain, this lack of effect is to be expected. It has previously been suggested that exercise induced pain could moderate exercise intensity or pacing strategy, which may affect the final outcome of performance (Mauger, 2013, 2014; Mauger, Jones, & Williams, 2010). Accordingly, by reducing perceived pain or increasing pain threshold, an athlete should be able to improve their performance. Indeed, reducing pain during exercise through the ingestion of analgesic drugs has been previously investigated (Foster, Taylor, Chrismas, Watkins, & Mauger, 2014; Mauger et al., 2014, 2010), and shown to be effective in improving performance in TTE, time trial and repeated sprint exercise. However, although analgesia is the primary effect of these drugs, it should be acknowledged that the observed performance improvement in these studies could be due other mechanisms (Mauger et al., 2014; Mauger & Hopker, 2013). For example, acetaminophen (paracetamol) elicits an antipyretic effect (Mauger et al., 2014) and has been shown to increase corticospinal excitability (Mauger & Hopker, 2013). Consequently, there is a need for studies to use interventions which moderate the central processing of pain, rather than changing the strength of the nociceptive signal. The use of neurophysiological techniques such as tDCS provides a method which may allow a viable means of administering analgesia with fewer unwanted effects (Mauger, 2013), and the findings of the current study provides an important methodological advancement in developing these techniques for exercise interventions. Indeed, developing an appropriate study design that solely mitigates pain perception during exercise is challenging.

To date, there is only one study investigating the effect of the tCDS on cycling performance. Contrary to our findings, Okano et al. (2015) demonstrated that a tDCS intervention did induce some minor improvements in performance (~4 %

peak power achieved in incremental test). The effect of tDCS on isometric force endurance has been investigated in two further studies with equivocal results (Cogiamanian, Marceglia, Ardolino, Barbieri, & Priori, 2007; Muthalib et al., 2013). These studies applied tDCS over the M1 before completing an isometric force time to exhaustion of the elbow flexors. Whilst Cogiamanian et al. (2007) demonstrated an improved TTE performance, no effect was found by Muthalib et al. (2013). It has to be taken in consideration that many differences including experimental design, exercise task and tDCS stimulation may be the cause of the divergent findings of these and the current study. In Okano et al study, participants performed a maximal cycling incremental test, rather than a TTE. In addition, the tDCS intervention was different in terms of duration (i.e., 20 min), and location (left temporal cortex). As suggested by the authors, anodal tDCS administration over the left temporal cortex might induce some pleasant sensations causing a reduction of exercise discomfort and perception of effort during the initial phase of the task. Thus, the longer duration or different targeted area of the brain (i.e., anodal on the left temporal cortex with cathodal on the contralateral supraorbital area) used by Okano et al. might explain the difference in performance between this and the current study. A further finding by Okano et al. was the significant difference in HR following tDCS, an effect they attributed to an increase in parasympathetic activity induced by stimulation of the left temporal cortex.

In the present study, we found no differences in cardiorespiratory response between the conditions (see Fig 28). However, the tDCS montage used in the current study may explain why no differences were observed in this case. The use of a single electrode montage in the current study may have led to changes in the brain which resulted in unwanted effects. With this particular electrode montage, the anode increases excitability in the M1, whereas the cathode reduces excitability of the DLPFC. This particular montage was chosen because it has consistently been shown to reduce experimental pain (Boggio et al., 2009; Boggio et al., 2008; Zandieh et al., 2013). However, because the DLPFC is important for cognitive function and emotional processing, decreasing the cortical excitability of this area may have impacted on endurance performance. Therefore, any benefits following M1 stimulation may be negated by the DLPFC cathodal stimulation. Additionally, the unilateral tDCS set-up on the motor cortex might not be beneficial for whole-body exercise, as this brain area is only related to the contralateral limb. As such, we recommend that future research should use an extracephalic montage, with cathodes placed on a non-brain area (such as the

shoulder). Finally, it should be acknowledged that tDCS stimulation modulates cortical activity in a relatively larger area than that targeted by the electrodes, as demonstrated in neuroimaging studies (Lang et al., 2005). Thus, whilst this study focused specifically on increasing the excitability of the M1, it is possible that the stimulation may have migrated to adjacent brain areas, and so we cannot rule out the possible effects of this on the exercise task.

Conclusion and perspectives

This is the first study investigating the analgesic effect of M1 tDCS on perceived pain during time to exhaustion exercise. No change in exercise-induced pain was evident following the tDCS intervention, which suggests that the processing of exercise-induced pain is very different from that of experimental pain induced by cold thermal stimuli. This may be representative of the different brain regions used in processing these different types of pain. This study provides valuable methodological advancement in developing appropriate montages for using tDCS in exercise-based research, and the findings suggest that future work utilises a bi-cephalic tDCS montage. If the focus is to reduce exercise-induced pain, stimulation of the DLPFC area, instead of the M1, should be considered.

Given the possible negative effect of extracephalic tDCS montage, further experiments should be performed to provide an optimal tDCS electrode montage for exercise. Accordingly, the next study will aim to clarify the optimal electrode montage to improve endurance performance and to investigate the neurophysiological and psychological mechanisms following tDCS.

CHAPTER 4

EXPERIMENTAL STUDY 2

Transcranial direct current stimulation improves isometric time to exhaustion of the knee extensors

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Abstract

Recently, research studies have applied the use of transcranial direct current stimulation (tDCS) to manipulate corticospinal excitability in order to improve endurance performance. Since there is no consensus on the standard placement of electrodes for improving endurance performance, we therefore tested the effect of two electrode montages. Nine subjects underwent a control (CON), placebo (SHAM) and two different tDCS configurations sessions in a double-blind and randomised design. In one tDCS session, the anodal electrode was placed over the left M1 and the cathodal on contralateral forehead (HEAD) while for the other montage, the anodal electrode was placed over the left M1 and cathodal electrode above the contralateral shoulder (SHOULDER). tDCS was delivered for 10 min at 2.0 mA, after which participants performed an isometric time to exhaustion (TTE) of the right knee extensors at 20% of the maximal voluntary contraction (MVC). Peripheral and central parameters were examined at baseline, after tDCS application and immediately after TTE. Heart rate (HR), ratings of perceived exertion (RPE), and leg muscle PAIN were monitored during the TTE. None of the central and peripheral parameters showed any difference between conditions after tDCS stimulation ($P > 0.05$). MVC significantly decreased after TTE ($P < 0.05$) whilst motor evoked potential area (MEP) increased after TTE ($P < 0.05$) independently of the experimental condition. TTE was longer in the SHOULDER in the SHOULDER condition compared to the HEAD, SHAM and CON conditions (219$\pm$136 s, 191$\pm$124 s, 173$\pm$114 s and 187$\pm$121 s, respectively) although HR and PAIN did not present any difference between conditions ($P > 0.05$). However, RPE slope was significantly lower in the SHOULDER condition compared to the HEAD, SHAM and CON conditions (1.21 $\pm$ 0.61, 1.52 $\pm$ 0.54, 1.64 $\pm$ 0.72, 1.57 $\pm$ 0.62 respectively). Our findings suggest that SHOULDER montage is more effective than HEAD montage to improve endurance performance.

The first study performed for this thesis in Chapter 3 investigated the effect of a well-established tDCS montage to relive pain on high intensity cycling performance. One of the most important questions raised from the previous study is that the cephalic montage might not be beneficial for exercise. Accordingly, the application of the extracephalic montage was proposed to be necessary for exercise studies, as the effect of the cathodal electrode on the DLPFC had potentially negated the positive effect of anodal stimulation over the motor cortex (cephalic montage). Therefore, in this chapter the effect of cephalic and extracephalic montage on exercise performance was compared in single limb exercise, in order to provide a montage model for the subsequent studies.

Muscle fatigue has been defined as an exercise-induced reduction in the maximal force production of the muscle, and can occur at any site of the neuromuscular system (Gandevia, 2001). Failure to generate output from the motor cortex (M1) can result in reduced muscle force – this is termed supraspinal fatigue and can occur during exercise involving both isometric and dynamic contractions (Gandevia, 2001; Søgaard, Gandevia, Todd, Petersen, & Taylor, 2006; Taylor et al., 1996). This has been observed to develop from exercise onset and continues until exhaustion along with peripheral mechanisms (Gandevia, 2001; Søgaard et al., 2006; Taylor et al., 1996).

There is evidence to suggest that the descending output from the motor cortex is not adequate during fatiguing exercise (Gandevia, 2001; Liu, Dai, Sahgal, Brown, & Yue, 2002; Taylor et al., 1996). In the study of Søgaard and colleagues (2006) the superimposed twitch evoked by TMS over motor cortex increased until exhaustion, indicating a suboptimal output from the motor cortex. Furthermore in the study of Liu et al., (2002) functional magnetic resonance imaging (fMRI) revealed a significant reduction in brain activation during the last 60 s of a sustained (125 s) handgrip maximal voluntary contraction (MVC).

If a suboptimal output from the motor cortex contributes to supraspinal fatigue, then any intervention which moderates this reduction could plausibly improve exercise performance. Anodal transcranial direct current stimulation (tDCS) of the M1 has reliably been shown to increase cortical excitability, and so this procedure may have the potential to attenuate the development of supraspinal fatigue. Recently, a series of experiments investigating the effect of tDCS prior to exercise have been conducted. In several studies, exercise performance appeared

to improve following tDCS stimulation (Cogiamanian et al., 2007; Okano et al., 2015; Williams et al., 2013), however other studies reported no effect (see chapter 3; Kan, Dundas, & Nosaka, 2013; Lampropoulou & Nowicky, 2013; Muthalib, Kan, Nosaka, & Perrey, 2013).

Aside from the absence of a placebo control in many of the above studies, a notable methodological difference is the use of a cephalic or extracephalic electrode montage. A cephalic electrode montage involves placing the anodal electrode over the M1 (or main target area) and the cathodal electrode (i.e. reference) placed over the contralateral prefrontal area (see chapter 3; Okano et al., 2015; Williams et al., 2013). An extracephalic set up places the cathodal electrode over the shoulder (Cogiamanian et al., 2007; Kan et al., 2013; Lampropoulou & Nowicky, 2013; Muthalib et al., 2013), rather than the contralateral area of the head, as the tDCS anode increases excitability over the area that it is placed, whereas the cathode decreases excitability. Therefore, in the studies which used a cephalic montage (for example, see chapter 3), the unwanted effects of decreased excitability in the brain area under the cathode may have negated the positive effects of the anodal stimulation. Using an extracephalic montage may avoid this problem and explain why exercise performance differences tend to be more apparent in the studies that use this approach (Cogiamanian et al., 2007).

The literature supporting the use of tDCS to moderate exercise performance is limited, with methodological differences contributing to apparent discrepancy in their findings. There is also a dearth of literature detailing changes in neuromuscular parameters following tDCS and exercise. Therefore, the purpose of the present study was to examine the effect of a tDCS M1 cephalic and extracephalic electrode montage on lower limb isometric exercise. Using TMS and peripheral stimulation to quantify changes in neuromuscular parameters, the study aimed to clarify the optimal electrode montage to improve endurance performance and detail any neuromuscular changes that paralleled this.

Methods

Participants. Nine recreationally active males (mean ± SD; age = 23.3 ± 2.9 yr, height = 179.8 ± 7.7 cm, weight = 76.2 ± 9.7 kg) participated in the present study. None of the participants had any history of cardiorespiratory, metabolic or mental disorder/disease or was taking any medication at the time of the study. Each participant gave their written informed consent and was informed about the

procedures of the study but not of the aims and hypothesis. All experimental protocols and procedures were approved by the local ethics committee. All tests were conducted in a temperature-controlled room (20°C, relative humidity 50 %), within 2-5 days of each other and at the same time of the day for each participant.

Experimental design. Each participant visited the laboratory on five different occasions. During the first visit, participants were familiarized with the laboratory and all the experimental procedures. In the 4 subsequent visits, using a double-blind, crossover and randomized experimental design, all participants underwent a control (CON), placebo (SHAM) and cephalic (HEAD) and extracephalic (SHOULDER) testing session.

Endurance task. Participants performed a submaximal isometric time to exhaustion (TTE) task of the right knee extensor muscles at 20% of their maximal voluntary contraction (MVC). During the test each participant received visual feedback on a computer monitor showing the target force. The task terminated when their force went below the required target value for more than 3 s. None of the participants were aware of the time elapsed during the test. Results of all the sessions were provided only after the completion of all visits. Participants' perception of effort was measured using the 15-point RPE scale (Borg, 1998) every 20 s of the TTE task. Leg muscle pain was assessed every 20 s by using a 10 point numerical scale (Cook et al., 1997). Heart rate (HR) was monitored continuously and averaged for every 20 s elapsed.

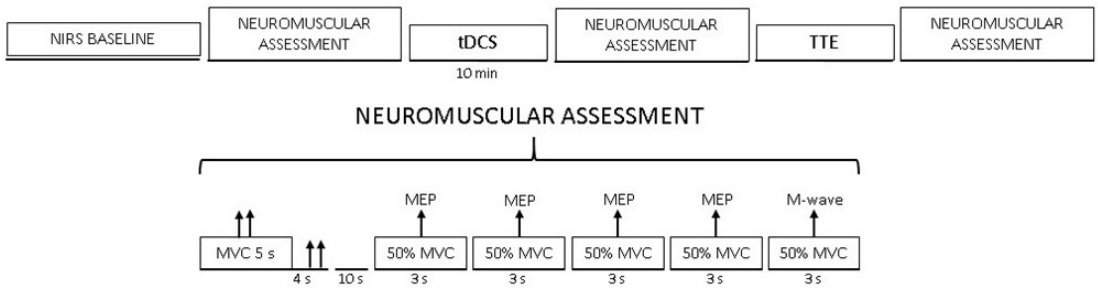

Fig 29. Overall view of the experimental protocol.

Maximal muscular wave (Mwave); motor evoked potential (MEP); maximal voluntary contraction (MVC); transcranial Direct Current Stimulation (tDCS); time to exhaustion (TTE).

Neuromuscular tests. After a brief, standardized warm-up with submaximal isometric contractions, all participants performed a 5 s MVC with superimposed

doublet stimulation, followed (4 s interval) by a resting potentiated doublet. The MVC produced during this test was used to calculate the participants 20% MVC used in the subsequent TTE task of that visit. Ten seconds after the MVC participants performed a series of four submaximal contractions at 50% of the MVC (3 s duration) with superimposed TMS and one with superimposed femoral stimulation. Each contraction was interspaced by 3 s. Neuromuscular assessment tests were performed prior to tDCS, post tDCS and immediately after the TTE task (see Fig 29).

Femoral nerve stimulation. TTranscutaneous electrically-evoked femoral nerve stimulation was delivered by using a high-voltage constant-current stimulator (model DS7 modified, Digitimer, Hertfordshire, UK). The femoral nerve was stimulated using a cathode surface electrode (Swaromed, Nessler Medizintechnik, Innsbruck, Austria) positioned over the femoral triangle while the anode electrode (Phoenix Healthcare Products Ltd., Nottingham, UK) was placed in the gluteal fold.

The stimulation intensity (mean current 288 ± 64 mA) was increased by 20 mA until the action potential (Mwave) demonstrated no further increase (Mmax) at rest and during submaximal 50% MVC contractions. The final intensity stimulation was then set at 130% M_{max}. Both Mmax and TMS intensities were determined at the beginning of each experimental session and was kept constant throughout that visit.

Mechanical recordings. All the experimental procedures were performed using on an isokinetic dynamometer (Cybex NORM isokinetic dynamometer, CMSi, Computer 267 Sports Medicine Inc., Stoughton, USA). All tests were performed with the right leg at a knee joint angle of 90° of flexion (0° = knee fully extended) and a hip angle of 90°. The set-up for each participant was recorded in the familiarisation session and kept constant in all subsequent visits. Mechanical signals were digitized on-line at a sampling frequency of 1 kHz using a computer, and stored for analysis with commercially available software (Acqknowledge 4.2 for MP Systems, Biopac Systems Inc., Goleta, USA).

Electromyographic recordings. Electromyography (EMG) of the vastus lateralis was recorded with two surface electrodes (Swaromed, Nessler Medizintechnik, Innsbruck, Austria) while the reference electrode was placed over the patella of the right knee. The skin was shaved and cleaned using alcohol swabs. Myoelectrical signals were amplified with a bandwidth frequency ranging from 10 Hz to 500 Hz (gain = 500), digitized on-line at a sampling frequency of 2 kHz using a computer, and stored for analysis with commercially available software

(Acqknowledge 4.2 for MP Systems, Biopac Systems Inc., Goleta, USA).

NIRS procedures. Brain oxygenation was monitored via near infrared spectroscopy using a portable device (Artinis, Zetten, The Netherlands). Two probes were placed on the left and right prefrontal cortex region of the forehead (Fp1 and Fp2, according to the international EEG 10-20 system) using a transmitter-receptor distance of 4 cm. NIRS data were recorded for four minutes at rest and were used as baseline. Subsequently, NIRS data were collected both during tDCS and the TTE task with a sampling frequency of 10 Hz.

Transcranial direct current stimulation (tDCS) procedure. Transcranial direct current stimulation was delivered by a direct current stimulator (TCT Research Limited, Hong Kong) using a pair of rubber electrodes in a 4x3 cm water-soaked synthetic sponge. Two different montages were used for the present investigation: 1) anodal placed over the left M1 with the cathodal placed above dorsolateral right prefrontal cortex (HEAD); 2) anodal placed over the left M1 with the cathodal was placed over the contralateral shoulder (SHOULDER). For the SHAM session, electrodes were placed in the same position for HEAD while in the control no electrodes were placed on the participant. During HEAD and SHOULDER sessions the current was applied with an intensity of 2.0 mA for 10 min, whereas during the SHAM session stimulation lasted 30 s and subsequently ramped down to no stimulation.

Data analysis. Peak force during the MVC of knee extensor muscles was considered as the peak torque attained during the MVC, while voluntary activation level (VAL) during the MVC was estimated according to the following formula:

$$VAL = 100 \cdot \left(1 - \frac{superimposed\ \ doublet\ \ amplitude}{potentiated\ \ doublet\ \ amplitude}\right)$$

The root mean square (RMS) of EMG was automatically calculated with the software and the peak-to-peak amplitude of the resting M-waves were calculated and averaged for the stimulations. The following parameters were also analysed: peak torque doublet, peak twitch.

EMG amplitude during the MVC was quantified as the RMS for a 0.5 s interval at peak torque (250 ms interval either side of the peak torque). The resting Mwave RMS (RMS_{MVC}/RMS_{Mwave}) then normalized maximal RMS (RMS_{MVC}) values, in order to take in account peripheral influences, including neuromuscular propagation and changes in impedance during the EMG recordings. The MEP area (MEP_{area}), was calculated and averaged for the four stimulations, and then normalized for the Mwave obtained during the 50% MVC contraction. MEP

amplitude ($MEP_{amp)}$, was calculated and averaged for the four stimulations, and then normalized for the $M_{max.}$ Cortical silent period (CSP) duration of the MEP was determined by the same experimenter from the onset of the MEP to the return of continuous EMG signal. Because of continuous measures, VL RMS was plotted as 0, 25, 50, 75 and 100 % of each TTE. 0% corresponded to the first 5s of the TTE while for 25, 50, 75 and 100 %, the signal was analysed and averaged for the last 5 s for each percentage.

NIRS data were averaged for the last 60 s during baseline measurement, while during tDCS administration NIRS data was averaged for the last 60 s every two min (i.e. min 2, 4, 6, 8 and 10). During exercise, data were averaged for 5 s respectively at the 0, 25, 50, 75 and 100 % of each TTE. The Beer-Lambert Law was used to calculate changes in tissue oxygenation. Relative concentration changes were measured from resting baseline for oxyhaemoglobin (ΔO_2Hb), deoxyhaemoglobin (ΔHHb), total haemoglobin (ΔtHb = O_2Hb + HHb) and haemoglobin difference (ΔHb diff = O_2Hb − HHb). ΔtHb was calculated to give an index of change in regional blood volume. Individual values of RPE, PAIN and HR obtained during the TTE were plotted against the absolute TTE time for each condition, and then the curve for each variable was mathematically fitted by a linear equation to obtain the slope.

Statistical analysis

All data are presented as mean ± SD. Assumptions of statistical tests such as normal distribution and sphericity of data were checked before running each individual statistical analysis. The effect of tDCS montage on TTE time and $B[La^-]$ were assessed by using one-way ANOVA with repeated measures. The same statistical analysis was performed to compare the slope of RPE, PAIN and HR obtained during the TTE. Fully repeated measures 4 x 3 way ANOVAs were used to test the effect of condition (HEAD, SHOUDLER, SHAM and CONTROL) and time (baseline, post-tDCS and post TTE) on MVC, VAL, Doublet, VL RMS during TTE, MEP_{area}/Mwave, and CSP. Three way 4 x 2 x 5 ANOVAs were used to test the effect of condition (HEAD, SHOUDLER, SHAM and CONTROL), prefrontal cortex side (left vs. right side) and time on ΔO_2Hb, ΔHHb, ΔHb diff, ΔtHb and TSI during tDCS stimulation. Three way 4 x 2 x 6 ANOVAs were used to test the effect of condition (HEAD, SHOUDLER, SHAM and CONTROL), prefrontal cortex side (left vs. right side) and time on ΔO_2Hb, ΔHHb,

ΔHb diff, ΔtHb and TSI obtained during the TTE. Bonferroni post hoc tests was used when appropriate. The α level was set at $P < 0.05$. Statistics were calculated using SPSS version 20.

Results

Performance and metabolic parameters. TTE was significantly longer ($F_{(3,24)} = 7.84$, $P < 0.001$) in the SHOULDER condition compared to the HEAD, SHAM and CON conditions (219±136 s, 191±124 s, 173±114 s and 187±121 s, respectively). This was accompanied by a significantly lower RPE slope in the SHOULDER condition ($F_{(3,24)} = 5.29$, $P < 0.006$). No significant differences between conditions were observed for $B[La^-]$ ($F_{(3,24)} = 0.06$, $P = 0.99$) or HR slope ($F_{(3,24)} = 0.031$, $P = 0.90$) or PAIN slope ($F_{(3,24)} = 0.50$, $P = 0.68$) (see Fig 30).

Neuromuscular parameters. MVC torque decreased significantly at exhaustion ($F_{(2,16)} = 24.85$, $P < 0.001$) but did not differ between conditions ($F_{(3,24)} = 0.68$, $P = 0.56$). RMS of VL increased over time ($F_{(3,24)} = 2.40$, $P < 0.001$) but did not differ between conditions ($F_{(3,24)} = 9.94$, $P = 0.38$) (see Fig 31)

Peripheral fatigue. Doublet amplitude decreased significantly only at exhaustion ($F_{(2,16)} = 36.92$, $P < 0.001$) but did not differ between conditions ($F_{(3,24)} = 0.70$, $P = 0.55$). Tw decreased only at exhaustion ($F_{(2,16)} = 36.92$, $P < 0.001$) but did not differ between conditions ($F_{(3,24)} = 0.70$, $P = 0.55$). Mamp at 50% MVC was significantly higher only at exhaustion ($P < 0.001$) but did not differ between conditions ($P = 0.28$). Marea at 50% MVC was significantly different only at exhaustion ($F_{(3,24)} = 10.21$, $P < 0.001$) but did not differ between conditions ($F_{(2,16)} = 2.14$, $P = 0.95$) (see Fig 31).

Central fatigue. Voluntary activation level decreased significantly only at exhaustion ($F_{(2,16)} = 15.27$, $P < 0.001$) but did not differ between conditions ($F_{(3,24)} = 1.19$, $P = 0.33$). RMS_{MVC}/RMS_{Mwave} of the vastus lateralis did not change over time ($F_{(2,16)} = 1.23$, $P = 0.85$) and did not differ between conditions ($F_{(3,24)} = 0.499$, $P = 0.68$) (see Fig 31).

Cortical excitability. MEP_{area} increased only at exhaustion ($F_{(2,16)} = 5.18$, $P = 0.018$) but did not differ between conditions ($F_{(3,24)} = 0.10$, $P = 0.96$). MEP_{area}/M_{area} ratio increased only at exhaustion ($F_{(2,16)} = 6.21$, $P < 0.01$) but did not differ between conditions ($F_{(3,24)} = 0.16$, $P = 0.91$). CSP increased only at exhaustion ($F_{(2,16)} = 5.48$, $P = 0.015$) but did not differ between conditions ($F_{(3,24)} = 0.87$, $P = 0.37$) (see Fig 31).

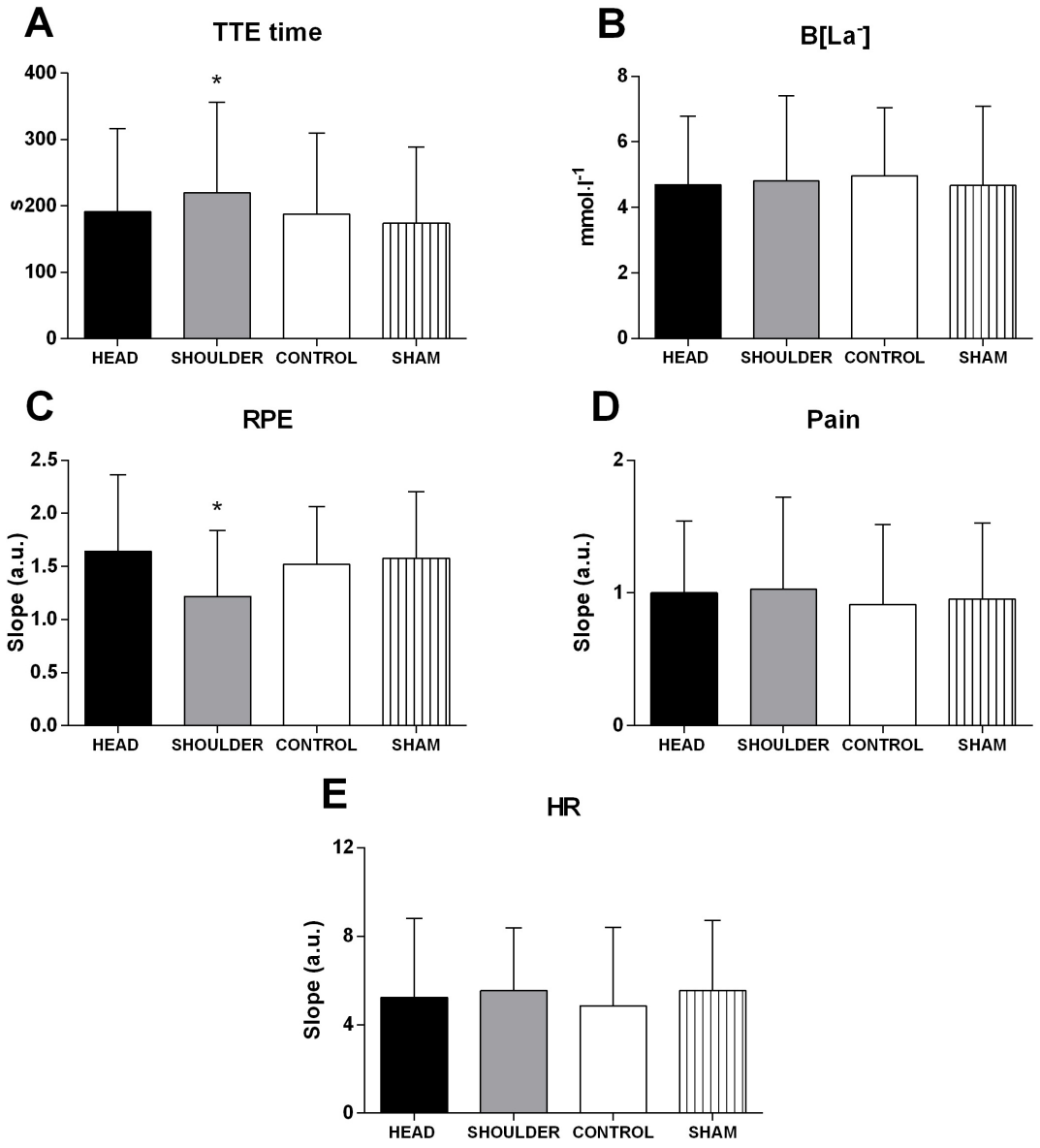

Fig 30. Physiological and perceptual response of all tests performed.

Panel A shows time to exhaustion (TTE) performance. Panel B shows blood lactate accumulation at exhaustion ($B[La^-]$). Panels C, D and E show respectively slope values of ratings of perceived exertion (RPE), muscle pain (Pain) and heart rate (HR) during exercise. *$P < 0.05$ significant from HEAD, CONTROL and SHAM. Data are presented as mean ± SD (n=9).

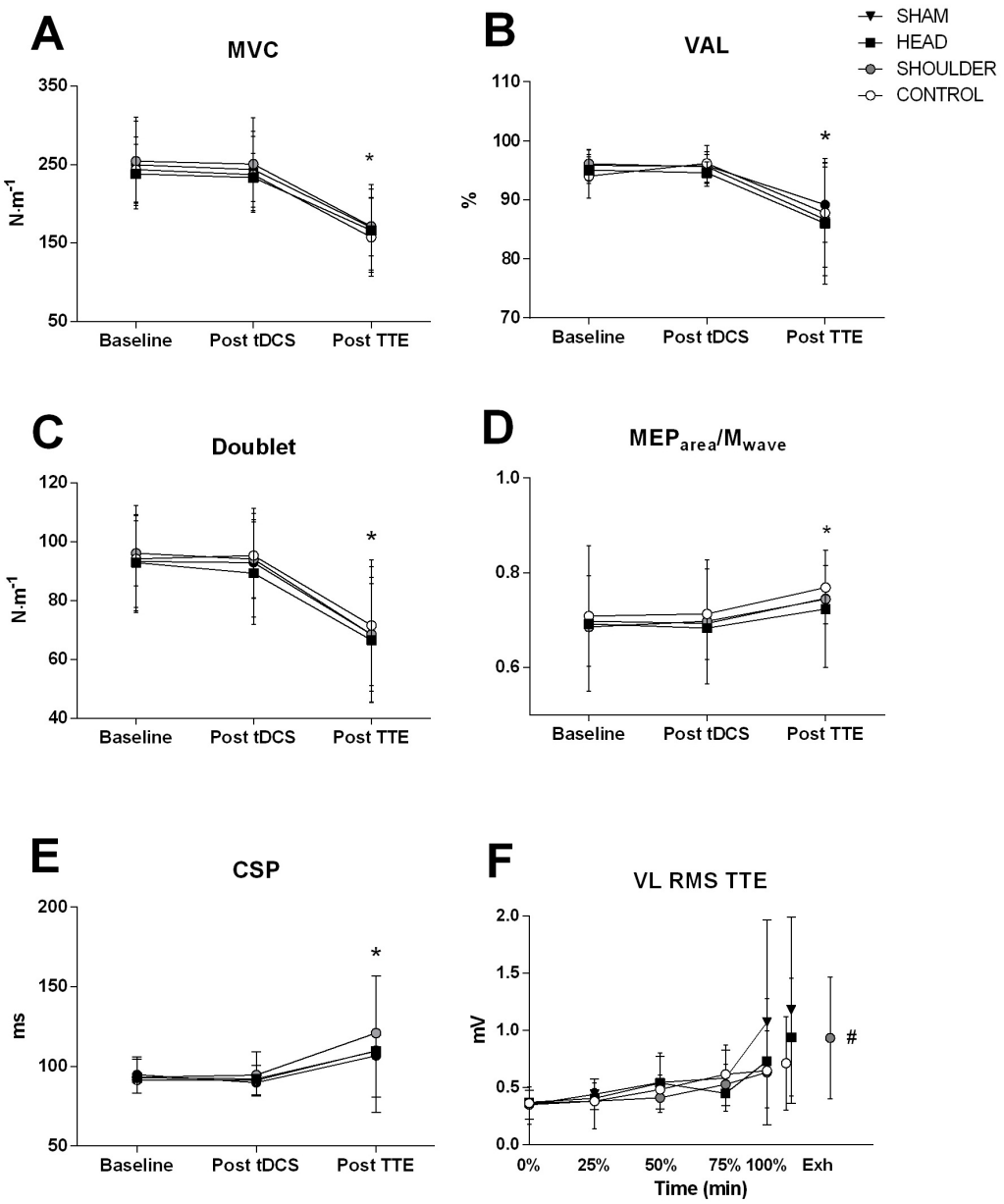

Fig 31. Overall response neuromuscular parameters during the various phases of the experiment.

Panel A Shows Maximal Voluntary Contraction (MVC); Panel B shows Voluntary Activation Level (VAL); Panel C shows peak torque of the Doublet; Panel D shows MEP_{area}/M_{wave} ratio; Panel E shows Cortical silent Period (CSP); Panel F shows root mean square of vastus lateralis (VL RMS) during time to exhaustion (TTE). * $P < 0.05$, significant from baseline and post tDCS; # $P < 0.05$, significant main effect of time. Data are presented as mean ± SD (n=9).

NIRS parameters during tDCS stimulation. ΔO_2Hb did not change over time ($F_{(4,32)} = 0.98$, $P = 0.42$) and no differences between conditions ($F_{(3,24)} = 0.30$, $P = 0.99$) or side ($F_{(1,8)} = 3.87$, $P = 0.85$) were found. ΔHHb did not change over time ($F_{(4,32)} = 0.92$, $P = 0.23$) and no differences between conditions ($F_{(3,24)} = 0.75$, $P = 0.39$) or side ($F_{(1,8)} = 0.62$, $P = 0.45$) were found. ΔtHb did not change over time ($F_{(4,32)} = 1.36$, $P = 0.77$) and no differences between conditions ($F_{(3,24)} = 0.29$, $P = 0.10$) or side ($F_{(1,8)} = 1.30$, $P = 0.28$) were found. ΔHbdiff did not change over time ($F_{(4,32)} = 2.58$, $P = 0.15$) and no differences between conditions ($F_{(3,24)} = 0.87$, $P = 0.32$) or side ($F_{(1,8)} = 0.02$, $P = 0.87$) were found. Tissue saturation index did not change over time ($F_{(4,28)} = 0.10$, $P = 0.63$) and no differences between conditions ($F_{(3,21)} = 0.83$, $P = 0.65$) or side ($F_{(1,7)} = 0.10$, $P = 0.75$) were found (see Table 2 and 3).

NIRS parameters during time to exhaustion. ΔO_2Hb increased over time ($F_{(5,40)} = 30.58$, $P < 0.001$) but no differences were observed between conditions ($F_{(3,24)} = 1.96$, $P = 0.24$) or side ($F_{(1,8)} = 0.04$, $P = 0.84$) were found. ΔHHb increased over time ($F_{(5,40)} = 38.11$, $P > 0.001$) and no differences between conditions ($F_{(3,24)} = 0.74$, $P = 0.43$) or side ($F_{(1,8)} = 2.88$, $P = 0.12$) were found. ΔtHb increased over time ($F_{(5,40)} = 21.13$, $P < 0.001$) and no differences between conditions ($F_{(3,24)} = 0.57$, $P = 0.55$) or side ($F_{(1,8)} = 1.14$, $P = 0.31$) were found. ΔHbDiff decreased over time ($F_{(5,40)} = 38.11$, $P < 0.001$) and no differences between conditions ($F_{(3,24)} = 0.74$, $P = 0.43$) or side ($F_{(1,8)} = 2.88$, $P = 0.12$) were found. Tissue saturation decreased over time ($F_{(5,40)} = 21.13$, $P < 0.003$) and no differences between conditions ($F_{(3,24)} = 0.57$, $P = 0.55$) or side ($F_{(1,8)} = 1.14$, $P = 0.31$) were found. (see tables 4-7).

Table 2. Changes of NIRS values from baseline in left and right prefrontal cortex during tDCS procedures in CONTROL and SHAM conditions.

	CONTROL					SHAM				
	min 2	**min 4**	**min 6**	**min 8**	**min 10**	**min 2**	**min 4**	**min 6**	**min 8**	**min 10**
					Left prefrontal cortex					
ΔTSI%	-0.10 ± 0.79	-0.08 ± 0.59	-0.14 ± 0.60	-0.48 ± 1.08	-0.27 ± 0.66	0.06 ± 2.18	0.06 ± 1.98	0.11 ± 1.96	0.25 ± 1.82	0.06 ± 1.74
ΔO_2Hb	-1.45 ± 6.14	-1.26 ± 5.95	-1.47 ± 5.80	-0.37 ± 5.14	-1.11 ± 5.72	-0.81 ± 3.42	-0.02 ± 3.81	-0.32 ± 3.66	-0.37 ± 3.70	-0.24 ± 3.68
ΔHHb	0.92 ± 3.21	0.97 ± 3.55	0.54 ± 3.05	0.68 ± 2.69	1.06 ± 2.30	1.01 ± 5.52	1.54 ± 5.79	1.16 ± 5.70	0.50 ± 5.78	0.57 ± 5.47
ΔtHb	-2.19 ± 4.56	-2.13 ± 4.52	-2.47 ± 4.39	-1.66 ± 2.54	-2.15 ± 4.21	1.19 ± 4.98	1.73 ± 5.10	1.49 ± 5.01	1.21 ± 5.39	1.10 ± 5.31
ΔHbDiff	0.52 ± 1.10	0.95 ± 1.33	0.84 ± 1.52	1.94 ± 3.09	1.19 ± 1.42	-1.37 ± 4.98	-0.55 ± 5.10	-0.85 ± 5.01	-0.90 ± 5.39	-0.84 ± 5.31
					Right prefrontal cortex					
ΔTSI%	0.40 ± 2.47	0.18 ± 2.26	0.24 ± 2.20	0.35 ± 2.08	0.23 ± 2.10	0.77 ± 2.36	0.59 ± 2.31	0.32 ± 2.31	0.35 ± 2.39	0.37 ± 2.53
ΔO_2Hb	0.68 ± 2.09	1.12 ± 2.70	0.75 ± 2.36	1.10 ± 2.78	1.36 ± 2.40	0.76 ± 5.53	1.33 ± 5.67	1.13 ± 5.45	0.90 ± 5.57	0.90 ± 5.38
ΔHHb	0.10 ± 1.5	0.00 ± 1.4	-0.15 ± 1.33	0.01 ± 1.26	0.04 ± 1.19	0.41 ± 1.29	0.49 ± 1.42	0.47 ± 1.48	0.41 ± 1.45	0.44 ± 1.30
ΔtHb	2.00 ±3.92	2.34 ± 4.42	1.82 ± 4.03	2.32 ± 4.29	2.62 ± 3.6	1.17 ± 6.17	1.82 ± 6.40	1.59 ± 6.24	1.34 ± 6.31	1.34 ± 5.98
ΔHbDiff	-0.33 ± 0.69	0.21 ± 1.98	0.00 ± 1.73	0.19 ± 1.71	0.42 ± 1.63	0.36 ± 5.14	0.84 ± 5.22	0.66 ± 4.98	0.51 ± 5.14	0.46 ± 5.06

Tissue saturation index (ΔTSI), oxyhaemoglobin (ΔO_2Hb), deoxyhaemoglobin (ΔHHb), total haemoglobin (ΔtHb) and haemoglobin difference (ΔHbDiff) during tDCS stimulation. *P < 0.05, significant main effect of time. Data are presented as means ± SD.

Table 3. Changes of NIRS values from baseline in left and right prefrontal cortex during tDCS procedures in HEAD and SHOULDER conditions.

	HEAD					SHOULDER				
	min 2	**min 4**	**min 6**	**min 8**	**min 10**	**min 2**	**min 4**	**min 6**	**min 8**	**min 10**
Left prefrontal cortex										
ΔTSI%	-0.89 ± 2.85	-0.50 ± 3.05	-0.22 ± 3.38	-0.24 ± 3.43	-0.24 ± 3.36	0.39 ± 2.75	0.31 ± 2.70	0.31 ± 2.91	0.27 ± 2.93	0.41 ± 2.53
ΔO_2Hb	-0.55 ± 4.42	-1.24 ± 5.57	-1.63 ± 7.18	-1.53 ± 7.30	-1.41 ± 7.36	-0.84 ± 5.33	-0.70 ± 5.10	-0.62 ± 5.05	-0.46 ± 5.16	0.62 ± 6.56
ΔHHb	1.63 ± 2.97	1.71 ± 4.16	1.85 ± 5.10	2.19 ± 5.23	2.94 ± 5.91	-1.98 ± 4.83	-1.57 ± 5.35	-1.42 ± 5.51	-1.28 ± 6.06	0.84 ± 9.73
ΔtHb	-0.15 ± 3.52	-0.79 ± 6.92	-1.73 ± 9.81	-1.56 ± 10.11	-1.14 ± 10.28	-2.24 ± 5.30	-1.21 ± 5.85	-1.58 ± 5.65	-1.39 ± 6.20	-0.95 ± 6.57
ΔHbDiff	0.42 ± 4.17	0.14 ± 4.39	-0.08 ± 5.01	-0.02 ± 5.10	-0.01 ± 5.15	-1.73 ± 5.30	-1.46 ± 5.32	-1.50 ± 5.36	-1.29 ± 5.55	-0.32 ± 6.53
Right prefrontal cortex										
ΔTSI%	0.71 ± 1.48	0.72 ± 1.52	1.34 ± 2.19	1.21 ± 2.79	1.28 ± 2.70	-1.29 ± 2.08	-1.45 ± 1.97	-1.35 ± 1.82	-1.13 ± 1.97	-0.70 ± 2.53
ΔO_2Hb	0.40 ± 4.31	0.81 ± 5.01	0.62 ± 6.23	0.85 ± 6.59	1.24 ± 7.45	-0.24 ± 6.53	0.18 ± 6.70	0.13 ± 6.67	0.31 ± 7.12	0.60 ± 7.32
ΔHHb	0.59 ± 2.37	0.34 ± 2.69	0.08 ± 3.33	-0.03 ± 3.64	0.36 ± 3.67	0.50 ± 1.37	0.29 ± 1.33	0.45 ± 1.49	0.43 ± 1.54	0.40 ± 1.72
ΔtHb	0.29 ± 6.19	0.42 ± 7.12	0.11 ± 9.04	0.27 ± 9.73	0.80 ± 10.62	-1.38 ± 5.57	-1.09 ± 5.69	-0.98 ± 5.82	-0.78 ± 6.35	1.34 ± 9.78
ΔHbDiff	1.09 ± 3.17	1.59 ± 3.72	1.54 ± 4.24	1.75 ± 4.29	2.40 ± 5.00	-2.39 ± 5.16	-1.66 ± 5.54	-1.88 ± 5.45	-1.65 ± 6.04	-1.35 ± 6.38

Tissue saturation index (ΔTSI), oxyhaemoglobin (ΔO_2Hb), deoxyhaemoglobin (ΔHHb), total haemoglobin (ΔtHb) and haemoglobin difference (ΔHbDiff) during tDCS stimulation. *P < 0.05, significant main effect of time. Data are presented as means ± SD (n=9).

Table 4. Changes of NIRS values from baseline in left and right prefrontal cortex during the isometric time to exhaustion in the CONTROL condition.

	CONTROL					
	0%	**25%**	**50%**	**75%**	**100%**	**EXH**
	Left prefrontal cortex					
ΔTSI%	-0.20 ± 0.90*	-0.81 ± 0.93*	-1.58 ± 1.42*	-1.81 ± 1.26*	-2.29 ± 1.63*	-2.48 ± 1.75*
ΔO_2Hb	7.20 ± 5.59*	9.69 ± 6.20*	11.50 ± 7.92*	13.66 ± 7.22*	15.24 ± 8.06*	15.74 ± 9.68*
ΔHHb	1.44 ± 1.60*	1.19 ± 1.52*	0.35 ± 1.49*	0.57 ± 1.71*	1.16 ± 1.84*	1.57 ± 2.19*
ΔtHb	8.65 ± 5.37*	10.88 ± 6.30*	11.84 ± 7.99*	14.23 ± 6.98*	16.40 ± 8.15*	17.31 ± 10.15*
ΔHbDiff	5.76 ± 6.24*	8.50 ± 6.47*	11.15 ± 8.14*	13.09 ± 7.83*	14.08 ± 8.38*	14.16 ± 9.75*
	Right prefrontal cortex					
ΔTSI%	0.13 ± 2.50*	-0.62 ± 2.63*	-0.83 ± 2.30*	-1.40 ± 2.80*	-2.80 ± 4.41*	-3.56 ± 4.57*
ΔO_2Hb	5.12 ± 5.72*	8.61 ± 7.17*	10.22 ± 9.83*	16.09 ± 10.41*	16.44 ± 8.80*	16.33 ± 9.00*
ΔHHb	0.66 ± 2.99*	0.37 ± 2.98*	-0.60 ± 3.54*	0.13 ± 3.00*	-0.08 ± 2.79*	-0.26 ± 2.91*
ΔtHb	5.78 ± 7.58*	8.99 ± 8.97*	9.62 ± 12.05*	16.22 ± 11.89*	16.36 ± 9.83*	16.07 ± 9.94*
ΔHbDiff	4.45 ± 5.09*	8.24 ± 6.34*	10.81 ± 8.55*	15.97 ± 9.67*	16.53 ± 8.60*	16.59 ± 8.95*

Tissue saturation index (ΔTSI), oxyhaemoglobin (ΔO2Hb), deoxyhaemoglobin (ΔHHb), total haemoglobin (ΔtHb) and haemoglobin difference (ΔHbDiff) during tDCS stimulation. *P < 0.05, significant main effect of time. Data are presented as means ± SD (n=9).

Table 5. Changes of NIRS values from baseline in left and right prefrontal cortex during the isometric time to exhaustion in the SHAM condition.

	SHAM					
	0%	**25%**	**50%**	**75%**	**100%**	**EXH**
			Left prefrontal cortex			
ΔTSI%	-3.26 ± 2.25*	-3.37 ± 2.77*	-3.73 ± 2.87*	-5.36 ± 2.41*	-5.13 ± 3.81*	-5.50 ± 3.49*
ΔO_2Hb	6.75 ± 7.31*	7.29 ± 6.54*	8.80 ± 7.09*	12.38 ± 6.52*	13.87 ± 7.39*	14.04 ± 7.90*
ΔHHb	2.24 ± 1.94*	1.40 ± 1.88*	1.17 ± 2.69*	0.87 ± 2.61*	1.12 ± 3.29*	1.28 ± 3.12*
ΔtHb	7.16 ± 5.64*	6.92 ± 5.14*	8.60 ± 6.45*	11.79 ± 7.46*	14.26 ± 9.28*	14.59 ± 9.50*
ΔHbDiff	2.69 ± 6.03*	4.12 ± 4.88*	6.27 ± 6.35*	10.04 ± 5.78*	12.03 ± 5.95*	12.03 ± 6.71*
			Right prefrontal cortex			
ΔTSI%	-1.74 ± 2.20*	-1.61 ± 2.21*	-1.92 ± 2.59*	-3.69 ± 3.54*	-4.23 ± 4.91*	-4.56 ± 6.70*
ΔO_2Hb	3.51 ± 5.32*	3.70 ± 5.41*	4.96 ± 5.95*	10.20 ± 7.60*	12.13 ± 6.05*	11.89 ± 6.53*
ΔHHb	1.01 ± 1.84*	0.16 ± 1.40*	-0.25 ± 1.65*	-0.20 ± 2.21**	-0.62 ± 2.46*	-0.59 ± 2.41*
ΔtHb	3.96 ± 4.26*	3.23 ± 4.55*	4.52 ± 5.35*	9.63 ± 8.56*	11.54 ± 6.68*	11.33 ± 7.07*
ΔHbDiff	4.82 ± 8.43*	5.81 ± 6.86*	7.91 ± 8.25*	12.92 ± 6.87*	15.67 ± 6.86*	15.39 ± 7.07*

Tissue saturation index (ΔTSI), oxyhaemoglobin (ΔO2Hb), deoxyhaemoglobin (ΔHHb), total haemoglobin (ΔtHb) and haemoglobin difference (ΔHbDiff) during tDCS stimulation. *P < 0.05, significant main effect of time. Data are presented as means ± SD (n=9).

Table 6. Changes of NIRS values from baseline in left and right prefrontal cortex during the isometric time to exhaustion in the HEAD condition.

	HEAD					
	0%	**25%**	**50%**	**75%**	**100%**	**EXH**
			Left prefrontal cortex			
ΔTSI%	-1.39 ± 1.97*	-1.68 ± 1.89*	-2.14 ± 2.16*	-2.58 ± 2.86*	-2.52 ± 2.73*	-3.10 ± 3.05*
ΔO_2Hb	1.96 ± 5.32*	3.75 ± 5.41*	7.19 ± 4.69*	7.37 ± 5.83*	10.56 ± 6.02*	10.54 ± 6.32*
ΔHHb	1.33 ± 3.06*	0.73 ± 2.51*	0.07 ± 2.59*	-0.02 ± 2.63*	0.19 ± 2.16*	-0.10 ± 3.94*
ΔtHb	5.52 ± 10.34*	6.70 ± 9.43*	9.48 ± 9.47*	10.69 ± 10.13*	12.96 ± 9.08*	12.67 ± 10.43*
ΔHbDiff	2.85 ± 7.71*	5.24 ± 6.71*	9.34 ± 5.80*	10.72 ± 6.07*	12.59 ± 6.39*	12.86 ± 6.63*
			Right prefrontal cortex			
ΔTSI%	-0.45 ± 3.48*	-0.39 ± 3.10*	-0.61 ± 3.41*	-1.14 ± 3.44*	-0.72 ± 4.38*	-1.11 ± 4*.
ΔO_2Hb	1.89 ± 8.15*	3.34 ± 7.48*	7.36 ± 6.36*	10.30 ± 6.23*	12.61 ± 5.06*	12.99 ± 9.22*
ΔHHb	0.64 ± 1.50*	0.04 ± 1.76*	-0.55 ± 2.23*	-0.74 ± 2.96*	-0.66 ± 2.56*	-1.49 ± 4.84*
ΔtHb	4.20 ± 8.10*	5.05 ± 7.60*	8.48 ± 6.10*	11.23 ± 6.36*	13.62 ± 6.59*	13.17 ± 12.58*
ΔHbDiff	-2.64 ± 8.18*	-0.59 ± 7.46*	4.02 ± 6.51*	7.14 ± 6.49*	9.38 ± 7.31*	10.59 ± 7.33*

Tissue saturation index (ΔTSI), oxyhaemoglobin (ΔO2Hb), deoxyhaemoglobin (ΔHHb), total haemoglobin (ΔtHb) and haemoglobin difference (ΔHbDiff) during tDCS stimulation. *P < 0.05, significant main effect of time. Data are presented as means ± SD (n=9).

Table 7. Changes of NIRS values from baseline in left and right prefrontal cortex during the isometric time to exhaustion in the SHOULDER condition.

	SHOULDER					
	0%	**25%**	**50%**	**75%**	**100%**	**EXH**
			Left prefrontal cortex			
ΔTSI%	0.17 ± 2.34*	0.06 ± 2.39*	0.12 ± 2.46*	-0.42 ± 2.83*	-0.45 ± 2.98*	-0.16 ± 2.97*
ΔO_2Hb	-0.29 ± 4.91*	2.00 ± 4.89*	5.78 ± 6.62*	4.84 ± 6.64*	6.30 ± 5.37*	6.48 ± 7.41*
ΔHHb	2.43 ± 1.74*	2.17 ± 1.96*	1.53 ± 2.78*	1.04 ± 3.31*	1.08 ± 2.93*	1.49 ± 3.48*
ΔtHb	8.81 ± 9.02*	10.83 ± 9.75*	13.98 ± 12.35*	12.55 ± 10.53*	14.04 ± 10.05*	14.64 ± 9.47*
ΔHbDiff	6.08 ± 4.86*	8.62 ± 4.89*	13.04 ± 6.36*	12.59 ± 6.77*	14.01 ± 5.84*	13.78 ± 6.16*
			Right prefrontal cortex			
ΔTSI%	-2.17 ± 8.46*	-2.05 ± 9.57*	-2.29 ± 11.29*	-2.92 ± 12.05*	-4.00 ± 12.59*	-4.54 ± 14.02*
ΔO_2Hb	0.33 ± 6.34*	2.63 ± 5.78*	6.45 ± 6.26*	8.66 ± 8.62*	11.51 ± 9.19*	11.23 ± 11.89*
ΔHHb	0.94 ± 4.59*	0.68 ± 3.81*	-0.11 ± 4.33*	-0.03 ± 4.45*	-0.01 ± 4.25*	0.31 ± 4.59*
ΔtHb	4.32 ± 8.69*	6.36 ± 7.28*	9.41 ± 9.61*	11.80 ± 8.02*	14.61 ± 8.82*	14.66 ± 9.11*
ΔHbDiff	4.79 ± 7.71*	7.34 ± 8.22*	11.97 ± 10.54*	14.20 ± 9.14*	16.99 ± 10.01*	16.39 ± 7.67*

Tissue saturation index (ΔTSI), oxyhaemoglobin (ΔO2Hb), deoxyhaemoglobin (ΔHHb), total haemoglobin (ΔtHb) and haemoglobin difference (ΔHbDiff) during tDCS stimulation. *P < 0.05, significant main effect of time. Data are presented as means ± SD (n=9).

Discussion

This is the first study showing an improvement of isometric TTE performance of the lower limbs after tDCS stimulation and further demonstrates that anodal tDCS over the M1 improves isometric endurance performance of the knee extensors. Our findings suggest that in order to improve lower limb endurance performance, an extracephalic electrode montage is more effective than cephalic montage.

Effect of tDCS on time to exhaustion performance and perceptual parameters

This study showed for the first time that only anodal tDCS stimulation with extracephalic montage improves isometric time to exhaustion performance of knee extensors. Following tDCS, an improvement in isometric endurance performance has been previously demonstrated in elbow flexor muscles (Cogiamanian et al., 2007; Williams et al., 2013) and these authors associated the improvement in performance with an augmented cortical excitability of the motor, premotor and somatosensory area and the consequent enhanced descending drive to the motoneuronal pool. However, it is important to note that two other studies showed no improvement in isometric performance following tDCS (Kan et al., 2013; Muthalib et al., 2013) which might be a consequence of different experimental designs.

In the current experiment, time to exhaustion performance was longer in the SHOULDER condition, where the anode was placed on the M1, with the cathode placed on the contralateral shoulder (thus avoiding any decreased excitability induced by the cathode). Perception of effort during the TTE task was significantly lower only in the SHOULDER condition, and therefore may explain the improvement in performance. It has previously been demonstrated that during prolonged sustained exercise, the increase in RPE reflects the augmented central motor drive to the motoneuronal pool to compensate the decline in force generating capacity of the muscle (Gandevia, 2001; Marcora et al., 2008; Søgaard et al., 2006). The relationship between RPE and central command has been previously demonstrated, providing strong evidence regarding the neurophysiological generation of RPE (de Morree et al., 2012; de Morree, Klein, & Marcora, 2014; Lafargue & Sirigu, 2006; Marcora et al., 2008). Thus, an increase in RPE reflects an increased magnitude of the central command during voluntary contraction involving the activation of motor and premotor areas of the brain (de Morree et al.,

2012, 2014; Lafargue & Sirigu, 2006). Consequently, anodal stimulation of the M1 may have facilitated supraspinal drive, thus reducing the central command required and therefore resulted in participants perceiving less effort for the same force produced. Manipulation of the activity of the M1 and premotor areas has previously been shown to influence RPE. Indeed, experiments involving repetitive Transcranial Magnetic Stimulation (rTMS) have shown that the alteration of the activity of M1 and premotor areas produces parallel changes in RPE (Takarada, Mima, Abe, Nakatsuka, & Taira, 2014), thus making participants perceive the exercise as harder or easier.

Effect of prolonged exhaustive isometric exercise on neuromuscular function

In line with previous experiments (Pageaux et al., 2013), prolonged isometric submaximal contraction of knee extensor induced a significant increase in muscle fatigue as demonstrated by the reduced MVC immediately after exhaustion. Our data demonstrates that the increase in muscle fatigue was caused by both peripheral and central mechanisms as supported by the decrement of Doublet, Tw and VAL. However, it should be noted that contrary to previous studies (Pageaux et al., 2013), the ratio RMS_{MVC}/RMS_{Mwave} EMG did change after exhaustion. This ratio has been previously used in different studies to detect any change of central parameters after exhaustion (Pageaux et al., 2015, 2013). However, conflicting results has meant that this metric has been criticised (Farina, 2006). Our data further confirm that the quantification and assessment of central fatigue should be instead be performed using the twitch interpolation technique (Gandevia, McNeil, Carroll, & Taylor, 2013). MEP_{area} and the MEP_{area}/M_{area} ratio increased at exhaustion when compared to baseline, thus demonstrating an increase in cortical excitability. Similar findings were shown in previous experiments involving both isometric and dynamic muscle contractions (Jubeau et al., 2014; Pageaux et al., 2015; Temesi et al., 2014). However, these findings contrast with the study of Gruet and colleagues (2014) where MEP did not change at exhaustion after an intermittent exhaustive isometric task of the knee extensors at 50% MVC when compared to baseline. These findings suggest that MEP response at exhaustion may differ according to the regime of the muscle contraction, thus showing task specificity. Similarly to previous studies (Gruet et al., 2014; Pageaux et al., 2015; Taylor et al., 1996) CSP duration significantly increased immediately after exercise. Lengthening of the CSP has been associated with the increase of intracortical inhibition of cortical and sub-cortical areas (Gandevia, 2001; Taylor et

al., 1996), impairment of the motoneuron responsiveness (McNeil, Giesebrecht, Gandevia, & Taylor, 2011) and stimulation of mechano-metabo sensitive muscle afferents (Hilty et al., 2011). However, in the current study, as CSP was not different between conditions it is unlikely that tDCS elicited an effect on these measures.

Effects of tDCS on neuromuscular parameters

To the best of our knowledge, this is the first study to investigate the effect of tDCS on VAL or during maximal contraction of knee extensors. tDCS administration appeared to elicit no effect on the neuromuscular response and consequently we did not find any change in either central or peripheral parameters. The effect of tDCS on maximal force production has mainly focused on upper limb muscles (i.e. elbow flexors) without any improvement in MVC (Cogiamanian et al., 2007; Kan et al., 2013; Lampropoulou & Nowicky, 2013), although none of these studies involved the super imposed stimulation technique during MVC to assess VAL. However, it is likely that these parameters would not be affected by acute administration of tDCS as they are already maximal, so any further increase in VAL or MVC might be not achievable. Indeed, as proposed by Khan et al (2013) and Hummel et al., (2006), tDCS does not further enhance motor function when there is little or no scope for potential improvement.

MEP parameters obtained by TMS have been extensively used as index of cortical excitability of the M1 following tDCS stimulation. An increase in cortical excitability supported by an increase in MEP response lasting up to 60 min (depending on the type and duration of stimulation) (Nitsche & Paulus, 2001) has been reliably shown following anodal tDCS stimulation both at rest and during submaximal contractions (Jeffery, Norton, Roy, & Gorassini, 2007; Madhavan & Stinear, 2010; Nitsche & Paulus, 2000; Michael A. Nitsche et al., 2005). Contrary to what was initially expected, in our experiment cortical parameters did not change following tDCS. It is likely that this inconsistency was caused by the different assessment protocol used or the muscles investigated. Experimental evidence regarding the excitability of the lower limb area of the motor cortex in the healthy individual is very limited with only a few studies demonstrating a modest effect of tDCS (Jeffery et al., 2007; Madhavan & Stinear, 2010; Tatemoto, Yamaguchi, Otaka, Kondo, & Tanaka, 2013). Jeffery and colleagues (2007) specified that stimulation of the leg area of the motor cortex might be less inclined to tDCS intervention compared to the hand area of the motor cortex because its

deeper location to the scalp. An additional cause might be the intensity chosen for the submaximal contractions. Isometric contractions at 50% of MVC have been previously used (Pageaux et al., 2015; Säisänen et al., 2008) to provide a more stable and consistent response of CSP (Säisänen et al., 2008). However, it has been shown that the largest MEP response occurs with a contraction at 50% MVC with no further increases observed beyond this (Goodall et al., 2009; Sidhu et al., 2009). Therefore, it might be possible that any changes to MEP response as a result of tDCS were masked as a result of the 50% MVC. However, as changes to MEP response have been already been reliably shown following tDCS, we chose to use a 50% MVC so that any potential changes to CSP could be more accurately quantified.

In the current study, CSP did not differ between each conditions. Few previous studies have investigated the effect of tDCS stimulation on CSP, with contrasting outcomes (Horvath, Carter, & Forte, 2014). To date, only study of Trambley et al., (Tremblay, Beaulé, Lepage, & Théoret, 2013) showed a decrease in CSP following anodal tDCS stimulation, which the authors attributed to a reduction of GABAB-related inhibition on the M1. In the study of Trambley et al. (2013), cortical response was assessed during 20% MVC of first dorsal interosseus following 20 min anodal tDCS stimulation. Therefore, it may be that the differing results may be caused by the duration of tDCS stimulation or muscle investigated.

The HEAD montage used in this experiment is the same used in numerous experiments to relieve pain (see chapter 3; Boggio et al., 2008; Kan et al., 2013; Lefaucheur et al., 2008). However, in accordance with previous findings related to pain and exercise performance (see chapter 3; Kan et al., 2013), this montage was not able to reduce exercise-induced pain. Kan et al., (2013) found no change in performance of a single joint isometric contraction (Kan et al., 2013), whilst in the study described in the chapter 3 no changes in high intensity cycling time to exhaustion were found. It should be noted that the nature of the pain stimulus induced to monitor the well-established analgesic effect of tDCS (Boggio et al., 2008; Lefaucheur et al., 2008) is very different to the nature of exercise-induced pain and this may explain the different findings. Indeed, whilst tDCS has been shown to reduce pain during a cold pressor test, no change in pain was found during exercise (see chapter 3). Furthermore, many other factors during exercise (including distraction and attention) might reduce the benefits of tDCS (see chapter 3). In addition to these factors, the cathodal electrode placed over the contralateral prefrontal area in the HEAD montage likely changed

the direction of electrical flow through the brain. Several experiments using computer based models have demonstrated that the propagation of the electrical field in the brain is mainly affected by the type and position of the electrodes over the scalp (Bai, Dokos, Ho, & Loo, 2014; Miranda, Mekonnen, Salvador, & Ruffini, 2013; Wagner et al., 2007). Accordingly, any possible benefits following anodal stimulation of the M1 may have been negated by the DLPFC cathodal stimulation. Therefore, in support of previous findings, it is unlikely that the observed changes in performance observed in the current study were related to analgesia, but rather a moderation of the participant's perception of effort.

Effect of tDCS and exercise on NIRS parameters

When activated, brain tissues require more oxygen and glucose availability which are supported by an increase in cerebral blood flow. Changes in cortical excitability during and following tDCS stimulation with subsequent increase in metabolism and regional blood flow are well documented (Lang et al., 2005; Paquette, Sidel, Radinska, Soucy, & Thiel, 2011). In our experiment, we used the NIRS technique over left and right prefrontal cortex to non-invasively monitor oxygen consumption both during tDCS stimulation and exercise. Contrary to previous findings, our data did not indicate any change in oxygen consumption during tDCS and no differences were found between the left and right prefrontal cortex when cathodal electrode was placed in the right prefrontal cortex. By using fNIRS technique, Merzagora and colleagues (Merzagora et al., 2010) documented an increase and decrease in oxygen consumption respectively during anodal and cathodal stimulation and is therefore in contrast to our data. Further study is therefore needed to confirm this effect (or lack of). For the NIRS response during exercise, our data are in agreement with previous findings (Muthalib et al., 2013; Rupp & Perrey, 2009), with no differences found between conditions. Analogous findings were reported by Muthalib et al. (2013) where anodal tDCS did not affect prefrontal oxygenation during isometric elbow flexor exercise. The lack of change in NIRS parameters between conditions is likely caused by the effect of exercise induced cerebral response overcoming any differences following tDCS stimulation.

This is the first study comparing the effect of tDCS electrode montages on neuromuscular, physiological and perceptual parameters of exercise performance of the knee extensor muscles. In summary, this study demonstrated that an extracephalic shoulder montage is more effective than a cephalic head montage in improving isometric TTE performance of the lower limb. As this performance improvement was paralleled by a reduced RPE, improved performance may be attributed to a facilitation in supraspinal drive, leading to a reduced central command required for the same force produced. This study provides important methodological and physiological guidance in developing appropriate techniques for the application of tDCS on exercise in the lower limbs.

The experimental findings provided from this study raised some questions about the ability of tDCS to improve exercise performance. Since the extracephalic montage has been demonstrated to improve exercise capacity and reduce perception of effort, it is plausible that bilateral stimulation of both M1 would elicit a similar effect. In accordance with these points, the study performed in Chapter 6 will verify and further investigate this hypothesis on high intensity cycling exercise.

CHAPTER 5

EXPERIMENTAL STUDY 3

The effect of anodal tDCS over left and right temporal cortex: a comparative study

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Abstract

Stimulation of the right and left anterior insular cortex, increases and decreases the cardiovascular response respectively, thus indicating the brain's lateralisation of the neural control of circulation. Previous experiments have demonstrated that transcranial direct current stimulation (tDCS) modulates the autonomic cardiovascular control when applied over the temporal cortex. Given the importance of neural control for a normal hemodynamic response, and the potential for the use of tDCS in the treatment of cardiovascular diseases, this study investigated whether tDCS was capable of modulating autonomic regulation. Cardiovascular response was monitored during a post exercise muscle ischemia (PEMI) test, which is well documented to increase sympathetic drive. A group of 12 healthy participants performed a PEMI test in a control, sham and two different anodal tDCS sessions over the left and right temporal cortex for 20 min at 2 mA. The cardiovascular response was measured both during the PEMI test and at rest during tDCS stimulation. Cardiovascular response was not affected at rest during tDCS stimulation (RIGHT = 70.40 ± 7.94 vs. 69.53 ± 7.89; LEFT = 67.42 ± 8.55 vs. 67.40 ± 6.08; pre/post tDCS respectively). A consistent cardiovascular response during PEMI test was observed in all conditions, but no significant differences ($P>0.05$) were found following tDCS stimulation. This is the first study comparing the cardiovascular response after tDCS stimulation of left and right temporal cortex both during exercise and at rest. In contrast with previous experiments, tDCS stimulation did not induce any change in the cardiovascular response.

The methodological aspect for tDCS and exercise are still to be clarified and a first progression has been provided in the study performed in the previous chapter. Section 1.1 of this thesis provided evidence to suggest that regulation of the cardiovascular system may be a limit (or at least regulator) of endurance performance. One of the few exercise-based tDCS studies (Okano et al., 2015) suggested that changes in cardiovascular response could explain the differences in endurance performance following tDCS. However, this study was not designed to examine changes in cardiovascular response and so this mechanism was not properly tested. The lack of knowledge regarding the effect of tDCS on cardiovascular response and its potential effect of exercise raised few questions about some methodological aspects. Accordingly, it is reasonable that stimulation of a specific brain area might alter cardiovascular response and therefore exercise capacity. This thesis chapter therefore sought to robustly examine the effect of tDCS on cardiovascular control.

During exercise, central command (a feed forward mechanism) and the exercise pressor reflex (a feedback mechanism) send signals that converge in the cardiorespiratory centres located in the medulla (Matsukawa, 2012; Mitchell et al., 1983; Williamson et al., 2006). Both mechanisms contribute to the shift of the sympathetic drive to stimulate cardiovascular response (Matsukawa, 2012; Williamson et al., 2006), resulting in an elevation of cardiac output (CO), systemic vascular resistance (SVR) and mean arterial blood pressure (Lewis et al., 1983b). Particular attention has been given to the neurocircuitry involved in the cardiovascular regulation during exercise. Cortical and subcortical areas of the brain such as the insula cortex (IC), anterior cingular cortex (ACC), thalamus, hypothalamus, amygdala and medial prefrontal region have been well documented as participating in the regulation of the cardiovascular system during exercise (Benarroch, 1993; Cechetto & Shoemaker, 2009; Williamson et al., 2006), with the ACC and IC primarily involved during the activation of the exercise pressor reflex (Basnayake, Green, & Paterson, 2012; M. Sander, Macefield, & Henderson, 2010; Williamson et al., 2006; Williamson, McColl, Mathews, Ginsburg, & Mitchell, 1999; Williams et al., 2013). In order to identify the level of cortical control of the heart, experiments involving deep brain stimulation have been performed. When stimulated, the left IC has been shown to decrease the cardiovascular response, while stimulation of the right IC has the opposite effect,

thus supporting the assumption of a cortical lateralization of the brain regarding cardiovascular control. In agreement, similar conclusions have been proposed in experiments involving patients affected by lesions on the left or right IC, epilepsy and post stroke damage (Oppenheimer, Kedem, & Martin, 1996; D. Sander & Klingelhöfer, 1994).

Given the specificity of some cortical areas in the control of the heart, the application of non-invasive techniques can be used to study their effect on the cardiovascular response. tDCS has previously used to relieve pain (Boggio et al., 2008) and treat other neurological or psychiatric disorders (Fregni et al., 2007). Moreover, its effects are not only limited to the targeted areas under the scalp but also to subcortical areas. In fact, studies involving anodal stimulation (which increases the activity of the targeted area) over the temporal cortex (TC) showed alteration of heart rate variability (HRV) (Montenegro et al., 2011) and reduction of heart rate (HR) during cycling exercise (Okano et al., 2015). Despite the promising evidence regarding the ability to manipulate a targeted brain area, the number of studies investigating the application of non-invasive brain techniques on the cardiovascular response is surprisingly very limited, with no studies comparing the effect of anodal tDCS on the right and left TC.

A recent review from Cogiamanian and colleagues (2010) proposed a novel therapy in the management of cardiovascular diseases by applying non-invasive brain stimulation techniques to patients. The regulation of cardiovascular control at rest or during exercise in both in healthy and clinical populations is important and thus non-invasive brain techniques might in part be used to manage cardiovascular problems such hypertension. Accordingly, given the potential benefits of tDCS in the treatment of cardiovascular diseases, we monitored multiple cardiovascular variables following tDCS over both left and right TC in a group of healthy volunteers. The aim of the present experiment was to elucidate whether the hypothesised tDCS induced alteration of sympathetic and parasympathetic activity might subsequently alter the cardiovascular response.

Methods

Participants and design. Participants and design: Twelve recreationally active, healthy volunteers (six males and six females), aged 21 ± 2 y, height 175 ± 11 cm and weight of 75 ± 17 kg were recruited. All participants were engaging in at least 3x30 min bout of exercise per week at the time of the study. None of the participants reported any history of cardiovascular, pulmonary or metabolic

disorders or were taking any medication during the study. All participants were asked to refrain from exercise, caffeine and alcohol intake in the 24 hours prior to each visit. The study was approved by the Institutional Ethics Committee (University of Kent) according to the Declaration of Helsinki. The study followed a single-blind, randomized cross-over experimental design, and participants visited the laboratory on five separate occasions at the same time of day, separated by at least 72 h. The protocol involved two post-exercise muscle ischemia (PEMI) sessions interspaced by 20 min of tDCS stimulation (Fig 32). All experiments were carried out in a temperature-controlled (20°C, humidity 50%), air-conditioned, quiet room.

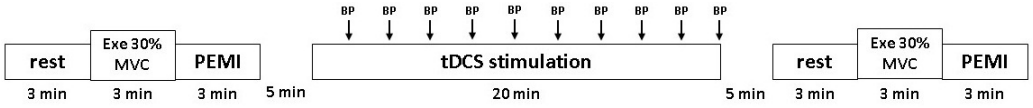

Fig 32. Overall view of experimental procedures performed during each experimental session.

Blood pressure, (BP); exercise (Exe); maximal voluntary contraction, (MVC); post exercise muscle ischemia (PEMI) test; transcranial direct current stimulation (tDCS).

Post exercise muscle ischemia (PEMI). Post exercise muscle ischemia (PEMI): This test involved a 3 min rest period, followed by 3 min of exercise consisting of dynamic rhythmic handgrip contractions at 30% of the participants' maximal voluntary contraction (MVC). The MVC was assessed at the start of each experimental visit and was recorded as the peak force achieved over 3 maximal handgrip contractions on hydraulic dynamometer (MAP 1.1; Kern & Sohn, Balingen, Germany). Rhythmic contractions were guided by an electronic metronome at a rate of 30 compressions/min. To obtain an estimation of central command (Williamson et al., 2006; Williamson, McColl, & Mathews, 2003), during the rhythmic contractions participants reported their rating of perceived exertion (RPE) at the end of each minute of exercise using the Borg scale (Borg, 1982). After 3 min of exercise, a cuff was rapidly inflated (<3 s) to 50 mmHg above exercise systolic pressure on the exercising arm using an automated pneumatic device (Hokanson E20 Rapid Cuff Inflator and AG101 Air Source, Bellevue, WA). The cuff was kept inflated for 3 min, after which it was then deflated. PEMI has been well documented to trap the metabolites in the exercising muscles to maintain

the stimulation of the metabo-receptors (Crisafulli et al., 2013; Roberto et al., 2012).

tDCS procedures. tDCS was delivered by a direct current stimulator (TCT Research Limited, Hong Kong) using a pair of humidified sponges (4x3 cm) in a water saline solution. Electric current was delivered at an intensity of 2 mA for 20 min. For the left condition (LEFT), the anodal electrode was applied over the left TC on the T3 area according to the international standards for EEG 10–20 system, with the cathodal electrode placed over the contralateral supraorbital area (Fp2). For the right condition (RIGHT), the anodal electrode was applied over the right TC on the T4 area, with the cathodal electrode placed over the contralateral supraorbital area (Fp3). For the SHAM condition, electrodes were applied in the same position as the LEFT, but stimulation lasted only 30 s after which it was rapidly ramped down. This induced the slight itching sensation which is commonly experienced during tDCS at the beginning of the stimulation, but has been shown to produce no cortical changes (Boggio et al., 2008; Mylius et al., 2012). No electrodes were placed during the control condition (CON) and instead participants sat quietly for 20 min.

Hemodynamic assessment. Stroke volume (SV), heart rate (HR), cardiac output (CO), SV/VET ratio (stroke volume/ventricular ejection time ratio) and SVR were monitored during all phases of the experiment with a transthoracic bioimpedance device (Physioflow PF05L1, Manatec, Petit-Ebersviller, France) that allows continuous, non-invasive monitoring of hemodynamic parameters. The method has been previously described by Charloux et al. (2000). Electrodes (Ambu Blue Sensor VL, Ambu A/S, Ballerup, Denmark) were placed over the chest in the V1 and V6 positions to the left ventricle to obtain an ECG signal, and then on the back in the midpoint of the spine corresponding to the same vertical position as the xiphoid process. Skin areas were shaved and cleaned in order to minimize electrical impedance. The PhysioFlow was calibrated during each experimental session before the tests. Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP) was measured every minute during PEMI, and every 2 min during tDCS stimulation. Arterial blood pressure parameters were obtained by an automated blood pressure device (Tango^{+}, SunTech Medical, Morrisville, NC) (Cameron et al., 2004; Hartwich et al., 2011; Pageaux et al., 2015) with a set of three electrodes placed in V2, V6 and RL positions. The cuff was placed on the left arm of the subject. Mean arterial blood pressure was calculated using the following equation:

$$MAP = \frac{(2 \cdot DAP) + SAP}{3}$$

Data and statistical analysis

All data are presented as mean ± SD. Beat-to-beat hemodynamic and RPE collected data were averaged for 3 min during both PEMI tests. Beat-to-beat hemodynamic collected data during the 20 min of tDCS stimulation were averaged for the last min every two min. Assumptions for statistical analyses such as normal distribution and sphericity of data were checked as appropriate before each analysis. Fully repeated measures 4x2x3 ANOVAs were used to monitor the effect of condition (control, sham, right TC and left TC), test (pre vs. post) and time (rest, exe and PEMI) on the hemodynamic and perceptive data collected during both PEMI tests. Fully repeated measures 4x10 ANOVAs were performed to monitor the effect of condition (control, sham, right TC and left TC) and time on the hemodynamic data collected during the 20 min of tDCS stimulation. Statistical analyses were followed by Bonferroni post-hoc when appropriate. Statistical significance was set as $P < 0.05$ in all cases. The Statistical Package for the Social Sciences (IBM, SPSS Statistics 20.0) was used to perform all analysis, and all test assumptions were met. All data are presented as means ± SD.

Results

None of the subjects presented any side effects during or after tDCS stimulation. All subjects reported feeling an itching sensation during the SHAM condition and none of the participants could tell the difference between SHAM and the actual tDCS stimulation. Tables 8 and 9 show absolute values of hemodynamic variables collected during all the phases of the experiment.

Hemodynamic response during PEMI. Statistical analysis did not show any differences regarding all the hemodynamic parameters between conditions during both PEMI. In details for HR ($F_{(3,33)} = 0.32$, $P = 0.80$), SV ($F_{(3,15)} = 0.22$, $P = 0.87$), CO ($F_{(3,15)} = 1.57$, $P = 0.23$), SV/VET ratio ($F_{(3,15)} = 1.57$, $P = 0.23$), SVR ($F_{(3,15)} = 1.74$, $P = 0.20$), SAP ($F_{(3,33)} = 1.07$, $P = 0.37$), DAP ($F_{(3,33)} = 0.55$, $P = 0.64$) and MAP ($F_{(3,33)} = 1.04$, $P = 0.38$). A normal hemodynamic profile response was observed during PEMI tests in all conditions. HR was elevated during exercise in all conditions compared to baseline and then returned to

resting values during the occlusion ($F_{(3,15)} = 2.32$, $P = 0.001$, Fig 33). SV ($F_{(3,15)} = 15.58$, $P = 0.02$), CO ($F_{(3,15)} = 148.49$, $P = 0.001$), SV/VET ratio ($F_{(3,33)} = 15.58$, $P = 0.001$), SAP ($F_{(3,33)} = 17.63$, $P = 0.001$), DAP ($F_{(3,33)} = 15.58$, $P = 0.001$), MAP ($F_{(3,33)} = 19.08$, $P = 0.001$) significantly rose compared to rest during both exercise and PEMI in all conditions ($P < 0.05$). SVR ($F_{(3,15)} = 18.39$, $P = 0.001$) significantly decreased during exercise and occlusion compared to rest state.

Hemodynamic response during tDCS stimulation. Statistical analysis did not show any differences regarding all the hemodynamic parameters between conditions during tDCS stimulation. In details for HR ($F_{(3,33)} = 2.20$, $P = 0.10$), SV ($F_{(3,15)} = 0.24$, $P = 0.98$), CO ($F_{(3,15)} = 0.72$, $P = 0.55$), SV/VET ratio ($F_{(3,15)} = 0.95$, $P = 0.43$), SVR ($F_{(3,15)} = 1.39$, $P = 0.28$), SAP ($F_{(3,33)} = 2.18$, $P = 0.10$), DAP ($F_{(3,33)} = 0.33$, $P = 0.79$) and MAP ($F_{(3,33)} = 1.56$, $P = 0.21$, see Fig 34).

RPE significantly rose during exercise in all conditions compared to baseline ($F_{(8,80)} = 129.02$, $P = 0.001$) and then returned to resting values during occlusion while no differences were found between conditions ($F_{(3,33)} = 1.00$, $P = 0.40$) or tests ($F_{(3,30)} = 0.92$, $P = 0.44$).

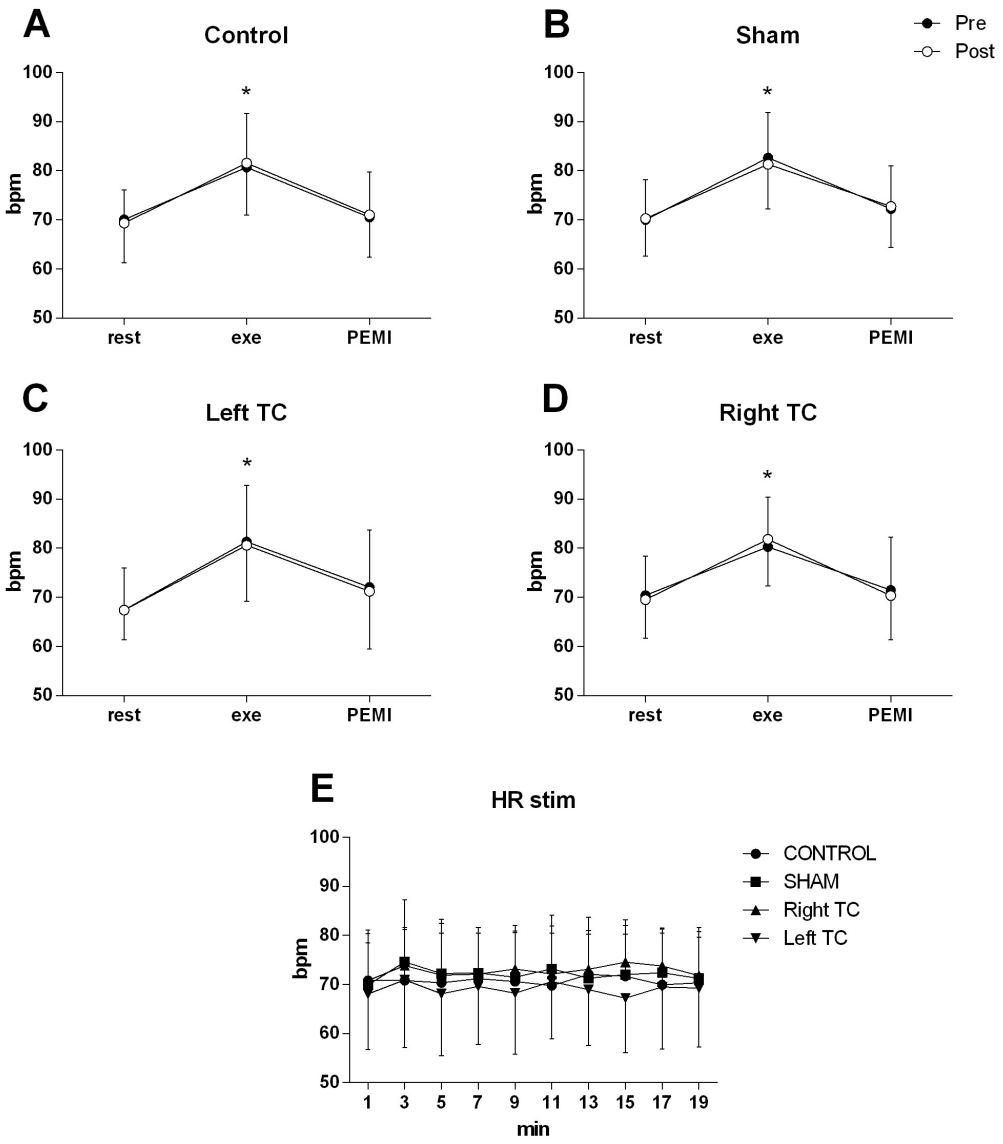

Fig 33. Time courses of heart rate response during the various phases of the experiment.

Panel A, B, C, D show time courses of heart rate (HR), at resting condition (rest), during exercise (exe) and post exercise muscle ischemia (PEMI) in all conditions. Panel E shows HR response during stimulation. Data were averaged over 3 min. * $P < 0.05$ vs. rest and PEMI. Data are presented as mean ± SD (n=12).

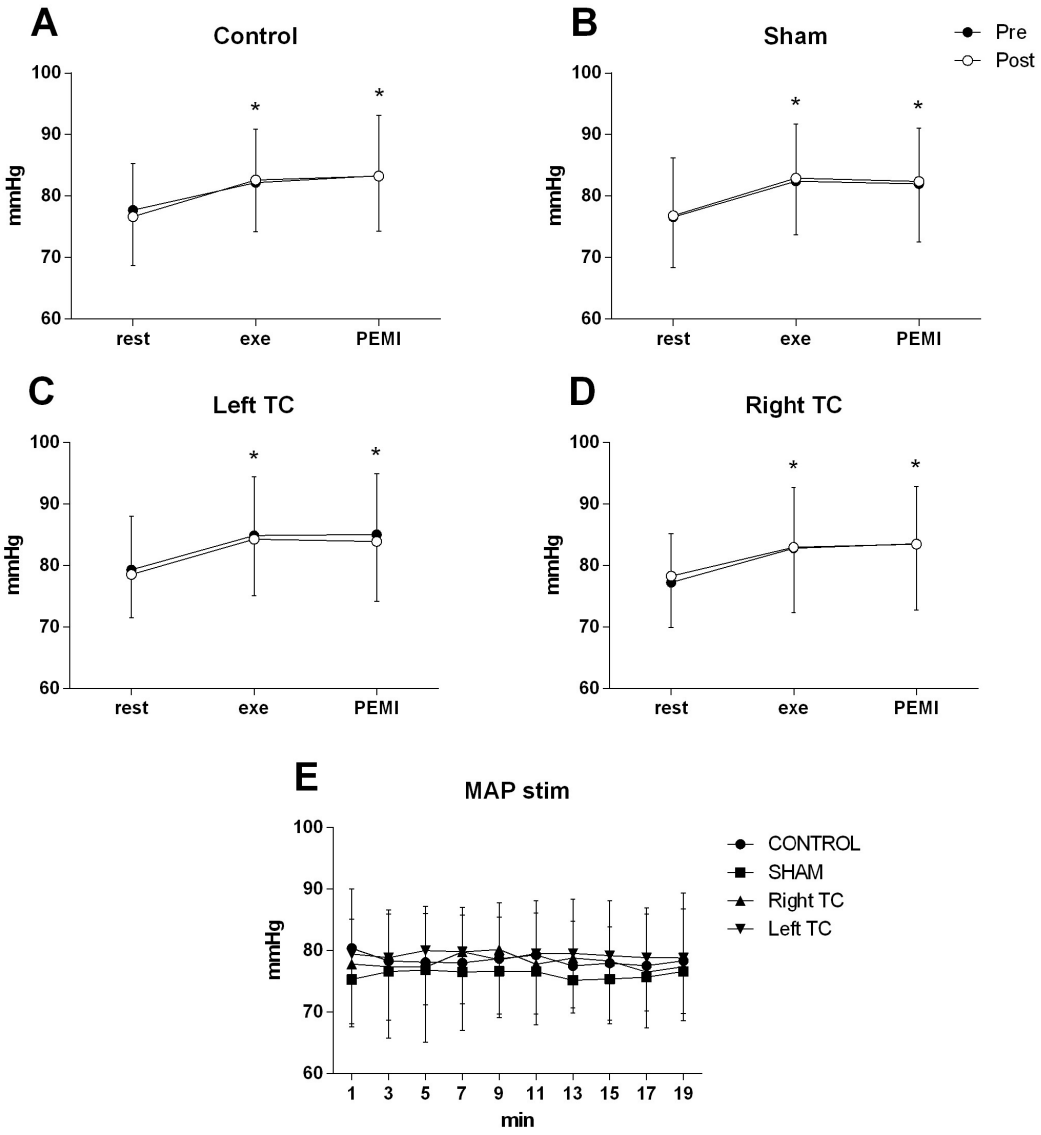

Fig 34. Time courses of mean arterial pressure during the various phases of the experiment.

Panel A, B, C, D show time courses of mean arterial pressure (MAP), at resting condition (rest), during exercise (exe) and post exercise muscle ischemia (PEMI) in all conditions. Panel H shows MAP response during stimulation. * $P < 0.05$ vs. rest. Data are presented as mean $\pm$ SD (n=12).

Table 8. Hemodynamic variables during rest, exe and PEMI periods in control and sham conditions.

	Control		Sham	
	Pre	**Post**	**Pre**	**Post**
SV (ml)				
Rest	83.54 ± 4.42	84.19 ± 5.78	82.20 ± 5.00	82.90 ± 6.58
Exe	92.28 ± 4.91*	93.79 ± 5.73*	94.65 ± 6.15*	93.46 ± 5.69*
PEMI	93.81 ± 6.33*	94.99 ± 4.488*	92.88 ± 4.33*	92.05 ± 4.07*
CO ($l \cdot min^{-1}$)				
Rest	6.06 ± 0.39	6.13 ± 0.62	5.84 ± 0.77	5.82 ±0.77
Exe	7.54 ±0.92*	7.63 ± 1.01*	7.61 ± 0.81*	7.56 ± 0.90*
PEMI	7.10 ±0.62*	7.25 ± 0.80*	6.99 ± 0.74*	6.96 ± 0.85*
SV/LVET				
Rest	0.26 ±0.02	0.26 ± 0.02	0.26 ± 0.02	0.26 ± 0.02
Exe	0.24 ±0.02*	0.24 ± 0.02*	0.24 ±0.02*	0.24 ± 0.01*
PEMI	0.25 ±0.02*	0.25 ± 0.02*	0.25 ±0.01*	0.25 ± 0.01*
SVR ($dyne \cdot s^{-1} \cdot cm^{5}$)				
Rest	1085.63 ± 97.73	1069.81 ± 154.30	1118.55 ± 270.22	1139.29 ± 184.45
Exe	945.64 ±140.28*	942.09 ± 146.24*	925.20 ± 204.24*	969.24 ± 153.02*
PEMI	1022.12 ±137.14	1002.72 ± 157.06	1062.09 ± 145.13	1060.21 ±178.84
SAP (mmHg)				
Rest	107.81 ±12.22	107.14 ± 11.86	106.13 ± 12.65	103.39 ± 7.63
Exe	114.22 ±13.36*	115.89 ± 14.44*	113.60 ± 16.64*	113.75 ± 9.13*
PEMI	114.89 ±16.14*	115.83 ± 16.52*	112.53 ± 16.32*	111.72 ± 8.38*
DAP (mmHg)				
Rest	62.69 ±7.74	61.39 ± 7.21	62.92 ± 9.51	63.53 ± 8.53
Exe	66.19 ±8.86*	66.00 ± 8.03*	67.88 ± 11.16*	67.50 ± 9.77*
PEMI	67.50 ±9.67*	66.94 ± 8.31*	67.89 ±11.06*	67.72 ± 10.69*

Resting condition (Rest); exercise (Exe); post exercise muscle ischemia (PEMI); stroke volume (SV); cardiac output (CO); systemic vascular resistance (SVR); stroke volume left ventricular ejection time ratio (SV/LVET); systolic arterial pressure (SAP); diastolic arterial pressure (DAP); * $P < 0.05$ vs. rest. Data are presented as means ± SD (n=12).

Table 9. Hemodynamic variables during rest, exe and PEMI periods in right TC and left TC conditions.

	Right TC		Left TC	
	Pre	**Post**	**Pre**	**Post**
SV (ml)				
Rest	82.89 ± 7.72	82.97 ± 7.12	79.99 ± 4.79	80.51 ± 3.19
Exe	94.82 ± 4.12*	94.62 ± 4.86*	94.40 ± 3.58*	95.65 ± 4.77*
PEMI	93.89 ± 3.59*	94.05 ± 5.13*	92.69 ± 3.31*	93.82 ± 4.43
CO ($l \cdot min^{-1}$)				
Rest	5.87 ± 0.87	5.85 ± 0.70	5.62 ± 0.25	5.63 ± 0.20
Exe	7.75 ± 0.81*	7.77 ± 0.81*	8.03 ± 0.58*	8.19 ± 0.50*
PEMI	7.02 ± 0.90*	6.98 ± 0.70*	7.62 ± 0.79*	7.58 ± 0.77*
SV/LVET				
Rest	0.26 ± 0.03	0.27 ± 0.02	0.25 ± 0.01	0.25 ± 0.01
Exe	0.24 ± 0.01*	0.24 ± 0.01*	0.24 ± 0.01*	0.25 ± 0.01*
PEMI	0.25 ± 0.01*	0.25 ± 0.02*	0.24 ± 0.01*	0.25 ± 0.01*
SVR ($dyne \cdot s^{-1} \cdot cm^{5}$)				
Rest	1146.86 ± 156.75	1180.20 ± 181.72	1245.08 ± 74.30	1191.61 ± 74.94
Exe	953.43 ± 106.04*	967.69 ± 131.45*	934.63 ± 20.22*	891.28 ± 25.09*
PEMI	1078.38 ±166.51	1062.87 ± 154.18	986.63 ± 97.48	1001.05 ± 98.81
SAP (mmHg)				
Rest	110.17 ± 15.33	109.75 ± 15.17	111.50 ± 14.14	109.39 ± 15.33
Exe	115.89 ± 20.71*	116.11 ± 21.20*	117.47 ± 19.55*	117.58 ± 19.52*
PEMI	116.25± 21.67*	114.94 ± 21.14*	116.89 ± 20.50*	115.86 ± 20.35*
DAP (mmHg)				
Rest	60.78 ± 6.77	62.56 ± 8.18	62.56 ± 8.52	63.81 ± 7.49
Exe	66.25 ± 8.70*	66.42 ± 11.86*	68.06 ± 7.93*	68.17 ± 7.85*
PEMI	67.22 ± 9.45*	66.25 ± 10.02*	68.50 ± 9.14*	68.58 ± 8.61*

Resting condition (Rest); exercise (Exe); post exercise muscle ischemia (PEMI); stroke volume (SV); cardiac output (CO); systemic vascular resistance (SVR); stroke volume left ventricular ejection time ratio (SV/LVET); systolic arterial pressure (SAP); diastolic arterial pressure (DAP); * $P < 0.05$ vs. rest. Data are presented as means ± SD (n=12).

Discussion

This study sought to elucidate whether tDCS of left and right TC caused changes to the cardiovascular response during rest, exercise and PEMI. We hypothesised that anodal tDCS of both left and right TC would alter the cardiovascular response by changing sympathetic and parasympathetic balance. However, the primary finding of the present study was that tDCS did not alter any of the functional cardiovascular parameters measured.

The hemodynamic profile observed during exercise and PEMI is in good agreement with previous findings (Crisafulli et al., 2006, 2013; Roberto et al., 2012). As expected, during exercise and PEMI, cardiac activity significantly increased compared to baseline showing a substantial increase in SV and CO with SAP, DAP and MAP while SVR and SV/VET decreased. These data further support the concept that metaboreflex activation achieved by PEMI is able to stimulate both central and peripheral cardiovascular response despite the absence of central command (Boushel, 2010; Crisafulli et al., 2006, 2013; Roberto et al., 2012). It should be noted that HR was not affected during the PEMI manoeuvre, and instead returned towards baseline. The likely reason for this response is due to the pronounced vagal tone, despite the persistent sympathetic activity (Stramba-Badiale et al., 1991; Tulppo, Mäkikallio, Seppänen, Airaksinen, & Huikuri, 1998).

Previous research has suggested a modulation of cardiovascular response following stimulation of a specific brain area using non-invasive techniques such as rTMS and tDCS. Yoshida et al. (2001) found a transient increase in HRV following low frequency rTMS over the vertex while Hong et al. (2002) showed a temporary reduction of blood pressure in rats following unilateral stimulation of motor cortex, thus supporting a potential activation of the para-sympathetic activity. More recently, Montenegro et al. (2011) showed an increase in HRV following anodal stimulation over the left TC while Okano et al. (2015) demonstrated a reduction of HR during exercise with increase in HRV following anodal during incremental cycling exercise. Both studies associated this behaviour with an enhanced para-sympathetic activity in healthy active subjects. These studies suggest that tDCS stimulation of the TC also induces alterations in subcortical brain areas, potentially due to the connection within cortico-neural networks (Augustine et al., 1996; Lang et al., 2005), and seems to have effect both at rest and during exercise. However, our results did not show any change in cardiovascular response during or after anodal tDCS stimulation. In support of our findings, Vandermeeren et al., (2010) failed to observe any significant variations

in HR or blood pressure between anodal, cathodal or sham tDCS in healthy subjects at rest, despite significant change of HRV indexes. It is likely that the inconsistency with previous studies and our current data involving tDCS stimulation can be explained by the different experimental protocol and the variables investigated. The study of Montenegro et al., (2011) only provides frequency domain parameters while no functional cardiovascular parameters were presented. Additionally the study performed by Okano and colleagues (2015) related the lower HR response following tDCS stimulation during exercise as consequence of an altered activation of the insular cortex. However, given that the test was a graded exercise test to exhaustion, and that participants were able to perform longer in the tDCS condition, it is likely that they were performing at different exercise intensities between conditions. This could be a likely explanation for the observed differences in HR.

Few previous studies investigating the effect of tDCS on the cardiovascular response have been performed (Montenegro et al., 2011; Okano et al., 2015; Vandermeeren et al., 2010), and consequently knowledge regarding the effect of tDCS on the cardiovascular response is limited. Compounding this is the difficulty in interpretation of previous results due to different experimental procedures used in these studies. Indeed, three main limitations are present in previous literature: 1) there are no studies comparing tDCS stimulation of both the left and right TC on the cardiovascular parameters, which would provide evidence regarding cortical lateralization of the brain in cardiovascular control. 2) Studies have been performed in the absence of a placebo controlled condition. 3) The cardiovascular parameters investigated and reported is limited and thus the exact effect on the cardiovascular system is uncertain.

To address this, we used a PEMI protocol which provides a unique opportunity to monitor and isolate the two main sympathetic systems regulating the cardiovascular responses (i.e. central command and metaboreflex), thus allowing a more in-depth analysis of any possible changes in cardiovascular response. To date, the only parameters used to assess the effect of tDCS on cardiovascular control have been MAP, HR and measures of HRV. In current experiment, the integration of variables such as SV, HR and SV/LVET provides a greater opportunity to examine potential tDCS induced change in parasympathetic and sympathetic balance on cardiac regulation.

Okano and colleagues (2015) found a significant reduction in HR during the first phases of a maximal incremental exercise test following anodal tDCS over left TC. Unfortunately, given the nature of the test performed, this protocol is

unlikely to be appropriate for the monitoring of tDCS effect due to the changes in exercise intensity. Indeed, a maximal incremental test implies a continuous increase in power output, which requires an increase in sympathetic drive to increase cardiac response to satisfy oxygen demand of the working muscles. These rapid changes in cardiovascular dynamics make interpretation of the effect of tDCS unclear. Rather, a constant load exercise should be performed to reduce these methodological limitations. The handgrip exercise performed during the PEMI in the current study was executed at the same absolute and relative workload, thus maintaining a stable sympathetic and parasympathetic balance unlike the study of Okano and colleagues (2015). Taken together, the setup used in the current study should be able to better monitor any cardiovascular changes induced by tDCS administration, with less methodological constraints.

Given the recent growing number of studies involving tDCS prior to exercise (see chapter 3; Cogiamanian et al., 2007; Muthalib et al., 2013; Okano et al., 2015), it is very important to understand its effect on the cardiovascular response (particularly in the exercise sciences) as any moderation of this has the potential to effect blood flow to the working muscles, and thus effect exercise capacity. Furthermore, the cardiovascular effects of non-invasive brain stimulation may be important for the treatment of chronic cardiovascular diseases such hypertension. The results of the current study suggest that an acute bout of tDCS stimulation over the left and right TC has no effect on cardiovascular parameters. Thus, the use of tDCS to treat cardiovascular disorders is questionable, and it is likely to have little impact on cardiovascular response if applied during/before exercise.

Conclusion

In conclusion, although the key brain areas related to autonomic cardiovascular control have been well established, the literature regarding the use of non-invasive brain stimulation techniques to modulate autonomic regulation demonstrate a lack of consistency in findings (Cogiamanian et al., 2010). The results of the current study suggest that anodal tDCS of the left and right TC does not affect functional cardiovascular response at rest, during excise and PEMI. Therefore, in light of the present and previous findings, the effect of tDCS on the cardiovascular response remains inconclusive.

CHAPTER 6

EXPERIMENTAL STUDY 4

Transcranial direct current stimulation improves cycling performance in healthy individuals

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Abstract

Changes in excitability induced by non-invasive brain stimulation of motor and premotor areas have been shown to alter perception of effort. Moreover, unilateral stimulation of the motor cortex by anodal transcranial direct current stimulation (tDCS) has been shown to improve endurance exercise. In the present investigation we monitored whether bilateral stimulation of the motor cortex can alter perception of effort during prolonged cycling exercise. Twelve healthy subjects were recruited and underwent a placebo (SHAM), anodal tDCS (ANODAL) and cathodal tDCS (CATHODAL) condition in a double-blind, randomised and counterbalanced experimental design. tDCS stimulation was administered by using two extracephalic montages with the active electrode placed over motor cortex and the reference electrode over the contralateral shoulder. Stimulation was delivered for 10 min at 2.0 mA. Neuromuscular parameters were examined at baseline and after tDCS stimulation to monitor whether tDCS induced changes in cortical excitability. After the neuromuscular assessment, a cycling time to exhaustion at 70% of peak power output (W_{max}) was performed. Heart rate (HR), ratings of perceived exertion (RPE), and leg muscle PAIN were monitored during the TTE while blood lactate was measured immediately after exhaustion. None of the peripheral parameters showed any difference between conditions after tDCS stimulation ($P = 0.74$) while cortical response significantly increased after ANODAL stimulation ($P < 0.001$). TTE was longer in the ANODAL condition compared to the CATHODAL and SHAM conditions (12.61 ± 4.65 min; 10.61 ± 4.34 min; 10.21 ± 3.47 min respectively) with significantly higher blood lactate concentration at exhaustion in the ANODAL condition ($P < 0.001$) compared to the CATHODAL and SHAM conditions (14.25 ± 4.51 mmol $\cdot$ l^{-1}; 10.91 ± 2.45 mmol $\cdot$ l^{-1}; 10.24 ± 2.43 mmol $\cdot$ l^{-1} respectively). No differences were found for HR ($P = 0.80$) and PAIN between conditions ($P = 0.27$). RPE was significantly lower in the ANODAL condition ($P < 0.001$). None of the monitored parameters was significantly affected in the SHAM and CATHODAL conditions. Our findings suggest that ANODAL stimulation improves cycling performance probably by increasing cortical excitability and thus reducing perception of effort during exercise.

The study detailed in the Chapter 4 confirmed the suggestions made in the discussion in Chapter 3 – that an extracephalic tDCS montage should be used in order to avoid the negative effect of the cathode. Chapter 4 provided evidence that tDCS is capable of reducing perception of effort and thus improve single-limb isometric time to exhaustion. According to what was hypothesised in chapter 4, for whole body exercise, both sides of the M1 would need to be stimulated. Accordingly, this study investigated whether a bilateral extracephalic tDCS montage was capable of producing similar results to those shown in Chapter 4.

Factors affecting exercise performance have been extensively studied and explained through multiple paradigms, although the predominant focus has been on physiological parameters (Joyner & Coyle, 2008). However, recently there has been growing attention given to the psychological aspects (McCormick, Meijen, & Marcora, 2015) with particular attention to the role of perception of effort (RPE) (Abbiss, Peiffer, Meeusen, & Skorski, 2015). RPE has been defined as the conscious sensation of how hard, heavy and strenuous a physical task is (Marcora, 2009) although the neurophysiological basis of RPE is still the matter of some debate. Indeed, several different models regarding the generation of RPE during exercise have been proposed (Marcora, 2009; Pires et al., 2011). One of the more eminent theories suggests that RPE is generated by processes related to the corollary discharge of the central motor command (Lafargue & Sirigu, 2006; McCloskey, 2011). Evidence in favour of the corollary discharge model derives from experimental procedures where the central motor command is needed to match the force required. This should lead to an increase in RPE, while a reduction in the force required should lead to a decrease in RPE (McCloskey et al., 1983). Therefore, this model proposes a direct link between the magnitude of central motor command and RPE. A significant correlation between RPE with motor and premotor areas during contraction of elbow flexors has been found (de Morree et al., 2012, 2014; McCloskey, 2011), thus providing strong neurophysiological evidence regarding the central generation of RPE. Similar findings have been also provided in whole body exercise under partial neuromuscular blockade (Gallagher et al., 2001; Marcora et al., 2008) and in pre-fatigued muscles (Marcora et al., 2008). with particular attention to the role of perception of effort (RPE). RPE has been defined as the conscious sensation of how hard, heavy and strenuous a physical task is although the neurophysiological basis of RPE is still

the matter of some debate. Indeed, several different models regarding the generation of RPE during exercise have been proposed. According to some authors, RPE is the result of peripheral signals arising from the muscle, heart and lungs which then converge at the brain. Contrarily one of the more eminent theories suggests that RPE is generated by processes related to the corollary discharge of the central motor command.

Central motor command involves activation of motor and premotor brain areas related to muscle contraction. Evidence in favour of the corollary discharge model derives from experimental procedures where the central motor command is needed to match the force required. This should lead to an increase in RPE, while a reduction in the force required should lead to a decrease in RPE. Therefore, this model proposes a direct link between the magnitude of central motor command and RPE. For example, in fatigued muscles an increase in central command is necessary to produce the required force/power and consequent increase in RPE. Furthermore, a significant correlation between RPE with motor and premotor areas during contraction of elbow flexors has been found, thus providing strong neurophysiological evidence regarding the central generation of RPE. Similar findings have been also provided in whole body exercise under partial neuromuscular blockade and in pre-fatigued muscles.

Interestingly, non-invasive brain stimulation techniques (such as rTMS) have been adopted to manipulate the activity of motor and premotor areas in healthy individuals (Goodall et al., 2013; Takarada, Mima, Abe, Nakatsuka, & Taira, 2014; Zénon, Sidibé, & Olivier, 2015). These experiments have demonstrated that alteration of the motor cortex (M1) can lead to alteration of RPE during movement execution. These experimental findings further demonstrated the relationship between the motor and premotor areas for the generation of RPE.

More recently, research has consistently shown an increase in M1 excitability following anodal tDCS stimulation prior to exercise. By increasing excitability in the motor and pre-motor areas via anodal stimulation, a facilitated supraspinal drive (and potentially reduced the central command) required for the same force produced could be expected. This could consequently lead to an individual perceiving less effort for the same force produced. As shown in Chapter 3 of this thesis, there is only one published study investigating the effect of tDCS stimulation of the M1 on exercise performance of the lower limbs during constant cycling exercise (Angius et al., 2015). In this study, no effect of tDCS of the left M1 on cycling performance was found. However, whole body exercise involves large muscle groups, and the left and right M1 activate the contralateral limb.

Therefore, in whole body tasks tDCS stimulation of both left and right motor cortex should be applied.

The results from previous experiments involving anodal tDCS on single limb exercise (Cogiamanian et al., 2007; Williams et al., 2013) and the study performed in Chapter 4 of this thesis, suggest that manipulation of whole body exercise performance by bilateral tDCS administration over the M1 is plausible. Therefore, the aims of this experiment were: 1) monitor the effect of bilateral administration of M1 on RPE during exercise; 2) monitor whether any alteration of RPE following tDCS administration can alter whole body exercise performance. These measurements should elucidate whether the hypothesised alteration in excitability of both sides of the M1 following tDCS administration could alter exercise performance.

Methods

Subjects. Twelve recreationally active participants (4 women and 8 men; mean ± SD, age: 24.4 ± 5.2 yr, height: 175.1 ± 12.2 cm, weight: 74.3 ± 17.8 kg) were recruited. None of the participants had any history of cardiorespiratory, metabolic or mental disease/disorder at the time of the study. All participants gave their written informed consent after being informed about the experimental procedures and aims of the experiment. All the procedures and the experimental protocol were approved by the local ethics committee. Each test was conducted at the same time of the day for each participant in a temperature-controlled room (20°C, relative humidity between 40-50%).

Experimental design. Participants visited the laboratory on four different occasions. In the first visit, participants were familiarized with the laboratory and all the experimental procedures. Additionally, they performed an incremental test on cycle ergometer (Lode, Excalibur Sport, Groningen, Netherlands) to establish individual peak power output (W_{max}). In this test, participants performed a 5 min warm up at 100 W, and the protocol started at 100 W and increased 5 W every 15 s^{-1} until exhaustion (i.e., the incapacity to maintain the cadence above 60 rpm).

In visits 2-4, using a double-blind, randomised, counter-balanced experimental design, participants underwent a placebo (SHAM), anodal tDCS stimulation (ANODAL) and cathodal tDCS stimulation (CATHODAL) session. (see Fig 35).

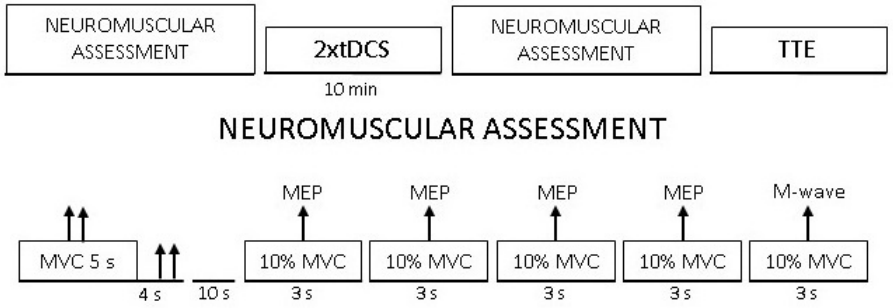

Fig 35. Overall view of the experimental protocol.

Maximal muscular wave (Mwave); motor evoked potential (MEP); maximal voluntary contraction (MVC); transcranial direct current stimulation (tDCS); time to exhaustion (TTE).

Endurance task. Participants performed a cycling time to exhaustion (TTE) test at 70% of the peak power output, which was previously assessed in the maximal cycling test during the first visit. The TTE test terminated when the participants was not able to maintain cycling cadence above 60 RPM for more than 5 s. Participants were not aware of the duration of the test and were continuously motivated during the test. Results of all the sessions were given after completion of all the experimental conditions.

Perception of effort (RPE) and leg muscle pain (PAIN) were measured using the 15-points RPE scale (Gunnar Borg, 1998) and a 10-point numerical scale (Cook, O'Connor, Eubanks, Smith, & Lee, 1997) after 30 s, at the end of each min and immediately after exhaustion in the TTE test. Heart rate (HR) was continuously monitored using a HR monitor (Polar RS400; Polar Electro Oy, Kempele, Finland) and averaged to provide data points to coincide with RPE and PAIN.

Neuromuscular tests. All neuromuscular assessments performed in this study were identical to those performed in Study 2 (Chapter 4, pages 114-119). However, the intensity of the submaximal contractions were performed at 10% of the MVC in this study. A schematic of the neuromuscular assessments is shown in Fig 35.

Transcranial magnetic stimulation (TMS). All magnetic stimulation assessments performed in this study were identical to those performed in Study 2 (Chapter 4, pages 114-119). The average stimulation intensity for this study was mean: 65 ± 4% of the maximum stimulator output.

Femoral nerve stimulation. All femoral nerve stimulation assessments performed in this study were identical to those performed in Study 2 (Chapter 4,

pages 114-119). The average stimulation intensity for this study was mean: 290 ± 71 mA.

Mechanical recordings. The procedures on the isokinetic dynamometer in this study was the same as those in Study 2 (Chapter 4, pages 114-119).

Electromyographic recordings. The procedures for electromyographic recordings in this study was the same as those in Study 2 (Chapter 4, pages 114-119).

Transcranial direct current stimulation procedures. tDCS was administered by a direct current stimulator (TCT Research Limited, Hong Kong) using two rubber electrodes (size: 4x3 cm) and water-soaked synthetic sponge. For the present experiment, tDCS stimulation was delivered with two different montages. In the ANODAL condition two anodal electrodes were placed over both sides of the motor cortex, while the two cathodal electrodes were placed on the contralateral shoulders. In the CATHODAL condition the two cathodal electrodes were placed over both sides of the motor cortex, with the two anodal electrodes placed on the contralateral shoulders. For the SHAM condition, the same set up of ANODAL was used. Stimulation intensity was set at 2.0 mA for 10 min, whereas during the SHAM session stimulation lasted 30 s and was subsequently ramped down to no stimulation.

Data analysis. Peak torque obtained during the MVC was used to calculate the peak force of knee extensors. Voluntary activation level (VAL) during the MVC was obtained according the following formula:

$$VAL = 100 \cdot \left(1 - \frac{superimposed\ doublet\ amplitude}{potentiated\ doublet\ amplitude}\right)$$

The EMG amplitude obtained during the MVC was quantified with the RMS for a 0.5 s interval during the peak torque (250 ms either side at the peak torque). The root mean square (RMS) of EMG was automatically calculated with the software. The following parameters were also obtained: peak-to-peak amplitude of the resting M-wave, peak torque Doublet and peak Twitch. The MEP area (MEP_{area}), was averaged for the four TMS stimulations at the 10% MVC and then normalized for the Mwave (MEP_{area}/Mwave) obtained during the 10% MVC contraction. MEP amplitude (MEP_{amp}), was calculated and averaged for the four stimulations, and then normalized for the M_{max}.

The MEP cortical silent period (CSP) was measured from the onset of the MEP to the return of EMG signal. The following MEP parameters were also calculated: MEP peak to peak amplitude (MEP_{amp}), MEP peak to peak duration (MEP_{dur}). The isotime data of RPE, PAIN and HR were calculated according

to the following: The shortest TTE was identified for each individual over the three visits and considered as 100% isotime. Subsequently, each value obtained at the final minute of the shortest TTE was compared at the equivalent minute of the longer visits. The respective 25%, 50% and 75% of isotime were obtained by multiplying the 100% isotime for 0.25, 0.50 and 0.75. Isotime values for 0% were attained by comparing values for the first full minute of each TTE test (Blanchfield et al., 2014).

Statistical analysis

All data are presented as mean ± SD. The normal distribution and sphericity of data were checked as appropriate. The effect of tDCS administration on time to exhaustion duration and B[La^-] were assessed by using one-way ANOVA with repeated measures. Fully repeated measures 3x6 ANOVAs were performed to test the effects of tDCS administration (ANODAL, CATHODAL and SHAM) and time on RPE, PAIN and HR during the time to exhaustion test. Fully repeated measures 3x2 ANOVAs were performed to test the effect of tDCS administration (ANODAL, CATHODAL and SHAM) and time (pre vs. post) on MVC, VAL, Doublet, MEP_{amp}, MEP_{dur}, MEP_{area}/Mwave ratio, CSP. Bonferroni post hoc tests were used when appropriate. Statistical significance was set at $P < 0.05$. Statistics analysis was performed by using SPSS version 20.

Results

All participants completed all the experimental sessions and none of them reported any side effect during or after tDCS administration. During tDCS administration, participants perceived a tingling sensation but none of them were able to distinguish any difference between SHAM, ANODAL and CATHODAL conditions.

W_{max} obtained during the maximal incremental test was 257 ± 58 W with a TTE power corresponding to 180 ± 40 W. TTE was significantly longer in the ANODAL condition ($F_{(1,11)} = 0.19$, $P = 0.003$) compared to the CATHODAL and SHAM conditions (12.61 ± 4.65 min; 10.61 ± 4.34 min; 10.21 ± 3.47 min respectively). B[La^-] was significantly higher in the ANODAL condition ($F_{(2,22)} = 11.28$, $P < 0.001$) compared to the CATHODAL and SHAM conditions (14.25 ± 4.51 mmol · l^{-1}; 10.91 ± 2.45 mmol · l^{-1}; 10.24 ± 2.43 mmol · l^{-1} respectively) (see Fig 36).

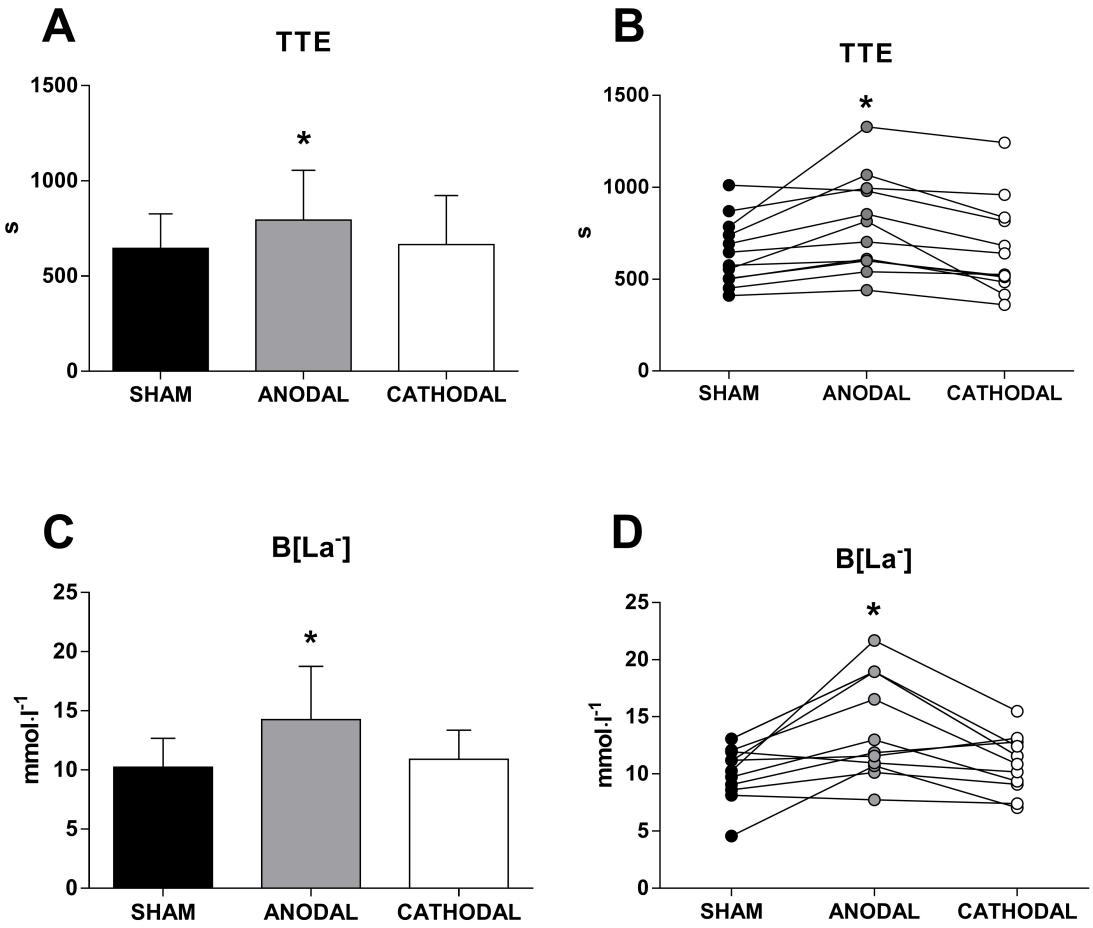

Fig 36. Performance result and physiological and perceptual response during exercise.

Panels A and B show time to exhaustion (TTE) performance, while panels C and D show blood lactate accumulation ($B[La^-]$) values at exhaustion. * $P < 0.05$, denotes significant difference from CATHODAL and SHAM conditions. Data presented as mean ± SD (n=12).

Physiological and perceptual parameters during exercise. RPE, PAIN and HR changed significantly over time (all main effect of time $P < 0.001$) but only RPE was affected by tDCS stimulation as it was significantly lower in the ANODAL condition ($F_{(2,22)} = 8.94$, $P < 0.001$) compared to the CATHODAL and SHAM conditions (see Fig 36).

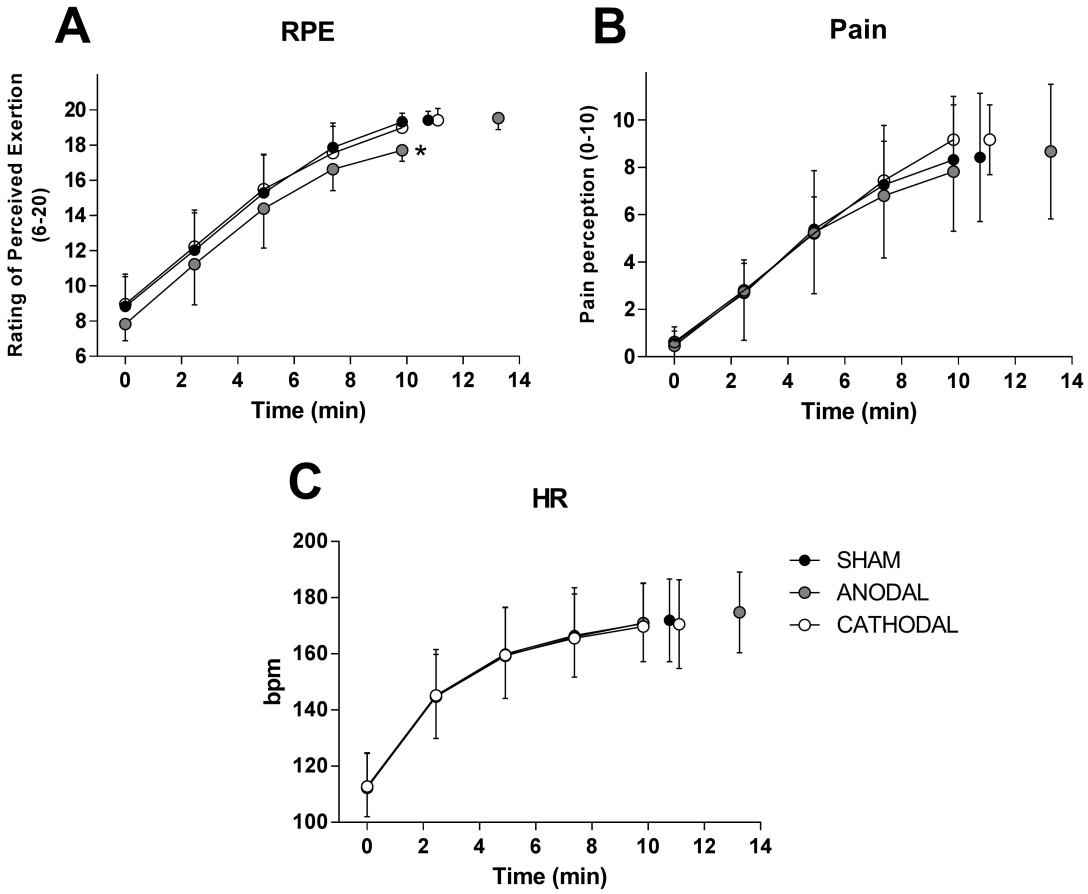

Fig 37. Neuromuscular response before and after tDCS stimulation.

Panel A shows time courses of rating of perceived exertion (RPE) during the TTE. Panel B shows time courses of pain perception (pain) during the TTE. Panel C shows time courses of heart rate (HR) during the TTE. * $P < 0.05$, denotes significant difference from CATHODAL and SHAM conditions. Data presented as mean ± SD (n=12).

Neuromuscular assessment. The statistical analysis did not observe any significant differences in neuromuscular function across each experimental session at baseline.

Neuromuscular response. No statistical difference was observed regarding MVC, VAL, Doublet, Tw, MEP_{dur} and CSP following tDCS or SHAM intervention ($P > 0.05$).

Corticospinal response. MEP_{amp}, MEP_{area}, MEP_{area}/Mwave ratio were significantly higher after tDCS stimulation in the ANODAL condition ($P < 0.05$), while no statistical differences were found regarding the CSP ($P > 0.05$) (see Fig 37 and 38).

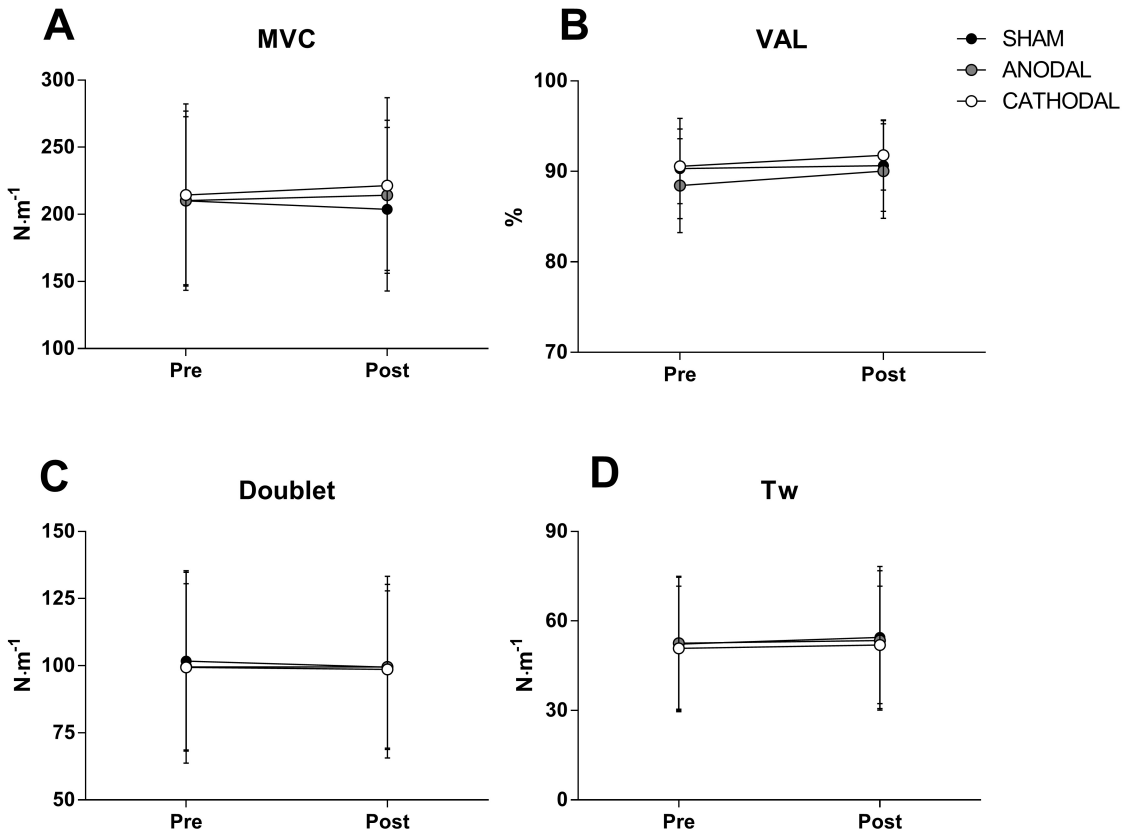

Fig 38. Cortical response before and after tDCS stimulation.

Panel A shows maximal voluntary contraction (MVC); Panel B shows voluntary activation level (VAL); Panel C shows peak torque of the doublet (Doublet); Panel D shows peak twitch (Tw). Data are presented as mean ± SD (n=12).

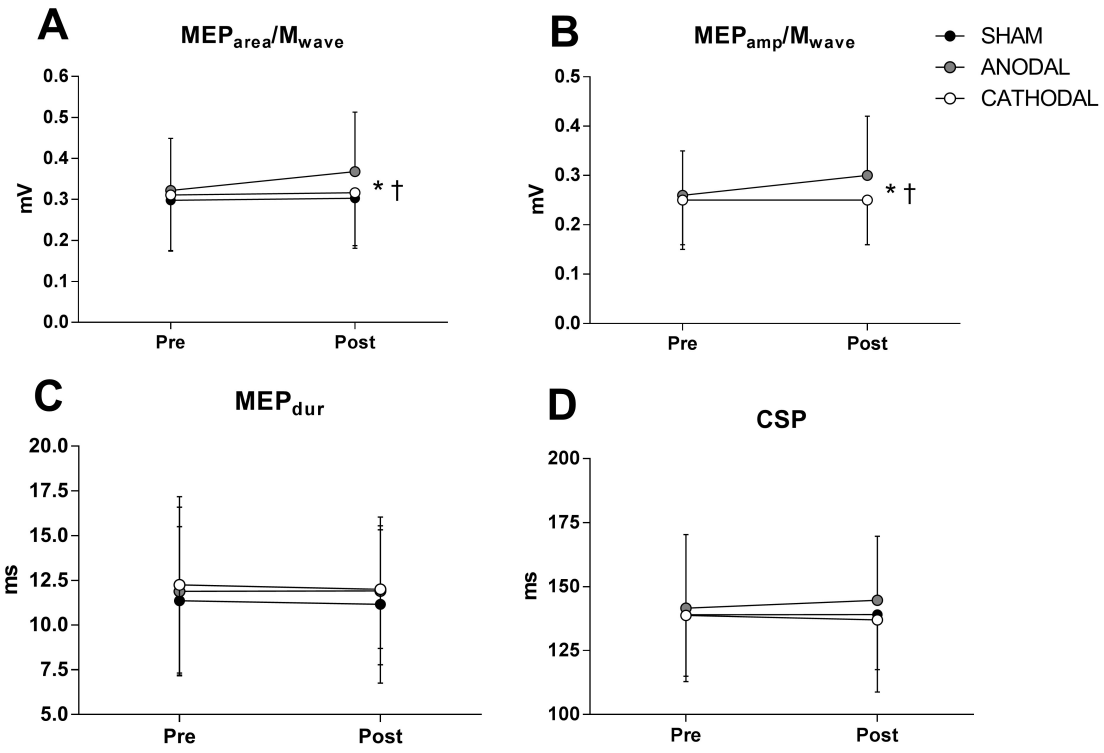

Fig 39. Cortical response before and after tDCS stimulation.

Panel A shows motor evoked potential area (MEP_{area}) muscular wave (Mwave) MEP_{area}/Mwave ratio; Panel B shows MEP peak to peak amplitude (MEP_{amp}) muscular wave (Mwave) ratio MEP_{amp}/Mwave ratio; Panel C shows MEP peak to peak duration (MEP_{dur}); Panel D shows MEP cortical silent period (CSP); * $P < 0.05$, denotes significant difference from CATHODAL and SHAM; † $P < 0.05$, denotes significant condition × time interaction. Data are presented as mean ± SD (n=12).

Discussion

The main aims of the present experiment were to test the hypothesis that bilateral tDCS stimulation of both motor cortices would; 1) change motor cortex excitability; 2) change perception of effort and 3) would alter cycling time to exhaustion performance. This study demonstrated that ANODAL bilateral tDCS stimulation of the M1 increases motor cortex excitability, decreases RPE for a given intensity and improves cycling time to exhaustion performance.

In line with previous experiments, cycling exercise significantly increased HR, B[La^-], RPE and PAIN over time (see chapter 3). As hypothesised, anodal tDCS stimulation improved exercise performance, likely as a consequence of the reduction in RPE. The decrease in RPE was most likely caused by the augmented cortical excitability of the M1, which was demonstrated by the increased MEP response following anodal tDCS stimulation. The increase in MEP response following anodal tDCS has been widely demonstrated in healthy individuals (Nitsche et al., 2007; Nitsche & Paulus, 2000, 2001). Our results suggest that for the same absolute power output, bilateral anodal stimulation of the M1 might facilitate the supraspinal drive, thus reducing the central command required and consequently leading to a reduction of RPE. It has been shown that during open loop exercise (time to exhaustion), supraspinal drive to the motoneuronal pool must increase in order to compensate the reduced force capacity of the muscles (Gandevia, 2001; Taylor & Gandevia, 2008). The neurophysiological link between central command and RPE has been supported by numerous experimental studies (de Morree et al., 2012, 2014; Williamson, 2010). Accordingly, any variations of the intensity of central command is reflected by parallel changes in RPE.

Previous studies detailing how changes to excitability of motor and premotor areas effect effort and force exerted support this supposition. The study of Zenon and colleagues (2015) showed a reduction of perception of effort after disruption of the M1 and somatory-sensory cortex area (SMA) by means of continuous theta burst stimulation (cTBS). Studies involving rTMS further support the link between motor and premotor area excitability and RPE. In particular, two other studies demonstrated that perception of force and effort can be manipulated when activity of the M1 is altered by administration of rTMS (Goodall et al., 2013; Takarada et al., 2014).

Several previous studies have been performed which demonstrate the effect of tDCS prior to exercise. However, major methodological differences between these studies have prevented a firm consensus on its effect. tDCS has been shown to improve isometric endurance performance of the elbow flexor muscles (Cogiamanian et al., 2007; Williams et al., 2013), and anodal stimulation of the left temporal cortex has been shown to improve maximal cycling power (by ~4%) and reduce RPE (Okano et al., 2015). Conversely, other studies have failed to find any improvement in performance (see chapter 3); Kan et al., 2013; Muthalib et al., 2013). The data from the current study suggests that for whole body exercise, an extracephalic set-up, involving 10 min at 2 mA bi-lateral stimulation

of the M1 elicits significant reduction in perceived exertion and a consequent 18% improvement in cycling time to exhaustion.

Our data are in line with, and further support the psychobiological model of endurance performance (Marcora & Staiano, 2010). According to this model, subjects stop exercising when they are not motivated to exercise or when RPE reaches maximal ratings (maximal exertion) (Marcora & Staiano, 2010). Indeed, in the current study, we observed reductions in RPE following anodal stimulation, which permitted participants to exercise longer. This model provides a valid explanation of the effects and importance of both physiological and psychological manipulation on endurance exercise in the absence of any differences in HR or PAIN response between conditions. As expected, HR increased with time until exhaustion, as a consequence of the activation of the central command and exercise pressor reflex (Smith, Mitchell, & Garry, 2006; Williamson et al., 2006). PAIN rose over time, most likely through the accumulation of muscle metabolites produced by exercise (e.g. H^+, K^+, La^- and prostaglandins) and demonstrated by the high B[La^-] obtained after exercise (O'Connor & Cook, 1999). However, no effect of tDCS on exercise induced PAIN were found. B[La^-] obtained in the ANODAL condition was significantly higher compared to other conditions, although this was most likely because of the longer exercise duration.

Neuromuscular response to tDCS stimulation

Neuromuscular assessment was performed as a manipulation check to monitor whether cortical parameters would be affected after tDCS stimulation. We did not find any changes in MVC or VAL following tDCS administration. MVC following tDCS has been previously investigated only in upper-limbs (Cogiamanian et al., 2007; Kan et al., 2013; Lampropoulou & Nowicky, 2013), where in-line with our data, no changes were found. In the current study, as with the MVC data, VAL did not present any change following tDCS stimulation. To the best of our knowledge there are no previous studies where VAL was monitored following tDCS stimulation. According to our data and what has been previously hypothesized by other authors, it is likely that tDCS has no effect on maximal force production and VAL as they are already maximal - the lack of effect of tDCS during maximal expressions of the neuromuscular parameters could thus be explained by the "ceiling effect". Accordingly, in agreement with previous authors, any further increase might not be possible because there is little or no potential improvement in the neuromuscular function in well-rested, normal con-

ditions (Hummel et al., 2005; Kan et al., 2013). In accordance with previous research, we found an increase in MEP response following anodal stimulation, thus suggesting an increase in cortical excitability of the M1. The increase in MEP response has been previously demonstrated following anodal stimulation both in healthy subjects and clinical populations (Nitsche et al., 2007; Nitsche & Paulus, 2000, 2001). MEP parameters have been extensively used as the main index to monitor changes in cortical excitability following tDCS (Nitsche et al., 2007; Nitsche & Paulus, 2000, 2001), and this therefore provides evidence that the tDCS intervention elicited the desired effect over the targeted area (M1).

In line with previous experiments, MEP response of lower limbs was greater after anodal stimulation of the M1 (Jeffery et al., 2007; Tatemoto et al., 2013) which further demonstrate a stimulatory effect of anodal tDCS. Contrarily, Madhavan et al., (2010) found only a modest effect of anodal stimulation on MEP response of the VL, and it was likely caused by the between subject variability.

A notable aside to this finding in the current study is the (lack of) relationship between cortical excitability and VAL. Our data demonstrated that despite an increased MEP response (and thus in cortical excitability), VAL was not affected. Similar findings were discussed by other authors (Gandevia, 2001; Gandevia et al., 1996) but the current data further supports that changes in VAL do not require an alteration of the cortical response, thus demonstrating a complex relationship between the two variables.

No changes in CSP were found following tDCS. CSP duration has been used as an index of intracortical inhibition (Tremblay et al., 2013; Ziemann et al., 2015). Compared to MEP response, relatively little evidence regarding the effect of tDCS on CSP is available, and that which does exist shows conflicting results (Horvath et al., 2014). One study showed an increase in CSP after cathodal stimulation in stroke patients (Horvath et al., 2014; Hummel et al., 2005) while another study did not show any change (Suzuki et al., 2012). Only one study showed a decrease in CSP after 20 min of anodal stimulation (Tremblay et al., 2013). The inconsistency might be related to the stimulation protocol used, muscle investigated and subjects tested, making findings difficult to compare.

Deficiency of cathodal stimulation on neuromuscular function and performance

Cathodal stimulation has been shown to reduce cortical excitability (Nitsche et al., 2007; Nitsche & Paulus, 2000) but contrary to what was expected in our

experiment, cathodal stimulation failed to induce any suppression of the cortical response or reduce exercise performance. Most of the previous tDCS research has monitored the effect of tDCS at rest or during submaximal contraction of the upper limbs or in small muscle groups (Cogiamanian et al., 2007; Kan et al., 2013; Khan, McNeil, Gandevia, & Taylor, 2011; Lampropoulou & Nowicky, 2013; Muthalib, Kan, Nosaka, & Perrey, 2013) which limits our knowledge of the potential effects of anodal and cathodal tDCS on the lower limbs. Speculatively, the lack of diminished cortical excitability following cathodal stimulation might be caused by the different neuroanatomical structure and orientation of the leg motor cortex. Indeed, previous research suggests that the leg motor cortex has less inhibitory circuits, with possibly different neuron orientation compared to the hand motor cortex (Jeffery et al., 2007; Tokimura et al., 2000).

Technical considerations

Our experiment is the first to demonstrate an improvement in constant load cycling performance and an improvement of cortical excitability of the knee extensor muscles as a result of tDCS. Contrary to the previous studies involving unilateral isometric exercise (Cogiamanian et al., 2007; Kan et al., 2013; Khan et al., 2011; Lampropoulou & Nowicky, 2013; Muthalib et al., 2013) our experiment involved the use of both lower limbs which required the stimulation of both motor cortices. The inconsistent findings across studies highlight the importance of optimizing the electrode placement over the scalp. Most notably, the inconsistency between results in previous tDCS experiments is likely largely due to the different electrode montages used. For example, any benefits following anodal stimulation of the M1 might be annulled if the cathodal electrode is placed on the opposite dorsolateral prefrontal cortex. Given the importance of various brain areas in the cognitive and physiological regulation (Carter et al., 1998; McCormick et al., 2015; Williamson, 2010) of exercise, placing the cathodal electrode on shoulder will likely improve experimental outcome and aid comparison between studies.

Conclusion

In conclusion, this study demonstrates that non-invasive anodal tDCS stimulation applied over both sides of the M1 can increase excitability of knee extensors

and improve cycling time to exhaustion performance in a group of healthy participants. Given the ability of the tDCS to target and alter the excitability of a specific brain area, further research should be performed to investigate the role of further brain areas on exercise performance.

CHAPTER 7

GENERAL DISCUSSION

7.0 Overall summary

There is no doubt that exercise performance is regulated by a large number of physiological systems, which differentially contribute according to the task performed. Accordingly, many models which explain endurance performance in these tasks and environments have been proposed. However, for the healthy individual, in normal conditions, over the last twenty years the focus on the systems which regulate exercise has changed. Researchers believed that exercise was mainly limited by the muscles, but recently this assumption has received less of a consensus from researchers. Indeed, a recent study from Marcora et al (2010) demonstrated that muscle fatigue does not solely limit prolonged endurance exercise, but rather that exercise performance is ultimately limited by the perception of effort. Noakes (2011) has also criticized the focus on peripheral factors and states the need to move beyond a brainless model of exercise physiology. It is only relatively recently that exercise sciences are starting to integrate peripheral structures with the brain in order to explain how performance is regulated. The central governor model (Noakes et al., 2005), psychobiological models of endurance exercise (Marcora & Staiano, 2010) and the anticipatory-RPE model (Tucker, 2009) propose that performance is centrally limited and regulated by the brain, and therefore these models are attracting the attention of a large number of people in the scientific community. To provide evidence for the role of the brain, many experiments have been performed involving brain imaging techniques such as fMRI (Scheef et al., 2012; Williamson et al., 1999) or monitoring of brain electrical activity with EEG (de Morree et al., 2012). These methods allow the investigation of how a specific brain area is related to exercise performance. However, although these experiments permit an understanding of which areas are involved during exercise, our knowledge regarding fatigue in whole body exercise is limited due to technical limitations of these devices and the largely correlative nature of the studies. Non-invasive techniques such rTMS and tDCS have been used to monitor the role of specific brain areas during exercise, and provide perhaps more scope to investigate the role of the brain as they provide the opportunity to perform an experimental manipulation of a brain area.

The main aim of the thesis was to investigate the effect of tDCS on exercise performance. Considering the recent interest and applications of tDCS in exercise science, this thesis represents a significant contribution in our understanding of the physiological mechanisms underlying tDCS and exercise. The studies per-

formed for this thesis have applied the most documented and reliable techniques present in the scientific literature to test and investigate the effect of tDCS on exercise performance. Therefore, underpinned by established mechanistic procedures, the experimental findings provided in these studies have further explored and explained part of the physiological mechanisms and potential benefits of tDCS on exercise performance. As a result, this thesis represents original work that makes a significant contribution to knowledge in this area of study.

In the Chapter 3, the experimental study examined the effect of tDCS on exercise-induced muscle pain and pain during a cold pressor test. Although tDCS is well recognized as a non-invasive technique to relieve pain (Boggio et al., 2008; Lefaucheur et al., 2008; Zandieh et al., 2013), this study was the first to investigate the potential analgesic effect of tDCS on exercise-induced muscle pain. Unlike other techniques, this approach reduces many methodological constraints which might confound experimental outcomes and thus permits a better isolation of the effect of pain on exercise. We found that the M1 tDCS montage does not induce any analgesic effect during high intensity cycling exercise, but reduces pain perception during a cold pressor test. Given the high accumulation of metabolites caused by the high intensity of exercise, an improvement in cycling performance was initially expected. However, the lack of efficacy of tDCS on exercise-induced pain perception resulted in no changes in performance. The different observed effect on perceived pain between the TTE and CPT task in this study provided an interesting insight regarding the application of tDCS and the psychological and physiological mechanisms involved in the generation of pain perception. This study further confirmed that this M1 tDCS montage effectively reduces pain, as confirmed in previous experiments (Boggio et al., 2008; Lefaucheur et al., 2008). Secondly (and more interestingly), the montage used for this experiment does not appear to reduce the perception of exercise-induced pain. Third, this study highlights that because of the complexity of whole-body exercise, the generation of pain during this is a complex psychophysiological process that deserves further investigation to understand its regulation. Consequently, the discrepancy of pain response following tDCS between the two tests is potentially contributed to by the direct attention to the painful stimuli (in the CPT) (Linton & Shaw, 2011). In support of this, the lack of analgesic effect of tDCS on exercise-induced pain was confirmed in the second experimental study, where with the same montage showed no effect during single leg isometric contraction. These findings suggest that tDCS of the M1 does not seem to moderate exercise-induced muscle pain in healthy subjects.

The study performed in Chapter 3 was important in terms of tDCS application for exercise science. It demonstrates that unlike single joint exercise, the use of a cephalic montage on whole body exercise might not be beneficial in terms of performance. This is partly caused by the potential negative effect of the cathodal electrode placed on the contralateral prefrontal area. Therefore, any benefits of anodal stimulation over the motor cortex might be negated by the cathodal electrode. Secondly, stimulating only one side of the motor cortex elicits a neural effect on only the contralateral limb. Therefore, an extracephalic montage with the cathodal electrodes placed on the shoulders and anodal stimulation on both motor cortex might be more appropriate for whole body exercise such as cycling, where both limbs are involved. It was decided that these possibilities would be followed up in the subsequent and final studies of this thesis.

The aim of the study performed in Chapter 4 was to compare two different tDCS montages in order to define the optimal montage to be applied in single limb exercise. A comparison of tDCS montages was necessary given the potential limitations of the cephalic montage, as shown in the previous Chapter. Furthermore, the decision to investigate the effect on lower limbs was important as it is the first step to exploring the application of tDCS on whole body exercise. Based on the results, the extracephalic montage seems to provide the best outcome in terms of performance and possibly confirms the negative effect of the cathodal electrode on another brain area. Maximal force capacity did not present any improvement following tDCS and therefore it seems that tDCS seems to provide effect on submaximal contraction rather than maximal. A significant reduction in perception of effort was observed together with an improvement in performance, which is likely caused by a facilitation of the supraspinal drive from motor cortex and therefore a perception of the exercise as easier. Accordingly, the extracephalic montage should be more appropriate for whole body exercise. The results of this study provide important methodological direction in developing an appropriate montage for the application of tDCS for exercise, and these findings were applied to whole body exercise in the final study of this thesis.

The study performed in Chapter 5 investigated the effect of tDCS on hemodynamic response following stimulation of the brain centres related to autonomic cardiovascular control. Hemodynamic response was shown to not be affected following anodal stimulation of both cortical areas. Despite the null result, this study further provided important knowledge regarding the effect of non-invasive brain stimulation on the cardiovascular response. Unlike previous experiments (Okano et al., 2015; Vandermeeren et al., 2010) cardiovascular response was mon-

itored using functional parameters, rather than heart rate spectrum frequency, and furthermore, this response was monitored at rest, during exercise and during post exercise muscle ischemia. This approach is of particular interest to sport scientists as these findings exclude the possibility of impairment (or facilitation) of the cardiovascular response when the temporal cortex is stimulated. The use of tDCS in the treatment of pathologies affecting the neural control of circulation has been proposed (Cogiamanian et al., 2010). It might be possible that in the presence of anatomical or functional lesions affecting the functionality of brain areas involved in the cardiovascular control, tDCS might have positive impact and potentially applied as therapy. However, given the lack of experiments in this area, further study is recommended to understand the application of tDCS to manipulate the cardiovascular response in clinical populations. cardiovascular response was monitored using functional parameters, rather than heart rate spectrum frequency, and furthermore, this response was monitored at rest, during exercise and during post exercise muscle ischemia. This approach is of particular interest to sport scientists as these findings exclude the possibility of impairment (or facilitation) of the cardiovascular response when the temporal cortex is stimulated. The use of tDCS in the treatment of pathologies affecting the neural control of circulation has been proposed . It might be possible that in the presence of anatomical or functional lesions affecting the functionality of brain areas involved in the cardiovascular control, tDCS might have positive impact and potentially applied as therapy. However, given the lack of experiments in this area, further study is recommended to understand the application of tDCS to manipulate the cardiovascular response in clinical populations.

The experimental study performed in Chapter 6 developed and applied some of the main principles obtained from the results obtained in Chapter 4, by using the extracephalic montage on both motor cortices. In order to examine the role of motor cortex excitability and its implication in the generation of perception of effort, anodal and cathodal stimulation were administered which would bring about a hypothesized increase and decrease respectively excitability of the M1. By improving cycling performance and decreasing perception of effort following anodal stimulation, the results of this study are hypothetically the most significant of the entire thesis. The results of these studies are in agreement with previous experiments manipulating the excitability of the motor cortex (Goodall et al., 2013; Takarada et al., 2014; Zénon et al., 2015), and further provide evidence in favour of the corollary discharge model for the generation of perception of effort. Furthermore, the relationship between changes in performance with a

parallel change in perception of effort strongly support the psychobiological model of endurance performance (Marcora & Staiano, 2010; Marcora et al., 2009). As previously proposed from other experiments (Marcora & Staiano, 2010; Marcora et al., 2009) the relationship between these two variables further support the importance of RPE in open loop exercises. The results of this experiment successfully demonstrated that whole body performance can be improved following brain stimulation. Nevertheless, more experiments should be performed to understand the neurophysiological and psychological mechanisms of tDCS during exercise.

tDCS, performance and perception of effort

In Chapter 4 and 6, the cortical and neuromuscular response was analysed following tDCS stimulation. Taken together, the findings from these studies (study 2 and 4) demonstrated that anodal tDCS improves both isometric and dynamic exercise performance in healthy individuals. However, tDCS seems to exert beneficial effects only on submaximal contraction, and not on maximal contraction, likely because these parameters are already maximal and any further increase is not achievable. Alongside the improved TTE, a significant reduction in RPE was also observed. tDCS has been demonstrated to increase or decrease resting membrane excitability of the targeted area according to the type of stimulation applied. It is possible that by reducing the resting membrane potential, the threshold required to depolarize the cell was lowered, therefore facilitating this chemical process (see Fig 40). An opposite effect would be hypothesized to occur following cathodal administration. However, as discussed in the previous chapters, the lack of effect of cathodal stimulation is possibly caused by the fewer inhibitory circuits or a different orientation of neurons in the leg compared to hand motor cortex (Jeffery et al., 2007; Tokimura et al., 2000). Considering the very limited research on this topic, further studies should be performed in this area.

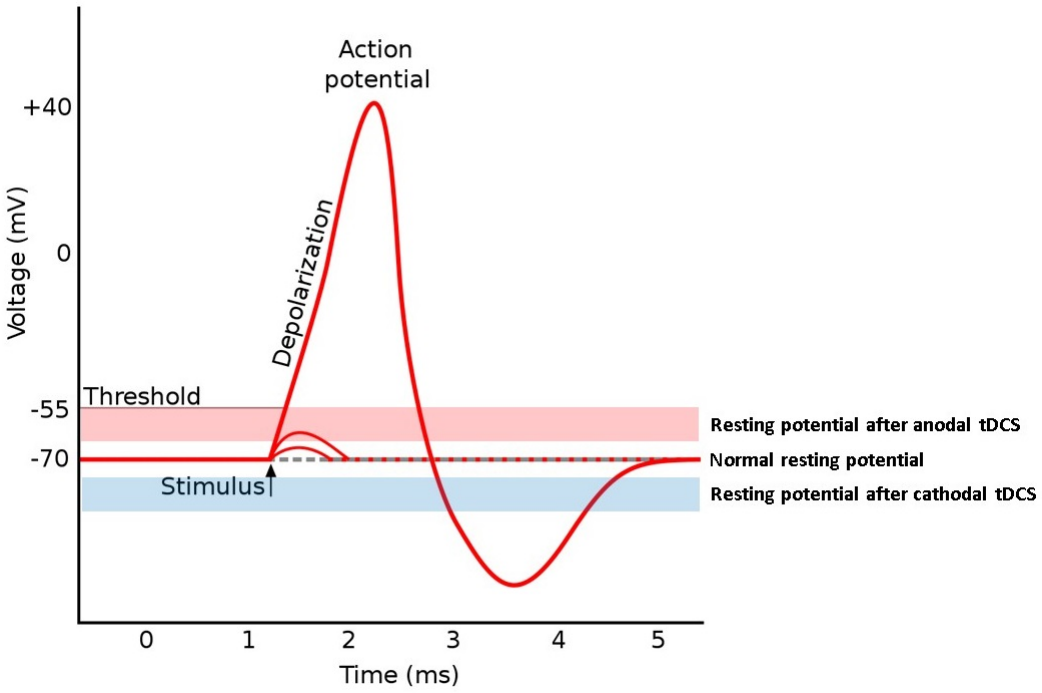

Fig 40. Effect of tDCS on resting membrane potential.

The picture shows the change in resting membrane potential following anodal tDCS (red) and cathodal stimulation (blue). The stimulus required to elicit a depolarization differs according to the stimulation applied.

In contrast, a significant change in cortical activity and perceptual response during exercise following anodal administration was observed. During submaximal contraction, it is likely that anodal stimulation facilitated the supraspinal drive, therefore requiring less central command for the same absolute force expressed (see Fig 41). In terms of exercise benefits, this was demonstrated by a significant decrease of RPE and increase in task duration. According to the data in this thesis, and previous experimental findings, this is the most reasonable explanation for the improvement in performance following anodal tDCS. However, given the recent interest in this field and the limited experimental research, further studies should be performed to understand the physiological mechanisms.

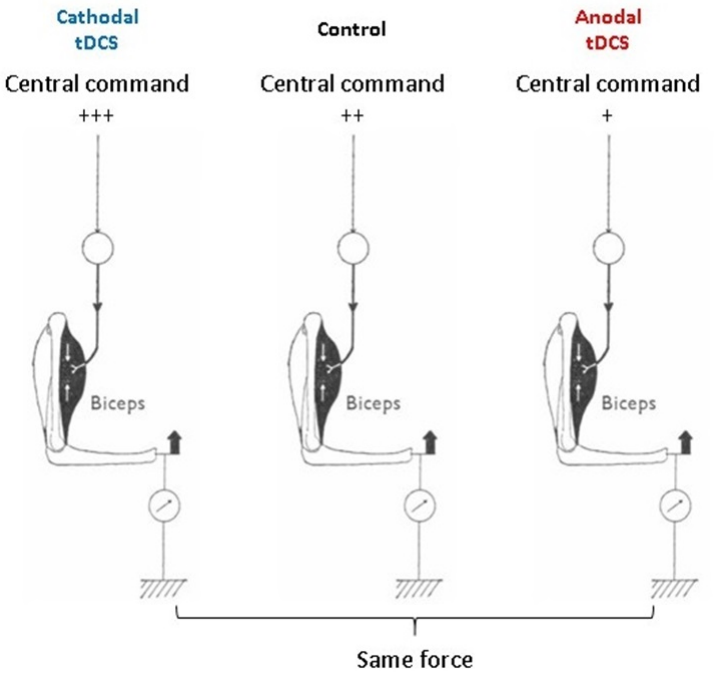

Fig 41. Hypothetical effect of tDCS on central command. Adapted from (Goodwin, McCloskey, & Mitchell, 1972).

The picture on the left shows the central command required to maintain the force after cathodal stimulation where motor cortex excitability is decreased, the magnitude of the central command is assumed +++. The picture in the center shows a control condition where no tDCS is applied over motor cortex, the magnitude of the central command is assumed +++. The picture on the right shows the central command required after anodal stimulation, the magnitude of the central command is assumed +.

It should be taken into account that the neuromuscular tests used for this thesis differ from the classic tests performed in tDCS experiments. This difference is due to the purpose of the test and the nature of the tasks performed. In classic tDCS experiments, TMS over motor cortex has been used as manipulation check to monitor the level of cortical excitability, by analysing the MEP response at rest or during brief submaximal contractions. In exercise science research however, the neuromuscular assessment is also performed during or after fatiguing tasks. These type of experiments require the normalization of the MEP response to the Mwave in order to take into account the peripheral factors influencing EMG signal. This approach permits a better understanding regarding the effect of tDCS on the neuromuscular system during exercise, and so the methods used in this thesis are the most appropriate in this context.

As proposed in previous chapters, the reduction in RPE during exercise could be the consequence of a change of the central command required to produce the same force. Since we did not measure brain activity during exercise we cannot provide any specific information on such a possible mechanism. Thus, the lack of knowledge regarding brain activity following tDCS during exercise might represent a limitation for this thesis and in the understanding of the underlying mechanism of tDCS.

It should be taken into account that experiments involving brain imaging such as fMRI or monitoring brain electrical activity by EEG present some limitations regarding the type of exercise that can be performed. Indeed, during dynamic whole body exercise, head motion must be minimised to avoid interference with neuronal activation patterns and the equipment used. To do so, an atypical body position (e.g. cycling in supine position) has been necessarily adopted which unfortunately does not replicate the normal exercise pattern and might potentially affect RPE. In light of the present limitations and lack of knowledge, further research and new integrative methodologies should be implemented to investigate the effect of tDCS on brain activity during exercise.

7.1 Conclusion and perspectives

The effect of brain stimulation techniques on exercise is beginning to attract considerable attention in exercise science. Considering the very limited amount of research regarding tDCS on exercise sciences, each study in this thesis potentially represents a substantial contribution in this field. By integrating all the experiments, this thesis provides new insights regarding the potential benefits of tDCS during exercise.

The role of supraspinal sites in the development of fatigue has been widely investigated, but only recently have research investigations focused on manipulating the activity of these areas to study and understand their importance on exercise. Few of these studies are designed to also explain the potential mechanisms underpinning any observed effect however. The studies of this thesis are designed in such a way as to demonstrate an effect and the underpinning mechanism.

Unlike previous experiments where tDCS has been applied on upper limbs (and therefore involving only small muscle groups), the current studies have largely focused on the application of tDCS on lower limbs. Therefore, these are the first studies to demonstrate the application and potential benefits of tDCS for whole body exercise tasks, which is more relevant both for exercise performance and the potential utility in clinical populations. One of the most important findings of the thesis is that anodal tDCS over the motor cortex is able to improve both dynamic and isometric performance of lower limbs. In particular, study two and four highlighted some interesting questions regarding the importance of motor cortex behaviour in the generation of perception of effort. Therefore, further research should be performed to understand the neurophysiological factors involved in the generation of perception of effort, and how tDCS may be used to moderate this.

The experimental findings provided from this thesis further demonstrate the potential applications of tDCS not only as method to enhance physical performance but also a method investigate important aspects of specific brain areas in exercise regulation and its role on exercise induced fatigue.

An important key concept provided in this thesis is the ability of tDCS to reduce the perception of effort and hence increase exercise capacity. According

to what was found in the study in Chapter 6, tDCS stimulation might be also be beneficial for enhancing athletic performance in self-paced exercise such as cycling time trials.

Repetitive tDCS sessions have been used and accepted as co-therapy for the treatment of various neurological diseases. To date there are no studies investigating the effect of multiple tDCS sessions on whole body exercise capacity either in healthy or clinical populations. According to what demonstrated in this thesis, multiple tDCS sessions might induce more benefits than a single session to improve exercise capacity. This approach might be advantageous in clinical populations to increase exercise adherence and improve the quality of life. In this particular population, the sensation of fatigue is common across a wide variety of neurological disorders and therefore it is plausible that a multiple tDCS intervention might beneficial in the reduction of exercise induced fatigue.

Unfortunately, the promising and interesting outcomes of tDCS on exercise performance have recently attracted various sport teams and companies specialised in neuroscience to develop tDCS stimulators for sport and domestic purposes. Unlike TMS equipment, tDCS devices are relatively small and easy to use and therefore an abuse by people not aware about its effects and application is likely. Since most of the literature is based on laboratory or clinical research, more work needs to be performed to explore its other potential applications.

In conclusion, this thesis provides an interesting and valuable advancement regarding the use of tDCS in exercise, but further studies are necessary to further investigate and understand the neurophysiological mechanisms involved following tDCS on exercise.

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Appendices

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Poster comunications

Transcranial current direct stimulation reduces cold pain perception but not acute muscle pain

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Stimulation of muscle pain receptors by release of algesic substances during high intensity exercise is the cause of acute muscle pain. Peripheral signals are processed in the brain and then perceived as pain sensation. Some authors have proposed that an athletes' ability to tolerate exercise-induced muscle pain could represent an important factor in long lasting, high intensity exercise (5, 6). Non-invasive techniques such as the transcranial direct current stimulation (tDCS) have been previously shown to relieve pain perception (1, 4), and so we investigated whether tDCS administration would lead to an improvement in exercise performance. Pain response was monitored during exercise (PAIN-EXE) and a cold pressor test (CPT), (PAIN-CPT) in two separate studies (A and B respectively). In study A, following full ethical approval, 9 participants performed a cycling time to exhaustion (TTE) at a 70% of their peak power output while in study B, 7 subjects underwent a CPT with an 8 min cut-off time. Both studies involved a control (CON), placebo (SHAM) and experimental (tDCS) session in a single-blind, randomised, counter-balanced design. tDCS stimulation for 10 min at 2.0 mA was delivered by placing anodal electrode above the left motor cortex (M1) with the cathodal electrode placed above dorsolateral right prefrontal cortex (1). Ratings of perceived exertion (RPE) were monitored during the TTE using Borg 6-20 scale. PAIN-EXE and PAIN-CPT were assessed using the 10 points numerical Cook scale (3). An isotime of 6 min, plus the final min, for both the TTE and CPT were used in order to include all participants in the subsequent analyses. A one-way ANOVA with repeated measures was used to assess TTE duration. Two-way ANOVA with repeated measures was used to analyse RPE, PAIN-EXE and PAIN-CPT data. All data are presented as means $\pm$ SD in Fig. 1.No significant differences ($p>0.05$) in exercise duration, RPE and PAIN-EXE were found in the TTE. However, PAIN-CPT in the tDCS session was significantly lower ($p<0.05$) compared with the other conditions (5.6 $\pm$ 2.8

CON, 6.0 ± 3.0 SHAM, 5.5 ± 2.7 tDCS).These findings demonstrate that tDCS is capable of inducing an analgesic effect in response to cold pain stimuli but not for exercise-induced muscle pain.

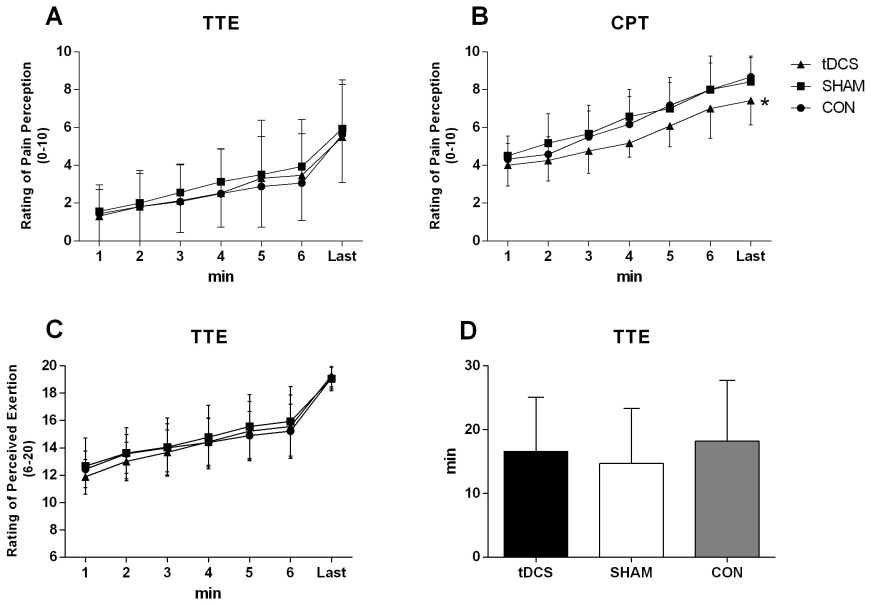

Fig 1. Time courses of rating of pain perception during CPT and TTE (panel A and B). Panel C shows time courses of RPE while panel D shows TTE duration. Values are presented as mean ± SD. * p< 0.05 respect to CON and SHAM condition.

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Oral comunications

Transcranial direct current stimulation improves isometric time to exhaustion performance of lower limbs

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Supraspinal fatigue is defined as the inability of the motor cortex (M1) to produce an adequate neural drive to excite and drive motoneurons adequately, and could contribute to the decrease in force production capacity (2). Recently, research studies have applied the use of transcranial direct current stimulation (tDCS) to manipulate corticospinal excitability in order to improve endurance performance (1). These interventions can be inhibitory (cathodal) or excitatory (anodal). Since there is no consensus on the standard placement of electrodes for improving endurance performance, we therefore tested the effect of two electrodes configurations. Nine subjects underwent a control (CON), placebo (SHAM) and two different tDCS configurations sessions in a double blind, randomised and counterbalanced design. In one tDCS session, the anodal electrode was placed over the left M1 and the cathodal on contralateral forehead (HEAD) while for the other montage, the anodal electrode was placed over the left M1 and cathodal electrode above the contralateral shoulder (SHOULDER). tDCS was delivered for 10 min at 2.0 mA, after which participants performed an isometric time to exhaustion (TTE) of the right knee extensors at 20% of the maximal voluntary contraction (MVC). Peripheral and central parameters were examined respectively by femoral nerve stimulation and M1 excitability via TMS at baseline, after tDCS application and immediately after TTE. Heart rate (HR), ratings of perceived exertion (RPE), and leg muscle PAIN were monitored during the TTE. A one-way ANOVA with repeated measures was used to assess TTE duration, while two-way ANOVA with repeated measures was used to analyse central and peripheral parameters, HR, PAIN, and RPE. None of the central and peripheral parameters showed any difference between conditions after tDCS stimulation

($p>0.05$). MVC significantly decreased after TTE ($p<0.05$) due to presence of central and peripheral fatigue, whilst motor evoked potential area (MEP) and cortical silent period increased after TTE ($p<0.05$) independently of the experimental condition. TTE was longer in the SHOULDER condition ($p<0.05$) although HR and PAIN did not present any difference between conditions ($p>0.05$). However, RPE was significantly lower in the SHOULDER condition ($p<0.05$). This is the first study showing an improvement of isometric TTE performance of the lower limbs after tDCS stimulation and further demonstrates that anodal tDCS over M1 improves isometric endurance performance of the knee extensors. Our findings suggest that SHOULDER montage is more effective than HEAD montage to improve endurance performance.

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ORIGINAL ARTICLE

The effect of transcranial direct current stimulation of the motor cortex on exercise-induced pain

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Abstract
Purpose Transcranial direct current stimulation (tDCS) provides a new exciting means to investigate the role of the brain during exercise. However, this technique is not widely used in exercise science, with little known regarding effective electrode montages. This study investigated whether tDCS of the motor cortex (M1) would elicit an analgesic response to exercise-induced pain (EIP).
Methods Nine participants completed a VO_{2max} test and three time to exhaustion (TTE) tasks on separate days following either 10 min 2 mA tDCS of the M1, a sham or a control. Additionally, seven participants completed 3 cold pressor tests (CPT) following the same experimental conditions (tDCS, SHAM, CON). Using a well-established tDCS protocol, tDCS was delivered by placing the anodal electrode above the left M1 with the cathodal electrode above dorsolateral right prefrontal cortex. Gas exchange, blood lactate, EIP and ratings of perceived exertion (RPE) were monitored during the TTE test. Perceived pain was recorded during the CPT.
Results During the TTE, no significant differences in time to exhaustion, RPE or EIP were found between conditions. However, during the CPT, perceived pain was significantly ($P < 0.05$) reduced in the tDCS condition (7.4 ± 1.2) compared with both the CON (8.6 ± 1.0) and SHAM (8.4 ± 1.3) conditions.
Conclusion These findings demonstrate that stimulation of the M1 using tDCS does not induce analgesia during exercise, suggesting that the processing of pain produced via classic measures of experimental pain (i.e., a CPT) is different to that of EIP. These results provide important methodological advancement in developing the use of tDCS in exercise.

Keywords Fatigue · Pain perception · Performance · tDCS · Exercise

Abbreviations

$B[La^{-1}]$	Blood lactate concentration
CON	Control condition
CPT	Cold pressor test
DLPFC	Dorsolateral prefrontal cortex
EIP	Exercise-induced pain
EXP	Experimental condition (tDCS intervention)
M1	Motor cortex
RPE	Rating of perceived exertion
tDCS	Transcranial direct current stimulation
TTE	Time to exhaustion

Introduction

Pain experienced during high intensity exercise is commonly believed to originate as a consequence of accumulation of muscle metabolites (e.g. H+, potassium, lactate and prostaglandins), produced as a result of anaerobic resynthesis of ATP (O'Connor and Cook 1999). Peripheral muscle nociceptors that detect exercise-induced metabolites are generally classified as group III and IV muscle afferents. These afferents originate from lower limbs, ascend via the dorsal columns and then project to various cortical and

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subcortical brain regions such as somatosensory cortex and ventroposterior lateral nucleus of the thalamus (Almeida et al. 2004; Brodal 1981), where the signal is then perceived as pain. The contribution of this exercise-induced pain to exercise performance has received little attention in experimental research (Mauger 2013). However, the wider contribution of afferent feedback, which rises in proportion of the metabolic demand, combined with multiple psychological and physiological systems (Noakes 2012; St Clair Gibson and Noakes 2004), has created significant debate and complexity regarding the understanding of endurance performance. It is difficult to uncouple afferent feedback and pain, as both travel through Type III and IV afferents, which may explain the limited number of studies which focus solely on changes in pain during exercise. In an attempt to explicate the role of afferent feedback (i.e., not pain specifically) in both regulation of work rate in self-paced exercise (Amann et al. 2009) and time to exhaustion tasks (Amann et al. 2011), a recent series of studies have used the opioid agonist fentanyl to prevent afferent feedback signals to reach cortical areas. As it has been suggested that afferent feedback limits central motor drive (Amann et al. 2011), blocking afferent feedback should result in an unimpaired central motor drive and a subsequent improvement in performance. However, because afferent feedback plays an important role for cardiovascular regulation (Kaufman 2012), performance in a time to exhaustion task was impaired (Amann et al. 2011) and performance in time trial type tasks was no different (Amann et al. 2009) after administration of fentanyl in these studies.

Whilst the studies of Amann et al. (2009, 2011) demonstrate the importance of afferent feedback for cardiovascular regulation during exercise, they are not able to explain how pain contributes to performance. Concomitant with afferent feedback during intense exercise is the stimulation of muscle nociceptors and the subsequent perception of pain and discomfort. This exercise-induced pain has been suggested to play an important role in work rate selection and thus consequently affect endurance performance (Mauger et al. 2010; Mauger 2014). However, as the sensation of pain during exercise is not only reliant on the noxious peripheral stimuli from skin and muscle nociceptors, but also the processing of this input in the primary sensorimotor cortex, secondary somatosensory cortex, anterior insular and cingulate cortex and thalamus (O'Connor and Cook 1999; Olesen et al. 2012), the effect of pain on endurance performance can be assessed by blocking the input or moderating the processing of it. Thus, many of the methodological difficulties associated with complete blockade of afferent feedback can be avoided or reduced. Several interventions that alter (i.e., increase or decrease) the sensation of pain at a peripheral level (moderating the pain signal before it reaches the brain) have been used to test this theory. These include: cuff occlusion of the exercising legs (Hollander et al. 2010), administration of analgesics (Mauger et al. 2010, 2014) and administration of algesic substances (Khan et al. 2011). However, studies which investigate the role of pain by reducing/increasing feedback during exercise might still present some methodological constraints (Mauger 2013). Therefore, methods which solely alter the central processing of pain would provide a useful means by which the pain–performance relationship can be tested.

In recent years, non-invasive modulation of cortical areas related to brain processing have been developed to relieve pain (Boggio et al. 2008), and thus provide a targeted method of inducing analgesia during exercise. Transcranial direct current stimulation (tDCS) provides a reliable, safe, non-pharmacological and non-invasive way to alter excitability of a targeted brain area (Nitsche et al. 2008), and therefore moderate the manner in which a given area of the brain processes a stimuli. The process of tDCS involves the passage of a weak electrical current through the brain between two electrodes, which can then alter resting membrane potential and consequently excite or inhibit the targeted brain area. Consequently, a targeted area of the brain can be moderated using a fully placebo controlled design, and avoiding unwanted side effects (such as disruption of the exercise pressor reflex). The benefits of this technique in the treatment of pain both in clinical populations and in healthy volunteers are well accepted (Boggio et al. 2008; Lefaucheur et al. 2008). However, because the processing of pain in the brain is complex, and will often depend on the type of pain experienced, the optimal tDCS electrode set-up for various types of pain is yet to be elucidated. Much of the tDCS pain research uses classical implementation of experimental pain (such as a cold pressor test) to assess analgesic efficacy, and for this type of pain, anodal tDCS of the M1 and cathodal over the contralateral prefrontal cortex proves most effective (Bachmann et al. 2010; Lefaucheur et al. 2008; Zandieh et al. 2013). In support of this M1 tDCS montage, studies which have monitored cerebral blood flow using positron emission tomography (PET) during motor cortex stimulation demonstrate that this stimulation indirectly effects pain areas such as thalamic and sub-thalamic nuclei (García-Larrea et al. 1997, 1999), and produces an overall analgesic effect. Consequently, as tDCS is only able to directly stimulate areas of the brain which are closer to the scalp, an electrode montage which stimulates the M1 may be able to indirectly moderate deeper brain areas involved in the processing of exercise-induced pain.

Processing of pain arising from a CPT primarily involves the thalamus, and specifically the ventral medial nucleus, which cortically projects to the insula and provides a specific network for the processing of thermal pain (Craig et al. 1994). However, there is also likely to

be a significant level of psychological processing, involving arousal, attention, memory, emotion and evaluation in response to CPT pain (Chen et al. 1998), which will involve cross processing in a number of different brain areas. Although brain mapping of particular areas involved in exercise-induced pain processing is yet to be attempted, it has been suggested that the primary sensorimotor cortex, secondary somatosensory cortex, anterior insular and cingulate cortex and thalamus are all involved (O'Connor and Cook 1999). When muscle pain has been experimentally induced, increased activation of the thalamus and basal ganglia has been reported (Peyron et al. 2000; Svensson et al. 1997; Wardman et al. 2014) showing an "overlap" of central processing of muscle pain and cold pain in the brain. Similarly to cold pain, because exercise also involves a multitude of other psychological processes, it is likely that mood, emotional and memory constructs also form an important part of EIP processing.

Although tDCS of the M1 likely provides some analgesic effect to experimental pain, it should be recognized that moderation of a brain area may cause a number of secondary effects. As the M1 is involved in instigating muscle contraction, excitability changes in this area may elicit motor effects which may alter exercise performance. Whilst there appear to be some positive effect for tDCS stimulation of the M1 on fine movements in small muscle groups (Reis and Fritsch 2011), its effect on exercise performance in the upper limbs remains equivocal (Lampropoulou and Nowicky 2013; Cogiamanian et al. 2007). There are currently no studies investigating the effect to tDCS stimulation of the M1 on exercise using the lower limbs.

Therefore, the aims of the current study were (1) to monitor whether the effect of a well-established analgesic tDCS intervention could reduce pain perception during a fixed high intensity cycling task, and (2) whether tDCS-induced analgesia would improve cycling time to exhaustion. As this tDCS intervention has been shown to reduce experimental pain, it was hypothesized that pain during exercise would be reduced and that this would consequentially improve cycling time to exhaustion.

Methods

Subjects

This investigation consisted of two separate studies (Part A and Part B). In the first study (Part A), 9 healthy recreationally active males (age: 23 ± 4 year, height: 179.7 ± 8.2 cm, mass: 75.4 ± 9.9 kg, $\dot{V}O_{2max}$: 48 ± 7 mL min^{-1} kg^{-1}) were recruited, while in the second study (Part B) 7 healthy recreationally active males (age: 23 ± 4 year, height: 179.7 ± 6.8 cm, weight: 75.11 ± 9.94 kg) were recruited. Six subjects participated in both studies. Each participant gave their written informed consent and was informed about the procedures of the study but not of the aims and hypothesis. Consent forms were approved by the School of Sport and Exercise Sciences local Ethics Committee (University of Kent). The present investigation was conducted according to the standards set by the World Medical Association of Helsinki. None of the volunteers had any history of cardiac or respiratory disease or were taking any medication at the time of the study. Tests were conducted at the same time of the day for each volunteer in a temperature-controlled room (20 °C, relative humidity 50 %). All participants refrained from intense exercise (48 h), alcohol (48 h), caffeine (6 h) and analgesic ingestion (6 h) prior to each visit.

Experimental design

Part A

Each participant visited the laboratory on 4 occasions, each separated by at least 48 h, but no more than 5 days.

Visit 1 The purpose of this visit was to familiarize the participants with all the procedures performed during the experimental protocol. In the same visit, they performed an incremental test on cycle ergometer (Lode, Excalibur Sport, Groningen, Netherlands) to establish maximal oxygen uptake ($\dot{V}O_{2max}$) and peak power output (W_{max}). After a 5-min warm up, the protocol started at 100 W and increased 5 W 15 s^{-1} until exhaustion (i.e., the incapacity to maintain the cadence above 60 rpm). $\dot{V}O_{2max}$ was considered as the attainment of at least two of the following criteria: (1) plateau of $\dot{V}O_2$ despite in workload (<80 mL min^{-1}), (2) respiratory exchange ratio (RER) above 1.10, and (3) heart rate (HR) within ±10 bpm of predicted maximum heart rate (calculated as 220—age). Following a 30-min rest period, participants completed a familiarization of the same time to exhaustion task that would be completed in the experimental visits.

Visits 2–4 Using a double-blind and randomized according to balanced permutations design, participants underwent a control (CON), placebo (SHAM) and experimental (EXP) session. They underwent 10 min of tDCS administration in the experimental (EXP) and SHAM tDCS (SHAM) condition, respectively (see "Transcranial direct current stimulation procedure"), while during the control condition, the participant was seated in a chair for 10 min. Two minutes after tDCS administration or control, participants performed a 5-min warm up at 100 W on the cycle ergometer, and then a time to exhaustion (TTE) at 70 % of W_{max} until they were unable to maintain their cadence above 60 rpm for more than 5 s.

During the incremental test (visit 1) and TTE tests, respiratory variables were monitored by an automated gas analyser (Cortex Metalyser 3B, Cortex GmbH, Leipzig, Germany), and heart rate (HR) by a telemetric device (Polar, FS1, Birmingham, United Kingdom). A 20-µl capillary sample of whole blood was taken at rest and immediately at the end of the TTE by pricking the volunteers' right thumb, collected blood was subsequently analyzed for lactate concentration (B[La^-]) by a laboratory lactate analyser (Super GL2, Dr. Müller Gerätebau, Germany). Rating of perceived exertion (RPE) was monitored during the TTE using Borg 6–20 scale (Borg 1998). Exercise-induced pain perception during the TTE was assessed using the validated 10-point numerical Cook scale (Cook et al. 1997). RPE and pain were recorded at predetermined intervals (varying between 1 and 3 min) so that knowledge of elapsed time would not affect participants reporting of these values.

Part B

Each participant visited the laboratory on 4 occasions, each separated at least by 48 h, but not more than 5 days.

Visit 1 The purpose of this visit was to familiarize the participants with the cold pressor test performed during the subsequent visits.

Visits 2–4 Using a single-blind, randomized, counterbalanced design; participants underwent a control (CON), placebo (SHAM) and experimental (EXP) session. They underwent the same tDCS procedures performed in Part A (see "Transcranial direct current stimulation procedure"). During these visits, participants underwent a cold pressor test (CPT) to investigate the effect of tDCS administration on pain perception and thus demonstrate that the tDCS set-up used in this study elicited an analgesic effect (manipulation check). Participants submerged their right hand into a container filled with iced water at a temperature between 0 and 1 °C, which was kept consistent between visits (±0.1 °C). During the measurements, participants were required to circulate their hand around the water to prevent the development of a microclimate around the skin. After each elapsed minute, participants were asked to report their perception of pain on a 10-point numerical scale (Cook et al. 1997). They were told to withdraw their hand from the water when the pain became too much to tolerate. If the participant had not already withdrawn their hand from the water, the experimenter terminated the test after 8 min had elapsed to prevent cold-induced damage. The participants were not aware of the 8-min cut-off time. During the CPT task, the participants faced a plain wall, with the experimenter standing out of sight and offering no encouragement in order to prevent any experimenter bias.

Transcranial direct current stimulation procedure

tDCS was delivered by a direct current stimulator (TCT Research Limited, Hong Kong) using a pair of rubber electrodes in a 4 × 3 cm water-soaked synthetic sponge. One electrode (anodal) was placed over the left motor cortex (M1) whereas the other electrode (cathodal) was placed above dorsolateral right prefrontal cortex (Boggio et al. 2008; Zandieh et al. 2013). Electrode positioning was made according to the 10–20 system for EEG placement to replicate the exact position for both experiments. This electrode montage has been previously shown to elicit an analgesic effect to experimentally induced pain (Boggio et al. 2008; Zandieh et al. 2013). In the experimental session, the current was applied with an intensity of 2.0 mA for 10 min, whereas during the SHAM session stimulation lasted 30 s and subsequently ramped down to no stimulation. This induced the slight itching sensation which is commonly experienced during tDCS at the beginning of the stimulation, but has been shown to produce no cortical changes (Boggio et al. 2008; Mylius et al. 2012). Participants were blinded as to the polarity of tDCS and the SHAM and EXP conditions. Following the study, participants stated that they were unable to tell the difference between the EXP and SHAM conditions.

Statistical analysis

All data are presented as mean ± SD. An isotime of 6 min plus the final min for both the TTE and CPT were used to include all participants' data in the subsequent analyses. Furthermore, RPE and pain during TTE were analyzed by 0, 25, 50, 75 and 100 % of total time. Gas and HR were averaged for each min during the TTE. Time to exhaustion duration and B[La^-] were assessed by using one-way ANOVA with repeated measures. Analysis of gas data, HR, RPE, pain during TTE and CPT was performed by using two-way ANOVA with repeated measures, followed by Bonferroni post hoc when appropriate. Difference in pain perception during the last min between CPT and TTE was assessed by using an independent *t* test. The normality assumption was checked using the Kolmogorov–Smirnov test, homogeneity of variance for ANOVA was checked by Levene's test. The α level was set at $P < 0.05$. Statistics were calculated using SPSS version 20.

Results

All participants completed the experimental protocols and none of them reported any adverse effect during or after tDCS stimulation or cold pressor test. All participants at the beginning of the tDCS perceived a tingling sensation,

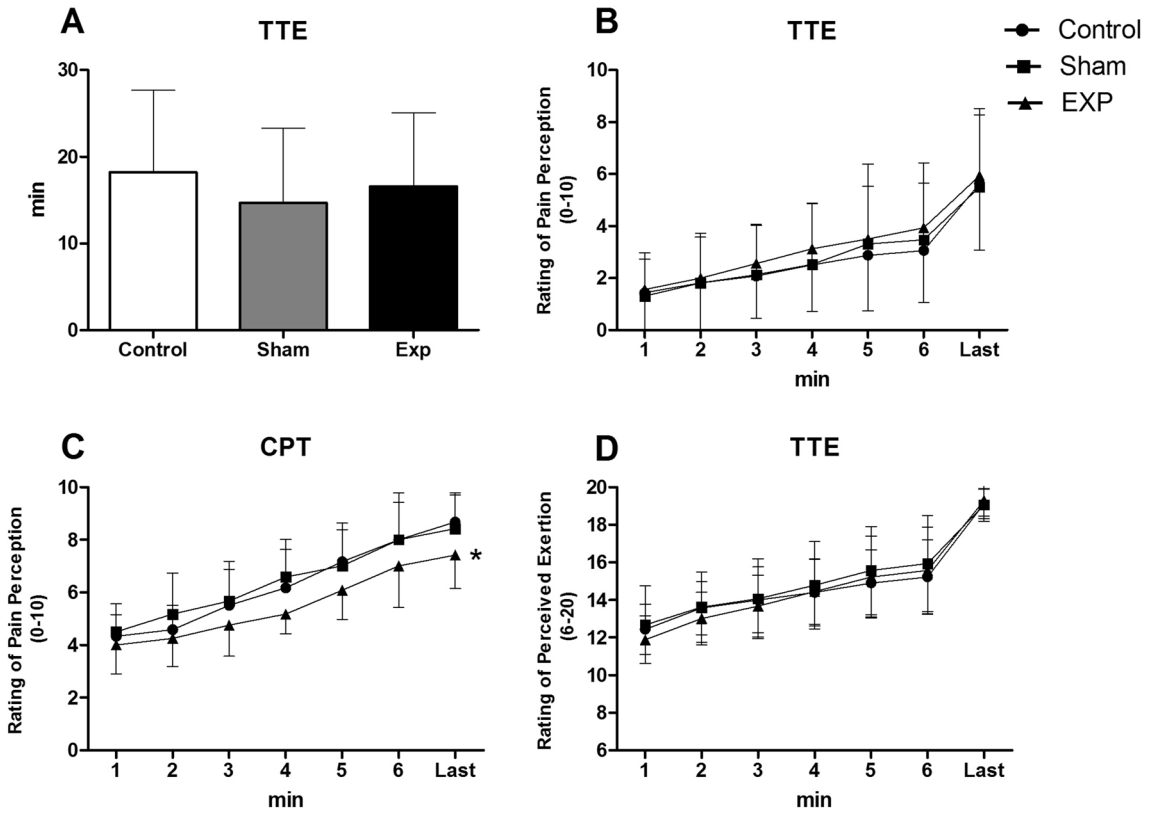

Fig. 1 *Panel* **a** shows time to exhaustion performance. *Panel* **b** shows time courses of pain perception during the time to exhaustion. *Panel* **c** shows time courses of pain perception during the cold pressor test and rating of perceived exertion during time to exhaustion are shown in *panel* **d**. Values are presented as mean ± SD. *$P < 0.05$ shows a significant main effect for condition

but no participants could distinguish between the EXP and SHAM conditions.

Part A

There were no significant differences ($P = 0.06$) in TTE time between EXP, SHAM and CON condition (16.58 ± 8.49; 14.68 ± 8.62; 18.22 ± 9.48 min, respectively). Pain and RPE increased during the TTE (main effect of time $P = 0.001$) but did not present any significant difference between the conditions at isotime ($P = 0.47$ and $P = 0.51$) or as percentage of total time ($P = 0.48$ and $P = 0.79$) (see Fig. 1). Heart rate, VO_2 and Ve increased during the TTE (main effect of time, $P = 0.001$) but did not present any difference between conditions ($P = 0.12$). Blood lactate collected after the TTE did not present any difference between the conditions ($P = 0.62$.) (see Fig. 2).

Part B

Pain reported during the CPT increased over time (main effect of time $P = 0.001$) and was significantly lower in the tDCS condition compared to SHAM and CON (main effect of condition, $P = 0.001$) while no difference in pain tolerance (time to remove hand) were found (see Fig. 1). The pain reported at the end of the CPT was significantly higher than the pain reported during the TTE ($P = 0.001$). In the CON condition, two participants reached the 8-min cut-off time, while in the tDCS and SHAM condition three participants reached the 8-min cut-off time.

Discussion

This is the first study to present data regarding tDCS M1 stimulation during whole-body exercise, and consequently provides important findings regarding the advancement for the use tDCS in exercise science.

This experiment aimed to assess whether a recognized tDCS montage that has been shown to induce analgesia to experimental pain would lead to (1) a reduction in exercise-induced pain, and (2) an improvement in cycling time to exhaustion. The main findings of the current study demonstrate that anodal tDCS over the primary motor cortex reduced pain perception during a cold pressor test in

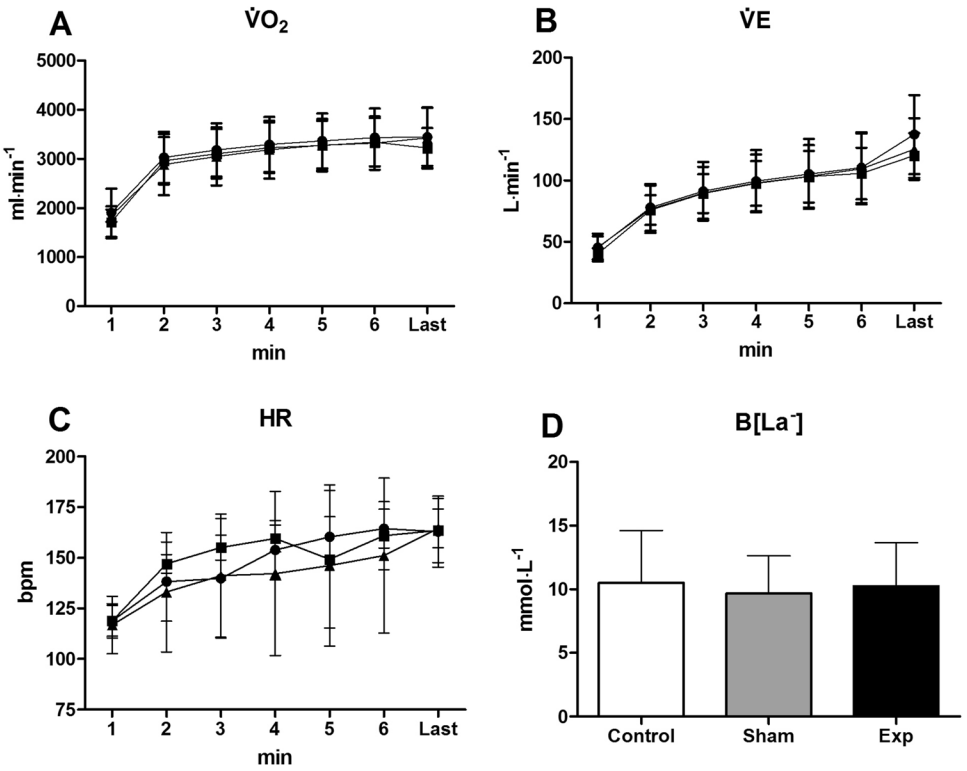

Fig. 2 Time courses of oxygen uptake (*panel* **a**), pulmonary ventilation (*panel* **b**), heart rate (*panel* **c**) and blood lactate (*panel* **d**) during the time to exhaustion. Values are presented as mean ± SD

healthy subjects, but did not change pain perception during a fixed high intensity cycling task.

In the present study, pain perception after anodal tDCS of the M1 was lower during the CPT compared to no stimulation (SHAM and CON conditions). This demonstrates that the tDCS intervention elicited an analgesic effect in response to the pain associated with cold thermal stimuli. These findings are in agreement with previous studies performed on healthy subjects where the tDCS intervention was able to evoke an analgesic effect during a cold pressor test (Zandieh et al. 2013) and in response to painful peripheral electrical stimulation (Boggio et al. 2008). This finding demonstrates a manipulation check for the intervention and that the established tDCS protocol used in this study did induce a central analgesic effect. However, while this form of analgesia moderated pain in the CPT, it did not affect pain perception during the exercise task. These findings demonstrate that the tDCS montage used in this study (anodal stimulation of M1, cathodal stimulation of the dorsolateral right prefrontal cortex) is not capable of producing an analgesic response to exercise-induced pain. As the neural pathways from nociception to the brain, and the processing of the pain signal within the brain are highly complex and are related to the type of pain (e.g., thermal, pressure, metabolic, etc.) (Millan 2002), this suggests that whilst the M1 (and moderation of it) is, at least indirectly (García-Larrea et al. 1997, 1999), important in the processing of cold pain, it has a limited role in the processing of exercise-induced pain.

Although not assessed in the current study, it is generally accepted that sensitivity to somatosensory inputs is reduced after cathodal tDCS administration to the motor and somatosensory cortex, probably because of the alteration of the resting membrane potential in the targeted area (Nitsche et al. 2008; Schestatsky et al. 2013). However, the analgesic effect observed in our study is unlikely to be caused by a reduction of activity in the somatosensory cortex, but rather through an alteration of the cold signaling pathway in the thalamus or insular cortex following anodal stimulation of the motor cortex (Zandieh et al. 2013). Indeed, investigations on animal models indicate an anatomical connection between the motor cortex with insula and thalamus (Schestatsky et al. 2013; Stepniewska et al. 1994), and so the effect of tDCS may be extended to other brain regions distant from the targeted area (i.e., spatial effect) as previously hypothesized by Zandieh et al. (2013). Therefore, the tDCS-induced analgesia demonstrated in the current study could be due to an inhibition of the nociceptive center at the ventroposterior and medial thalamic nuclei via corticothalamic pathway, which would have a greater antinociceptive action for thermal pain signaling (Stepniewska et al. 1994; Zandieh et al. 2013).

With regard to the lack of analgesic effect of M1 tDCS on exercise-induced pain, it has been shown that different populations of afferent fibers process cold and mechanical

stimuli (Olesen et al. 2012). Therefore, whilst M1 tDCS stimulation reduces thermal and electrical pain, according to the results from the current study, stimulation of this brain area produces no such effect for exercise-induced pain. It has been suggested that the important areas for pain processing during exercise include the primary sensorimotor cortex, secondary somatosensory cortex, anterior insular and cingulate cortex and thalamus (O'Connor and Cook 1999). tDCS stimulation of the M1 has been proposed to induce acute analgesia through a corticothalamic inhibition of epicritic (consistent with type III afferents) and nociceptive sensation at the VPL and VPM thalamic nuclei (Boggio et al. 2008). However, as skeletal muscle is more densely populated by type IV afferents, which are more consistent with a gradual build-up of pain which is dull, burning and aching in nature (O'Connor and Cook 1999), it may be that tDCS over the M1 elicits little analgesic effect to this type of pain. There is a strong emotional response to exercise-induced pain, which is likely important in its classification in terms of the unpleasantness. tDCS stimulation of the dorsolateral prefrontal cortex (DLPFC) has been shown to correlate negatively with the perception of pain (Lorenz et al. 2003) and reduce the emotional response to pain (Boggio et al. 2009), likely through a modulation of brain structures including the anterior cingulate cortex, insula and amygdala. Consequently, future studies should use tDCS to moderate the DLPFC during exercise to assess its role in the processing of exercise-induced pain.

The pain arising from intense exercise presents a unique set of circumstances which makes its processing unique. Firstly, the pain arising from the CPT was rated as 'very strong pain' (Cook scale value of 7.4–8.6), whereas the rating for the TTE task was that of 'strong pain' (Cook scale value of 5.5–6). Therefore, it may be the case that the TTE task did not elicit levels of pain high enough for an analgesic effect to be detected. This may be in part due intense exercise stimulating the body's inherent analgesic system, including the release of endogenous opioids and growth factors, an activation of brain controlled supraspinal nociceptive inhibitory mechanisms and the release of catecholamines (Nijs et al. 2012), all of which are likely to mitigate the strength of the pain signal reaching the brain, or the processing of it. Thus, the additive effect of tDCS may not supplement this already powerful natural analgesic response to exercise. Additionally, it is well known that one of the requisites of pain perception is the direct attention to the stimuli, and so distraction from the pain sensation can reduce reporting of pain (Linton and Shaw 2001). So, it is likely that during the CPT participants focused solely on the nociceptive stimuli, while during the TTE, attention was more focused on the exercise task (Linton and Shaw 2001). Subjective experience represents a significant portion component of pain processing (Linton and Shaw 2001) and participants (although familiarized in this study) are not usually experienced with the unusual nociceptive stimuli which a CPT elicits. Consequently, participants may tend to report a higher rating of pain compared to experienced stimuli such as muscle pain.

In the current study, there was no improvement in TTE duration following tDCS compared to the SHAM and CON conditions. Because the tDCS intervention did not induce analgesia to exercise-induced pain, this lack of effect is to be expected. It has previously been suggested that exercise-induced pain could moderate exercise intensity or pacing strategy, which may affect the final outcome of performance (Mauger 2013, 2014; Mauger et al. 2010). Accordingly, by reducing perceived pain or increasing pain threshold, an athlete should be able to improve their performance. Indeed, reducing pain during exercise through the ingestion of analgesic drugs has been previously investigated (Foster et al. 2014; Mauger et al. 2010, 2014), and shown to be effective in improving performance in TTE, time trial and repeated sprint exercise. However, although analgesia is the primary effect of these drugs, it should be acknowledged that the observed performance improvement in these studies could be due other mechanisms (Mauger and Hopker 2013; Mauger et al. 2014). For example, acetaminophen (paracetamol) elicits an antipyretic effect (Mauger et al. 2014) and has been shown to increase corticospinal excitability (Mauger and Hopker 2013). Consequently, there is a need for studies to use interventions which moderate the central processing of pain, rather than changing the strength of the nociceptive signal. The use of neurophysiological techniques such as tDCS provides a method which may allow a viable means of administering analgesia with fewer unwanted effects (Mauger 2013), and the findings of the current study provides an important methodological advancement in developing these techniques for exercise interventions. Indeed, developing an appropriate study design that solely mitigates pain perception during exercise is challenging. To date, there is only one study investigating the effect of the tCDS on cycling performance. Contrary to our findings, Okano et al. (2013) demonstrated that a tDCS intervention did induce some minor improvements in performance (~4 % peak power achieved in incremental test). The effect of tDCS on isometric force endurance has been investigated in two further studies with equivocal results (Cogiamanian et al. 2007 and Muthalib et al. 2013). These studies applied tDCS over the M1 before completing an isometric force time to exhaustion of the elbow flexors. Whilst Cogiamanian et al. (2007) demonstrated an improved TTE performance, no effect was found by Muthalib et al. (2013). It has to be taken in consideration that many differences including experimental design, exercise task and tDCS stimulation may be the cause of the divergent findings of these and the current study. In Okano et al study, participants performed

a maximal cycling incremental test, rather than a TTE. In addition, the tDCS intervention was different in terms of duration (i.e., 20 min), and location (left temporal cortex). As suggested by the authors, anodal tDCS administration over the left temporal cortex might induce some pleasant sensations causing a reduction of exercise discomfort and perception of effort during the initial phase of the task. Thus, the longer duration or different targeted area of the brain (i.e., anodal on the left temporal cortex with cathodal on the contralateral supraorbital area) used by Okano et al. might explain the difference in performance between this and the current study. A further finding by Okano et al. was the significant difference in HR following tDCS, an effect they attributed to an increase in parasympathetic activity induced by stimulation of the left temporal cortex. In the present study, we found no differences in cardiorespiratory response between the conditions (see Fig. 2, panel a, b and c). However, the tDCS montage used in the current study may explain why no differences were observed in this case.

The use of a single electrode montage in the current study may have led to changes in the brain which resulted in unwanted effects. With this particular electrode montage, the anode increases excitability in the M1, whereas the cathode reduces excitability of the DLPFC. This particular montage was chosen because it has consistently been shown to reduce experimental pain (Boggio et al. 2008; Zandieh et al. 2013). However, because the DLPFC is important for cognitive function and emotional processing, decreasing the cortical excitability of this area may have impacted on endurance performance. Therefore, any benefits following M1 stimulation may be negated by the DLPFC cathodal stimulation. Additionally, the unilateral tDCS set-up on the motor cortex might not be beneficial for whole-body exercise, as this brain area is only related to the contralateral limb. As such, we recommend that future research should use an extracephalic montage, with cathodes placed on a non-brain area (such as the shoulder). Finally, it should be acknowledged that tDCS stimulation modulates cortical activity in a relatively larger area than that targeted by the electrodes, as demonstrated in neuroimaging studies (Lang et al. 2005). Thus, whilst this study focussed specifically on increasing the excitability of the M1, it is possible that the stimulation may have migrated to adjacent brain areas, and so we cannot rule out the possible effects of this on the exercise task.

Conclusion and perspectives

This is the first study investigating the analgesic effect of M1 tDCS on perceived pain during time to exhaustion exercise. No change in exercise-induced pain was evident following the tDCS intervention, which suggests that the processing of exercise-induced pain is very different from that of experimental pain induced by cold thermal stimuli. This may be representative of the different brain regions used in processing these different types of pain.

This study provides valuable methodological advancement in developing appropriate montages for using tDCS in exercise-based research, and the findings suggest that future work utilizes a bi-cephalic tDCS montage, with a focus on the DLPFC area.

Conflict of interest None.

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