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Effects of Transcranial Direct Current Stimulation on Exercise Tolerance and Related Psychophysiological Responses

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List of abbreviations

$[La^-]_b$ = Blood lactate concentration

$\Delta[La^-]_b$ = Blood lactate accumulation

η_p^2 = Partial eta squared

BDNF = Brain-derived neurotrophic factor

Ca^{2+} = Calcium

CO_2 = Carbon dioxide

cTBS = Continuous theta burst stimulation

DC = Direct current

DCS = Direct current stimulation

DLPFC = Dorsolateral prefrontal cortex

EEG = Electroencephalography

EMG = Electromyography

GABA = Gamma-aminobutyric acid

GET = Gas exchange threshold

HD-tDCS = High-definition transcranial direct current stimulation

H^+ = Hydrogen

HCO_3^- = Bicarbonate

HD-EMG = High-density electromiography

HR = Heart rate

LTP = Long-term potentiation

M1 = Primary motor cortex

MEP = Motor evoked potential

MVC = Maximal voluntary contraction

NIBS = Non-invasive brain stimulation

NMDA = N-methyl-d-aspartate

O_2 = Oxygen

$PETCO_2$ = End-tidal carbon dioxide partial pressure

$PETO_2$ = End-tidal oxygen partial pressure

PFC = Prefrontal cortex

PMC = Premotor cortex

PO_{peak} = Peak power output

$\dot{Q}$ = Cardiac Output

RCP = Respiratory compensation point

RMT = Resting motor threshold

RPE = Rate of perceived exertion

RPM = Revolutions per minute

SMA = Supplementary motor area

SV = Stroke volume

tDCS = Transcranial direct current stimulation

TC = Temporal cortex

TMS = Transcranial Magnetic Stimulation

TTE = Time to exhaustion

UPDRS = Parkinson's Disease Rating Scale

$\dot{V}CO_2$ = Carbon dioxide production

$\dot{V}E$ = Pulmonary Ventilation

$\dot{V}O_2$ = Oxygen uptake

$\dot{V}O_{2max}$ = Maximal oxygen uptake

$\dot{V}O_{2peak}$ = Peak of oxygen uptake

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Abstract

During fatiguing exercise, a myriad of peripheral and central physiological perturbations occurs in the brain and in the central nervous system, which impair the capability to produce force or power through muscle contractions. The capacity to tolerate such conditions is a determinant factor for exercise capacity in healthy and clinical populations. Transcranial direct current stimulation (tDCS), which involves the transmission of a weak electrical current through the scalp for several minutes, is a form of non-invasive brain stimulation that aims to alter the level of cortical excitability of the stimulated area. It has been proposed as an ergogenic tool to counteract central fatigue mechanisms and alter the physiological responses and perceived exertion of healthy individuals during different types of exercise, including ones requiring whole-body dynamic contractions. Conflicting results emerge from the literature, often due to a lack of tDCS and exercise protocol standardization. Furthermore, a precise cause-effect relationship and the related mechanisms have yet to emerge. Therefore, two studies were conducted in this dissertation. In Study 1, twelve participants were enrolled and visited the laboratory on four experimental trials after a preliminary visit for cardiorespiratory and anthropometrics assessments. They performed, in a randomized and counterbalanced order, two constant work-rate cycling in the heavy domain (Δ15%) and two in the severe domain (Δ75%) following a 20 min of either anodal (2 mA) or sham primary motor cortex (M1) tDCS session. No significant differences in exercise tolerance and related psychophysiological responses were found between real and sham conditions. In Study 2, a similar design was employed to assess differences in exercise tolerance and related psychophysiological responses, especially the rate of perceived exertion, during constant work-rate cycling in the heavy domain (Δ25%), following a 10 min 1 mA of either anodal, cathodal, or sham tDCS of the supplementary motor area (SMA). No significant differences in all

studied parameters were found between the conditions. The findings suggest that one session of M1 or SMA tDCS stimulation is incapable of affecting exercise tolerance and related psychophysiological responses during exercise involving dynamic contraction of large muscle mass in young, healthy, active males. More studies are needed to compare different protocols and investigate the neurophysiological rationale for exercise capacity enhancement through tDCS.

1. Introduction

1.1 Historical Perspective of Transcranial Direct Current Stimulation

1.1.1 Brief History of Electrical Stimulation

The use of electrical stimulation to treat different conditions finds its origins in ancient times. It is reported that ancient populations used live torpedo fish to provoke a strong discharge on the head for headache pain relief, which was later proposed as a treatment for epilepsy (Priori, 2003). The existence of electricity was classified for the first time by the English scientist William Gilbert in his book "The Magnete", and Luigi Galvani first demonstrated the response to electricity shocks in animals with his experiments. In light of the first electrophysiology studies of the world, Giovanni Aldini then proposed electrostimulation as a treatment for patients with psychiatric conditions (Parent, 2004). Almost two centuries passed before successfully get the first experiments capable of stimulating the brain noninvasively through the application of electrodes on the intact scalp (Gualtierotti & Paterson, 1954) and proposing those types of brain stimulation for neuromuscular physiological measurements in research and clinical environments (Merton et al., 1982). Advances in the electrical engineering field during the twentieth century allowed pulsed stimulations to be employed. The use of electrical stimulation to induce a sleep-like state was introduced and later classified for the treatment of insomnia, anxiety, and depression. Before the advent of chemical anesthetics, attempts were conducted with electro-anesthesia for surgery application, but several side effects and concerns led to the abandonment of this application. The direct current (DC) was the first typology of neurostimulation employable by neurophysiologists, leading to increased spontaneous activity of pyramidal neurons following anode placement above the cortex and reduced following cathode placement (Purpura & McMurtry, 1965), an effect on cortical excitability that persisted for several minutes after the cessation of direct current (Bindman et al.,

1964). For the first time, long-term potentiation (LTP) of excitatory synaptic transmission was also demonstrated (Bliss & Lømo, 1973). After that, modern studies on applying polarizing currents in treating neuropsychiatric disorders started (Redfearn et al., 1964). For some decades, the studies focused mainly on what now is called extracephalic montage, with the active electrode positioned on the supraorbital, occipital, or temporal area and the reference electrode on the legs, hand, mastoid bone, or collarbone. Metallic electrodes were generally considerably smaller than what is employed now in conventional transcranial direct current stimulation (tDCS), and current intensity was also lower than what is generally employed now (Knotkova et al., 2019). The duration of early tDCS for clinical studies utilized several sessions, accumulating several hours of sessions, each of which could last from 4.5 to 11 hours. (Redfearn et al., 1964)

1.1.2 Modern Transcranial Direct Current Stimulation

Nowadays, modern tDCS is one of the most investigated neuromodulation techniques for the treatment of several different neurological and psychiatric conditions (Fregni et al., 2006; Nitsche et al., 2009; Schlaug et al., 2008) and for the improvement of cognitive and physical performance of healthy individuals (Angius et al., 2017). tDCS consists of inducing an electric field into the brain, measured in Volts per meter (V/m), produced by at least two electrodes, of which at least one is placed on the scalp and connected to the stimulation device. tDCS administration allows the manipulation of different parameters, such as current density, which is determined by the intensity of the delivered current divided by the size of the electrodes (mA/cm^2), the duration of stimulation, which is generally of 10-20 min, and the electrode position, which determines the electric field and the brain target of the stimulation protocol. The versatility of tDCS represents a strength point for clinical application. However, as discussed later, it is considered one of the main reasons for results inconsistency in the scientific literature, especially in exercise performance studies. Conventional tDCS

protocols used in human studies (duration ≤ 40 min, intensity ≤ 4 mA) are considered safe and have not produced any reports of severe or irreversible injury (Bikson et al., 2016). In a recent study involving 1109 participants (including also alternated transcranial direct current stimulation and transcranial random noise stimulation) from four different institutions, skin sensations were reported as the most common adverse effect (Sheffield et al., 2022). Common adverse effects are characterized by itching, tickling, or light burning sensations under the electrode, especially in the first minute of the stimulation. tDCS clinical and exercise studies need to employ blinding procedures to avoid participants being aware of whether they are assigned to a control group, in the case of parallel design studies, or when the actual stimulation is or is not delivered in cross-over design studies. To achieve good blinding, the usual approach is to apply a sham stimulation protocol, which consists of ramping up at the start and down at the end of the stimulation period as in the actual stimulation, but only for a few seconds to avoid provoking the neurophysiological effects of tDCS (Knotkova et al., 2019). Despite this remaining the most reliable blinding protocol, it is not always guaranteed, especially for stronger current stimulations and studies that employ repeated measures stimulations (Opitz et al., 2015; Sheffield et al., 2022)

1.2 Neurophysiology of Transcranial Direct Current Stimulation

1.2.1 Neurophysiological Effects of Direct Current Stimulation

The complexity of the tDCS neurophysiological effect is a debated argument nowadays. In addition to the difficulty of predicting the electric field, some uncertainty arises from the different effects of current administration between single neurons and the neuronal network level. With the external application of current from crayfish and lobsters, while controlling the synaptic input, the difference in potential membrane changes based primarily on the orientation of the current relative to the neurons between active and silent neurons was shown, and the authors laid then to the foundation of modern tDCS neurophysiological mechanisms (Terzuolo & Bullock, 1956). When the membrane voltage is far from the threshold for the action potential generations, it has been demonstrated that anodal stimulation (current that flows from the apical to the basal dendrites) tends to depolarize the soma of hippocampal slices. In contrast, cathodal (current that flows from the basal to the apical dendrites) tends to hyperpolarize it (Jefferys, 1981). This change linearly depends on the electric field magnitude (Bikson et al., 2004). The following results at the cellular level also confirmed the crucial role of the orientation of the current relative to the neurons; when the electric field is applied perpendicular to the neuron, it cannot significantly polarize the somatic membrane. Later findings confirmed how dendritic locations seem to be a critical factor for the actual polarity-based tDCS effect: at least in the animal model hippocampus *in vitro*, cathodal and anodal DC enhanced LTP of apical dendrites and basal dendrites, respectively (Kronberg et al., 2017). Since the induction of only a tiny current is not sufficient to directly induce action potentials in the neurons, it would be reasonable to think of tDCS as an ineffective tool for brain stimulation. However, the

crucial point is that as opposed to *in vitro* conditions, in which cortical neurons result in high input resistance, a hyperpolarized membrane potential, and lack of spontaneous firing, neurons *in vivo* show a natural depolarization state and continuous membrane potential fluctuations due to the spontaneous synaptic activity (Destexhe et al., 2003). Computational models also predicted that the high-conductance state of *in vivo* neurons gives out to the neurons the capability of synaptic input discrimination and responsiveness in the amplitude, spatial, and temporal domains (Destexhe et al., 2003). The first findings of transcranial electrical stimulation on *in vivo* anesthetized animals showed the polarity dependence of anodal/cathodal stimulation, emphasizing how the application longer than 5 min induced long-lasting changes in neuronal firing rates and also observed a linear relationship between the magnitude of the administered current and firing rate changes (Bindman et al., 1964; Creutzfeldt et al., 1962; Purpura & McMurtry, 1965). On the other hand, the level of activity may imply suppression of the effect of the transcranial stimulation exactly because the total membrane resistance is smaller than during inactivity, reducing the external current ability to significantly alter the membrane potential at a single neuronal level (Paulus & Rothwell, 2016). The central part of neurophysiological investigations focused on the effects of somatic polarization, demonstrating the polarity-dependent effects described above. Generally, anodal tDCS is expected to produce somatic depolarization of pyramidal cortical neurons, while cathodal produces hyperpolarization instead. However, recent investigations on tDCS cellular targets suggested that other cellular levels may be involved. A statistical model mimicking neurotransmitter depletion during an *in vitro* experiment on rat primary motor cortex showed that the DC might also involve afferent axons (Rahman et al., 2013). Furthermore, axon pathways' potentiation seemed to depend on their terminal pointing: terminals pointed toward the anode were potentiated, while axon pathways with terminals pointed toward the cathode were inhibited (Kabakov et al., 2012; Knotkova et al., 2019). tDCS administration in *vivo* animal models also observed increases in Ca^{2+} levels of astrocytes origins (Monai et al., 2016). Although the basic neurophysiological mechanisms underlying tDCS are

now well understood and replicated in several different studies, both *in vitro* and *in vivo* experiments, numerous mechanisms at different levels are yet to be understood. They are now challenging the classic general assumption of polarity-dependent effects of tDCS. In summary, the literature exhibits a relatively precise scenario about the effect of weak electric fields on single neurons. When the neurons are in a high conductance state, somatic action potential generation is affected, with anodal stimulation augmenting the firing rate and decreasing the required time to reach the threshold. In contrast, cathodal stimulation has the opposite effect. Nonetheless, there are still many open questions about the effect of tDCS on single neurons. The symmetrical difference of excitatory and inhibitory effects of anodal and cathodal current, respectively, has been questioned in the last years because of the different effects that different neuronal compartments encounter when the DC is administered, of which some are still partially unknown, such as axons and dendrites. Last, the effect of tDCS on non-neuronal type cells is a field that needs further exploration and understanding. Thus, advancements in the interaction between those cells and tDCS may be a critical aspect of the knowledge of the human brain transcranial stimulation field. (Knotkova et al., 2019).

1.2.2 Effects of Direct Current Stimulation on Neuronal Synapses and Populations

Although the effects of different forms of current stimulation on single neuron levels are relatively straightforward and well documented, moving to the synapse and neuronal population level complicates the picture. Of course, to induce any relevant clinical and applied brain function modifications through tDCS, the aim must be to act on neuronal populations rather than at a single neuronal level. Several studies attempted to measure evoked responses of neuronal populations during or after the application of weak currents, also combining with prediction models, providing information about the synaptic currents on post-synaptic neurons, adding new insights on the physiological mechanisms of tDCS long-lasting effects, and questioning the

tDCS effects predictions based on single neuron experiments. 15 min of extracellular DCS to *in vitro* animal primary motor cortex (M1) has been observed to induce long-term plasticity that requires activity-dependent brain-derived neurotrophic factor (BDNF) secretion when administered in combination with low-frequency stimulation, which induces an increase of synaptic activity different from the conventional n-methyl-d-aspartate (NMDA) receptor activation, induced long-term plasticity that requires BDNF, effect that disappeared when the experiment was repeated on genetically modified animals with blockade of those receptors. (Fritsch et al., 2010). These results were also confirmed by a similar experiment in the CA3-CA1 hippocampus, which reported the inability of DCS to induce LTP without the ongoing activity of synaptic plasticity; the authors speculated on the DCS influence on molecules involved in the LTP process (NMDA receptor and Ca^{2+} channels), or the formation of activity-dependent molecular tags. (Ranieri et al., 2012). Thus, DCS administered on in vitro M1 and hippocampus samples would like as a response modulator to the subsequent protocol of synaptic potentiation, highlighting the possible role of tDCS as a therapeutical tool in several pathological conditions in which LTP is reduced, as well as motor and cognitive learning in healthy individuals. In recent decades, tDCS *in vivo* experiments have also been conducted simultaneously. For example, two experiments on animal models reported that anodal tDCS was capable of increasing the propagation speed of induced cortical spreading depression (a mechanism considered responsible for migraine and also involved in epilepsy and stroke), and cathodal tDCS diminished the threshold for epileptic seizure generation (Liebetanz et al., 2006). Prolonged anodal and cathodal M1 tDCS on *in vivo* animal models have also been proved to increase and decrease forelimbs motor evoked potentials (MEP), respectively, even 10 min after the end of the stimulation (Cambiaghi et al., 2010). The same polarity-dependent aftereffects were observed when the tDCS was administered to the visual and the somatosensory cortex (Márquez-Ruiz et al., 2012). The role of NMDA and BDNF receptors as primary and essentially responsible for the enhancement of LTP induced by tDCS administration

has also been confirmed by an *in vivo* study in animal models, which also reported a long-lasting LTP potentiation effect 24 hours and one week after the cessation of the stimulation in the hippocampus, and the effects had also been confirmed in a different study testing mice on behavioral task (Rohan et al., 2015).

1.2.3 Neurophysiology of Transcranial Direct Current Stimulation in Humans

Differently from other brain stimulation techniques (i.e., transcranial alternating current stimulation, transcranial random noise stimulation), the intrinsic factor of tDCS is that the induced polarization shift described above is constantly sustained during the entire time of the stimulation, without changing due to the change of stimulation waveform, and this is considered the central and unique characteristics of tDCS, which may be the reason of its role in clinical settings and the motor and cognitive learning fields. Neurophysiological effects of tDCS can be divided into acute- and after-effects. As mentioned above, the acute effects are based primarily on the membrane polarization shift of the targeted cells involved for the entire stimulation time. The flow from outside the neuron into it will result in local membrane hyperpolarization, and the flow from inside to outside will result in local membrane depolarization instead. Thus, any neuron exposed to an extracellular direct current will have a hyperpolarized and depolarized compartment, respectively (Bikson et al., 2004). Hence, the DCS effects on the soma have historically characterized the anodal/excitatory and cathodal/inhibitory tDCS rationale. However, the reasons for attention on tDCS depend on the reported effects that outlast the stimulation when administered for a sufficiently prolonged time. When the DC is administered for some minutes, polarity-specific lasting changes in cortical excitability occur, leading to altered synaptic plasticity (Nitsche & Paulus, 2000). Combining tDCS and different classes of central nervous system active drugs allowed the understanding of the underlying mechanism related to synaptic plasticity in humans. Several studies have investigated the interactions of tDCS with plasticity drivers and modulators. When

tDCS is administered in combination with carbamazepine or flunarizine, which blocks the voltage-gated ion channels, the after-effect of anodal tDCS on cortical excitability measured by transcranial magnetic stimulation (TMS) evoked potentials is abolished. When dextromethorphan, which blocks the NMDA receptors, is combined with tDCS, both anodal and cathodal after-effects are abolished (Liebetanz, 2002; Nitsche et al., 2003). Correspondingly, when D-Cycloserine, an NMDA agonist, is administered, the long-lasting effects of both anodal and cathodal tDCS are enhanced (Nitsche et al., 2004). Magnetic resonance spectroscopy reported that both anodal and cathodal tDCS decreases gamma-aminobutyric acid (GABA) concentration in the cortex, and glutamate concentrations are reduced by the latter (Stagg et al., 2009). Dopamine precursor administration combined with tDCS has shown a non-linear dose relationship between the pharmacological and tDCS interventions, reporting an alteration in dopamine activity after tDCS (Fresnoza et al., 2014). Acute nicotine administration in non-smokers subject abolished the excitatory and delayed the inhibitory effects of anodal and cathodal tDCS, respectively, and cholinergic activation by the cholinesterase inhibitor rivastigmine abolished LTP plasticity induced by anodal tDCS (Kuo et al., 2017). A single dose of serotonin reuptake inhibitor citalopram enhances and prolongs the LTP plasticity induced by anodal tDCS and converts LTD plasticity induced by cathodal tDCS into LTP plasticity (Nitsche et al., 2009). Overall, the literature agrees with the assumption that the plasticity effects induced by tDCS in humans act at different drivers and neuromodulator levels. Some of these require more investigations to reach a mechanistic understanding of tDCS effects in humans.

1.2.4 Regional Effects of Transcranial Direct Current Stimulation in Humans

Provoking MEP with transcranial magnetic impulses delivered through the motor cortex allows us to reliably measure the corticospinal excitability of the motor cortex. Thus, M1 is the most investigated brain region in tDCS studies. As mentioned above, a few seconds of stimulation can modulate corticospinal excitability, but the aftereffect

appears only if the tDCS is sustained for a few minutes and persists up to some hours when the administration exceeds 9 min (Nitsche & Paulus, 2000). However, the dose-response relationship of motor cortex tDCS is still partly unclear, and some studies reported that 20 min of 2 mA cathodal tDCS and 26 min of 1mA cathodal tDCS respectively enhanced and reduced MEP amplitudes, thus reporting a partial conversion of the commonly accepted polarity-dependent effects (Batsikadze et al., 2013). This reversion of the effect is plausibly related to the enhancement of Ca^{2+} concentrations for cathodal tDCS and Ca^{2+} overflow during prolonged anodal tDCS (Lisman, 2001). When repeated within 1 hour, the after-effect seems to last more than 24 hours (Katia Monte-Silva et al., 2013; Monte-Silva et al., 2010). Numerous studies also investigated the effects of tDCS on the motor cortex through other methods: motor cortex activity is influenced by tDCS, and the modulation of event-related desynchronization of mu rhythm seems to be polarity dependent (Kasashima et al., 2012; Matsumoto et al., 2010). It is also possible to assess cortical excitability by measuring cerebral blood flow and oxygenation levels using different methods. The first study that investigated the effects of tDCS showed a significant decrease in blood oxygenation level-dependent after 5 min of cathodal tDCS and a non-significant increase after 5 min of anodal tDCS (Baudewig et al., 2001). Positron emission tomography studies showed alterations in brain blood flow under resting conditions after 10 min of anodal and cathodal tDCS at rest. However, the effect was suppressed when a movement task was executed during the assessment (Lang et al., 2005). Later findings showed instead a significant increase in blood flow changes after 20 min of anodal tDCS compared to sham during stereotyped hand movements (Jang et al., 2009), and the results during resting condition tDCS were also confirmed with a higher sensitivity method (Zheng et al., 2011). A recent meta-analysis reported that tDCS is capable of altering functional near-infrared spectroscopy outcomes in young adults (<25 y) but not in middle-aged (25-38 y) and older adults (> 60 y) (Figeys et al., 2021). Although the majority of the studies investigating the effects of tDCS on cortical excitability focused on the upper limb, especially in hand muscles, several studies

highlighted the changes in cortical excitability also in the lower limb with both TMS (Jeffery et al., 2007) and functional magnetic resonance imaging assessments (Kim et al., 2012). Overall, evidence from the last decades suggests a polarity-dependent effect on motor cortex cortical excitability and activity. The primary effect involves subthreshold membrane polarization, while the after-effects seem to be primarily related to changes in glutamatergic synaptic strength and GABAergic activity reduction; besides, some studies suggest an impact on neuromodulators that has yet to be fully clarified.

1.3 Applications of Transcranial Direct Current Stimulation in Clinical Populations

1.3.1 Transcranial Direct Current Stimulation Applications in Neuropsychological Disorders

The interest in the employment of different types of cerebral stimulation for the treatment of psychiatric conditions traces back centuries. In the last decades, there has been an increase in the clinical application of different forms of non-invasive brain stimulation (NIBS) techniques, including tDCS. This necessity arises because pharmacology therapy success is not always established, and many patients become resistant to treatment pharmacotherapy (Brunoni et al., 2021). Compared to other types of stimulation techniques, such as TMS or electroconvulsive therapy, tDCS is cheaper, easier to use, and portable, and can be a valid alternative in patients who cannot receive drug treatments; in addition, tDCS is a more localized treatment compared to antidepressant drugs. Thus, the interest in tDCS clinical relevance in several cognitive and psychophysiological conditions has grown significantly in recent decades. By referring to randomized controlled trials that investigated the effects of tDCS as monotherapy on major depression disorder, the results are in contrast with each other. Several studies employed different modalities of tDCS (5 to 15 sessions over 1 to 3 weeks, 20 min, 1 to 2 mA) and revealed positive effects in mood and depression scales compared to the sham or control groups (Boggio et al., 2008; Fregni et al., 2006; Salehinejad et al., 2015). Otherwise, similar randomized controlled trials conducted in the same period revealed no significant effects of active tDCS compared to sham (Loo et al., 2010, 2018; Palm et al., 2012). The conflicting results are likely explainable by the heterogeneity of employed protocols and participant conditions, in addition to the

acceptance of patients with concomitant use of antidepressant drugs (Brunoni et al., 2021). A study randomized 120 antidepressant-free patients reported that active tDCS as monotherapy was more effective than sham, that there was no significant difference between the tDCS and sertraline groups, and that the combination of active tDCS and sertraline was superior to all other groups. (Brunoni et al., 2013). In a second randomized controlled trial by the same group, the authors found that twenty-two sessions (30 min, 2 mA) over three weeks and then once a week for another seven weeks, the results reported that tDCS was not inferior to the drug. (Brunoni et al., 2017). Looking at the meta-analysis that investigated the effects of tDCS on depression disorder, the findings report a favorable trend in the tDCS effectiveness. Only one meta-analysis reports no significant difference between sham and active tDCS (Berlim et al., 2013), while others reveal a superiority of active tDCS versus sham (Kalu et al., 2012; Razza et al., 2020). Also, individual patient data meta-analyses, which employ the statistical analysis of individual rather than aggregated data, reported a significant effect with small to medium effect size of active tDCS on remission and depressive symptoms, which is, however, lower than the clinical effects of antidepressant drugs. (Brunoni et al., 2016). NIBS techniques have also emerged for the treatment of schizophrenia in pharmacological refractory patients. In most studies, the treatment consisted of ten sessions of 20 min 2 mA tDCS. The results are in contrast with each other. Several studies reported a significant decrease in the hallucination rating scale after active tDCS (Kantrowitz et al., 2019), while others found no significant effect on the same outcome (Fitzgerald et al., 2014; Fröhlich et al., 2016). Despite the controversial results evinced by randomized controlled trials, one systematic review involving fifteen studies suggests an overall significant effect of tDCS on positive symptoms, negative symptoms, and hallucinations (Chan & Han, 2020), while another meta-analysis reported that only the group that received at least ten sessions of tDCS twice-daily showed a significant effect in auditory hallucination symptoms reduction. (Jiang et al., 2022). tDCS is a promising new tool for the treatment of schizophrenia. However, conflicting results exist, and larger randomized clinical trials are needed to

confirm or dispute the effectiveness of tDCS in this clinical population. Because of the changes in prefrontal cortex neuroplasticity induced by long-lasting drug additions, tDCS has emerged as a potential treatment for different drug addiction conditions. The results in alcohol use disorders are unclear. Most of the studies in this area investigated different modalities of dorsolateral prefrontal cortex (DLPFC) tDCS. Even some single-session research reported a decrease in craving score after active tDCS compared to sham (Boggio et al., 2008) and an improvement in frontal cortex function (Nakamura-Palacios et al., 2012). When administered for multiple sessions, another study reported similar results in craving scores (Den Uyl et al., 2017). However, several other investigations reported a non-significant effect of tDCS in modulating cravings and relapse in alcohol abuse patients. Evidence is still limited, and two meta-analyses reported a non-significant effect of tDCS on craving and relapses (Mehta et al., 2024; Mostafavi et al., 2020). Several randomized controlled trials demonstrated a significant effect of active DLPFC tDCS on tobacco use disorder participants, reporting a general diminishing of cigarette consumption in short-term cigarette consumption and craving (Fecteau et al., 2014; Meng et al., 2022), as well as in the long-term (De Souza Brangioni et al., 2018). Despite there being reasonable evidence that DLPFC tDCS can impact tobacco consumption and craving, subgroups meta-analysis revealed a non-significant effect of active tDCS compared to sham both after single and multi-session tDCS interventions (Mehta et al., 2024). The impact of tDCS on other severe drug additions has been investigated through the years, such as opioid or cannabis additions. In this case, the lack of consistency in the results is broadened by the relatively few studies conducted, which makes meta-analysis unfeasible. Up to date, tDCS is classified as “probably effective or ineffective” in the treatment of these conditions, and more studies demonstrating the effect of tDCS on larger and homogenous samples, which indicate specific stimulation protocols for different types of drug addictions still necessitate to be identified (Lefaucheur et al., 2017).

1.3.2 Transcranial Direct Current Stimulation Applications in Neurological Disorders with Motor Impairments

Different neurological conditions are associated with impaired motor function and reduced autonomy in daily living activities. It is well-established and confirmed by a meta-analysis involving 112 studies that after a stroke, the affected hemisphere exhibits lower levels of cortical excitability than the unaffected limb in the acute and chronic phases (McDonnell & Stinear, 2017). Therefore, the interest in the clinical application of modern tDCS in this population has grown considerably in the last two decades because of the well-known capacity of this technique to modulate cortical excitability levels in humans. Upper extremity function is one of the primary outcomes assessed in stroke rehabilitation clinical studies. The first report on the possibility of promoting recovery after a stroke reported increased levels of hand function and cortical excitability in patients who combined M1 anodal tDCS and motor rehabilitation compared to the sham group (Hummel, 2005). Later, also cathodal tDCS was reported to reduce cortical excitability levels of the unaffected hemisphere to favor a balance between hemispheres was investigated, and similar positive results in hand motor function were reported (Boggio et al., 2008; Fregni et al., 2005). All the mentioned studies were conducted with a sample size < 10. Specifically, to treat stroke conditions, bi-hemispheric tDCS has emerged as a promising protocol of electrode positioning; it consists of placing the anode on the affected limb and the cathode electrode on the unaffected one to upregulate cortical excitability on the former and downregulate it on the latter at the same time. The first clinical studies employing this protocol revealed significant effects on upper extremity function (Bolognini et al., 2011; Lindenberg et al., 2010). On the other hand, other investigations reported opposite results. Investigations in patients with similar or more severe conditions reported no differences in upper extremity function between anodal tDCS on the affected limb, cathodal tDCS on the unaffected limb, and sham combining the stimulation with robotic-assisted rehabilitation (Hesse et al., 2011). A large randomized controlled trial administered five daily sessions on the second day from

stroke onset to fifty participants and reported no significant difference in the hand motor function improvement between anodal tDCS on the affected hemisphere and sham groups (Rossi et al., 2013). Similar results were observed in prolonged interventions of combined rehabilitation strategies and tDCS, which showed no significant difference in upper limb extremity function between anodal or bi-hemispheric tDCS and sham groups (Triccas et al., 2015). A few of these studies also employed follow-up assessments to investigate the possible long-term effect of tDCS intervention. The results are in contrast with each other. Only one study reported significant changes in upper extremity function at the follow-up (Di Lazzaro et al., 2014), while four studies reported opposite results (Allman et al., 2016; Hesse et al., 2011; Rossi et al., 2013; Triccas et al., 2015). Some investigations also employed lower extremity function assessments in acute and chronic patients, and the same results were obtained. Anodal tDCS (15-20 min, 1-2 mA) administration for at least ten sessions on the primary motor cortex or primary somatosensory cortex combined with conventional physiotherapy, robot-assisted training, or functional training resulted in significant improvement in lower extremity function compared with sham condition. (Cha et al., 2014; Chang et al., 2015). Other randomized controlled trials reported opposite results, although utilizing methodologies of lower limb investigation (Geroin et al., 2011; Yi et al., 2016). Sixty-seven studies for 1729 participants about the effect of tDCS in stroke recovery were included in a recent meta-analysis, which highlighted the high heterogeneity regarding trial design, therapy variables, and participant characteristics. This review concluded that insufficient evidence supports using tDCS in clinical practice to improve active daily living and upper and lower extremity function in stroke patients (Elsner et al., 2020). The authors also evidenced the importance of optimizing stimulation parameters in future clinical studies and conducting more extensive randomized controlled trials. Parkinson's disease is among the most studied neurological pathologies investigated in neurology. Different therapeutic strategies have been investigated in the last decades, such as pharmacological and deep and NIBS. While unable to resolve the disease completely,

all the interventions present benefits and disadvantages in multiple aspects (Brunoni et al., 2021; Malvea et al., 2022). Parkinson's disease symptoms are multifaceted and involve different brain areas and functions, affecting the motor system as well as other cognitive functions. Thus, because the tDCS characteristics act on different levels and cause long-lasting effects at different layers beyond the simple excitation/inhibition effects on the stimulated area, the possibility as a treatment tool for Parkinson's disease individuals has been considerably investigated in recent times. The first randomized controlled trial on tDCS effects on Parkinson's disease reported significant improvements in different motor functions assessed through the Parkinson's Disease Rating Scale (UPDRS), reaction time, and the Purdue Pegboard Test, only after one session of M1 anodal stimulation, compared to sham and DLPFC. The authors also reported a positive but not significant strong correlation between the motor-evoked potential and the amplitude increase after M1 anodal tDCS and the UPDRS improvements (Fregni et al., 2006). In another randomized controlled trial with a parallel design employing eight sessions of M1 and prefrontal anodal tDCS in the treatment group and sham tDCS in the control group, the treatment group reported a reduced time performance in the 10 meters walking test and improvement of dyskinesias after the treatments, which lasted up to 3 months. However, during all the timepoint measurements, UPDRS, reaction time, physical and mental well-being, and self-assessed mobility remained unchanged between the intervention and the parallel group (Benninger et al., 2010). Similar investigations have been conducted through the years employing different modalities of interventions, combining tDCS administration with physical therapy (Kaski et al., 2014), dual-task tests (Criminger et al., 2018; Swank et al., 2016), and neurophysiological assessments (Cosentino et al., 2017). Two meta-analyses concluded that regardless of whether the brain area is stimulated, there is no significant evidence of short-term effects on motor functions and dyskinesias following tDCS (Elsner et al., 2016; Oliveira et al., 2022). As often happens in tDCS clinical investigations, the heterogeneity of participant characteristics

and the wide variety of tDCS protocols, stimulated brain areas, and research designs make it difficult to delineate a conclusive assumption.

1.4 Transcranial Direct Current Stimulation Applications in Healthy Individuals to Improve Exercise Performance and Tolerance

1.4.1 General View of tDCS Effects on Exercise Performance in Healthy Participants

Exercise physiologists investigate and seek ergogenic aids to improve different types of physical performance because increasing the capacity of individuals to perform and tolerate more exercise may lead to higher levels of physical health and improved specific sports performance. Of course, the ethical aspects are of paramount interest, and researchers must place the health and safety of individuals in the first place. The range of legit interventions is broad, from supplementation to target specific physiological mechanisms (i.e., creatine, nitric oxide, etc.) to psychological interventions (i.e., meditation, self-talk, etc.). Of course, the brain plays a crucial role during exercise in many respects, from regulating autonomic responses to processing high-level cognitive information in the prefrontal cortex (PFC) to driving the input for voluntary muscle contractions in the primary motor area. Unsurprisingly, the interest in the exercise physiology and performance field grew rapidly with the emergence of an easy-to-use, safe, and relatively low-cost tool that induces reliable neurophysiological effects such as tDCS. Because of the neurophysiological effects of tDCS demonstrated in human brains, it is unsurprising that M1 was the first and the most stimulated area in tDCS studies investigating effects on physical exercise. Coherently with tDCS clinical studies, DLPCF has been investigated several times in exercise studies, mainly because of its role in cognitive processes and inhibitory control. The main two investigated exercise science areas are isolated isometric muscle contraction strength and endurance and large muscle mass whole-body endurance exercise. Other types of exercise, such as multi-joint maximal strength and endurance,

and other physical capacities, such as sprint ability, flexibility, and balance, have also been investigated.

1.4.2 Transcranial Direct Current Stimulation Effects on Single-Joint Isometric Exercise Tolerance

The first study of 24 healthy participants compared anodal and cathodal tDCS of the right motor cortex and a control group with no stimulation with elbow flexor maximal voluntary contraction (MVC) and 35% time to exhaustion (TTE) pre and post-tDCS, resulting in a TTE less decrease in the post anodal tDCS condition compared to cathodal and control, suggesting that anodal tDCS could increase TTE time because of changes in cortical excitability (Cogiamanian et al., 2007). Interestingly, a similar study on 11 participants reported similar results employing a cross-sectional design with anodal tDCS and sham. However, the authors reported no causal effects of changes in cortical excitability on time-to-exhaustion performance. (Abdelmoula et al., 2016). Two other studies repeated the same study design and reported no effects of anodal tDCS on elbow flexor TTE (Kan et al., 2013; Muthalib et al., 2013), and a similar study on elbow flexor stimulating the premotor cortex (PMC) or PFC in separate sessions also showed no effects of anodal tDCS on TTE (Radel et al., 2017). Angius and colleagues investigated the effects of tDCS on the lower limb neuromuscular function and reported an increase in TTE in sustained knee extensions when the cathode electrode was positioned on the shoulder but not on the contralateral supraorbital area as in conventional montage (Angius et al., 2016). Otherwise, similar studies employing conventional and high-definition tDCS (HD-tDCS) targeting M1 (Flood et al., 2017, p. 201) and left DLPFC (Denis et al., 2019, p. 201) showed no effect of anodal stimulation. The same results variability between studies exists for dynamic isokinetic and isotonic exercise. A study found increased torque production during isokinetic exercise at different velocities after temporal cortex (TC) and M1 stimulation (Washabaugh et al., 2016), but studies with similar designs and protocols

failed to replicate the results and showed no significant differences between anodal tDCS and sham conditions (Ciccone et al., 2019; Maeda et al., 2017).

1.4.3 Transcranial Direct Current Stimulation Effects on Whole-Body Exercise Tolerance

The interest in tDCS ergogenic effects has also expanded to large muscle mass and whole-body endurance exercise, which is more ecological and related to exercise tolerance and performance in sports and exercise activities. The first study on this area reported increased peak power and time to exhaustion in incremental ramp tests after 20 min at 2 mA of TC anodal tDCS in 10 cyclists (Okano et al., 2015), while the first study targeting M1 investigated the effects on a TTE trial at 80% of peak power (Vitor-Costa, Nilo Massaru Okuno, et al., 2015). These two studies, in addition to the one conducted by Angius and colleagues that showed improved TTE and reduction of RPE during TTE test at 70% of peak power after bilateral M1 extracephalic tDCS (Angius et al., 2017), opened the way to a period rich in different studies aiming to confirm the effects of tDCS on endurance tolerance and performance in recreationally active healthy participants and competitive athletes of various levels. Other studies targeting M1 as the stimulation site reported improved running (Park et al., 2019) and cycling (Sidhu, 2021) performance. Otherwise, a series of investigations from other authors reported fewer promising results in different modalities of exercise regardless of the site of stimulation and the protocol of exercise (Baldari et al., 2018; Barwood et al., 2016; Holgado et al., 2019)

1.4.4 Current Limitations of Transcranial Direct Current Stimulation Studies on Whole-Body Exercise Tolerance

A large number of studies investigated the possibility of enhancing endurance performance with different modalities of exercise. Concerning healthy participants,

some studies employed ramp incremental tests only in different stimulation conditions (Baldari et al., 2018; Okano et al., 2015). While incremental exercise tests are fundamental to assess maximal oxygen uptake ($\dot{V}O_{2max}$), ventilatory thresholds, lactate thresholds, and respective power or speed, TTE trials offer a more specific way of assessing the capacity of individuals to tolerate fatiguing exercise involving large-muscle mass dynamic contraction (e.g., cycling). Furthermore, with TTE trials, it is possible to inspect physiological differences during steady-state exercise at different intensities or conditions (e.g., external intervention) at the same intensity. In the context of tDCS studies on endurance performance, some studies employed TTE trials at different intensities to measure differences between different protocol stimulations. The first study showing the possibility of enhancing exercise tolerance through tDCS reported increased TTE at 80% of the peak power output (PO_{peak}) attained in a ramp incremental test, which increased significantly after M1 anodal tDCS (20 min, 2 mA, anode: vertex (Cz), cathode: inion) compared to cathodal tDCS and sham, without reporting significant differences in the rate of perceived exertion (RPE), knee extensors activity, and heart rate (HR) over time (Vitor-Costa, Nilo Massaru Okuno, et al., 2015). On the other hand, in a following study investigating anodal tDCS effects on hot environments at 75% PO_{peak} after 25 min of cycling at 55% PO_{peak}, TTE was unaffected by tDCS as well as HR and RPE (Barwood et al., 2016). A similar study employed bilateral extracephalic tDCS, administering the current through two active electrodes on C3 and C4, following the 10-20 EEG guidelines, and reference electrodes on the shoulders. Results showed that participants significantly increased TTE and blood lactate concentration ($[La^-]_b$) after anodal tDCS compared to sham and cathodal tDCS in a 70% PO_{peak} trial and reduced RPE in the anodal condition. Interestingly, this was the first tDCS and endurance tolerance study employing cortical excitability assessment through TMS, which revealed increased MEP after anodal tDCS (Angius et al., 2018). A similar increase in exercise tolerance has been reported in a study of 11 participants cycling at 80% or PO_{peak} following anodal tDCS on the M1 right-hand hotspot without changes in HR and RPE. Interestingly, cortical

excitability assessments showed contrasting results: cortical excitability measured through MEP remained unchanged after anodal tDCS while decreased after the sham session. Short intracortical inhibition increased significantly in both conditions over time, showing a significant increase of anodal tDCS compared to sham. After exhaustion, MEP increased significantly only in the sham condition, while the opposite was expected, and short intracortical inhibition was greater in the sham session compared to anodal tDCS (Sidhu, 2021). The discrepancy between the results of the studies seems to be irrespective of the level of physical training of the participants. A recent study investigated differences between M1 anodal HD-tDCS (20 min, 2.4 mA), conventional anodal tDCS (20 min, 2 mA, anode: Cz; cathode: inion), and sham on endurance athletes ($\dot{V}O_{2max}$: 60.13 ± 4.91 mL · kg · min^{-1}; PO_{peak}: 340 ± 53.2) in TTE trials at 80% of PO_{peak}, reporting no differences between all conditions for TTE as well as HR and RPE (da Silva Machado et al., 2021). All the studies above employed a fixed percentage of PO_{peak} between 70% and 80% to test the endurance tolerance of participants. Although prescribing the exercise intensity during the experimental trials based only on the PO_{peak} may suggest correct protocol standardization between participants, it might instead represent a limitation because it fails to account for individual variations in metabolic boundaries that demarcate transitions between exercise intensity domains. When exercising in the heavy intensity domain (also termed "moderate" in the physical activity guidelines terminology), which refers to intensities above the lactate threshold or the gas exchange threshold (GET), levels of [La^-] constantly remain above resting values, and $\dot{V}O_2$ stabilizes after 10-20 min at a level above the GET. The upper boundary of the heavy domain is generally considered the highest intensity or speed that still allows steady-state maintenance, and it is called, based on the method of measurement, critical power, respiratory compensation point (RCP), or maximal lactate steady state (Baldari & Guidetti, 2000; Billat et al., 2003; Jones & Vanhatalo, 2017; Keir et al., 2018). Different methods have been developed to precisely identify this intensity, such as all-out testing protocols or 30 min constant power trials to identify the points where the steady state is no longer maintained. From

a cardiorespiratory point of view, the secondary ventilatory threshold, or RCP, is considered the closest identifiable point to the upper limit of the heavy domain in a ramp incremental exercise test (Keir et al., 2018). When exercising above the upper limit of the heavy domain, maintaining a physiological steady state is no longer possible, and the $\dot{V}O_{2max}$ levels are attained rapidly relative to the exercise intensity, leading to exhaustion in a shorter time. The physiological differences between exercise domains are also reflected in different fatigue mechanisms. While central fatigue measured via voluntary activation seems to occur during heavy as well as severe domain exercise (Iannetta et al., 2022), single-joint studies reported a major presence of peripheral fatigue when exercise was performed above the critical torque, but no statistical differences of central fatigue between intensities above and below the critical torque (Burnley et al., 2012). In healthy young individuals, the percentages of PO_{peak} relatives to the GET range between 45-73% for men and 49-74% for women, while the range for RCP is 69-96% for men and 73-94%, respectively (Iannetta et al., 2020). Because of this high variability generally observed across participants, individuals at the same PO_{peak} percentage might exercise in different exercise domains, leading to possible different mechanisms of fatigue during the TTE trials, creating more heterogeneity regarding the relative individual intensity and fatigue mechanisms. This can, in turn, result in a confounding factor in understanding the possible effect of tDCS on reducing fatigue and increasing exercise tolerance.

1.5 Neuromuscular Fatigue and Transcranial Direct Current Stimulation

1.5.1 Interactions Between Cortical Excitability and Fatiguing Exercise

Fatigue is defined as any induced loss of muscle or muscle group contraction capacity to generate for or power induced by prolonged exercise (Enoka & Duchateau, 2008; Taylor et al., 2016). The processes contributing to muscle fatigue are multifaceted and occur at different levels of the motor pathway (Gandevia, 2001), and ultimately impact the ability of the muscle contractile mechanisms to produce force. The processes that occur at or distal to the neuromuscular junction are collectively termed peripheral fatigue (Allen et al., 2008; Debold et al., 2016), while processes within the central nervous system taking place in premotor and motor regions of the brain (Tanaka & Watanabe, 2012) and/or within the corticospinal motor pathway (McNeil et al., 2011; Weavil et al., 2015) which diminish neural drive to the muscle are termed central fatigue (Taylor et al., 1996). When central fatigue occurs, greater synaptic input into the motor cortex and/or spinal motoneurons is needed to maintain the muscle force required to sustain a given task (Mazzocchio et al., 1994). If the increase in neural drive from the brain or spinal motoneurons is not possible or insufficient to sustain the given task, the recruitment of motor units by the central nervous system diminishes, compromising the force of power production (Taylor et al., 2016), and then exercise capacity. The method to assess cortical excitability of the primary motor area is by producing MEP by delivering transcranial magnetic impulses (Rossini et al., 1991). Different TMS protocols exist, and the most commonly used in the tDCS literature is the resting motor threshold (RMT), which consists of finding the lowest possible stimulation intensity to evoke a muscular contraction. Thus, a bigger amplitude in the MEP response delivering the baseline RMT intensity stimulation after an intervention

(e.g., M1 anodal tDCS) would represent higher levels of cortical excitability. Regardless, the methods used to investigate cortical, spinal, and peripheral excitability in neuromuscular studies involving different types of contractions and exercise generally utilize techniques that compare electromyography (EMG) response resulting from stimulations at different sites of the motoneuronal pathway. During single-joint contractions at a fixed torque, the increase in muscle activation enhances cortical excitability (McNeil et al., 2011; Taylor et al., 1997; Weavil et al., 2015), although when the EMG is kept constant during submaximal fatiguing contractions, corticospinal excitability is reduced (McNeil et al., 2011). Corticospinal excitability remains unchanged during exhaustive whole-body exercise characterized by significant central and peripheral fatigue (Sidhu et al., 2012). A prevalence of inhibition over facilitation is observed during continuous large muscle mass dynamic contractions like cycling, evidenced by the absence of increased corticospinal excitability during constant load TTE trials at relatively high intensities (Weavil et al., 2016). Specifically, fatigue-related depression of cortical and/or motoneuronal excitability may reduce the excitatory effects of increased muscle activation (Martin et al., 2006; Sidhu et al., 2013; Weavil et al., 2016). Because the well-known effect of tDCS in the human cortex is the modulation of excitability of targeted area, especially its increase after several minutes of anodal stimulation, the most recurrent hypothesis in tDCS and exercise literature proposes the enhancement, or decrease in case of cathodal tDCS, of the targeted brain area would be the possible mechanism of changes in exercise tolerance or performance, modifying different factors that can affect physical capacities. For example, the reason why M1 is the most investigated area with anodal stimulation resides in the hypothesis that enhancing M1 cortical excitability can counteract the decrease of neuronal drive and voluntary activation at a supraspinal level induced by central fatigue, leading to increased endurance. Another proposed mechanism is that modulating DLPFC excitability can modify inhibitory control, thus, a more remarkable ability to endure physical and psychological fatigue during fatiguing exercise. To better understand whether tDCS can be employable and valuable

in the context of physical exercise, especially fatiguing contractions and multi-joint activities, it is essential to address the relationship between those tasks and the excitability of the neuron pool at different levels of the motoneuronal pathway. These neurophysiological mechanisms represent the reason for most motor cortex studies investigating possible enhancing effects on exercise tolerance and endurance with the administration of – mostly anodal - tDCS.

1.5.2 Impact of Transcranial Direct Current Stimulation on Perceived Effort during Whole-body Endurance Exercise

In addition to neurophysiological modifications induced by prolonged exercise, other psychological and physiological mechanisms concur with the capacity of individuals to tolerate fatigue. Among these, the perception of effort, defined as the sensation of strenuousness of a physical task (Borg, 1998), is considered a central limit of exercise tolerance and performance (S. Marcora, 2009). It has been demonstrated in several studies that manipulating the perception of effort during exercise can dramatically change exercise capacity and tolerance during prolonged whole-body activities. This can be achieved with different interventions, such as listening to music (Marques et al., 2024), self-talk (Blanchfield et al., 2014), and cognitive reappraisal (Giles et al., 2018). Despite the neurophysiological basis of effort perception still being debated in the scientific community, most evidence seems to support the presence of internal signals that arise from centrifugal motor commands, which influence perception (Pageaux, 2016). During fatiguing exercises, a myriad of processes simultaneously occurs at different levels. At a peripheral level, metabolic and mechanical perturbation can impair the intrinsic muscle capacity to contract or the neuromuscular junction to induce action potentials due to the accumulation of metabolites. At the same time, other processes can also impair the central nervous system to optimally drive the signal from the premotor and motor cortex through the central nervous system, leading to a decline in force generation capacity. Of course, much evidence demonstrates the

importance of tolerating fatiguing exercises, which depends not only on the neuromuscular system but also on the brain interpretation of muscular and cardiopulmonary peripheral afferent signals, in addition to inputs from regions "upstream" of the primary motor cortex. When sensory feedback is blocked during voluntary movement by ischemic nerve blockade of large afferents, there are no detectable decreases in movement-related activation in the somatosensory cortex concurrently to increase activation of the non-primary sensory-motor cortex (Christensen et al., 2007). One of these regions is the supplementary motor area (SMA), which is in the dorsomedial frontal cortex and contributes to ~10% of all corticospinal cells. Although the SMA function is yet to be fully understood, several reports in monkeys and humans have shown that SMA (and also pre-SMA) neurons fire before voluntary movement initiation (Nachev et al., 2008). EEG recordings in humans also showed a negative potential centered over the SMA and the pre-SMA before the movement onset, supporting the theory on the role of SMA on willing movement planning (Nachev et al., 2008). Also, this area seems to be affected by fatiguing exercise: SMA activity measured through functional magnetic resonance imaging increases during intermittent isometric isolated muscle fatiguing exercises and decreases when volitional exhaustion is reached. (Benwell et al., 2006; Liu et al., 2003; Van Duinen et al., 2007). Furthermore, cortical excitability measures assessed with TMS reported decreased SMA excitability after incremental cycling exercise until exhaustion (Coco et al., 2016). Different investigations showed an association between perceived effort and the magnitude of central motor command, suggesting the existence of brain pathways responsible for the generation of exertion perception (Williamson et al., 2006). In a neurophysiological study exploring the generation of effort during handgrip exercise, Zenon and colleagues reported a significant decrease in effort perception when the SMA was disrupted with continuous theta-bursts stimulation (cTBS) compared to M1 and a control site (Zenon et al., 2015). This is likely the most accurate study investigating the central generation of effort sensation, which indicates the SMA as a key node for the perception of effort generation. In some

of the already mentioned tDCS and exercise studies stimulating M1, but not all, a significantly lower RPE was reported after administering anodal tDCS (Angius et al., 2018). In that study, authors suggested that M1 cortical excitability changes could consequently impact perceived effort and exercise tolerance. Although this argument seems to be in contrast with the neurophysiological findings on the role of SMA on the generation of effort sensation, the fact that conventional tDCS employing relatively big electrodes is unlikely to stimulate a specific brain area with a high focality must be considered. It is then possible that SMA cortical excitability was also impacted when different RPE was reported for different stimulation conditions during whole-body and single-joint fatiguing exercises. Only a few studies have investigated the functional impact of SMA tDCS in different fields related to motor learning and reaction time. Employing a specific montage with a small electrode (8.1 cm^2) placed 1.8 cm anterior to the vertex and a large electrode (51 cm^2) on the forehead and stimulating at 1 mA intensity for 10 min, it has been shown differences in motor behavior based on tDCS polarity, anodal SMA tDCS, and cathodal tDCS significantly reduced and increased reaction time in a wrist extension go-task compared to sham up to 40 min after the stimulation ends, respectively (Carlsen et al., 2015). Other investigations revealed that SMA tDCS increased motor and visuomotor learning (Vollmann et al., 2013). Overall, SMA tDCS has been shown to produce functional changes in different types of motor performance. However, its effect on large muscle mass dynamic contraction endurance tolerance and related rate of perceived exertion has never been investigated.

Aims and hypothesis

Study 1: To investigate the potential differences of primary motor area anodal tDCS effects on exercise tolerance and/or related psychophysiological responses during constant work-rate exercise at heavy and severe intensity domains in healthy young male adults. We hypothesized that the contrasting results in the literature might arise from incorrect standardization of exercise intensity in previous studies and that an intensity-dependent effect of tDCS on exercise tolerance and related psychophysiological responses would exist.

Study 2: To investigate the effects of supplementary motor area tDCS on exercise tolerance and psychophysiological responses, particularly perceived effort, during constant work-rate exercise at the heavy intensity domain in healthy, active young male adults. We hypothesized that tDCS would alter the perceived effort experienced during prolonged whole-body exercise, thus leading to differences in exercise tolerance and psychophysiological responses to exercise.

2. General Methods

2.1.1 Participants, inclusion criteria, and general instructions

All participants involved in the study were students from the University of Pavia or residents living in Pavia or nearby. All participants were male volunteers and were contacted by the staff involved during the data collection. To meet inclusion criteria, all the participants had to be enrolled in regular physical exercise without any medical diagnosis and with authorization from a sports physician to practice physical exercise and competitive activities. None of the participants were smokers or users of dietary supplements or medications. Before enrolling in the study as volunteers, participants were informed of the aims and purposes of the research, the potential benefits for themselves and the community resulting from the experiments, the tDCS effects and associated potential side effects, and their freedom to leave the study at any moment. Thereafter, written informed consent was obtained from all participants before the commencement of the study. Furthermore, participants performed all the sessions at the same time of the day (± 2 h), separated by at least three days, in a temperature-controlled room (21-23°). Participants were also asked to arrive in the laboratory well-rested and fully hydrated, at least 3 hours postprandial, and refrain from alcohol, caffeine, and exercise training for at least 24 hours before each visit.

2.1.2 Familiarization Session and Incremental Ramp Exercise Test

During the first session of Study 1 and 2, participants arrived at the laboratory for preliminary assessments and procedures. Volunteers were illustrated and familiarized with the experimental equipment employed during the data collection. After demographic and anthropometrics data recording, participants mounted the cycle ergometer (Excalibur Sport, Lode, Netherlands) for vertical and horizontal handlebar and settled adjustments. Simultaneously, participants started cycling at different intensities ranging from 50 to 200 W and were asked to identify the most comfortable position and cadence between 70 and 90 revolutions per minute (RPM). After some

minutes, the cycle ergometer configuration was saved, and the preferred cadence was recorded. Participants were accurately informed that position and cadence would remain unchanged for the rest of the assessment and experimental sessions. After returning to baseline levels, participants performed a ramp-incremental test to task failure, preceded by 3 min of baseline recording and 3 min of warm-up at 20 W. The intensity increment was selected to ensure a test duration of at least 7 min (Midgley et al., 2008). In Study 1, which involved highly trained participants who were regularly enrolled in cycling activities, volunteers performed a 30 $W \cdot min^{-1}$ ramp incremental test: after the warm-up, the cycle-ergometer was automatically set at 50 W, and the cycle ergometer power increased by 1 W every 2 s. In study 2, which involved young active males less accustomed to using cycle ergometers, the increment was set to 25 $W \cdot min^{-1}$. During the test, participants were asked to maintain the preferred cadence shown in the cycle ergometer monitor for the entire duration and were restricted from seeing time and intensity. The test was set to automatically interrupt when the cadence fell 10 RPM below the preferred cadence for more than 3 continuous seconds (Bailey et al., 2009). During the entire test, oxygen consumption (Quark RMR, Cosmed, Italy) and heart rate (HR; Polar H10, Polar, USA) were continuously measured, and researchers verbally encouraged participants to reach volitional exhaustion.

2.1.3 Determination of Heavy and Severe Intensity Domains of Exercise

After the incremental ramp test, maximal and submaximal cardiorespiratory and performance parameters were determined offline. The oxygen consumption peak ($\dot{V}O_{2peak}$) was determined as the rolling average of the last 30 s of breath-by-breath data recorded during the incremental ramp test (Zhang et al., 2021). The GET, or estimated lactate threshold, is considered the first boundary between the moderate and the heavy intensity domains (Beaver et al., 1986; Whipp, 1996) and represents the transition from the moderate domain, where O_2 and CO_2 are respectively consumed and produced at the same rate, to the heavy domain, in which additional CO_2 is

produced because of the binding between H^+ ions with HCO_3^-, reflected in an increase of $\dot{V}CO_2$ relative to $\dot{V}O_2$ (Keir et al., 2022). Therefore, the GET was identified as the breakpoint observed in $\dot{V}O_2$ versus $\dot{V}CO_2$ and $\dot{V}O_2$ versus $\dot{V}_E$ relationships in the ramp incremental exercise test. The GET was then confirmed by checking abrupt changes of the ventilatory equivalents for $\dot{V}O_2$ ($\dot{V}E/\dot{V}O_2$) and $\dot{V}CO_2$ ($\dot{V}E/\dot{V}CO_2$), and the partial pressures of end-tidal O_2 ($PETO_2$) and CO_2 ($PETCO_2$) (Keir et al., 2022). The second submaximal threshold that can be individualized in a ramp incremental exercise test is the respiratory compensation point (RCP), also termed the second ventilatory threshold, and is considered the ventilatory response of the transition from the heavy domain to the severe domain, where H^+ ions concentration starts to raise because of the inability of the bicarbonate buffering system to produce enough CO_2, which lead to hyperventilation and a consequent drop of arterial CO_2 pressure. This point was identified with the second breakpoint in the $\dot{V}E$ versus $\dot{V}O_2$ relationship and then confirmed by the second and more rapid rise of $\dot{V}E/\dot{V}O_2$ and a deviation of $\dot{V}E/\dot{V}CO_2$ relationship versus $\dot{V}O_2$, in addition to an abrupt rise and fall of PETO2 and $PETCO_2$, respectively (Keir et al., 2022). Because of the delay in ventilatory response compared with the increase in power during a ramp incremental test due to the mean response time, two-thirds of the minute ramp increase was deducted from the power corresponding to GET $\dot{V}O_2$ values and from PO_{peak} (Poole & Jones, 2012). Thus, 20 W because of the 30 W increment and 17 W because of the 25 W increment every minute were deducted in Studies 1 and 2, respectively. In Study 1, the intensities relative to the severe and heavy domains were then defined as 75% (Δ75) and 15% (Δ15) of the difference between the GET intensity and PO_{peak}. In Study 2, 25% (Δ25) was selected as heavy domain intensity. Finally, the retrieved severe and heavy intensities were checked to ensure being above and below the RCP, respectively. Participants were unaware of any of these values until the end of the last experimental session.

2.1.4 Constant Work-Rate Exercise Test

In a real endurance context, physical performance is generally measured as the time to cover a prefixed distance (e.g., time trials, Amann et al., 2008). Although this is the most accurate and direct outcome representing performance, employing time trial tests in the research process can be challenging, especially in cross-sectional studies where different conditions are assessed within the same participant. Time trials intrinsically allow participants to change pace throughout the tests, which becomes a confounding factor when comparing physiological and psychological responses between different conditions (Thomas et al., 2012). Furthermore, a similar level of sensitivity between time-to-exhaustion in constant work-rate trials and time trials has been previously reported (Amann et al., 2008). For this reason, exercise tolerance has been assessed as the time of exhaustion in constant work-rate tests in severe (Study 1) and heavy (Study 1 and 2) intensities. In Study 1, rather than exercise tolerance in the heavy domain, our interest was to observe potential differences in physiological and perceived effort responses induced by the administration of tDCS. Thus, the duration of the heavy-intensity exercise was limited to 30 min. All the experimental sessions were conducted in a randomized and counterbalanced order to avoid the impact of learning effects throughout the studies. To prevent the impact of different pedaling frequencies on time to exhaustion and physiological responses (Vercruyssen et al., 2005), participants were allowed to see the cadence in real-time through the cycle ergometer monitor and asked to perform all the experimental sessions at the same cadence chosen in the familiarization phase. The constant-power output cycling trials were performed at randomly assigned intensities. Upon arrival at the laboratory, participants were comfortably seated and asked to rest for 10 min before receiving either sham or real tDCS (read Chapter 2.1.10 for details). After equipment preparation, participants

performed the constant-work rate test at the prescribed intensity previously described (Figures 1 and 2).

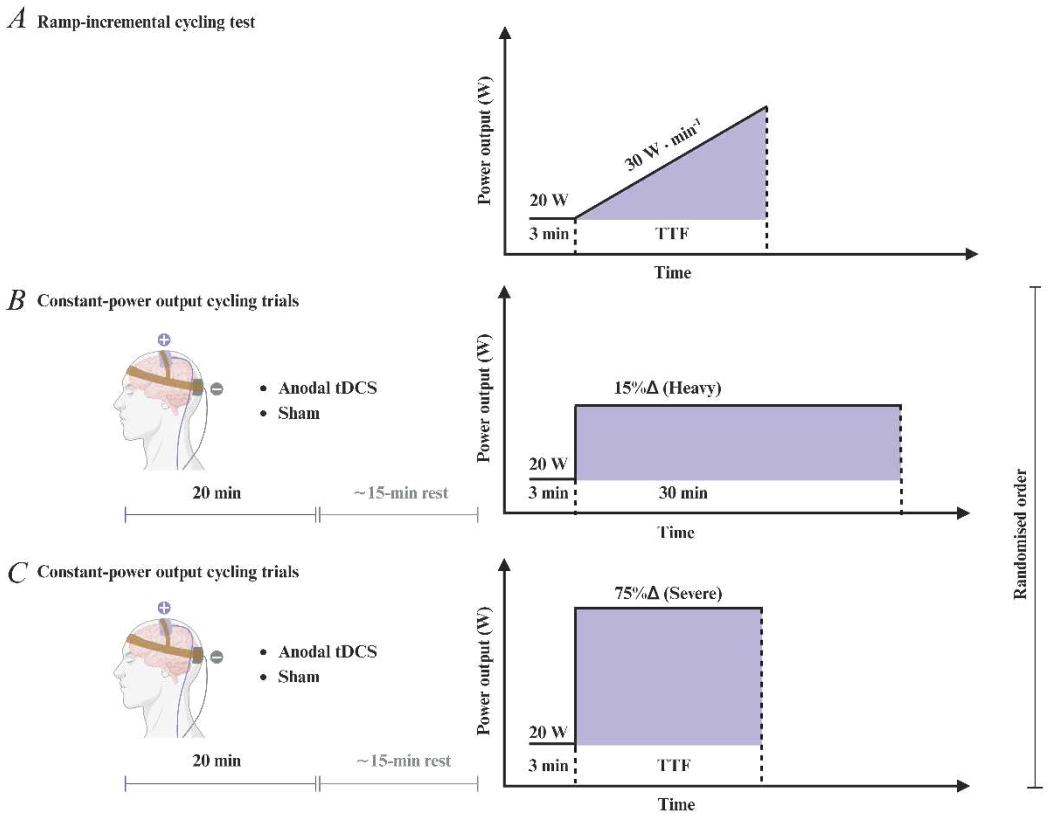

Figure 1. Study 1 design. Participants were recruited for a total of five sessions. One ramp-incremental exercise session and four experimental trials. Two 30 min heavy- and two time-to-exhaustion severe-intensity constant work-rate tests following 20 min 2 mA of either anodal or sham tDCS. TTF = Time-to-fatigue.

Time-to-exhaustion was recorded when participants voluntarily disengaged from the task or failed to maintain the cadence within the target range despite verbal encouragement with the same criteria of the ramp-incremental test already described. Before each trial, participants were asked to cycle at their self-selected cadence (±5 RPM) and were reminded of the task failure criteria.

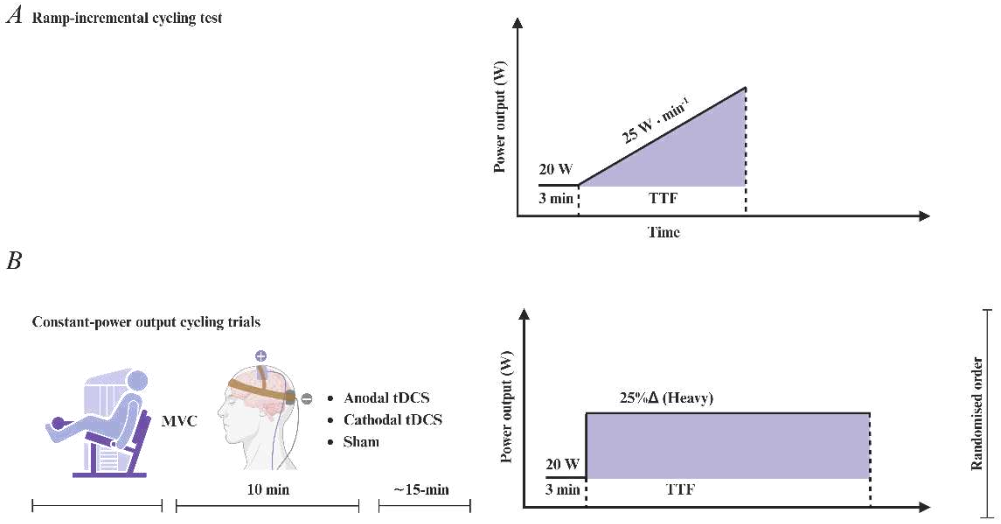

Figure 2. Study 2 design. Participants were recruited for a total of four sessions. One ramp-incremental exercise session and three experimental heavy-intensity constant work-rate tests following 10 min 1 mA of either anodal, cathodal, or sham tDCS. MVC = Maximal voluntary contraction; TTF = Time-to-fatigue.

2.1.5 Measurement and Data Processing of Pulmonary Gas Exchange and Ventilation

After several seconds of continuous exercise, skeletal muscles need to rely on oxidative mechanisms to sustain the required production of energy successfully; this happens for any typology of muscle contraction and becomes especially observable when large muscles are engaged, especially during dynamic large muscle mass and whole-body exercise. During incremental exercise tests, oxygen consumption shows a linear relationship with other performance parameters, such as ergometer mechanical power or treadmill speed, psychological and perceptual parameters, such as RPE, and physiological parameters, such as HR (American College of Sports Medicine et al., 2022). During constant work-rate exercise, individuals continuously exercise at the same intensity, and those relationships change. For example, an actual and complete oxygen consumption steady state without power or speed changes can be maintained only when exercising in the moderate domain below the GET. Above the GET and

below the RCP, which is the heavy domain, the oxygen consumption continuously and slowly increases, even without changes in mechanical power or speed. In contrast, in the severe domain, above the RCP, oxygen consumption reaches $\dot{V}O_{2max}$ level generally in a few minutes (Jones et al., 2019). It has also been shown that in steady-state conditions, the relationship between oxygen consumption and other parameters, such as HR and rate of perceived exertion, is not linear (Ferri Marini et al., 2022, 2024). Therefore, monitoring cardiorespiratory parameters during exercise testing is of paramount interest to control and observe the general responses to endurance exercise. Two main methods exist, and the standard procedures consist of sampling and analyzing the expired breath air during the exercise. One consists of collecting the air of several breaths in a mixing chamber, which is then sampled to measure the oxygen concentration every relatively long-time window. The mixing chamber methods generally provide more stable and smoothed data that is less susceptible to intensity changes. The second method consists of directly sampling and analyzing the air breath-by-breath. This method is generally considered more sensible to changes in exercising intensity, showing more variability, which is usually managed by applying rolling and time averages to the data. During the ramp-incremental test and the four constant-power output cycling trials, pulmonary gas exchange and ventilation were measured breath-by-breath using an automated metabolic cart (Quark CPET, Cosmed, Italy). The inspired and expired airflow volume was measured with a digital transducer turbine, while a paramagnetic O_2 analyzer and an infrared CO_2 analyzer measured gas concentrations. The breath-by-breath system allowed for continuous sampling of volume and concentration via a capillary line connected to the turbine. O_2 and CO_2 gas analyzer were calibrated before each test with a gas mixture of known concentration (16% O_2, 5% CO_2, and balance N_2), and the turbine was calibrated with a 3-L syringe (Hans Rudolph, Kansas City, USA) following the manufacturer's instructions. The turbine and the capillary were connected to a dedicated facemask secured to participants. After ramp-incremental and constant work-rate tests, breath-by-breath pulmonary ventilation and gas exchange were examined. Values over three standard

deviations from the local mean were considered errant breaths and removed (Lamarra et al., 1987). Breath-by-breath gas exchange and ventilation were then averaged into 10 s bins. In Study 1, during heavy-intensity exercise trials, $\dot{V}_E$, $\dot{V}O_2$, and $\dot{V}CO_2$ were calculated as the 30 s rolling averaged before the end of the 3 min baseline, and 30 s before the 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th}, and 30^{th} min of exercise, while in the severe intensity exercise trials, pulmonary ventilation and gas exchange data were calculated in the same way at the end of the 3 min warm up, and 20s before the 30^{th}, 60^{th}, 90^{th}, 120^{th}, 150^{th}, and 180^{th} s of exercise, as well as task failure. In Study 2, which employed constant work-rate time to exhaustion in the heavy-intensity domain, all data were normalized with a linear interpolation method to ensure consistency in data length across all tests. Data at 0, 20, 40, 60, 80, and exhaustion (100%) to allow comparisons without the implementation of missing data imputation techniques.

2.1.6 Measurement and Data Processing of Central Hemodynamics

Central cardiovascular mechanisms are crucial to increase $\dot{V}O_2$ when exercising, in addition to oxygen extraction capacity from the muscles. It is estimated that $\dot{V}O_{2max}$ is limited in a range between 70-85% from the maximal cardiac output ($\dot{Q}$), which is the product of HR and stroke volume (SV), representing the capacity of the cardiovascular system to increase blood flow and delivery O_2 when exercising. Increased $\dot{Q}$ is associated with increased $\dot{V}O_{2max}$ in endurance-trained individuals, and higher SV, which is the amount of blood ejected every beat, is the main discriminant in cardiovascular characteristics between sedentary and trained, healthy individuals of the same age. Both HR and SV increase during exercise to maintain the required amount of O_2 needed by the muscle to contract. The gold standard of $\dot{Q}$ measurement and other techniques involve invasive measurement that inserts a specific artery catheter connected to a temperature sensor (Pugsley & Lerner, 2010). The employment of these measurement techniques can be challenging, if not unfeasible, in a context

that requires dynamic whole-body exercise at very high intensities. A non-invasive method to assess cardiac output consists of measuring changes in transthoracic impedance during cardiac ejection with a specific impedance cardiograph (PhysioFlow Q-link, Menatec Biomedical, France), which has been proven to be reliable during both resting and exercise (Charloux et al., 2000; Richard et al., 2001). This technology is reliable for estimating SV by detecting transthoracic impedance changes during the cardiac cycle, which reflects the changes in the volume and velocity of aortic blood flow (Moshkovitz et al., 2004). This indirect measure of SV can then be used to determine $\dot{Q}$ (Siebenmann et al., 2015). Three pairs of electrodes (Ambu® BlueSensor, Ambu A/S, Denmark) were positioned on the chest following the manufacturer's instructions after gently skin-scraping and alcohol cleaning. Two pairs of electrodes were positioned at the supraclavicular fossa at the left base of the neck and next to the spine at the xiphoid level. An additional pair of electrodes were positioned at the center of the sternum and the left second rib following the manufacturer's instruction for exercise hemodynamic recordings. The electrode placement was followed by 5 min of resting, during which participants remained seated and immobile on a chair. Blood pressure of the brachial artery was then measured using an automated sphygmomanometer (Connex Spot Monitor, Welch Allyn Inc, USA). Blood pressure and anthropometric data were inserted into the PhysioFlow software to run an autocalibration based on 30 beats in complete resting conditions. SV, HR, and $\dot{Q}$ data were recorded continuously during constant work-rate tests with a 10 s temporal average.

2.1.7 Collection and Data Processing of Blood Lactate

Blood samples (20 µL) were collected from the earlobe into a capillary tube and analyzed after for [La-]b with an automated lactate analyzer (Biosen C-Line, EKF Diagnostic GmbH, Germany). Blood samples were obtained at baseline and after one, three, and 5 min after exhaustion during the severe-intensity exercise trials in Study 1

and after exhaustion in the heavy-intensity conditions in Study 2. Peak $[La^-]_b$ was determined as the highest $[La^-]_b$ measured during the post-test period (Beneke & Alkhatib, 2014), and blood lactate accumulation ($\Delta[La^-]_b$) was calculated as the difference between peak and baseline $[La^-]_b$ (Bailey et al., 2009).

2.1.8 High-Density Surface Electromyography Measurement and Data Processing

Electrical skeletal muscle activity provides crucial information on how the central and peripheral nervous systems finely control movement by activating the skeletal muscles, including the strength and timing of muscular contractions and how they respond to perturbance conditions such as fatiguing exercise. Different EMG techniques exist to measure skeletal muscle electrical activity. The most accurate consists of inserting a needle or a fine wire with an electrode on the tip into the muscle belly (intramuscular EMG) and directly recording the signal produced by the motor units in the surrounding area. On the other hand, intramuscular EMG, especially needle, is generally not implemented during exhaustion dynamic exercise because of possible pain influence in tolerance and performance, in addition to possible artifacts induced by the movements. Skeletal muscle activity can also be measured non-invasively by positioning a pair of electrodes directly on the skin. In recent years, high-density surface electromyography (HD-EMG) has continuously emerged as an evolution of conventional surface EMG. Instead of only two electrodes, HD-EMG generally employs one or more arrays of electrodes that need to be positioned in the same direction as the muscle fibers and allow the measurement of motor unit properties, such as muscle fiber conduction velocity CV that are not possible with conventional surface or intramuscular EMG (Merletti et al., 2008). Studies 1 and 2 both employed HD-EMG recordings in a monopolar configuration using an 8 x 4 multichannel array with 10 mm inter-electrode distance (Muovi, OTBiolettronica, Italy) with the vertical midline of the matrix positioned at two-thirds of the distance between the patella and the anterior iliac spine with an inclination of ∼20 degrees

relative to the line between the lateral border of the patella and the superior iliac spine (Barbero et al., 2012). A wrapped tie on the right ankle with a male clip connector was used as the reference. The skin was accurately prepared with an abrasive paste (Ac cream, Spes Medica, Italy) and cleaned with water to improve conductance. The matrix was attached to the skin through an adhesive foam, connected to the electromyograph, and secured with micropore tape and elastic bends to avoid possible detachment and movement during the exercise. The HD-EMG sampling rate was 2000 Hz, data were continuously transmitted via Bluetooth to the synchronization station (Syncstation, OTBioelettronica, Italy, Torino), attached in turn to a computer, and finally recorded with the dedicated software (OTBiolab+, OTBioelettronica, Italy). At the end of all the constant work-rate tests, the positions of the HD-EMG array were marked with a surgical marker pen, and participants were asked to keep it visible until the following experimental session to guarantee placement in the same position for successive experimental trials. HD-EMG data were first band-passed filtered (20-450 Hz) with a fourth-order, zero-lag Butterworth filter. Each channel was then visually inspected and removed if it exhibited a poor signal-to-noise ratio or artifacts (Hamard et al., 2023). Single differentials were calculated between adjacent filters in a proximo-distal direction and rectified to estimate myoelectric activity (Avrillon et al., 2021), and the average between the new 28 differential channels was then computed. The EMG amplitude was finally over 10 s intervals following a 0.5 s moving average. In Study 1, the baseline level was set as the highest value in the last 30 s of the 3 min warm-up cycling at 20 W, and data during both the severe- and heavy-intensity constant work rate was normalized accordingly. In Study 2, three MVCs were performed before initiating the experimental protocol, and myoelectric activity data of the constant work-rate trials were normalized to the highest values recorded in the MVC.

2.1.9 Measurement of Rate of Perceived Exertion

Physiological phenomena, such as cardiorespiratory, cardiovascular, and neuromuscular responses, are considered objective measures. Obtaining precise and reliable measures can be challenging, but although they can be influenced, the outcomes are independent of subjective interpretation from the assessed individuals. Regardless, many factors that directly influence physical performance, such as emotions or perceived effort, are intrinsically subjective, and any measurement must involve, if not solely, subjective measurement. The most used scale to measure perceived effort during exercise is the 6-20 Borg's scale, a numerical scale in which numbers are anchored to words to represent the perceived effort (6: no effort; 7: extremely light; 9: very light; 11: light; 13: somewhat hard; 15: hard; 17: very hard; 19: extremely hard; 20: maximal exertion). As previously suggested (Borg, 1998), participants were instructed to report how heavy and strenuous the exercise was at the given moment, combining all the sensations provoked by the exercise without concentrating only on leg pain or hyperventilation. The definition of perceived exertion was read to participants before each test, along with a set of instructions on how to use the 6-20 Borg's scale (Dasilva et al., 2011). All participants were asked to rate their conscious sensation of how hard, heavy, and strenuous the physical task was (Marcora, 2011) at baseline, at the end of the warm-up, every second minute during the constant work rate tests, and at exhaustion in both Study 1 and 2.

2.1.10 Transcranial Direct Current Stimulation Administration

In Study 1, the 10-20 EEG system was adopted for electrode placement: the center of the anode electrode (5 x 7 cm) was positioned on the Cz region ~3.5 cm of each side of M1 - while the cathode was on the occipital protuberance. This montage was used in the first study, which reported a significant improvement in exercise tolerance during 80% PO_{peak} constant work-rate tests. (Vitor-Costa, Nilo Massaru Okuno, et al.,

2015). To ensure an impedance level below 5 ohms and ensure good conductance, the electrode sponges were wrapped in a saline solution (0.9 NaCl), and a conductive gel was applied over the electrode correspondent area of the scalp (NeuroConn gel, Germany). In the anodal condition, the current was delivered with a portable apparatus (DC Stimulator, NeuroConn Inc, Germany) at 2mA for 20 min, with 30 s ramp up and down at the beginning and end of the stimulation, respectively. To blind participants from the sham condition, the electrodes were placed at the same positions, but the stimulator only delivered ramp-up and ramp-down phases to induce typical tickling sensations that often occur at the start and end of real tDCS (Gandiga et al., 2006). In Study 2, the first electrode (3 x 3 cm) was placed 1.8 cm in a rostral direction relative to Cz, while the second electrode (5 x 10 cm) was positioned in the center of the forehead. This method has been proven with the implementation of TMS to stimulate the SMA and reliably change performance in reaction time (Carlsen et al., 2015). The small electrode was plugged into the anode and cathode for anodal and cathodal tDCS, respectively. Because of different electrode sizes, the current intensity was set to 1 mA (current density: 0.156 mA/cm^2), and the stimulus was administered for 10 min with 15 s of ramp-up and ramp-down for anodal and cathodal tDCS. As in Study 1, the sham condition employed only the ramp-up and ramp-down phases and the start and the end of the stimulation period.

2.1.11 Statistical Analysis

Data are presented as means ± SD unless stated otherwise. Normality and sphericity of data were controlled with Shapiro-Wilk and Mauchly's tests, respectively. Degrees of freedom were adjusted with Greenhouse-Geisser when the assumption of sphericity was violated. Two-way repeated measures ANOVA were used to assess differences in physiological responses to both heavy- and severe-intensity cycling exercise across

conditions and over time. In case of significant differences, Bonferroni post-hoc correction was applied to identify specific differences between conditions at specific time points. Effect sizes were calculated as partial eta squared (η_p^2) and Cohen's d_z for omnibus and pairwise comparison tests and reported with their corresponding *P* values. Small, medium, and large effect sizes estimates were considered utilizing boundaries of ≤ 0.02, ≤ 0.13, and ≤ 0.26 for η_p^2, and ≤ 0.20, ≤ 0.50, and ≤ 0.80 for Choen's d_z (Cohen, 2013). In Study 1, TTE and $\Delta[La^-]_b$ were assessed using the paired-sample t-test between sham and anodal tDCS. A linear mixed model was employed to assess differences in RPE between different conditions. In Study 2, one-way repeated measures ANOVA were used to assess statistical differences between sham, anodal, and cathodal tDCS. Bonferroni post-hoc correction was applied in case of significant differences between conditions. Statistical analyses were performed using a commercially available statistical package (SPSS version 29.0; IBM, USA), with statistical significance set at $P < 0.05$.

3. Results

3.1 Study 1

3.1.1 Severe Intensity Domain

Twelve participants completed the severe-intensity domain experimental sessions.

Table 1 presents participants' age, anthropometric, physiological, and performance characteristics. Time-to-exhaustion trials lasted 262 ± 56 s and 258 ± 59 for sham and anodal conditions, respectively, showing no statistical difference in exercise tolerance [$t_{(11)} = 0.492$, $P = 0.633$, $d_z = 0.142$]. Similarly, no significant difference has been observed for $\Delta[La^-]_b$ [$t_{(11)} = -0.548$, $P = 0.595$, $d_z = 0.158$] between sham (13.4 ± 2.1) and anodal (13.6 ± 2.1) conditions (Figure 3).

Figure 4 shows pulmonary ventilation and gas exchange responses during constant-work rate exercise in the severe domain. Two-way repeated measures ANOVA revealed no effect of stimulation condition for $\dot{V}E$ [$F_{(1, 11)} = 0.226$, $P = .644$, $\eta_p^2 = .020$], $\dot{V}O_2$ [$F_{(1,11)} = .061$, $P = .809$, $\eta_p^2 = .006$], and $\dot{V}CO_2$ [$F_{(1, 11)} = .012$, $P = .914$, $\eta_p^2 = .001$]. Similarly, time × condition interaction revealed no significant differences for $\dot{V}E$ [$F_{(3.003, 33.035)} = 1.985$, $P = .135$, $\eta_p^2 = .153$], $\dot{V}O_2$ [$F_{(2.570, 28.269)} = .201$, $P = .868$, $\eta_p^2 = .018$], and $\dot{V}CO_2$ [$F_{(2.203, 24.235)} = . 0.366$, $P = .717$, $\eta_p^2 = .032$]. A significant effect of time was revealed for $\dot{V}E$ [$F_{(1.722, 18.943)} = 209.195$, $P = .644$, $\eta_p^2 = .950$], $\dot{V}O_2$ [$F_{(.443, 26.870)} = 171.175$, $P < .001$, $\eta_p^2 = .940$], and $\dot{V}CO_2$ [$F_{(1.870, 20.570)} = 230.916$, $P < .001$, $\eta_p^2 = .955$].

Figure 5 shows central hemodynamic responses during severe-intensity constant work rate for sham and anodal conditions. No significant differences were observed between sham and anodal conditions for HR [$F_{(1, 11)} = 3.358$, $P = .094$, $\eta_p^2 = .234$], SV [$F_{(1, 9)} = 0.105$, $P = .753$, $\eta_p^2 = .012$], and $\dot{Q}$ [$F_{(1, 9)} = 0.007$, $P = .936$, $\eta_p^2 = .001$]. Similarly, the interaction between time and condition revealed no significant differences for HR [$F_{(1.477, 16.243)} = 1.173$, $P = .319$, $\eta_p^2 = .096$], SV [$F_{(2.074, 18.662)} = 0.392$, $P = .689$, $\eta^{p2} =$

.042], and $\dot{Q}$ [$F_{(2.876,\ 25.883)}$ = 0.682, P = .565, η_p^2 = .070]. Differences between timepoints was revealed for HR [$F_{(1.785,\ 19.637)}$ = 109.560, P < .001, η_p^2 = .909] and $\dot{Q}$ [$F_{(2.060,\ 18.536)}$ = 29.759, P < .001, η_p^2 = .768], while no differences were observed for SV [$F_{(2.419,\ 21.772)}$ = 1.235, P = .316, η_p^2 = .121].

Figure 6 shows EMG and RPE responses during severe-intensity constant work-rate exercise. No significant differences between conditions were observed for EMG [$F_{(1,\ 11)}$ = 0.007, P = .936, η_p^2 = .001] and RPE [$F_{(4,64.6)}$ = 0.664, P = .418]. Similarly, no time × condition interactions were observed for EMG [$F_{(2.761,\ 30.368)}$ = 1.058, P = .377, η_p^2 = .088] and RPE [$F_{(3,\ 64.6)}$ = .319, P = .812]. Effects of time were observed for both EMG [$F_{(2.547,\ 28.019)}$ = 217.433, P < .001, η_p^2 = .952] and RPE [$F_{(4,66.3)}$ = 430.377, P < .001].

	Mean	SD
Age (yrs)	27.3	4.9
Weight (kg)	76.3	6.5
$\dot{V}O_{2peak}$ (mL ·kg^{-1} · min^{-1})	50.6	8.3
PO_{peak} (W)	371.8	44.5
Heavy intensity (W)	216.2	42.0
Heavy intensity (%PO_{peak})	57.8	4.8
Severe intensity (W)	312.3	48.1
Severe intensity (%PO_{peak})	83.8	1.9

Table 1: Age, anthropometric, physiological, and performance characteristics of Study 1 participants. $\dot{V}O_{2peak}$ = Oxygen consumption peak; PO_{peak} = Peak power output; SD = Standard deviation.

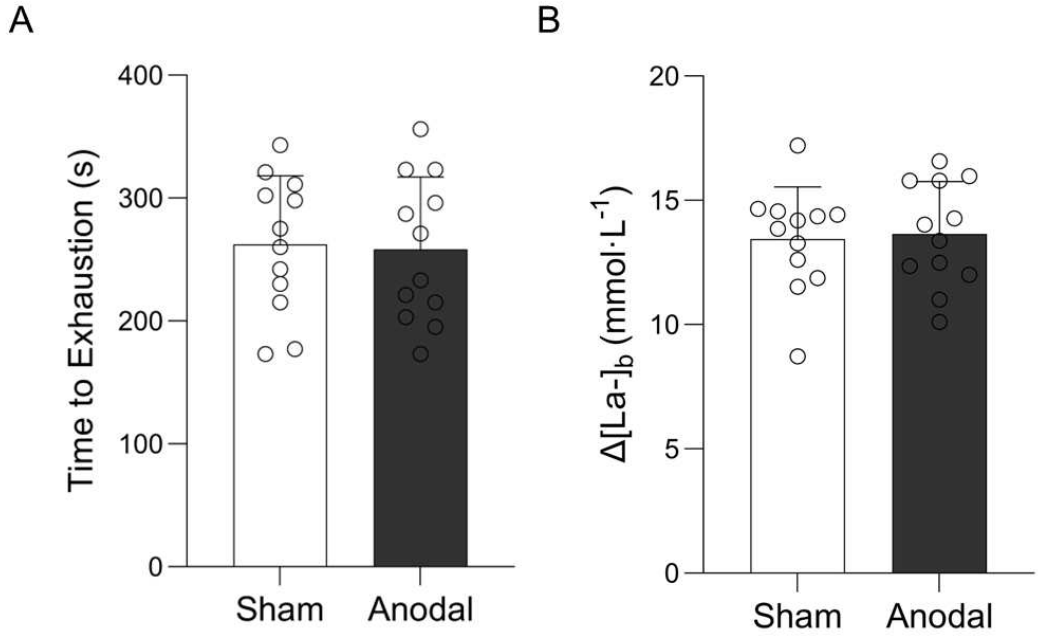

Figure 3. Time-to-exhaustion (A) and blood lactate accumulation (B) of severe-intensity constant work-rate tests of sham (white bar) and anodal (grey bar) conditions. Error bars represent standard deviation. Δ[La-]b = Blood lactate accumulation.

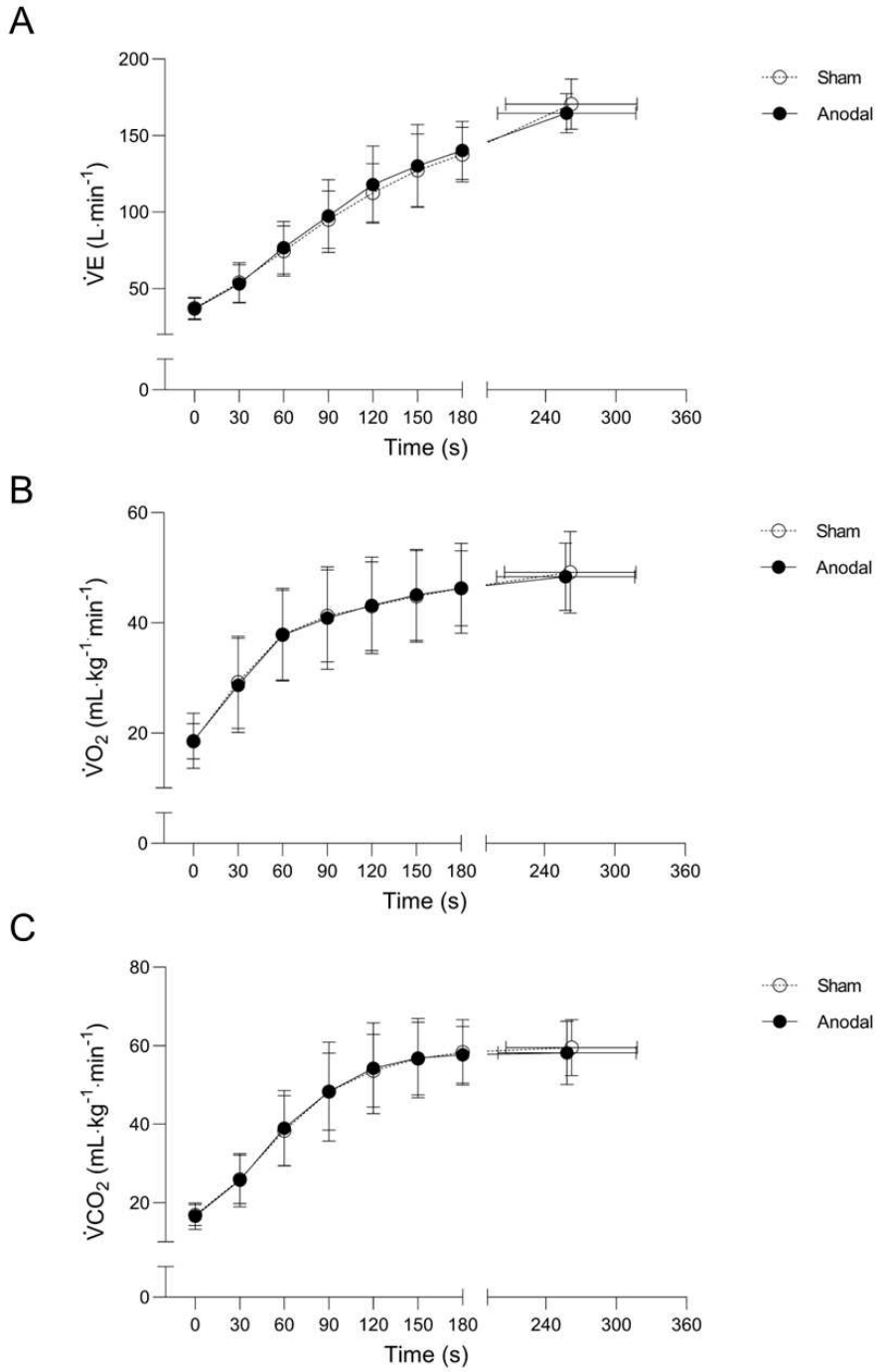

Figure 4: Pulmonary ventilation (A), oxygen consumption (B), and carbon dioxide production (C) responses during severe-intensity time-to-exhaustion constant work-rate tests for sham (empty circles) and anodal (black circles) tDCS. Error bars represent standard deviation. $\dot{V}E$ = Pulmonary ventilation; $\dot{V}O_2$ = Oxygen consumption; $\dot{V}CO_2$ = Carbon dioxide production.

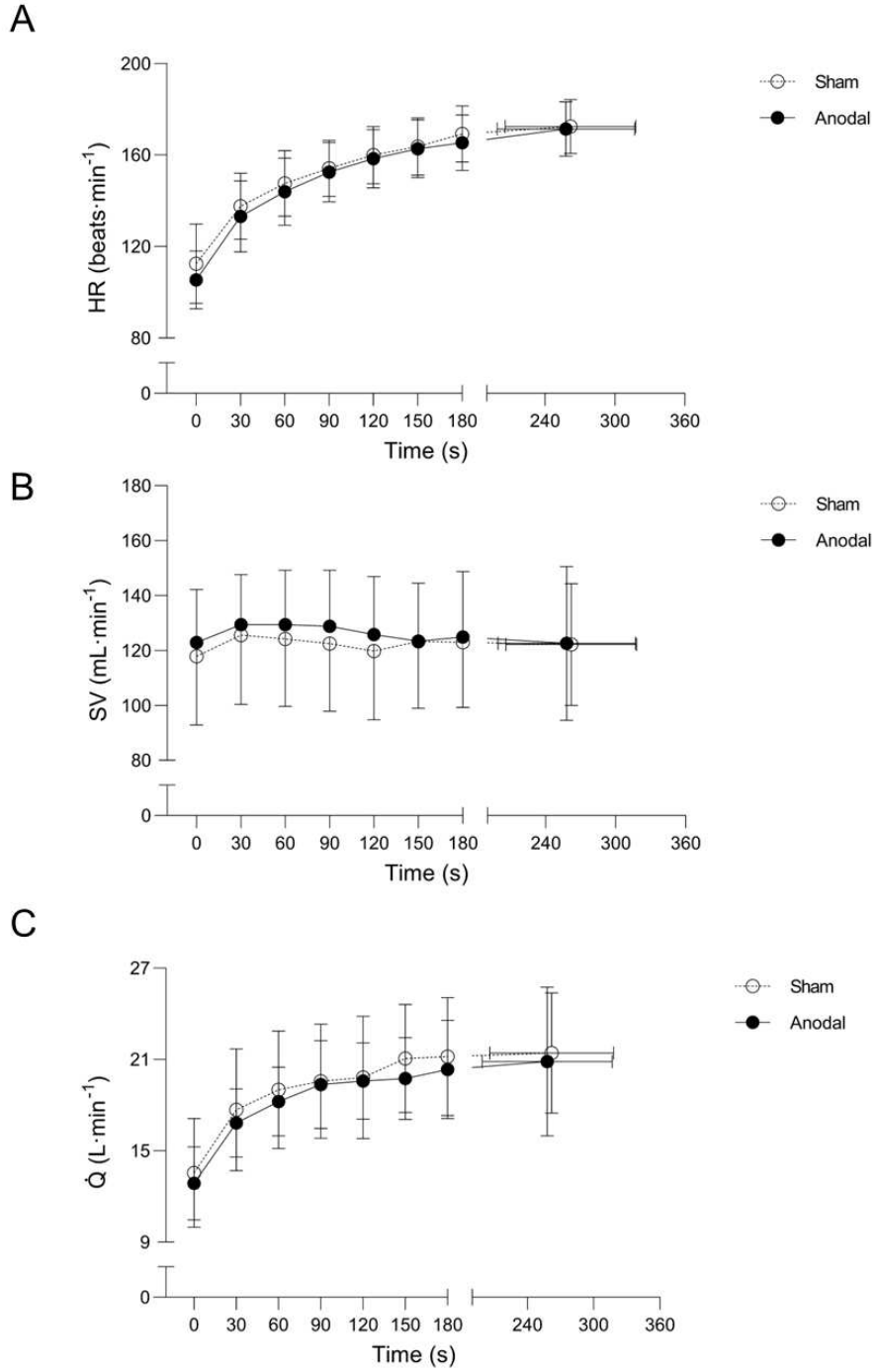

Figure 5: Heart rate (A), stroke volume (B), and cardiac output (C) responses during severe-intensity time-to-exhaustion constant work-rate tests for sham (empty circles) and anodal (black circles) tDCS. Error bars represent standard deviation. HR = Heart rate; SV = Stroke volume; $\dot{Q}$ = Cardiac output.

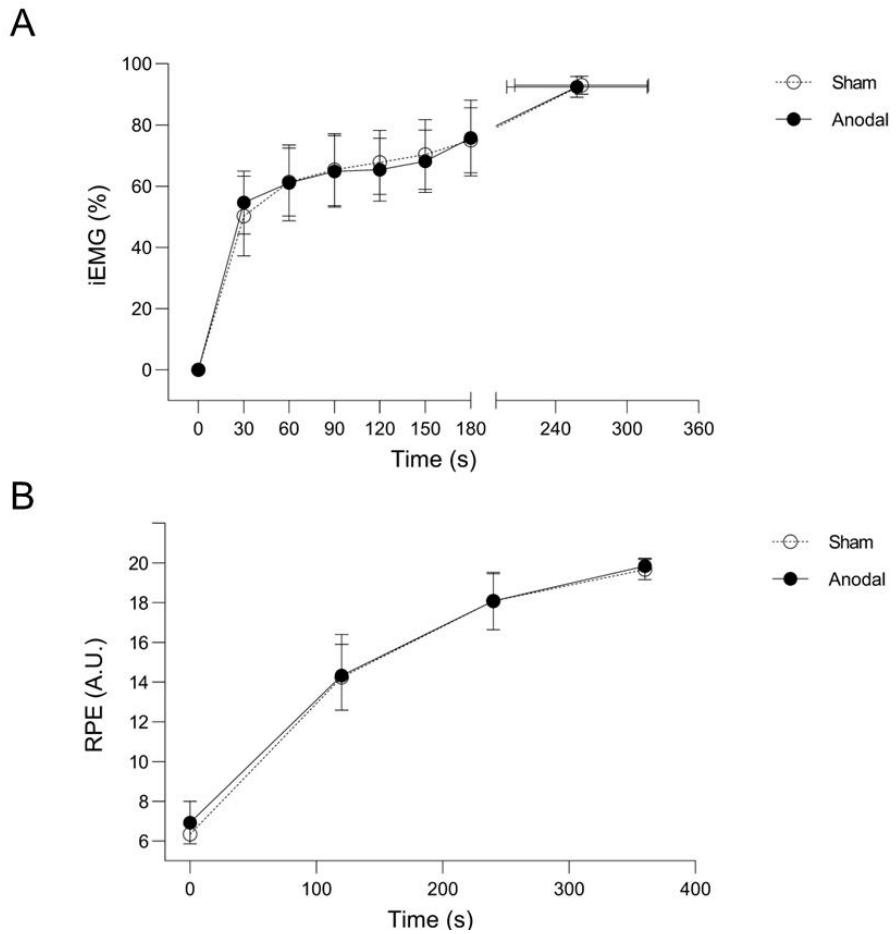

Figure 6: Electromyographic activity (A) and rate of perceived exertion (B) responses during severe-intensity time-to-exhaustion constant work-rate tests for sham (empty circles) and anodal (black circles) tDCS. Error bars represent standard deviation. iEMG = Integrated electromyography; RPE = Rate of perceived exertion.

3.1.2 Heavy intensity domain

Ten participants completed the heavy-intensity domain experimental sessions.

Figure 7 shows pulmonary ventilation and gas exchange in the 30 min heavy-intensity constant work-rate tests. No differences were revealed between conditions for $\dot{V}E$ [$F_{(1, 9)} = 0.016$, $P = .902$, $\eta_p^2 = .002$], $\dot{V}O_2$ [$F_{(1, 9)} = 0.004$, $P = .952$, $\eta_p^2 = .000$], and $\dot{V}CO_2$

[$F_{(1, 9)}$ = 0.004, P = .950, η_p^2 = .000]. Significant interactions between time and conditions were observed for $\dot{V}E$ [$F_{(1, 9)}$ = 0.016, P = .902, η_p^2 = .002] and $\dot{V}CO_2$ [$F_{(2.961, 26.652)}$ = 3.14, P = .042, η^2 = .259], while non-significant interaction was observed for $\dot{V}O_2$ [$F_{(2.439, 21.950)}$ = 2.48, P = .098, η^2 = .216]. Adjustments for multiple comparisons with Bonferroni correction revealed a significant difference at baseline levels between sham and anodal conditions for $\dot{V}E$ (MD = 4.157, SE = .1381, P = .015) and $\dot{V}CO_2$ (MD = 2.001, SE = .827, P = .039). A significant effect of time was revealed for $\dot{V}E$ [$F_{(1.185, 10.668)}$ = 56.21, P < .001, η_p^2 = .862], $\dot{V}O_2$ [$F_{(1.473, 13.256)}$ = 61.20, P < .001, η_p^2 = .872], and $\dot{V}CO_2$ [$F_{(1.291, 11.623)}$ = 86.31, P < .001, η_p^2 = .906].

Figure 8 shows central hemodynamics responses in the heavy-intensity exercise. No condition differences were reported for HR [$F_{(1, 9)}$ = 0.173, P = .687, η_p^2 = .019], SV [$F_{(1, 9)}$ = 0.920, P = .363, η_p^2 = .093], and $\dot{Q}$ [$F_{(1, 9)}$ = 1.38, P = .271, η_p^2 = .133]. Similarly, no time × condition interactions were observed for HR [$F_{(1.366, 12.290)}$ = 0.196, P = .741, η_p^2 = .021], SV [$F_{(6, 54)}$ = 0.81, P = .569, η_p^2 = .082], and $\dot{Q}$ [$F_{(3.431, 30.882)}$ = 1.41, P = .256, η_p^2 = .136]. Significant differences between time points were observed for HR [$F_{(1.785, 19.637)}$ = 109.560, P < .001, η_p^2 = .909], SV [$F_{(2.419, 21.772)}$ = 1.235, P = .316, η_p^2 = .121], and $\dot{Q}$ [$F_{(2.060, 18.536)}$ = 29.759, P < .001, η_p^2 = .768].

Figure 9 shows EMG and RPE responses during heavy-intensity constant work-rate exercise. No differences between sham and anodal conditions were observed for EMG [$F_{(1, 11)}$ = 0.007, P = .936, η_p^2 = .001] and RPE [$F_{(4,81)}$ = 0.388, P = 0.535]. Similarly, no interactions between time and condition were observed for EMG [$F_{(2.761, 30.368)}$ = 1.058, P = .377, η_p^2 = .088] and RPE [$F_{(4, 81)}$, = 0.400, P = 0.808]. Time effect was instead observed for both EMG [$F_{(2.547, 28.019)}$ = 217.433, P < .001, η_p^2 = .952] and RPE [$F_{(4, 81)}$ = 184.221, P < .001].

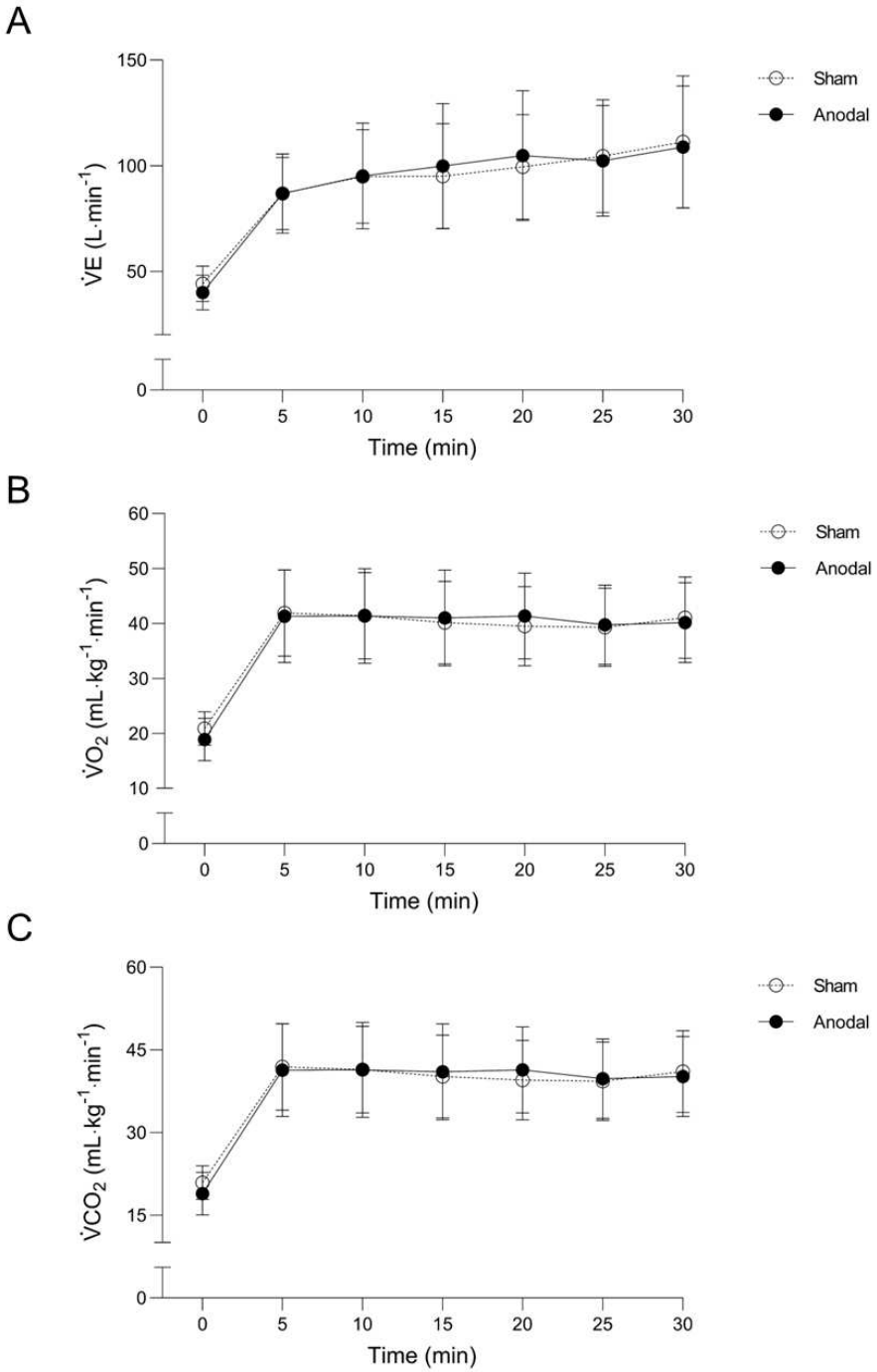

Figure 7. Pulmonary ventilation (A), oxygen consumption (B), and carbon dioxide production (C) responses during 30 min heavy-intensity constant work-rate tests for sham (empty circles) and anodal (black circles) tDCS. Error bars represent standard deviation. $\dot{V}E$ = Pulmonary ventilation; $\dot{V}O_2$ = Oxygen consumption; $\dot{V}CO_2$ = Carbon dioxide production.

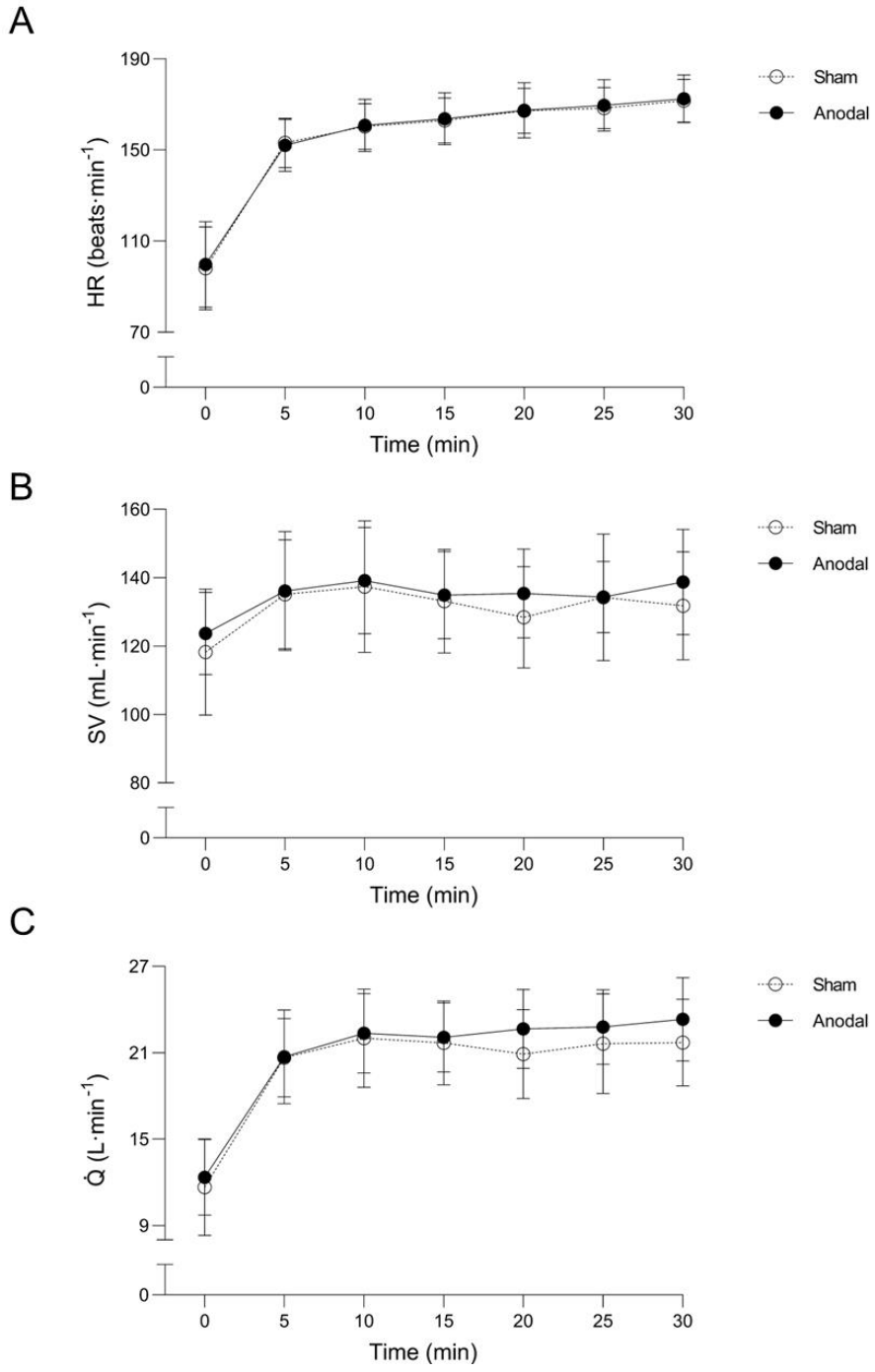

Figure 8: Heart rate (A), stroke volume (B), and cardiac output (C) responses during 30 min heavy-intensity constant work-rate tests for sham (empty circles) and anodal (black circles) tDCS. Error bars represent standard deviation. HR = Heart rate SV = Stroke volume; $\dot{Q}$ = Cardiac output.

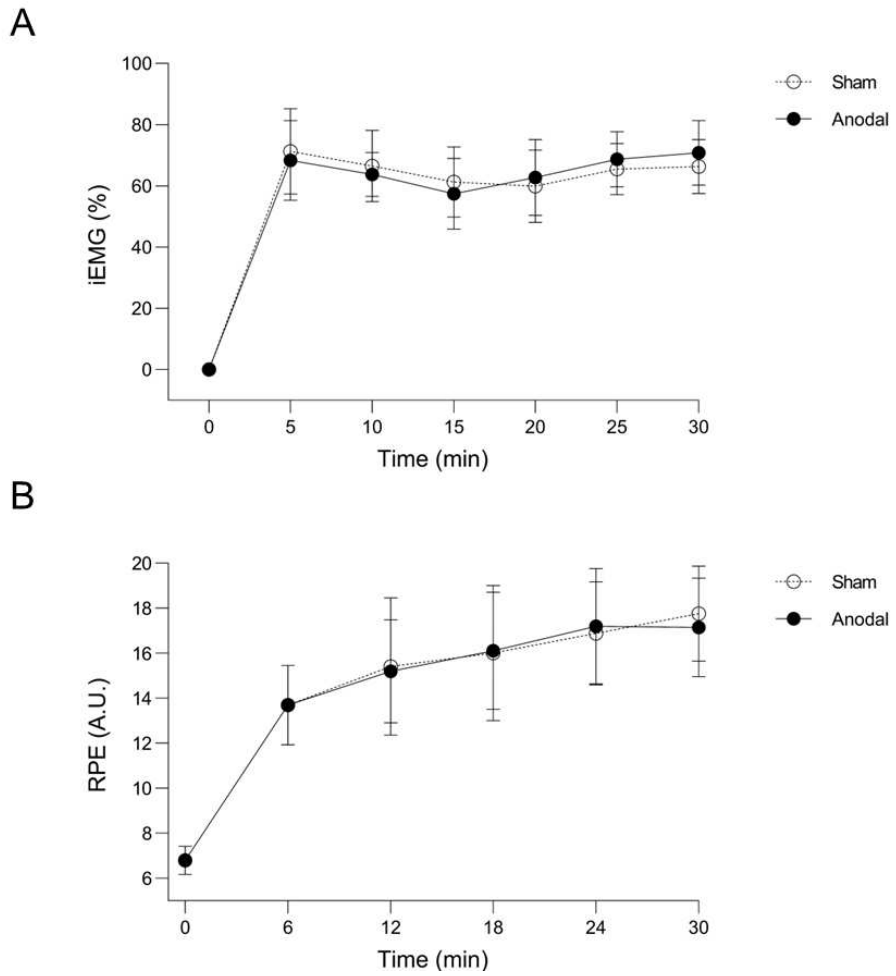

Figure 9. Myoelectric activity (A) and rate of perceived exertion (B) responses during 30 min heavy-intensity constant work-rate tests for sham (empty circles) and anodal (black circles) tDCS. Error bars represent standard deviation. RPE is shown every six minutes for clarity. iEMG = Integrated electromyography; RPE = Rate of perceived exertion.

3.2 Study 2

Twelve participants completed the heavy-intensity domain constant work-rate exercise following sham, anodal, and cathodal tDCS on the SMA. Table 2 presents participants' age, anthropometric, physiological, and performance characteristics.

Time to exhaustion lasted 30.8 ± 7.8 s, 31.2 ± 11.4 s, and 30.8 ± 12.0 s for sham, anodal, and cathodal tDCS, respectively. One-way repeated measures ANOVA reported no significant differences between the three stimulation conditions [$F_{(2,22)}$ = .030, P = .971, η_p^2 = .003]. Similarly, $\Delta[La^-]_b$ was 6.2 ± 2.0, 6.2 ± 2.1, and 6.1 ± 2.5 for sham, anodal, and cathodal tDCS, respectively, with no significant differences between conditions [$F_{(2,22)}$ = .003, P = .0997, η_p^2 = 0] (Figure 10).

Figure 11 shows pulmonary ventilation and gas exchange responses during constant-work rate exercise in the heavy domain. Two-way repeated measures ANOVA showed no significant differences between brain stimulation condition for $\dot{V}E$ [$F_{(2, 22)}$ = 0.347, P = .710, η_p^2 = .031], $\dot{V}O_2$ [$F_{(2, 22)}$ = 0.218, P = .806, η_p^2 = .019], $\dot{V}CO_2$ [$F_{(2, 22)}$ = 0.122, P = .886, η_p^2 = .011]. Similarly, no interaction between time and condition was observed for $\dot{V}E$ [$F_{(2.018, 22.193)}$ = 0.745, P = .487, η_p^2 = .063], $\dot{V}O_2$ [$F_{(1.550, 17.048)}$ = 0.463, P = .589, η_p^2 = .040], and $\dot{V}CO_2$ [$F_{(1.961, 21.566)}$ = 0.455, P = .636, η_p^2 = .040]. Effects of time was observed for $\dot{V}E$ [$F_{(1.321, 14.535)}$ = 91.318, P < .001, η_p^2 = .892], $\dot{V}O_2$ [$F_{(1.782, 19.602)}$ = 141.987, P < .001, η_p^2 = .928], and $\dot{V}CO_2$ [$F_{(1.373, 15.105)}$ = 83.030, P < .001, η_p^2 = .883].

Figure 12 shows central hemodynamic responses during heavy-intensity constant work-rate trials. No differences were observed between brain stimulation conditions for HR [$F_{(2, 22)}$ = 0.360, P = .702, η_p^2 = .032], SV [$F_{(2, 20)}$ = 1.660, P = .215, η_p^2 = .142], and $\dot{Q}$ [$F_{(2, 20)}$ = 1.561, P = .234, η_p^2 = .135]. Similarly, no time × condition interactions were revealed by the two-way repeated measures ANOVA for HR [$F_{(1.993, 21.922)}$ =

2.030, $P = .155$, $\eta_p^2 = .156$], SV [$F_{(2.910, 29.101)} = 1.255$, $P = .308$, $\eta_p^2 = .112$], and $\dot{Q}$ [$F_{(3.462, 34.625)} = 1.714$, $P = .176$, $\eta_p^2 = .146$]. Time effect was revealed for HR [$F_{(1.453, 15.979)} = 79.784$, $P < .001$, $\eta_p^2 = .879$], SV [$F_{(2.479, 24.787)} = 3.452$, $P = .039$, $\eta_p^2 = .257$], and $\dot{Q}$ [$F_{(2.332, 23.320)} = 22.162$, $P < .001$, $\eta_p^2 = .689$].

Figure 13 shows the myoelectric activity and RPE responses during heavy-intensity constant work-rate trials. No differences were observed between conditions for both EMG [$F_{(2, 22)} = 0.158$, $P = .855$, $\eta_p^2 = .014$] and RPE [$F_{(2, 22)} = 0.249$, $P = .782$, $\eta_p^2 = .022$]. Similarly, no significant interaction between time and condition was revealed for EMG [$F_{(2.397, 26.370)} = 0.909$, $P = .431$, $\eta_p^2 = .076$] and RPE [$F_{(3.996, 43.956)} = 0.249$, $P = .873$ $\eta_p^2 = .027$]. ANOVA revealed a time effect for RPE [$F_{(2.691, 29.597)} = 216.611$, $P < .001$, $\eta_p^2 = .952$], showing no time effect in myoelectric activity [$F_{(2.094, 23.037)} = 1.895$, $P = .172$, $\eta_p^2 = .147$].

	Mean	SD
Age (yrs)	21.5	1.6
Weight (kg)	74.6	10.7
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	42.9	3.6
PO_{peak} (W)	294.9	3.6
Heavy intensity (W)	165.0	23.8
Heavy intensity (%PO_{peak})	56.0	3.8

Table 2. Age, anthropometric, physiological, and performance characteristics of Study 2 participants. $\dot{V}O_{2peak}$ = Oxygen consumption peak; PO_{peak} = Peak power output; SD = Standard deviation

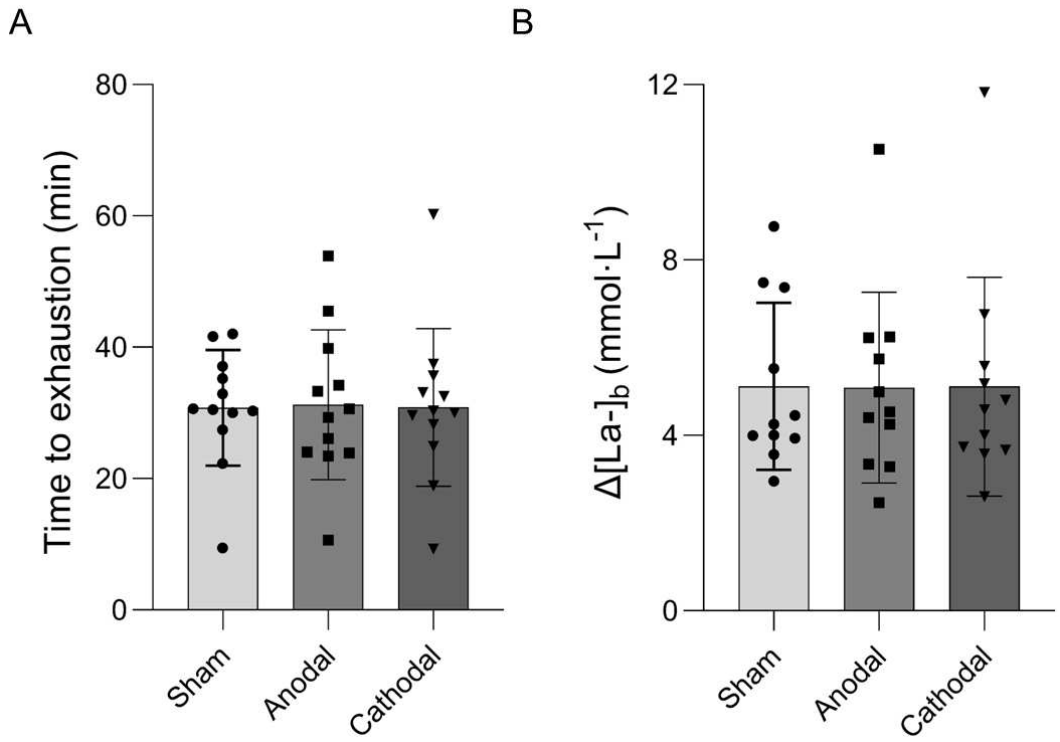

Figure 10. Time-to-exhaustion (A) and blood lactate accumulation (B) of heavy-intensity constant work-rate tests of sham (circles), anodal (squares), and cathodal (triangles) conditions. Error bars represent standard deviation. Δ[La-]b = Blood lactate accumulation.

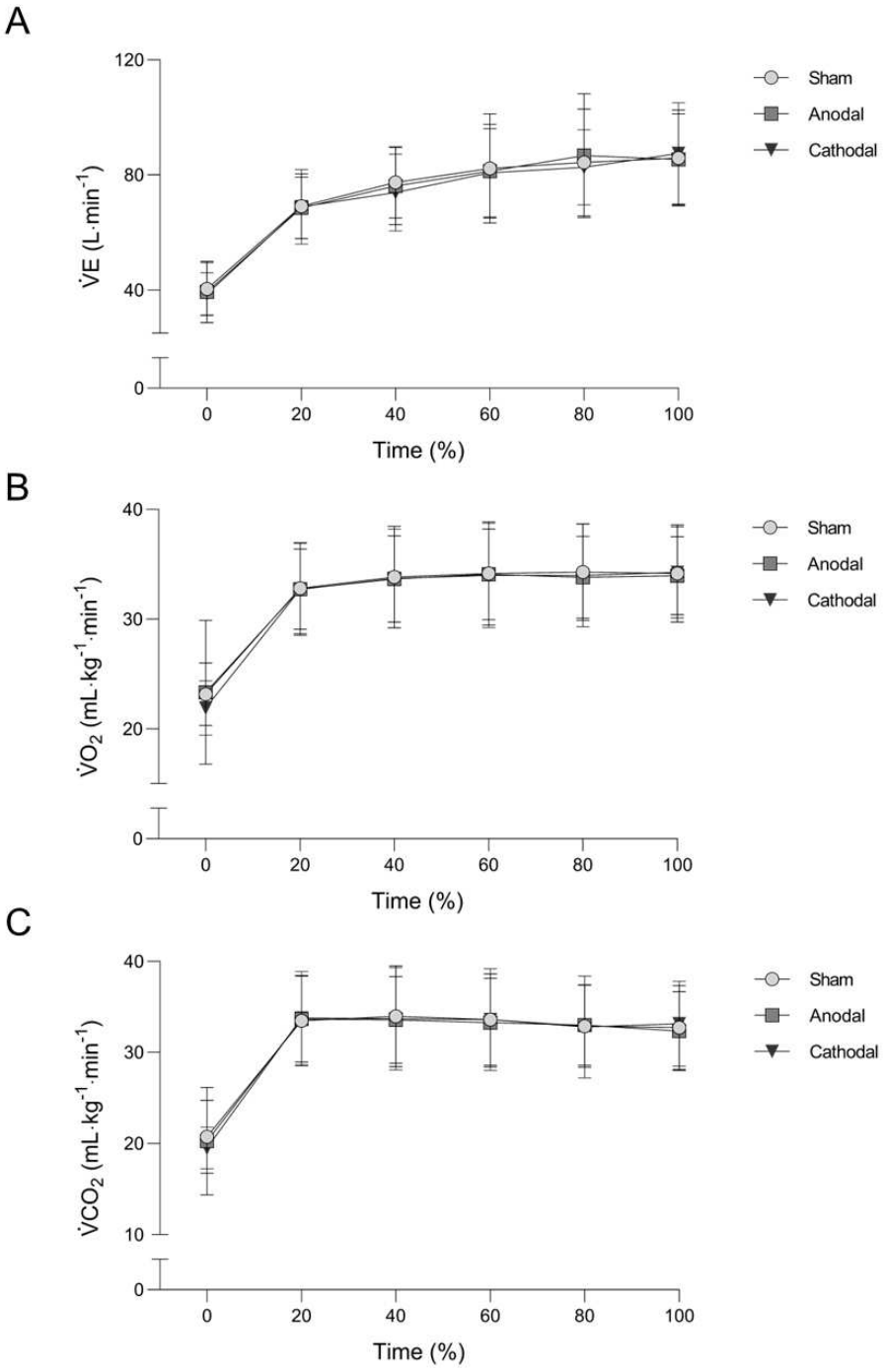

Figure 11. Pulmonary ventilation (A), oxygen consumption (B), and carbon dioxide production (C) responses during heavy-intensity time-to-exhaustion constant work-rate tests for sham (circles), anodal (squares) and cathodal (triangles) tDCS. Error bars represent standard deviation. $\dot{V}E$ = Pulmonary ventilation; $\dot{V}O_2$ = Oxygen consumption; $\dot{V}CO_2$ = Carbon dioxide production.

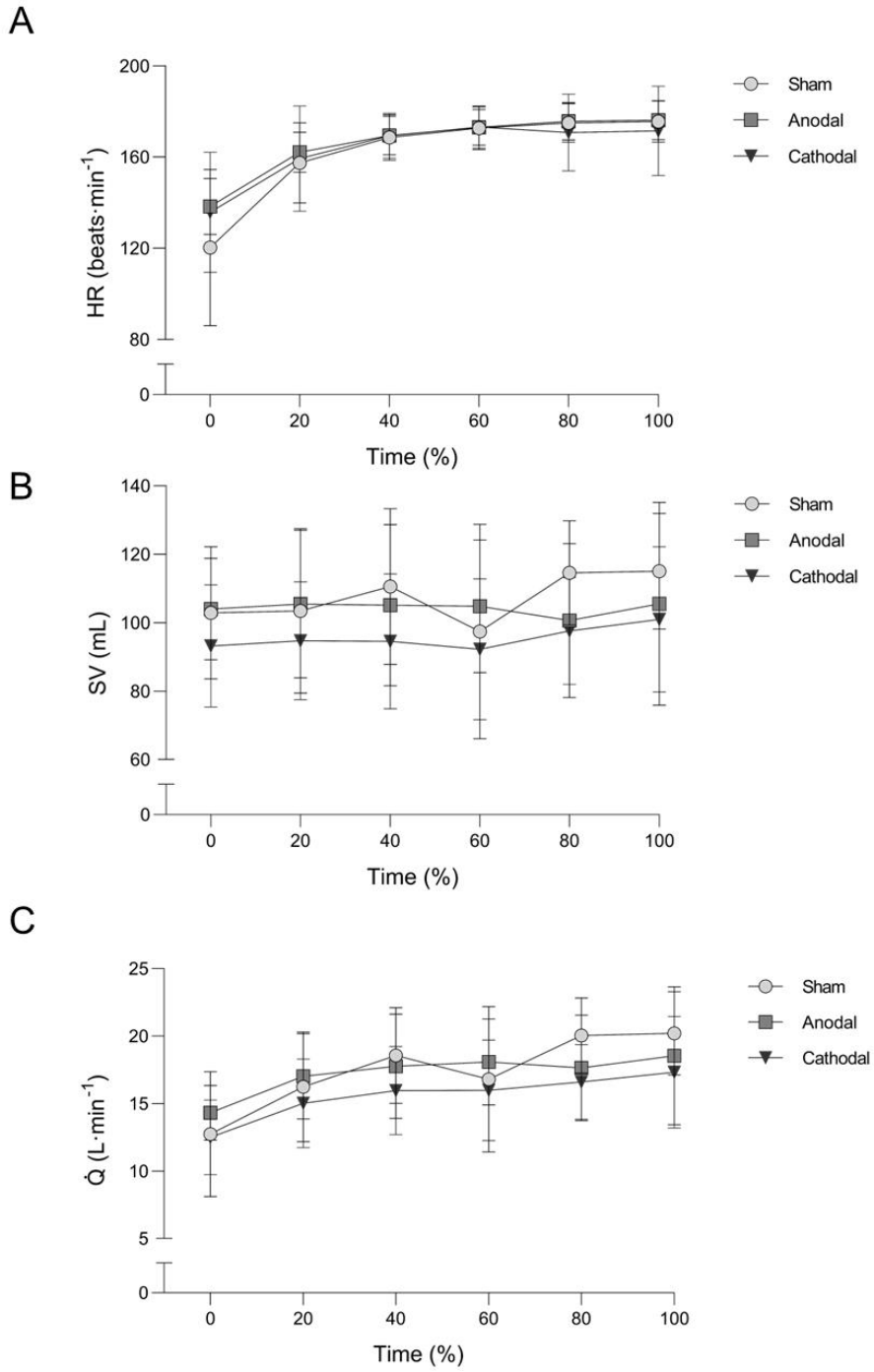

Figure 12: Heart rate (A), stroke volume (B), and cardiac output (C) responses during heavy-intensity time-to-exhaustion constant work-rate tests for sham (circles), anodal (squares) and cathodal (triangles) tDCS. Error bars represent standard deviation. HR = Heart rate; SV = Stroke volume; $\dot{Q}$ = Cardiac output.

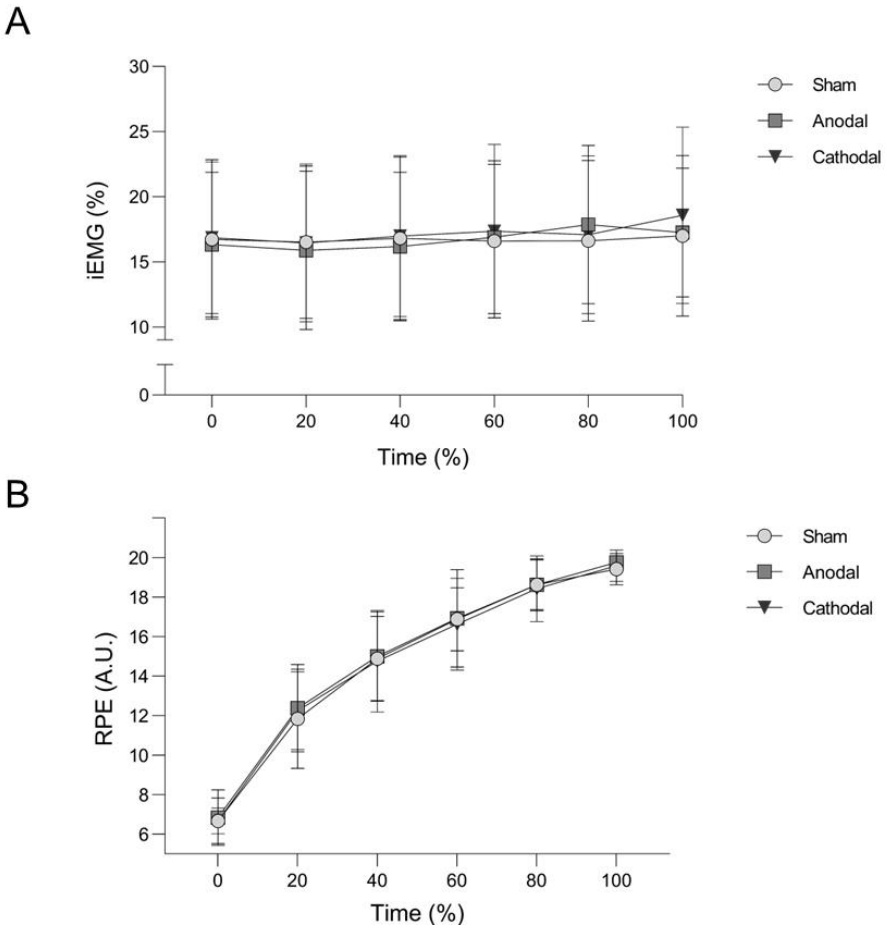

Figure 13. Myoelectric activity (A) and rate of perceived exertion (B) responses during heavy-intensity time-to-exhaustion constant work-rate tests for sham (circles), anodal (squares), and cathodal (triangles) tDCS. Error bars represent standard deviation. iEMG = Integrated electromyography; RPE = Rate of perceived exertion.

4. Discussion

Exploring new ways of increasing the exercise capacity and exercise performance of different populations is one of the most intriguing and essential challenges in exercise and sports science. Because it is increasingly evident how brain processes are crucial in healthy and diseased populations, the interest in neuromodulation to optimize exercise capacity has significantly increased in the past years. Due to its safety, relative ease of use, and affordability, tDCS is undoubtedly the most investigated neuromodulation tool in this sense. The electric field generated by tDCS modulates neural activity depending on the current polarity (Nitsche et al., 2008). In the short term, during the stimulation, the membrane resting potential of neurons under the anode is expected to depolarize, while near the cathode is expected to hyperpolarize (Stagg & Nitsche, 2011). In the long term, following the stimulation, the tDCS effect is supposed to induce long-term potentiation and long-term depression under the anode and the cathode, respectively (Nitsche et al., 2005). Thus, anodal stimulation increases cortical excitability levels of the stimulated area, while cathodal stimulation diminishes it. The most used method to assess cortical excitability in tDCS classical studies was identifying the lowest intensity necessary to evoke a short-latency electromyography response in a resting muscle, called motor-evoked potential (MEP), and changes in the MEP amplitude reflect changes in the cortical and spinal excitability levels of the involved neurons (Hallet, 2000; Hallet, 2007). Other measures employing TMS, such as cortical silent period, intracortical inhibition and facilitation, short interval cortical inhibition, functional magnetic resonance imaging, and EEG power spectrum, can be used. A systematic review that included in the analysis numerous different methods of cortical excitability assessment reported how tDCS seems to alter only MEP measures systematically, and, interestingly, the magnitude of the effect is decreased with time and the advancement in technologies and techniques, addressing this change to the improvement in overall signal to noise ratio of the equipment employed in the studies (Horvart et al., 2015). Furthermore, with TMS only, it is unfeasible to address changes at different sites of the corticomotoneuronal pathway. Still, it is also necessary to administer stimulations at the spinal level, with the

implementation of cervicomedullary or thoracic MEPs, and with supramaximal stimulation at the peripheral nerve. Even though it is reasonable that the administration of tDCS at the supraspinal level provokes changes in MEPs depending on the cortical excitability, only normalizing cortical and spinal MEPs to the maximal peripheral stimulation allows the chance to detect that potential change (Butler et al., 2003, Amann et al., 2022). Only a few studies addressed changes in cortical excitability after M1 tDCS administration, normalizing the MEPs to the maximal peripheral stimulation, with conflicting results. Two studies reported no differences between a single session of anodal and sham tDCS when measuring the MEP amplitude or area as a percentage of the maximal peripheral stimulation in the knee extensors (Angius et al., 2016; Kristiansen et al., 2021), while after four days of either anodal or sham tDCS, the normalized MEP amplitude recorded in the wrist extensors increased in the anodal condition, also increasing maximal voluntary contraction (Frazer et al., 2016). Two different scenarios are plausible. First, it is possible that one single session of tDCS is not sufficient to induce cortical and/or spinal changes that can acutely reflect exercise tolerance, some indications from neurophysiological investigations (Frazer et al., 2016) and different studies on functional capacities in both healthy (Jaberzadeh & Zoghi, 2022) and clinical (Andrade et al., 2017) populations suggest a cumulative effect of cortical excitability modulation when tDCS is administered for several days. Second, the leg area of the motor cortex may be less susceptible to cortical excitability changes compared to the upper limb following tDCS, at least with conventional tDCS montages. Despite the lack of evidence, it is plausible that the electric field generated by the tDCS in these studies hardly reached the neurons of the leg area representation, which are located more deeply (Litcher et al., 2007; Meier et al., 2008). A recent computational work simulating different montages showed how the montage C1-C2, with 5 x 5 $cm^{2,}$ can be the more appropriate to reach the deepest region of the lower-limb motor area (Hamaijama et al., 2023). Despite all these considerations, the main concern on tDCS's potential to systematically increase exercise tolerance and impact fatigue during exercise still needs further elucidation from a neuromuscular point of

view. The most proposed mechanism of action in previous tDCS studies on exercise science is the exercise capacity enhancement induced by the increased neuromuscular drive, which is, in turn, derived from the increased cortical excitability measured with MEP. In a recent review, there is no complete consensus on the relationship between motor cortex excitability and fatigue, which seems to be affected by exercise modality and cortical excitability measurement (Amann et al., 2022). During whole-body fatiguing exercise, evidence from the literature suggests that when considering the fatiguing effect on neural drive, MEP size decreases compared to baseline and non-fatiguing exercise (Sidhu et al., 2012; Weavil et al., 2016), but not the silent period, which is another marker of cortical excitability (Sidhu et al., 2017; Sidhu et al., 2018). Furthermore, a recent investigation that combined high-density electromyography and stimulation at different sites of the motor pathway suggested that changes in cortical excitability during and after fatiguing isometric contractions in two different intensities are the cause of increased motor unit firing rates (Angius et al., 2024), and this agree with other several investigations that suggest how changes in cortical excitability are more likely a consequence or a concurrent effect of fatigue, rather than its cause (Gandevia, 2001). Although central fatigue is a critical aspect of physical tolerance and is strictly correlated with cortical excitability, evidence from the last decades of neurophysiological fatigue studies suggests the unlikelihood of a direct cause-effect of cortical excitability on central fatigue, consequently, the rationale for reducing exercise-induced neuromuscular fatigue by increasing cortical excitability is arguable. More investigations are needed to better establish the relationship between fatigue and cortical excitability.

In Study 1, we explored the possibility that the discrepancies from different studies of the last years were attributable to the lack of exercise protocol standardization. All the previous investigations that explored tDCS-induced performance improvements during whole-body exercise employed incremental ramp tests or constant work-rate

tests at a fixed percentage relative to the PO_{peak}. There are indications that the intensity and the duration of the exercise lead to the distinct presence of central and peripheral fatigue, or at least, to a preponderance of one or the other. Short and high-intensity locomotor exercise lead to pronounced peripheral fatigue, while longer durations to a greater presence of central fatigue (Place et al., 2004; Thomas et al., 2015, 2016), and the peripheral and central fatigue presence is dependent on the exercise domain (Black et al., 2017; Burnley et al., 2012). Because intensity prescription based on fixed percentages of PO_{peak} could lead to exercise in different domains for different participants because of interindividual variability of ventilatory and metabolic thresholds (Iannetta et al., 2020), we hypothesized that tDCS effects on exercise tolerance and related psychophysiological responses could be dependent on the exercise domain. Regardless, no differences have been revealed between real and sham tDCS in both heavy and severe domains of exercise, suggesting that the tDCS protocol employed in Study 1 was incapable of altering any physiological or RPE responses. The results from Study 1 agree with most part of the existing literature that investigated tDCS effects on exercise tolerance. Regardless of whether the performance (i.e., time-to-exhaustion) occurs in the real stimulation compared to the sham condition, the physiological responses were unaltered during the exercise in terms of $\dot{V}O_2$ and HR (Angius et al., 2018; Baldari et al., 2018; Barwood et al., 2016; Vitor-Costa, Okano, et al., 2015). A second mechanism proposed in the literature is the potential capability of tDCS administration to alter the perception of effort during sustained physical exercise and to improve exercise tolerance or performance accordingly. Psychological motivation to sustain the effort is considered a crucial aspect of exercise tolerance (S. M. Marcora & Staiano, 2010; Wright, 2008), and it has already been demonstrated that the different modalities of perceived exertion manipulation can significantly impact individuals' ability to sustain different types of effort (Blanchfield et al., 2014; Giles et al., 2018; Marques et al., 2024). The rate of perceived exertion is defined as the subjective level of strenuousness of the exercise (Borg, 1998), and the neurophysiological generation and/or the interpretation of this sensation is still debated

in the literature. Perceived effort is likely the integration of peripheral signals from the III/IV group afferents (Amann et al., 2010; Bergstrom et al., 2015) and the central motor command (De Morree et al., 2012). A cTBS study suggested that perceived effort particularly relies on circuits upstream of M1, especially the SMA (Zenon et al., 2015). In Study 2, we tested the hypothesis that administering tDCS on the SMA would alter perceived exertion and physiological and exercise tolerance accordingly during cycling exercise in healthy, active young adults. Although a few studies about SMA tDCS and physical performance have been published, this is the first to investigate its potential effects on an exercise involving large muscle mass and dynamic contractions. Similarly to Study 1, no significant differences have been observed between different conditions on exercise tolerance, reflected as time-to-exhaustion, and the other related psychophysiological variables. Surprisingly, both anodal and cathodal tDCS on the SMA were not capable of altering the RPE response compared to the sham condition. Some previous studies revealed a decrease in RPE during time-to-exhaustion isometric exercise after an extracephalic montage tDCS (Angius et al., 2016) and during cycling after bicephalic montage tDCS (Angius et al., 2018). In any case, whether the RPE modulation was the cause of exercise capacity enhancement or vice versa is not inferable from these reports. Furthermore, the results from Study 2 are similar to several other studies employing different types of tDCS that showed unaltered RPE responses during exercise (Baldari et al., 2018; Barwood et al., 2016; Holgado et al., 2019). Previous studies reported how SMA tDCS modulated reaction time (Carlsen et al., 2015) and visuomotor learning (Vollmann et al., 2013). The main reason to explain the absence of effects in RPE and exercise tolerance may reside in the differences between tDCS and cTBS with TMS, which have been previously reported as capable of altering the perception of effort of participants during a handgrip exercise (Zenon et al., 2015). Although cTBS has been reported to provoke similar cortical excitability effects of cathodal tDCS (Di Lazzaro & Rothwell, 2014), it is possible that the different electric fields generated by the two techniques led to a different magnitude of cortical excitability modulation. Another possibility to explain the absence may reside in the

different types of exercise employed in this study. During continuous locomotor exercise that involves large muscle mass dynamically, the RPE response can be affected by breath rhythm and peripheral muscle sensations (Bergstrom et al., 2015). It is then possible that the SMA cortical excitability modulation was not sufficient to impact the RPE reported during exercise. The two studies involved participants with a moderate-to-good level from a cardiorespiratory point of view. In Study 1, the average level of $\dot{V}O_{2peak}$ and PO_{peak} assessed in the ramp incremental test shows a "good" to "excellent" level based on the ACSM guidelines (American College of Sports Medicine et al., 2022), while in Study 2, participants are classified in the "fair" category. However, none of the participants were competitive cycling athletes in the period of data collection. Thus, we think that a strength point of the studies is that they are extensible to a wide spectrum of healthy, active general populations. In contrast, one limitation of the studies is the absence of female participants, making it impossible to explore potential sex differences. In the past years, several randomized controlled trials and systematic reviews with meta-analysis have been published (Chinzara et al., 2022; Maudrich et al., 2022; Shyamali Kaushalya et al., 2022), despite the overall conclusion from the authors favor a small effect of anodal tDCS compared to sham on endurance performance, these findings are challenged by the difficulties of subgrouping the meta-analysis for both exercise and tDCS protocols. The employment of studies that stimulated different brain areas (i.e., M1 and DLPFC) and assessed performance or tolerance in markedly different ways (i.e., time-to-exhaustion and time trials) increases the risk of misinterpreting published results in addition to hiding the comprehension of the underlying mechanisms that, if exist, would make tDCS a potential ergogenic tool for increase exercise tolerance or performance in healthy young adults.

5. Conclusions

The studies included in the present dissertation have explored the potential ergogenic effects of two different transcranial direct current stimulation protocols during whole-body exercise involving large muscle mass. In Study 1, a conventional anodal tDCS protocol on the primary motor cortex was employed in combination with a constant work-rate cycling trial in the heavy and severe domains of intensity, and no significant differences were observed between real and sham tDCS. In Study 2, a novel approach was investigated, where anodal, cathodal, and sham tDCS were administered on the supplementary motor area to alter the effort perception during a constant work-rate cycling test in the heavy-intensity exercise domain, showing no significant differences between experimental conditions. In conclusion, a single session of tDCS over the primary motor cortex or supplementary motor area seems incapable of increasing exercise tolerance and altering psychophysiological responses to constant work exercise trials at different intensities in healthy young adults. Although the current findings may have implications for neuroscientists and exercise professionals, future studies are needed to further explore the ergogenicity of alternative electroceutical approaches, including different tDCS modes of administration, during whole-body dynamic exercise protocols involving large muscle mass in different exercise domains.

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