Neuroscience 339 (2016) 363–375

TRANSCRANIAL DIRECT CURRENT STIMULATION IMPROVES ISOMETRIC TIME TO EXHAUSTION OF THE KNEE EXTENSORS

L. ANGIUS,[a] B. PAGEAUX,[b] J. HOPKER,[a] S. M. MARCORA[a] AND A. R. MAUGER[a]*

[a] *Endurance Research Group, School of Sport and Exercise Sciences, University of Kent, Chatham Maritime, UK*

[b] *Laboratoire INSERM U1093, Université de Bourgogne, Dijon, France*

Abstract—Transcranial direct current stimulation (tDCS) can increase cortical excitability of a targeted brain area, which may affect endurance exercise performance. However, optimal electrode placement for tDCS remains unclear. We tested the effect of two different tDCS electrode montages for improving exercise performance. Nine subjects underwent a control (CON), placebo (SHAM) and two different tDCS montage sessions in a randomized design. In one tDCS session, the anodal electrode was placed over the left motor cortex and the cathodal on contralateral forehead (HEAD), while for the other montage the anodal electrode was placed over the left motor cortex and cathodal electrode above the shoulder (SHOULDER). tDCS was delivered for 10 min at 2.0 mA, after which participants performed an isometric time to exhaustion (TTE) test of the right knee extensors. Peripheral and central neuromuscular parameters were assessed at baseline, after tDCS application and after TTE. Heart rate (HR), ratings of perceived exertion (RPE), and leg muscle exercise-induced muscle pain (PAIN) were monitored during the TTE. TTE was longer and RPE lower in the SHOULDER condition ($P < 0.05$). Central and peripheral parameters, and HR and PAIN did not present any differences between conditions after tDCS stimulation ($P > 0.05$). In all conditions maximal voluntary contraction (MVC) significantly decreased after the TTE ($P < 0.05$) while motor-evoked potential area (MEP) increased after TTE ($P < 0.05$). These findings demonstrate that SHOULDER montage is more effective than HEAD montage to improve endurance performance, likely through avoiding the negative effects of the cathode on excitability.

Key words: tDCS, exercise, performance, brain stimulation.

INTRODUCTION

Muscle fatigue, defined as an exercise-induced reduction in the maximal force/power production of a muscle group (Gandevia, 2001), is known to be associated with changes at or distal to the neuromuscular junction (i.e. peripheral fatigue) and/or failure to recruit the active muscle group (i.e. central fatigue) (Gandevia, 2001). However, because the process of fatigue also involves multiple structures of the cortico-spinal tract, it is also important to recognize that a failure to generate output from the motor cortex (M1) can also result in reduced muscle force – this is termed supraspinal fatigue (Gandevia et al., 1996; Taylor et al., 1996; Taylor and Gandevia, 2008). Supraspinal fatigue can occur during exercise involving both isometric and dynamic contractions (Taylor et al., 1996; Gandevia, 2001; Søgaard et al., 2006) which has been observed to develop from exercise onset and continues until exhaustion along with peripheral parameters (Taylor et al., 1996; Gandevia, 2001; Søgaard et al., 2006). Non-invasive techniques such as the transcranial magnetic stimulation (TMS) have been extensively used to stimulate the M1 during muscle contraction in order to investigate the impact of supraspinal fatigue during exercise (Taylor et al., 1996; Gandevia, 2001; Søgaard et al., 2006). However, despite a significant number of studies and the development/refinement of non-invasive methods, the physiological mechanisms of supraspinal fatigue are still not well established (Gandevia, 2001; Taylor and Gandevia, 2008). However, there is evidence to suggest that the descending output from the M1 is not adequate during fatiguing exercise (Taylor et al., 1996; Gandevia, 2001; Liu et al., 2002). In the study of Søgaard and colleagues (2006) the superimposed twitch (Tw) evoked by TMS over M1 increased until exhaustion, indicating a suboptimal output from the M1. Furthermore, in the study of Liu et al. (2002) functional magnetic resonance imaging (fMRI) revealed a significant reduction in brain activation during the last 60 s of a sustained (125 s) handgrip maximal voluntary contraction (MVC). If a suboptimal output from the M1 contributes to supraspinal fatigue, then any intervention which moderates this reduction could plausibly improve exercise performance. Anodal transcranial direct current stimulation (tDCS) of the M1 has reliably been shown to increase cortical excitability (Nitsche and Paulus, 2001),

*Corresponding author. Address: School of Sport and Exercise Sciences, University of Kent, Chatham Maritime, Kent ME4 4AG, UK. Fax: +44-0-1634-888890.

E-mail address: l.mauger@kent.ac.uk (A. R. Mauger).

Abbreviations: BLa^-, blood lactate; CSP, cortical silent period; EMG, electromyography; Hb diff, hemoglobin difference; HHb, deoxyhemoglobin; HR, heart rate; MEP, motor-evoked potential; MVC, maximal voluntary contraction; NIRS, near-infrared spectroscopy; O_2Hb, oxyhemoglobin; PAIN, exercise-induced muscle pain; RMS, root mean square; RPE, rating of perceived exertion; SMA, supplementary motor area; tDCS, transcranial direct current stimulation; tHb, total hemoglobin; TTE, time to exhaustion; Tw, twitch; VAL, voluntary activation level; VL, vastus lateralis.

http://dx.doi.org/10.1016/j.neuroscience.2016.10.028

and so this procedure may have the potential to attenuate the development of supraspinal fatigue. Briefly, tDCS involves the application of a weak electrical current through the brain between two electrodes, which consequently excite (i.e. anodal stimulation) or inhibit (i.e. cathodal stimulation) the targeted brain area by altering resting membrane potential (Nitsche and Paulus, 2001; Nitsche et al., 2005). Recently, a series of experiments investigating the effect of tDCS prior to exercise have been conducted. In several studies, endurance performance appeared to improve following tDCS stimulation (Cogiamanian et al., 2007; Williams et al., 2013; Okano et al., 2015), however other studies reported no effect (Kan et al., 2013; Lampropoulou and Nowicky, 2013; Muthalib et al., 2013; Angius et al., 2015). The variation in changes to exercise performance arising from tDCS are potentially a consequence of different experimental and methodological set up. Aside from the absence of a placebo control in many of the above studies, a notable methodological difference is the use of a cephalic or extracephalic electrode montage. A cephalic electrode montage involves placing the anodal electrode over the M1 (or main target area) and the cathodal electrode (i.e. reference) placed over the contralateral prefrontal area (Williams et al., 2013; Angius et al., 2015; Okano et al., 2015). An extracephalic set up places the cathodal electrode on the opposite shoulder (Cogiamanian et al., 2007; Kan et al., 2013; Lampropoulou and Nowicky, 2013; Muthalib et al., 2013), rather than the contralateral area of the head. This is because the tDCS anode increases excitability over the area that it is placed, whereas the cathode decreases excitability. Therefore, in the studies which used a cephalic montage (Angius et al., 2015), the unwanted effects of decreased excitability in the brain area under the cathode may have negated the positive effects of the anodal stimulation. Using an extracephalic montage may avoid this problem and explain why exercise performance differences tend to be more apparent in the studies that use this approach (Cogiamanian et al., 2007; Kan et al., 2013). In addition to differences in electrode location, there are notable discrepancies between exercise-based tDCS studies with regard to the relative participant fatigue state in which tDCS is applied. Indeed, tDCS has been administered in a pre-fatigued state (Abdelmoula et al., 2016; Cogiamanian et al., 2007), during exercise (Williams et al., 2013) or at rest (Angius et al., 2015). These differences are relevant because a rapid and adaptive change in the corticospinal response has been shown to depend on the exercise intensity and exercise duration (Gandevia, 2001), which will dictate the level of central and/or peripheral fatigue in the individual. Therefore, brain response and exercise performance might change according to the ‘‘state” of the corticospinal tract prior to tDCS administration, thus making the effect of tDCS and study comparisons unclear.

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

EXPERIMENTAL PROCEDURES

Ethical approval

For the present investigation, each participant was informed about the procedures of the study but not of the aims and hypothesis. Written informed consent was given by all participants. Study ethics were approved by the School’s Research Ethics Advisory Group (approval number Prop82_2012_13), which conformed to the standards set by the Declaration of Helsinki.

Participants

Nine recreationally active males (mean ± SD; age = 23 ± 2 year, height = 179 ± 7 cm, weight = 76 ± 9 kg) participated in the present study. None of the participants had any history of cardiorespiratory, metabolic or mental disorder/disease or was taking any medication at the time of the study. Each participant gave their written informed consent and was informed about the procedures of the study but not of the aims and hypothesis. All experimental protocols and procedures were approved by the local ethics committee. All tests were conducted in a temperature-controlled room (20 °C, relative humidity 50%), within 2–5 days of each other and at the same time of the day for each participant. Each participant was informed about the procedures of the study but not of the aims and hypothesis. Throughout the experiment, all participants were asked to keep their normal eating behaviors and refrain from vigorous exercise (24 h prior), drinking alcohol (48 h), caffeine (8 h prior) and analgesics (6 h prior) prior to any test occasion.

Experimental protocol

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

Endurance task (time to exhaustion test; TTE)

To assess endurance performance, participants performed a submaximal isometric TTE task of the right

knee extensor muscles at 20% of their MVC, which was performed during each visit. During the TTE each participant received visual feedback on a computer monitor showing the target force. The task terminated when their force went below the required target value for more than 3 s. None of the participants were aware of the time elapsed during the test and results of all the sessions were provided only after the completion of all visits. Participants' perception of effort was measured using the 15-point rating of perceived exertion (RPE) scale (Borg, 1998) every 20 s of the TTE task. Leg muscle pain was assessed every 20 s by using a 10-point numerical scale (Cook et al., 1997). Heart rate (HR) was monitored continuously and averaged for every 20 s elapsed. Blood lactate concentration ($B[La^-]$) was obtained by collecting a 10 μl samples of capillary blood immediately after the TTE task. Each sample was then analyzed after the completion of all experimental procedures to determine the lactate concentration (Biosen; EFK Diagnostics, London, UK).

Neuromuscular tests

After a brief, standardized warm-up with submaximal isometric contractions, all participants performed a 5-s MVC with superimposed doublet stimulation, followed (4-s interval) by a resting potentiated doublet. The MVC produced during this test was used to calculate the participants' 20% MVC used in the subsequent TTE task of that visit. Ten seconds after the MVC participants performed a series of four submaximal contractions at 50% of the MVC (3 s duration) with superimposed TMS and one with superimposed femoral stimulation. Each contraction was interspaced by 3 s. Neuromuscular assessment tests were performed prior to tDCS, post tDCS and immediately after the TTE task (see Fig. 1).

TMS procedure

TMS was used to assess the level of cortical excitability of the M1. The stimulation site was determined by a TMS stimulator (Magstim 2002, The Magstim Company Ltd, Whitland, UK) with the concave double coil (110 mm diameter) placed over the contralateral M1 of the exercising leg. The stimulation site was determined when the largest motor-evoked potential (MEP) response of the vastus lateralis (VL) was obtained together with a small MEP response (< 10%) of the antagonist muscle (biceps femoris, BF). Once the site was determined, this was marked on the participant's scalp with a marker pen. After determining the stimulation site, stimulus intensity was set according to the highest MEP response elicited during a 3-s submaximal contraction at 50% MVC. To determine this, stimulation intensity commenced at 45% and was subsequently ramped up in increments of 5% until a plateau in MEP response was observed. Procedures for stimulation location and intensity were performed for each participant at the beginning of each visit. The intensity of stimulation across participants and visits was 63 ± 8% of the maximum stimulator output.

Femoral nerve stimulation

Transcutaneous electrically-evoked femoral nerve stimulation was delivered by using a high-voltage constant-current stimulator (model DS7 modified, Digitimer, Hertfordshire, UK). The femoral nerve was stimulated using a cathode surface electrode (Swaromed, Nessler Medizintechnik, Innsbruck, Austria) positioned over the femoral triangle while the anode electrode (Phoenix Healthcare Products Ltd., Nottingham, UK) was placed in the gluteal fold. The stimulation intensity (mean current 288 ± 64 mA) was increased by 20 mA until the action potential (M-wave) demonstrated no further increase (M_{max}) at rest and during submaximal 50% MVC contractions. The final intensity stimulation was then set at 130% M_{max}. Both M_{max} and TMS intensities were determined at the beginning of each experimental session and were kept constant throughout that visit.

Mechanical recordings

All the experimental procedures were performed on an isokinetic dynamometer (Cybex NORM isokinetic dynamometer, CMSi, Computer 267 Sports Medicine Inc., Stoughton, USA). All tests were performed with the right leg at a knee joint angle of 90° of flexion

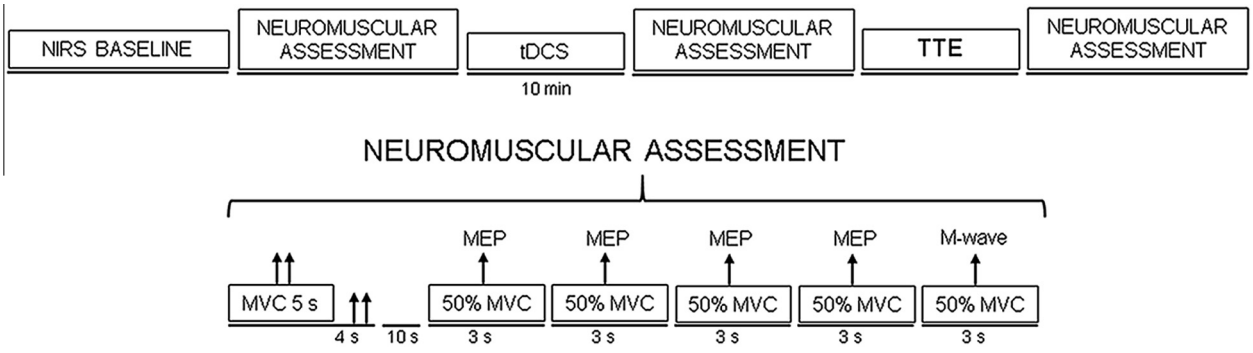

Fig. 1. Overall view of the experimental protocol. Maximal muscular wave (M_{wave}); motor-evoked potential (MEP); maximal voluntary contraction (MVC); transcranial direct current stimulation (tDCS); time to exhaustion (TTE).

(0° = knee fully extended) and a hip angle of 90°. The set-up for each participant was recorded in the familiarization session and kept constant in all subsequent visits. Mechanical signals were digitized on-line at a sampling frequency of 1 kHz using a computer, and stored for analysis with commercially available software (Acqknowledge 4.2 for MP Systems, Biopac Systems Inc., Goleta, USA).

Electromyographic recordings

Electromyography (EMG) of the VL was recorded with two surface electrodes (Swaromed, Nessler Medizintechnik, Innsbruck, Austria) while the reference electrode was placed over the patella of the right knee. The skin was shaved and cleaned using alcohol swabs. Myoelectrical signals were amplified with a bandwidth frequency ranging from 10 Hz to 500 Hz (gain = 500), digitized on-line at a sampling frequency of 2 kHz using a computer, and stored for analysis with commercially available software (Acqknowledge 4.2 for MP Systems, Biopac Systems Inc., Goleta, USA).

Near-infrared spectroscopy (NIRS) procedures

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

tDCS procedure

tDCS was delivered by a direct current stimulator (TCT Research Limited, Hong Kong) using a pair of rubber electrodes in a 4×3-cm water-soaked synthetic sponge. Two different montages were used for the present investigation: (1) anodal placed over the left M1 with the cathodal placed above dorsolateral right prefrontal cortex (HEAD); (2) anodal placed over the left M1 with the cathodal was placed over the shoulder (SHOULDER). For the SHAM session, electrodes were placed in the same position for HEAD while in the control no electrodes were placed on the participant. During HEAD and SHOULDER conditions the current was applied with an intensity of 2.0 mA for 10 min, whereas during the SHAM condition stimulation lasted 30 s and subsequently ramped down to no stimulation.

Data analysis

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

$$VAL = 100 \cdot (1 - \text{superimposed doublet amplitude} / \text{potentiated doublet amplitude})$$

The root mean square (RMS) of the EMG signal was automatically calculated with the software and the peak-to-peak amplitude of the resting M-waves were calculated and averaged for the stimulations. The following parameters were also analyzed: peak torque doublet, peak Tw. EMG amplitude during the MVC was quantified as the RMS for a 0.5-s interval at peak torque (250-ms interval either side of the peak torque). Maximal RMS values obtained during the MVC (RMS_{MVC}) were normalized by the resting M-wave RMS (RMS_M) to obtain the RMS_{MVC}/RMS_M ratio in order to take into account peripheral influences, including neuromuscular propagation and changes in impedance during the EMG recordings. The MEP area (MEP_{area}), was calculated and averaged for the four stimulations, and then normalized for the M-wave obtained during the 50% MVC contraction. Cortical silent period (CSP) duration of the MEP was determined by the same experimenter from the onset of the MEP to the return of continuous EMG signal (Säisänen et al., 2008). Because of continuous measures, VL RMS was plotted as 0%, 25%, 50%, 75% and 100% of each TTE. 0% corresponded to the first 5 s of the TTE while for 25%, 50%, 75% and 100%, the signal was analyzed and averaged for the last 5 s for each percentage. NIRS data were averaged for the last 60 s during baseline measurement, while during tDCS administration NIRS data were averaged for the last 60 s every two min (i.e. min 2, 4, 6, 8 and 10). During exercise, data were averaged for 5 s respectively at the 0%, 25%, 50%, 75% and 100% of each TTE. The Beer–Lambert Law was used to calculate changes in tissue oxygenation. Relative concentration changes were measured from resting baseline for oxyhemoglobin (ΔO_2Hb), deoxyhemoglobin (ΔHHb), total hemoglobin ($\Delta tHb = O_2Hb + HHb$) and hemoglobin difference (ΔHb diff $= O_2Hb - HHb$). ΔtHb was calculated to give an index of change in regional blood volume. Individual values of RPE, exercise-induced muscle pain (PAIN) and HR obtained during the TTE were plotted against the absolute TTE time for each condition, then the curve for each variable was mathematically fitted by a linear equation to obtain the slope.

Statistical analysis

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

Three-way $4 \times 2 \times 6$ ANOVAs were used to test the effect of condition (HEAD, SHOUDLER, SHAM and CONTROL), prefrontal cortex side (left vs. right side) and time on ΔO_2Hb, ΔHHb, ΔHb diff, ΔtHb and TSI obtained during the TTE. Bonferroni post hoc tests was used when appropriate. The α level was set at $P < 0.05$. Statistics were calculated using SPSS version 20.

RESULTS

TTE was significantly longer ($F(3,24) = 7.84$, $P \leqslant 0.001$, $\eta_p^2 = 0.49$) in the SHOULDER condition compared to the HEAD, SHAM and CON conditions (219 ± 136 s, 191 ± 124 s, 173 ± 114 s and 187 ± 121 s, respectively). This was accompanied by a significantly lower RPE slope in the SHOULDER condition ($F(3,24) = 5.29$, $P \leqslant 0.006$, $\eta_p^2 = 0.88$) (see Fig. 2). No significant differences between conditions were observed for $B[La^-]$ ($F(3,24) = 0.06$, $P = 0.99$, $\eta_p^2 = 0.00$) (4.81 ± 206, 4.70 ± 210, 4.67 ± 241, 4.97 ± 2.07 mmol l^{-1} respectively for SHOULDER, HEAD, SHAM and CON) or HR slope ($F(3,24) = 0.031$, $P = 0.90$, $\eta_p^2 = 0.03$) (5.36 ± 2.49, 5.60 ± 3.62, 5.53 ± 3.11 and 4.85 ± 3.54 respectively for SHOULDER, HEAD, SHAM and CON) or PAIN slope ($F(3,24) = 0.50$, $P = 0.68$, $\eta_p^2 = 0.05$) (1.02 ± 0.69, 1.00 ± 0.53, 0.95 ± 0.57 and 0.91 ± 0.60 respectively for SHOULDER, HEAD, SHAM and CON).

Neuromuscular parameters

MVC torque decreased significantly at exhaustion ($F(2,16) = 24.85$, $P \leqslant 0.001$, $\eta_p^2 = 0.75$) but did not differ between conditions ($F(3,24) = 0.68$, $P = 0.56$, $\eta_p^2 = 0.07$). RMS of VL increased over time ($F(3,24) = 2.40$, $P \leqslant 0.001$, $\eta_p^2 = 0.87$) but did not differ between conditions ($F(3,24) = 0.68$, $P = 0.38$, $\eta_p^2 = 0.07$) (see Fig. 3).

Peripheral fatigue

Doublet amplitude decreased significantly only at exhaustion ($F(2,16) = 36.92$, $P \leqslant 0.001$, $\eta_p^2 = 0.82$) but did not differ between conditions ($F(3,24) = 0.70$, $P = 0.55$, $\eta_p^2 = 0.08$). Tw decreased only at exhaustion ($F(2,16) = 36.92$, $P \leqslant 0.001$, $\eta_p^2 = 0.82$) but did not differ between conditions ($F(3,24) = 0.70$, $P = 0.55$, $\eta_p^2 = 0.08$). M_{amp} at 50% MVC was significantly higher only at exhaustion ($F(3,24) = 11.09$, $P \leqslant 0.001$, $\eta_p^2 = 0.58$) but did not differ between conditions ($F(3,24)$, $P = 0.28$, $\eta_p^2 = 0.14$). M_{area} at 50% MVC was significantly different only at exhaustion ($F(3,24) = 10.21$, $P \leqslant 0.001$, $\eta_p^2 = 0.56$) but did not differ between conditions ($F(3,24)$, $P = 0.95$, $\eta_p^2 = 0.21$) (see Fig. 3).

Central fatigue

VAL decreased significantly only at exhaustion ($F(2,16) = 15.27$, $P \leqslant 0.001$, $\eta_p^2 = 0.99$) but did not differ between conditions ($F(3,24) = 1.19$, $P = 0.33$, $\eta_p^2 = 0.13$). RMS_{MVC}/RMS_{Mwave} of the VL did not change over time ($F(2,16) = 1.23$, $P = 0.85$, $\eta_p^2 = 0.13$) and did not differ between conditions ($F(3,24) = 0.499$, $P = 0.68$, $\eta_p^2 = 0.05$).

Cortical excitability

MEP_{area} increased only at exhaustion ($F(2,16) = 5.18$, $P \leqslant 0.018$, $\eta_p^2 = 0.39$) but did not differ between conditions ($F(3,24) = 0.10$, $P = 0.96$, $\eta_p^2 = 0.01$). MEP_{area}/M_{area} ratio increased only at exhaustion ($F(2,16) = 6.21$, $P \leqslant 0.01$, $\eta_p^2 = 0.43$) but did not differ between conditions ($F(2,16) = 6.21$, $P = 0.91$, $\eta_p^2 = 0.01$). CSP increased only at exhaustion ($F(2,16) = 5.48$, $P \leqslant 0.015$, $\eta_p^2 = 0.40$) but did not differ between conditions ($F(3,24) = 0.49$, $P = 0.37$, $\eta_p^2 = 0.98$) (see Fig. 3).

NIRS parameters during tDCS stimulation

ΔO_2Hb did not change over time ($F(4,32) = 0.98$, $P = 0.42$, $\eta_p^2 = 0.27$) and no differences between conditions ($F(3,24) = 0.30$, $P = 0.99$, $\eta_p^2 = 0.55$) or side ($F(1,8) = 3.87$, $P = 0.85$, $\eta_p^2 = 0.41$) were found. ΔHHb did not change over time ($F(4,32) = 0.92$, $P = 0.23$, $\eta_p^2 = 0.25$) and no differences between conditions ($F(3,24) = 0.75$, $P = 0.39$, $\eta_p^2 = 0.18$) or side ($F(1,8) = 0.62$, $P = 0.45$, $\eta_p^2 = 0.10$) were found. ΔtHb did not

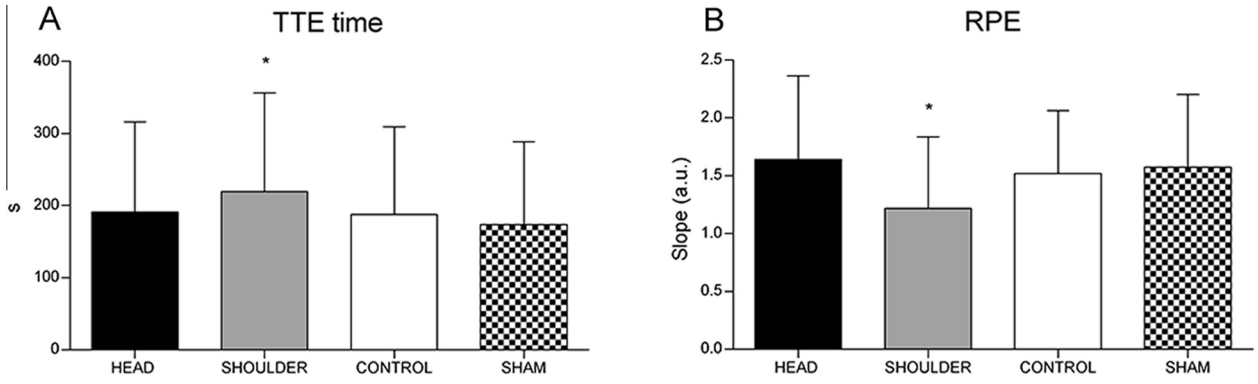

Fig. 2. Physiological and perceptual response of all tests performed. Panel A shows time to exhaustion (TTE) performance. Panel B shows the slope values of ratings of perceived exertion (RPE). $^*P \leqslant 0.05$ significant from HEAD, CONTROL and SHAM. Data are presented as mean ± SD ($n = 9$).

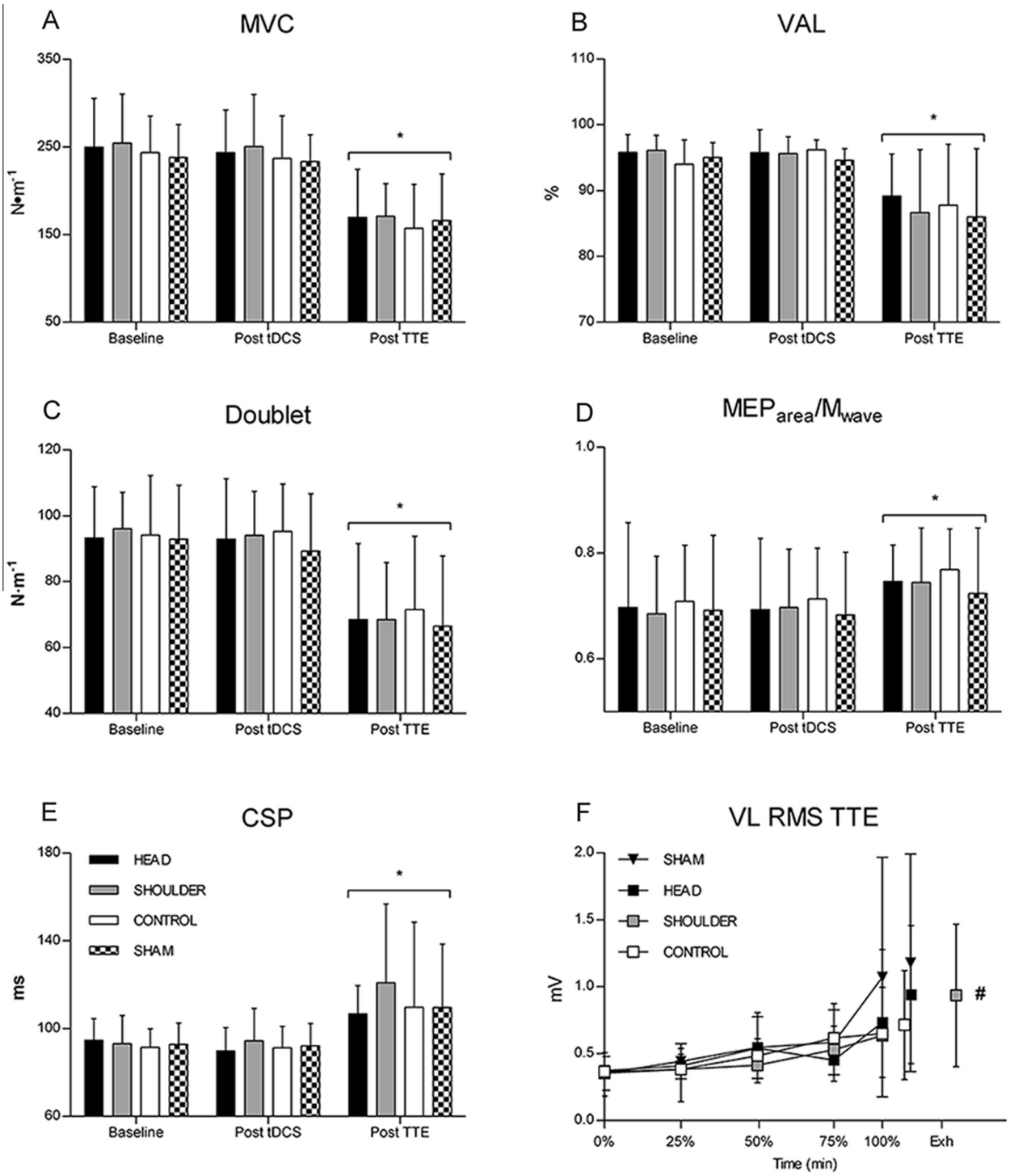

Fig. 3. Overall response neuromuscular parameters during the various phases of the experiment. Panel A shows maximal voluntary contraction (MVC); Panel B shows voluntary activation level (VAL); Panel C shows peak torque of the doublet; Panel D shows MEP_{area}/M_{wave} ratio; Panel E shows cortical silent period (CSP); Panel F shows root mean square of vastus lateralis (VL RMS) during time to exhaustion (TTE). $^{*}P \leqslant 0.05$ significant from baseline and post tDCS; $^{\#}P < 0.05$, significant main effect of time. Data are presented as mean ± SD ($n = 9$).

change over time ($F(4,32) = 1.36$, $P = 0.77$, $\eta_p^2 = 0.37$) and no differences between conditions ($F(3,24) = 0.29$, $P = 0.10$, $\eta_p^2 = 0.09$) or side ($F(1,8) = 1.30$, $P = 0.28$, $\eta_p^2 = 0.17$) were found. ΔHbdiff did not change over time ($F(4,32) = 2.58$, $P = 0.15$, $\eta_p^2 = 0.65$) and no differences between conditions ($F(3,24) = 0.87$, $P = 0.32$, $\eta_p^2 = 0.21$) or side ($F(1,8) = 0.02$, $P = 0.87$, $\eta_p^2 = 0.53$) were found. Tissue saturation index did not change over time ($F(4,28) = 0.10$, $P = 0.63$, $\eta_p^2 = 0.06$) and no differences between conditions ($F(3,21) = 0.83$, $P = 0.65$, $\eta_p^2 = 0.20$) or side ($F(1,7) = 0.10$, $P = 0.755$, $\eta_p^2 = 0.05$) were found (see Tables 1 and 2).

Table 1. Changes of NIRS values from baseline in left and right prefrontal cortex during tDCS procedures in CONTROL and SHAM conditions

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

Tissue saturation index (ΔTSI), oxyhemoglobin (ΔO_2Hb), deoxyhemoglobin (ΔHHb), total hemoglobin (ΔtHb) and hemoglobin difference (ΔHbDiff) during tDCS stimulation. Data are presented as means ± SD.

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

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

Tissue saturation index (ΔTSI), oxyhemoglobin (ΔO_2Hb), deoxyhemoglobin (ΔHHb), total hemoglobin (ΔtHb) and hemoglobin difference (ΔHbDiff) during tDCS stimulation. Data are presented as means ± SD (n = 9).

NIRS parameters during TTE

ΔO_2Hb increased over time ($F(5,40) = 30.58$, $P \leqslant 0.001$, $\eta_p^2 = 1.00$) but no differences were observed between conditions ($F(3,24) = 1.96$, $P = 0.24$, $\eta_p^2 = 0.44$) or side ($F(1,8) = 0.04$, $P = 0.84$, $\eta_p^2 = 0.05$) were found. ΔHHb increased over time ($F(5,40) = 38.11$, $P > 0.001$, $\eta_p^2 = 1.00$) and no differences between conditions ($F(3,24) = 0.74$, $P = 0.43$, $\eta_p^2 = 0.18$) or side ($F(1,8) = 2.88$, $P = 0.12$, $\eta_p^2 = 0.32$) were found. ΔtHb increased over time ($F(5,40) = 21.13$, $P \leqslant 0.001$, $\eta_p^2 = 1.00$) and no differences between conditions ($F(3,24) = 0.57$, $P = 0.55$, $\eta_p^2 = 0.15$) or side ($F(1,8) = 1.14$, $P = 0.31$, $\eta_p^2 = 0.15$) were found. ΔHbDiff decreased over time ($F(5,40) = 38.11$, $P \leqslant 0.001$, $\eta_p^2 = 0.10$) and no differences between conditions ($F(3,24) = 0.74$, $P = 0.43$, $\eta_p^2 = 0.18$) or side ($F(1,8) = 2.88$, $P = 0.12$, $\eta_p^2 = 0.32$) were found. Tissue saturation decreased over time ($F(5,40) = 21.13$, $P = 0.003$, $\eta_p^2 = 0.10$) and no differences between conditions ($F(3,24) = 0.57$, $P = 0.55$, $\eta_p^2 = 0.15$) or side ($F(1,8) = 1.14$, $P = 0.31$, $\eta_p^2 = 0.15$) were found. (see Tables 3–6).

DISCUSSION

This is the first study showing an improvement in isometric endurance performance of the lower limbs following tDCS stimulation. Our findings suggest that in order to improve lower limb endurance performance, an extracephalic electrode montage is more effective than cephalic montage.

Effect of tDCS on isometric endurance performance and perceptual parameters

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

In the current experiment, isometric endurance performance was longer in the SHOULDER condition, where the anode was placed over the M1 and the cathode placed on the shoulder (thus avoiding any decreased excitability of right prefrontal cortex induced by the cathode). A potential explanation for this improvement in isometric endurance performance is perception of effort during the TTE task, which increased more slowly over time in the SHOULDER condition. It has previously been proposed that during sustained exercise, the increase in perception of effort over time reflects, at least in part, the increase in activity of premotor and/or motor areas of the brain (i.e. central motor command) necessary to compensate the decline in force-generating capacity of the neuromuscular system (de Morree et al., 2012, 2014; Marcora et al., 2008). This proposal is based on evidence supporting the hypothesis that the sensory signals for perception of effort are corollary discharges from premotor and/or motor areas of the brain (Marcora, 2009; Takarada et al., 2014; Zénon et al., 2015).

In our experiment, two different reasons might explain the reduction in RPE in the SHOULDER condition. The first is that anodal stimulation of the M1 facilitated the descending drive to the muscle, thus reducing activity of premotor areas and participants perceiving less effort for the same force produced. In support of this, previous experimental findings have demonstrated that manipulation of the activity of the M1 and supplementary motor area (SMA) influenced perception of effort. In accordance with this, the study of Takarada and colleagues (2014) demonstrated that suppression of the activity of the M1 by repetitive TMS (rTMS) increases perception of effort, thus making participants perceive the

Table 3. Changes of NIRS values from baseline in left and right prefrontal cortex during the isometric time to exhaustion in the CONTROL condition

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

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

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

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

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

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

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

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

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

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

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

voluntary contraction as harder. Furthermore, another study performed by Zénon et al. (2015) demonstrated that disrupting neural activity in SMA and M1 led to a significant alteration of perception of effort. In the HEAD condition, the positive effect of anodal stimulation over M1 on perception of effort and isometric endurance performance

could have been counteracted by a negative effect of cathodal stimulation over the right dorsolateral prefrontal cortex. This brain area is involved in mood and emotion regulation (Ochsner et al., 2002), and it may be part of a system-regulating exercise performance (Robertson and Marino, 2016). Therefore, it is plausible that its cathodal stimulation may negatively affect endurance performance as recently proposed by Angius and colleagues (2015) where cycling TTE was not affected following tDCS stimulation with the same HEAD montage used in this study. While a facilitated descending drive to the muscle is perhaps the most likely explanation for the observed effect on RPE and TTE in the SHOULDER condition, this hypothesis should be approached with some caution given that there was no apparent effect on cortical excitability following tDCS observed in this study (see Fig. 3). However, this is likely due to specific neuromuscular assessment protocols used in the current study, or that the muscles in the leg were the target for stimulation, as tDCS of the M1 is well-established to increase M1 excitability (Nitsche and Paulus, 2000; Nitsche et al., 2005; Jeffery et al., 2007; Madhavan and Stinear, 2010). This discussion is expanded below in the section '*Effects of tDCS on neuromuscular parameters*'. It should also be noted that the benefits of the anodal tDCS stimulation could have been extended to other areas of the brain (i.e. spatial effect), including the cortical brain areas such as the SMA, premotor cortex and somatosensory areas, or sub-cortical brain areas such as red nucleus and reticular formation (Lang et al., 2005). The data from the current study cannot confirm whether this may have occurred or the potential functional significance of such an effect, but the potential spatial effect of tDCS on other brain areas should not be discounted in explaining the observed ergogenic effect in this study.

Effect of prolonged exhaustive isometric exercise on neuromuscular function

In line with previous experiments (Pageaux et al., 2013), prolonged isometric submaximal contraction of knee extensor induced a significant increase in muscle fatigue as demonstrated by the reduced MVC immediately after exhaustion. Our data demonstrate that the increase in muscle fatigue was caused by both peripheral and central mechanisms as supported by the decrement of Doublet, Tw and VAL. However, it should be noted that contrary to previous studies (Pageaux et al., 2013), the ratio RMS_{MVC}/RMS_{Mwave} EMG did change after exhaustion. This ratio has been previously used in different studies to detect any change of central parameters after exhaustion (Pageaux et al., 2013, 2015). However, conflicting results have meant that this metric has been criticized (Farina, 2006). Our data further confirm that the quantification and assessment of central fatigue should instead be performed using the Tw interpolation technique (Gandevia et al., 2013). In the current study, MEP_{area} and the MEP_{area}/M_{area} ratio increased at exhaustion when compared to baseline, thus demonstrating an increase in cortical excitability at exhaustion. Similar findings were shown in previous experiments involving both isometric and dynamic muscle contractions (Jubeau et al., 2014; Temesi et al., 2014; Pageaux et al., 2015). However, these findings contrast with the study of Gruet and colleagues (2014) where MEP did not change at exhaustion after an intermittent exhaustive isometric task of the knee extensors at 50% MVC when compared to baseline. These findings suggest that MEP response at exhaustion may differ according to the regime of the muscle contraction, thus showing a task specificity. Similarly to previous studies (Taylor et al., 1996; Gruet et al., 2014; Pageaux et al., 2015), CSP duration significantly increased immediately after exercise. Lengthening of the CSP has been associated with the increase of intracortical inhibition of cortical and sub-cortical areas (Taylor et al., 1996; Gandevia, 2001), impairment of the motoneuron responsiveness (McNeil et al., 2011) and stimulation of mecano–metabo-sensitive muscle afferents (Hilty et al., 2011). However, in the current study, as CSP was not different between conditions it is unlikely that tDCS elicited an effect on these measures.

Effects of tDCS on neuromuscular parameters

To the best of our knowledge, this is the first study to investigate the effect of tDCS on VAL or during maximal contraction of knee extensors. tDCS administration appeared to elicit no effect on the neuromuscular response and consequently we did not find any change in either central or peripheral parameters. The effect of tDCS on maximal force production has mainly focused on upper limb muscles (i.e. elbow flexors) without any improvement in MVC (Cogiamanian et al., 2007; Kan et al., 2013; Lampropoulou and Nowicky, 2013), although none of these studies involved the super imposed stimulation technique during MVC to assess VAL. However, it is likely that these parameters would not be affected by acute administration of tDCS as they are already maximal, so any further increase in VAL or MVC might be not achievable. Indeed, as proposed by Khan et al. (2013) and Hummel et al. (2006), tDCS does not further enhance motor function when there is little or no potential improvement. MEP parameters obtained by TMS have been extensively used as index of cortical excitability of the M1 following tDCS stimulation. An increase in cortical excitability supported by an increase in MEP response lasting up to 60 min (depending on the type and duration of stimulation) (Nitsche and Paulus, 2001) has been reliably shown following anodal tDCS stimulation both at rest and during submaximal contractions (Nitsche and Paulus, 2000; Nitsche et al., 2005; Jeffery et al., 2007; Madhavan and Stinear, 2010). Contrary to what was initially expected, in our experiment cortical parameters did not change following tDCS. It is likely that this inconsistency was caused by the different assessment protocol used or the muscles investigated. Experimental evidence regarding the excitability of the lower limb area of the M1 in the healthy individual is very limited with only a few studies demonstrating a modest effect of tDCS (Jeffery et al., 2007; Madhavan and Stinear, 2010; Tatemoto et al., 2013). Jeffery and colleagues (2007) specified that stimulation of the leg area of the M1 might be less inclined to tDCS intervention compared to the hand area of the M1 because it has a deeper location to

the scalp. However, the fact that endurance performance was improved in the current study suggests that tDCS did elicit an effect on the M1. An additional cause might be the intensity chosen for the submaximal contractions in the neuromuscular tests. Isometric contractions at 50% of MVC have been previously used to provide a more stable and consistent response of CSP (Säisänen et al., 2008; Pageaux et al., 2015). However, it has been shown that the largest MEP response occurs with a contraction at 50% MVC with no further increases observed beyond this (Goodall et al., 2009; Sidhu et al., 2009). Therefore, it might be possible that any changes to MEP response as a result of tDCS were masked as a result of the 50% MVC. As changes to MEP response have been already reliably shown following tDCS, we chose to use a 50% MVC so that any potential changes to CSP could be more accurately quantified. In the current study, CSP did not differ between each condition. Few previous studies have investigated the effect of tDCS stimulation on CSP, with contrasting outcomes (Horvath et al., 2014). To date, only the study of Tremblay et al. (2013) showed a decrease in CSP following anodal tDCS stimulation, which the authors attributed to a reduction of GABAB-related inhibition on the M1. In the study of Tremblay et al. (2013), cortical response was assessed during 20% MVC of first dorsal interosseus following a 20-min anodal tDCS stimulation. Therefore, it may be that the differing results may be caused by the duration of tDCS stimulation or the muscle investigated.

The HEAD montage used in this experiment is the same used in numerous experiments to relieve pain (Boggio et al., 2008; Lefaucheur et al., 2008; Kan et al., 2013; Angius et al., 2015). However, in accordance with previous findings related to pain and exercise performance (Kan et al., 2013; Angius et al., 2015), this montage was not able to reduce exercise-induced pain. Kan et al. (2013) found no change in performance of a single joint isometric contraction, while Angius et al. (2015) found no change in high-intensity cycling TTE. It should be noted that the nature of the pain stimulus induced to monitor the well-established analgesic effect of tDCS (Boggio et al., 2008; Lefaucheur et al., 2008) is very different to the nature of exercise-induced pain and this may explain the different findings. Indeed, while tDCS has been shown to reduce pain during a cold pressor test, no change in pain was found during exercise (Angius et al., 2015). Furthermore, many other factors during exercise (including distraction and attention) might reduce the benefits of tDCS (Angius et al., 2015).

In addition to the above factors, the cathodal electrode placed over the contralateral prefrontal area in the HEAD montage likely changed the direction of electrical flow through the brain. Several experiments using computer-based models have demonstrated that the propagation of the electrical field in the brain is mainly affected by the type and position of the electrodes over the scalp (Wagner et al., 2007; Miranda et al., 2013; Bai et al., 2014). Accordingly, any possible benefits following anodal stimulation of the M1 may have been negated by the dorso lateral prefrontal cortex (DLPFC) cathodal stimulation. Therefore, in support of previous findings, it is unlikely that the observed changes in performance observed in the current study were not related to analgesia, but rather a moderation of the participant's perception of effort.

Effect of tDCS and exercise on NIRS parameters

When activated, brain tissues require more oxygen and glucose availability, which are supported by an increase in cerebral blood flow. Changes in cortical excitability during and following tDCS stimulation with subsequent increase in metabolism and regional blood flow are well documented (Lang et al., 2005; Paquette et al., 2011). In our experiment, we used the NIRS technique over left and right prefrontal cortex to non-invasively monitor oxygen consumption both during tDCS stimulation and exercise. Contrary to previous findings, our data did not indicate any change in oxygen consumption during tDCS and no differences were found between the left and right prefrontal cortex when the cathodal electrode was placed over the right prefrontal cortex. By using fNIRS technique, Merzagora and colleagues (2010) documented an increase and decrease in oxygen consumption respectively during anodal and cathodal stimulation and is therefore in contrast to our data. Further study is therefore needed to confirm this effect (or lack of). For the NIRS response during exercise, our data are in agreement with previous findings (Rupp and Perrey, 2009; Muthalib et al., 2013), with no differences found between conditions. Analogous findings were reported by Muthalib et al. (2013) where anodal tDCS did not affect prefrontal oxygenation during isometric elbow flexor exercise. The lack of change in NIRS parameters between conditions is likely caused by the effect of exercise-induced cerebral response overcoming any differences following tDCS stimulation. The distance between the tDCS stimulation site (M1) and the site monitored by NIRS is also a likely reason for the lack of observed effect of NIRS parameters monitored in this study. However, difficulties in obtaining NIRS data from the M1 area (compounded by the requirement of the anodal tDCS electrode placement above the M1) necessitated the placement of the NIRS probe over the prefrontal area. While this study suggests that NIRS placement over the prefrontal area when tDCS is used to stimulate the M1 is perhaps not warranted, this technique may still have some utility when tDCS is used to stimulate the prefrontal cortex.

CONCLUSION

This is the first study comparing the effect of different tDCS electrode montages on neuromuscular, physiological and perceptual parameters of exercise performance of the knee extensor muscles. In summary, this study demonstrated that an extracephalic shoulder montage is more effective than a cephalic head montage in improving isometric endurance performance of the lower limb. This performance improvement was paralleled by a reduced perception of effort. This study provides important methodological and physiological guidance in developing appropriate techniques for the application of tDCS on exercise in the lower limbs.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgment—The authors want to thank Mr. Eric Clark for his support in the acquisition of the data.

REFERENCES

Abdelmoula A, Baudry S, Duchateau J (2016) Anodal transcranial direct current stimulation enhances time to task failure of a submaximal contraction of elbow flexors without changing corticospinal excitability. Neuroscience 13:94–103.

Angius L, Hopker JG, Marcora SM, Mauger AR (2015) The effect of transcranial direct current stimulation of the motor cortex on exercise-induced pain. Eur J Appl Physiol 115:2311–2319.

Bai S, Dokos S, Ho K-A, Loo C (2014) A computational modelling study of transcranial direct current stimulation montages used in depression. NeuroImage 87:332–344.

Boggio PS, Zaghi S, Lopes M, Fregni F (2008) Modulatory effects of anodal transcranial direct current stimulation on perception and pain thresholds in healthy volunteers. Eur J Neurol 15:1124–1130.

Borg G (1998), Borg's Perceived Exertion and Pain Scales. Human Kinetics.

Cogiamanian F, Marceglia S, Ardolino G, Barbieri S, Priori A (2007) Improved isometric force endurance after transcranial direct current stimulation over the human motor cortical areas. Eur J Neurosci 26:242–249.

Cook DB, O'Connor PJ, Eubanks SA, Smith JC, Lee M (1997) Naturally occurring muscle pain during exercise: assessment and experimental evidence. Med Sci Sports Exerc 29:999–1012.

de Morree HM, Klein C, Marcora SM (2012) Perception of effort reflects central motor command during movement execution. Psychophysiology 49:1242–1253.

de Morree HM, Klein C, Marcora SM (2014) Cortical substrates of the effects of caffeine and time-on-task on perception of effort. J Appl Physiol 117:1514–1523.

Farina D (2006) Interpretation of the surface electromyogram in dynamic contractions. Exerc Sport Sci Rev 34:121–127.

Gandevia SC (2001) Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 81:1725–1789.

Gandevia SC, Allen GM, Butler JE, Taylor JL (1996) Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. J Physiol 490(Pt 2):529–536.

Gandevia SC, McNeil CJ, Carroll TJ, Taylor JL (2013) Twitch interpolation: superimposed twitches decline progressively during a tetanic contraction of human adductor pollicis. J Physiol 591:1373–1383.

Goodall S, Romer LM, Ross EZ (2009) Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. Exp Physiol 94:995–1004.

Gruet M, Temesi J, Rupp T, Levy P, Verges S, Millet GY (2014) Dynamics of corticospinal changes during and after high-intensity quadriceps exercise. Exp Physiol 99:1053–1064.

Hilty L, Lutz K, Maurer K, Rodenkirch T, Spengler CM, Boutellier U, Jäncke L, Amann M (2011) Spinal opioid receptor-sensitive muscle afferents contribute to the fatigue-induced increase in intracortical inhibition in healthy humans. Exp Physiol 96:505–517.

Horvath JC, Carter O, Forte JD (2014), Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be). Front Syst Neurosci 8 Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3901383/ [Accessed November 9, 2015].

Hummel FC, Voller B, Celnik P, Floel A, Giraux P, Gerloff C, Cohen LG (2006) Effects of brain polarization on reaction times and pinch force in chronic stroke. BMC Neurosci 7:73.

Jeffery DT, Norton JA, Roy FD, Gorassini MA (2007) Effects of transcranial direct current stimulation on the excitability of the leg motor cortex. Exp Brain Res 182:281–287.

Jubeau M, Rupp T, Perrey S, Temesi J, Wuyam B, Levy P, Verges S, Millet GY (2014) Changes in voluntary activation assessed by transcranial magnetic stimulation during prolonged cycling exercise. PLoS One 9:e89157.

Kan B, Dundas JE, Nosaka K (2013) Effect of transcranial direct current stimulation on elbow flexor maximal voluntary isometric strength and endurance. Appl Physiol Nutr Metab 38:734–739.

Khan B, Hodics T, Hervey N, Kondraske G, Stowe AM, Alexandrakis G (2013) Functional near-infrared spectroscopy maps cortical plasticity underlying altered motor performance induced by transcranial direct current stimulation. J Biomed Opt 18:116003.

Lampropoulou SI, Nowicky AV (2013) The effect of transcranial direct current stimulation on perception of effort in an isolated isometric elbow flexion task. Mot Control 17:412–426.

Lang N, Siebner HR, Ward NS, Lee L, Nitsche MA, Paulus W, Rothwell JC, Lemon RN, Frackowiak RS (2005) How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? Eur J Neurosci 22:495–504.

Lefaucheur J-P, Antal A, Ahdab R, Ciampi de Andrade D, Fregni F, Khedr EM, Nitsche M, Paulus W (2008) The use of repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) to relieve pain. Brain Stimulat 1:337–344.

Liu JZ, Dai TH, Sahgal V, Brown RW, Yue GH (2002) Nonlinear cortical modulation of muscle fatigue: a functional MRI study. Brain Res 957:320–329.

Madhavan S, Stinear JW (2010) Focal and bi-directional modulation of lower limb motor cortex using anodal transcranial direct current stimulation. Brain Stimulat 3:42.

Marcora S (2009) Perception of effort during exercise is independent of afferent feedback from skeletal muscles, heart, and lungs. J Appl Physiol 1985(106):2060–2062.

Marcora SM, Bosio A, de Morree HM (2008) Locomotor muscle fatigue increases cardiorespiratory responses and reduces performance during intense cycling exercise independently from metabolic stress. Am J Physiol Regul Integr Comp Physiol 294: R874–R883.

McNeil CJ, Giesebrecht S, Gandevia SC, Taylor JL (2011) Behaviour of the motoneurone pool in a fatiguing submaximal contraction. J Physiol 589:3533–3544.

Merzagora AC, Foffani G, Panyavin I, Mordillo-Mateos L, Aguilar J, Onaral B, Oliviero A (2010) Prefrontal hemodynamic changes produced by anodal direct current stimulation. NeuroImage 49:2304–2310.

Miranda PC, Mekonnen A, Salvador R, Ruffini G (2013) The electric field in the cortex during transcranial current stimulation. NeuroImage 70:48–58.

Muthalib M, Kan B, Nosaka K, Perrey S (2013) Effects of transcranial direct current stimulation of the motor cortex on prefrontal cortex activation during a neuromuscular fatigue task: an fNIRS study. Adv Exp Med Biol 789:73–79.

Nitsche MA, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 527(Pt 3):633–639.

Nitsche MA, Paulus W (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 57:1899–1901.

Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, Fricke K, Liebetanz D, Lang N, Antal A, Paulus W, Tergau F (2005) Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol 568:291–303.

Ochsner KN, Bunge SA, Gross JJ, Gabrieli JDE (2002) Rethinking feelings: an FMRI study of the cognitive regulation of emotion. J Cogn Neurosci 14:1215–1229.

Okano AH, Fontes EB, Montenegro RA, Farinatti P.de TV, Cyrino ES, Li LM, Bikson M, Noakes TD (2015) Brain stimulation modulates the autonomic nervous system, rating of perceived exertion and performance during maximal exercise. Br J Sports Med 49:1213–1218.

Pageaux B, Marcora SM, Lepers R (2013) Prolonged mental exertion does not alter neuromuscular function of the knee extensors. Med Sci Sports Exerc 45:2254–2264.

Pageaux B, Angius L, Hopker JG, Lepers R, Marcora SM (2015) Central alterations of neuromuscular function and feedback from group III–IV muscle afferents following exhaustive high-intensity one-leg dynamic exercise. Am J Physiol Regul Integr Comp Physiol 308:R1008–1020.

Paquette C, Sidel M, Radinska BA, Soucy J-P, Thiel A (2011) Bilateral transcranial direct current stimulation modulates activation-induced regional blood flow changes during voluntary movement. J Cereb Blood Flow Metab 31:2086–2095.

Robertson CV, Marino FE (2016) A role for the prefrontal cortex in exercise tolerance and termination. J Appl Physiol 120:464–466.

Rupp T, Perrey S (2009) Effect of severe hypoxia on prefrontal cortex and muscle oxygenation responses at rest and during exhaustive exercise. Adv Exp Med Biol 645:329–334.

Säisänen L, Pirinen E, Teitti S, Könönen M, Julkunen P, Määttä S, Karhu J (2008) Factors influencing cortical silent period: optimized stimulus location, intensity and muscle contraction. J Neurosci Methods 169:231–238.

Sidhu SK, Bentley DJ, Carroll TJ (2009) Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. Muscle Nerve 39:186–196.

Søgaard K, Gandevia SC, Todd G, Petersen NT, Taylor JL (2006) The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. J Physiol 573:511–523.

Takarada Y, Mima T, Abe M, Nakatsuka M, Taira M (2014) Inhibition of the primary motor cortex can alter one's ''sense of effort'': effects of low-frequency rTMS. Neurosci Res 89:54–60.

Tatemoto T, Yamaguchi T, Otaka Y, Kondo K, Tanaka S (2013), Anodal Transcranial Direct Current Stimulation over the Lower Limb Motor Cortex Increases the Cortical Excitability with Extracephalic Reference Electrodes. In: Converging Clinical and Engineering Research on Neurorehabilitation (Pons JL, Torricelli D, Pajaro M, eds), pp 829–834 Biosystems & Biorobotics. Springer Berlin Heidelberg. Available at: http://link.springer.com/chapter/10.1007/978-3-642-34546-3_135 [Accessed November 9, 2015].

Taylor JL, Gandevia SC (2008) A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. J Appl Physiol 104:542–550.

Taylor JL, Butler JE, Allen GM, Gandevia SC (1996) Changes in motor cortical excitability during human muscle fatigue. J Physiol 490(Pt 2):519–528.

Temesi J, Rupp T, Martin V, Arnal PJ, Féasson L, Verges S, Millet GY (2014) Central fatigue assessed by transcranial magnetic stimulation in ultratrail running. Med Sci Sports Exerc 46:1166–1175.

Tremblay S, Beaulé V, Lepage J-F, Théoret H (2013) Anodal transcranial direct current stimulation modulates GABAB-related intracortical inhibition in the M1 of healthy individuals. NeuroReport 24:46–50.

Wagner T, Fregni F, Fecteau S, Grodzinsky A, Zahn M, Pascual-Leone A (2007) Transcranial direct current stimulation: a computer-based human model study. NeuroImage 35:1113–1124.

Williams PS, Hoffman RL, Clark BC (2013) Preliminary evidence that anodal transcranial direct current stimulation enhances time to task failure of a sustained submaximal contraction Hug F, ed. PLoS One 8:e81418.

Zénon A, Sidibé M, Olivier E (2015) Disrupting the supplementary motor area makes physical effort appear less effortful. J Neurosci 35:8737–8744.

(Accepted 7 October 2016)
(Available online 14 October 2016)