Lack of an effect of transspinal and transcranial direct current stimulation on performance, perception of effort, and physiologic function in humans

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ABSTRACT

Introduction: For nearly two decades, direct current stimulation has been used to attempt to alter cognitive function, particularly in individuals who exhibit symptoms of mental illness. More recently, human performance enhancement has been a target of direct current stimulation. Direct current stimulation may be administered through non-invasive cutaneous transcranial or transspinal stimulation. Where altered motor cortex excitability or altered cortical activity are potential targets of transcranial stimulation, transspinal stimulation may affect spinal and supraspinal neuron function. However, the body of research related to the effects of transcranial and transspinal direct current stimulation on human performance is small and the mechanisms that may explain the effects are debated or unclear. **Methods:** One transcranial and two transspinal direct current studies were completed. The first study investigated the effects of transcranial direct current stimulation of the temporal lobe on high intensity work capacity and heart rate variability. The second project analyzed the potential modulatory effects of transspinal direct current stimulation on motor unit function and perception of effort. The third study quantified the effects of transspinal direct current stimulation on cycling time to exhaustion and perception of effort. **Conclusions:** In all studies, there was no effect of direct current stimulation condition, anodal or cathodal, on any measure of human performance, perceived exercise intensity, or physiologic function. Therefore, more research is required to determine how to maximize the efficacy of direct current stimulation with respect to acute modulation of human performance.

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Review of literature

INTRODUCTION

Over the past twenty years, hundreds of studies have investigated the use of transcranial direct current stimulation to alter brain function. The most commonly cited mechanism by which direct current stimulation influences brain function is through modulation of neuron resting membrane potentials via the interaction of electrolytes and electrical fields. Researchers have suggested this mechanism may explain the alterations in motor cortex excitability, perception of exercise effort, and exercise endurance observed after transcranial direct current stimulation. In the past few decades, researchers have become interested in determining if direct current stimulation techniques targeting the spinal neurons can also modulate neuromuscular function.

If transspinal direct current stimulation (tsDCS) can modulate motor unit (MU) behavior in humans, it is most likely to affect the amount of torque produced by a given quantity of central drive, the relative reliance on high versus low threshold MUs, or both. For example, if a tsDCS intervention increases the recruitment threshold of low-threshold MUs and decreases the recruitment threshold of high threshold MUs, tsDCS may facilitate the recruitment of typically difficult to recruit MUs and decrease the firing rates of low-threshold MUs. Such neuromuscular modulatory effects may be applicable to athletes, military personnel, or those suffering from spasticity.

ANIMAL STUDIES

Aguilar, Pelucchi, Dilena, Oliviero, Priori, and Foffani (2011)

Researchers anesthetized adult rats before surgically placing one sub-dermal direct current stimulation electrode over the area covering T9-T10 vertebrae and another sub-dermal electrode on the anterior abdominal area. Two different stimulation conditions, anodal and cathodal, were investigated. During the anodal condition, the anodal electrode was placed over the thoracic spine while the cathode electrode was place over the abdomen. The opposite orientation was utilized for the cathodal condition. Spontaneous firing rates and firing amplitudes as well as hindpaw stimulation-evoked afferent responses from neurons in the gracile nucleus and somatosensory cortex were analyzed prior to, during, and after 15 minutes of 1.26 mA/cm^2 current density transspinal direct current stimulation (tsDCS). Researchers found the anodal condition to increase spontaneous firing rates but decreased evoked stimuli responses in both the gracile nucleus and somatosensory cortex, whereas the cathodal response resulted in decreased spontaneous firing rates and increased evoked responses. These findings suggest anodal stimulation may blunt the signaling of afferent stimuli to cortical centers, whereas cathodal stimulation may enhance such signaling. However, it is unclear why spontaneous firing and evoked responses diverged.

Ahmed (2011)

Sub-dermal tsDCS at a current density of 383.20 mA/cm^2 was delivered to adult mice for 3 minutes using either an anodal or cathodal montage. For the anodal condition, the anode electrode was placed over the spinal column spanning from T10 to L1 and the cathode electrode was placed on the lateral abdominal area; the cathodal condition had electrode sites switched. Before, during, and after stimulation, cortically-elicited cortical triceps surae muscle twitch

torque and spontaneous tibial nerve firing were elicited using electrical stimulation. It was observed that, in the cathodal condition twitch force was increased during stimulation but decreased after stimulation, whereas the opposite response was observed in the anodal condition. However, during stimulation in both anodal and cathodal conditions spontaneous firing frequency and amplitude of the tibial nerve increased. The author suggested cathodal tsDCS may optimize corticospinal activity during stimulation and depress corticospinal activity after stimulation.

Ahmed (2016)

Sub-dermal tsDCS at a current density of 33.0 mA/cm^2 was delivered to adult mice using either an anodal or cathodal montage. For the anodal condition, the anode electrode was placed over the spinal column spanning from T13 to L6 and the cathode electrode was placed on the abdominal area; the cathodal condition had electrode sites switched. The researcher measured tibialis anterior and triceps surae force responses after 20 minutes of tsDCS. Also, sciatic nerve potential firing was analyzed for rhythmicity using autocorrelograms during voluntary walking at fast and slow speeds during 40-seconds bouts of anodal or cathodal stimulation. It was observed that cathodal tsDCS resulted in enhanced rhythmicity and anodal tsDCS decreased rhythmicity during the fast walking condition. The researcher also observed spontaneous firing rates of smaller alpha motor neurons to increase in the cathodal tsDCS condition but decrease in the anodal tsDCS condition; the opposite occurred in larger alpha motor neurons. The modulation of larger alpha motor units were hypothesized to be due to changes in spontaneous pre-synaptic activity whereas modulation of small alpha motor units to be due to changes in pre-synaptic

inputs. The author suggests walking changes were due to tsDCS altering sensory input gain and that cathodal magnifies afferent sensory input whereas anodal decreases sensory input gain.

SUPRASPINAL MODULATION IN HUMANS

Bocci, Barloscio, Vergari, Di Rollo, Rossi, Priori, and Sartucci (2015)

The researchers measured first dorsal interosseus and tibialis anterior low- and high-frequency magnetic doublet motor evoked potential (MEP) amplitudes as well as silent periods from 4 women and 6 men in anodal, cathodal, and sham tsDCS conditions. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 20 minutes at a current density of 0.071 mA/cm^2 with montage locations covering T9-T11 and on the right shoulder. MEPs and silent periods (measured during half of maximal effort contractions) were observed before, immediately after the start of stimulation, and one hour post stimulation. The researchers reported no effect of tsDCS condition on silent period in response to low-frequency (10 ms between pulses) motor cortex stimulation, but reported increased post-stimulation MEP amplitudes following cathodal tsDCS and decreased MEP amplitudes following anodal tsDCS.

Niérat, Similowski, and Lamy (2014)

Niérat and colleagues measured hemidiaphram magnetic motor evoked potentials (MEPs) as well as spontaneous tidal volume from 13 women and 9 men and in anodal, cathodal, and sham tsDCS conditions. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, cutaneous tsDCS was delivered for 15 minutes at a current density of 0.071 mA/cm^2

with montage locations covering C3-C5 and 35 cm^2 area just below the cervicomental angle (anterior neck). MEPs and tidal volume were measured before, immediately after the start of stimulation, 7.5 minutes into stimulation, immediately post stimulation, and 15 minutes post stimulation. Relative to the sham condition, hemidiaphram MEP area increased due to tsDCS and responses remained elevated for at least 15 minutes post stimulation. Interestingly, the researchers also reported cathodal tsDCS to increase spontaneous tidal volume. The authors suspect a spinal mechanism was responsible for the findings.

SPINAL MODULATITION

Bocci, Vannini, Torzini, Mazzatena, Vergari, Cogiamanian, Priori, and Sartucci (2014)

The researchers measured MEP silent periods and motor unit number estimation (MUNE) in the abductor digiti minimi (ulnar nerve stimulation) and abductor pollicis brevis (median nerve stimulation) from 6 women and 6 men in anodal, cathodal, and sham tsDCS conditions. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 20 minutes at a current density of 0.071 mA/cm^2 with montage locations covering T9-T11 and the right shoulder in one experiment and C6-T1 and the right shoulder in another experiment. MEP silent periods (measured during 20% of maximal effort contractions) were observed before, immediately after the start of stimulation, and one hour post stimulation. Cathodal tsDCS increased MUNE for at least an hour post stimulation in both cervical spine and thoracic spine experiments. Accordingly, cathodal stimulation also decreased silent periods post-stimulation. If the silent period (the period of time following a MEP where there is no muscle activity) decreases, this suggests the neural pathway from the motor cortex to

the alpha motor neurons is more easily excitable. Furthermore, as MUNE is a measure of how many motor units are active in response to submaximal nerve-evoked potentials, an increase in MUNE suggests the alpha motor units are more easily excitable. As a whole, these data suggest it is possible for tsDCS to affect not only motor units near the spinal electrode, but also motor units distal the spinal electrode. Also, the MEP changes suggest supraspinal modulation may not be completely ruled out as an affected site, in addition to the clearly observed spinal modulation.

Hubli, Dietz, Schrafl-Altermatt, and Bolliger (2013)

The researchers measured surface electrical amplitudes (spinal reflexes) from the tibialis anterior immediately following left tibial noxious nerve stimulation in 17 healthy individuals and 17 individuals with complete spinal cord injuries (injury sites ranging from C3 to T6) in anodal, cathodal, and sham tsDCS conditions as well as a locomotion condition (70% unloaded body mass walking at 2 km/hr on a treadmill). For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 20 minutes at a current density of 0.056 mA/cm^2 with montage locations covering T11-T12 and on the left shoulder. Spinal reflexes were observed before, immediately after the end of the stimulation or walking, and 20 minutes post stimulation or walking. For healthy subjects, researchers reported no notable effects of tsDCS. However, reflex amplitudes in subjects with spinal cord injury increased by up to 84% after anodal tsDCS. The researchers concluded that anodal stimulation might be able to excite spinal circuitries.

Kuck, Stegeman, van der Kooij, and van Asseldonk (2018)

The researchers measured brain derived neurotrophic factor (BDNF) genotype and motor unit excitability (H-reflex amplitude) in ten healthy adults during anodal, cathodal, and sham tsDCS conditions. The researchers utilized two different montages. One montage included an anode electrode centered over T11 and cathode electrode on the left shoulder. Another montage included electrode locations 7 cm above and below T11, with one condition with the cathode electrode in the superior position and anode electrode in the inferior position and another condition with the opposite orientation. In all three of these configurations, tsDCS was delivered for 15 minutes at 2.5 mA (electrode sizes were not reported so it was not possible to calculate current density). Peak-to peak H-reflexes and M-waves were observed before, 2 and 9 minutes into stimulation, immediately post stimulation, and 30 minutes post stimulation. Results showed no difference in motor unit excitability modulation between the two BDNF genotypes (Val/Met and Val/Val). However, the dual-spine montage with inferior cathodes resulted in decreased H-reflex amplitudes up to 30 minutes post stimulation.

Lamy, Ho, Badel, Arrigo, and Boakye (2012)

The researchers measured electrically elicited motor unit excitability (H-reflex) responses from the soleus muscles of 17 adults in the experimental conditions (anodal and cathodal) and 5 adults in the sham condition. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 15 minutes at a current density of 0.071 mA/cm^2 with montage locations covering T9-T12 and on the right shoulder. Tibial nerve elicited peak-to peak H-reflexes and M-waves were observed before, 2 and 9 minutes into stimulation, immediately post stimulation, and 15 minutes post stimulation. The researchers reported anodal stimulation to

increase H-reflex amplitude although p-values were close to but not less than 0.05. The authors report being uncertain if potential modulation was due to spinal or supraspinal mechanisms.

Lamy and Boakye (2013)

The researchers measured motor unit excitability in 17 val/met (heterozygous) and 17 met/met (homozygous) BDNF genotype adults after anodal tsDCS. Previous transcranial direct current stimulation research found BDNF genotype to influence the likelihood an individual can be affected by direct current stimulation. tsDCS was delivered for 15 minutes at a current density of 0.071 mA/cm^2 with montage locations covering T9-T12 and on the right shoulder. Tibial nerve elicited peak-to peak H-reflexes and M-waves were observed before, 2 and 9 minutes into stimulation, immediately post stimulation, and 15 minutes post stimulation. The authors report that Val/Met individuals are less likely show increased motor unit excitability in response to anodal tsDCS than Val homozygotes.

Winkler, Hering, and Straube (2010)

The researchers measured soleus motor unit excitability via H-reflex and M-wave responses from 7 or 6 males and 2 or 3 females (one subject was not included in the results due to nerve stimulation tolerance issues) in anodal, cathodal, and sham tsDCS conditions. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 15-20 minutes at a current density of 0.063 mA/cm^2 with montage locations 2 cm left of T11 and over the left infraclavicular region (40 cm^2 electrodes). Soleus motor unit excitability measures (H-reflexes) were observed before, immediately after the start of stimulation, and 15 minutes post stimulation. The researchers reported anodal tsDCS to increase

and cathodal tsDCS to decrease H-reflex peak-to-peak amplitudes for at least 15 minutes post-stimulation. The authors hypothesized these findings may be explain by modulation of afferent synaptic function, which may be due to a change in neurotransmitter release to a change in a down-stream neuron's response to a given quantity of neurotransmitter concentration.

SPINAL AND SUPRASPINAL MODULATION

Albuquerque, Campêlo, Mendonça, Agusto, Fontes, de Mattos Brito, Monte-Silva (2018)

The researchers measured first dorsal interosseus MEP (magnetic stimulation) amplitude, medial gastrocnemius H-reflex amplitude, and biceps femoris nocioceptive reflex via sural nerve electrical stimulation from 6 women and 6 men in anodal, cathodal, and sham tsDCS conditions. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 20 minutes at a current density of 0.071 mA/cm^2 with montage locations covering T11-T12 and on the right shoulder. Dependent variables were measured pre, immediately after, 30 and 60 mins post stim and 20 mins of treadmill walking at 64-76% maximal heart rate. The authors reported anodal tsDCS reduced corticospinal excitability (MEP amplitude), motor unit excitability (H-reflex amplitude), and nocioceptive reflex area for up to 30 minutes post tsDCS, whereas cathodal tsDCS only increased nocioceptive reflex area. The researchers proposed that anodal tsDCS decreases spinal cord excitability whereas cathodal tsDCS may only increase polysynaptic reflex responses without affecting mono-synaptic responses.

Bocci, Marcelia, Vergari, Cognetto, Cogiamanian, Sartucci, and Priori (2015)

The researchers measured MEP amplitudes from abductor digiti minimi and abductor halluces muscles from 14 subjects and also measured H-reflex amplitudes of the previously mentioned muscles as well as the soleus in 8 subjects using anodal and cathodal tsDCS conditions. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 20 minutes at a current density of 0.071 mA/cm^2 with montage locations covering T9-T11 and on the right shoulder. MEP and H-reflex amplitudes were observed before, immediately post stimulation, and 30 minutes post stimulation. Hand muscles were not affected by tsDCS condition. However, in the lower-limb muscles, anodal tsDCS increased resting motor threshold, whereas cathodal tsDCS increased MEP area. Furthermore, the modulatory effects lasted at least 30 minutes post stimulation. As upper-limb muscles were not affected by tsDCS, these data suggest the effects of tsDCS may be localized to the neurons near the active electrode site.

Cogiamanian, Vergari, Pulecchi, Marceglia, and Priori (2008)

The researchers measured lumbar, cervico-medullary, and cortical somatosensory electrically evoked potential amplitudes from tibial and median nerves from 6 women and 6 men in anodal and sham tsDCS conditions. For the anodal (anode over the spine) conditions, tsDCS was delivered for 15 minutes at a current density of 0.071 mA/cm^2 with montage locations centered over T10 and on the right shoulder. Somatosensory evoked potentials, bipolar amplitude responses to tibial or median nerve stimulation at different points along the spinal column, were observed before, immediately after the start of stimulation, and 20 minutes post stimulation. The data suggested anodal tsDCS depressed tibial nerve evoked amplitude responses at the cervico-

medullary level, but no other evoked responses were affected by tsDCS. These data suggest tsDCS acts only on spinal neurons near the spinal tsDCS electrode.

Dongés, D'Amico, Butler, and Taylor (2017)

The researchers measured corticospinal excitability and motor unit excitability via evoked muscle activation through stimulation of the motor cortex (MEP), cervicomedullary descending tract, H-reflex amplitudes from 5 women and 7 men in cathodal and sham tsDCS conditions. These data allowed researchers to determine which levels of the central nervous system may be affected by tsDCS. For the cathodal (cathode over the spine) condition, tsDCS was delivered for 20 minutes at a current density of 0.10 mA/cm^2 with montage locations covering C6-T1 and under the cervicomental angle along the midline of the neck. CMEPs were measured from flexor carpi radialis and biceps brachii muscles whereas MEPs also included the first dorsal interosseus; H-reflexes were measured in flexor carpi radialis muscles. MEPs and CMEPs were measured pre, immediately post, and 10, 20, and 30 minutes post stimulation, whereas H-reflex measurements were taken pre stimulation and 0-20 and 20-40 minutes post stimulation. Cathodal tsDCS did not differentially affect CMEP, MEP, or H-reflex responses relative to the sham condition. The authors suggest a more longitudinal montage may be more efficacious, but also hint that a file-drawer effect may be suppressing null findings regarding the efficacy of tsDCS.

Lim and Shin (2011)

The researchers measured corticospinal excitability and motor unit excitability via MEP and H-reflex amplitudes in the flexor carpi radialis muscles of 9 women and 3 men in anodal,

cathodal, and sham tsDCS conditions separated by 48 hours. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 20 minutes at a current density of 0.08 mA/cm^2 with montage locations at the center of C7 and below the cervicomental angle. MEP and H-reflex amplitudes were observed before, immediately post stimulation, and 1 and 2 hours post stimulation. Both anodal and cathodal tsDCS increased corticospinal excitability (MEP amplitude) without affecting H-reflex amplitude. Moreover, these modulatory effects lasted for at least 2 hours. These data suggest that cervical spine tsDCS affected motor cortex excitability without affecting alpha motor neuron excitability.

Murray, Tahayori, and Knikou (2018)

The researchers measured corticospinal excitability and motor unit excitability via MEP and H-reflex amplitudes in the tibialis anterior muscles from 10 women and 12 men in seated and supine anodal, cathodal, and sham tsDCS conditions. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 30 minutes at a current density of 0.391 mA/cm^2 with montage locations covering T10-T12 and on the thigh. MEP and H-reflex amplitudes were observed before, 0-15 minutes post stimulation, and 30-45 minutes post stimulation. Both cathodal and anodal tsDCS increased corticospinal excitability (MEP amplitudes), but cathodal tsDCS elicited in the supine position resulted in the largest increase in motor unit excitability. Furthermore, motor unit excitability was decreased after cathodal tsDCS but not affected by anodal tsDCS. The authors suggested cathodal tsDCS currents may be more powerful than anodal tsDCS currents and that interneuron spontaneous activity modulation and corticospinal neuron resting membrane potential modulation may be responsible these findings.

PAIN MODULATION

Cogiamanian, Vergari, Schiaffi, Marceglia, Ardolino, Barbieri, and Priori (2011)

The researchers measured reported perceived pain and biceps femoris reflex amplitude response to evoked painful stimuli from the right sural nerve as well as H-reflex amplitudes from the soleus muscles of 5 women and 6 men during anodal and sham tsDCS conditions. Painful stimuli consisted of an initial 5-stimuli train being delivered at 200 Hz five times , once every 5-20 seconds. For the anodal (anode over the spine) condition, tsDCS was delivered for 15 minutes at a reported current density of 0.071 mA/cm^2 (calculated current density is 0.057 mA/cm^2) with montage locations at the center of T10 and on the right shoulder. Dependent variables were observed before, immediately post stimulation, and 30 minutes post stimulation. Compared to the sham condition, the anodal condition blunted the H-reflex amplitude in response to painful stimuli, in spite of no difference in reported perceived pain between conditions. The authors suggest this finding may suggest anodal tsDCS affected interneuronal networks.

Meyer-Frißem, Haag, Schmidt-Wilcke, Magerl, Pogatzki-Zahn, Tegenthoff, and Zahn (2015)

The researchers measured perceived pain from pinpricks of different force levels during anodal (12 subjects) and sham (12 subjects) tsDCS conditions. For the anodal (anode over the spine) condition, tsDCS was delivered for 15 minutes at a current density of 0.071 mA/cm^2 with montage locations covering T10-T12 and on the right shoulder. Perceived pain from pinpricks was observed before, immediately post stimulation, and 30 and 60 minutes post stimulation. Relative to the sham condition anodal tsDCS decreased perceived pain ratings to high-threshold painful pinpricks but not low threshold pricks, and these effects lasted for at least one hour.

Perrotta, Bolla, Anastasio, Serrao, Sandrini, Pierelli (2016)

The researchers measured reported perceived pain and biceps femoris reflex amplitude response to evoked painful stimuli from the right sural nerve. Painful stimuli consisted of an initial 5-stimuli train being delivered at 200 Hz five times over a 2.5-second period. Perceived pain was reported on a 0-10 scale with 10 being unbearable. Five women and 5 men participated in anodal, cathodal, and sham tsDCS conditions. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 15 minutes at a current density of 0.057 mA/cm^2 with montage locations at the center of T10 and on the right shoulder. Perceived pain and reflex responses to evoked potentials were observed before, 15 minutes, 30 minutes, and 60 minutes post stimulation. In comparison to cathodal tsDCS, anodal tsDCS decreased perceived pain as well as muscle reflex responses to painful stimuli for at least 60 minutes. The authors hypothesize that anodal tsDCS affects the excitability of sensory neurons. They also bring up the interesting conflict in opposing effects of tsDCS on motor neurons and nocioceptive neurons with little evidence to explain this apparent phenomenon.

Truini, Vergari, Biasiotta, La Cesa, Gabriele, Di Stefano, Cambieri, Cruccu, and Priori (2011)

The researchers measured perceived pain and pain tolerance duration from 8 health women and 9 health men in anodal and cathodal tsDCS conditions separated by 48 hours. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered at a current density of 0.071 mA/cm^2 with montage locations at the center of T10 and on the right shoulder. Stimulation began 15 minutes before the start of the cold pressor test and continued for the duration of the cold pressor test. The cold pressor test consisted of subjects immersing their right foot in 0.4±0.16°C water; pain threshold was measured as the time passed

before subjects indicated feeling pain and pain tolerance was measured as the total duration subjects were able to keep their foot immersed in the ice bath. Anodal tsDCS did not affect pain threshold, but pain tolerance was enhanced relative to cathodal tsDCS. The authors hypothesized that anodal tsDCS blocks impulse conduction in some axons.

PERFORMANCE MODULATION

Sasada, Endoh, Ishii, and Komiyama (2017)

The researchers measured 30-second cycle ergometer sprint (Wingate test) work capacity of 6 women and 17 men in anodal and sham tsDCS conditions. For the anodal (anode over the spine) and condition, tsDCS was delivered for 15 minutes at a current density of 0. 0857 mA/cm^2 with montage locations covering T11-L1 and on the right shoulder. A 5-minute warmup was followed by the stimulation condition, a 3-minute warmup, and then the 30-second sprint. Although peak power did not differ between conditions, subjects performed 2.4% more work in the cathodal condition relative to the sham condition.

Berry, Tate, and Conway (2017)

The researchers measured countermovement jump ground reaction force variables from 3 women and 9 men in anodal, cathodal, and sham tsDCS conditions. For anodal (anode over the spine) and cathodal (cathode over the spine) conditions, tsDCS was delivered for 20 minutes at a current density of 0. 0833mA/cm^2 with montage locations covering T11-T12 and on the sides of the umbilicus. Subjects performed sets of 5 jumps (20 seconds between jumps) prior to stimulation, immediately post stimulation, 20 minutes post stimulation, 60 minutes post

stimulation, and 180 minutes post stimulation. Anodal tsDCS resulted in multiple positive performance differences between conditions, most notably, work was less attenuated in the anodal condition. As intra-set jump performance did not change, it is most likely that tsDCS affected countermovement jump performance by attenuating central fatigue. These are the first data to suggest tsDCS can affect performance in humans.

SUMMARY

Although performance and pain data suggest tsDCS may be able to affect applied outcomes such as vertical jump and cycling performance as well as perception of pain, many inconsistencies exist in the body of research regarding the mechanisms behind tsDCS efficacy. For example, Two studies have observed anodal tsDCS to reduce MU excitability (Cogiamanian 2011, Albuquerque 2018), three studies have reported anodal tsDCS to increase MU excitability (Lamy 2012, Lamy 2014, Winkler 2010), and four studies have suggested anodal tsDCS does not affect MU excitability (Bocci 2014, Lim 2011, Kuck 2018, Murray 2018).

Explanations as to why there are a high percentage of inconsistent findings between tsDCS studies are purely speculative. However, the total body of literature is small and most studies differ in one or more of the following: current density, stimulation duration, electrode montage sites, and dependent variable. Therefore, many more tsDCS studies will need to be published before a better understanding of tsDCS efficacy and the mechanisms responsible for tsDCS efficacy.

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Transcranial Direct Current Stimulation of the Temporal Lobe Does Not Affect High Intensity Work Capacity

ABSTRACT

Stimulation of the left insular cortex may affect heart rate variability (HRV) and exercise effort perception. These studies investigated the effects transcranial direct current stimulation (tDCS) and electrode orientation on HRV and repeated maximal knee extensions. In study 1, following sham stimulation, anodal left temporal lobe stimulation, or anodal right temporal lobe stimulation, 10 male and 10 female subjects (age=21.0±1.5 yr) completed 50 maximum isokinetic extensions at $180°s^{-1}$. There was a significant effect of stimulation condition on HRV for only one (SD2; $p=.037$; $\eta^2=.159$) of five HRV metrics. There was no significant effect on isokinetic fatigue percent or isokinetic work (all $p\geq.278$; all $\eta^2\leq.065$). It has been proposed that placing the cathode electrode on the shoulder, may differentially affect tDCS. Therefore, in study 2, the effects of electrode orientation on tDCS-induced changes in HRV was assessed in ten healthy females and eight healthy males (21.6±2.5 yr) who completed cephalic, extracephalic, and sham trials. In the cephalic montage, the anode was placed over the left temporal lobe and the cathode was placed over right prefrontal cortex. In the extracephalic montage, the cathode was placed on the shoulder on the same side of the body as the anode. Neither cephalic nor extracephalic montages affected HRV (all $p\geq.152$; all $\eta^2\leq.105$). These data suggest that anodal tDCS of the insular cortex has little effect on HRV, and does not improve high-intensity exercise performance in the current population. Therefore, anodal tDCS applied over the left temporal lobe is not recommended for high intensity performance enhancement.

INTRODUCTION

Electroencephalography data suggests the insular cortex may play a large role in processing exercise sensory information and communicating with the motor cortex (15). If the insular cortex plays a large role in informing other areas of homeostatic imbalance, as some researchers suggest (25), as exercise is sustained at high intensities, altering insular cortex activity may positively or negatively affect high effort exercise performance. For example, intraoperative stimulation of the left insular cortex causes a shift towards increased parasympathetic cardiovascular modulation (29). Previous studies have reported that the insular cortex may be non-invasively affected via transcranial direct current stimulation (tDCS), as anodal tDCS over the left temporal lobe has resulted in increased heart rate variability (HRV; perhaps reflecting a shift towards increased parasympathetic modulation, decreased sympathetic modulation, or both), attenuated perception of effort, and attenuated fatigue during exercise (24, 28). This means that if something is capable of affecting the activity of the insular cortex and associated autonomic outflow, we likely would expect to see changes in HRV. However, some studies that have used similar stimulation methods as Monetenegro et al. (24) and Okano et al. (28) have found no effect of anodal tDCS over the left temporal lobe on perception of effort, exercise endurance, or HRV (4, 14); indeed one study found impaired work capacity (6). Furthermore, it is yet to be determined if stimulation of the right insular cortex results in impaired work capacity [presumably via a change (increase) in perception of effort in the opposite direction as left temporal lobe stimulation] and a shift towards decreased parasympathetic modulation, increased sympathetic modulation, or both, which may result in decreased HRV. Nonetheless, there are commercially available tDCS devices that are marketed as performance-enhancing devices.

Studies that have investigated the effects of tDCS on corticospinal excitability have used both cephalic (contralateral supraorbital) (2, 23, 40) and extracephalic (shoulder) (1, 10, 18) cathode locations as a part of their stimulation montages (ie electrode positioning). It has been proposed that placing the cathode electrode on the shoulder, instead of over the prefrontal cortex (contralateral supraorbital), may result in different effects (2, 10) as any cathode that is placed over a brain region may affect the function of that region of the brain near the cathode, whereas an extracephalically-located cathode is thought to avoid this issue. Certainly, studies have shown that when tDCS is administered over the pre-frontal cortex, tDCS can affect risk-taking behaviors (12) and affect perception of effort and work capacity (22). Thus, it is plausible that when a cephalic montage utilizes a prefrontal cortex cathode location, that cathode may confound the effects of the anodal stimulation. Furthermore, research has shown that cephalic and extracephalic montages result in the delivery of different current densities (3, 26) as greater between-electrode distances result in deeper-penetrating electrical fields (3, 26), but the electrical field intensities may be suppressed (3). Thus, cephalic and extracephalic tDCS montages may differ in their ability to affect the insular cortex, which is located deeper in the brain than typical tDCS targets. It should be noted that some concern has been expressed regarding the potential of extracephalic montages to affect the autonomic functions of the brain stem function (37), however, data suggests that tDCS administered with extracephalic cathodes does not substantially affect the brainstem (30, 37).

Therefore, the purpose of study 1 was to determine if anodal stimulation of the left insular cortex enhances Thorstensson fatigue test (35), work capacity, and HRV and if stimulation of the right insular cortex decreases work capacity and HRV. After the completion of study 1, study 2 investigated the effect left temporal lobe anodal tDCS using cephalic and

extracephalic cathode locations on HRV. Because direct stimulation of the left insular cortex results in a shift towards parasympathetic cardiovascular dominance (29), if anodal tDCS over the left temporal lobe does increase HRV, it is likely that tDCS can be used to target the insular cortex. If cephalic and extracephalic montages have no effect on HRV, the efficacy of tDCS on non-superficial brain areas may be questionable, and it may not be able to affect exercise intensity perception. If cephalic and extracephalic montages differentially affect HRV, researchers may need to more carefully select their cathode electrode placement when investigating the efficacy of tDCS.

METHODS

Study 1

Experimental Approach to the Problem

Subjects visited the lab on four occasions, one familiarization visit and three randomized single-blind experimental trials with cross-over (figure 1). Subjects refrained from strenuous exercise in the 24 hours prior to each experimental visit. At least 4 days separated each visit and experimental visits two and three commenced at a time of day within ± 1 hour of the beginning of their first experimental visit. For female subjects, researchers did not control for menstrual cycle phase. However, research suggests that menstrual phase does no significantly affect strength or fatigability of muscles (17). No questionnaire was administered to determine if subjects experienced residual fatigue or soreness from prior testing sessions. However, data suggests that subjects can recover from similar concentric-only isokinetic fatiguing tasks in as little as two days (20). During the familiarization visit, subjects were exposed to the relatively uncommon task of isokinetic knee extensions. This visit decreased the likelihood of the learning effect occurring during experimental trials. During each of the three experimental trials, after ten minutes of sitting in a dark room, subjects received one of three 30-minute tDCS treatments: Sham, left temporal lobe anodal stimulation (Para), or right temporal lobe anodal stimulation (Symp). For the duration of each treatment, electrocardiographic (ECG) data was continuously sampled at 500 Hz. Post treatment, subjects performed 50 maximal effort knee extensions at $180°•s^{-1}$. External torque and dynamometer lever arm position voltages were continuously sampled for the duration of each experimental trial using custom-written LabVIEW (National Instruments Corporation, Austin, TX) programs.

Subjects

Ten healthy males and ten healthy females (age=21.0±1.5 yr; height=173.6±11.8 cm; mass=71.2±14.2 kg; mean±SD) participated the study. Exclusion criteria included current or past neuromuscular or musculoskeletal injuries as well as any metal implants above the neck (besides common orthodontic braces). The subjects were recreationally active as defined by an exercise frequency between two and four times per week. Each subject signed an informed consent document before completing a health history questionnaire. The study was reviewed and approved by the University Human Subjects Committee.

Procedures

tDCS

Two different tDCS montages were implemented (figure 2). The Para and Sham montages followed the methods reported by previous studies that attempted to affect the insular cortex with anodal tDCS (4, 6, 24, 28), where the anode is placed over T3 and cathode is placed over Fp2. The Symp montage was similar except the electrodes were placed on opposite sides of the head (figure 2). Electrode positions followed the 10-20 system (19): 1) the distance from the anion to nasion to the inion was measured (distance A), 2) marks were made at 10% of distance A above both the naison and the inion and at 50% of distance A (C_z), 3) the distance from the left preauricular point or the right preauricular to C_z was measured (distance B), 4) a mark was placed at 10% of distance B above the preauricular point of interest (left side = T3, right side = T4), 5) a mark was placed on the forehead at 10% of the distance from the nasion through either T3 (Fp1) or T4 (Fp2) to the inion. Prior to the placement of electrodes, electrode placements were marked, 5 x 5 cm sponges were soaked with 0.15 M saline solution, and those sponges

were placed over the stimulating electrodes. Electrodes were held in place by firmly wrapped self-adherent flexible wrap (Sensi-Wrap, Dynarex, Orangeburg, NY). For each trial, a 30-second ramp up to a constant current of 2.00 mA was delivered using a direct current stimulator (TCT Research Limited, Hong Kong), which has been used in previous research (2). During the sham condition, the bicephalic montage was used, and the stimulator was turned off after the 30-seconds of stimulation, which is commonly utilized in tDCS sham conditions (2, 24, 28). These sham methods were utilized to mimic those used in previous insular cortex stimulation studies (2, 24, 28). However, data suggests these sham methods may not effectively blind subjects who participate in a repeated measures study (27) and that the use of off-target stimulation or the use of topical anesthetic may be more efficacious in masking subjects differential perceptions to sham and non-sham tDCS conditions (11). The researchers were not blinded to experimental conditions. For non-sham conditions, the stimulation continued for 30 minutes.

HRV

Prior to data collection, isopropyl alcohol was used to clean the areas of skin where electrodes (Red Dot Foam Monitoring Electrodes, 3M, St Paul, MN) would be placed for bipolar ECG data collection. One electrode was placed below the right clavicle, just medial to the anterior deltoid. A second electrode was placed along the left mid-axillary line, in the fifth intercostal space. The reference electrode was placed over the spinous process of the first cervical spine bone. During each trial, continuous ECG data were amplified using UFI Model 2122i Bioamplifier (UFI Instruments, Morrow Bay, CA) and sampled with a four-channel NI-9215 analog input module (National Instruments Corporation, Austin, TX), which sampled at 500 Hz. All raw ECG signals were collected using custom data acquisition programs written

with LabVIEW programming software (National Instruments, Austin, TX). The digitized ECG signals were stored on computer disk for subsequent analysis.

To analyze HRV, the ECG signals were processed using a custom LabVIEW program that incorporated automated R-wave peak detection. To ensure the accuracy of all detected R-waves, the program also allowed cursor-driven manual editing where visual inspection indicated potential peak detection errors (38, 39). The resulting inter-beat interval (RR_i; msec) data were used to quantify HRV using two time domain and three frequency domain variables. For the time domain variables, the root mean square of successive differences (RMSSD) was used to assess short-term HRV (7) and long-term HRV was quantified using the standard deviation of the perpendicular distances between each xy coordinate and a line perpendicular to the line of identity that goes through the centroid of a Poincaré plot (SD2), which has been mathematically described by Brennan et al. (5). For the frequency domain variables, RR_i data were interpolated, and resampled at 10 Hz. The resulting data were processed using a Hamming window and discrete Fourier transform to create frequency power spectra. The spectra were integrated between specified frequencies, to quantify high- and low-frequency power. The natural log of the power between 0.04-0.15 Hz was used to describe low frequency power (LF). The natural log of the power between 0.15-0.40 Hz was used to describe high frequency power (HF). The ratio of LF to HF was also calculated (LF/HF). The inclusion of multiple HRV metrics served to increase the likelihood of observing any autonomic function changes. For example, although changes in HF or RMSSD may indicate a change in vagal tone (7), there is not a perfect relationship between HF and RMSSD (8). Similarly, total HRV may be indicated by both LF (7) and SD2 (5), but there is not a perfect relationship between the two metrics (16). Furthermore, there is no single metric that can be confidently used to estimate sympathetic modulation (7), but

if a vagal metric such as HF or RMSSD does not change and a total HRV metric such as SD2 does change, it is likely that the change in HRV has been primarily driven by sympathetic modulation.

Isokinetic Protocol

Subjects were seated and secured into a Biodex System 3 Pro Isokinetic Dynamometer (Biodex Medical Systems Inc, Shirley, NY) with their right knee joint in-line with the dynamometer arm axis-of-rotation, the bottom of the dynamometer arm pad approximately five centimeters above the foot, and a hip angle of 100°. Dynamometer ROM bottom stop was set at arm angle of 90° (perpendicular to the floor) and the top stop was set at full knee extension (knee angle reference = 180°). The dynamometer was operated in isokinetic mode at a velocity of $180°•s^{-1}$. For each repetition, subjects performed voluntary knee extensor contractions from the bottom stop to the top stop, and then they relaxed as their leg fell from full extension back to the bottom stop. Subjects warmed up by completing five submaximal repetitions at the testing velocity. After resting for at least 30 seconds, subjects began performing maximal effort knee extensions. To increase the likelihood of measuring true maximal effort knee extensions, strong verbal encouragement was provided during all maximal effort knee extensions. Fatigue responses were quantified using two general methods: 1) calculation of the Fatigue Index (35), and 2) calculation of the mean torque integral across all 50 repetitions. Fatigue Index was calculated using the following formula: [(highest three rep average-final three rep average)/ highest three rep average]*100.

Signal Processing

Using a custom-written LabVIEW program, torque, position, and velocity data were simultaneously sampled at a rate of 10k Hz. Using a 4th order zero lag Butterworth digital filter, torque data were low pass filtered at 6 Hz. Velocity data were low pass filtered at 15 Hz. Position data were low pass filtered at 100 Hz. Using the peaks and troughs of the position signal, each repetition was segmented into concentric and passive lowering phases. Torque repetition data were captured by including data between the trough and peak of the position signal and by calculating the torque integral, or the area under the torque curve, over the full concentric range of motion of each repetition.

Data Analysis

All statistical analyses were performed using SPSS version 22. For each of the HRV variables (RMSSD, SD2, LF, HF, and LF/HF) and the fatigue variables (Fatigue Index and the sum of torque integrals) a 3-level (Sham vs Para vs Symp) repeated measures ANOVA was performed. The Huynh-Feldt correction was used to compensate for violations of sphericity, where appropriate. Effect sizes were calculated as partial eta squared values (η_p^2). For post-hoc comparisons, dependent t-tests with the Bonferroni adjustment for multiple comparisons was used. Alpha was set at .05. All data are presented as means and SD, unless otherwise noted.

Study 2

Experimental Approach to the Problem

Each subject completed three different trials, where the between-trial difference was tDCS stimulation condition: Cephalic, Extracephalic, and Sham. The three trials were counterbalanced and separated by at least two days (figure 1). The second and third experimental

trials began at a time of day within ± 1 hour of the beginning of the first experimental trial. For each trial, after subjects were prepared for tDCS and ECG collection, subjects were taken to a quiet and dark room, where they sat for 45-minutes of ECG data collection. The first 15 minutes (pre) was spent without tDCS or sham tDCS, the next 20 minutes (online) included tDCS or sham tDCS, and the last 10 minutes (post) were spent without tDCS or sham tDCS. To replicate the methods from Montenegro et al. (24), the last 5-minutes of ECG data from the pre, online, and post phases were used for HRV analysis. Such methods previously captured changed in HRV, indicative of insular cortex stimulation, due to tDCS. This method allows for the same duration of data to be compared between pre, online, and post periods of each trial. Furthermore, excluding the beginning of each trial phase may have minimized some of the initial change in mental state that could have inherently occurred due to subjects being aware of changes between the end of one phase and the beginning of another phase. However, it should be known that we also analyzed data over the full duration of each intervention interval (pre, online, and post) and the results were very similar to those reported below.

Subjects

Ten healthy females and eight healthy males were included in the analysis (21.6±2.5 yr; 172.1±7.2 cm; 71.3±17.4 kg; mean±SD). These subjects were not the same subjects from Study 1. Subjects reported participation of 4.9±4.5 hours of weekly aerobic exercise, and one female subject was a Division 1 soccer player. All subjects were between the ages of 18 and 30, non-obese (body mass index less than 28 kg/m^2), non-smokers, free of known metabolic and cardiovascular diseases, not prescribed to any stimulant, depressant, or anti-depressant drugs, not pregnant, free of pacemakers or any metal non-dental implant above the neck, and free from a

history of epilepsy or seizures. Subjects refrained from alcohol and caffeine consumption in the 24 hours prior to each trial, but diet was not controlled in any other way. All subjects signed an informed consent document that was approved by the University Human Subjects Committee.

Procedures

tDCS

Two different tDCS montages were implemented (figure 2). The Cephalic montage followed the methods reported by previous studies that attempted to affect the insular cortex with anodal tDCS (4, 6, 24, 28), where the anode is placed over T3 and cathode is placed over Fp2. The Extracephalic montage was similar to that used by Cogiamanian et al. (10) where the cathode was placed on the shoulder on the same side of the body as the anode, which was still placed over T3. All other stimulation procedures were the same as in Study 1, except the stimulation occurred for 20-minutes, and ECG was monitored for the 15 minutes before and 10 minutes after the online stimulation.

HRV

All ECG signals were acquired using the same methods as described in Study 1. HRV was described using the same methods as reported in Study 1. It is important to note that the analysis of SD2 and RMSSD would have yielded identical p-values as the standard deviation of consecutive normal beat-to-beat intervals (SDNN) and the standard deviation of the perpendicular distances between each xy coordinate and the line of identity in the Poincaré plot (SD1), respectively (5, 9). Thus, the most commonly reported HRV metrics were analyzed in both studies.

Data Analysis

All statistical analyses were performed using SPSS version 22. For each of the HRV variables (RMSSD, SD2, LF, HF, and LF/HF) a 3 (pre vs online vs post) by 3 (Cephalic vs Extracephalic vs Sham), repeated measures ANOVA was performed. The Huynh-Feldt correction was used to compensate for violations of sphericity. Effect sizes were calculated as partial eta squared values (η_p^2) and 95% confidence intervals were calculated as warranted. Alpha was set at .05. All data are presented as means and SD, unless otherwise noted.

RESULTS

Study 1

HRV

The mean RMSSD, SD2, LF, HF, and LF/HF values are reported in table 1. All values were in the normal physiologic range. For all HRV metrics other than SD2 [$F(2,38)=3.599$; $p=0.037$], the repeated measures ANOVA revealed no significant effect of condition [RMSSD $F(2,38)=1.323$; LF $F(2,38)=.975$; HF $F(2,38)=.801$; LFHF $F(2,38)=.630$; all p-values ≤ 0.278]. Individual responses for each HRV metric are depicted in figures 3 and 4.

Fatigue

The mean Fatigue Index and torque integral (work) across all repetitions, for each condition, can be seen in table 2. Although not reported here, electromyographic signal amplitudes of these subjects did not systematically decrease across the test, which indicates the subjects were likely engaging maximally throughout the test. The repeated measures ANOVA revealed no significant effect of condition on Fatigue Index [$F(2,38)=0.760$; $p=.475$; $\eta_p^2=.038$] or mean torque integral [$F(2,38)=0.732$; $p=.488$; $\eta_p^2=.037$]. Individual responses can be seen in figure 5.

Study 2

RMSSD

The 3 x 3 repeated measures ANOVA revealed no significant interaction between trial and time [$F(3.323,56.492)=1.783$; $p=.156$; $\eta_p^2=.095$], no significant main effect for time [$F(1.610,27.376)=0.683$; $p=.483$; $\eta_p^2=.039$], and no significant main effect for trial

[F(2,34)=1.117; p=.339; η_p^2=.062]. Mean responses are displayed in table 3; individual responses are displayed in figure 6.

SD2

The 3 x 3 repeated measures ANOVA revealed no significant interaction between trial and time [F(4,68)=0.923; p=.456; η_p^2=.052], no significant main effect for time [F(2,34)=2.535; p=.094; η_p^2=.130], and no significant main effect for trial [F(2,34)=1.990; p=.152; η_p^2=.105]. Mean responses are displayed in table 3; individual responses are displayed in figure 6.

LF

The 3 x 3 repeated measures ANOVA revealed no significant interaction between trial and time [F(4,68)=0.294; p=.881; η_p^2=.017], no significant main effect for time [F(2,34)=1.056; p=.359; η_p^2=.058], and no significant main effect for trial [F(2,34)=1.020; p=.371; η_p^2=.057]. Mean responses are displayed in table 3; individual responses are displayed in figure 7.

HF

The 3 x 3 repeated measures ANOVA revealed no significant interaction between trial and time [F(4,68)=0.660; p=.622; η_p^2=.037), no significant main effect for time [F(1.595,27.111)=0.337; p=.669; η_p^2=.019], and no significant main effect for trial [F(2,34)=0.430; p=.654; η_p^2=.025]. Mean responses are displayed in table 3; individual responses are displayed in figure 7.

LF/HF

The 3 x 3 repeated measures ANOVA revealed no significant interaction between trial and time [$F(4,68)=0.163$; $p=.957$; $\eta_p^2=.009$], no significant main effect for time [$F(1.574,26.750)=0.257$; $p=.722$; $\eta_p^2=.015$], and no significant main effect for trial [$F(2,34)=0.357$; $p=.690$; $\eta_p^2=.022$]. Mean responses are displayed in table 3; individual responses are displayed in figure 7.

DISCUSSION

The purposes of this study were to investigate the effect of temporal lobe anodal tDCS on repeated maximal effort work capacity and HRV. The findings of Study 1 indicated the specific anodal tDCS administration methods utilized in the study affected one (SD2) of the five HRV metrics reported. In contrast to Okano et al. (28) and Montenegro et al. (24), of particular note is that in both studies 1 and 2, there were no significant differences in RMSSD or any frequency domain variable. Thus, the efficacy of tDCS using the T3-FP2 montage on insular cortex excitation is questionable, and if the montage did affect the insular cortex, it did not affect performance in the 50 repetition maximal effort knee extension task. In Study 2, the addition of pre and post stimulation data and a different montage resulted in data that suggest 20 minutes of 2 mA tDCS does not affect the insular cortex. Accordingly, it is unlikely that temporal lobe anodal tDCS is capable of affecting short-duration repeated maximal effort work capacity.

Previous research suggests the insular cortex is involved in exercise perception of effort via communication with the motor cortex (15). The study by Okano et al. (28) supports the idea that anodal tDCS can affect the insular cortex by enhancing cycling peak power output and a delaying in perception of effort increases during an incremental cycling test in trained cyclists. However, using the same montage, Barwood et al. (4) found no effect of anodal tDCS on RPE, cycling time to exhaustion, or pacing profile in recreationally trained males. Furthermore, Cantreva et al. (6) have reported that anodal tDCS applied over C3, which is slightly closer to the center of the skull than T3, results in decreased work capacity as indicated by impaired performance of three sets of 10 knee extension-flexion repetitions. There are slight methodological differences between these studies, such as stimulation current [2 mA for Okano et al. (28) and 1.5 mA for Barwood et al. (4)] and anode electrode placement differing by a few

centimeters. However, with the conflicting performance data and the existence of such few studies, the efficacy of anodal tDCS to stimulate the insular cortex should be scrutinized. Furthermore, a recent publication suggesting that typical tDCS methods minimally affect the brain supports this call for greater scrutiny (36).

Certainly, researchers have reported a wide variety of effects of tDCS using the T3 – Fp2 montage. The Montenegro et al. (24) study, which reported no effect of anodal tDCS on HRV in non-athletes and a shift towards sympathetic dominance in athletes, was the first study to suggest that anodal tDCS over T3 can affect the insular cortex. The results of Okano et al. (28) supported the hypothesis that anodal tDCS over T3 can affect the insular cortex, as their results showed a parasympathetic shift in HRV. However, other unpublished dissertation data from the Okano lab suggest that anodal tDCS over T3 has no effect on HRV in middle-aged women with hypertension (14). Surprisingly, a study by Gomes et al. (14) found that cathodal tDCS over T3 caused a shift in HRV towards parasympathetic balance. As the tDCS methods between these two Okano group studies were nearly identical, and the only difference was population, it seems plausible that different populations result in different tDCS outcomes. However, the lack of effects in HRV due to tDCS in the current studies suggests that anodal tDCS does not stimulate the insular cortex. Furthermore, although not presented here, when the delta values from the pre-post HRV (RMSSD, SD2, and LFHF) are regressed against hours of aerobic activity performed per week, the resulting R^2 values were less than .046. Although hours of aerobic activity is not the best indicator of fitness, the clear lack of any trend suggests that it is unlikely that fitness influenced the efficacy of the stimulation employed in the current study. It should also be noted that there was a NCAA Division 1 soccer player in the study, and their cephalic montage condition resulted in an increase in LF/HF, which conflicts with the results from the Montenegro

et al. study (24). Thus, few studies have used anodal tDCS over T3 and measured HRV, and the findings from these studies question the efficacy of the ability of anodal tDCS to stimulate the insular cortex, which is located deeper than cortical areas such as the motor cortex.

Barwood et al. (4) suggest that one explanation for the variance in the use of the T3 – Fp2 montage may be due to a confounding effect of the cathode, which, when placed over Fp2, is located over the prefrontal cortex. Evidence does suggest that when tDCS is administered over the prefrontal cortex, tDCS can affect risk-taking behaviors (12), which could affect exercise effort or pacing strategy. Furthermore, a recent publication showed that pre-frontal cathodal tDCS resulted in increased perception of effort with lower elbow flexion work capacity with a ten-repetition maximum load (22). Indeed, tDCS studies that have aimed to use anodal stimulation to affect the motor cortex have attempted to address this issue by using extracephalic montages (1, 10, 18, 21, 31). However, the lack of consistent findings also applies to these studies, as they have conflicting reports on the efficacy of anodal tDCS to increase corticospinal excitability. Furthermore, some studies have reported improvements in exercise endurance with (10) and without (1) concomitant enhancement of cortical excitability. Just as the T3 – Fp2 montage studies have some methodological differences, these motor cortex – extracephalic montage studies also have methodological differences where stimulation duration ranges from 10-15 minutes, current delivery ranges from 1-2 mA, and extracephalic cathode electrode location is either ipsilateral or contralateral to the anode. These seemingly minor details, such as extracephalic electrode location, have already been shown to have the potential to affect the current density that is delivered to the brain (26). Perhaps these differences and other physiological differences result in differing quantities of current reaching the target area. For HRV, this may mean that if tDCS can indeed affect the insular cortex, individuals whose insular

cortex is stimulated a little may experience a shift towards parasympathetic dominance, where those who experience larger insular cortex stimulation may exhibit little to no change in HRV. This is because HRV increases as parasympathetic input increases, until HRV reaches a plateau, where greater increases in parasympathetic input decreases HRV (13).

The current series of studies are not without interpretation limitations. One confounding influence may be due to variability in catecholamine responses between visits. For example, tDCS may simultaneously affect an off-target brain region that influences arousal and circulating catecholamine concentrations, or a subject may randomly have different states of arousal between visits. Certainly research supports the relationship between circulating catecholamine concentrations and HRV (32). Another variable that has been observed to influence HRV is menstrual cycle phase (34). Similarly, ovarian steroids have been observed to affect cortical excitability (33). Thus, it is likely that additional random HRV variance was introduced to the study as menstrual cycle phase was not controlled nor included as a covariate. The efficacy of tDCS to affect female subjects is under-researched, which is highlighted by the fact that most of the tDCS studies closely related to the current studies only included male subjects (4, 23, 24, 28), one included all female subjects (14) and one included a single female subject (6). With this lack of female data, it is currently impossible to determine if there are any sex-related efficacy differences in the ability of temporal lobe tDCS to affect the insular cortex. Thus, future tDCS studies should aim to determine if there are sex differences in the efficacy of tDCS and if the efficacy is affected by menstrual cycle phase.

PRACTICAL APPLICATIONS

In conclusion, the current data suggests that is unlikely that the specific anodal tDCS administration methods utilized in this study are capable of affecting the insular cortex, as indicated by a lack of a consistent change in HRV measures or a change in repeated maximal effort work capacity. Thus, the use of anodal tDCS of the temporal lobe to influence exercise capacity is of questionable value. However, the area of tDCS and exercise performance is still a relatively young area of research and there are many physiologic variables and methodologic variables that may account for some of the inconsistent findings in the literature. Certainly, these discrepancies clearly highlight the need for more research in the area of tDCS and exercise performance, as the idea that tDCS can modulate certain areas of the brain is an exciting proposition to athletes and coaches.

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Figure 1. Experimental methods and timing described for Study 1 and Study 2. In Study 1, a different experimental condition of Sham, sympathetic montage (Symp), or parasympathetic montage (Para) was completed during each experimental trial, before the maximal effort isokinetic knee extension performance test. In Study 2, a different experimental montage of Sham, Cephalic, or Extracephalic was implemented during each experimental trial.

Figure 2. A representation of the electrode placement locations for the anode (gray circle) and cathodes (black circles). The dashed vertical line (A) connects the nasion and the inion. The inner circle crosses marks that are equal to 10% of the distance of A from the nasion and 10% of the distance of A from the inion. The horizontal dashed line (B) connects the left and right preauricular points. For the Sham, Para, and cephalic montages, the anode was located at T3 and the cathode was located at Fp2, which is at a position 5% of the distance from A. For the Symp montage, the anode was located at T4 and the cathode was located at Fp1. For the extracephalic montage, the anode was placed on T3 and the cathode was placed on the ipsilateral anterior deltoid (solid black circle).

Figure 3. Study 1 individual heart rate variability responses during 30-minutes of Sham, parasympathetic (Para), and sympathetic (Symp) stimulation conditions. Metrics include the root mean square of successive differences (RMSSD) and SD2. Each data point contains values from a pair of two conditions for one subject. The dashed line is the line of identity, where data would lie if the two conditions resulted in the same values; data above the dashed line have greater variability in the y condition than the x condition and the opposite is true for data below the dashed line. Exact mean and SD values can be found in table 1.

Figure 4. Study 1 individual heart rate variability responses during 30-minutes of Sham, parasympathetic (Para), and sympathetic (Symp) stimulation conditions. Metrics include the natural log of low frequency power (LF), and the natural log of high frequency power (HF), and the ratio of LF to HF (LF/HF). Each data point contains values from a pair of two conditions for one subject. The dashed line is the line of identity, where data would lie if the two conditions resulted in the same values; data above the dashed line have greater variability in the y condition than the x condition and the opposite is true for data below the dashed line. Exact mean and SD values can be found in table 1.

Figure 5. Study 1 mean repeated knee extension torque integral (average work) and Fatigue Index (FI; percent change across 50 repetitons) responses following 20-minutes of each of the Sham, parasympathetic (Para), and sympathetic (Symp) stimulation conditions. Each data point contains values from a pair of two conditions for one subject. The dashed line is the line of identity, where data would lie if the two conditions resulted in the same values; data above the dashed line have greater variability in the y condition than the x condition and the opposite is true for data below the dashed line. Exact mean and SD values can be found in table 1.

Figure 6. Study 2 mean (thick black lines) and individual (thin lines) time-domain heart rate variability values during the last five minutes of pre-condition rest (Pre), the condition (Cond), and post-condition (Post), for each of the three experimental conditions (Sham, Extracephalic, and Cephalic). Exact mean and SD values can be found in table 3.

Figure 7. Study 2 mean (thick black lines) and individual (thin lines) frequency-domain heart rate variability values during the last five minutes of pre-condition rest (Pre), the condition (Cond), and post-condition (Post), for each of the three experimental conditions (Sham, Extracephalic, and Cephalic). Exact mean and SD values can be found in table 3.

Figure 1.

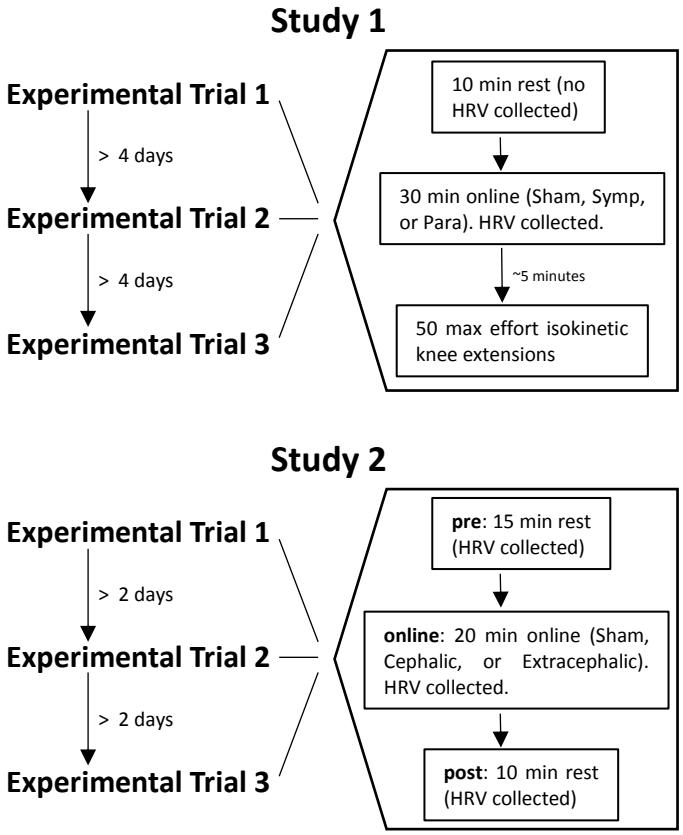
Study 1
Experimental Trial 1
> 4 days
Experimental Trial 2
> 4 days
Experimental Trial 3
10 min rest (no HRV collected)
30 min online (Sham, Symp, or Para). HRV collected.
~5 minutes
50 max effort isokinetic knee extensions
Study 2
Experimental Trial 1
> 2 days
Experimental Trial 2
> 2 days
Experimental Trial 3
pre: 15 min rest (HRV collected)
online: 20 min online (Sham, Cephalic, or Extracephalic). HRV collected.
post: 10 min rest (HRV collected)

Figure 2.

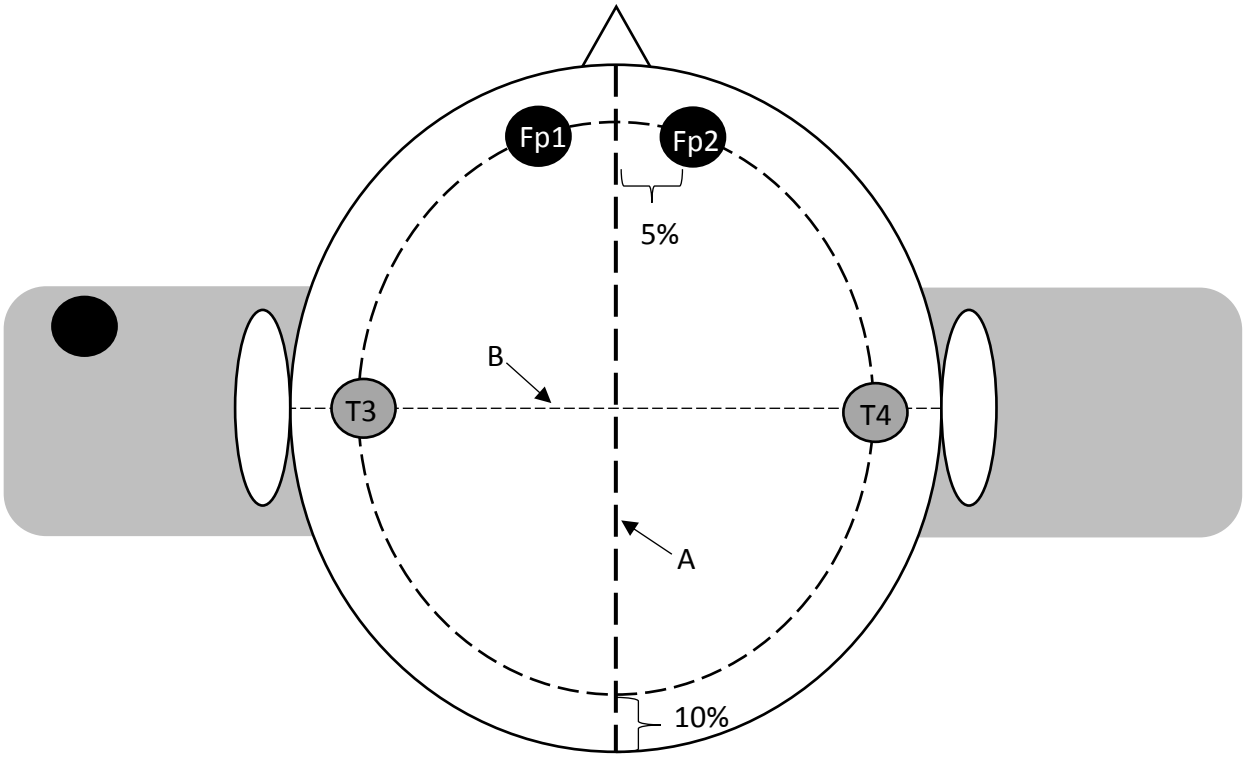

Figure 3.

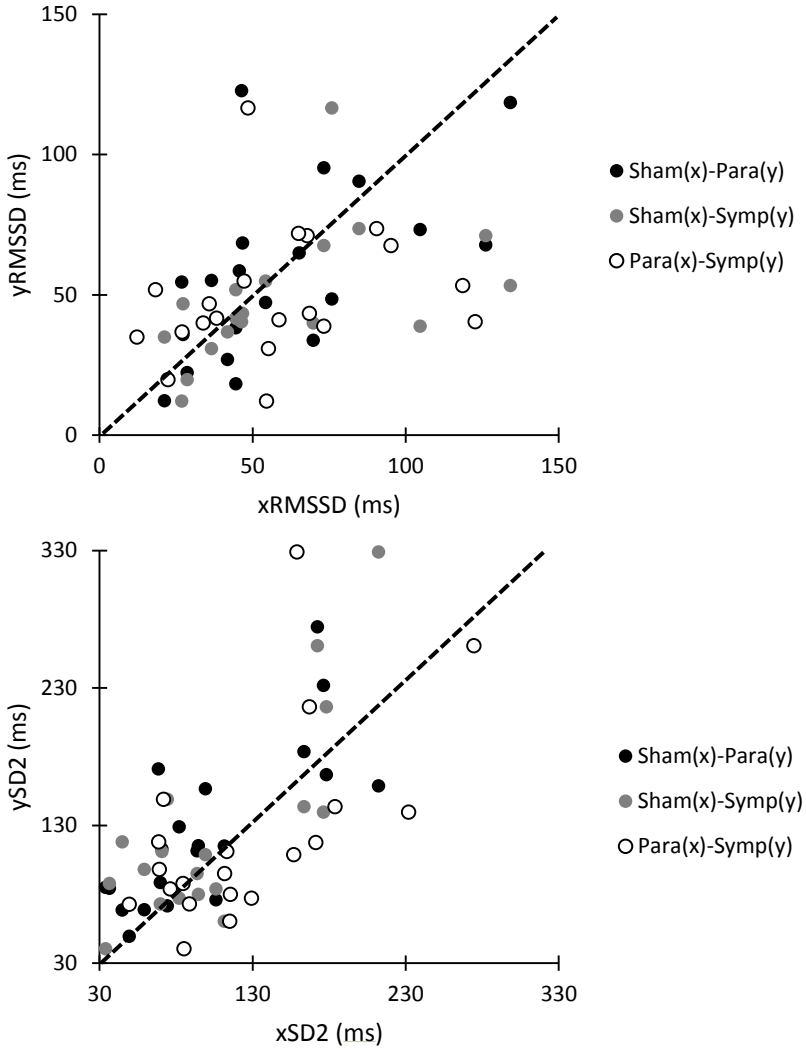
150
100
50
0
yRMSSD (ms)
0
50
100
150
xRMSSD (ms)
Sham(x)-Para(y)
Sham(x)-Symp(y)
Para(x)-Symp(y)
330
230
130
30
ySD2 (ms)
30
130
230
330
xSD2 (ms)
Sham(x)-Para(y)
Sham(x)-Symp(y)
Para(x)-Symp(y)

Figure 4.

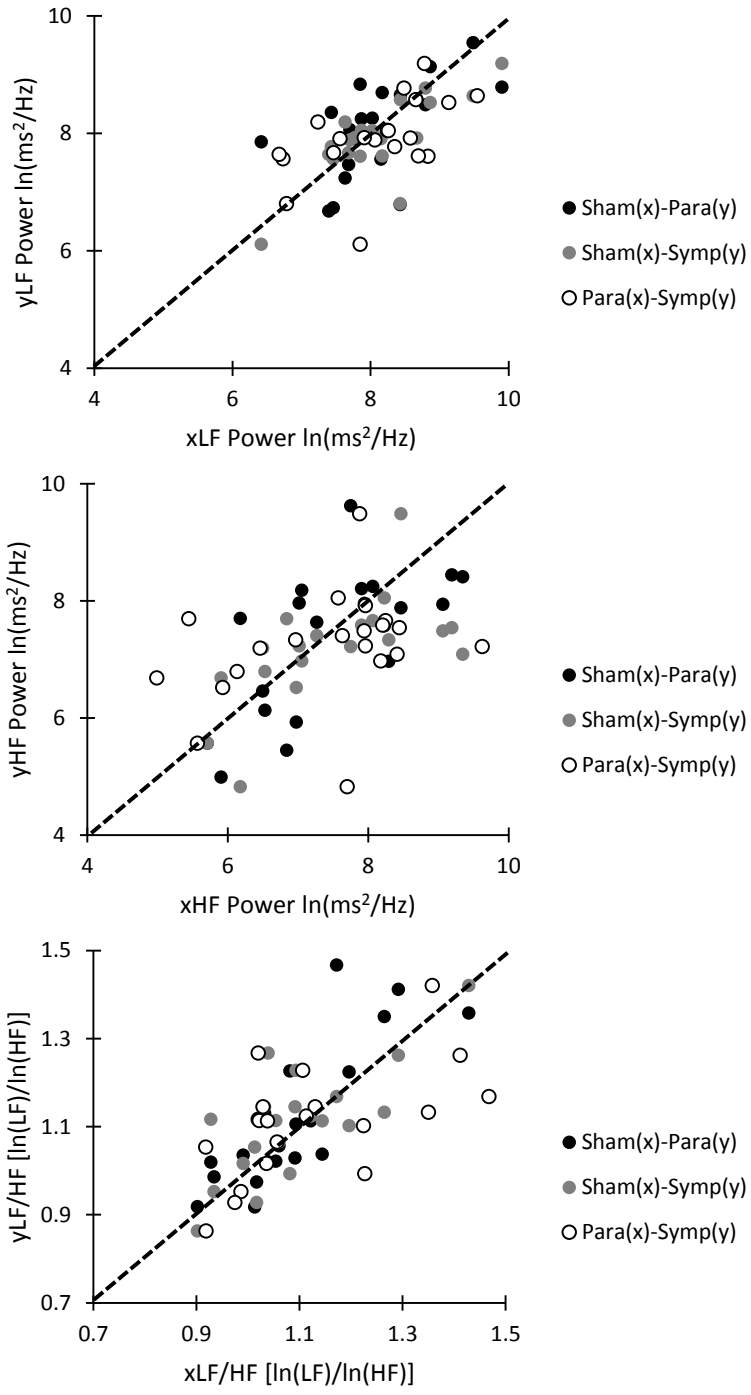
10
8
6
4
4
6
8
10
yLF Power ln(ms²/Hz)
xLF Power ln(ms²/Hz)
Sham(x)-Para(y)
Sham(x)-Symp(y)
Para(x)-Symp(y)
10
8
6
4
4
6
8
10
yHF Power ln(ms²/Hz)
xHF Power ln(ms²/Hz)
Sham(x)-Para(y)
Sham(x)-Symp(y)
Para(x)-Symp(y)
1.5
1.3
1.1
0.9
0.7
0.7
0.9
1.1
1.3
1.5
yLF/HF [ln(LF)/ln(HF)]
xLF/HF [ln(LF)/ln(HF)]
Sham(x)-Para(y)
Sham(x)-Symp(y)
Para(x)-Symp(y)

Figure 5.

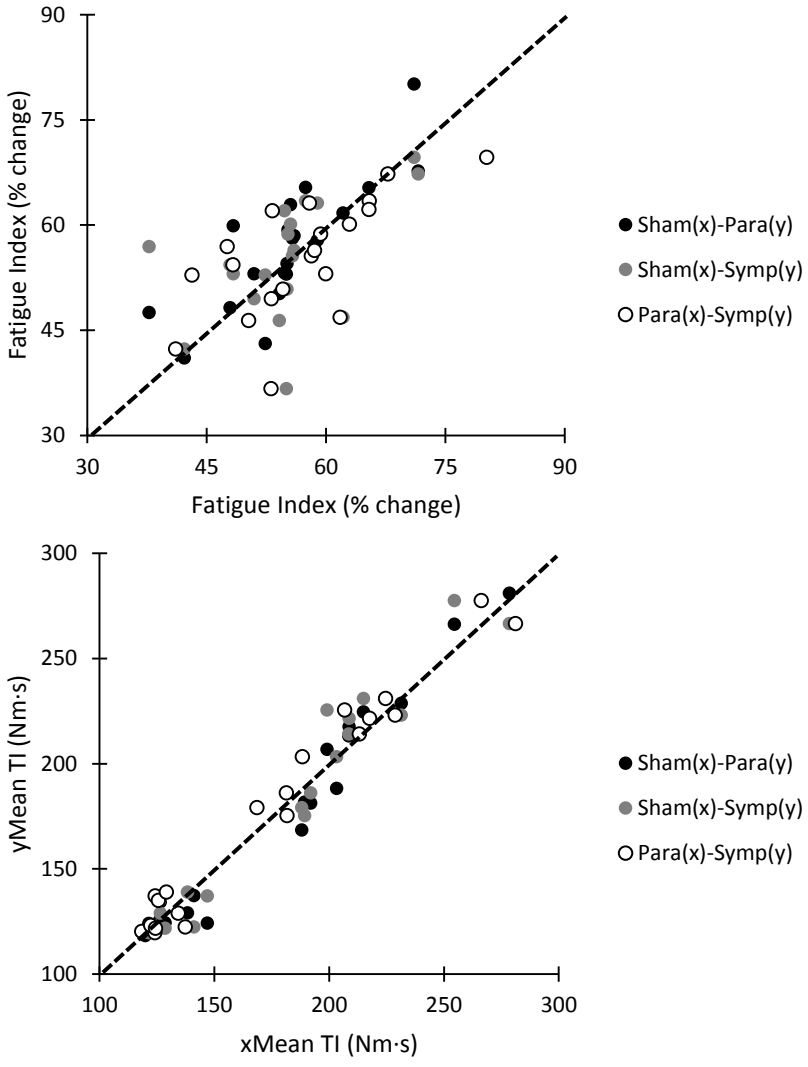
90
75
60
45
30
Fatigue Index (% change)
30
45
60
75
90
Fatigue Index (% change)
Sham(x)-Para(y)
Sham(x)-Symp(y)
Para(x)-Symp(y)
300
250
200
150
100
yMean TI (Nm·s)
100
150
200
250
300
xMean TI (Nm·s)
Sham(x)-Para(y)
Sham(x)-Symp(y)
Para(x)-Symp(y)

Figure 6.

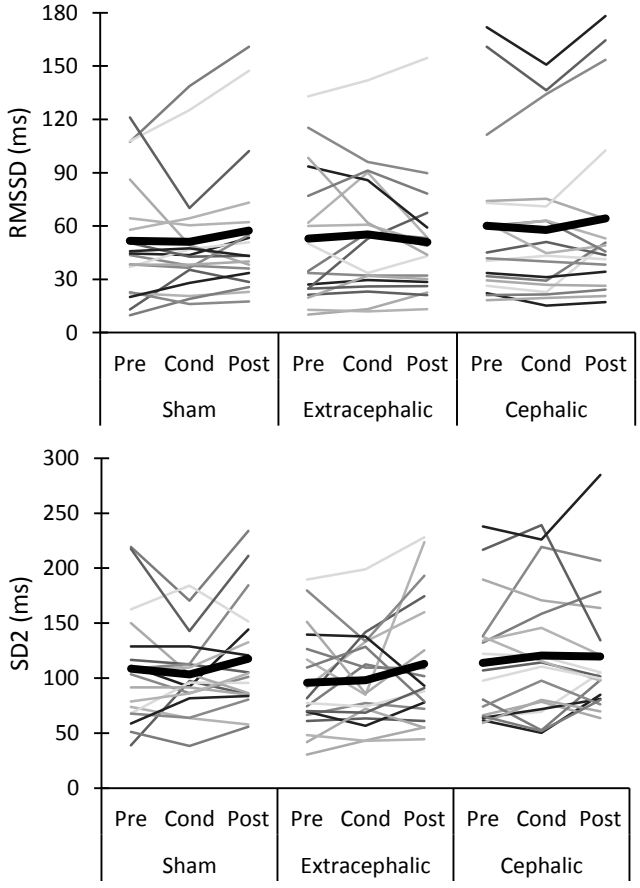
RMSSD (ms)
180
150
120
90
60
30
0
Pre Cond Post
Sham
Pre Cond Post
Extracephalic
Pre Cond Post
Cephalic
SD2 (ms)
300
250
200
150
100
50
0
Pre Cond Post
Sham
Pre Cond Post
Extracephalic
Pre Cond Post
Cephalic

Figure 7.

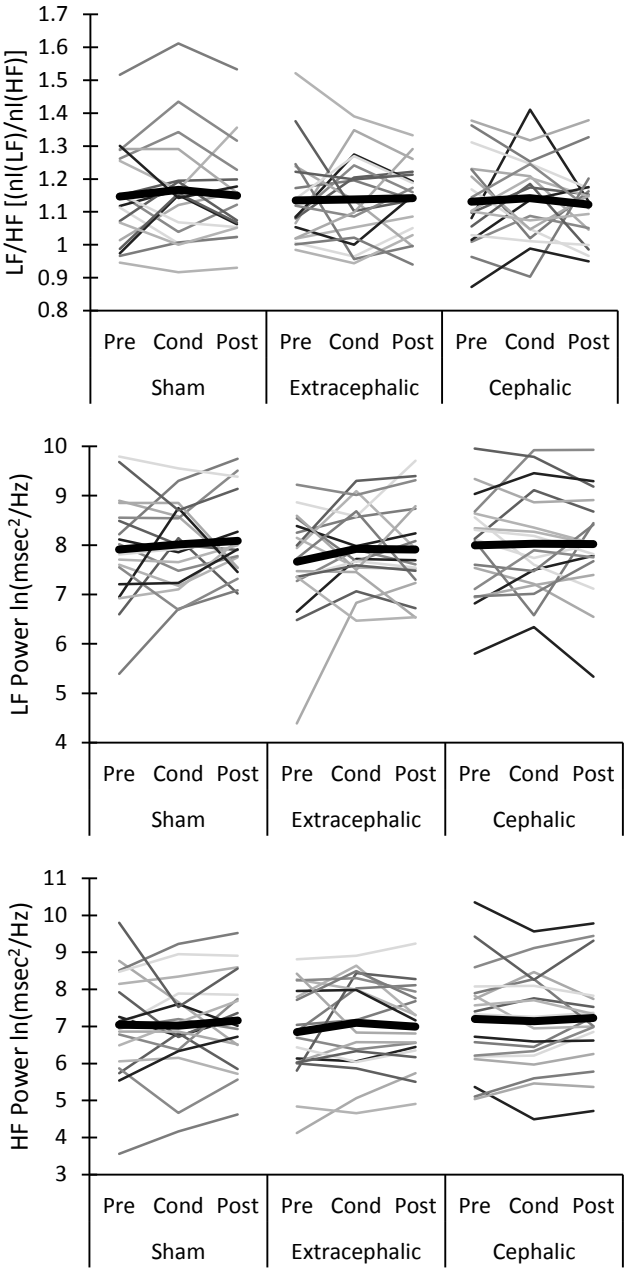
LF/HF [(nl(LF)/nl(HF)]
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
Pre Cond Post
Sham
Pre Cond Post
Extracephalic
Pre Cond Post
Cephalic
LF Power ln(msec²/Hz)
10
9
8
7
6
5
4
Pre Cond Post
Sham
Pre Cond Post
Extracephalic
Pre Cond Post
Cephalic
HF Power ln(msec²/Hz)
11
10
9
8
7
6
5
4
3
Pre Cond Post
Sham
Pre Cond Post
Extracephalic
Pre Cond Post
Cephalic

Table 1. Study 1 heart rate variability (HRV) measures (mean±SD) during 30-minutes of Sham, parasympathetic (Para), and sympathetic (Symp) stimulation conditions. Metrics include the root mean square of successive differences (RMSSD), SD2, the natural log of low frequency power (LF), the natural log of high frequency power (HF), and the ratio of LF to HF (LF/HF). Confidence intervals for each measurement are also included (95% CI). *statistically greater than sham (p=0.021; 95% CI=3.475 to 49.089)

	Condition	HRV	95% CI	p	η_p^2
RMSSD	Sham	59.9±32.2	45.8 to 74.0		
	Para	57.6±31.1	44.0 to 71.3	0.278	0.065
	Symp	49.3±22.8	39.3 to 59.3		
SD2	Sham	99.8±53.1	76.5 to 132.0		
	Para	126.1±58.8*	100.3 to 151.8	0.037	0.159
	Symp	123.2±71.0	92.1 to 154.3		
LF	Sham	8.10±.79	7.7 to 8.5		
	Para	8.09±.81	7.7 to 8.5	0.382	0.049
	Symp	7.92±.67	7.6 to 8.2		
HF	Sham	7.51±1.08	7.0 to 8.0		
	Para	7.36±1.21	6.8 to 7.9	0.442	0.04
	Symp	7.21±.93	6.8 to 7.6		
LF/HF	Sham	1.09±.13	1.0 to 1.1		
	Para	1.12±.16	1.0 to 1.2	0.497	0.032
	Symp	1.11±.13	1.1 to 1.2		

Table 2. Study 1 mean repeated knee extension torque integral (average work) and Fatigue Index (FI; percent change across 50 repetitons) responses following 20-minutes of each of the Sham, parasympathetic (Para), and sympathetic (Symp) stimulation conditions. Data are presented as means±SDs.

Condition	Mean TI (Nm•s)	FI (%Δ)
Sham	177.0±48.6	55.4±8.2
Para	174.8±51.8	57.0±9.1
Symp	177.5±52.4	55.4±8.4

Table 3. Study 2 heart rate variability measures (mean±SD) during the last five minutes of pre-condition rest (Pre), the condition (Online), and post-condition (Post), for each of the three experimental conditions (Sham, Extracephalic, and Cephalic).

	Condition	Pre	Online	Post
RMSSD	Sham	51.7±33.5	51.1±33.0	57.4±40.5
	Extracephalic	52.8±37.0	55.3±34.7	50.9±33.0
	Cephalic	60.1±45.3	57.7±42.2	64.3±50.4
SD2	Sham	108.6±51.8	103.4±36.2	117.7±50.3
	Extracephalic	95.8±46.2	98.1±41.5	112.9±58.4
	Cephalic	113.8±55.3	120.6±60.8	119.5±56.5
LF	Sham	7.91±1.09	8.01±.85	8.08±.83
	Extracephalic	7.67±1.10	7.93±.80	7.91±.96
	Cephalic	8.00±1.02	8.03±1.06	8.02±1.06
HF	Sham	7.05±1.45	7.02±1.28	7.16±1.28
	Extracephalic	6.85±1.30	7.10±1.30	6.99±1.09
	Cephalic	7.20±1.44	7.14±1.36	7.23±1.33
LF/HF	Sham	1.15±.15	1.17±.17	1.15±.14
	Extracephalic	1.14±.14	1.14±.14	1.14±.11
	Cephalic	1.13±.14	1.14±.13	1.12±.11

Effects of Online and Post-online Transspinal Direct Current Stimulation on Perception of Effort and Neuromuscular Behavior

ABSTRACT

Recently, researchers have become interested in the prospect of utilizing direct current stimulation to affect spinal neurons in a similar manner as direct current stimulation has been suggested to affect cerebral neurons. This study investigated the effects of non-invasive thoracic transspinal direct current stimulation (tsDCS) on perceived exertion, maximal strength, and motor unit behavior during isometric knee extensions in humans. During anodal, cathodal, and sham stimulation conditions, participants performed isometric knee extensions at 40% of their maximal strength, maximal effort contractions (MVC), and reported their perception of effort (RPE) during and post stimulation. For MVCs, there was no significant interaction between condition and time ($p=0.90$) or significant main effects for condition ($p=0.12$) or time ($p=0.29$). For RPE, there was no significant interaction between condition and time ($p=0.87$) or main effect of condition ($p=.083$). Motor unit behavior derived from surface electromyographic data decomposition (recruitment threshold, mean firing rate, and action potential size) was also not significantly affected by tsDCS condition (lowest $p=0.15$). Therefore, thoracic spine and lower abdominal montage delivering a current density of 0.071 mA/cm^2 for 20 minutes likely does not affect spinal or supraspinal neuron function during voluntary muscle contractions in humans. Therefore, more research is needed to investigate the efficacy of tsDCS and which stimulation methods may and may not modulate human function.

INTRODUCTION

Muscle torque development depends on the torque-generating properties of the muscle fibers, the excitability and behavior of the spinal neurons that directly or indirectly affect alpha motor neuron function, and magnitude of central drive. Acute transcranial direct current stimulation has been proposed to affect the excitability of cerebral neurons primarily through modulation of resting membrane potentials, thus making them either more likely (increased resting membrane potential) or less likely to fire (decreased resting membrane potential). However, research has not yielded consistent findings supporting this proposition. Recently, researchers have become interested in determining if direct current stimulation can be used to affect spinal neurons in a similar manner as cerebral neurons are proposed to be affected. Specifically, research suggests that transspinal direct current stimulation (tsDCS) may directly alter alpha motor neuron excitability and affect afferent pathways that ascend up spinal neurons to supraspinal brain centers. If spinal neuron excitability is affected by tsDCS, the central drive necessary to evoke a given amount of motor neuron activation may change. Such potential changes may be independent of any effect tsDCS may have on afferent feedback modulation. If tsDCS can indeed affect motor neuron excitability, affect afferent feedback to modulate perception of effort, or both, tsDCS may be of interest for those looking to attenuate hypertonia (spasticity), enhance exercise program adherence, and enhance athletic or military performance.

Research regarding the efficacy of tsDCS to modulate alpha motor neuron function has yielded conflicting results, but numerous potential benefits have been identified. Animal data suggest anodal stimulation decreases the firing rates of smaller motor units (MU) and increases the firing rates of larger motor units; cathodal stimulation increases the firing rates of smaller

motor units and decreases the firing rates of larger motor units (2). Also suggestive of excitability modulation, additional animal data suggest cathodal tsDCS increases twitch torque during stimulation and decreases twitch torque post stimulation and anodal tsDCS achieves the opposite (1). Conversely, human data suggests that alpha motor neuron excitability via H-reflex measurement may (30) or may not (12) be influenced by tsDCS. Importantly, the proposal that small neurons and large neurons diverge in their response to DCS necessitates analysis methods that allow for the consideration of MU recruitment threshold (a function of MU neuron size) to be included in the analysis of MU excitability.

If tsDCS can modulate MU behavior in humans, it is most likely to affect the amount of torque produced by a given level of central drive, the relative reliance on high versus low threshold MUs, or both. For example, if a tsDCS intervention increases the recruitment threshold of low-threshold MUs and decreases the recruitment threshold of high threshold MUs, tsDCS may facilitate the recruitment of typically difficult to recruit MUs and decrease the firing rates of low-threshold MUs. Such alterations in MU behavior would provide strong evidence that tsDCS can have a meaningful effect on peripheral neuromuscular function. However, no study has investigated the effects of tsDCS on MU behavior during voluntary contractions in humans. Therefore, the purpose of this study was to investigate the effects of online and post-online tsDCS on MU behavior during isometric knee extensions in humans.

METHODS

Participants

Eighteen healthy adults (7 male, 11 female; 175.4 ± 11.2 cm; 67.9 ± 12.5 kg; 21.8 ± 2.6 y; mean ± SD) participated in the study. One participant was removed from the study due to a nauseous feeling elicited during their first experimental visit, which happened to be a sham condition. Participant exclusion criteria included: being older than 30 or younger than 18, been diagnosed with cardiovascular disease, being a smoker, having a non-dental metal implant, having a neuromuscular or musculoskeletal condition which impairs their ability to perform knee extensions, being pregnant, and having a history of epilepsy or seizures. Each subject signed an informed consent document before completing a health history questionnaire. The study was reviewed and approved by the University Human Subjects Committee.

Experimental Design

Participants visited the lab on four occasions: one familiarization visit and three single-blinded and counterbalanced experimental visits. Participants were instructed to refrain from lower-body exercise and alcohol in the 48 hours prior to each experimental visit and also asked to refrain from caffeine consumption the day of each experimental visit. All experimental visits occurred at approximately the same time of day (+/- one hour), were separated by at least 47 hours and no more than approximately one week. During the familiarization visit, participants performed three maximal voluntary isometric knee extension contractions (MVCs) and practiced ramped contractions held at 40% of MVC torque. During each of the three experimental visits, participants performed a trapezoidal ramped isometric knee extension that increased and decreased at a rate of 10% of MVC per second and held at 40% MVC for 20 seconds followed

by a MVC before, 10 minutes and 20 minutes into the stimulation condition, and 10 and 20 minutes after the end of the stimulation condition (figure 1). The three stimulation conditions were comprised of sham, anodal, and cathodal montages.

Transspinal Direct Current Stimulation

All stimulation montages consisted of 5 x 7 cm sponge electrodes placed over the 9^{th}-11^{th} thoracic spine vertebrae and the umbilicus. Previous research suggests electrodes placed in the aforementioned regions results in an electrical field concentrated in the mid-low spine (25), where alpha motor neurons controlling the lower-limb musculature are located. Furthermore, the first study to investigate the effects of tsDCS on performance in humans included a similar thoracic spine and low stomach electrode montage (5). Thoracic spine sponge placement was determined by centering the top electrode border (long side of the rectangle electrode) over the spine at the level of the xiphoid process, which was conveniently the level at which the heart rate monitor straps rested. For anodal and sham conditions, the anode electrode was placed over the spine; for cathode conditions, the cathode was placed over the spine. Sponge electrodes were soaked in 0.15 M saline solution before being affixed by Transpore tape (3M, St. Paul, MN). Anodal and cathodal stimulation consisted of a 30-second ramp up to a constant current of 2.5 mA, which was delivered using a neuroConn DC- STIMULATOR PLUS (Ilmenau, Germany) for 20 minutes (figure 1). The current density over the spine electrode for anodal and cathodal conditions was 0.071 mA/cm^2. For sham conditions, the current ramped up to 2.5 mA over 15 seconds and remained on for 15 more seconds. These sham methods were utilized to mimic those used in previous direct current stimulation studies.

Data Collection

During experimental visits, electromyographic (EMG) signals were acquired from the right vastus lateralis (VL) during isometric knee extensions on a Biodex System 3 Pro Isokinetic Dynamometer (Biodex Medical Systems Inc, Shirley, NY) performed with the dynamometer arm perpendicular to the floor. On experimental visits, prior to the completion of the first knee extension, researchers shaved and cleaned the participants' skin over the right VL and left patella using isopropyl alcohol and Transpore tape (3M, St. Paul, MN). Next, Transpore tape was used to fix one EMG sensor over the VL muscle belly 50% of the distance from the anterior superior iliac spine to the lateral patella (14), after which a second EMG sensor was placed approximately 1 cm proximal to the first sensor, along the same iliac spine-patella line. A reference electrode (Red Dot Foam Monitoring Electrodes, 3M, St Paul, MN) was placed over the clean left patella. Each EMG sensor contained four pairs of bipolar electrodes and was specifically built for the purpose of surface EMG signal decomposition to detect individual MU firings. During each knee extension, each of the eight bipolar signals was sampled at 20 kHz.

During each trapezoidal contraction, participants received real-time torque visual feedback overlaid on a target template. Knee extension torque data were sampled at 20 kHz and were synchronized with EMG data collection.

Immediately following each 40% MVC ramped contraction, participants reported their rate of perceived exertion by drawing a mark along a visual analog scale [figure 2; Ueda (29)].

Data Extraction

The highest of the three maximal knee extensions during the familiarization visit was used as the MVC value for which all ramped contractions for all visits referenced. Therefore, all

ramped contractions contained steady forces that were 40% of the highest MVC performed during the familiarization visit. For both familiarization and experimental visits, MVC torque was identified by finding the highest 250 ms mean torque by means of custom LabVIEW software (LabVIEW 2016, National Instruments, Austin, TX, USA). During ramped contractions, the middle 5-second window used to collect MU mean firing rate data was used to collect mean torque over the same window (plateau torque) and was also quantified relative to the highest familiarization MVC. This data was collected to ensure plateau torque values were not systematically different between conditions.

RPEs were quantified by manually measuring the placement of the participant mark on each 20 cm visual analog scale and converting that value to a zero to ten scale, to maintain the qualities of a continuous scale but convey the data in a more familiar number scale. The scale was a flat line with no marks other than dots at the beginning and end of the 20 cm line. Therefore, a mark placed in the middle of the 20 cm line would correspond to an RPE of 5. Since five 40% MVC tracings were completed during each experimental visit, five RPEs were also measured. A new number scale was utilized for each tracing; participants were unable to view their previous RPE ratings.

Surface EMG data collected during ramped contractions were decomposed to detect firings of individual MUs using Delsys EMGworks software (Delsys, Boston, MA). This software is based on previous validation research (11, 23). Firings from MUs with at least 90% accuracy were extracted using a 2-second Hanning window. Using custom LabVIEW software, and each MU was characterized using the following metrics: recruitment threshold (RT), mean firing rate (MFR), and MU action potential size (MUAP). RT was quantified as the mean % MVC over the 0.1 ms epoch following the first detected firing. MFR was the mean pulses per

second during the middle five seconds of the 20-second steady force segment. MUAP size was calculated using the mean peak-to-peak amplitude of the waveforms identified by EMGworks from each of the four bipolar signals.

Data Analysis

It is not possible to detect all active MUs during any contraction, but firing rates of MUs are highly dependent on alpha motor neuron size, which necessitates each MU be analyzed with respect to its RT or MUAP size. Therefore, to account for the unpredictability in which MUs were detected by the EMGworks software, relationships between the three MU variables (RT, MFR, and MUAP) were created to extract slopes and y-intercepts, which generally describe MU behavior but are less susceptible to spurious findings of raw MU analyses (13, 27). For example, there is a linear negative relationship between MFR and RT, but if by chance, only low RT MUs are detected, it would appear as if MFRs are high when MFR may not have been altered. Slopes and y-intercepts of MFR-RT relationship (figure 3) can elucidate if MUs recruited at lower and higher RTs have had their MFRs systematically altered. Slopes and y-intercepts of MUAP-RT relationships indicate if MUs recruited at lower and higher RTs innervate less or more muscle fibers. Finally, slopes and y-intercepts of MFR-MUAP relationships were used to determine if and how the firing rates of smaller and larger MUs changed. As the accuracy of the three aforementioned pairs of y-intercepts and slopes are likely to be influenced by the recruitment range of observed MUs and the quantity of MUs observed, only contractions that yielded at least ten MUs with a recruitment range of at least 10% MVC were statistically analyzed. For similar reasons, the RT of the first (RT_{MIN}) and last recruited MU (RT_{MAX}) was included as a covariate in each analysis.

All statistical analyses were performed using R version 3.2.3 (26). Alpha was set at 0.05. To determine if stimulation condition had an effect on RPE, MVC, plateau torque, or MFR-RT, MUAP-RT, and MFR-MUAP slope or y-intercept values, restricted maximum likelihood linear mixed effects models were fitted using lme4 (4); p-values were obtained using the lmerTest package (16). With lmer, each of the eight variables of interest were separately modeled with participant as a random effect, and experimental condition (sham, anodal, and cathodal) and time (pre, 10 min, 20 min, 30 min, and 40 min) as fixed effects; all models other than the RPE and MVC models also included RT_{MIN} and RT_{MAX} as fixed effects. Experimental condition and time were treated as a categorical variables; all other variables were treated as continuous. Therefore, models were:

lmer(RPE, MVC, or plateau torque ~ 1 + condition*time + (1 | participant ID), REML=TRUE)

lmer(MU variable ~ 1 + RT_{MIN} + RT_{MAX} + condition*time + (1 | participant ID), REML=TRUE)

To improve model fit for the MUAP-RT slope model, slopes were log-transformed. As one participant needed further RPE instruction during their first experimental visit, that RPE data from that visit was not included in the RPE analysis. Due to poor MU yield (less than ten MUs identified) or poor MU recruitment range (less than 10% MVC), only 498 (540 possible) sets of MU data were included in the analyses. Fixed effects were analyzed for significant main effects and interactions (experimental condition X time) via F tests using Satterthwaite's method of estimating denominator degrees of freedom (16).

RESULTS

For MVCs, there was no significant interaction between condition and time ($p=0.90$). Likewise, there were no significant main effects for condition ($p=0.12$) or time ($p=0.29$). Individual responses can be seen in figure 4; means and SDs can be seen in table 1.

For RPE, there was no significant interaction between condition and time ($p=0.87$) or main effect of condition ($p=.083$). However, there was a significant main effect of time ($p<.001$) where RPEs for Min 0 contractions were less than all other contractions (highest $p=0.016$) and Min 10 contractions were less than Min 40 contractions ($p=0.0014$). Individual responses can be seen in figure 5; means and SDs can be seen in table 2.

The 489 signal decompositions yielded 14,144 individual MUs, resulting in a mean of 28.9 ± 8.5 MUs included in each MU relationship. The mean RT_{MIN} and RT_{MAX} were 9.2 ± 6.2 and 30.7 ± 8.7 %MVC, respectively.

For MUAP-RT slopes, there was no significant interaction between condition and time ($p=0.12$). Furthermore, there were no significant main effects for condition ($p=0.14$) or time ($p=0.074$). However, there were significant main effects for RT_{MIN} ($p<0.001$) and RT_{MAX} ($p<0.001$), where larger RT_{MIN} values resulted in more positive slopes and larger RT_{MAX} values resulted in more negative slopes. Mean responses are displayed in table 3.

For MUAP-RT y-intercepts, there was no significant interaction between condition and time ($p=0.1510$). Furthermore, there were no significant main effects for condition ($p=0.22$) or time ($p=0.34$). However, there were significant main effects for RT_{MIN} ($p<0.001$) and RT_{MAX} ($p<0.001$), where larger RT_{MIN} values resulted in smaller intercept values and larger RT_{MAX} values resulted in larger intercept values. Mean responses are displayed in table 3.

For MFR-RT slopes, there was no significant interaction between condition and time ($p=0.99$). Furthermore, there were no significant main effects for condition ($p=0.25$) or time ($p=0.82$). However, there were significant main effects for RT_{MIN} ($p<0.001$) and RT_{MAX} ($p<0.001$), where larger RT_{MIN} values resulted in more negative slopes and larger RT_{MAX} values resulted in more positive slopes. Mean responses are displayed in table 4.

For MFR-RT y-intercepts, there was no significant interaction between condition and time ($p=0.97$). Furthermore, there were no significant main effects for condition ($p=0.20$) or time ($p=0.58$). However, there were significant main effects for RT_{MIN} ($p<0.001$) and RT_{MAX} ($p<0.001$), where larger RT_{MIN} values resulted in larger intercept values and larger RT_{MAX} values resulted in smaller intercept values. Mean responses are displayed in table 4.

For MFR-MUAP slopes, there was no significant interaction between condition and time ($p=0.99$). Furthermore, there were no significant main effects for condition ($p=0.15$) or time ($p=0.99$). Likewise, there were no significant main effects for RT_{MIN} ($p=0.83$) or RT_{MAX} ($p=0.45$). Mean responses are displayed in table 5.

For MFR-MUAP y-intercepts, there was no significant interaction between condition and time ($p=0.85$). Furthermore, there were no significant main effects for condition ($p=0.19$), time ($p=0.79$), or RT_{MIN} ($p=0.090$). However, there was a significant main effect for RT_{MAX} ($p=0.0011$), where larger RT_{MAX} values resulted smaller intercept values. Mean responses are displayed in table 5.

DISCUSSION

The purpose of this study was to investigate the online and post-online effects of tsDCS on MU behavior and perception of effort during isometric knee extensions in humans. Our data suggest the implemented tsDCS methods, a thoracic spine and lower abdominal montage delivering a current density of 0.071 mA/cm^2 for 20 minutes, does not significantly affect maximal knee extension strength or MU behavior both online and up to 20 minutes post stimulation. Also, the similarity between tsDCS conditions in RPE across time suggests the tsDCS methods utilized in this study likely did not significantly modulate the ascending transmission or supraspinal interpretation of afferent feedback related to knee extension intensity. A secondary finding from this study was that RT_{MIN} and RT_{MAX} heavily influence MU behavior variables, which suggests that future MU behavior research should attempt to account for the recruitment range of observed MUs when investigating changes in MU firing rates.

One potential site of tsDCS modulation is at the alpha motor neuron level. Published in 2016, a study by Ahmed et al. presented data that outlined diverging responses between smaller and larger alpha motor neuron cells of mice in response to online anodal and cathodal tsDCS (2). Specifically, this study observed anodal stimulation to increase the firing rates of larger MUs and decrease the firing rates of smaller MUs, whereas cathodal stimulation resulted in the opposite pattern. However, the mechanism(s) responsible for these findings are not known. The tsDCS methods utilized in the current study did not produce any systematic change in MU behavior. Aside from the obvious human versus mouse difference, the current density delivered in the Ahmed et al. study was 3.3 A/m^2, which is over 400 times greater than the current density utilized in the current study and over 80 times greater than the highest reported current density delivered in a human tsDCS study (22). With delivered current densities of 0.391 mA/m^2, the

Murray et al. current densities were approximately 5.5 times larger than the densities delivered in the current study, but Murray et al. observed alpha MU excitability to decrease after cathodal stimulation and not change after anodal stimulation (22). As Murray et al. also utilized a different montage (thoracic spine and thigh instead of thoracic spine and low abdomen) and quantified MU modulation using involuntary passive methods (H-reflex), it is reasonable to hypothesize that the difference in cathodal tsDCS MU modulation efficacy between the current study and the Murray et al. study can be attributed to differences in stimulation methods. Another possible explanation for the disparate results between studies is that tsDCS MU modulatory effects may be irrelevant during voluntary contractions due to a potentially overwhelming amount of excitatory stimuli from central drive. Further, the findings of Murray et al. are in not in line with the mice data as the mice data suggests cathodal tsDCS has an overall inhibitory effect on alpha MU excitability (2).

Inconsistent findings are commonplace in the existing body of research regarding the effects of tsDCS on MU excitability in humans. Two studies have observed anodal tsDCS to reduce MU excitability (3, 10), three studies have reported anodal tsDCS to increase MU excitability (17, 18, 30), and four studies have suggested anodal tsDCS does not affect MU excitability (8, 15, 19, 22). Interestingly, studies utilizing nearly identical stimulation methods (the greatest difference being 15 versus 20 minutes of stimulation) yielded three different anodal tsDCS conclusions (3, 8, 17, 18). Findings regarding the efficacy of cathodal tsDCS are comparably inconclusive. Three studies have observed cathodal tsDCS to reduce MU excitability (15, 22, 30), one study has reported cathodal tsDCS to increase MU excitability (8), and four studies have suggested cathodal tsDCS does not affect MU excitability (12, 17-19). It is difficult to reconcile the vast quantity of inconsistent findings between studies as most studies differ in

one or more of the following: current density, stimulation duration, electrode montage sites, and dependent variable. However, as previously mentioned, even studies utilizing nearly identical methods have yielded opposing results (3, 8, 17, 18). Thus, the DCS community may benefit from studies which attempt to reconcile the inconsistent findings in the literature.

Another reported mechanism of tsDCS-induced performance modulation is by mitigating central fatigue in situations where central drive is inadequate (5). This may be possible through modulation of afferent feedback gain or by modulation of motor cortex excitability. Although the current study did not find any effect of tsDCS on MU behavior, RPE, or MVC, RPE and MVC could have been independently affected by ascending or supraspinal neuron modulation. It is possible that tsDCS could have affected MVC or RPE through changes in motor cortex excitability, making the production of a given quantity of central drive feel easier. However, the body of research addressing the potential for tsDCS to induce supraspinal changes in motor cortex excitability is conflicting. The only study to report changes in muscle responses evoked through cortical stimulation (increased after both anodal and cathodal stimulation) that could not be explained by changes in MU excitability was Lim and colleagues (19). However, Donges et al. (2017) utilized nearly identical methods, aside from utilizing a 25% higher current density, did not include an anodal condition, and found no effect of cathodal stimulation on any portion of the corticospinal tract (12). Furthermore, Lim et al. identify the lack of known physiologic mechanisms to explain their findings (19). Despite these discrepancies, modeling data suggests that thoracic-shoulder montages are most likely to create electrical fields that affect the spine superior the spinal electrode (25). Although the physiologic mechanism(s) behind this proposal are speculative, such montages may be most likely to affect afferent feedback gain through affecting ascending interneuron neurotransmitter release or axonal conductance (28).

Interestingly, all of the tsDCS studies that have addressed pain modulation have utilized thoracic-shoulder montages and suggest anodal thoracic spine stimulation attenuates perception of pain via changes in ascending spinal neuron function, not supraspinal function. When pain afferent neurons are activated they have been shown to increase MU excitability but simultaneously decrease motor cortex excitability (20), which means our null RPE finding may have been due to competing spinal and supraspinal changes that negated each other. However, this is unlikely as we did not observe any alteration in MU behavior.

A finding from this study that should be considered by future researchers who analyze individual MU data is that all slopes except for MFR-MUAP slopes were significantly affected by RT_{MIN} and RT_{MAX}. For example, we observed that when very few low-threshold MUs (high RT_{MIN}) are recorded, contractions resulted in biased data suggesting that higher threshold MUs were not all that much larger than lower threshold MUs. This means that if two contractions happen to yield MUs that vastly differ in RT of the earliest or latest recruited MUs, the MU firing properties may seem to differ, but it would be difficult to be confident in this potential difference. The necessity to analyze MU data in a manner similar to the methods implemented in the current study are due to: 1) the potential for non-voluntary passive MU function to not translate to alterations during active voluntary contractions, 2) the fact that, generally, more difficult to recruit large MUs fire slower than the easier to recruit small MUs, and 3) the inability to detect all active MUs in any contraction, which means a contraction may, by chance, yield a disproportionate amount of high or low threshold MUs. Therefore, the quantification of MUAP-RT, MFR-RT, and MFR-MUAP slopes and intercepts allow researchers to determine if MUs of a particular size or RT have had their firing rates altered. The inclusion of RT_{MIN} and RT_{MAX} in statistical models allows researchers to decrease the noise that inherently occurs due to MU

observation being largely influenced by pure chance, as the ideal scenario of MU detection across the entire range of RTs often does not occur. The utilization of these methods were particularly necessary in the current investigation, as animal data had yielded diverging responses in small and large MUs, which may mask the effects in studies that do not differentiate between changes in small and large MUs in some capacity. Therefore, if possible, future researchers investigating the effect of tsDCS on neuromuscular function should include individual MU data analyzed similarly to the methods utilized in this study.

The limitation of this study was the utilization of thoracic spine and lower abdomen montage. It is plausible that other montages may have resulted in different findings and other montages, particularly the thoracic spine and shoulder montage, should be investigated by future studies. The abdomen site was used in the current study because the first study to document enhanced performance due to tsDCS utilized a similar thoracic-abdomen montage (5). Also, it is clear that animal studies are delivering utilizing much higher current densities, which may account for the numerous effects reported in mice studies. Researchers have safely delivered current densities as high as 0.391 mA/m^2 in humans (22), and although these magnitudes are lower than densities used in mice studies, future researchers may consider utilizing much higher densities than the most commonly used 0.071 mA/m^2 density (3, 6-9, 17, 18, 21, 24, 28), which was chosen to be used in the current study due to its proven lack of adverse events.

CONCLUSIONS

In conclusion, our data suggests that a thoracic spine and lower abdominal montage delivering a current density of 0.071 mA/cm^2 for 20 minutes likely does not affect spinal or supraspinal neuron function. Therefore, these stimulation methods should not be used if acute performance enhancement is desired. Although, recent tsDCS data suggests a thoracic spine and shoulder montage and slightly higher current density may be more likely to acutely enhance performance, the tsDCS literature body as a whole is inconsistent. As the body of research regarding the efficacy of tsDCS is still very young, more research is necessary to determine which tsDCS methods may consistently affect neuromuscular function and performance.

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Figure 1. Timing of measurements during anodal, cathodal, and sham transspinal direct current stimulation conditions. Sets of measurements were taken once prior to initiation of the stimulation condition, as indicated by the shaded region. Maximal effort isometric contractions are represented by thick solid lines, 40% isometric trapezoidal tracings are represented by thin solid lines, and perceived exertion measurements are represented by thin dotted lines.

Figure 2. Visual analog rating of perceived exertion scale

Figure 3. Example of data yielding slope (-0.3951) and y-intercept (21.835) values from motor unit recruitment threshold s(torque at which the motor unit was recruited) and MFRs (mean firing rate during the middle of the 40%MVC plateau) data collected during a trapezoidal contraction. Each point represents an individual motor unit.

Figure 4. Individual participant maximal voluntary isometric knee extension torque (MVC) values across all time points during anodal (blue), cathodal (pink), and sham (green) visits. Time point 0 represents the pre-stimulation measurement, time points 10 and 20 were taken 10 and 20 minutes into the stimulation condition, and time points 30 and 40 were taken 10 and 20 minutes post stimulation.

Figure 5. Individual participant rating of perceived exertion (RPE) values across all time points during anodal (blue), cathodal (pink), and sham (green) visits. Time point 0 represents the pre-stimulation measurement, time points 10 and 20 were taken 10 and 20 minutes into the stimulation condition, and time points 30 and 40 were taken 10 and 20 minutes post stimulation.

Figure 1.

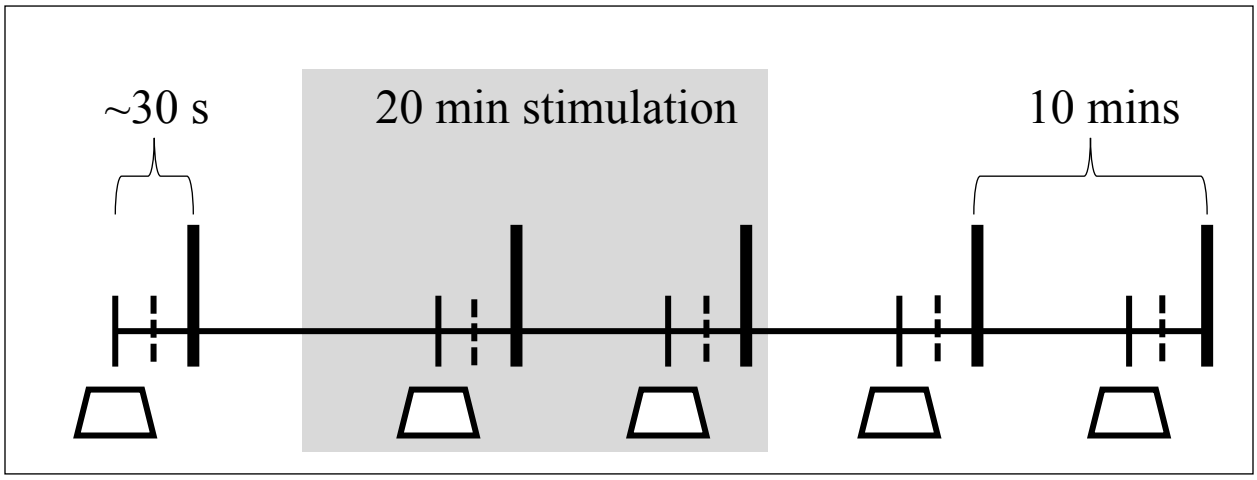


Figure 2,

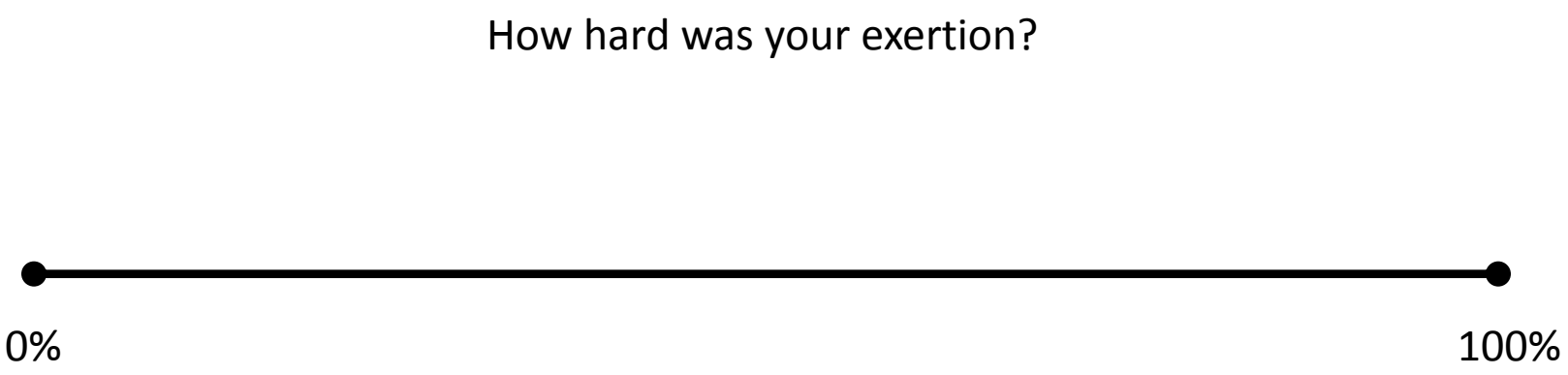
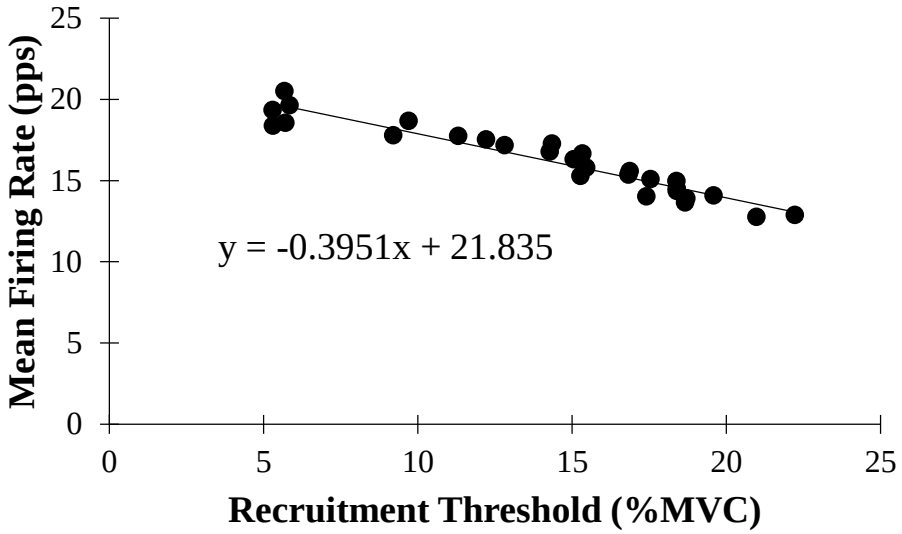


Figure 3.


Mean Firing Rate (pps)
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y = -0.3951x + 21.835
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5
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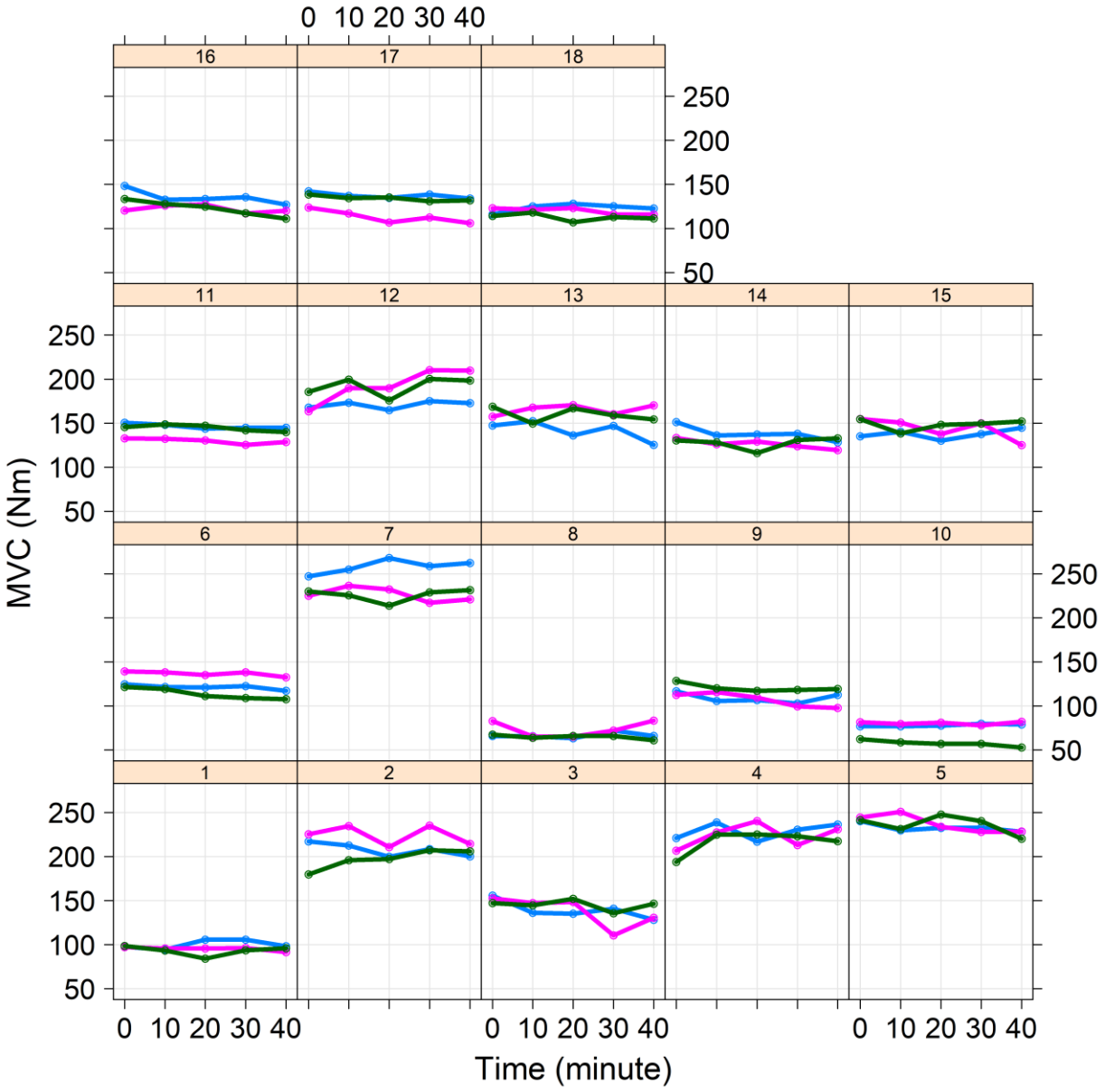
25
Recruitment Threshold (%MVC)

Figure 4.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MVC (Nm)
Time (minute)
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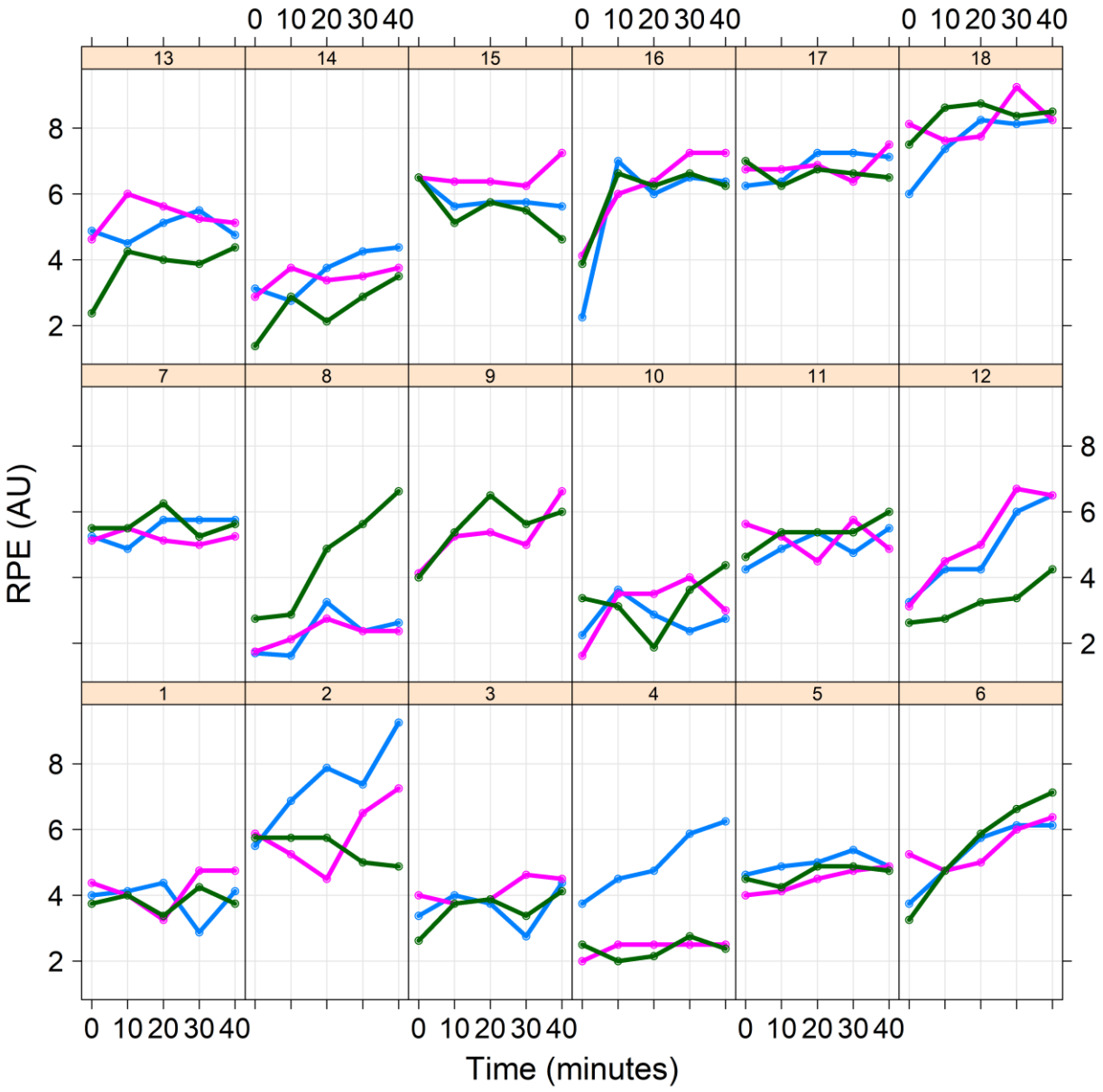
Figure 5.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RPE (AU)
Time (minutes)

Table 1. Mean±SD maximum voluntary isometric knee extension torque (MVC) values during anodal, cathodal, and sham conditions. Time point 0 represents the pre-stimulation measurement, time points 10 and 20 were taken 10 and 20 minutes into the stimulation condition, and time points 30 and 40 were taken 10 and 20 minutes post stimulation.

	MVC (Nm)				
Condition	Min 0	Min 10	Min 20	Min 30	Min 40
Sham	146.72±48.17	145.68±51.63	144.03±53.29	145.60±54.38	143.87±52.96
Anodal	151.17±51.92	148.90±53.97	146.38±52.62	149.76±52.31	146.00±53.87
Cathodal	148.64±48.49	151.20±55.52	148.13±53.36	144.61±53.33	144.87±52.73

Table 2. Mean±SD rate of perceived exertion (RPE) values following 40% isometric trapezoidal knee extensions during anodal, cathodal, and sham conditions. Time point 0 represents the pre-stimulation measurement, time points 10 and 20 were taken 10 and 20 minutes into the stimulation condition, and time points 30 and 40 were taken 10 and 20 minutes post stimulation.

	RPE (AU)				
Condition	Min 0	Min 10	Min 20	Min 30	Min 40
Sham	4.10±1.74	4.63±1.65	4.87±1.85	4.98±1.52	5.20±1.49
Anodal	4.16±1.44	4.82±1.50	5.24±1.53	5.24±1.77	5.57±1.74
Cathodal	4.44±1.78	4.83±1.45	4.79±1.45	5.32±1.68	5.44±1.79

Table 3. Mean±SD slopes and y-intercepts from motor unit action potential (MUAP) size versus recruitment threshold relationships during anodal, cathodal, and sham conditions. Time point 0 represents the pre-stimulation measurement, time points 10 and 20 were taken 10 and 20 minutes into the stimulation condition, and time points 30 and 40 were taken 10 and 20 minutes post stimulation.

MUAP-RT slope (mV/%MVC)						
Condition	Min 0	Min 10	Min 20	Min 30	Min 40	r (median)
Sham	0.004545±0.003846	0.004730±0.004612	0.004382±0.003452	0.004937±0.005149	0.004452±0.003664	0.800541
Anodal	0.004422±0.004112	0.004182±0.003268	0.004303±0.003743	0.004057±0.002690	0.003694±0.003651	0.8021915
Cathodal	0.004383±0.003052	0.004613±0.003383	0.004751±0.003719	0.004359±0.004147	0.004536±0.003911	0.7983335

MUAP-RT y-intercept (mV)						
Condition	Min 0	Min 10	Min 20	Min 30	Min 40	r (median)
Sham	-0.01049±0.06443	-0.01825±0.09198	-0.007047±0.05677	-0.02331±0.04945	-0.01497±0.06994	0.800541
Anodal	-0.01986±0.08544	-0.01313±0.06662	-0.01717±0.07406	-0.006168±0.09102	-0.005588±0.06019	0.8021915
Cathodal	-0.01148±0.05816	-0.01103±0.05709	-0.01213±0.06371	-0.01189±0.1040	-0.01436±0.07111	0.7983335

Table 4. Mean±SD slopes and y-intercepts from MFR (mean firing rate during the middle of the 40%MVC plateau) versus motor unit recruitment threshold (torque at which the motor unit was recruited) relationships during anodal, cathodal, and sham conditions. Time point 0 represents the pre-stimulation measurement, time points 10 and 20 were taken 10 and 20 minutes into the stimulation condition, and time points 30 and 40 were taken 10 and 20 minutes post stimulation.

MFR-RT slope (pps/%MVC)						
Condition	Min 0	Min 10	Min 20	Min 30	Min 40	r (median)
Sham	-0.4022±0.1227	-0.3901±0.1349	-0.4210±0.1322	-0.4055±0.1079	-0.4246±0.1659	-0.9352625
Anodal	-0.3993±0.1330	-0.3936±0.1225	-0.3906±0.1091	-0.4154±0.1500	-0.3928±0.1330	-0.942468
Cathodal	-0.4177±0.1567	-0.3924±0.1030	-0.4297±0.1648	-0.3916±0.1314	-0.4078±0.1567	-0.937693

MFR-RT y-intercept (pps)						
Condition	Min 0	Min 10	Min 20	Min 30	Min 40	r (median)
Sham	24.27±4.20	24.48±4.06	25.11±4.09	24.93±3.97	25.68±5.50	-0.9352625
Anodal	24.68±4.29	24.63±4.33	24.59±3.75	24.91±5.56	25.14±5.28	-0.942468
Cathodal	24.78±5.27	23.89±3.54	24.75±5.22	24.70±4.79	25.37±5.53	-0.937693

Table 5. Mean±SD slopes and y-intercepts from MFR (mean firing rate during the middle of the 40%MVC plateau) versus motor unit action potential size (MUAP) relationships during anodal, cathodal, and sham conditions. Time point 0 represents the pre-stimulation measurement, time points 10 and 20 were taken 10 and 20 minutes into the stimulation condition, and time points 30 and 40 were taken 10 and 20 minutes post stimulation.

MFR-MUAP slope (pps/mV)						
Condition	Min 0	Min 10	Min 20	Min 30	Min 40	r (median)
Sham	-77.80±24.04	-72.66±25.64	-76.05±19.74	-73.50±24.20	-74.62±24.84	-0.805178
Anodal	-77.76±27.78	-76.41±26.52	-78.06±25.92	-79.57±27.26	-77.37±22.79	-0.807972
Cathodal	-72.77±24.80	-73.97±26.21	-73.71±25.04	-73.04±28.33	-73.52±22.02	-0.803879

MFR-MUAP y-intercept (pps)						
Condition	Min 0	Min 10	Min 20	Min 30	Min 40	r (median)
Sham	22.21±2.04	21.89±2.02	22.72±2.02	22.05±2.48	22.20±2.19	-0.805178
Anodal	21.67±1.88	21.75±1.92	21.88±1.88	21.94±2.64	21.83±2.45	-0.807972
Cathodal	21.65±2.38	21.69±1.77	21.66±2.07	22.15±3.31	22.08±2.28	-0.803879

Effects of Transspinal Direct Current Stimulation on Cycling Perception of Effort and Time to Exhaustion

ABSTRACT

In the past decade, researchers have investigated the efficacy of transspinal direct current stimulation (tsDCS) on central nervous system and afferent neuron function in humans. In the past year, data has suggested it may be possible for such tsDCS-induced changes in neuromuscular function to enhance performance. This study utilized non-invasive thoracic spine tsDCS to determine if cycling performance and perception of effort (RPE) could be modulated by tsDCS. In three different stimulation conditions, anodal, cathodal, and sham, participants cycled at 80% of their maximal aerobic capacity until exhaustion and reported their RPE every minute. From this period, researchers compared the RPE responses over the first three minutes and time to exhaustion. There was no significant difference in time to exhaustion between anodal (408 ± 121 s), cathodal (413 ± 168 s), and sham (440 ± 189 s) conditions ($p=0.58$). There was no significant difference in RPE from minutes 1-3 (collapsed across time) between anodal (12.9 ± 2.4 AUs), cathodal (13.3 ± 2.2 AUs), and sham (12.9 ± 2.1 AUs) conditions ($p=0.51$). These data suggest tsDCS condition did not influence cycling performance or perception of effort during high-intensity cycling. Therefore, thoracic spine and lower abdominal montage delivering a current density of 0.071 mA/cm^2 for 20 minutes likely does not affect high-intensity cycling work capacity. Therefore, more research is needed to investigate the efficacy of tsDCS and which stimulation methods may and may not enhance human performance.

INTRODUCTION

The ability to sustain a given work rate depends on the properties of muscle fibers, the behavior of the spinal neurons that directly or indirectly affect alpha motor neurons, and central drive. Previous studies have used transcranial direct current stimulation to in an attempt to alter performance by targeting the insular or motor cortex, as the insular cortex likely plays an important role in effort perception and the motor cortex generates the excitatory stimuli that ultimately results in the recruitment of alpha motor neurons. However, efficacy discrepancies exist, particularly with respect to the ability of direct current stimulation to affect the insular cortex (3, 10, 24). Mechanisms that support the use of transcranial direct current stimulation to enhance performance include altered neuronal excitability and altered spontaneous neuronal activity, resulting in increased motor cortex excitability and altered perception of effort. Researchers have begun to utilize similar direct current stimulation techniques at the spinal level in an attempt to alter acute neuromuscular function. Although the efficacy of transspinal direct current stimulation (tsDCS) has yielded similarly inconsistent findings related to neuromuscular variables, much fewer studies have investigated the effect of tsDCS on human performance.

In 2017, the first study to investigate the effect of tsDCS on performance in humans found anodal stimulation at the thoracic spine to attenuate the decrement in countermovement vertical jump performance over the course of hours (5). The researchers suggested anodal tsDCS affected performance by attenuating central fatigue as sets of jump efforts were spaced out over three hours and within-set jump performance did not decrease. Interestingly, the only other performance study found that cathodal stimulation enhanced the amount of work completed in a 30-second Wingate test (29). Although only two studies exist, the inconsistency of anodal versus cathodal stimulation in regards to performance may be due to the potential of tsDCS to act at

numerous neuromuscular sites and the difference in the physiological demands of jumping and Wingate tests.

Data from human subjects suggest that tsDCS may affect human function through modulation of spinal (6, 14, 17, 18, 34) and supraspinal mechanisms (6, 23). Spinal mechanisms include modulation of motor unit (MU) excitability (2, 11, 15, 17, 18, 22, 34), afferent neuronal activity (32), and spinal motor programs [mice data; (1)]. Supraspinal mechanisms include alterations in motor cortex excitability and acute altered regional connectivity (30). In support of afferent feedback modulation, Perrotta and colleagues (27) as well as Meyer-Friessem (20) observed decreased perceived pain (sural nerve stimulation-evoked in Perrotta et al. and pinprick-evoked in Meyer-Friessem et al.), following anodal tsDCS. Importantly, the types of neurons largely responsible for pain feedback (group III and IV neurons) are also responsible for peripheral fatigue feedback (33). However, the finding that cathodal, not anodal, stimulation enhanced Wingate work makes it difficult to confidently attribute enhanced Wingate work to altered group III/IV afferent activity and may suggest performance changes were more likely explained by motor unit or cortical modulation.

Although not all studies have found tsDCS to affect human function (12), the few and inconsistent findings clearly identify a need for tsDCS research in humans. As tsDCS may affect both spinal and supraspinal function, performance changes may be detected through alterations in work capacity, perception of effort, or both. As previous research suggests cycling at 80% of aerobic power induces central fatigue (25), this task may allow researchers to determine if tsDCS affects performance and if any change in performance may be explained by spinal or supraspinal mechanisms through the quantification of perceived exertion, cardiovascular stress, and time to exhaustion. This task will, for the first time, determine if tsDCS performance changes may be at

least partially explained by changes in perceived exertion or exercise economy. Therefore, purpose of this study was to investigate the effects of transspinal direct current stimulation on cycling time to exhaustion, perception of effort, cardiovascular variables.

METHODS

Participants

Eighteen healthy adults (9 male, 9 female; 171.1 ± 9.0 cm; 68.0 ± 11.8 kg; 22.0 ± 2.5 y; 42.8 ± 11.0 ml O_2/kg/min; 230.6 ± 64.8 W maximum aerobic power; mean ± SD) participated in the study. Participant exclusion criteria included: being older than 30 or younger than 18, having been diagnosed with cardiovascular disease, being a smoker, having a non-dental metal implant, having a neuromuscular or musculoskeletal condition which impairs their ability to pedal a cycle ergometer at maximal effort, being pregnant, and having a history of epilepsy or seizures. Each subject signed an informed consent document before completing a health history questionnaire. The study was reviewed and approved by the University Human Subjects Committee.

Experimental Design

Subjects visited the lab on four occasions: one aerobic capacity visit and three single-blinded and counterbalanced experimental visits. Participants were instructed to refrain from lower-body exercise and alcohol in the 48 hours prior to each experimental visit and also asked to refrain from caffeine consumption the day of each experimental visit. All experimental visits occurred at approximately the same time of day (+/- one hour), were separated by at least 47 hours and no more than approximately one week. During the aerobic capacity visit, participants performed a graded cycle ergometer test to determine maximal aerobic work capacity. During each of the three experimental visits, participants were exposed to a tsDCS condition for 5 minutes prior to and throughout the duration of their cycling bout. The cycling bout included a 3-minute 50 W warm-up followed by 80% of maximum cycle aerobic power until exhaustion. The three tsDCS conditions were comprised of sham, anodal, and cathodal montages (figure 1).

Maximal Aerobic Capacity Testing

At least approximately 48 hours prior to the first experimental visit, participants had their height and weight measured before being fitted to an electronically-braked cycle ergometer (Lode, Groningen, Netherlands). After the participant was fitted, seat height, handlebar height, and handlebar length were recorded for use during experimental visits. For all visits, participants wore athletic shoes which were secured to pedals with toe-clip straps. Before the graded exercise test began, a researcher showed the participant how to report rating of perceived exertion (RPE) through the use of a large visual version of Borg's 6-20 scale (9), which allowed participants to point at their RPE values during their cycling bouts. Graded exercise tests began with a three-minute warm-up at 50 W, after which the work rate increased by 25 W every minute. Forty-five seconds into each minute stage, participants reported their RPE. This not only aided in their familiarization to the RPE scale, but assisted in determining if the aerobic capacity test was valid. The graded exercise test concluded when participants could no longer pedal. The work rate of the last completed one-minute stage was determined to be the maximal aerobic work rate. For the duration of the aerobic capacity test and all experimental visits, a Polar FT7 Heart Rate Monitor (Polar Electro, INC, Lake Success, NY, USA) was used to measure heart rate. Additionally, participants wore a nose clip and breathed through a mouthpiece and one-way breathing valve (Hans Rudolph Inc., Shawnee, KS) to enable the measurement of metabolic gasses and subsequent calculation of oxygen consumption variables through the use of a metabolic cart and software (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Sandy, UT). An aerobic capacity test was determined to be valid if it yielded data that met two of the four validity indicators (within 10 beats per minute of age-predicted max HR, RPE of at least 17, respiratory equivalent ration of at least 1.1, and a change of less than 150 mL O_2 in a stage)

identified by the American College of Sports Medicine Guidelines (19). All participants met at least two of these criteria.

Experimental Visits

At the start of each visit, body mass, room temperature and humidity were recorded for purposes of determining if body mass, temperature, or humidity were systematically different in any condition. Five minutes after the beginning of each stimulation condition, participants transitioned into the 3-minute warm-up and then into 80% of maximal aerobic power. The trial ended when the participant's cadence dropped below 60 revolutions per minute for five consecutive seconds (25). Forty-five seconds into each minute stage, participants reported their RPE. In addition to RPE, researchers documented time to exhaustion (TTE) and heart rate at exhaustion. Heart rate at exhaustion (HR_{END}) was calculated as the highest 15-second-averaged heart rate over the last minute of the trial. This heart rate measure was used as an indicator of the magnitude of aerobic stress the participant was willing to attain.

Transspinal Direct Current Stimulation

All stimulation montages consisted of 5 x 7 cm sponge electrodes placed over the 9^{th}-11^{th} thoracic spine vertebrae and the umbilicus. Previous research suggests electrodes placed in the aforementioned regions results in an electrical field concentrated in the mid-low spine (26), where neurons controlling the lower-limb musculature are located. Furthermore, the first study to investigate the effects of tsDCS on performance included a similar thoracic spine and abdominal electrode montage (5). Thoracic spine sponge placement was determined by centering the top electrode border (long side of the rectangle electrode) over the spine at the level of the xiphoid

process, which is where the heart rate monitor was centered. For anodal and sham conditions, the anode electrode was placed over the spine; for cathode conditions, the cathode was placed over the spine. Sponge electrodes were soaked in 0.15 M saline solution before being affixed by Transpore tape (3M, St. Paul, MN). Anodal and cathodal stimulation consisted of a 30-second ramp up to a constant current of 2.5 mA, which was delivered using a neuroConn DC-STIMULATOR PLUS (Ilmenau, Germany) until participants reached exhaustion. Thus, the current density over the spine electrode for anodal and cathodal conditions was 0.071 mA/cm^2. For sham conditions, the current ramped up to 2.5 mA over 15 seconds and remained on for 15 more seconds. These sham methods were utilized to mimic those used in previous insular cortex stimulation studies.

Data Analysis

All statistical analyses were performed using R version 3.2.3 (28). Alpha was set at 0.05. Due to the confounding effect of different exercise durations, RPE was analyzed from the first three minutes of each TTE bout, as all TTE bouts lasted longer than three minutes. To determine if stimulation condition had an effect on RPE, HR_{END}, or TTE, or if body mass, room temperature or room humidity varied between conditions, restricted maximum likelihood linear mixed effects models were fitted using the lme4 package (4); p-values were obtained using the lmerTest package (16). With lmer, each of the three variables of interest were separately modeled using participant as a random effect, and experimental condition (sham, anodal, and cathodal), as a fixed effect. Therefore, models were:

lmer(variable ~ 1 + condition + (1 | participant ID), REML=TRUE)

Fixed effects were analyzed for significant main effects via F tests using Satterthwaite's method of estimating denominator degrees of freedom (16).

RESULTS

There was no significant difference in body mass between anodal (67.91 ± 11.99 kg), cathodal (67.76 ± 11.89 kg), and sham (67.89 ± 12.04 kg) conditions (p=0.73). There was no significant difference in room temperature between anodal (22.1 ± 1.4°C), cathodal (23.0 ± 2.0°C), and sham (22.3 ± 1.8°C) conditions (p=0.30). There was no significant difference in room humidity percentage between anodal (32.7 ± 7.4), cathodal (36.1 ± 8.3), and sham (35.7 ± 8.7) conditions (p=0.38).

Individual TTE responses are displayed in figure 2. There was no significant difference in TTE between anodal (408 ± 121 s), cathodal (413 ± 168 s), and sham (440 ± 189 s) conditions (p=0.58). Individual HR_{END} responses are displayed in figure 3. As with TTE, there was no significant difference in HR_{END} between anodal (183.2 ± 10.7 bpm), cathodal (184.1 ± 10.6 bpm), and sham (183.7 ± 10.5 bpm) conditions (p=0.90). Individual RPE responses are displayed in figure 4. There was no significant difference in RPE from minutes 1-3 (collapsed across time) between anodal (12.9 ± 2.4 AUs), cathodal (13.3 ± 2.2 AUs), and sham (12.9 ± 2.1 AUs) conditions (p=0.51).

DISCUSSION

The purpose of this study was to investigate the effects of tsDCS on cycling time to exhaustion and determine if changes in perception of effort or cardiovascular variables may explain tsDCS efficacy. Our data suggest the implemented tsDCS methods, thoracic spine and abdominal stimulation at a current density of 0.071 mA/cm^2 does not significantly affect cycling TTE at 80% of maximal aerobic power. These data combined with the lack of a difference in HR_{END} between conditions suggests participants pushed themselves to a similar level of aerobic stress across experimental visits and cycling economy was not systematically affected by the tsDCS methods utilized in this study. This finding, combined with the consistency between tsDCS conditions in RPE throughout the first three minutes of the TTE, trials suggests the tsDCS methods utilized in this study likely did not modulate motor unit behavior or cortical function in a manner that systematically affected performance.

Unlike the current study, the two previous studies that investigated the effects of tsDCS on performance in humans found some effect of stimulation condition (5, 29). The first published study (5) showed an anodal thoracic spine and abdominal montage, similar to the montage utilized in the current study, to mitigate the decline in countermovement vertical jump power over the course of three hours. The authors suggested this effect was due to the mitigation of central fatigue, which must have been responsible for the decrement in performance as jump power decreased across the four clusters of jumps performed over three hours post stimulation condition. However jump power did not decline within each cluster of five jumps performed with only 20 seconds of rest between jumps, where peripheral fatigue would have manifested, had it influenced jump performance at all. Unfortunately, Berry et al. did not include a cathodal tsDCS condition in their countermovement jump study. The second study (Sasada et al.), did

include both cathodal and anodal tsDCS conditions using a thoracic-shoulder montage. However, these results suggested the cathodal condition enhanced performance as indicated by the greater quantity of work during a 30-second Wingate performed three minutes following cathodal stimulation when compared to both anodal and sham conditions. As Berry et al. did not include a cathodal condition in their countermovement jump study, it is not known if cathodal tsDCS would have also positively affected countermovement jump performance, although the current study utilized a similar montage as the Berry et al. study and did not find any effect of cathodal tsDCS on cycling TTE. Although highly speculative, the approximately 3% difference in current density, abdominal versus shoulder "reference" electrode placement difference, and the exercise test differences may account for the inconsistent performance change findings between the current study and those of Berry et al., and Sasada et al.

There are two general physiologic sites where modulation from tsDCS may result in enhanced performance. One is at the spinal level through either alpha motor neuron excitability changes or through afferent feedback modulation. Perhaps most obviously, tsDCS may affect performance through altering motor neuron thresholds, making it easier or more difficult to achieve maximal muscle activation. However, if this change did occur, it would plausibly also affect RPE through alteration in central drive requirements necessary to activate motor units. Another physiologic site that may be modulated by tsDCS is the motor cortex. Data suggests that tsDCS may, through unclear mechanisms, affect motor cortex excitability (22). Therefore, if an exercise task is limited by the inability to produce a quantity of central drive, tsDCS may be able to affect performance by making it more difficult or easier to maximize central drive.

To speculate on the efficacy of tsDCS in regards to the findings of the current study and the two other tsDCS studies that have investigated human performance modulation, it may be

useful to determine if the performance task from each study is likely limited by central fatigue, peripheral fatigue, or both. In the current study, our exercise task may have been limited by both central and peripheral fatigue (25, 31), which differs from the Berry et al. study that clearly did not result in any detrimental amount of peripheral fatigue. However, Sasada et al. utilized an exercise task that likely was limited by both central and peripheral fatigue (13), which is similar to the limitations of the exercise task used in the current study. Therefore, without mechanism(s) speculation, it is possible that the thoracic-shoulder montages are more efficacious in affecting exercise tasks that are limited by both central and peripheral fatigue (assuming the 3% difference in current density is negligible).

One concern that has been raised in the general neuroscience field is the inability to reproduce the findings of neuroscience studies (21). Since only two tsDCS investigations into human performance modulation have been published prior to production of the current manuscript, it is difficult to determine how replicable the performance outcomes will be. The relatively new area of the efficacy of tsDCS on motor unit excitability and motor cortex excitability has not yielded consistent findings. For example, studies utilizing nearly identical anodal tsDCS stimulation methods to modulate motor unit excitability (the greatest difference was a 5-minute difference in stimulation duration) yielded all three possible conclusions: 1) no effect (7), 2) increased excitability (17, 18), and 3) decreased excitability (2). The prospect of non-invasive stimulation being a viable option to acutely alter neuromuscular function is very exciting, but the inconsistent findings in the young body of literature warrants skepticism and more research to elucidate the inconsistent findings in the literature.

One of the limitations of this study was that cycling was performed under online tsDCS conditions; most studies stimulate before measuring post-online effects. Although online tsDCS

methods have previously affected pain tolerance (32), and animal data (8) suggests tsDCS may only be efficacious online, online and post-online tsDCS may elicit different physiologic responses. However, this is the first study to investigate the effects of online tsDCS on performance; unpublished data from our lab suggest post-online stimulation utilizing the same stimulation methods does not affect RPE or motor unit function of the vastus lateralis during isometric knee extensions at 40% of maximal torque. Another limitation is that verbal encouragement was given near the end of trials to maximize the effort of participants, which could have masked the potential effects of tsDCS on TTE.

PRACTICAL APPLICATIONS

In conclusion, our data suggests that thoracic spine and abdominal direct current stimulation using a current density of 0.071 mA/cm^2 does not significantly affect cycling performance longer than three minutes and less than fifteen minutes. Therefore, these results do not indicate that stimulation methods should be used in an attempt to acutely enhance high intensity cycling performance. However, as recent tsDCS data that utilizes a thoracic spine and shoulder montage and slightly higher current density suggests certain tsDCS methods may be able to enhance high-intensity cycling performance. As the body of research regarding the efficacy of tsDCS is still very young, more research is necessary to determine which tsDCS methods may affect human performance and what performance measures may be modulated by tsDCS.

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Figure 1. Timing of measurements during anodal, cathodal, and sham transspinal direct current stimulation (tsDCS) conditions.

Figure 2. Individual cycling to exhaustion durations in anodal, cathodal, and sham transspinal direct current stimulation conditions.

Figure 3. Individual maximal heart rate values (15-second averages) at the end of each cycling to exhaustion test during anodal, cathodal, and sham transspinal direct current stimulation conditions.

Figure 4. Individual mean rating of perceived exertion values from the first three minutes of each cycling to exhaustion test during anodal, cathodal, and sham transspinal direct current stimulation conditions.

Figure 1.

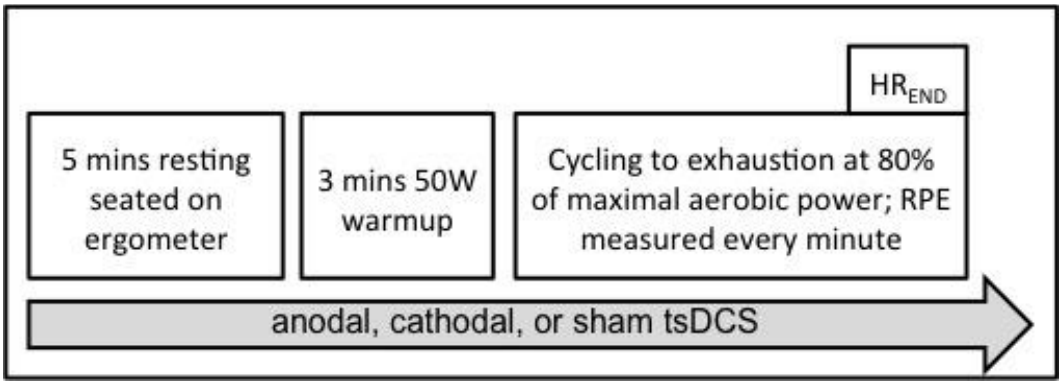

HR$_{END}$
5 mins resting seated on ergometer
3 mins 50W warmup
Cycling to exhaustion at 80% of maximal aerobic power; RPE measured every minute
anodal, cathodal, or sham tsDCS

Figure 2.

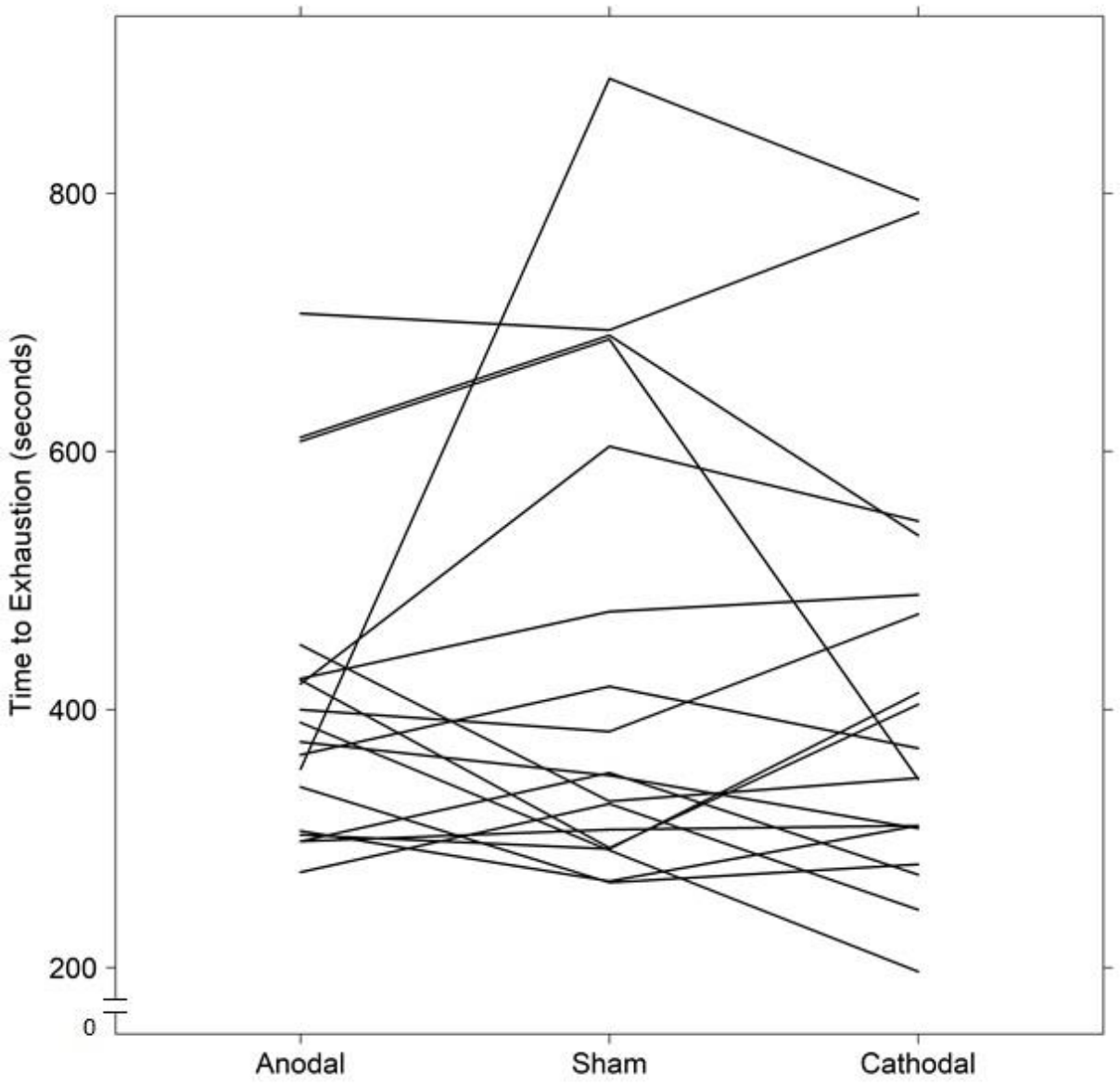

Time to Exhaustion (seconds)
800
600
400
200
0
Anodal
Sham
Cathodal

Figure 3.

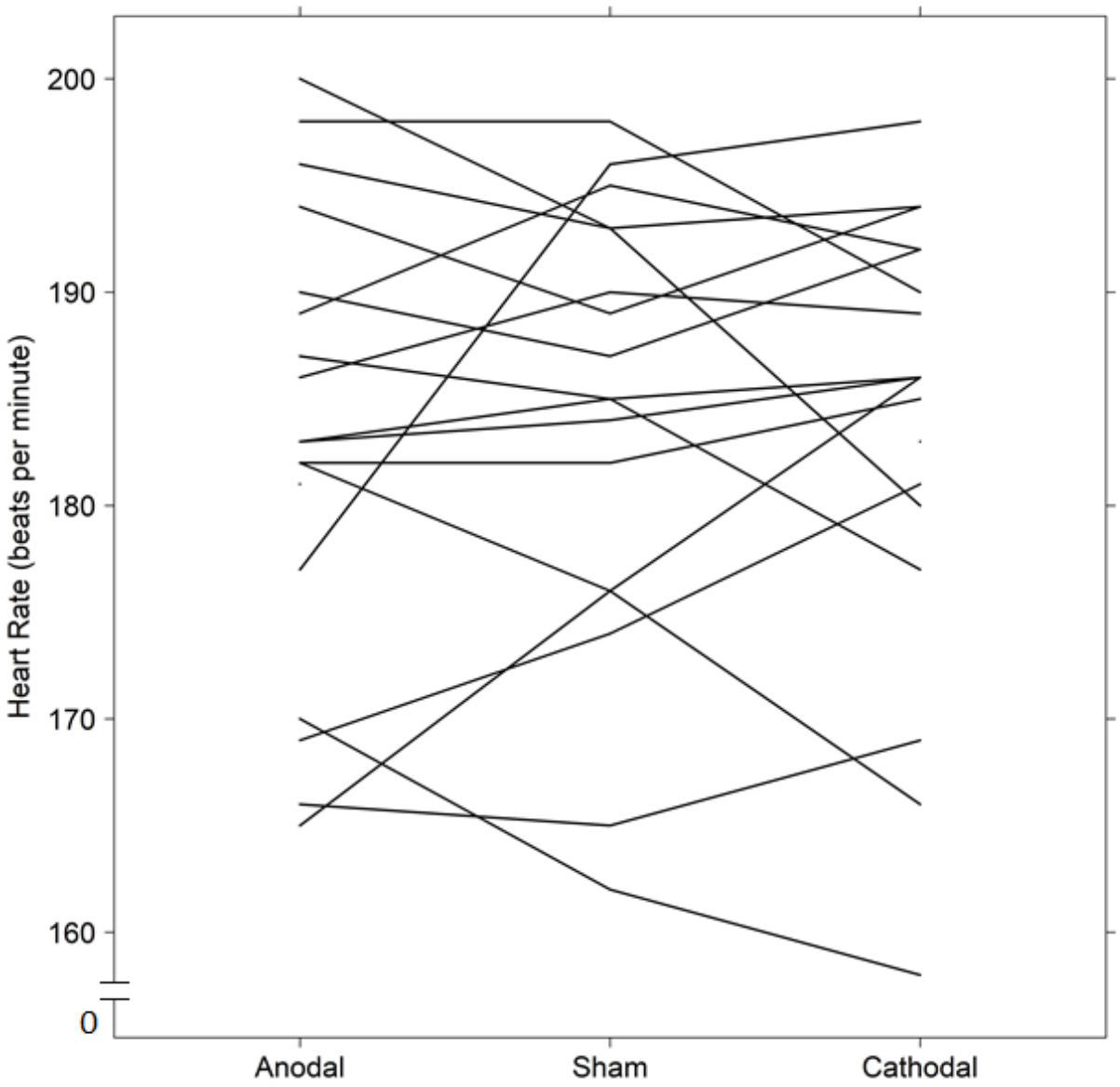
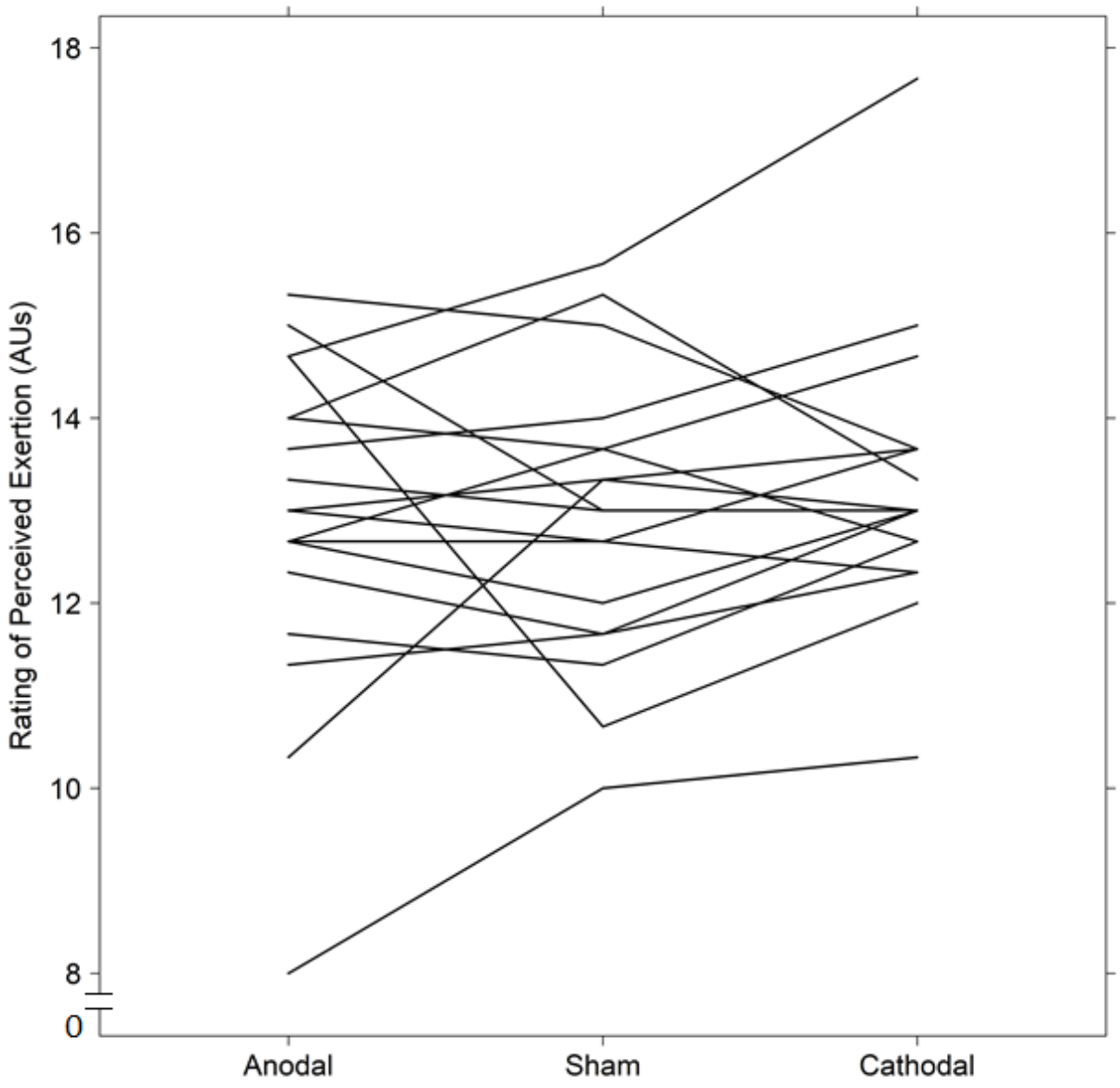

200
190
180
170
160
0
Heart Rate (beats per minute)
Anodal
Sham
Cathodal

Figure 4.


18
16
14
12
10
8
0
Rating of Perceived Exertion (AUs)
Anodal
Sham
Cathodal