The Effects of tDCS on Intermuscular Coherence During Speaking in Healthy Younger and Older Adults

by

Althea Feil

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Department of Communication Sciences and Disorders

University of Alberta

Abstract

Background. Research in neuroplasticity has focused recently on the preservation of cognitive and motor functions in aging adults. Previous studies have shown that non-invasive neurostimulation can mitigate age-related changes in speech motor control. Specifically, transcranial direct current stimulation (tDCS) has been shown to improve performance on cognitive, language, and motor tasks in older adults. For example, anodal tDCS over Broca's area increases speech rate and articulatory accuracy during recitation of tongue twisters. tDCS can be delivered off-line (before a task) or on-line (during a task), which results in differential changes in underlying neural mechanisms. Little is known about off-line tDCS and neuromodulation of speech motor control in typically aging adults. **Methods.** Thirty healthy younger adults (18-43yrs) and 30 healthy older adults (54-77yrs) recited tongue twisters pre- and post-13 minutes (1mA) tDCS over Broca's area (FC5-10/20 system). Participants were randomly assigned to receive anodal, cathodal, or sham stimulation. Effects on intermuscular coherence between perioral muscles and intercostal and oblique chest wall muscles were evaluated. **Results.** tDCS did not modulate the strength of perioral or chest wall intermuscular coherence in either younger or older adults. However, tDCS influenced where the peak coherence frequency occurred, the lag or timing between motor unit firing of paired muscles and the overall similarity between paired muscle signals. Only one significant anode and cathode post-stimulation effect (i.e., timing of motor unit firing) was found for the perioral muscles in older adults. Based on individual responses and group data analyses, it appears that most of the modulatory effects were found for chest wall muscles following cathodal tDCS. Cathodal tDCS appeared to affect where peak coherence frequencies occurred, timing of motor unit firing as well as similarity between intercostal and oblique muscle signals. Whereas these effects were observed primarily in the high frequency bandwidth (i.e., 60-110 Hz) the patterns of post-cathodal tDCS changes appeared to be age dependent. **Conclusion.** Strength of coherence (i.e., peak coherence amplitude) remained stable pre-post stimulation for both younger and older adults. The significant effects on measures of peak coherence frequency, cumulant density (lag) and cross-correlation coefficient (similarity) indicate that these measures may be more sensitive to stimulation than peak coherence amplitude. Additionally, the significant modulatory effects found for cathodal stimulation add to the body of literature examining long-term-depression like effects of tDCS. The results of this study expand our understanding of the effects of off-line tDCS on intermuscular coherence of perioral and chest wall muscles during a highly complex speech motor control task (i.e., tongue twisters) in younger and older adults. The results may help guide future studies examining the effects of tDCS on intermuscular coherence in healthy adults.

Preface

This thesis is an original work by Althea Feil. The research project, of which this thesis is part, was led by professors Carol A. Boliek and Jacqueline Cummine. The present study received research ethics approval from the University of Alberta Research Ethics Board, Project Name: *The Effects of Transcranial Direct Current Stimulation on Speech and Language Processing in Healthy Adults*; Pro00055070, 08 March 2017. The overall design of the study was developed by Drs. Boliek and Cummine in the context of neuromodulation and speech motor control (NSCERC Discover Grants: Boliek, PI; Cummine, PI). I was responsible for assisting in the recruitment and running of the older adults in the present study. The data analysis, as well as the following paper were completed by myself, with guidance provided from professor Carol A. Boliek. No part of this thesis has been previously published.

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Background Information

Introduction

Normal aging in humans is associated with neuromotor and physiological declines, which accelerate between the ages of 50 to 60 years (Booth, Weeden, & Tseng, 1994). By the time a person is 80 years old, almost 50% of his or her motor units, muscle fibers, muscle mass and muscle strength has been lost (Booth et al., 1994; Larsson, Grimby, & Karlsson, 1979; Rogers & Evans, 1993). Aging also is linked to decreases in cortical grey and white matter volume (Seidler et al., 2010). For example, white matter deterioration has been observed in aging adults, which may impair conduction of neural signals across the brain (Davis et al., 2009). In addition to the neuroanatomical declines, there appears to be a reduction in glutamate uptake capacity (Segovia, Porras, Del Arco, & Mora, 2001). Glutamate is a neurotransmitter present in excitatory synapses that may be involved in both motor and cognitive performance (Segovia, et al., 2001). Reduction in glutamate is therefore associated with declines in cognition (e.g., declarative memory) and motor tasks (e.g., spatial navigation tasks) (Morrison & Baxter, 2012; Segovia et al., 2001). The reduction in the uptake capacity for glutamate observed in older adults may be responsible for changes in synaptic connectivity (Morrison & Baxter, 2012; Pakkenberg et al., 2003). This is especially important to speech motor control due to the complexity of the speech mechanism and its coordinative movements. Although researchers have examined the relationship between aging and cognition (Seidler et al., 2010) as well as the relationship between aging and motor function (Summers, Kang, & Cauraugh, 2016), little is known about the impact on aging, in the context of speech motor control.

Speech requires coordination of respiratory, laryngeal and supralaryngeal subsystems by using approximately 100 muscles and 200 coordinative movements (Ackerman, 2008). As speed and accuracy of movement declines in typically aging adults (Ketcham, Seidler, Van Gemmert, & Stelmach, 2002; Salthouse & Somberg, 1982), changes in speech may occur such as slower speaking and reading rates (Torre & Barlow, 2009). In addition to the declines in motor function associated with normal aging, declines in cognitive processing also have been observed in typically aging adults (Torre & Barlow, 2009). Specifically, a decline in cognitive processing speed has been observed in older adults (Glisky, 2007) and may be related to declines in motor function. However, other than slower processing and production rates, speech and language remain largely intact in older healthy adults (Glisky, 2007).

According to the United Nations Population Fund (2015), 12.3% of the global population is 60 years or older, and that number is expected to reach 22% by the year 2050. With an increasingly aging population, there is an increased interest in finding opportunities to slow down the aging process through non-invasive behavioural interventions such as nutrition and exercise (Mattson, Chan, & Duan, 2002) as well as non-invasive neurostimulation techniques such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) (Gutchess, 2014).

Researchers studying activity-dependent neuroplasticity have focused recently on the preservation of cognitive and motor functions in healthy aging adults (Gow et al., 2012). If cognitive performance can be altered during healthy aging (Fertonani, Brambilla, Cotelli, & Miniussi, 2014), mitigating or delaying age-related declines may maintain or enhance functioning in older adults. Results from preliminary work have demonstrated that non-invasive neurostimulation techniques can reduce age-related cognitive changes (Kar & Wright, 2014). In particular, tDCS has been shown to improve performance on a word recall task (Sandrini et al., 2014), increase accuracy in naming famous faces and landmarks (Ross, McCoy, Coslett, Olson,

& Wolk, 2011), and positively influence dexterous manual performance on finger tapping tasks (Heise et al., 2014). Whereas tDCS has been shown to improve performance on these cognitive, language, and motor tasks in older adults (Summers et al., 2016), the effects of tDCS on speech motor control in this population are not well understood.

The purpose of the present study was to gain insight about the effects of tDCS on complex speech motor tasks (i.e., tongue twisters) in healthy older adults. Although this research involved only healthy adults, any facilitative effects of tDCS on motor speech control could later be applied in neurorehabilitation settings.

Transcranial Direct Current Stimulation (tDCS)

Transcranial Direct Current Stimulation is considered a non-invasive neurostimulation technique. Electrodes are placed on top of the scalp and a low-level direct current, usually 1-2 mA (Been, Ngo, Miller, & Fitzgerald, 2007), is delivered. There are three types of tDCS: anodal, cathodal, and sham. Anodal tDCS has been found to increase neural excitability in the region to which it is applied, whereas cathodal stimulation appears to decrease neural excitability (Kadosh, 2013). In anodal tDCS, the current exits the electrode and enters the more superficial layers of the brain, while in cathodal tDCS, the current exits the brain and enters the electrode (Kadosh, 2013). Altering neural excitability has been shown to alter both cognitive (Kadosh, 2013) and motor functions (Summers et al., 2016) in typical adults and those with disorders.

Mechanisms Underlying On-line vs. Off-line Stimulation

Transcranial direct current stimulation can be delivered off-line (i.e., before a task) or on-line (i.e., during a task) and it is believed that different underlying neural mechanisms cause associated behavioural changes (Stagg & Nitsche, 2011). Researchers have shown that there are differences in the behavioural changes observed between on-line and off-line stimulation, proposing that the application of tDCS during on-line stimulation shifts the resting membrane potential of superficial interneurons (i.e., neurons closest to the skull), which then alters neuronal excitability (Summers et al., 2016). There appears to be a shift in the initial membrane potential to a longer-term-like synaptic plasticity during off-line stimulation (Summers et al., 2016). The induction of off-line effects is believed to be dependent on membrane depolarization during anodal stimulation, whereas it is unknown exactly what role membrane polarization changes play in inducing off-line effects in cathodal stimulation (Stagg & Nitsche, 2011). It has been proposed that processes similar to long-term potentiation and long-term depression, in which glutamatergic (*N*-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors are modulated, play a role in off-line anodal and cathodal stimulation (Stagg & Nitsche, 2011). Researchers also have shown that anodal stimulation can lead to reductions in GABA concentration, which may correlate to motor learning and motor memory processes (Kim, Stephenson, Morris, & Jackson, 2014). A reduced capacity to modulate GABA-mediated inhibitory processes in older adults is thought to be associated with age-related declines in cognitive and motor function (Gleichmann, Chow, & Mattson, 2011; Levin, Fujiyama, Boisgontier, Swinnen, & Summers, 2014). As such, tDCS is thought to be a potential means of altering inhibitory activity in older adults (Heise et al., 2014).

There has been a limited amount of research on the effects of off-line tDCS in the healthy aging population, especially with regards to muscle control involved in speech motor tasks. Of interest to the aims of the present study is preliminary research showing that when anodal tDCS is applied over the left dorsolateral prefrontal cortex, significant increases in speech rate and articulatory accuracy during recitation of tongue twisters are observed in healthy adults (Fiori, Cipollari, Caltagirone, & Marangolo, 2014). Additionally, tDCS had more prominent effects in older adults compared to younger adults when anodal stimulation was applied over the primary motor cortex in a task involving right hand motor functions required for activities of daily living (Hummel et al., 2010).

Little is known about the application of off-line tDCS, affecting long-term potentiation-like effects (Stagg & Nitsche, 2011; Fertonani et al., 2014) on speech motor control in typically aging adults. These effects have not been fully explored in healthy aging adults, particularly in regards to how tDCS may affect expressive language tasks. One way to objectively evaluate the impact of stimulation on neuromuscular control of speech is through intermuscular coherence measurements.

Intermuscular Coherence

Intermuscular coherence (IMC) is a measure of the relationship between the neuronal signals driving two sets of muscles by recording and analyzing their electromyographic (EMG) discharge (Grosse, Cassidy, & Brown, 2002). Coherence analysis is based on squared correlation of oscillations from two separate physiological signals in the frequency domain (Grosse et al., 2002). In the case of intermuscular coherence, measurements are derived from multiple EMG recordings over paired muscle groups. By measuring the correlation between two signals in the same frequency bandwidth, we can obtain a coherence value between 0 and 1. This value indicates the strength of correlated oscillatory activity between different signal sources at a particular frequency (Grosse et al., 2002). A value of 0 indicates no linear relationship between signals, whereas a value of 1 indicates a perfect linear relationship between signals. Thus, when two signals exhibit a significant frequency-specific linear relationship they are said to be coherent. IMC analysis is derived from paired EMG signals, making it particularly useful for investigating the underlying mechanisms driving coordination of disparate muscle areas.

Whereas the mechanisms that underlie intermuscular coherence continue to be discussed (Boonstra, 2013), intermuscular coherence is believed to represent common descending oscillatory drive to disparate muscle areas during coordinated muscle activity (Grosse et al., 2002). Norton and Gorassini (2006) studied individuals with incomplete spinal cord injury and analyzed intermuscular coherence between leg muscles in the 24-40 Hz bandwidth, which is the range thought to indicate common drive to two muscles from corticospinal units. They found that individuals with moderate volitional motor strength in their leg muscles had greater coherence than individuals with absent or weak leg muscle strength. When the 5-18 Hz bandwidth, which is thought to indicate common drive from spinal inputs, was analyzed these same researchers found that coherence did not change in either group. It is thought that improvements in motor function of the leg muscles are mediated in part by corticospinal drive to these muscles as confirmed by TMS over the motor cortex and an increase in limb motor evoked potentials associated with motor improvements in these same individuals (Norton & Gorassini, 2006).

Grosse et al. (2002) proposed that the frequency at which oscillatory coupling occurs may indicate the origin of control and therefore serve as a potential indicator of physiological discharges to muscles and a means to characterize functional neuromuscular control networks. Studies of both typical (Maurer, von Tscharner, & Nigg, 2013; Jaiser, Baker, & Baker, 2016) and clinical populations (Hansen et al., 2005; Fisher, Zaaimi, Williams, Baker, & Baker, 2012) have contributed to this conceptualization of IMC. Studies of intermuscular coherence between limb muscles have found synchronized oscillations are primarily observed in two bandwidths: the beta bandwidth or β-band (approximately 15-30 Hz) and the gamma bandwidth or γ-band (approximately 30-60 Hz) (Maurer et al., 2013; Jaiser et al., 2016). Coherent oscillations in beta and gamma bandwidths have been reported between muscles of the speech mechanism, including muscles of the jaw (Smith & Denny, 1990), strap muscles of the neck (Stepp, Hillman, & Heaton, 2011) and respiratory muscles of the chest wall (Smith & Denny, 1990; Denny & Smith, 2000; Tomczak, Greidanus, & Boliek, 2013). Coherence in muscles of the speech mechanism has been found during non-speech (e.g., chewing, controlled deep breathing), speech (e.g., reading aloud), and speech-like tasks (e.g., reading silently). Furthermore, coherence between 60-110 Hz has been found in the chest wall muscles (Bruce & Ackerson, 1986; Smith & Denny, 1990). For example, Smith and Denny (1990) studied coherence in the 60-110 Hz range and found significant chest wall coherence during deep breathing (i.e., inhaling and exhaling in time with a visually-presented waveform). However, when subjects read a passage aloud, coherence was significantly reduced in the 60-110 Hz range. Denny and Smith (2000) replicated these results in a study comparing 60-110 Hz coherence during spontaneous speech and controlled deep breathing. This shift away from high frequency coherence during speech tasks is theorized to be a result of a change in neuromuscular control of the respiratory system to meet the differing demands of breathing during speech production, whereby speech and non-speech breathing tasks are thought to be controlled independently by oscillations of different frequencies (Smith & Denny, 1990; Denny & Smith, 2000). Moreover, high frequency oscillations appear to be unique to the respiratory system and produced primarily during non-speech breathing, suggesting that coherence detected in these higher frequencies implicates known contributions from brainstem central pattern generators for respiration (Bruce & Ackerson, 1986; Smith & Denny, 1990).

Intermuscular coherence in speech also appears to be sensitive to task demands (Stepp et al., 2011) and has been shown to change based on the types of speaking tasks employed (Smith & Denny, 1990; Stepp et al., 2011). As tasks increase in difficulty, a reduction in β-band coherence has been found (Maurer et al., 2013; Stepp et al., 2010). Researchers also have studied the coordination of chest wall muscles and found differences in intermuscular coherence as a function of lung volume excursion. Beta band coherence decreased in tasks requiring greater lung volume excursions than would typically be used in speech (Tomczak, et al., 2013).

Changes in IMC appear also to be a function of the linguistic nature of the task (i.e., speech vs. non-speech). IMC increased in the 20-60 Hz range during non-speech tasks (i.e., chewing) when compared with speech and jaw clenching tasks (Smith & Denny, 1990). To date, the study by Smith & Denny (1990) may be the only one to investigate intermuscular coherence of speech muscles innervated by cranial nerves via the corticobulbar tract. In a study involving the strap muscles of the neck, Stepp et al. (2011) found IMC to modulate as a function of task demands (i.e., coherence was lower for non-speech tasks such as singing than for typical speech). These studies seem to suggest that increased task complexity leads to decreases in intermuscular coherence.

Based on the apparent sensitivity of IMC to non-speech and speech tasks, the measure may be sensitive to changes in neuromuscular modulation following tDCS. Previous work has shown that the application of anodal tDCS over the motor cortex can result in increased intermuscular coherence between peripheral muscles used in skilled and/or strength-based tasks by inducing short-term plasticity in corticomotor neural networks (Power et al., 2006). Tomczak et al. (2013) used transcranial direct current stimulation to modulate IMC in speech and non-speech tasks, finding that anodal tDCS increased chest wall intermuscular coherence between intercostals and oblique muscles in non-speech tasks using large lung volumes (i.e., vital capacity and maximum duration phonation). To date, studies that have examined the effects of tDCS over Broca's area primarily have measured behaviours such as verbal fluency and accuracy (Cattaneo, Pisoni, &

Papagno, 2011; Fertonani et al., 2014). To our knowledge, no studies have examined the effects of tDCS on muscles of articulation (e.g., orbicularis oris) nor have they evaluated the effects of tDCS on IMC in healthy older adults.

Intermuscular Coherence and Aging

Speech motor control is based on numerous complex coordinative movements of the speech mechanism. Based on what we know about physical and neural aging, it is expected that older adults will exhibit a lower degree of intermuscular coherence than their younger counterparts. This expectation is based on what we know about the typical aging process including: (*a*) a reduction in glutamate uptake capacity and related declines in cognitive and motor performance observed in older adults (Segovia, et al., 2001), and (*b*) a reduction of neural signal conduction related to decreases in cortical white matter volume observed in older brains (Davis et al., 2009). Also worth noting is that intermuscular coherence appears to change based on the difficulty of the task performed (Maurer et al., 2013; Stepp et al., 2010). Because typically aging adults are expected to exhibit reductions in speed and accuracy of motor speech movements, we can argue that by creating a more "difficult" speech task (i.e., tongue twisters), age-related changes in coherence may be expected.

Study Aims

The purpose of this study was to examine the effects of anodal and cathodal off-line tDCS on intermuscular coherence during a highly complex speech motor control task (i.e., tongue twisters) in younger and older adults. We used perioral (i.e., left-right orbicularis oris) intermuscular coherence and chest wall (i.e., intercostals and obliques) intermuscular coherence as dependent measures of age and stimulation condition related change. The prediction was that anodal stimulation over the left frontal cortex, specifically Broca's area (FC5-10/20 system) would increase intermuscular coherence of both perioral and chest wall muscle groups in both younger and older adults, whereas cathodal would weaken or lower intermuscular coherence. Additionally, it was predicted that anodal tDCS would improve performance in older adults so that it approximated the performance of younger adults in pre-stimulation conditions. Sham stimulation was predicted to have no significant change in performance for either age group. Using data obtained from the same 60 participants as the current study, Freitag (2017) measured tongue twister accuracy, vocal reaction time and rate of speech. The aforementioned behavioural data will be briefly reported in this paper and intermuscular coherence data will be interpreted in the context of these behavioural outcomes.

Research Question 1. Does tDCS over the left frontal cortex, specifically Broca's area (FC5-10/20 system) have an effect on speech motor control as measured by intermuscular coherence?

Hypothesis 1.1: Following anodal tDCS over the left frontal cortex, it was expected that both younger and older adults would experience a transient increase in intermuscular coherence of left-right orbicularis oris and intercostal-oblique muscle groups.

Hypothesis 1.2: Following cathodal tDCS over the left frontal cortex, it was expected that both younger and older adults would experience a transient decrease in intermuscular coherence of left-right orbicularis oris and intercostal-oblique muscle groups.

Hypothesis 1.3: Following the sham condition, it was expected that for both younger and older adults there would be no significant change in intermuscular coherence.

Research Question 2. Does tDCS over the left frontal cortex, specifically Broca's area (FC5-10/20 system) have a differential effect on speech motor control as a function of age?

Hypothesis 2.1: In the post-anodal condition, it was expected that peak intermuscular coherence of older adults would approximate pre-stimulation conditions of the younger adults.

Hypothesis 2.2: In the cathodal condition, it was expected that peak intermuscular coherence would be significantly different in pre- and post-conditions between younger and older adults.

Hypothesis 2.3: In both the pre- and post-sham condition, it was expected that there would be significant peak coherence differences between the younger and older adults.

Methods

Participants

Two groups of healthy adults (i.e., younger and older) were recruited for the study. The younger group consisted of 30 participants (age range: 18-43; mean age = 26.97 ± 6.04 years; sex = 8 men). The older group consisted of 30 participants (age range: 54-77 years; mean age = 66.37 ± 6.83 years; sex = 7 men). All participants reported normal or corrected to normal vision. Participants did not have any language and/or speech impairment, reading disorder, attention deficit disorder, history of stroke, epilepsy or migraines. Participants also did not have any acute or chronic muscle conditions or surgeries affecting the head, neck, chest or abdomen. All participants were right-handed, English first-language speakers who met the requirements of the screening for tDCS. Informed consent was obtained from each participant upon admission to the study as approved by the Health Research Ethics Board at the University of Alberta. Each participant received a small honorarium for participation. All data were collected in the Speech Physiology Laboratory, Corbett Hall at the University of Alberta.

Partway through the recruitment and testing of the older adults, hearing and cognitive assessments were added to the study protocol. The purpose of these assessments was to provide an additional description of participants. All the younger adults and 7 of the older adults had already completed the study when these assessments were added. A total of 23 of 30 older adults completed these assessments. The hearing assessment involved using a portable audiometer and testing hearing at 40 dB HL at 1,000 Hz, 2,000 Hz, and 4,000 Hz for left and right ears. Three tones were presented to each ear at each frequency. Some participants did not respond to certain tones but were able still to functionally interact with examiners with no overt signs of being deaf or hard of hearing.

The cognitive assessment used was an adapted version of the Mini Mental State Exam (MMSE). The adapted version used for the purposes of this study excluded one question relating to which county the participants lived in. Since the location of this study, the University of Alberta, is not part of a specific county or comparable district, this question was removed. As such, a score of 24/29 points was required to pass the MMSE. No participant scored less than 24. The average score was 28.39, the standard deviation was 1.01, and the range was 26-29.

tDCS Stimulation Procedure

A randomized control trial (single-blind) was implemented to evaluate effects of each tDCS stimulus condition. Participants from each group (i.e., younger and older) were randomly assigned to one of the following conditions: (*1*) anodal tDCS, (*2*) cathodal tDCS, or (*3*) sham tDCS (i.e., no stimulation) over FC5. Ten participants from each age group received each condition. Participants were blind to experimental conditions. The skin on each participant's scalp and right shoulder was prepared for tDCS application by cleaning with light abrasion to reduce skin impedance. In both anodal and cathodal conditions, tDCS set to 1 mA was applied with a pair of sponge electrodes (5 cm x 4 cm) soaked in saline solution (0.9 % (36g/4L) concentration) for a total of 13 minutes. The calculated current density for this protocol was 0.05 mA/cm^2 (Nitsche et al., 2008). The active electrode was placed on the left precentral gyrus, specifically region FC5, based on the 10-20 electrode positioning system (Jasper, 1958). The reference electrode was extracephalic and placed over the right upper arm. In the sham condition, current was temporarily delivered through the scalp using the same "ramp up" protocol as in true stimulation with the total ramp up and ramp down period lasting a total of 1 minute. This was repeated at the end of the sham stimulation period. This procedure served to make the sham condition perceptually identical to the anodal and cathodal conditions (Gandiga, Hummel, & Cohen, 2006) thus, blinding the participant to experimental condition. Participants sat in front of a computer during stimulation and typed numbers with a keyboard as they came onto the computer monitor. The number-typing task was selected because it was unrelated to the motor speech task (i.e., tongue twisters) assessed after the stimulation period.

Experimental Task

Thirty tongue twisters, controlled for length and lexical qualities (See Appendix A), were used. Tongue twisters were presented using E-Prime software, presented visually on a computer screen. In each experimental task, participants' responses were recorded. Each participant was asked to complete the tongue twister task immediately before stimulation and then again immediately after stimulation. A randomized set of 15 tongue twisters (Appendix A) was used during the recitation task before stimulation and a matched set was used for recitation immediately following stimulation. Participants were asked to read each tongue twister as quickly and accurately as possible.

Surface Electromyographic Recordings

Surface electromyography (sEMG) was used to record activity from the muscles of the perioral muscles (i.e., left and right orbicularis oris) via 8 small paired (left, right) electrodes placed on the upper (n=4 electrodes) and lower (n=4 electrodes) lips. sEMG recordings were also obtained from the chest wall over intercostal and oblique muscle groups on the right side of the body. Paired electrodes (Kendal Soft-E H69P, Tyco Healthcare Group, Mansfield, MA) were placed over the sixth intercostal space and oblique muscle regions, 2 cm apart (center-to-center) and oriented parallel to fiber direction. Intercostal electrodes were placed ventrally 8-10 cm from midline, and oblique electrodes were placed at a midpoint between the anterior superior iliac spine and the caudal border of the rib cage. According to the protocol used by Tomczak, et al. (2013), this placement configuration avoids the intercartilaginous region of the rib cage while enhancing ventral-dorsal EMG placement. In order to increase signal-to-noise ratio and reduce power line interference occurring at 50 or 60 Hz, a third electrode was placed on the clavicle to serve as a reference signal. All EMG signals were amplified (Grass P511, Quincy, MA), band-pass filtered (3-3000 Hz), and sampled at 10,000 Hz. A multichannel data acquisition system (PowerLab 16SP ML795; ADInstruments, Colorado Springs, CO) was used to collect signals, which were saved to a computer using LabChart software (v5.5.6; ADInstruments).

Acoustic and Kinematic Recordings

Acoustic and chest wall kinematic (inductance plethysmography, Ambulatory Monitoring, Ardsley, NY) recordings were used in the offline analyses for selecting the expiratory limb of the breath groups associated with tongue twister productions. Acoustic signals were used to verify that the speech waveform was associated with tongue twister production versus other miscellaneous conversation.

Video Recordings

Video recordings (Canon ZR60 Camcorder) were collected during the entire study protocol to ensure that offline analyses only included data that were free from extraneous limb and trunk movements. A second microphone was attached to each participant’s clothing and acoustic signals were digitized (10 kHz) along with the video signals to ensure an acoustic record of the study was available during analyses.

Data Analysis

Intermuscular Coherence

Intermuscular coherence (IMC) was analyzed for the expiratory limb of speech only, as determined by the derived calibrated lung volume signal (i.e., summing the rib cage and abdomen kinematic signals) (Tomczak et al., 2013). Specifically, the production window, which encompassed the entire tongue twister, was isolated using the expiratory breath group(s) associated with the tongue twister (i.e., from peak inspiration to end of expiration). Figure 1 shows an exemplar of the raw signals and signal selection for coherence analyses.

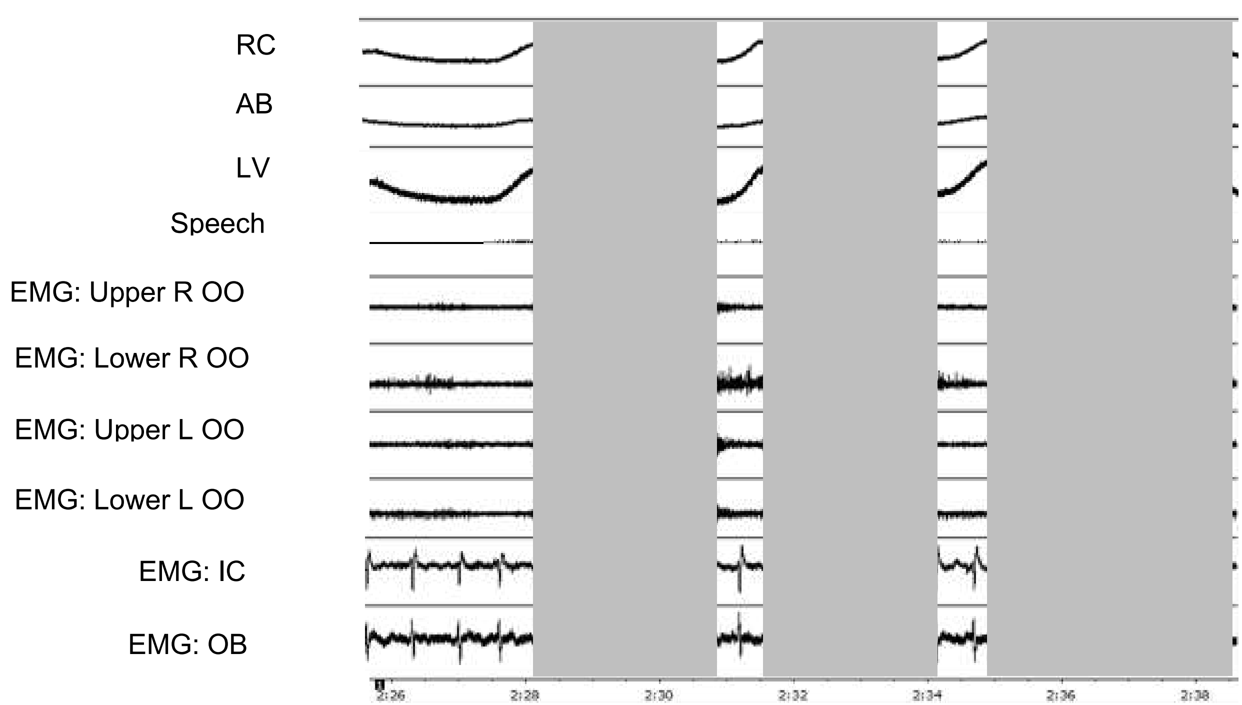

Figure 1. Exemplar of raw signals and signal selection for coherence analyses. Shaded regions represent isolated segments (from peak inspiration to end of expiration). LV [lung volume; i.e., rib cage (RC) and abdomen (AB) kinematics combined after calibration] was used as a guide for selecting segments.

If a participant recited the tongue twister on more than one breath, all breaths were isolated separately and included in analysis. The average total length of the concatenated data used for analysis is listed in Table 1.

Condition	Young								Old							
	Duration (s)				Segments				Duration (s)				Segments			
	ICOB		LO-RO		ICOB		LO-RO		ICOB		LO-RO		ICOB		LO-RO	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Anode	41	41	81	82	99	99	198	199	40	40	79	81	97	99	194	198
Cathode	39	40	77	80	94	97	189	196	39	38	78	75	95	92	190	184
Sham	38	40	73	81	94	99	178	198	40	41	79	81	97	99	194	199

Table 1. Mean duration and corresponding number of segments used in coherence calculations for intercostal-oblique (IC-OB) and left- vs right-orbicularis oris (LO-RO) coherence during the expiratory limb of speech production. Values have been averaged across time (pre- and post-stimulation) and condition (anode, cathode, and sham) in younger and older adults.

Within the expiratory limb of speech, IMC was evaluated in low (15-35 Hz), mid (40-59 Hz), and high (60-110 Hz) frequency bands using MatLab (MathWorks, Natick, MA). IMC was evaluated in two sets of muscle pairings: (a) left upper and lower orbicularis oris (LO) and right upper and lower orbicularis oris (RO) and (b) intercostal (IC) and oblique (OB). Peak IMC calculations are depicted in Equation 1. All coherence values were transformed into *Fisher Z* values prior to analysis.

$$MSC = \left|C_{xy}(w)\right|^2 = \frac{\left|G_{xy}(w)\right|^2}{G_{xx}(w) \cdot G_{yy}(w)}$$

Equation 1. Intermuscular Coherence calculation where MSC = magnitude squared coherence; $G_{xx}(w)$ and $G_{yy}(w)$ = averaged power spectra of the x and y muscles of interest, for a given frequency (w); G_{xy} = averaged cross-power spectrum of x and y signals at frequency w (Halliday et al., 1995).

In addition to peak coherence, we derived three additional measures from the paired muscle signals. First, we determined the frequency where peak coherence occurred in each bandwidth of interest (i.e., peak frequency). Second, we calculated the cumulant density of the two EMG signals of interest. To do this, we used an inverse Fourier transform (i.e., cross-correlation of the paired muscle signals). Cumulant density allows us to define the lag between the two signals in the time domain. Third, we calculated the cross-correlation coefficient. Higher coefficient values indicate a greater degree of similarity between the two EMG signals of interest. Cumulant density (lag) and cross-correlation coefficient values (similarity) characterized the EMG signals from paired muscles of interest across the entire frequency range studied (i.e., 15-110 Hz). Whereas hypotheses were appropriate for peak amplitude of coherence based on previous studies, these three additional measures were exploratory and used to further describe the nature of the relationship between the two muscle groups of interest and whether or not they would be sensitive to age and stimulation condition.

Statistics

We applied a series of paired samples *t*-tests to answer the *a prioi* hypotheses in the present study. A *p* value of ≤ 0.05 indicated statistical significance. Here we are evaluating the impact of tDCS on neuromuscular modulation in a group of older adults for the first time in the context of *Phase I* treatment research (Robey, 2004). Given the *Phase I* level of study, we elected to apply a liberal statistical function (i.e., no correction for multiple comparisons) commensurate with acceptable *Type I* error tolerances for this phase level. Paired samples *t*-tests also were used to detect age or condition effects on measures of peak frequency, cumulant density, and cross-correlation coefficient. Prior to applying statistical analyses, outliers more than three standard deviations from the mean were removed from the data (10 participants had one outlier value each).

Results

Before reporting the coherence results of this study, it is worth briefly summarizing the behavioural outcomes of the study performed by Freitag (2017), which utilized the same 60 participants as the present study. Doing so will allow for interpretation of the physiological coherence findings in the context of behavioural measures.

Freitag (2017) measured vocal reaction time, accuracy and rate of speech during recitation of tongue twisters. Reaction time was defined as the amount of time between the presentation of the tongue twister and the participant's audible response. Accuracy was calculated as the percentage of words produced correctly (i.e., accurate pronunciation, no added or corrected speech sounds, no word repetitions). Speech rate (i.e., syllables per second) was calculated only on accurately produced tongue twisters. Freitag found no significant effects for type of stimulation nor interactions between age and stimulation type on vocal reaction time, accuracy, or rate of speech. A significant effect of age on rate of speech was found, revealing that on average older adults spoke slower than younger adults. This is in line with previous speech research (Torre & Barlow, 2009). Additionally, there was a significant effect of time (i.e., pre- vs. post-stimulation) on reaction time, revealing that both age groups exhibited increased reaction times following stimulation, regardless of type of stimulation. This finding may have been the result of a practice effect. Statistical analysis revealed also a trend of participants decreasing in accuracy following stimulation, which is postulated to have been the result of fatigue.

Chi square analyses of individual responders were performed also, revealing one statistical trend for the comparison of reaction time across age groups based on stimulation condition. Reaction times trended towards improvement in this Chi square test. As well, participants trended towards improvement in the cathodal condition more than in anodal or sham. Freitag (2017) postulated that this indicated possibly a differential effect of cathodal stimulation.

Present Study

Research Question 1. Does tDCS over the left frontal cortex, specifically Broca's area (FC5- 10/20 system) have an effect on speech motor control as measured by intermuscular coherence?

Hypothesis 1.1: Following anodal tDCS over the left frontal cortex, it was expected that both younger and older adults would experience a transient increase in intermuscular coherence of left-right orbicularis oris and intercostal-oblique muscle groups.

Hypothesis 1.2: Following cathodal tDCS over the left frontal cortex, it was expected that both younger and older adults would experience a transient decrease in intermuscular coherence of left-right orbicularis oris and intercostal-oblique muscle groups.

Hypothesis 1.3: Following the sham condition, it was expected that for both younger and older adults there would be no significant change in intermuscular coherence.

Paired samples *t*-tests performed based on *a priori* Hypotheses 1.1-1.3 are described below. The four measures of coherence taken for each muscle group pairing are shown in Figures 2-6.

Peak Coherence Amplitude

Paired samples *t*-tests revealed no significant increase in peak coherence amplitude for either age group or muscle pairing in the anodal condition in any of the three frequency bandwidths, contrary to *a priori* Hypothesis 1.1 (Figs. 2 and 3). *t*-tests revealed no significant decrease in peak coherence amplitude for either age group or muscle pairing in the cathodal condition in any of the three frequency bandwidths, contrary to *a priori* Hypothesis 1.2 (Figs. 2 and 3). *t*-tests revealed no significant change in peak coherence amplitude for either age group or muscle pairing in the sham condition for any of the three frequency bandwidths, as per *a priori* Hypothesis 1.3 (Figs. 2 and 3).

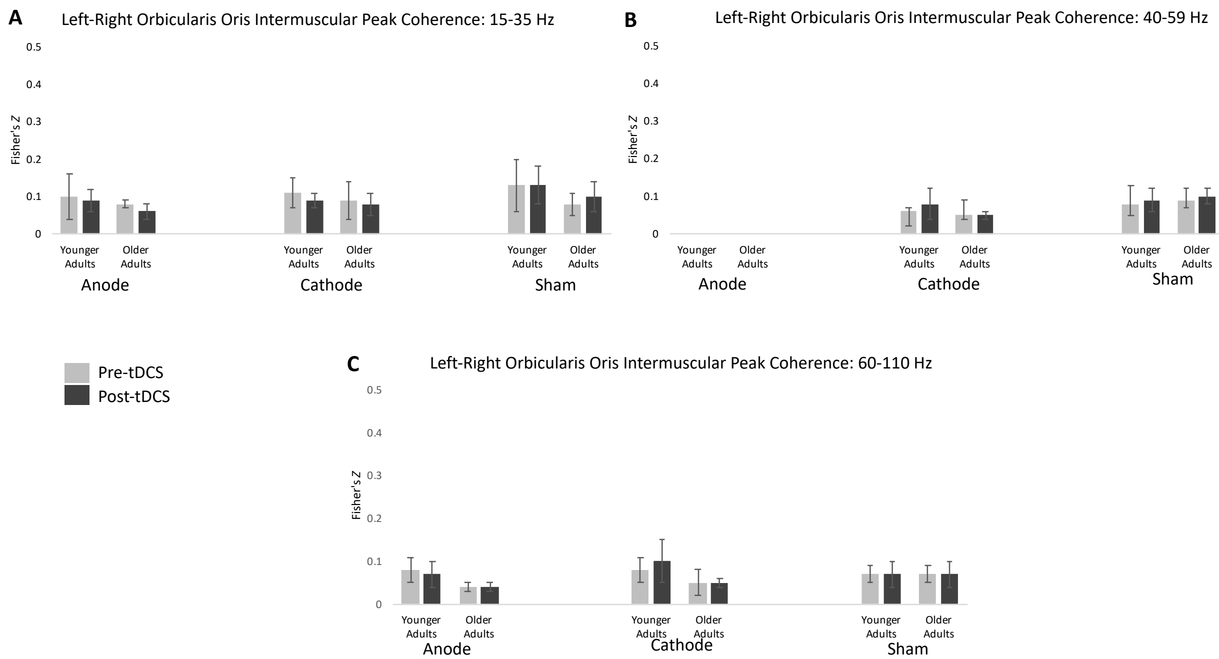

Figure 2. Peak amplitude of left vs. right obicularis oris in the low (A), mid (B), and high (C) frequency bandwidths for younger and older adults, pre- and post-stimulation. Error bars represent one standard deviation.

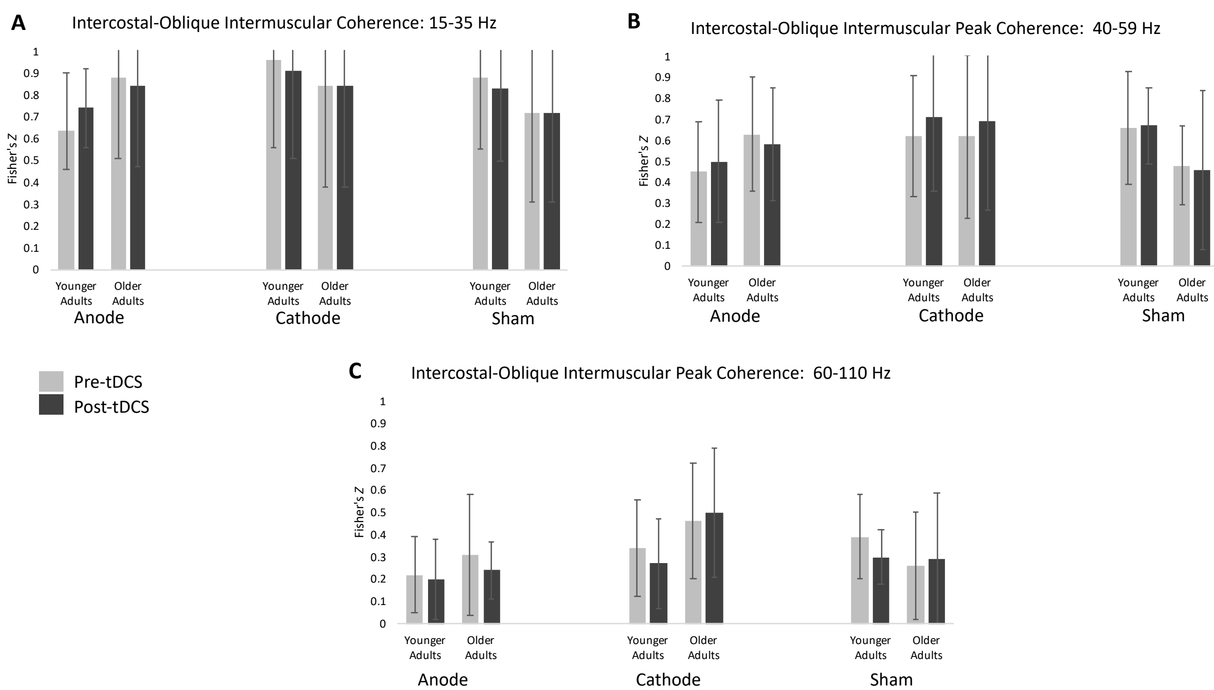

Figure 3. Peak amplitude of the chest wall muscles in the low (A), mid (B), and high (C) frequency bandwidths for younger and older adults, pre- and post-stimulation. Error bars represent one standard deviation.

Peak Frequency

Within group comparisons using paired samples *t*-tests revealed no significant increase in peak frequency for either age group or muscle pairing in the anodal condition in any of the three frequency bandwidths. No significant within group differences in peak frequency were found for the perioral muscles in any of the three frequency bandwidths in the cathodal condition (Fig. 4). However, in the cathodal condition, *t*-tests revealed a significant increase in peak frequency of the chest wall muscle groups (Fig. 5C) for both younger (t = 2.358, df = 7, p = 0.05, two-tailed) and older (t = 3.162, df = 5, p = 0.03, two-tailed) adults in the high (60-110 Hz) frequency bandwidth. Paired samples *t*-tests revealed no significant change in peak frequency for either age group or muscle pairing for the sham condition (Figs. 4 and 5).

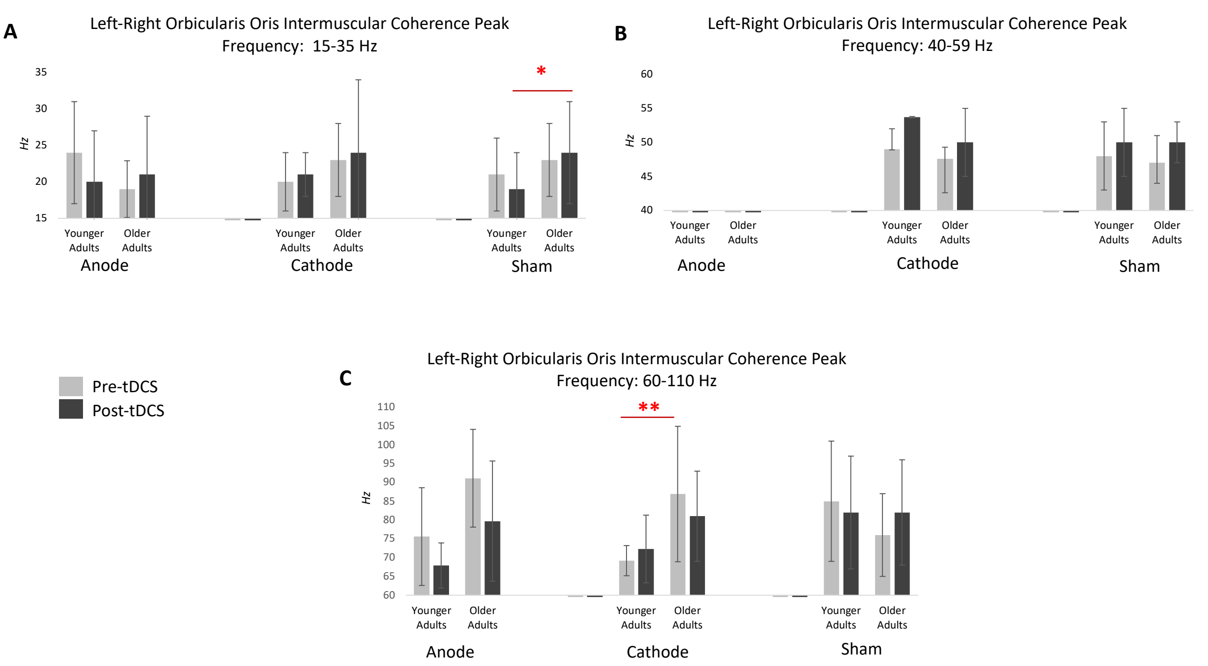

Figure 4. Peak frequency of left vs. right obicularis oris in the low (A), mid (B), and high (C) frequency bandwidths for younger and older adults, pre- and post-stimulation. Error bars represent one standard deviation. Asterisks represent significant difference at $p \leq 0.05$.

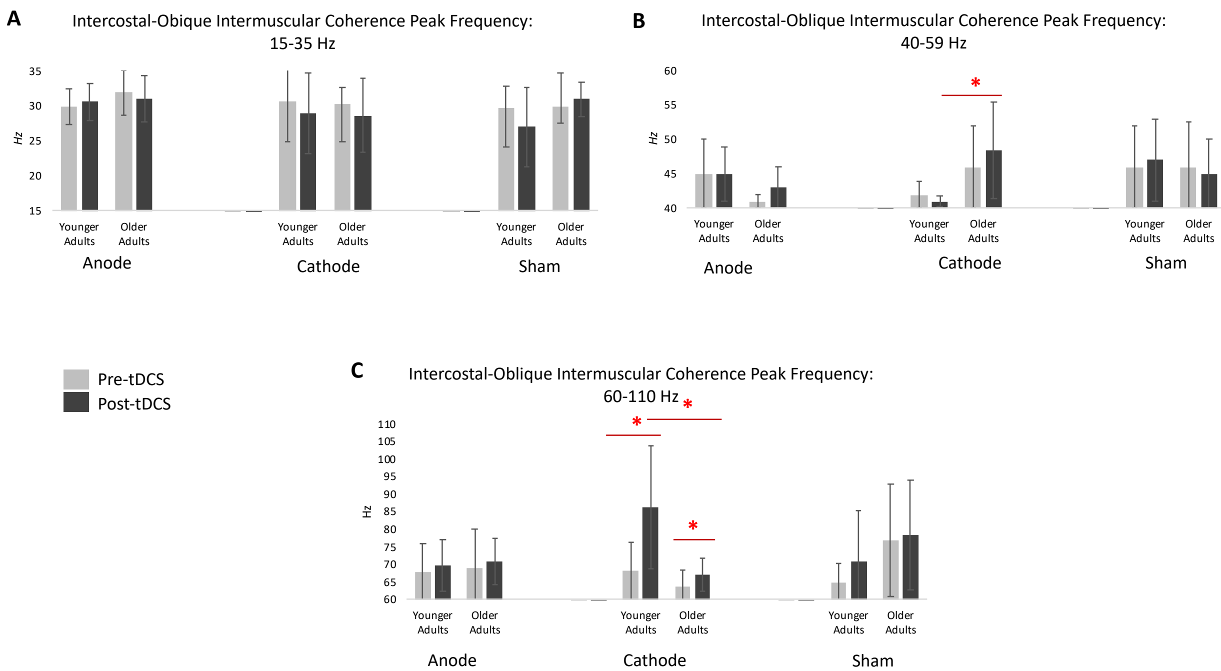

Figure 5. Peak frequency of chest wall muscles in the low (A), mid (B), and high (C) frequency bandwidths for younger and older adults, pre- and post-stimulation. Error bars represent one standard deviation. Asterisks represent significant difference at $p \leq 0.05$.

Cumulant Density

As can be seen in Figure 6A and 6C, paired samples *t*-tests revealed no significant difference in cumulant density for either age group or muscle pairing in the anodal condition in any of the three frequency bandwidths. *t*-tests revealed no significant change in cumulant density for either age group or muscle pairing in the cathodal condition in any of the three frequency bandwidths. *t*-tests revealed no significant change in cumulant density for either age group or muscle pairing in any of the three frequency bandwidths for the sham condition.

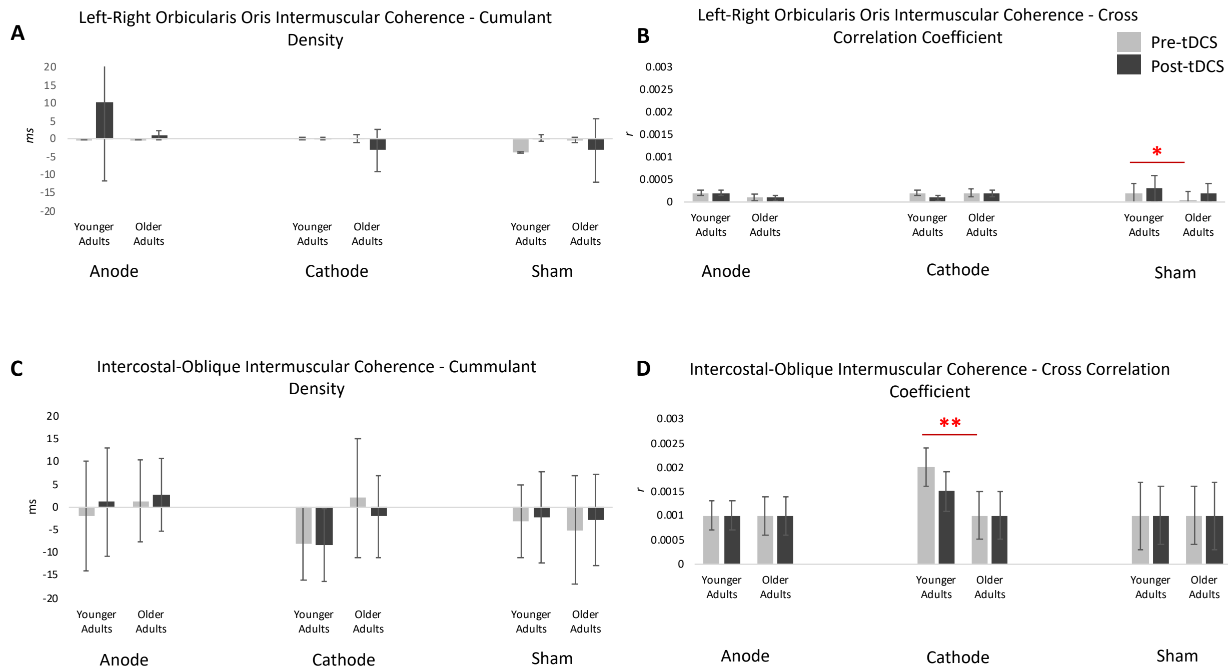

Figure 6. Cumulant density and cross-correlation coefficient for left vs. right obicularis oris (A, B) and muscles of the chest wall (C, D) in younger and older adults, pre- and post-stimulation. Error bars represent one standard deviation. Asterisks represent significant difference at $p \leq 0.05$.

Cross-Correlation Coefficient

As can be seen in Figure 6B and 6D, *t*-tests revealed no significant increase in cross-correlation coefficient for either age group or muscle pairing in the anodal condition in any of the three frequency bandwidths. *t*-tests revealed no significant decrease in cross-correlation coefficient for either age group or muscle pairing in the cathodal condition in any of the three any frequency bandwidths. *t*-tests revealed no significant change in correlation coefficient for either age group or muscle pairing for the sham condition.

Research Question 2. Does tDCS over the left frontal cortex, specifically Broca's area (FC5- 10/20 system) have a differential effect on speech motor control as a function of age?

Hypothesis 2.1: In the post-anodal condition, it was expected that peak intermuscular coherence of older adults would approximate pre-stimulation conditions of the younger adults.

Hypothesis 2.2: In the cathodal condition, it was expected that peak intermuscular coherence would be significantly different in pre- and post-conditions between younger and older adults.

Hypothesis 2.3: In both the pre- and post-sham condition, it was expected that there would be significant peak coherence differences between the younger and older adults.

Paired samples *t*-tests performed based on *a priori* Hypotheses 2.1-2.3 are summarized below. The four measures of coherence taken for each muscle group pairing are displayed in Figures 2-6. For peak coherence amplitude, no age differences were found for either perioral or chest wall muscle groups pre- or post-stimulation. Thus, it was not possible to test whether or not following anode stimulation in older adults approximated younger adult values pre-stimulation (i.e., $p > 0.05$). This was also the case for cathode and sham conditions for peak coherence amplitude. However, *t*-tests revealed a significant difference in peak coherence frequency between younger and older adults in the post-cathodal condition in the mid (40-59 Hz) frequency bandwidth ($t = 2.454$, df = 7, $p = 0.044$, two-tailed) as well as in the high (60-110 Hz) frequency bandwidth ($t = 3.042$, df = 6, $p = 0.023$, two-tailed) for the muscles of the chest wall (Figure 6B and 6C). In addition, *t*-tests revealed a significant difference in cross-correlation coefficient value between younger and older adults in the pre-cathodal condition ($t = 3.038$, df = 9, $p = 0.014$, two-tailed) for the muscles of the chest wall (Figure 6D). This difference was not detected post-cathodal stimulation because younger adults approximated the older adult values after receiving this stimulation condition.

Individual Responses to Stimulation by Age Group

Previous research has shown that when given the same type of tDCS there may be significant differences in how individual participants respond, particularly after a single administration of stimulation (Fricke et al., 2011). In consideration of individual responses to stimulation we looked at the number of individuals within each age group, who increased or decreased peak coherence amplitude, peak coherence frequency, cumulant density, and cross-correlation coefficient values following anodal, cathodal, or sham stimulation. Chi square analyses based on categorical assignment of increase or decrease were run on the *a priori* probability model of equal likelihood of either category.

The results of these analyses showed that there were no significant patterns of responses for younger adults in coherence measures associated with left-right orbicularis oris. However, older adults exhibited significant patterns of response for cumulant density (delay) ($\chi2 = 8.3$, df = 2, $p = 0.02$). Figure 7 shows that in the anode condition, 90 percent of older individuals demonstrated a decrease in cumulant density value whereas in the cathodal condition, 70 percent of individuals demonstrated an increase in value. The sham condition revealed a near even split (i.e., 60/40% increase/decrease; respectively). Table 2 shows an exemplar contingency table used to calculate the above significant Chi Square result.

			Delay_mstwister		
			Increase	Decrease	Total
Condition	Anode	Count	1_a	9_b	10
		% within Condition	10.0%	90.0%	100.0%
		% within Delay_mstwister	7.1%	56.3%	33.3%
		% of Total	3.3%	30.0%	33.3%
	Cathode	Count	7_a	3_a	10
		% within Condition	70.0%	30.0%	100.0%
		% within Delay_mstwister	50.0%	18.8%	33.3%
		% of Total	23.3%	10.0%	33.3%
	Sham	Count	6_a	4_a	10
		% within Condition	60.0%	40.0%	100.0%
		% within Delay_mstwister	42.9%	25.0%	33.3%
		% of Total	20.0%	13.3%	33.3%
Total		Count	14	16	30
		% within Condition	46.7%	53.3%	100.0%
		% within Delay_mstwister	100.0%	100.0%	100.0%
		% of Total	46.7%	53.3%	100.0%

Table 2. Exemplar contingency table used to calculate the significant Chi Square finding for cumulant density of left-right orbicularis oris in older individuals.

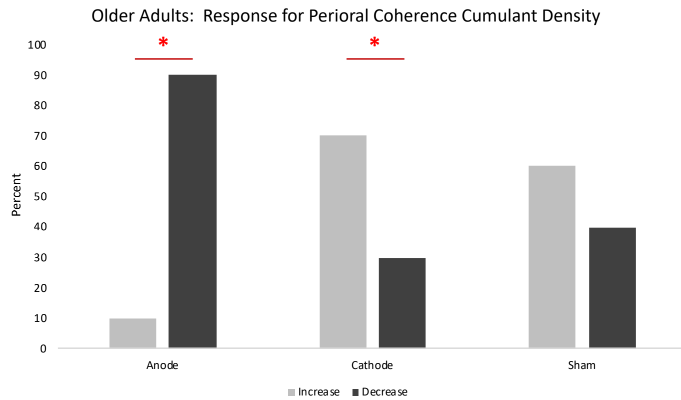

Figure 7. Percent of older individuals who showed an increase or decrease in cumulant density values between perioral muscles following each stimulation condition. * $p < 0.02$.

Three significant patterns were found for coherence measures of chest wall muscle groups. For younger adults, a statistical trend for differences in responses was found for cross-correlation coefficient measures (Figure 8). In the anode condition, 80 percent of individuals demonstrated an increase in coefficient values whereas in the cathodal condition, 70 percent of individuals demonstrated a decrease in values ($\chi 2 = 5.61$, df = 2, $p = 0.06$). Sham condition revealed a near even split (i.e., 40/60% increase/decrease; respectively).

Older adults demonstrated significant differences in responses in peak coherence frequency in the mid frequency bandwidth (40-59 Hz) (Figure 9). In the anode condition, 90 percent of individuals demonstrated an increase in peak frequency values whereas in the cathodal condition, 90 percent of individuals demonstrated a decrease in values ($\chi 2 = 14.12$, df = 2, $p = 0.001$). Sham condition revealed a non-significant split (i.e., 30/70% increase/decrease; respectively).

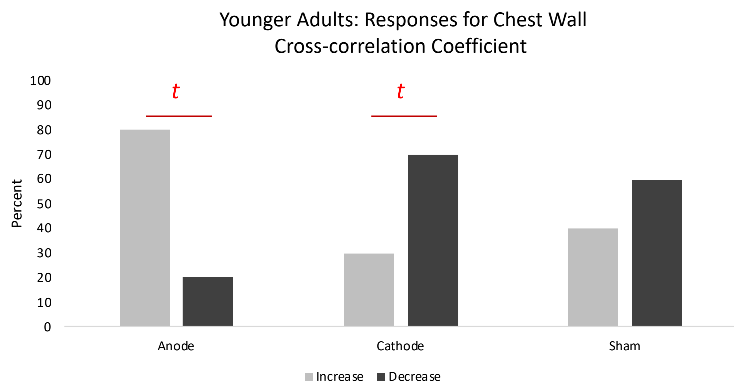

Figure 8. Percent of younger individuals who showed an increase or decrease in chest wall cross-correlation coefficient values between chest wall muscle groups following each stimulation condition. $t = p < 0.06$.

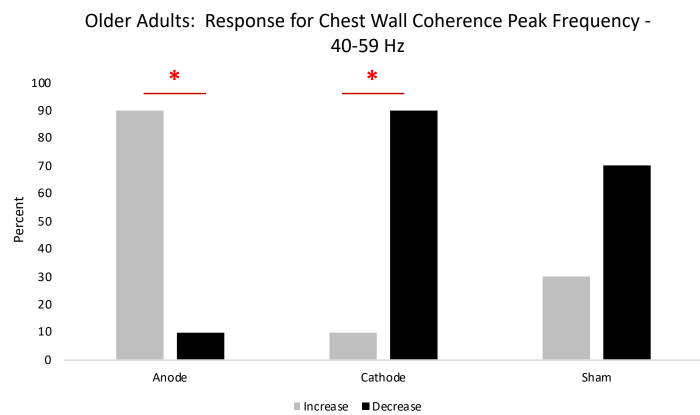

Figure 9. Percent of older individuals who showed an increase or decrease chest wall coherence peak frequency values between chest wall muscle groups following each stimulation condition. $* = p < 0.001$.

Discussion

The aim of the study was to examine whether or not a single application of transcranial direct current stimulation (tDCS) would significantly impact neuromuscular modulation of perioral or chest wall muscles during a complex speaking task (i.e., tongue twisters). Moreover, we wanted to know if tDCS would have a differential effect on healthy older individuals who are undergoing neuroanatomic and neurochemical changes associated with typical aging. A group of 30 healthy younger adults (age range: 18-43; mean age = 26.97 ± 6.04 years; sex = 8 men) and 30 healthy older adults (age range: 54-77 years; mean age = 66.37 ± 6.83 years; sex = 7 men) served as participants. Participants from each group were randomly assigned to one of the following tDCS (1 mA, 13 minutes) conditions: (*1*) anodal tDCS, (*2*) cathodal tDCS, or (*3*) sham tDCS (i.e., no stimulation) over FC5 (i.e., Broca's area; 10-20 system). Participants were asked to read a set of tongue twisters as quickly and accurately as possible before stimulation and a second, matched set of tongue twisters, immediately following stimulation. Surface electromyography (sEMG) was used to capture muscle activity from perioral muscles (i.e., right and left orbicularis oris) and chest wall muscles (i.e., intercostal and obliques). Four measures representing elements of neuromuscular modulation were taken, including, (*a*) intermuscular peak coherence amplitude (i.e., strength of muscle coordination), (*b*) peak coherence frequency, (*c*) cumulant density (i.e., lag between paired muscle motor unit firing) and (*d*) cross-correlation coefficient (i.e., similarity of muscle activation signals) across frequency bandwidths representing cortico-muscular drive (Grosse et al., 2002; Norton & Gorassini, 2006).

Based on group and individual responses to tDCS, several significant observations can be made. Our primary findings suggest that tDCS did not modulate the *strength* of perioral or chest wall intermuscular coherence (i.e., peak coherence amplitude) in either younger or older individuals. However, tDCS did influence where the peak coherence frequency occurred, the lag or timing between motor unit firing of paired muscles (i.e., cumulant density), as well as overall similarity between paired muscle signals (i.e., cross-correlation coefficient). Interestingly, most of these modulatory effects were found following cathodal tDCS and, as expected, differentiated younger from older adults.

Peak Coherence Amplitude

Our data indicated that the strength of coherence or peak coherence amplitude remained stable pre-post stimulation for both younger and older individuals. We anticipated that the strength of intermuscular coherence during a complex speech task such as reading tongue twisters would be susceptible to tDCS similar to that reported previously in limbs (Power et al., 2006). However, our results are in line with Tomczak and colleagues (2013), who found that anodal tDCS increased area of intercostal-oblique intermuscular coherence on tasks requiring large lung volume excursions (i.e., vital capacity and maximum duration phonation) but had no influence on coherence for tasks carried out in the midrange of the vital capacity (i.e., resting tidal breathing and passage reading). Here, participants were asked to read tongue twisters one at a time, which can easily be done in one or two breath groups initiated in the midrange of vital capacity and with a requirement of lung volumes between 10 to 20 percent of vital capacity. Thus, our tongue twister task would be similar to lung volume events associated with the passage reading task described in Tomczak et al. (2013). Peak coherence amplitudes in older individuals were predicted to increase as a function of anodal tDCS and decrease with cathodal tDCS because of a potentially more vulnerable neurological system (Heise et al., 2014; Gleichmann et al., 2011; Levin et al., 2014; Segovia, et al., 2001) and based on observed changes in behavioural measures (i.e., articulatory speed and accuracy) following tDCS (Fertonani et al., 2014; Fiori et al., 2014). However, peak coherence amplitude was not sensitive to stimulation condition in our group of older adults and did not support the behavioural findings reported by Fiori and colleagues (2014). In particular, Fiori and colleagues found changes in speed and accuracy following tDCS on spoken tongue twisters in healthy adults. However, their task required participants to recite each tongue twister after an auditory presentation. Thus, their task required a significant cognitive and memory load in contrast to the reading task in the present study, which might account for different results.

In addition, with the exception of a statistical difference found for cross-correlation coefficient values of the chest wall muscle groups in the pre-cathode condition (likely due to a randomization effect), no significant differences between age groups were found pre-stimulation. This is in contradiction to Hypotheses 2.2 and 2.3, which predicted that there would be significant differences in peak intermuscular coherence between younger and older adults in the pre-cathode and pre-sham conditions.

Supplemental Measures of Neuromodulation

Measures of peak coherence frequency, cumulant density, and cross-correlation coefficient revealed interesting patterns of response to tDCS in both younger and older adults. These results will be discussed in terms of differential responses to anode and cathode tDCS followed by those variables that were responsive to cathode tDCS only. Taken together, these supplemental measures of muscle activity may shed light on possible post-tDCS neuromuscular modulatory effects.

Differential Responses to Anode vs. Cathode Stimulation. Individual responses to tDCS, within the younger age group, showed a trend indicating that anode tDCS increased cross-correlation coefficient values whereas cathode decreased these values for the intercostal-oblique muscle groups. Increases in cross-correlation coefficient values indicated that sEMG signals from these muscle pairings became more similar following anodal tDCS in contrast to becoming less similar following cathodal tDCS. These findings are in line with what we predicted and support the basic principles of excitatory-inhibitory effects of anode and cathode stimulation, respectively (Stagg & Nitsche, 2011; Summers et al., 2016). More specifically, changes in the number of young individuals who demonstrated an increase in degree of signal similarity following anode tDCS may be related to the induction of off-line effects believed to be dependent on membrane depolarization during anodal stimulation, whereas membrane polarization may have played a role in reducing chest wall EMG signal similarity for those individuals receiving cathodal tDCS (Stagg & Nitsche, 2011; Summers et al., 2016).

Individual responses to tDCS by older adults showed two interesting patterns. First, the lag or delay in motor unit firing between left and right orbicularis oris appeared to be sensitive to stimulation condition. Specifically, following anodal tDCS, a significant number of individuals exhibited a decrease in cumulant density, suggesting that the right-sided aspect of the orbicularis oris muscle was more likely firing (i.e., receiving input from the corticobulbar tract) before its left-sided counterpart. Thus, perhaps an indication of enhancing the contralateral corticobulbar pathway. In contrast, following the cathodal condition, cumulant density increased, which might indicate that the left-sided aspect of the orbicularis oris was more likely to fire or receive input from the corticobulbar tract before its right counterpart. If cathodal stimulation over the left hemisphere motor area is inhibitory, it might be that the right hemisphere increases its support for the motor command, thus enhancing the contralateral left-sided orbicularis oris muscle. Importantly, individuals exhibited a change in lag between left and right orbicularis oris following both active tDCS conditions, which may indicate depolarization or polarization effects on the motor pathway controlling muscles of articulation (i.e., excitation of the left hemisphere during anode tDCS and inhibition of the left hemisphere during cathode stimulation) (Heise et al., 2014; Stagg & Nitsche, 2011; Summers et al., 2016). This finding was only apparent in the older adults and the only stimulation-related finding associated with muscle activation patterns of the orbicularis oris.

The second pattern of significance found for older adults was observed in individual responses to tDCS measured by peak coherence frequency for chest wall muscles in the 40-59

Hz bandwidth. Following anode tDCS, a significant number of older individuals exhibited an upward shift in frequency (Hz) relative to where peak coherence occurred before stimulation. The opposite observation was true for older adults following cathodal tDCS. These shifts upwards and downwards in peak frequency may indicate some perturbation of cortico-muscular modulation based on the gamma bandwidth where these shifts are observed (Grosse et al., 2002; Norton & Gorassini, 2006) and the type of stimulation delivered (Nitsche, et al., 2008).

Notably, the changes in physiological measures observed in older adults in the present study align with the changes in behavioural measures Freitag (2017) observed in older adults. Freitag found that older adults spoke on average slower than younger adults. This is in line with the research that suggests the decrease in glutamate uptake capacity expected in typically aging adults results in slower speech rates (Torre & Barlow, 2009). The shifts in lag in the perioral muscles of older adults in the present study suggest that depolarization or polarization effects, acting on neurotransmitters GABA and glutamate, may be at play.

Responses to Cathodal Stimulation. Group findings (i.e., inferential statistics) for muscles of the chest wall, indicated that cathodal tDCS had an effect on peak frequency especially in the high-frequency bandwidth (60-110 Hz) for both younger and older adults. It is interesting that cathode tDCS perturbed (i.e., increased) peak frequency in the high bandwidth for intercostal-oblique muscle pairs. These particular chest wall muscle groups play a significant role in speech breathing including making fine adjustments for loudness variation, pauses, and length of utterance (Hixon, Mead & Goldman, 1976). Previous work has demonstrated that EMG-EMG coherence for muscles of respiration also are characterized in the high frequency bandwidth (60-110 Hz) and may represent neuromuscular drive not only from the cortex, but also from brainstem circuitry (Carr et al., 1994; Denny and Smith, 2000; Smith and Denny, 1990).

The fact that for both groups, peak frequency increased along the frequency continuum following cathodal tDCS may indicate a relative shift in balance between cortically driven neuromuscular control (i.e., lower frequencies of this bandwidth) and brainstem central pattern generators (i.e., presumably higher frequencies of this bandwidth) (Smith and Denny, 1990). This would be in line with the potential polarization effects of cathodal tDCS over the motor cortex and possibly resulting long-term-depression like changes (Stagg & Nitsche, 2011). More specifically, because the present experimental protocol used off-line cathodal tDCS, these observations may be related to modulation decreases in both glutamate and GABA (Stagg et al., 2009) but others have found no change in either neurotransmitter following cathodal tDCS (Kim et al., 2014).

The effect of cathodal tDCS appeared to be much greater in younger adults as indicated by significant group differences post-stimulation (Fig. 5C). This response difference may be related to age distinctions in (*a*) neuroanatomic features (Davis, et al., 2009; Kennedy and Raz, 2005; Rosano et al., 2008; Seidler et al., 2010; Sullivan et al., 2010), (*b*) synaptic connectivity (Davis et al., 2009; Morrison and Baxter, 2012; Pakkenberg et al., 2003, Segovia et al., 2001), and (*c*) level of CNS neurotransmitters (Mattay et al., 2002; Morrison and Baxter, 2012; Segovia et al., 2001).

The other significant result following cathodal tDCS was found for younger adults and chest wall muscular activity. This group showed a significant decrease in cross-correlation coefficient values following cathodal tDCS, which indicates that the two EMG signals were significantly different following stimulation. Once again, this is in line with other studies showing a disruption of behaviour following depolarizing effects of cathodal tDCS and long-term-depression like changes (Stagg & Nitsche, 2011). The significant group differences found for cross-correlation coefficient values of the chest wall muscle groups pre-cathode (Figure 6D) are believed to reflect randomization effects.

Additional Findings. All but one of our significant effects were found for chest wall muscles. As can be seen in Figures 2 and 3, peak coherence amplitude was lower for perioral muscles than for muscles of the chest wall. Whereas chest wall muscles are controlled by the corticospinal tract, orbicularis oris is controlled by the corticobulbar tract. It is possible that the differing effects of intermuscular coherence on these two muscle group pairings are caused by the different neuromuscular pathways. However, Denny and Smith (2000) evaluated intermuscular coherence in corticobulbar tract muscles and found that coherence did not change as a result of innervation origin. Thus, the differences in coherence amplitude observed as well as susceptibility to tDCS, may be more related to function as opposed to differences in neuromuscular innervation. Whereas the perioral muscles may intermittently activate depending on the speech sounds being produced, the chest wall muscles of expiration are activated consistently throughout the breath group, which may impact the strength of coherence found in these muscles groups. It is possible also that neighbouring facial muscles acted on the orbicularis oris during the production of some speech tokens and thus, may have impacted coherence strength.

Studies have evaluated the effects of tDCS on chest wall muscles (Tomczak et al., 2013), which are part of the respiratory subsystem of speech, but to our knowledge no studies have yet evaluated the effects of tDCS on muscles belonging to the articulatory subsystem (e.g., orbicularis oris). Since we found only one significant effect of tDCS on left and right orbicularis oris but several effects on chest wall muscles, the results of the present study may indicate that chest wall muscles (and by extension, the respiratory subsystem of speech) are more sensitive to neuromodulation techniques such as tDCS than perioral muscles (and by extension, the articulatory subsystem of speech), particularly when examining measures of peak coherence frequency, cumulant density (lag), and cross-correlation coefficient values.

Experimental Considerations

There are several experimental factors that need to be considered in the context of the present findings. The fact that the experiment did not yield robust results for stimulation condition or age as expected, may be due to several experimental features, which should be considered when designing future tDCS experiments. These elements include, (*a*) current density, (*b*) dose of stimulation (i.e., strength of current, duration of stimulation), (*c*) single session vs. repeated exposure, (*d*) state-dependency, and (*e*) on-line vs. off-line; training vs. no-training paradigms.

Current Density. It is possible that the current density (in this case, 0.05 mA/cm^2) was insufficient to cause significant changes in intermuscular coherence. Dedoncker, Brunoni, and Baeken (2016) reported that participants who received higher current density had higher response accuracy on cognitive tasks. It is possible that by increasing the current density, either by increasing the amperes administered or decreasing the electrode sponge size, more robust stimulation-related observations would have been realized. Thus, future studies should consider increasing current density when evaluating the effects of tDCS on intermuscular coherence in healthy adults.

Dose of Stimulation. An ineffective dose (i.e., strength of current or duration of stimulation) may account for the lack of robust findings related to stimulation condition or age in the present study. Cuypers et al. (2013) found that an increase in current intensity led to an improvement in motor performance. The current intensity used in the present study was 1 mA, the ampere value commonly used in anodal tDCS studies evaluating limb motor performance (Cuypers et al., 2013). Similarly, when evaluating the effects of tDCS on a tongue twister recitation task in healthy adults, Fiori and colleagues (2014) administered 2 mA on-line tDCS and found significant improvements in speech rate and articulatory accuracy. Important to consider is the varying evidence for increasing current intensity. In a study by Teo, Hoy, Daskalakis, Fitzgerald, and Mcclintock (2011), participants received 20 minutes of either *(a)* 1 mA anodal tDCS, *(b)* 2 mA anodal tDCS, or *(c)* sham tDCS to the left dorsolateral prefrontal cortex while completing a working memory task. No significant improvement in accuracy of participant responses was found between the sham, 1 mA, or 2 mA conditions, indicating that current intensity did not affect performance.

The duration of stimulation in the present study (i.e., 13 minutes) also may have affected results. Summers et al. (2015) reported through completing a meta-analysis, that tDCS duration across studies ranged from 6-37.5 minutes. With such varying stimulation durations found in the literature, future studies of the effects of tDCS may consider systematically altering stimulation duration. Interestingly, there exists evidence that increasing the duration of tDCS may not necessarily increase its efficacy. Indeed, one study found that increasing duration of anodal tDCS from 13 minutes to 26 minutes decreased neuronal excitability in healthy adults (Monte-Silva et al., 2013).

Single-Session vs. Repeated Exposure. Another possibility is that single-session tDCS such as that used in the present study, may be less effective than tDCS delivered over repeated sessions. In a previous study, participants received anodal tDCS over the primary motor cortex for five consecutive days resulting in increased acquisition of a motor skill (i.e., a visual isometric pinch task), which the researchers proposed was potentially a result of overnight consolidation (Reis et al., 2009). Furthermore, there exists evidence to suggest that neurons can regulate their own excitability relative to network activity or stimulation and that this regulation (known as “homeostatic plasticity”) depends on the time interval between stimulation protocols. In a study by Fricke et al. (2011), researchers compared the effects of a single session of tDCS with the effects of an identical tDCS session that was preceded by a preconditioning session. Fricke and colleagues found that five minutes of tDCS affected excitability for a period of five minutes, whereas ten minutes of tDCS caused the effects to last longer. However, administering two, five-minute sessions of tDCS, 30 minutes apart, yielded results comparable to the single five-minute stimulation session. Interestingly, when two five-minute sessions of tDCS were administered with a three-minute break between, the second stimulation period had the opposite effect of a single five-minute stimulation session. The findings of Fricke et al. (2011) and the concept of “homeostatic plasticity” suggest that it may be worthwhile for future studies evaluating the effects of tDCS on motor control to implement repeated exposure protocols.

State-Dependency. There is evidence in the literature showing that the effects of neurostimulation are reliant on what is known as state-dependency. The theory of state-dependency suggests that any external stimulus applied to the brain is dependent on the state of the brain at the time of stimulation. Silvanto and Pascual-Leone (2008) investigated the interaction between effects of transcranial magnetic stimulation (TMS) and the pre-stimulation state of the brain region (i.e., baseline cortical activation) and concluded that, in agreement with the theory of state-dependency, the impact of an external stimulus is determined not only by the features of said stimulus but also by the pre-stimulation state of the brain region itself. Moreover, Silvanto, Muggleton, and Walsh (2008) suggested that the neural populations affected by TMS can be controlled for by modifying neural activation states before applying stimulation, thus allowing for the selective targeting of specific neural regions. Acquiring a measurement of baseline state for each participant's brain prior to tDCS application was outside the scope of the present study. However, taking the findings of Silvanto et al. (2008) into consideration, the theory of state-dependency shows promise for improving the effects of neurostimulation techniques in future studies.

On-line vs. Off-line, Training vs. No-Training Paradigms. There is emerging evidence in the current body of tDCS literature that stimulation effects may be influenced by motor or cognitive activities undertaken during or after stimulation. In the present study, participants sat in front of a computer during stimulation and typed numbers with a keyboard as they came onto the computer monitor. There appears in the tDCS literature to be a relationship between the effects of stimulation and the cognitive or motor tasks completed during (i.e., on-line) or after (i.e., off-line) stimulation. Some studies have shown that tasks performed during or after stimulation negatively impact the effects of stimulation, whereas other studies have reported a positive impact of task on stimulation effects.

Antal, Terney, Poreisz, and Paulus (2007) found that when participants performed a cognitive task (i.e. a questionnaire about language, geography, history, and mathematics) during stimulation, the effects of anodal and cathodal stimulation were canceled out. Moreover, Antal et al. (2007) found that performing a motor task (i.e., pushing a ball around) during stimulation decreased the effects of both anodal and cathodal stimulation, indicating that the simple motor activity performed on-line had a negative impact on the effects of tDCS. Other studies have shown also that tasks completed before or after stimulation may interfere with stimulation (See Miyaguchi, Onishi, Kojima, & Sugawara, 2013; Thirugnanasambandam, Sparing, Dafotakis, & Meister, 2011; and Quartarone et al., 2004).

On the other hand, there exists evidence that tasks completed during stimulation may positively impact the effects of tDCS. Gill, Shah-basak, and Hamilton (2015) reported that when participants were asked to perform a cognitively demanding task during stimulation, they performed with more accuracy and greater speed on an off-line cognitive task. Given that tasks undertaken during or after tDCS can potentially interfere with or enhance the effects of stimulation, it is possible that the on-line number-typing task used in the present study affected the results of stimulation. The number-typing task was selected because it was unrelated to the motor speech task assessed after the stimulation period. It is possible then that incorporating a priming task (e.g., having participants perform a tongue twister-like task) during stimulation could have led to more robust findings in the present study. Future studies may find it beneficial to adjust for the type of task performed during stimulation.

Limitations

Here we address the limitations of the present study, first pertaining to statistical analyses, then stimulation parameters, and finally the additional measures of intermuscular coherence.

Statistical Analyses

Recent research has shown that individual participants may respond differently to tDCS (Fricke et al., 2011). For this reason, we believed it was valuable to take into consideration individual responses to stimulation. In the present study, individual responses were included in statistical analyses. Individual response was considered a dichotomous variable (i.e., the individual either increased or decreased on the coherence variable of interest). As such, we were limited to non-parametric statistics in the form of Chi Square analyses. Furthermore, as is the case with nominal data of this nature, the degree to which an individual increased or decreased was not taken into consideration.

The research undertaken in the present study was classified as *Phase I* level treatment research (Robey, 2004), which allowed for the use of somewhat liberal statistical methodology. We did not correct for multiple comparisons in our *t*-tests, for example. Because the intent of this study was exploratory in nature, liberal statistical methods were appropriate. However, in future studies examining the effects of tDCS on intermuscular coherence in older adults and/or perioral muscles, more rigorous analyses should be applied so as to reduce the possibility of *Type I* errors.

Stimulation Parameters

As addressed earlier in this paper, there are several parameters which could be adjusted when designing tDCS experiments for future studies. These include increasing current density (Dedoncker et al., 2016; Teo et al., 2011), modifying the duration of stimulation (Summers et al., 2015; Monte-Silva et al., 2013), utilizing repeated exposure to tDCS (Reis et al., 2009), evaluating state-dependency (Silvanto, Muggleton, & Walsh, 2008) and implementing a training protocol (Gill et al., 2015).

Additional Measures of Intermuscular Coherence

The three additional measures of intermuscular coherence evaluated in this study (i.e., peak coherence frequency, cumulant density or lag, and cross-correlation coefficient value) were exploratory in nature. They were intended to assist in describing the nature of the relationship between the muscles of the chest wall and perioral muscles and whether these muscle groups are sensitive to stimulation condition or age. Whereas these measures did yield significant findings, it is still unclear exactly what these findings infer in regards to specific mechanisms of neuromuscular modulation.

Future Directions

The results of the present study reveal some insights into cathodal stimulation. Notably, most of the modulatory effects found in this study were in post-cathode conditions. The significant changes in peak coherence frequency found may indicate potential polarization effects of cathodal stimulation over the motor cortex, possibly resulting in long-term-like depression (Stagg & Nitsche, 2011). However, while there exists some evidence that cathodal tDCS is related to decreases in the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter GABA (Stagg et al., 2009), other research finds no change in either neurotransmitter following cathodal stimulation (Kim et al., 2014). Given the inconsistent results of cathodal stimulation in the tDCS literature, further research is needed.

Future studies examining the effects of tDCS on coherence of the chest wall and perioral muscles in older and younger adults should consider also adjusting stimulation parameters such as current density, dose of stimulation (i.e., strength of current, duration of stimulation), single session vs. repeated exposure, training vs. no-training protocols, as well as evaluating state dependency in order to further examine stimulation or age effects of tDCS on peak coherence amplitude.

Conclusions

The results of the present study expand our understanding of the effects of off-line tDCS on coherence of perioral and chest wall muscles during a highly complex speech motor control task (i.e., tongue twisters) in younger and older adults. The significant modulatory effects found for cathodal stimulation contribute to the existing body of literature on tDCS and intermuscular coherence. The fact that strength of coherence (peak coherence amplitude) remained stable pre-post-stimulation for both younger and older healthy individuals indicates that measures such as peak coherence frequency, cumulant density (lag), and cross-correlation coefficient (similarity) may be more sensitive to stimulation than peak coherence amplitude in these populations. Additionally, the lack of significant results for anodal stimulation in the present study suggest that future tDCS paradigms should consider increasing current density, adjust dosage of stimulation (i.e., duration), consider single session vs. repeated exposure, and/or evaluate state dependency to further examine stimulation and age effects of tDCS on intermuscular coherence. Further research examining cathodal stimulation is necessary to better understand its long-term-like depression effects on typically aging adults.

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Appendix A

Tongue Twisters Set 1	*Tongue Twisters Set 2*
Brief beastly beach breezes	Brad’s burned bran buns
Fine fresh free fish	Chef’s sooty shoe soles
Floyd’s fourth floor fort	Chop shops stock chops
Gloria’s Greek green gloves	Five frantic fat frogs
Kick six sticks quick	Flea-free fruit flies
Luke’s ducks like lakes	Kate takes Tate’s cake
Luther’s moose’s loosest tooth	Larry’s really rarely leery
Plastic potted pansy plants	Proper copper coffee pot
Santa’s short suit shrunk	Richard’s wretched ratchet wrench
Shops seldom sell shellfish	Simon’s minimum cinnamon synonym
Swizzle scissors sizzle thistles	Six thick thistle sticks
Three short sword sheaths	This sister’s sixth zither
Trish's ritzy Irish wristwatches	Three tree twigs twine
Which wished which wish	Which Swiss witch switched?
Willy’s real rear wheel	Whistle softer thistle sifter

The above tongue twisters were adapted from literature on speech production (Bressman & Irish, 2014; Dell, Burger, & Svec, 1997; Schwartz, Saffran, Block, & Dell, 1994; Sobkowiak, 1990), from an interest article (Plunkett, 2013), and from a collection of online tongue twisters (“Twister,” 2015). The design of the study required that each tongue twister combine four syntactically possible English words. Some tongue twisters from the sources listed here were modified to meet these criteria. Tongue twisters were sorted into two sets based on speech sounds to match for level of difficulty.