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Effects of training and lung volume levels on voice onset control and cortical activation in singers

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Effects of training and lung volume levels on voice onset control and
cortical activation in singers

Nicholas A. Barone

A dissertation submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

for the degree of

Doctor of Philosophy

Department of Communication Sciences and Disorders

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Dedication

For my father, who put aside his own aspirations of getting a Ph.D. to provide for me a future where I could get mine. Thank you.

Acknowledgements

First, to my mentor, Dr. Christy Ludlow, you helped forge me into a better, more science-minded researcher, and pushed me to put aside my “passions and feelings” to find the story the data has to tell, for it is far more exciting. For that, I am sincerely grateful. Thanks also to my Dissertation Committee, Dr. Lincoln Gray and Dr. Rory DePaolis. You were generous with your time, patience, and advice which helped keep me calm and focused throughout this process. Thank you to my graduate research partners, Grace Baillie, Anna Louthan, and Nora Colman for generously assisting me. A warm thanks to Dr. Carol Dudding for providing mentorship in teaching. Special gratitude to Dr. Erin Kamarunas, a friend and colleague throughout this process. To my fellow doctoral students, I was glad to go through this process with you all.

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ABSTRACT

Singers need to counteract respiratory elastic recoil at high and low lung volume levels (LVLs) to maintain consistent airflow and pressure while singing. Professionally trained singers modify their vocal and respiratory systems creating a physiologically stable and perceptually pleasing voice quality at varying LVLs. In manuscript 1, we compared non-singers and singers on the initiation of a voiceless plosive followed by a vowel at low (30% vital capacity, VC), intermediate (50% VC), and high (80% VC) LVLs. In manuscript 2, we examined how vocal students (singers in manuscript 1) learn to control their voice onset at varying LVLs before and after a semester of voice training within a university program. Also examined were the effects of training level and LVLs on cortical activation patterns between non-singers and singers (manuscript 1), and within vocal students before and after training (manuscript 2) using fNIRS. Results revealed decreased control of voice onset initially in singers prior to training as compared to non-singers, but significant improvements in initial voice onset control after training, although task difficulty continued to alter voice physiology throughout. Cortical activation patterns did not change with training but continued to show increased activation during the most difficult tasks, which was more pronounced after training. Professionally trained techniques for consistent, coordinated voice initiation were shown to alter voice onset following plosive consonants with training. However, in non-singers and, as performance improved in singers after training, cortical activation remained greatest during the tasks at low LVLs when difficulty was highest.

I. INTRODUCTION

Singers are often trained to onset voicing and produce voice qualities that are both perceptually pleasing and aerodynamically efficient at varying lung volume levels (LVLs). The goal of most professional voice training is determined perceptually by a voice teacher to approach an “ideal voice quality” for a specific genre (e.g. opera, belt, etc.). Specifically, in manuscript 1 we examined differences in physiological vocal behaviors and cortical activation patterns between untrained singers and freshman vocal performance students (vocal students). In manuscript 2 we examined the same vocal students’ before and after their first semester of training within a university vocal performance/education program.

Physiological behaviors of voice onset

Human speech and vocalization are integral parts of the communication process and rely on cortical control of the respiratory system (Smith & Denny, 1990). During quiet respiration, speech, and singing, the forces of elastic recoil are present particularly during expiration at high LVLs and to a reduced degree at low LVLs (Sundberg, Leanderson, Von Euler, & Knutsson, 1991). Speech respiration supports the inspiration of adequate LVLs, up to 70-80% of vital capacity (VC), to speak long utterances while maintaining adequate length of expiration (Huber, 2008; Watson & Hixon, 1985). To achieve this, speakers must overcome the forces of elastic recoil that include gravity, relaxation of the external intercostal muscles and diaphragm, and restoration of the chest wall to its resting shape. Speech expiration involves counteractive checking forces (active inhibition) of the primary muscles of inspiration (diaphragm and external intercostal muscles) to prevent rapid loss of air volume early in a phrase. This is required primarily at high LVLs. At lower LVLs, expiratory airflow depends upon activation of muscles of

forced expiration. The part of speech most susceptible to the effects of elastic recoil is the initial onset of voice, particularly when initiating speech with voiceless plosives as they require the release of a burst of air before the vocal folds come together to begin voicing.

All speakers learn to control these forces effectively for the production of speech; however, for professionally trained singers, this process is highly refined. Singers use substantially larger LVLs than speakers to produce long intricate phrasing, sometimes utilizing nearly 100% VC (Watson & Hixon, 1985; Watson, Hixon, Stathopoulos, & Sullivan, 1990). The greater the LVL above the resting expiratory level, the larger the elastic recoil forces. For that reason, singers must use considerably greater counteractive checking behaviors to overcome elastic recoil at higher LVLs. Voice onset (or vocal attack), is an important characteristic of singing usually involving plosives and vowels. Professional singers, therefore, need to learn to control the onset of voicing of consonants and vowels at varying LVLs to produce a consistent voice quality throughout the sung phrase.

Singing training

Professional voice training is aimed at enhancing the singing voice and should distinguish trained singers from non-singers (Åkerlund & Gramming, 1994; Brown, Morris, Hicks, & Howell, 1993; Brown, Rothman, & Sapienza, 2000; Mendes, Rothman, Sapienza, & Brown, 2003). Postural changes, breathing techniques, abdominal support, vocal function exercises, and articulatory training are thought to help achieve an optimal voice quality (Mendes et al., 2003). For finer control of elastic recoil forces of the thorax at high LVLs, singers aim to use both the primary and supportive muscles of respiration (i.e., diaphragm, external intercostal muscles, transverse abdominal and oblique

abdominal muscles) to stabilize the chest wall during voice onset using the “lean in” technique, part of the *appoggio* tradition of voice training (Miller, 1986; Miller, 1997; Robbin, 2014; Sonninen, Laukkanen, Karma, & Hurme, 2005; Sundberg et al., 1991).

Trained singers’ learn to control the abdominal wall using abdominal muscles, such as the external oblique abdominal muscles to support voicing throughout the sung phrase at varying LVLs. This has been documented using electromyography (EMG) (Pettersen & Westgaard, 2004; Pettersen, 2005; Sundberg et al., 1991; Watson, Hoit, Lansing, & Hixon, 1989), and respiratory kinematics (Sonninen et al., 2005; Sundberg et al., 1991; Thomasson, 2003a; Thomasson & Sundberg, 1997; Thomasson & Sundberg, 1999; Thorpe, Cala, Chapman, & Davis, 2001; Watson & Hixon, 1985; Watson et al., 1990). Several phenomena such as ‘tracheal pull’ (or lowering the larynx) (Iwarsson & Sundberg, 1998; Shipp, Morrissey, & Haglund, 1983) and increasing subglottal pressure (Iwarsson, Thomasson, & Sundberg, 1996, 1998) may alter voice quality, pitch, and loudness at high and low LVLs (Sundberg et al., 1991; Watson & Hixon, 1985; Watson et al., 1990; Watson, Ciccica, & Weismer, 2003).

In vocal pedagogy, another main focus of training is on the onset of voicing, sometimes referred to as vocal attack (Alderson, 1979; Miller, 1986; Miller, 1997). Vocal attack is described as the skillful coordination of stabilized subglottal pressure and appropriate tension of the vocal folds to produce a “clean, crisp initial sound” (Miller, 1997, P. 1). Abdominal support training (trained in the *appoggio* tradition) may also affect voice onset coordination at times (Miller, 1997; Sonninen et al., 2005), in which activation of the primary and auxiliary musculature of respiration is thought to stabilize vocal tract pressures, to prevent excessive airflow at voice onset, especially at high LVLs.

Physiological measures of voice onset

There are two measures of voice onset coordination: voice onset time (VOT) of voiced and voiceless plosives, measured from the plosive release to vocal fold vibration onset (Borden, Baer, & Kenney, 1985; Lisker & Abramson, 1964), and vocal attack time of vowels from the airflow modulation as the onset to vocal fold contact for vibration (Baken & Orlikoff, 2000; Orlikoff, Deliyski, Baken, & Watson, 2009). Research has shown that subglottal pressure, airflow, laryngeal height, and VOT change significantly in speakers and singers at low, intermediate, and high LVLs (Hoit, Solomon, & Hixon, 1993; Iwarsson et al., 1996; Iwarsson, Thomasson, & Sundberg, 1998; McCrea & Morris, 2005; Sundberg et al., 1991; Watson & Hixon, 1985; Watson et al., 1990; Watson et al., 2003). Differences in aerodynamic and acoustic measures have also been observed between trained and untrained singers (Brown et al., 2000; Griffin, Woo, Colton, Casper, & Brewer, 1995; Sonninen et al., 2005).

Iwarsson and colleagues (1998) examined how changes in LVL affected glottal flow measures in untrained subjects at high (90%) and low (20%) LVLs. The authors found that subglottal pressure and peak-to-peak airflow were significantly greater at high LVL as compared to low LVL. Reducing LVL will indubitably reduce the forces of elastic recoil causing a drop in both peak-to-peak airflow and subglottal pressure. Åkerlund and Gramming (1994) compared classically trained and untrained female singers and found no difference in subglottal pressure when singing under the same conditions. However, at high sound pressure level and fundamental frequency, subglottal pressure was significantly higher in trained singers. In another study when untrained singers were compared with trained singers no difference in subglottal pressure was found, although the maximum

airflow declination rate on the glottal pulse was significantly higher in the trained singers (Sulter & Wit, 1996).

Intraoral pressure is the air pressure behind the closed lips during plosive production. If the velum is raised to close the nasal cavity and the vocal folds are open the intraoral pressure is the same as the pressure throughout the vocal system (Rothenberg, 1973). The current studies examined the intraoral pressure in non-singers and trained singers prior to the release of the initial plosive at the initial onset of plosives at different LVLs. Singers must contend with increased initial intraoral pressure so that the plosive opening at onset does not result in an explosion of air before initiating voicing at high LVLs. Trained singers likely learn to control the initial intraoral pressure on vowel and plosive onsets by regulation of oral, tracheal, and lung pressure through checking of respiratory elastic recoil (Miller, 1997; Thomasson, 2003b).

Another aspect of voice production that may change with differences in LVL is VOT (Hoit et al., 1993). Traditionally, VOT has been used to determine differences in voicing of plosive consonants (Abramson & Lisker, 1970; Lisker & Abramson, 1964). VOT was longer at higher LVLs in speech (Hoit et al., 1993). McCrea and Morris (2005, 2007a, 2007b) examined the differences in VOT between singers and controls with somewhat inconclusive results. Male singers had significantly longer VOT after /p/ than untrained singers, while in another study trained female singers had slightly shorter VOT times after /p/ onset. A schwa preceded the /p/ in the female study and not in the male study (e.g. “a peak at the peacock”) which may account for different results.

The initiation of vowel onsets at high lung volumes is a primary focus in vocal performance training (Miller, 1986; Miller, 1997), which can be measured with vocal

attack time (Baken & Orlikoff, 2000; Orlikoff et al., 2009). Singers are thought to initiate voice without extensive airflow or abrupt vocal fold closure on vowels (e.g., hard glottal onset) (Miller, 1986). Carryover from this extensively trained vocal behavior in singers may be seen when voiceless plosives precede vowel onsets after the initial burst of air. The current research measured the time interval from airflow modulation to vocal fold contact initiation for voice (AMCI) to quantify the timing of voice initiation after the release of the plosive on the syllable /pi/.

In the first manuscript we examined the effects of LVLs on voice onset coordination in vocal performance/education majors (vocal students) versus untrained controls. We tested the hypothesis that as initial LVL increased, integrated airflow, intraoral pressure, VOT, and AMCI would increase in singers (pre-trained vocal students) and non-singers. In the second manuscript we examined the effects of vocal training in a university program on voice onset coordination at different LVLs in vocal students. We tested the hypotheses that aerodynamic and temporal measure of voice onset would be decreased between high and low LVLs after the first semester of training in vocal students.

Changes in cortical activation patterns

Changes in brain function can alter singing behavior. Brain function and structure is altered with training of a behavior, development, modifications in sensory or motor function, or use/lack of use (Draganski & May, 2008; Ludlow et al., 2008). Adaptive changes in brain function may result in cortical map reorganization, alterations in synaptic strength and neuronal excitability, and/or modulations in neurovascular coupling (for review see: Ludlow et al., 2008; Nudo, 2006; Nudo, 2011). One method of inducing

neural plastic changes in the central nervous system is through motor skill learning/training (Cooke & Bliss, 2006; Dayan & Cohen, 2011; Doyon & Benali, 2005; Draganski & May, 2008; Draganski et al., 2004; Gaser & Schlaug, 2003; Jensen, Marstrand, & Nielsen, 2005; Kleim, Barbay, & Nudo, 1998; Kleim et al., 2004; Perez, Lungholt, Nyborg, & Nielsen, 2004; Ungerleider, 1995; Ungerleider, Doyon, & Karni, 2002).

Skilled motor function is accomplished through practice and repetition, as well as with feedback involving procedural/non-declarative (implicit) memory and knowledge of results (explicit feedback and memory) (Okano, Hirano, & Balaban, 2000; Wulf, Lee, & Schmidt, 1994). Repetitions and practice can alter how a person performs a skilled motor behavior (Green & Bavelier, 2008; Schmidt, Lange, & Young, 1990; Schmidt & Bjork, 1992). Changes in behavior that persist for days, weeks, months, or years can be distinguished from immediate adaptive changes brought about by transient perturbations (Draganski & May, 2008; Salmoni, Schmidt, & Walter, 1984). Brain activity may also alter with task difficulty, as increased task difficulty can result in greater and more diffuse cortical responses within task specific regions of the brain (Dove, Pollmann, Schubert, Wiggins, & von Cramon, 2000; Gould, Brown, Owen, & Howard, 2003; Sunaert, Van Hecke, Marchal, & Orban, 2000).

Measures of change in cortical functioning, connectivity and neurovascular coupling can be made using functional magnetic resonance imaging (fMRI) (Draganski & May, 2008; May, 2011). Several fMRI studies have compared groups with different levels of experience (e.g. laypersons or novices, amateurs, and professionals) to determine cortical activation differences as a result of experience and showed that

increased skill results in increased cortical activation and cortical map reorganization (Gaser & Schlaug, 2003; Kleber, Veit, Birbaumer, Gruzelier, & Lotze, 2010; Maguire et al., 2000; Münte, Altenmüller, & Jäncke, 2002; Pantev et al., 2003; Rosenkranz, Williamon, & Rothwell, 2007). Other longitudinal studies have investigated changes within subjects before and after training which showed that learning a task results in an initial increase in cortical activation and cortical map reorganization (Draganski & May, 2008; Draganski et al., 2004; May, 2011; Thomas & Baker, 2013; Ungerleider, 1995).

Musicians have been shown to have cortical difference associated with motor skill learning due to their early training, which typically begins in childhood and is fostered by extensive practice and training throughout their lifespan (Gaser & Schlaug, 2003; Kleber et al., 2010; Münte et al., 2002). With classification and comparison of distinct populations, from untrained to professional, increases in cortical activation patterns have been associated with greater skill level in musicians (Gaser & Schlaug, 2003; Kleber et al., 2010; Münte et al., 2002; Pantev et al., 2003).

Most of the research on use-dependent cortical changes in musicians has focused on classically trained instrumentalists comparing non-musicians with musicians (Gaser & Schlaug, 2003; Luo et al., 2012; Pantev et al., 2003; Rosenkranz et al., 2007; Schneider et al., 2002). Very little is known, however, about cortical changes in the vocal system associated with skilled training. A comparison of brain activation between untrained singers, vocal students, and professional singers found increased activation in vocal students and professional singers compared to untrained singers in the somatosensory strip bilaterally, dorsolateral prefrontal cortex, inferior parietal cortex (angular gyrus), and the left temporal pole (Kleber et al., 2010). Differences between professional singers

and vocal students involved increased activation in the right S1 and M1 in professional singers. Activation common to all groups was found in the bilateral lateral premotor cortex and primary auditory cortex. The authors suggested that the recruitment of right M1 and S1 areas in professional singers was related to increased articulatory and laryngeal feedback demands (Kleber et al., 2010). Increased activation in the auditory cortex corresponding to training has been reported in musicians and singers (Pantev et al., 1998; Pantev, Lappe, Herholz, & Trainor, 2009; Zarate & Zatorre, 2008).

The increased activation in the voice production and perception areas of the right and left hemispheres in singers seems to be in direct contrast to previous studies of the effects of skilled motor learning on cortical activation patterns. Single-unit recording studies in primates and fMRI studies of skilled motor learning (e.g., learning finger tapping sequences) have shown that early in learning there is an increase in cortical activation, followed by a decrease in firing as training progresses and the learned behavior becomes more habitual (Chen & Wise, 1995; Ojakangas & Ebner, 1992; Toni, Krams, Turner, & Passingham, 1998). Why vocal training might result in increased cortical activity both in early learning (vocal students) and following extensive training (professional singers) is unknown.

Functional magnetic resonance imaging (fMRI) provides a mechanism to assess changes in cortical activation. Blood oxygenation level dependent (BOLD) magnetic resonance changes are secondary to increases in neuronal firing in a neural substrate. Functional near-infrared spectroscopy (fNIRS) is a non-invasive method using optical wave length transfer in the cortical layer to measure changes in blood oxygenation/deoxygenation. Changes in blood oxygenation levels over time can be

tracked with fNIRS and strongly correlates with the fMRI BOLD response (Boas, 2004; Gagnon et al., 2012; Strangman, Culver, Thompson, & Boas, 2002; Ye, Tak, Jang, Jung, & Jang, 2009).

Through fNIRS analysis, changes in blood oxygenation levels can be examined over time in relation to behavioral events. By event-related averaging from numerous trials, activation patterns can be observed in blood oxygenation level changes in different cortical regions can be related to behavior (Hull, Bortfeld, & Koons, 2009; Kober et al., 2014; Plichta, Heinzl, Ehli, Pauli, & Fallgatter, 2007; Strangman et al., 2002). Motion artifacts can interfere with fMRI measures during analysis of brain activation in response to speech and voice stimuli. Continuous wave fNIRS allows for examination of hemodynamic changes in blood oxygenation level over time, which occurs following a behavior and is not as susceptible to motion artifact that occur during speech (Birn, Cox, & Bandettini, 2004; Friston et al., 1998; Henson, Price, Rugg, Turner, & Friston, 2002; Ishikuro et al., 2014; Kober & Wood, 2014; Sato et al., 2011; Wriessnegger, Kurzmann, & Neuper, 2008).

The purpose of the current research was to examine brain activation levels during the initial voice onset at different LVLs, a demanding control task for professional singers. Increased levels of brain activation have been found in professional singers compared to vocal students and untrained matched controls in the prefrontal, premotor region, inferior somatosensory and auditory regions of the brain (Kleber et al., 2010). However it remains unknown what changes will occur within singers as they are trained. In manuscript 1, we examined the effects of changes in LVL on cortical activation patterns in singers and non-singers. We hypothesized that (1) activation patterns in the

premotor, auditory, and somatosensory regions would be greater at low and high LVLs than at intermediate LVLs in both untrained and trained singers prior to training, and (2) that singers would have greater overall cortical activation than non-singers. We also examined, in the second manuscript, the effects of training on cortical activation patterns in vocal performance students before and after their first semester of university voice training in the western classical style (i.e. opera). Given the previous findings by Kleber et al. (2010), we hypothesized that following training cortical activation patterns would be increased in the right hemisphere compared to pre-training levels.

Title

Voice production at low lung volume levels create significant challenges for singers

Running header

LVL EFFECTS ON CORTICAL ACTIVATION IN SINGERS

Abstract

When professional singers sing at very high and very low lung volume levels (LVLs) they must check the forces of elastic recoil to coordinate voice onset of plosives and vowels. Through training singers become highly skilled at initiating voice at different LVLs, yet little is known about how they learn to do this. The relationship between voice initiation at different LVLs and cortical activation patterns in bilateral perisylvian regions of the cortex in singers has yet to be determined. We compared singers and non-singers on the initiation of a voiceless plosive followed by a vowel at low (30% vital capacity, VC), intermediate (50% VC), and high (80% VC) LVLs. fNIRS was used to quantify cortical activation patterns to compare singers and non-singers during initial voice onset. The singers were first year university vocal performance students, and the non-singers had no singing practice or training. At each LVL from 30% VC to 80% VC, the singers had longer time intervals between airflow modulation to vocal fold contact to initiate phonation after plosive release than non-singers ($p = .004$), and both groups showed greater integrated airflow and intraoral pressure. The cortical activation patterns were similar between the two groups, with reduced changes in cortical activation at 80% VC compared to 30% VC and 50% VC in all cortical locations for the early hemodynamic response. Hemodynamic responses at low LVL showed the highest and most diffuse cortical activation in both groups. Untrained and trained singers had the most difficulty coordinating voice initiation at low LVLs.

KEYWORDS: singers, voice onset time, lung volume levels, respiratory control, vocal fold vibration, intraoral pressure, fNIRS, hemodynamic response, cortical activation, training effects, skill effects, task difficulty

Introduction

Human speech and singing are integral parts of the voice system and involve learned cortical control of the respiratory system (1). During speech and singing, the forces of elastic recoil are present during expiration at high lung volume levels (LVLs) (2). Cortically driven speech respiration allows for the inspiration of adequate LVLs, up to 60-80% vital capacity (VC), to speak long or loud utterances while maintaining adequate duration of expiratory airflow for speech (3-5). To achieve this, speakers must overcome the *forces of elastic recoil* that include gravity, relaxation of the external intercostal muscles and diaphragm, and restoration of the chest wall to its resting shape (6).

Speech expiration is achieved through the generation of counteractive checking forces of the primary muscles of inspiration (diaphragm and external intercostal muscles). At higher LVLs the checking of expiratory elastic recoil forces may be achieved by the continued contraction of inspiratory muscles. At lower LVLs, the checking of inspiratory elastic recoil forces may be achieved through controlled activation of muscles of forced expiration (e.g. internal intercostals and abdominal muscles). Speech onsets with voiceless consonants followed by vowels are susceptible to loss of airflow on the plosive release of air before the vocal folds come together to begin voicing.

Trained singers learn highly refined respiratory checking techniques to control airflow and voice quality throughout the sung phrase at very high and very low LVLs (7). Postural changes, breathing techniques, abdominal support, vocal function exercise, and articulatory training are thought to help achieve respiratory control and optimal voice production at any LVL (8). One of the main areas of study has been abdominal support

(8-11). Singers have been shown to control the abdomen during singing (e.g. abdominal support) through electromyographic (2, 12, 13) and respiratory kinematic studies (2, 7, 12, 14, 15). Singers use these techniques to overcome natural phenomena at higher LVLs such as tracheal pull depressing the larynx (16, 17) and increasing subglottal pressure (18, 19) which can negatively impact airflow and voice quality. Therefore, focus in vocal training is the closure of vocal folds at the onset of voicing (pedagogically termed *vocal attack*) at varying LVLs (11, 20).

Research has shown that subglottal pressure, airflow, laryngeal height, and voice onset time (VOT) change significantly in speakers (18, 19, 21, 22) and singers (2, 7, 9, 23, 24) at low and high LVLs. Differences in aerodynamic and acoustic measures have been found between trained and untrained singers (14, 25, 26). Subglottal pressure and airflow voicing significantly increase from low to high LVLs (18). At low LVLs, near resting expiratory level, subglottal pressure and airflow are controlled by active expiration (6). No differences in subglottal pressure have been found between singers and non-singers (27, 28). Significantly greater peak airflow was observed when singers used trained techniques (such as abdominal support) when compared to not using trained techniques (25). Others have determined that VOT increases as LVL increases in speakers (22). Although comparisons of the effects of training on VOT in singers compared with non-singers were inconclusive, singers had significantly longer VOT, when singing was compared to speaking (29-31).

Vocal attack time is the interval between the onset of acoustic modulation due to airflow and vocal fold contact for phonation onset (32). Initial voice onset at high LVLs is highly trained for vowels and consonants in singers (11, 20). For the current study we

measured the interval between the onset of *airflow modulation* after plosive release to vocal fold *contact* for voice *initiation* (AMCI) to measure changes in voice control at different LVLs.

Singing a new piece of music or performing a novel singing task adds difficulty to the singing task and results in changes in the LVL characteristics within singers (33). Singers will often start phrasing at higher than normal LVLs and end phrasing at lower than normal LVLs, often well below resting expiratory level (where elastic recoil is at equilibrium, (33-35). Singing or speaking at very high LVLs increases the amount of respiratory recoil forces singers have to contend with, and if unchecked can result in aerodynamic and physiological changes in the vocal system (2, 9, 18, 19, 21-23, 29). However, it remains unknown how singers learn to control voice onsets during difficult vocal tasks.

Changes related to task difficulty can be observed in brain function (36-38). Increased task difficulty is associated with greater and more diffuse cortical responses within the involved regions of the brain; for example when comparing a difficult task (speed discrimination task) with an easy task (fixation only) (36). Differences between singers with differing degrees of training have also been reported using functional magnetic resonance imaging (fMRI) which measures the blood oxygenation level dependent (BOLD) hemodynamic response to determine levels of cortical activation within the brain (39). Greater brain activation was found in voice students and professional singers as compared to untrained singers in bilateral somatosensory, dorsolateral prefrontal cortex, inferior parietal cortex (angular gyrus), and the left temporal pole. Common activation was noted in bilateral premotor cortex and primary

auditory cortex between groups (39). On the other hand, increased activation in the auditory cortex related to training was found in musicians and singers (40-42).

To measure cortical activation for speech and singing using fMRI, event-related sparse sampling following a behavior have been used to avoid scanner noise and motion artifact (43). Functional near-infrared spectroscopy (fNIRS) is a non-invasive method that measures changes in blood oxygenation in the most superficial layer of the cortex over time. The hemodynamic response measured with fNIRS strongly correlates with the fMRI BOLD response (44-46). Although fNIRS has less spatial resolution (~1 cm), participants can be seated or standing during recording, and continuous fNIRS event related allows sampling over time at 50 Hz.

The purpose of this study was to examine the effects of voice initiation at different LVLs on cortical responses, which involve differences in task difficulty. Trained singers learn to check the forces of elastic recoil to coordinate an efficient voice onset after plosive release at high and low LVLs. Little is known, however, of how singers learn to control voice onset after plosives at different LVLs. Brain activity patterns likely relate to task difficulty with increases in cortical activity and more diffuse activation as task difficulty increases (36-38). We measured the integrated airflow, intraoral pressure, VOT, and airflow modulation to contact initiation time (AMCI) during voice initiation, as well as cortical activation patterns in premotor, auditory, and somatosensory areas of the cortex at low, intermediate, and high LVLs. As singers are trained to control voice onset after plosives, we hypothesized that: (1) as LVL increased the VOT, AMCI, airflow, and intraoral pressure would increase in singers and non-singers, (2) all participants would have greater cortical activation while performing the

more difficult tasks of singing at low and high LVLs, and (3) singers would have greater overall cortical activation than non-singers.

Materials and Methods

Participants

Volunteer singers and non-singers were recruited from the James Madison University student population through flyers and via in-class presentations. Inclusion criteria included: right handedness, normal hearing, speech, and voice function. Exclusion criteria included: presence or history of voice disorder, history of smoking, current diagnosis or treatment for reflux, current diagnosis of a breathing disorder, diagnosis of a neurological disease, and/or diagnosis or medical treatment for psychiatric disorders (besides depression). Singers were freshmen enrolled in the vocal education and/or vocal performance program. Non-singers were not vocal performance or vocal education majors and had no a history of choral or private voice training. All participants recruited for the study gave written informed consent. The institutional review board for James Madison University approved the protocol.

Procedures

Instrumentation and calibration: PowerLab 16/SP hardware, running LabChart Pro version 7.3.7 was used for data acquisition for all voice and respiratory kinematic measures (ADInstruments, Inc., Colorado Springs, CO). A sampling rate of 20 kHz was used to record all data except the respiratory kinematic channels which were sampled at 1 kHz. Prior to testing, all equipment was calibrated or normalized. A vented

pneumotachometer mask was used to measure airflow (Glottal Enterprises, Syracuse, NY, Model MS110). An air pressure transducer was used to measure intraoral pressure measures (Glottal Enterprises, Syracuse, NY, Model MS110). Airflow and spirometric measures (Universal Ventilation Meter, UVM; Vacu-Med, Ventura, CA) were calibrated using MCU-4 Calibrator (Glottal Enterprises, Syracuse, NY) and interpolated from voltage to liters/second (l/s) in LabChart. Intraoral pressure was calibrated using the MCU-4 Calibrator and converted to cm/H₂O. Vocal fold contact was detected via electroglottograph (EGG) transducers connected to an EG-2 two-channel EGG amplifier (Glottal Enterprises, Syracuse, NY). Respiratory kinematics were transduced using the Inductotrace inductive plethysmograph (Ambulatory Monitoring Inc., Ardsley, NY). The Inductotrace amplifier levels were set to one for both the ribcage (RC) and abdomen (AB) and the DC signals were recorded in LabChart.

Voice physiology measures setup and recording: Participants were positioned standing 3-4ft from a computer monitor set at eye level with their head and back stabilized against a secured equipment cart and cushioned pole raised to 7 ft. The monitor provided realtime biofeedback of participants' respiratory kinematics during testing. A stand holding the pneumotachometer mask was set up leaning against the face of a participant at the appropriate level to ensure a proper seal. A small tube was sealed through the mask and placed between the lips into the mouth for determining intraoral pressure during lip closure (47, 48). The stand holding the mask allowed the participant to relax between trials and aided in sealing the mask during trials. A Sony ECM-55B condenser microphone was sealed in the mask to record the speech signal (Sony, Japan).

The EGG transducers were secured to the neck at the level of the thyroid cartilage and connected to an EG-2 EGG amplifier to transduce vocal fold contact.

Participants were also fitted with Inductobands around the abdomen at the level of the umbilicus and the ribcage inferior to the axilla (49). The AB, RC, and combined sum of AB plus RC movements were recorded in separate channels in LabChart. During LV normalization, subjects performed 3 vital capacity (VC) maneuvers, while breathing through the spirometer. For the VC maneuver, subjects were instructed to inspire as much air as possible before exhaling all the air out of the lungs. This normalization technique allowed for comparing LVL based on the VC maneuver. Due to substantial drift of respiratory kinematics throughout testing and the need for realtime biofeedback of respiratory movements for testing target acquisition, we normalized the combined sum signal to percent vital capacity (%VC) for each individual prior to each condition. The combined sum signal was converted from voltage to %VC in LabChart and displayed in realtime on the monitor to provide biofeedback of %VC to participants during testing.

fNIRS setup and recording: A continuous wave fNIRS system (NIRSOptix TechEn, Milford, MA, model CW6), sampling at 50 Hz, was used to measure blood oxygenation and deoxygenation patterns in the inferior premotor cortex (premotor), inferior somatosensory cortex, and auditory cortex (posterior-superior temporal gyrus; pSTG) of the right and left hemispheres (Table 1 and Fig. 3). The fNIRS system uses 690 nm and 830 nm wavelengths of near-infrared light to measure concentration changes in oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (HbR) by means of a modified Beer-Lambert Law (50). We used 4 emitters and 6 detectors (Fig. 3). Each emitter-detector pair was 3 cm apart and measured the oxygenation in cortical substrate at

the mid-point between the pairs. The near-infrared light emitted from the optodes was modulated between 6.4 to 12.6 kHz with an interval of 200 Hz to reduce cross-talk contamination between channels (51). The 690 nm laser optodes were calibrated to 12 mW average power and the 830 nm laser optodes were calibrated to 6 mW average power.

Neuronavigation for identifying Talairach coordinates: Participants' heads were fitted to a female high-resolution brain anatomical MRI template using the BrainSight Neuronavigational System version 1 (Rogue Research, Montreal, QC). Following MRI fitting, the placement for fNIRS emitters and detectors were marked on the scalp to the areas corresponding to the premotor, somatosensory, and auditory regions of the left and right hemispheres of the brain according to previously determined Talairach coordinates (Table 1) based on (39, 52, 53). The coordinates for the areas of interest were in millimeters along the left-right (x), anterior-posterior (y), and superior-inferior (z) axes. The fNIRS emitters and detectors were then placed on the scalp prior to testing using two flexible plastic templates and the optodes secured to the head using Coban self-adhering wrap.

Production task performance

All participants produced /pi/ at 262Hz (middle C) 30 times at 3 specific %VC conditions (80%VC, 50%VC, and 30%VC, presented in that order for all participants). A go signal (green light) was used to signal experimental trial onset and completion. Every fifth trial there was a rest trial in which the go signal was not lit, for a total of 35 experimental and rest trials per condition. Before each %VC condition, participant's conducted a VC maneuver to normalize the sum trace into %VC to account for any drift

in the signal caused by postural adjustments or movement affecting the Inductobands between trials. The signal was scaled from 0-100 %VC. Participants monitored the go signal light mounted on the computer screen along with realtime biofeedback of %VC and a highlighted target line at 30, 50, or 80% VC condition in LabChart (Fig. 1).

After several practice trials using the biofeedback to monitor their breathing to attain the target %VC and produce the singing task after go signal was lit, participants received formal instructions and a reference pure tone at 262Hz (middle C) before testing began. LabChart and fNIRS recordings were started at approximately the same time and testing began after a 30 second rest. When the go signal was turned on, participants checked their body position with their head and back firmly against the cart and pole and pressed the pneumotachometer mask firmly against their face, not moving their face out to the mask. They inspired above the target %VC and then exhaled until they were within the target. They then sang /pi/ and held the vowel until the go signal light was turned off 3 seconds after voice onset (Fig. 1). Participants were instructed to sing /pi/ in their best voice quality as if they were “starting an aria.” The task was repeated 30 times with 5 rest trials.

Participants were instructed to remain still and relaxed during the inter-trial intervals and only move at the go signal to set-up for that trial. Participants were instructed to move if needed (e.g. cough, swallow, stretch, etc.) before the go signal was on, rather than during the inter-trial intervals to avoid motion artifact during a hemodynamic response. If a participant swallowed when the go signal came on, the timer was reset and the trial repeated. If there was drift noted in the biofeedback signal at any

time during the condition, testing was stopped and a VC maneuver was conducted to rescale the sum signal to 0-100% VC before continuing the condition.

Data processing

Physiological measures: All signal processing and measurement calculations were conducted in LabChart. The four voice physiology measures were as follows:

Voice onset time (VOT) was defined as the time interval from initiation of plosive release to the onset of voicing (54, 55). The first derivative of the EGG signal was calculated using a window width of 33 points; no drift in the signal was detected. VOT was measured from the onset of airflow at plosive release to the first peak of the first derivative of the EGG signal (adapted from 54) indicating the first instance of vocal fold contact (56). The VOT, in milliseconds (ms), were collected for each of the 30 repetitions of a condition and then averaged for each participant (Fig. 2).

Airflow modulation to contact initiation (AMCI) was defined as the time interval from onset of modulation of the airflow after plosive release to the onset of vocal fold contact (adapted from 32). The first derivative of the airflow signal was calculated using a window width of 33 points, and the time interval from first periodic peak (modulation) of the derived airflow signal (within $\pm 1SD$) to the first peak of the first derivative of the EGG signal was determined for each trial using Peak Analysis in LabChart. The mean AMCI interval (ms) was calculated across 30 trials in each condition for each participant (Fig. 2).

Integrated airflow was defined as the area under the curve in the raw airflow signal, from the onset of airflow after the plosive release to the periodic modulation of the

first derivative of the airflow signal. Measures in milliliters (ml) were collected and averaged for each participant for each condition (Fig. 2).

Intraoral pressure was defined as the peak pressure before the initial plosive release was smoothed at 33 points to determine the absolute peak measurement from baseline using Peak Analysis in LabChart. The peak was confirmed using the 1st derivative of the raw intraoral pressure signal calculated in a separate channel in LabChart. All peaks within a condition were averaged for each participant for each condition (Fig. 2).

All physiologic data collected were transferred to a spreadsheet for data analysis.

fNIRS data processing: Hemodynamic Evoked Responses 2 v.1.5.2 (HomER2; www.nmr.mgh.harvard.edu) was used for data analysis. The raw acoustic signal, airflow signal, intraoral pressure signal, and go signal were recorded as auxiliary inputs in the fNIRS system. The onset times for each trial were identified for both LabChart and HomER2 using linear interpolation so that the timing in the two separate systems was synchronized.

Expiratory onset was used to mark experimental trial onset. Expiratory onset was defined as the onset of expiration following the go signal and prior to singing onset (Fig. 1). Expiratory onset was determined in LabChart when the 1st derivative of the sum signal crossed zero indicating a transition from inspiration to expiration after the go signal. Trials were eliminated from analysis if: there was excessive movement (e.g. throat clear, swallow, cough, etc.) observed 10 seconds before or after the go signal, or expiratory onset occurred before the go signal was lit.

After the expiratory onset marker times were inputted into HomER2, the raw fNIRS signals were processed for analysis. Three distinct sources of noise in fNIRS were removed. Instrumental noise was removed using a low-frequency bandpass filter set to 0.5 kHz (57). Experimental noise, usually related to motion artifact, was removed using a principle component analysis (PCA) filter for all channels (57). After checking the signals for the presence of the cardiac artifact to confirm data quality following acquisition, a 0.010 kHz high-frequency bandpass filter was used reduce the impact of physiological artifact. As motion artifact from temporalis muscle contraction due to jaw motion can interfere with fNIRS recordings, the mask was on a stand securely pressed against the face and chin to reduce jaw movement (52, 57, 58).

The optical signals were converted to optical density in HomER2. The relative concentrations of HbO, HbR, and total hemoglobin were then derived in HomER2 for analysis. Trials were identified using expiratory onset markers and event-related averaging of 20 s epochs (5 s before stimulation markers and 15 s after) were calculated for each %VC condition and plotted by channel and side (Fig. 3). A minimum of 10 trials was required for each epoch.

The HbO and HbR signals were visually examined to check for an inverse relationship between them to indicate a cortical response and not physiological artifact (Fig. 5) (59). The data for HbO and HbR cortical responses from fNIRS analysis in the premotor, somatosensory, and auditory regions of the right and left hemispheres (6 locations) were exported to a spreadsheet. Time windows of interest were: baseline: -5 to -1 seconds, peak 1 between 2 to 6 seconds; and peak 2 between 6 to 13 seconds. The peak amplitude of each peak for the 6 HbO channels was determined to be the highest or

lowest point (depending on the direction of the peak) in the HbO signal in each condition (30% VC, 50% VC, and 80% VC; Fig. 4). Baseline measures were calculated from the averaged HbO signal from -5 to -1s of the curve. Raw HbO data were then converted into a Z-score using the formula, $Z = (\text{peak amplitude} - \text{mean baseline}) / \text{baseline SD}$. This conversion provided a normalized measure of amplitude change from baseline.

Several interval markers occurring within each 20 s epoch were identified for analysis including: go signal onset, voice onset, HbO peak 1, voice offset, HbO peak 1 offset, and HbO peak 2. The go signal recording in LabChart was used to identify go signal onset as the first moment of positive divergence from zero at the outset of each epoch. Voice onset was the EGG signal time identified for calculating AMCI and VOT. Voice offset was identified at the end of each trial as the final peak in the 1st derivative of the EEG signal indicating the offset of vocal fold vibration. During peak 1 amplitude detection between 2 and 6 s, the peak 1 onset time was identified. Between 4 and 8 s the absolute negative peak 1 offset time and amplitude was determined for peak 1 offset. Within the final 6 to 13 s window, the time at the absolute second peak was identified. All interval markers identified in LabChart (go signal onset, expiratory onset, voice onset, and voice offset) were time synchronized using linear interpolation to fNIRS so that all interval markers were in a common time signature. All times were recorded into a spreadsheet.

Statistical analysis

All physiological voice measures were analyzed using SPSS version 22 (IBM) and SYSTAT version 13 (Crane Software International Ltd). The general linear model (GLM) was a two-factor mixed Analysis of Variance (ANOVA) with a between subjects

factor (singers vs. non-singers) and a within subjects factor (%VC conditions) to examine main effects and interactions. Post-hoc Fisher's least significant differences (LSD) comparisons were calculated for significant results to determine where the significant differences occurred. Alpha was set a priori at 0.0125 (.05/4) after Bonferroni correction for 4 analyses, one for each voice measure.

The fNIRS data for the first and second peaks (peak 1 and peak 2) in the HbO signals were converted into the Z-score of change. The GLM was a four-factor mixed ANOVA with three within subjects factors (%VCs, side, and location) and one between subjects factor (singers and non-singers) was computed for peak 1 and peak 2 with Bonferroni correction for 2 analyses ($\alpha = .025$). Post-hoc Fisher's LSD comparisons were calculated for significant results.

The time intervals between the go signal and expiratory onset, and expiratory onset and voice onset were examined. The GLM was a two-factor mixed ANOVAs for within-subjects factors of 3 %VC conditions between the two groups. Alpha was Bonferroni adjusted a priori to .025 to account for the two time intervals examined, the average time from the go signal to expiratory onset, and from expiratory onset to voice onset. Post-hoc Fisher's LSD comparisons were calculated for significant results.

Results

The singers were 10 female freshman vocal performance and/or vocal education majors prior to their first semester of vocal training, and the non-singers were 10 untrained female controls. Singers (mean age 18.2 years) had an average of 3.1 years (range .25 to 9 years) of private vocal training and 7.2 years (range 2 to 13 years) of

choral training All singers were sopranos trained in the western classical style (opera). Of the 12 singers recruited and consented, 2 were excluded from the study due to a current diagnosis of reflux disease. Non-singers (mean age 20.2) had no history of private or choral training and all who gave written consent completed the study.

Reliability

Inter-rater and intra-rater reliability were determined using the 5 data points per trial used to calculate the 4 physiological voice measures and the stimulation markers for fNIRS (time at: airflow onset, airflow modulation, first peak of EGG, go signal on, and expiratory onset; Fig. 1 and 2). A total of 1,800 data points were compared for Kappa agreement between and within investigators averaged across the 5 equally weighted data points for 20% of the data.

Inter-rater analysis revealed an average Kappa agreement = .898, $p < .0001$ (range .774 to 1.00) indicating very good agreement between investigators (60). Kappa agreement for intra-rater analysis revealed an averaged Kappa = .871, $p < .0001$ (range .757 to 1.00), also demonstrating very good agreement within the primary investigator.

Physiological voice measure results

A Greenhouse-Geisser correction was calculated for AMCI, intraoral pressure, and airflow which did not meet the assumption of sphericity. All other statistical assumptions were met.

VOT did not show a %VC effect, $F(2, 34) = .45$, $p = .64$, $\eta^2 = .01$ (Table 2) nor a group effect, $F(1, 17) = .22$, $p = .62$, $\eta^2 = .02$. A non-significant trend in the interaction of %VC and group, $F(2, 34) = 3.515$, $p = .04$, $\eta^2 = .17$, indicated that singers may have had increased VOT from 30% VC to 80% VC while non-singers did not (Fig. 5A).

Results for AMCI revealed a Greenhouse-Geisser corrected significant % VC effect, $F(1.29, 22.00) = 7.80, p = .007, \eta^2 = .24$, and a significant group effect for ACMI, $F(1, 17) = 10.82, p = .004, \eta^2 = .39$, which indicated that singers had significantly longer AMCI times than non-singers. ACMI showed an interaction between % VC task and group effects, $F(1.29, 22.00) = 7.74, p = .007, \eta^2 = .39$ (Table 2, Fig. 6B).

Post-hoc Fisher's LSD corrected pairwise comparisons of the simple effects of the interaction showed that the difference between singers and non-singers varied between LVLs. Singers had significantly longer AMCI times than non-singers only for the 80% VC condition ($p = .002, d = 1.78$). There were no significant differences between singers and non-singers for either 30 or 50% VC. Singers also exhibited significant differences between 30 and 80% VC ($p < .001, d = 1.68$), as well as between 50 and 80% VC ($p = .002, d = 1.23$). Non-singers showed no significant differences between % VC conditions (Table 2). Approximately 63% of the within-subjects variability in AMCI was explained by the LVL effect ($\eta^2 = .24$) and the interaction between singer and non-singers and LVLs ($\eta^2 = .39$).

Intraoral pressure revealed a Greenhouse-Geisser corrected significant % VC effect, $F(1.43, 21.48) = 15.57, p < .001, \eta^2 = .75$ (Table 2). Neither the group effect, $F(1, 15) = .34, p = .571, \eta^2 = .02$, nor the interaction between % VC and group effects was significant, $F(2, 30) = .05, p = .90, \eta^2 = .00$ (Fig. 5C). Post-hoc pairwise comparisons of the estimated marginal means revealed that intraoral pressure significantly increased from 30 to 80% VC ($p = .001, d = 1.43$) as well as from 50 to 80% VC ($p = .001, d = 1.05$) (Table 2.). Effects % VC accounted for nearly 75% ($\eta^2 = .75$) of the within-subjects variance in intraoral pressure.

Integrated Airflow had a Greenhouse-Geisser corrected significant % VC effect, $F(1.54, 27.34) = 6.394, p = .009, \eta^2 = .26$. There was no significant group effect, $F(1, 18) = .265, p = .613, \eta^2 = .01$, nor an interaction between % VC and group, $F(1.54, 27.34) = .581, p = .564, \eta^2 = .02$ (Table 2, Fig. 5D). Post-hoc pairwise comparisons of estimated marginal means for % VC conditions showed integrated airflow for 80% VC was significantly greater than 50% VC ($p = .011, d = .62$) and 30% VC ($p = .012, d = .91$).

fNIRS results

There was a within-subjects main effect of % VC on cortical Z-scores on peak 1, $F(2, 28) = 14.833, p < .001, \eta^2 = .24$, no between-subjects group effect on peak 1, $F(1, 14) = .059, p = .812, \eta^2 = .00$ (Fig. 6, Fig. 7), no side effects, $F(1, 14) = 3.881, p = .069, \eta^2 = .01$, and no location effects, $F(1, 14) = 3.869, p = .069, \eta^2 = .01$. There were no significant interactions. Post-hoc pairwise comparisons determined that significant differences were present between 30% VC ($M = 11.335$) and 80% VC ($M = -3.542, p < .001, d = 1.94$), as well as between 50% VC ($M = 8.644$) and 80% VC conditions ($p = .003, d = 1.66$). Results of analysis of cortical activation patterns for peak 2 revealed no significant effects or interactions.

Onset intervals

There was a significant % VC effect on the time from the go signal to expiratory onset, $F(2, 36) = 38.507, p < .0001, \eta^2 = .64$. There were no group effects, $F(1, 9) = .732, p = .403, \eta^2 = .04$, and no interaction, $F(2, 36) = 3.309, p = .048, \eta^2 = .04$. Post-hoc analysis of % VC effect revealed that at 80% VC it took significantly longer to get to expiratory onset than at 50% VC ($p < .0001, d = 3.0$) or 30% VC ($p < .0001, d = 3.65$) (Fig. 8).

There was a significant effect of %VC on the time interval from expiratory onset and voice onset, $F(2, 36) = 16.331, p < .0001, \eta^2 = .39$, as well as an interaction between %VC and group, $F(2, 36) = 7.088, p = .003, \eta^2 = .17$. Group did not have an effect on the time interval, $F(1, 18) = 1.575, p = .456, \eta^2 = .30$ (Fig. 8). Post-hoc analysis of the simple effects of the interaction showed that at 30% VC non-singers took significantly longer than singers to onset voice after beginning to exhale to the target %VC ($p = .014, d = 1.29$). Also, the length of time it took non-singers to reach expiratory onset significantly decreased from 30 to 50% VC ($p = .011, d = 1.57$) and to 80% VC ($p < .0001, d = 1.88$). At 50% VC, singers took significantly longer to reach expiratory onset than at 80% VC ($p = .001, d = 2.06$).

Discussion

We examined the effects of LVL (based on %VC) and training on physiological voice measures and cortical activation patterns in singers and non-singers. As LVL increased from low to high, integrated airflow and intraoral pressure significantly increased in all participants, and AMCI increased only in the singers. Voice onset time did not vary between groups or across LVLs. Cortical activation patterns were similar between groups with significant LVL effects showing reduced peak amplitude of peak 1 at 80% VC compared to 50% VC and 30% VC. Cortical activation patterns did not vary by side or location. Peak 2 amplitude showed no significant main effects or interactions.

The production task allowed for the investigation of both physiological voice behaviors and cortical responses to voice production at different LVLs. Our production task was normalized to each participant based on VC before each trial to account for positive and negative drift in the signal. Realtime biofeedback was used to target different

LVLs across participants. By using event-related processing we were able to examine cortical activation associations with voice initiation at different LVLs.

We sought to obtain a comprehensive understanding of the effects of LVL and training on the initial onset of voice (pedagogically termed vocal attack). Although postural changes and abdominal support have been extensively examined in singers, very little focus has been on initial voice onset. Few studies have specifically examined aerodynamic features of the initial voice onset in singing although its importance in singing training is difficult to overstate. Miller (1997) emphasizes the need to develop skillful coordination of initial voice onset to achieve a good sound, while others have emphasized trained singers' need to create consistent flow and precise control over constantly changing subglottal pressure (7, 61). We measured aerodynamic parameters of voice production (integrated airflow and intraoral pressure) along with temporal aspects of voice onset (VOT and AMCI) to provide the most insight into the coordination of voice onset.

Voice onset time (VOT), the time from release of the airflow of the plosive to the onset of vocal fold vibration has been previously examined in singers (29-31). Here, measures of VOT showed no significant main effects from changes in LVLs. Our findings differ from results of a study examining the effects of LVL on VOT in male speakers with no history of voice training where VOT significantly decreased with decreasing LVL (22). The investigators suggest that VOT decreased as the participant approached low LVLs due to a need to conserve air. Others suggested that at low LVLs, subglottal pressure and airflow are reduced making voice onset more difficult initiate (18, 24). This is where the longer times between peaks in the hemodynamic response and onset of voice at 30%VC would have substantiated greater difficulty at low LVLs and agree with other suggestions.

Airflow modulation to contact initiation (AMCI) was our measure of voice onset (vocal attack) of vowels after plosive release. The onset of modulation of the airflow signal is due to the approximation of the vocal folds as they close to initiate voicing (32). The significant interaction between the trained singer and non-singer group and LVL showed that, at high LVL, singers had significantly longer AMCI times than non-singers. We also determined that as singers' LVL increased, their AMCI times significantly increased while non-singers did not alter their AMCI times between LVLs (Table 2, Fig, 6B).

Singers make a conscious effort to improve their voice control during training, which likely accounts for their longer AMCI times. However, they apparently had more difficulty efficiently controlling the onset of voicing after the plosive release when there was greater positive intraoral pressure likely due to increased elastic recoil at high LVL. This evidence appears to indicate that training resulted in singers taking a longer time to close their vocal folds at high LVL. Non-singers do not consciously change their voice control while singing and therefore showed no differences between AMCI times at low, intermediate, or high LVL (Table 2). Longer AMCI times at higher LVLs may be due to increased subglottal pressure and flow making vocal fold closure more unstable when initiating voice at high LVLs. As our trained singers were enrolled as freshmen in university singing training, further study with continued training would determine if skill level within singers would result in reduced AMCI time at high LVLs. AMCI was the only measure that clearly showed a significant training effect and may be an important measurement for understanding voice onset with singing training.

Intraoral pressure significantly increased from 30 to 50 to 80% VC. As intraoral pressure is an indirect measure of vocal tract pressure when the lips are closed, the velum is elevated and the vocal folds are open, it should be related to subglottal pressure even though we were not able to measure subglottal pressure during repeated plosives (47, 48). Our findings of increased intraoral pressure with increased LVLs are in agreement with investigations into LVL effects on subglottal pressure in untrained singers (18, 19). However, previous studies found no significant effect of LVL on subglottal pressure in professional trained singers (24). Thomasson (24), proposed that singers have more independent accurate control over vocal loudness, the main physiological voice parameter controlled by subglottal pressure (62), and therefore have more independent control over subglottal pressure. However, the trained singers in our study showed the same LVL effects on intraoral pressure as untrained singers. As our singers were entering vocal performance training at the university, they may not have yet developed the skills of professional singers in controlling vocal tract pressure prior to voice onset.

In a previous study, trained singers using abdominal support techniques while singing at soft, normal, and loud intensities exhibited increased intraoral pressure with increased intensity, as compared to those singing without using training techniques (25). However, others comparing classically trained female with untrained female singers found no significant differences in subglottal pressure between groups (27). Trained singers, however, used significantly higher intraoral pressure when singing at high intensity and pitch, demonstrating that subglottal pressure will vary with voice intensity (28). Our findings showing no differences in intraoral pressure between trained and untrained singers may also be due to a lack of increases in vocal intensity during singing.

Airflow measures have been shown to increase, along with intraoral pressure with increases in LVL (18, 19). Investigators have shown that peak to peak flow (l/s) and glottal pulse (l/s) significantly increased from low to high LVLs. We determined that LVL influenced the amount of airflow loss during plosive release. This may explain why AMCI increased with LVL. If greater flow loss occurred with increased LVL, then the time to initiate vocal fold contact may have taken longer when airflow levels were higher. These trends make sense for untrained singers since they are not trained to use advanced counteractive checking techniques to reduce elastic recoil effects on pressure and flow which increase at higher LVLs (6). However, the increase in AMCI with LVLs occurred in the singers and not in the untrained group, while airflow and intraoral pressure increased to a similar degree in both groups with increased LVLs. We are therefore left with conclusion that singing training effectively reduced the ability of singers in this study to control closure of their vocal folds at higher LVLs.

Relating voice production with cortical activation

Our production acquisition protocol was initially devised to allow event-related averaging to examine cortical activation in relation to voice initiation at target LVL. Therefore, we allowed for adequate inter-trial-intervals (12 to 18 s) to measure hemodynamic responses after a voice production task lasting 3 s. In addition, the production task had to be adequately difficult at high and low LVLs to reveal the effects of task difficulty on voice and respiration coordination on cortical activation.

As a consequence, participants were forced to delay voice onset after they reached a target LVL before beginning vocalization. When we examined the hemodynamic responses in the pilot data, we noticed that the HbO had a peak (peak 1) that began at or directly after

voice onset, followed by a dip in the signal before rising again to a second peak that usually started 4-6 s after voice onset, but peaked at 8-9 s after voice onset (Fig. 4).

Peak 1 may reflect cortical activation related to preparation for voice task production which involved reaching a specific LVL and initiating expiration prior to voice onset which occurred 0.8- 2.4 s after expiration onset (Fig 9.). Therefore we used expiratory onset prior to voicing as the event onset which effectively placed the onset of peak 1 hemodynamic response within the acceptable range of 1-2 s past stimulation onset, with the peaks occurring 2.5-6.5 s after event onset (63). Having determined that expiratory onset was well related to the peak 1 response, we were able to examine blood oxygenation levels related to task onset.

fNIRS: We found that differences between singing at high and lower LVLs had a significant effect on peak 1 showing reduced blood oxygenation changes at 80%VC. No group effects, side (right vs. left hemisphere), or location (auditory, somatosensory, or premotor), nor interaction effects were observed in the results. However, contrary to our expectations, increases in LVL were associated with lower activation in the specific areas of the perisylvian region of the lateral cortex. The lower LVLs of 30 and 50%VC had higher levels of cortical activation than at 80%VC, which corresponds with greater respiratory and laryngeal control of respiratory preparation for voice onset at low LVLs.

It is possible that the smaller increase in HbO during the 80% LVL condition in both non-singers and singers may be the result of similar processes. If subjects take less time to prepare to vocalize after expiratory onset at 80%VC, the peak 1 hemodynamic response may be less pronounced. Though they reached expiratory onset significantly faster at 30%VC, they then took significantly longer to exhale to the target, which was

mostly active exhalation below resting expiratory level. At 80% VC, from the go-signal onset, they took longer to inspire above 80% VC; however once they were above the target and began expiration, they rapidly attained the target LVL (Fig. 8). As they took longer from the expiratory onset, which was shown to be a more accurate marker than the go signal of the hemodynamic response in peak 1, to onset of voice at 30 and 50% VC, then the brain activation may be greater. As respiration is more difficult to control at low LVLs than at high LVLs, the greater cortical activation levels are likely reflecting task difficulty at 30% VC.

At 80% VC the process of exhalation is totally passive, entirely depending on counteractive checking of the forces of elastic recoil with no active expiratory force required to onset and maintain voicing (6, 64). Expiring at high lung volumes is a relative easy task and does not require a significantly greater cognitive load. 80% VC is not much higher than the LVLs achieved when shouting or speaking loudly (4, 5). The reduced task difficulty is reflected in the decreased HbO at 80% VC in both singers and non-singers.

At 50% VC the singers began assisting passive expiration to compensate for reduction in elastic recoil forces and pressure as the chest cavity closes in on respiratory thoracic equilibrium (e.g. resting expiratory level) (6). Expiratory onset also marks the point at which many of our participants exhibited respiratory patterns consistent with speech and singing respiration (Fig. 8). At 50% VC we saw positive cortical activity at peak 1 during voice preparation. The cortical activity associated with respiration and voice behaviors becomes apparent in the significantly greater positive HbO signal (Fig. 7B).

80% VC required the least cortical effort, or cortical load, to perform and may even be the preferred LVL to initiate singing (64, 65). It follows, then, that 30% VC may be the

most difficult production task as it is below reported resting expiratory levels (~37%VC) for singers and speakers (5, 6, 23, 64). Speakers rarely drop below resting expiratory level when speaking (5, 6) and singers mostly terminate voicing just at or above resting expiratory level (34, 64). Cortical activation at 30%VC was significantly higher than 80%VC, but it was not significantly greater than 50%VC ($p = .211$).

Possible Limitations

The methods for testing LVL conditions was not counterbalanced to control for possible order effect. The fact that our findings are concurrent with other research using different protocols helps to alleviate that concern. Future research on this topic, however, should counterbalance the %VC conditions to better control for order or fatigue effects which could have affected our measurements. We did not control intensity or change it; therefore, increases in intensity may have been caused by some of the voice measures, especially intraoral pressure and integrated airflow. Finally, we cannot be sure what singing and/or respiratory behavior peak 2 in the hemodynamic response reflects as it did not show any task effects. Further examination of the interval markers may shed light on the peak 2 cortical response.

Conclusions

Initial voice onset coordination at varying LVLs is a highly trained vocal skill often of primary importance to singers and voice teachers. Through the combination of several modes of measurement, we were able to examine characteristics of respiration, voice onset, and cortical control, not seen before in the voice literature. By combining physiological temporally-based parameters and aerodynamic characteristics of the voice while exploring the underlying changes that were elicited in the brain, we have been able to present a

comprehensive view of voice onset coordination. We showed that training may reduce singers' ability to rapidly and consistently close the vocal fold after plosive onsets at high LVLs and that non-singers may be less skilled at controlled expiration past resting expiratory level. We confirmed previous reports that increasing LVL significantly increases intraoral pressure and the volume of air released on plosives, but that the increase in pressure and flow caused by high elastic recoil forces, does not equate to increased vocal difficulty. Cortical measures actually show that it may take significantly less effort and skill to phonate at high LVLs than at low LVLs, and that singing at low LVLs may increase the complexity of otherwise simple vocal tasks. Further examination is necessary, but we showed that cortical activation at high LVLs was lower than when conducting the same behavior at low LVLs, likely reflecting differences in task difficulty. The effects of training have to be investigated further, as our professional singers may change in their vocal preparation and production with further training and professional experience.

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Tables

Table 1

Talairach coordinates for regions of interest examined using fNIRS (39, 52, 53)

	Regions	x	y	z
Left	Inferior Premotor area	-53	7	23
	Primary inferior Somatosensory Cortex (somatosensory - orofacial)	-57	-23	12
	Posterior superior temporal gyrus (Auditory)	-60	-34	16
Right	Inferior Premotor area	53	7	23
	Primary inferior Somatosensory Cortex (somatosensory - orofacial)	57	23	12
	Posterior superior temporal gyrus (Auditory)	64	34	12

Table 2

Results of mixed 2-factor ANOVA of the %VC effects on physiological voice measures, with means for singers and non-singers.

Measure	Group	Average (sd)			% VC Effect		
		30% VC	50% VC	80% VC	df	F	p
VOT (ms)	Non-singers	99.55 (23.14)	99.62 (37.67)	91.58 (31.14)	2,34	.453	.640
	Singers	96.01 (31.67)	101.63 (37.66)	113.15 (39.67)			
AMCI (ms) [†]	Non-singers	4.51 (2.74)	4.3 (3.47)	4.45 (1.8)	2,34	7.80	.000*
	Singers	7.34 (2.83)	8.81 (4.47)	14.52 (8.27)			
intraoral pressure (cm H ₂ O)	Non-singers	6.59 (1.87)	7.08 (1.91)	8.77 (2.36)	2,30	15.57	.000*
	Singers	6.96 (2.11)	7.68 (2.1)	9.39 (2.47)			
Integrated Airflow (ml)	Non-singers	38.05 (17.91)	40 .01 (20.06)	50.83 (27.51)	2,36	6.861	.007*
	Singers	39.01 (31.84)	47.95 (42.68)	67.89 (50.67)			

* Significant %VC effect. [†] Significant Group effect and interaction.

Figure Legends

Figure 1.

Event-related design. Example of screen participants used during production task showing respiratory biofeedback (%VC) with target guidelines for target LVL acquisition and go signal. For this example (80% VC), go signal was turned on, participant inspired above target LVL (Expiratory Onset), controlled expiration until in target range and then vocalized until signal was turned off.

Figure 2.

The method for calculating integrated airflow, intraoral pressure, VOT, and AMCI. Integrated airflow is the area under the curve from plosive release to airflow modulation. Intraoral pressure is the peak of the pressure signal before plosive release. VOT is measured from the onset of airflow (denoting the point of plosive release) to the peak of the first derivative of the first glottal pulse indicating initial vocal fold contact (54). AMCI measured from the first periodic peak (± 1 SD) of the first derivative of the airflow signal (airflow modulation after plosive release) to the peak of the first derivative of the first glottal pulse (66).

Figure 3.

fNIRS optode placement. The red letters are emitters and the blue numbers are detectors. The brain regions measured are the green circles on the black lines between the emitters and detectors. A3 measures the dorsolateral premotor area, B2 – orofacial/laryngeal somatosensory area, and B1 – posterior superior temporal gyrus auditory area.

Figure 4.

fNIRS output (HbO and HbR) for Right Auditory area at 50% VC singer condition in a averaged across all participants with +/- 1 SE error bars. Baseline derived from an average of -5 s to -1 s for calculating Z-scores of change. The orange line is the go signal onset, the black line a 0 represents the expiratory onset, the green line represents the average onset of voicing (~2 s after expiratory onset), and the red line is voice offset. Peak 1 (P1) and peak 2 (P2) of the HbO signal are identified, along with P1 offset (O1). HbR inverse relationship HbO is present.

Figure 5.

Box plots of the group for each of the four physiological voice measure by % VC.

Figure 6.

Box plots of cortical activation patterns show groups by % VC for all activity to examine LVLs effect by group for peak 1 (A) and peak 2 (B) at 30, 50, and 80% VC.

Figure 7.

Box plots comparing a 6 cortical locations, combined by group, at the LVL examined.

Below are examples of the auditory hemodynamic response on the left side that correlate with the boxplot for left auditory each LVL condition.

Figure 8.

A and B are plots of the time from go signal onset to first (orange) expiratory onset and then to voice onset (Blue). Data is presented as means in seconds and (SD). The brackets are indicating the interval from expiratory onset to voice onset. Note that it takes significantly longer for both groups to achieve target at 80% VC, but then achieve their target and onset voicing relatively quickly. At 30% VC those characteristics flip and singers and non-singer reach expiratory onset relatively quickly and it takes them a relatively longer time than 80% VC to achieve target LVL to begin singing (non-singers significantly longer than singers).

Figures

Figure 1.

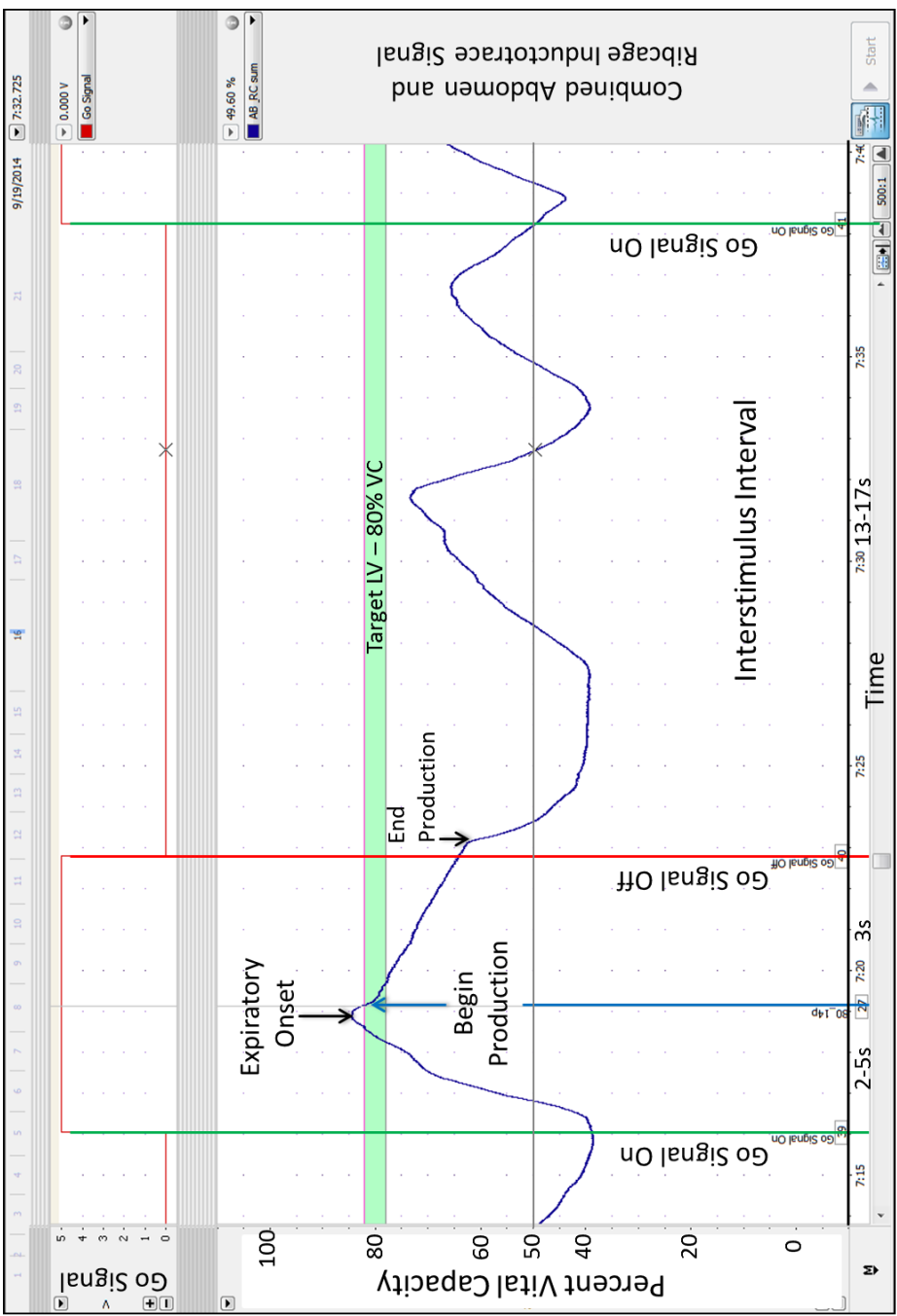


Figure 2.

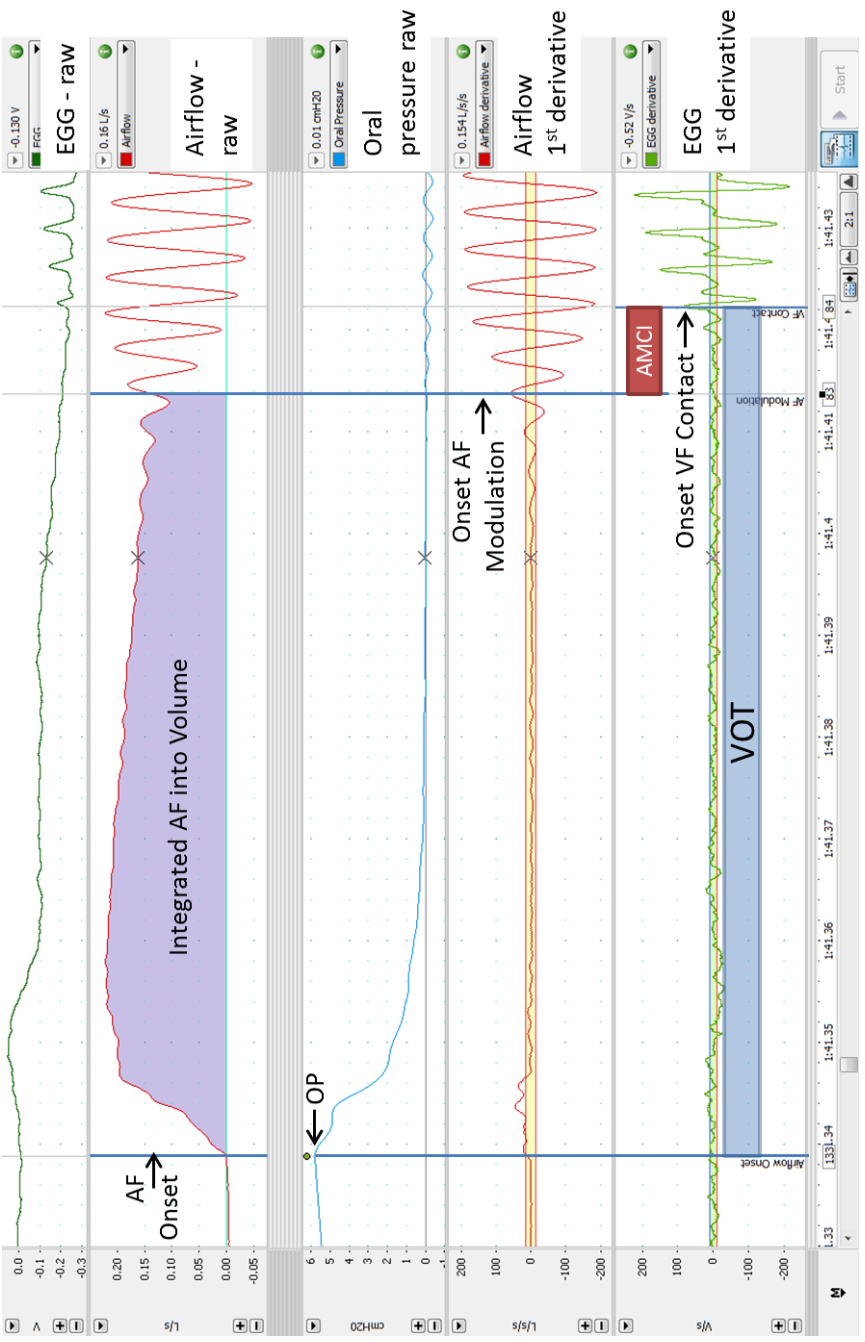


Figure 3.

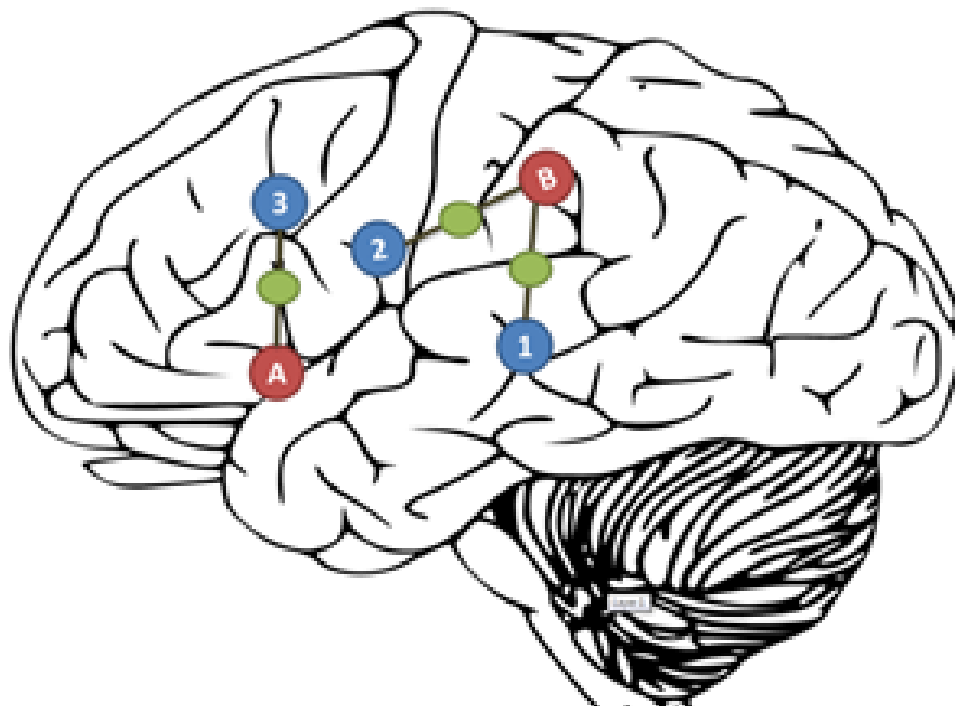


Figure 4.

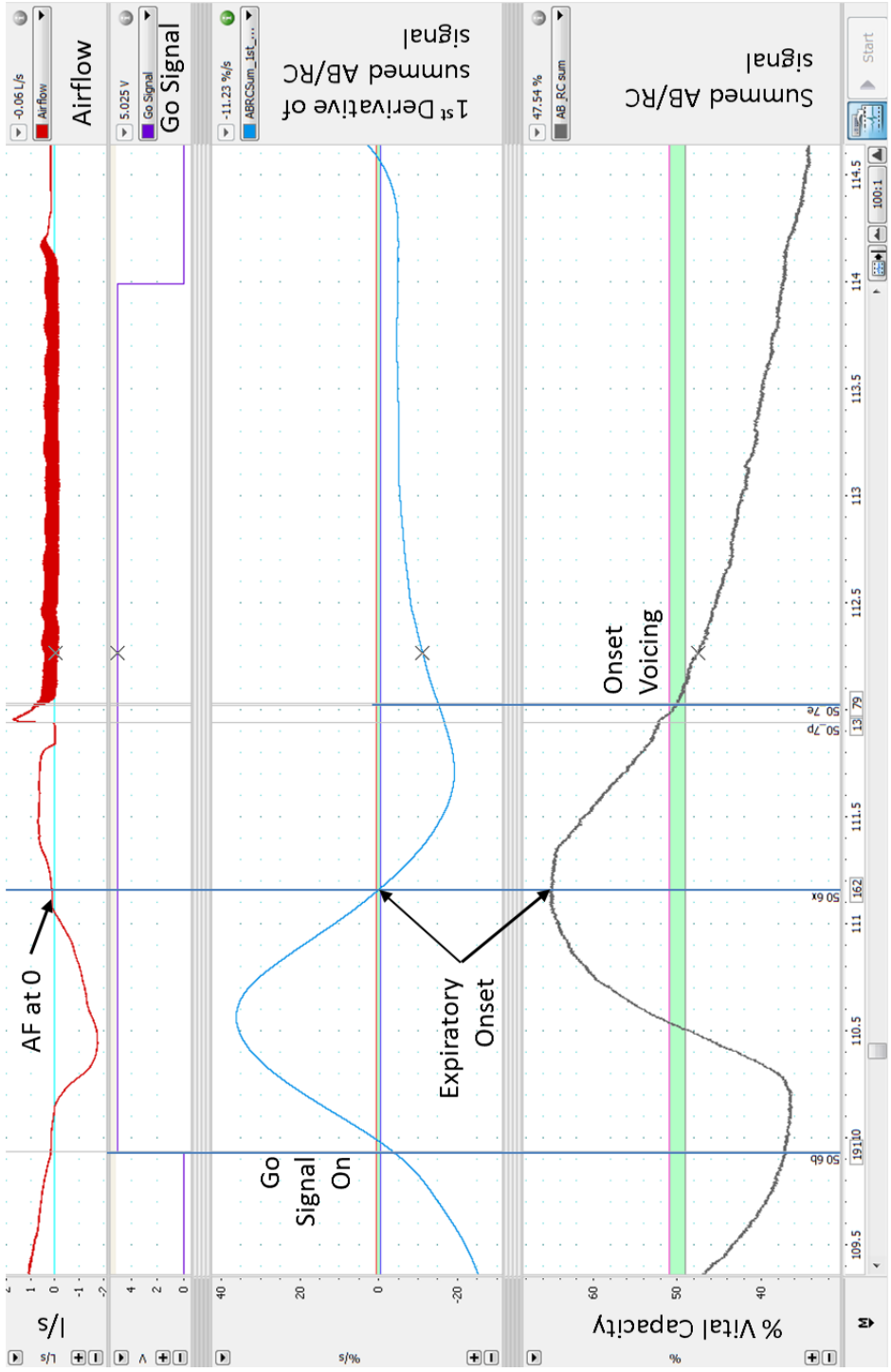


Figure 5.

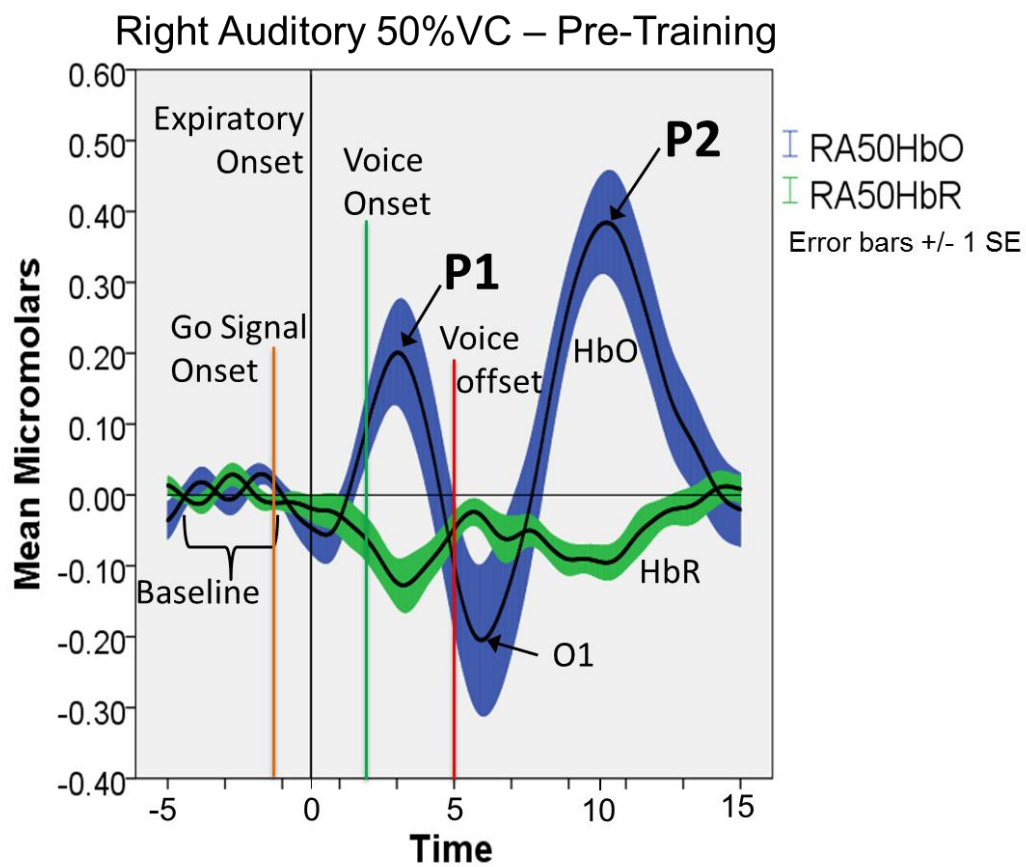


Figure 6.

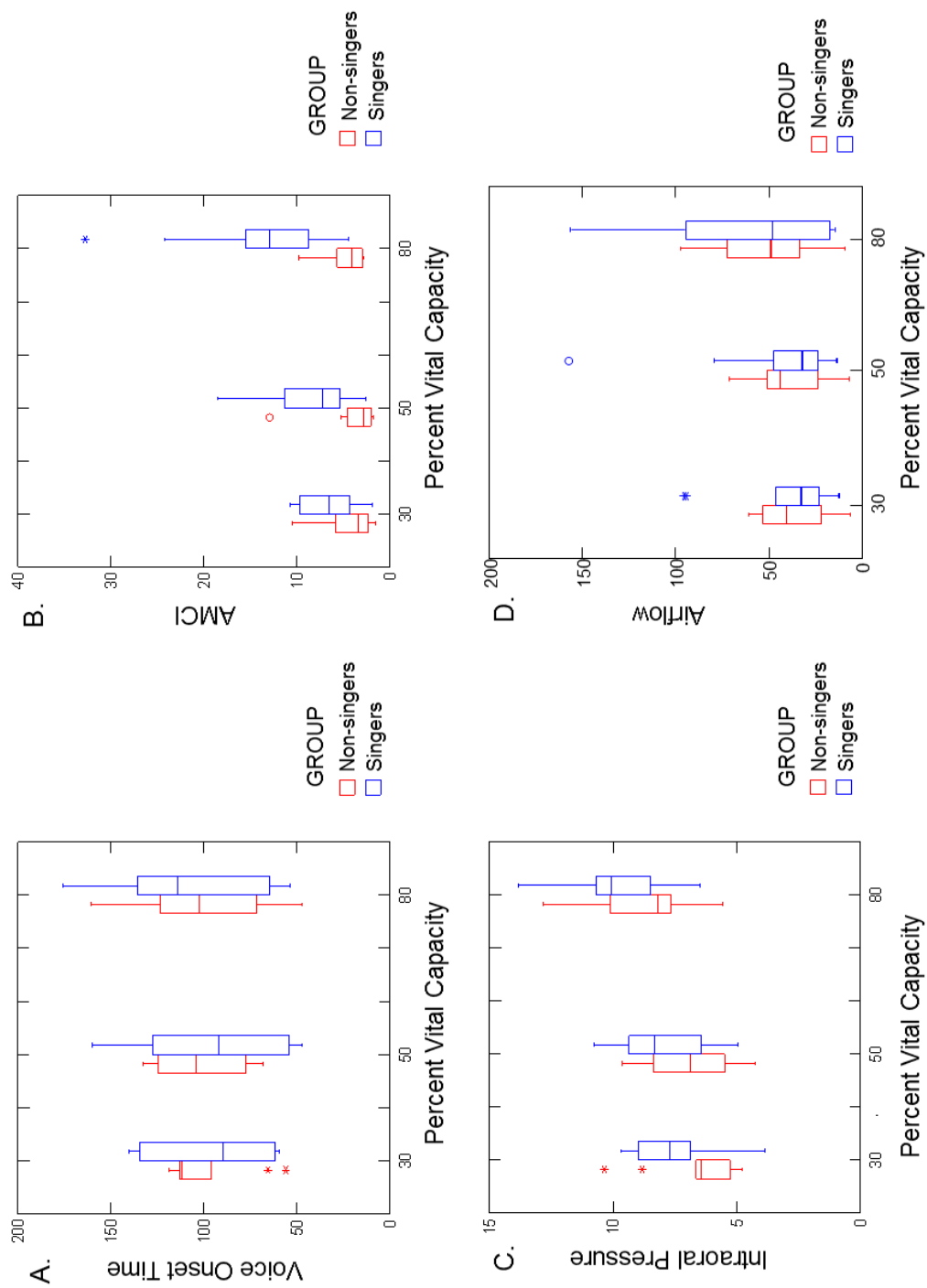


Figure 7.

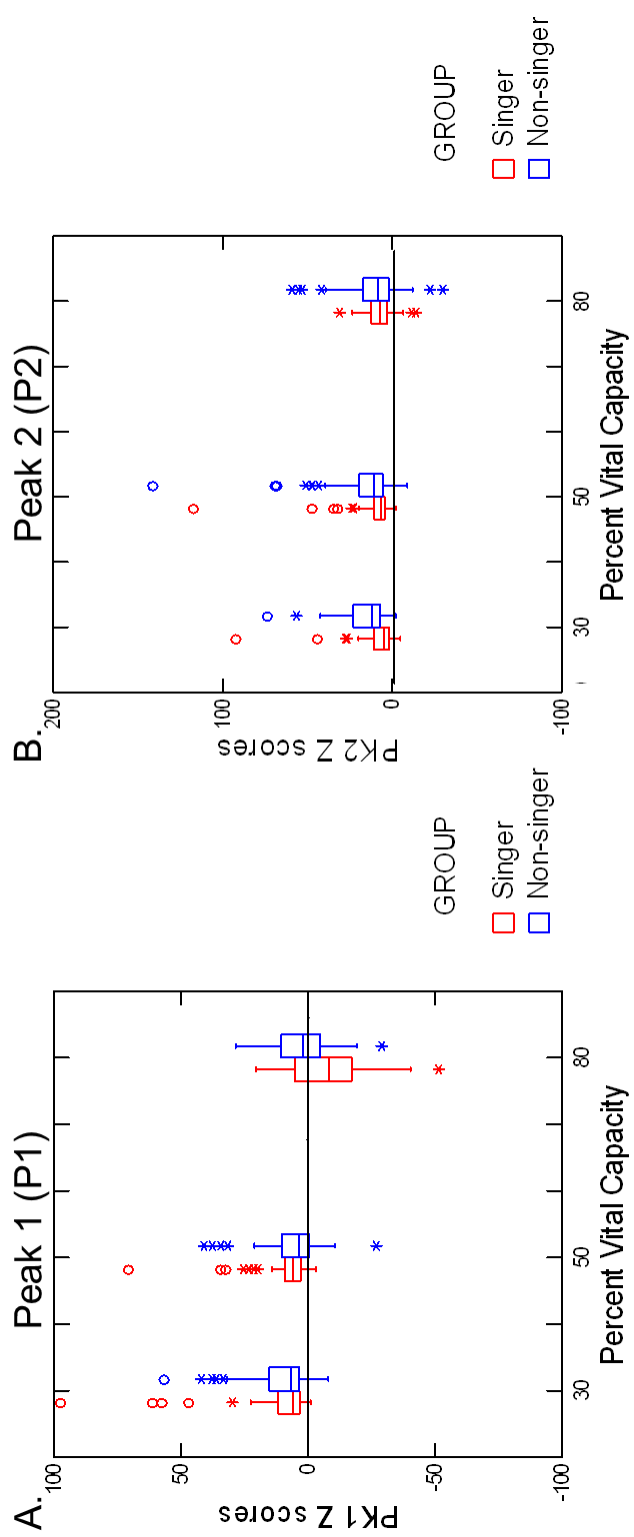


Figure 8.

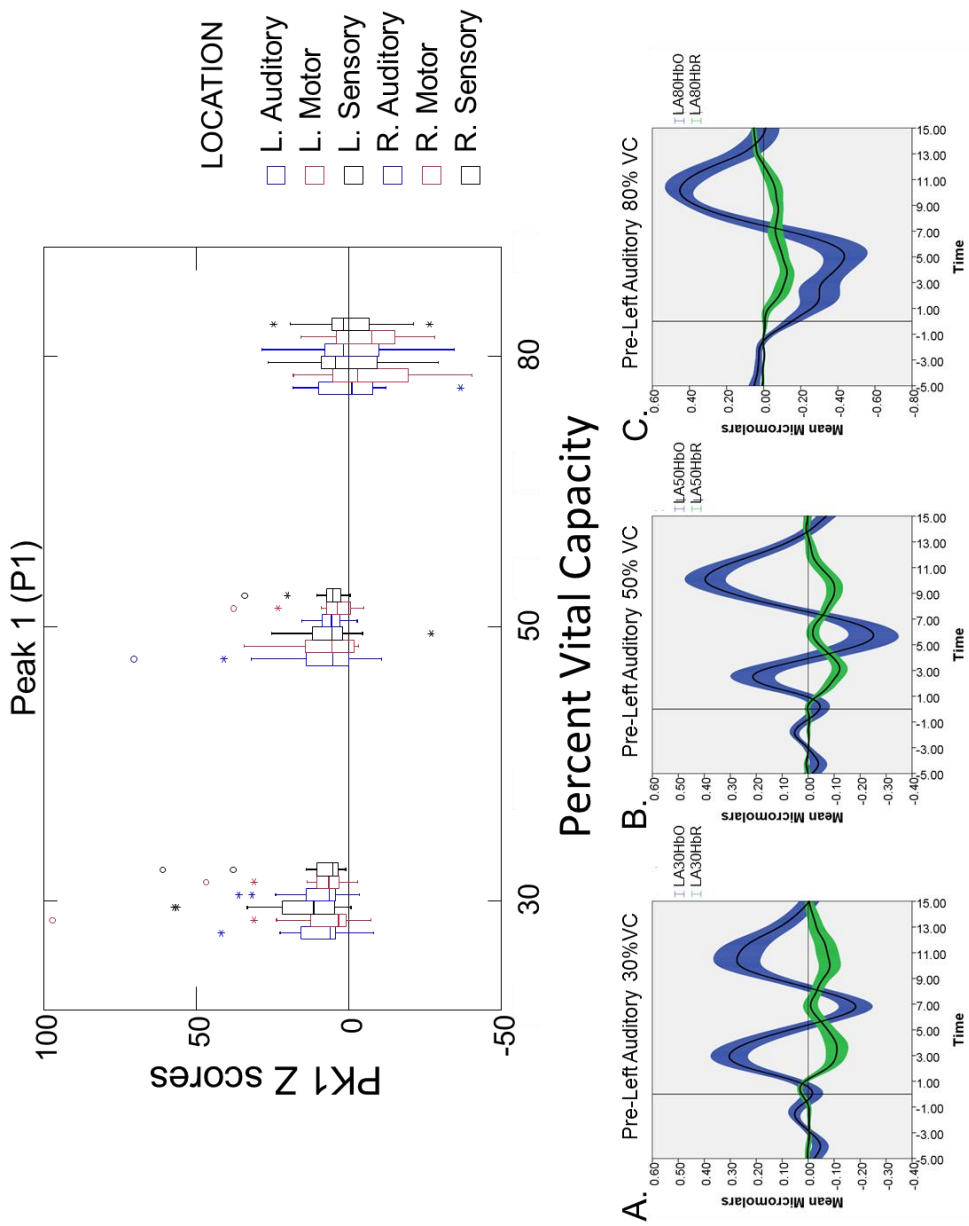
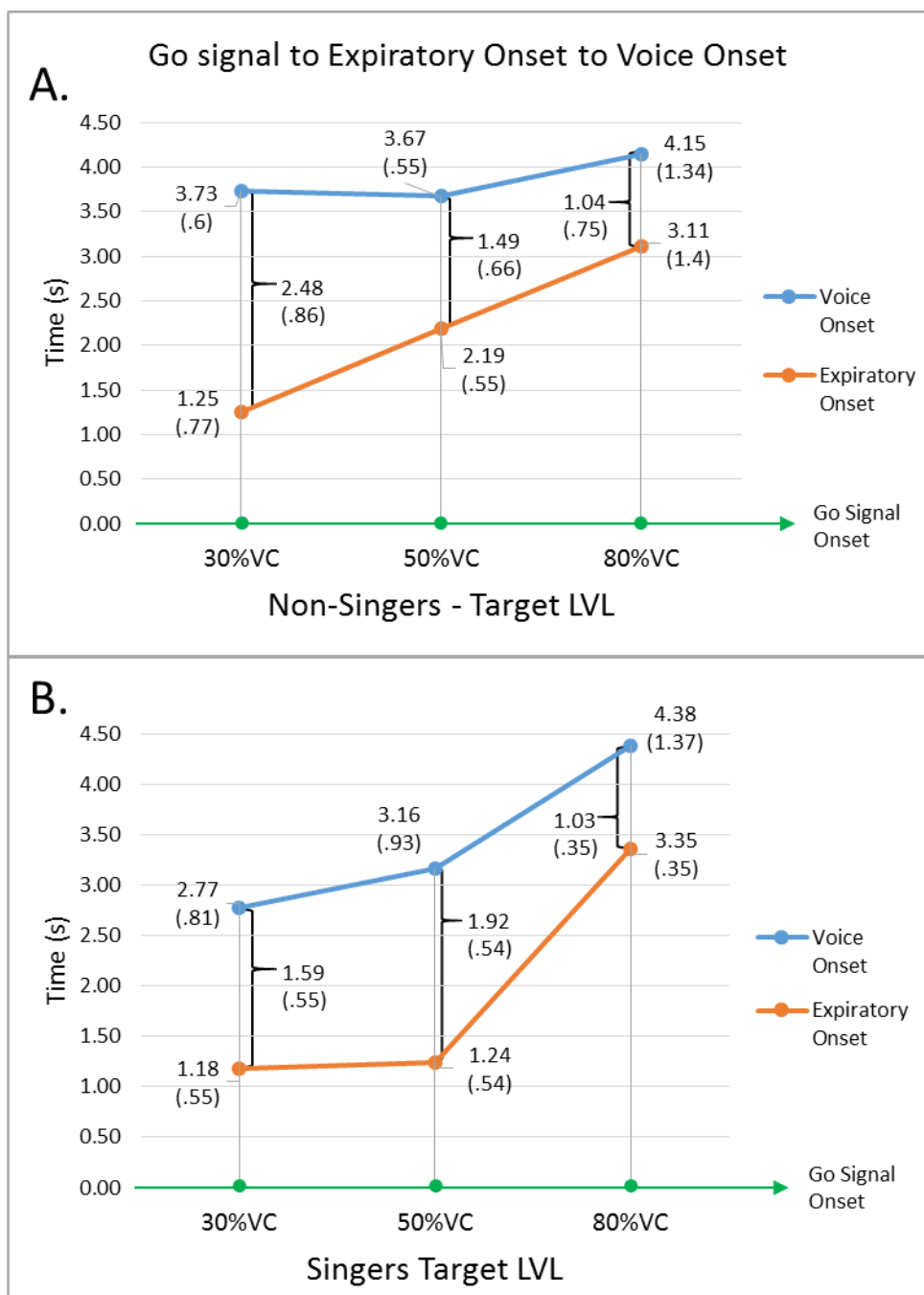


Figure 9.



Title

Training effects on cortical activation at different lung volume levels in singers

Running head

TRAINING AND CORTICAL ACTIVATION IN SINGERS

Abstract

Singers need to counteract respiratory elastic recoil at high and low lung volume levels (LVLs) to maintain consistent airflow and pressure while singing. Professionally trained singers modify their vocal and respiratory systems creating a physiologically stable and perceptually pleasing voice quality at varying LVLs. We examined how vocal students learn to control their voice onset at varying LVLs. Also examined were the effects of training on cortical activation patterns within vocal students using fNIRS. Results revealed significant improvements in initial voice onset control after training ($p = .011$) although task difficulty continued to alter voice physiology. Cortical activation patterns did not change with training but continued to show increased activation during the most difficult tasks, which was more pronounced after training. Professionally trained techniques for consistent, coordinated voice initiation were shown to alter voice onset following plosive consonants with training. However, as performance improved cortical activation remained greatest during the tasks at low LVLs when difficulty was greatest.

Keywords: fNIRS, cortical activation, hemodynamic response, training effects, skill effects, task difficulty voice physiology, voice onset coordination, lung volume, vocal fold vibration, intraoral pressure

Introduction

Professional voice training is aimed at enhancing the singing voice and should distinguish trained singers from non-singers (1-4). Postural changes, breathing techniques, abdominal support, vocal function exercises, and articulatory training are thought to help achieve optimal voice quality control. Most training techniques, however, are based on the voice teacher's perceptual determinations of an "ideal" voice quality, and the teacher trains vocal students to achieve that perceptual ideal. There is very little biofeedback available for voice teachers and students, and little is known of how vocal students actually learn how to produce perceptually pleasing and physiologically efficient voice qualities.

As long as human's have communicated through speech and song, we have relied on our ability to cortically control the respiratory system to overcome the elastic forces of expiratory recoil (5, 6). Speech and singing breathing is highlighted by rapid inhalation followed by the slow, controlled release of air allowing for sufficient lung volume levels (LVLs) to be achieved, up to 70-80% of vital capacity (%VC) for speakers (7, 8) and as high as 90-100% VC for singers (9), to produce long utterances (9, 10). Singers have been shown to sing at very high and very low LVLs, sometimes utilizing nearly all of their VC (9, 11). The higher the LVL from resting expiratory level (elastic equilibrium of the thorax), the greater the forces of elastic recoil (12); therefore singers are often trained in advanced counteractive checking behaviors to overcome the increased levels of elastic recoil (6, 13-16).

Several other natural phenomena occur with the inhalation of large LVLs, such as lowering the larynx (17, 18) and increasing subglottal pressure (19). These phenomena

may alter voice quality, pitch, and loudness at high and low LVLs (6, 9, 11, 20). For finer control of elastic recoil forces of the thorax at high LVLs, singers aim to use both the primary and supportive muscles of respiration (i.e., diaphragm, external intercostal muscles, transverse abdominal and oblique abdominal muscles) to stabilize the chest wall during voice onset using the “lean in” technique, part of the *appoggio* tradition of voice training (6, 13-16). Pedagogically termed abdominal support, expanding and holding the abdomen to support and stabilize voice onset and voicing throughout the sung phrase, has been documented using electromyography (EMG) (6, 21-23), and respiratory kinematics (6, 9, 11, 15, 24-27).

Although initiating voice onset at high LVLs creates several factors that can impact speech, speakers still routinely start speaking at 70-80% VC when talking loudly or shouting (7, 8). On the contrary, speakers rarely range down past resting expiratory level (~30-40% VC), at which thoracic equilibrium is achieved (7, 8, 12). Normally speakers will stay in the range of 40-60% VC as it is the most efficient and avoids the significantly increased forces of respiratory elastic recoil at the far ends of our total % VC causing subglottal pressures as high as 40 cm H₂O and as low as -40 cm H₂O (12). Given that resting expiratory levels are in the bottom third of our total VC, nearly 2/3 of the total VC remains exploitable for communication with passive forces (elastic recoil) which is very efficient.

Singers consistently use large LVLs to support large breath group volumes (LVLs expired per sung phrase) between 30-50% VC, to sustain long phrasing and increased intensity, routinely initiating phrasing at 70-80% VC and terminating vocalization between 30-50% VC (11, 25). Female singers use larger breath group volumes than male

singers, though women still prefer to end phrasing at or slightly above resting expiratory, and they therefore will increase the inspiratory volume rather than decrease expiratory volume below resting expiratory level while singing (11, 25). This evidence suggests that inspiratory recoil initiated at low LVLs (below resting expiratory level) and the subsequent negative subglottal pressure it produces (12) require increased effort and control as they are more difficult to overcome than elastic forces and positive subglottal pressure produced at high LVLs.

In vocal pedagogy, another main focus of voice training is on vocal fold closure at the onset of voicing, termed “vocal attack” (13, 14, 28). The skilled manipulation of airflow with gradual vocal fold closure to initiate vibration is thought to produce a “clean, crisp initial sound” (13). Abdominal support training (taught via the tradition of *appoggio*) may also affect voice onset coordination which, in turn, may affect subglottal pressure, airflow, voice onset time (VOT) for consonant onsets, as well as gradual initiation of vowel onsets (13, 15). Activation of the primary and auxiliary musculature of respiration is thought to stabilize intraoral pressure, which reduces excessive airflow at voice onset, especially at high LVLs, and may improve voice onset coordination.

Performing using very high and low LVLs, which adds a level of difficulty to a speech or singing task, have been shown to significantly change airflow measures, laryngeal height, VOT, and airflow modulation to contact initiation (AMCI) time in speakers and singers (6, 9, 11, 19, 20, 29-31). Performance task difficulty also increases when singers are learning a new piece of music or performing a novel singing task that results in the use of larger breath group volumes and reduced termination volumes (% VC when sung phrase is ended), well below resting expiratory level (32) (recoil equilibrium

~38% VC (25, 32, 33). Skilled voice training also results in significant changes in acoustic, aerodynamic, and physiological voice measures (2, 15, 34). Little is known, however, of how singers learn to control voice onset coordination to create physiologically efficient and perceptually pleasing voice qualities.

Changes in brain activation patterns occur with training and at different levels of task difficulty (35-49). Skilled motor training induces neural plastic changes in the central nervous system (35-46). Measurements of changes in cortical functioning, connectivity, and neurovascular coupling in humans can be measured using functional magnetic resonance imaging (fMRI) (45, 50). Several fMRI studies have conducted cross-sectional comparisons between groups with different levels of experience (e.g. laypersons or novices, amateurs, and professionals) to determine cortical activation differences as a result of experience (46, 51-55). Other longitudinal studies have investigated changes within subjects before and after training (37, 44, 45, 50, 56). Results of these studies indicate that learning a new task effectively increases cortical activation initially and results in cortical map reorganization in task specific areas of the brain following training/learning.

Musicians have emerged as an ideal population to examine use-dependent cortical changes (46, 52, 53, 57, 58). Very little is known, however, about cortical changes in the vocal system associated with skilled training. A comparison of brain activation between laymen, vocal students, and professional singers found increased activation in vocal students and professional singers compared to laymen in the somatosensory strip bilaterally, dorsolateral prefrontal cortex, inferior parietal cortex (angular gyrus), and the left temporal pole (51). Differences between professional singers and vocal students

involved increased activation in the right S1 and primary motor cortex in professional singers. Activation common to all groups was found in bilateral premotor cortex and primary auditory cortex. The authors suggested that the recruitment of the right sensorimotor area in professional singers was related to increased articulatory and laryngeal feedback demands (51). Increased activation in the auditory cortex corresponding to training has been reported in musicians and singers (59-61). Within subjects changes in cortical activation related to voice training have not been investigated.

The increased activation in the voice production and perception areas of the right and left hemispheres in singers seems to be in direct contrast to previous studies on the effects of skilled motor learning on cortical activation patterns. Single-unit recording studies in primates and fMRI studies of skilled motor learning (e.g., learning finger tapping sequences) have shown that early in learning there is an increase in cortical activation, followed by a decrease in firing as training progresses and the learned behavior becomes more habitual (62-64). Why voice training may result in increased cortical activity both early in learning (vocal students) and late in learning (professional singers) is unknown.

fMRI provides a mechanism to assess changes in cortical activation. The effects of increased neuronal firing in a neural substrate can be measured indirectly through blood oxygenation level dependent (BOLD) magnetic resonance changes. Functional near-infrared spectroscopy (fNIRS) is non-invasive and measures the optical wave length transfer in the cortical layer. Changes in blood oxygenation levels over time can be tracked with fNIRS and strongly correlates with the fMRI BOLD response (65-68).

Through analysis of the hemodynamic response with fNIRS, changes in blood oxygenation levels are examined over time in relation to behavioral events. By averaging numerous trials, activation patterns can be observed in blood oxygenation level changes in different cortical regions (65, 69-71). Motion artifacts can interfere with fMRI measures during analysis of brain activation in response to speech and voice stimuli. Continuous wave fNIRS allows for examination of changes in blood oxygenation level over time, and is not limited to sparse sampling as there is no noise associated with fNIRS (72-78).

Professional singers' enhanced voice quality may be related to respiratory, aerodynamic, phonatory, and resonatory/articulatory adjustments as a result of training, which in turn may result in cortical changes. However, little is known about how singers learn to control voice onset coordination, as well as the effects of voice training on physiological, aerodynamic, and cortical activation measures within vocal students. The current study examined the effects of voice training and LVLs on physiological (VOT and AMCI) and aerodynamic (airflow and intraoral pressure) vocal behaviors and cortical activation patterns in freshmen vocal students during initiation of /pi/ before and after their first semester of professional voice training within a university vocal performance/education program. We hypothesize that 1) after voice training, physiological and aerodynamic voice measures will not differ significantly between different LVLs; 2) training will result in increased right hemisphere activation across different LVLs, and 3) right and left activation will not differ as vocal students become more skilled at voice onset control.

Materials and Methods

Subjects recruitment

Volunteer female vocal students enrolled as freshmen in the James Madison University School of Music vocal performance and/or vocal education programs were recruited for the study through flyers and in-class presentations. Subjects had to be right-handed with normal hearing, and enrolled to receive individual voice training in the western classical style (opera) from the voice faculty within the School of Music at James Madison University. Exclusion criteria included: presence or history of a voice disorder, history of smoking, current diagnosis or treatment for reflux, current diagnosis of a breathing disorder, diagnosis of a neurological disease, and/or diagnosis or medical treatment for psychiatric disorders (besides depression). All subjects gave informed consent on the James Madison University Institutional Review Board (IRB) approved protocol.

Procedures

This was a within-subjects repeated measures event-related design (Fig. 2). Pre- and post-training experimental testing sessions were identical. Subjects were recruited before or at the start of individualized voice training session with Voice faculty. All subjects produced /pi/ at 262Hz (middle C) 30 times at 3 specific LVLs (% VC: 80% VC, 50% VC, and 30% VC) at the start and end of voice training. Between experimental testing sessions, subjects received individual voice training and small group voice training from the voice faculty at James Madison University for 13 weeks, referred to as voice training.

Voice training: Voice training was conducted by 1 of 7 voice faculty members in the school of music at James Madison University in the western classical style (opera). All freshman vocal students, regardless of previous training, start in “Level 1” and are trained using a common set of four vocal technique goals. Training consisted of a one hour lesson per week with a one hour joint master class (group lesson with other freshman) per week for 13 weeks. They were also required to practice for a minimum of 10 hours per week (79).

Integral to the current students’ training were the vocal technique goals to “demonstrate 1) acceptable vocal posture and 2) appropriate breathing techniques to 3) produce a clear ringing tone” (79). These goals were of a high priority in the core curriculum for freshman vocal students. Acceptable breathing techniques, trained using the *appoggio* tradition and abdominal support, were thought by the voice teachers to allow singers to perform coordinated, aerodynamically stable voice onsets at high lung volumes ($> 70\%$ VC). Trained techniques are mainly tested perceptually by voice teachers via auditory feedback and tactile feedback of the abdomen and trunk. Vocal students were trained to extend their “breath/resonance coordination to a higher level, and begin to feel the breath/resonance balance necessary for more extreme ranges” of lung volume (80).

Instrumentation and calibration: PowerLab 16/SP hardware, running LabChart Pro version 7.3.7 was used for data acquisition for all voice and respiratory kinematic measures (ADInstruments, Inc., Colorado Springs, CO). A sampling rate of 20 kHz per second was used to record all data except the respiratory kinematic channels which were sampled a 1 kHz. Prior to testing, all equipment was calibrated or normalized. A vented

pneumotachometer mask was used to measure airflow (Glottal Enterprises, Syracuse, NY, Model MS110). An air pressure transducer was used to measure intraoral pressure (Glottal Enterprises, Syracuse, NY, Model MS110). Airflow and spirometric measures (Universal Ventilation Meter, UVM; Vacu-Med, Ventura, CA) were calibrated using the MCU-4 Calibrator (Glottal Enterprises, Syracuse, NY) and interpolated from voltage to liters/second (l/s) in LabChart. Intraoral pressure was calibrated using the MCU-4 Calibrator and converted to cm H₂O. Vocal fold contact was detected via electroglottograph (EGG) transducers connected to an EG-2 two-channel EGG amplifier (Glottal Enterprises, Syracuse, NY). Respiratory kinematics were transduced using the Inductotrace inductive plethysmograph (Ambulatory Monitoring Inc., Ardsley, NY). The Inductotrace amplifier levels were set to one for both the ribcage (RC) and abdomen (AB) and the DC signals were recorded in LabChart.

Voice physiology measures setup and recording: Subjects were positioned standing 3-4ft from a computer monitor set at eye level with their head and back stabilized against a secured equipment cart and cushioned pole raised to 7 ft (Fig. 1). The monitor provided realtime biofeedback of subjects' respiratory kinematics during testing. A stand holding the pneumotachometer mask was set up leaning against the face of a subject at the appropriate level to ensure a proper seal. A small tube was sealed through the mask and placed between the lips into the mouth for determining intraoral pressure during lip closure (81, 82). The stand holding the mask allowed the subject to relax between trials and aided in sealing the mask during trials. A Sony ECM-55B condenser microphone was sealed in the mask to record the speech signal (Sony, Japan). The EGG

transducers were secured to the neck at the level of the thyroid cartilage and connected to an EG-2 EGG amplifier to transduce vocal fold contact.

Subjects were also fitted with Inductobands around the abdomen at the level of the umbilicus and the ribcage inferior to the axilla (Fig. 1.) (83). The AB, RC, and combined sum of AB plus RC movements were recorded in separate channels in LabChart. During LV normalization, subjects performed 3 vital capacity (VC) maneuvers, while breathing through the spirometer. For the VC maneuver, subjects were instructed to inspire as much air as possible before exhaling all the air out of the lungs. This normalization technique allowed for comparing LVL based on the VC maneuver. Due to substantial drift of respiratory kinematics throughout testing and the need for realtime biofeedback of respiratory movements for testing target acquisition, we normalized the combined sum signal to percent vital capacity (%VC) for each individual prior to each condition. The combined sum signal was converted from voltage to %VC in LabChart and displayed in realtime on the monitor to provide biofeedback of %VC to subjects during testing.

fNIRS setup and recording: A continuous wave fNIRS system (NIRSOptix TechEn, Milford, MA, model CW6), sampling at 50 Hz, was used to measure blood oxygenation and deoxygenation patterns in the inferior premotor cortex (premotor), inferior somatosensory cortex, and auditory cortex (posterior-superior temporal gyrus) of the right and left hemispheres (Table 1 and Fig. 4). The fNIRS system uses 690 nm and 830 nm wavelengths of near-infrared light to measure concentration changes in oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (HbR) by means of a modified Beer-Lambert Law (84). We used 4 emitters and 6 detectors (Fig. 4). Each

emitter-detector pair was 3 cm apart and measured the oxygenation in cortical substrate at the mid-point between the pairs. The near-infrared light emitted from the optodes were modulated between 6.4 to 12.6 kHz with an interval of 200 Hz to reduce cross talk contamination between channels (85). The 690 nm laser optodes were calibrated to 12 mW average power and the 830 nm laser optodes were calibrated to 6 mW average power.

Neuronavigation for identifying Talairach coordinates: Subjects' heads were fitted to a female high-resolution brain anatomical MRI template using the BrainSight Neuronavigational System version 1 (Rogue Research, Montreal, QC). Following MRI fitting, the placement for fNIRS emitters and detectors were marked on the scalp to the areas corresponding to the premotor, somatosensory, and auditory regions of the left and right hemispheres of the brain according to previously determined Talairach coordinates (Table 1) based on (51, 70, 86). The coordinates for the areas of interest were in millimeters along the left-right (x), anterior-posterior (y), and superior-inferior (z) axes. The fNIRS emitters and detectors were then placed on the scalp prior to testing using two flexible plastic templates and the optodes secured to the head using Coban self-adhering wrap.

Task Performance

During both pre- and post-training testing sessions, subjects produced /pi/ at 262Hz (middle C) 30 times during three percent vital capacity (% VC) conditions (80% VC, 50% VC, and 30% VC, presented in that order for all subjects). A go signal (green light) was used to signal experimental trial onset and completion. Every fifth trial there was a rest trial in which the go signal was not lit, for a total of 35 experimental and

rest trials per condition. Before each %VC condition, subject's conducted a VC maneuver to normalize the sum trace into %VC to account for any drift in the signal caused by postural adjustments or movement affecting the Inductobands between trials. The signal was scaled from 0-100 %VC. Subjects monitored the go signal light mounted on the computer screen along with realtime biofeedback of %VC and a highlighted target line at 30, 50, or 80% VC condition in LabChart (Fig. 2).

After several practice trials using the biofeedback to monitor their breathing to attain the target %VC and produce the singing task after go signal was lit, subjects received formal instructions and a reference pure tone at 262Hz (middle C) before testing began. LabChart and fNIRS recordings were started at approximately the same time and testing began after a 30 second delay. When the go signal was turned on, subjects checked their body position with their head and back firmly against the cart and pole and pressed the pneumotachometer mask firmly against their face, not moving their face out to the mask. They inspired above the target %VC and then exhaled until they were within the target. They then sang /pi/ and held the vowel until the go signal light was turned off 3 seconds after voice onset (Fig. 1 and Fig. 2). Subjects were instructed to sing /pi/ in their best voice quality as if they were "starting an aria." The task was repeated 30 times with 5 rest trials

Subjects were instructed to remain still and relaxed during the inter-trial intervals and only move at the go signal to set-up for that trial. Subjects were instructed to move if needed (e.g. cough, swallow, stretch, etc.) before the go signal was on, rather than during the inter-trial intervals to avoid motion artifact during a hemodynamic response. If a subject swallowed when the go signal came on, the timer was reset and the trial repeated.

If there was drift noted in the biofeedback signal at any time during the condition, testing was stopped and a VC maneuver was conducted to rescale the sum signal to 0-100% VC before continuing the condition.

Data processing

Physiological measures: All signal processing and measurement calculations were conducted in LabChart (Fig. 3). The four voice physiology measures were as follows:

Voice onset time (VOT) was defined as the time interval from initiation of plosive release to the onset of voicing (87, 88). The first derivative of the EGG signal was calculated using a window width of 33 points; no drift in the signal was detected. VOT was measured from the onset of airflow at plosive release to the first peak of the first derivative of the EGG signal (adapted from 87) to the first instance of vocal fold contact (89). VOT results, in milliseconds (ms), were collected for each of the 30 repetitions of a condition and then averaged for each subject (Fig. 3).

Airflow modulation to contact initiation (AMCI) was defined as the time interval from onset of modulation of the airflow after plosive release to the onset of vocal fold contact (adapted from 90). The first derivative of the airflow signal was calculated using a window width of 33 points and the time interval from the first periodic peak (modulation) of the derived airflow signal (within $\pm 1SD$) to the first peak of the first derivative of the EGG signal was determined for each trial using Peak Analysis in LabChart. The mean AMCI interval (ms) was calculated across 30 trials in each condition for each subject (Fig. 3).

Integrated airflow was defined as the area under the curve in the raw airflow signal, from the onset of airflow after the plosive release to the periodic modulation of the

first derivative of the airflow signal. Measures in milliliters (ml) were collected and averaged for each subject for each condition (Fig. 3).

Intraoral pressure was defined as the peak pressure before the initial plosive release was smoothed at 33 points to determine the absolute peak measurement from baseline using Peak Analysis in LabChart. The peak was confirmed using the 1st derivative of the raw intraoral pressure signal calculated in a separate channel in LabChart. All peaks within a condition were averaged for each subject for each condition (Fig. 3).

fNIRS data processing: Hemodynamic Evoked Responses 2 v.1.5.2 (HomER2; www.nmr.mgh.harvard.edu) was used for data analysis. The raw acoustic signal, airflow signal, intraoral pressure signal, and go signal were recorded as auxiliary inputs in the fNIRS system. The onset times for each trial were identified for both LabChart and HomER2 using linear interpolation so that the timing in the two separate systems was synchronized.

Expiratory onset was used to mark experimental trial onset. Expiratory onset was defined as the onset of expiration following the go signal and prior to singing onset (Fig. 2). Expiratory onset was determined in LabChart when the 1st derivative of the sum signal crossed zero indicating a transition from inspiration to expiration after the go signal. Trials were eliminated from analysis if: there was excessive movement (e.g. throat clear, swallow, cough, etc.) observed 10 seconds before or after the go signal, or expiratory onset occurred before the go signal was lit.

After the expiratory onset marker times were input into HomER2, the raw fNIRS signals were processed for analysis. Three distinct sources of noise in fNIRS were

removed. Instrumental noise was removed using a low-frequency bandpass filter set to 0.5 kHz (91). Experimental noise, usually related to motion artifact, was removed using a principle component analysis (PCA) filter for all channels (91). After checking the signals for the presence of the cardiac artifact to confirm data quality following acquisition, a 0.010 kHz high-frequency bandpass filter was used reduce the impact of physiological artifact. As motion artifact from temporalis muscle contraction due to jaw motion can interfere with fNIRS recordings, the mask was on a stand securely pressed against the face and chin to reduce jaw movement (70, 91, 92).

The optical signals were converted to optical density in HomER2. The relative concentrations of HbO, HbR, and total hemoglobin were then derived in HomER2 for analysis. Trials were identified using expiratory onset markers, and event-related averaging of 20 s epochs (5 s before stimulation markers and 15 s after) were calculated for each %VC condition and plotted by channel and side (Fig. 5). A minimum of 10 trials was required for each epoch.

The HbO and HbR signals were visually examined to check for an inverse relationship between them to indicate a cortical response and not physiological artifact (Fig. 4 and Fig. 5) (93). The data for HbO and HbR cortical responses from fNIRS analysis in the premotor (pM), somatosensory (S1), and auditory (A1) regions of the right and left hemispheres (6 locations) were exported to a spreadsheet. Time windows of interest were: baseline: -5 to -1 seconds, peak 1 between 2 to 6 seconds; and peak 2 between 6 to 13 seconds. The peak amplitude of each peak for the 6 HbO channels were determined to be the highest or lowest point (depending on the direction of the peak) in the HbO signal in each condition (30% VC, 50% VC, and 80% VC; Fig. 5). Baseline

measures were calculated from the averaged HbO signal from -5 to -1s of the curve. Raw HbO data were then converted into a Z-score using the formula, $Z = (\text{peak amplitude} - \text{mean baseline}) / \text{baseline SD}$. This conversion provided a normalized measure of amplitude change from baseline.

Several interval markers occurring within each 20 s epoch were identified for analysis including: go signal onset, voice onset, HbO peak 1, voice offset, HbO peak 1 offset, and HbO peak 2 (Fig. 5). The go signal recording in LabChart was used to identify go signal onset as the first moment of positive divergence from zero at the outset of each epoch. Voice onset was the EEG signal time identified for calculating AMCI and VOT. Voice offset was identified at the end of each trial as the final peak in the 1st derivative of the EEG signal indicating the offset of vocal fold vibration. During peak 1 amplitude detection between 2 and 6 s, the peak 1 onset time was identified. Between 4 and 8 s the absolute negative peak 1 offset time and amplitude were determined for peak 1 offset. Within the final 6 to 13 s window, the time at the absolute second peak was identified. All interval markers identified in LabChart (go signal onset, expiratory onset, voice onset, and voice offset) were time synchronized using linear interpolation to fNIRS so that all interval markers were in a common time signature. All times were recorded into a spreadsheet.

Statistical analysis

SPSS version 22 (IBM) and SYSTAT version 13 (Crane Software International Ltd) were used to analyze all data. Training (pre-training and post-training) and task effects (30% VC, 50% VC, and 80% VC), and their interaction were determined for each of the 4 physiological measures via general linear model (GLM) two-way repeated

Analysis of Variance (ANOVA) tests. Significant results were further examined using post-hoc Fisher's least significant differences (LSD) corrected pairwise comparisons. For the physiological hypotheses, alpha was Bonferroni corrected to 0.0125 to account for 4 analyses ($.05/4$).

A GLM four-way repeated ANOVA was determined to examine % VC effects (30% VC, 50% VC, and 80% VC), pre- vs. post-training effects, effects of side (right vs. left) and location effects (pM, S1, and A1), as well as any interactions on Z-scores of change of first and second peak of the HbO signal with Bonferroni correction for 2 analyses ($\alpha=.025$). Post-hoc Fisher's LSD adjusted pairwise comparisons of the estimated marginal means were explored for significant results.

Results

Subjects

Of the 16 subjects recruited for the study, 10 female vocal students (mean age 18.2 years) completed all phases of the study. Two of the 4 excluded subjects were eliminated from the study following the pre-training phase due to a current diagnosis of reflux which was not disclosed during screening. The remaining 2 subjects excluded from analysis failed to complete the post-training testing. All included subjects were freshman sopranos enrolled in the School of Music Vocal Performance and/or Vocal Education programs at James Madison University. All were recruited before beginning their first semester of voice training by the voice faculty in the western classical style (opera). Prior to university training, subjects had an average of 3.1 years (0.25 to 9 years) of private voice training and 7.2 years (2 to 13 years) of choral training. There was an average of 9 weeks (64.27 days, range 51 to 77 days) of training between testing. University training, mainly perceptually-based, consisted of a one hour private lesson once a week, a one hour joint lesson once a week, and a minimum of 10 hours of practice per week for 13 weeks.

Reliability

Inter-rater and intra-rater reliability were determined in LabChart using the 5 data points (airflow onset, airflow modulation, first peak of EGG, go signal, and expiratory onset; Fig. 2 and Fig. 3) per trial used to calculate the 4 physiological voice measures and the stimulation markers for fNIRS. A total of 1,800 measurements were compared for Kappa agreement between and within investigators averaged across the 5 equally weighted data points for 20% of the data.

Inter-rater analysis revealed an average Kappa = .898, $p < .0001$ (range .774 to 1.00) indicating very good agreement between investigators (94). Kappa agreement for intra-rater analysis revealed an averaged Kappa = .871, $p < .0001$ (range .757 to 1.00), also demonstrating excellent agreement within the primary investigator.

Physiological voice measure results

All variables met the assumption of Sphericity except AMCI for %VC effect ($\chi^2(2) = 10.742, p = .005$), therefore a Greenhouse-Geisser correction was performed. Post-hoc Fisher's LSD adjusted pairwise comparisons of the estimated marginal means were explored for significant results.

Voice onset time: A non-significant trend for %VC effect, $F(2, 18) = 3.41, p = .056, \eta^2 = .10$, no training effect, $F(1, 9) = .81, p = .77, \eta^2 = .00$, and no significant interaction between %VC and training, $F(2, 18) = 1.02, p = .38, \eta^2 = .02$, was found in VOT (Fig. 6A).

Airflow Modulation to Contact Initiation: Greenhouse-Geisser corrected results revealed a significant %VC effect, $F(1.15, 10.34) = 9.78, p = .001, \eta^2 = .29$, a significant training effect, $F(1, 9) = 10.25, p = .011, \eta^2 = .17$, and a significant interaction between %VC task and training, $F(2, 18) = 6.544, p = .007, \eta^2 = .05$, on AMCI (Fig. 6B).

The simple effects of the interaction for AMCI were examined through post-hoc LSD corrected pairwise comparisons of the estimated marginal means. The AMCI time prior to training was significantly longer than after training, but for only the 80% VC condition ($p = .005, d = .97$). AMCI times pre- and post-training did not significantly differ for either 30% VC ($p = .053$) or 50% VC ($p = .093$). Differences in AMCI time

between 30% VC and 80% VC were significant only prior to training ($p = .006$, $d = 1.26$). Following training, differences between %VC conditions were not significant (Fig. 6B).

Intraoral pressure: There was a significant % VC effect for intraoral pressure, $F(2, 14) = 13.09$, $p = .001$, $\eta^2 = .36$, no significant differences between pre and post training were observed, $F(1, 7) = .06$, $p < .817$, $\eta^2 = .00$, and no significant interaction between %VC task and training, $F(2, 14) = .32$, $p < .732$, $\eta^2 = .01$ (Fig. 6C).

Post-hoc pairwise comparisons of the main % VC effect revealed that subjects had significantly greater intraoral pressure during 80% VC ($M = 9.439$ cm H₂O) than when performing at 30% VC ($M = 6.666$ cm H₂O, $p = .002$, $d = 1.28$). Neither differences between 30 and 50% VC ($p = .017$, $d = .51$), or between 50 and 80% VC were significant ($p = .075$, $d = .74$) (Fig. 6C).

Integrated airflow: There was a significant % VC effect for airflow, $F(2, 18) = 7.247$, $p = .005$, $\eta^2 = .15$, the group effect was not significant, $F(1, 9) = .772$, $p = .402$, $\eta^2 = .04$, nor was the interaction significant between % VC task and training, $F(2, 18) = .176$, $p = .840$, $\eta^2 = .00$. Post-hoc comparisons indicated that 80% VC was greater than 30% VC ($p = .01$, $d = .73$) (Fig. 6D).

fNIRS results

All variables met the assumption of Sphericity. Post-hoc Fisher's LSD adjusted pairwise comparisons of the estimated marginal means were explored for significant results.

The results of the 4-way repeated ANOVA for peak 1 amplitude revealed no significant training effect, $F(1, 7) = .998$, $p = .919$, $\eta^2 = .00$ (Fig. 7). There was a significant % VC, $F(2, 14) = 9.409$, $p = .003$, $\eta^2 = .34$ (Fig. 7), and a significant location

effect, $F(2, 14) = 16.429$, $p < .0001$, $\eta^2 = .03$ (Fig. 8). There was a non-significant trend for a difference between right and left side cortical activation, $F(1, 7) = 5.265$, $p = .055$, $\eta^2 = .01$. There was a significant interaction between %VC task and location, $F(4, 28) = 6.833$, $p = .001$, $\eta^2 = .01$ (Fig. 7).

The significant differences between locations were dependent on target %VC condition. Examination of simple effects of the interaction through Fisher's LSD corrected pairwise comparisons revealed that at the 30%VC condition S1 had significantly greater cortical activation than pM ($p = .003$, $d = 1.78$). At 80%VC S1 had significantly greater cortical activation than pM ($p = .005$, $d = .75$) and A1 ($p = .032$, $d = .30$), and A1 also had significantly greater activation than pM ($p = .014$, $d = .51$). Locations did not differ for the 50%VC condition.

There was a significant main effect of location on cortical activation for peak 2, $F(2, 18) = 9.106$, $p = .003$. There were no %VC, side, or training effects on P2. Post-hoc analysis revealed that pM had significantly less overall activation than S1 ($p = .013$, $d = .71$).

Discussion

Professional singers' enhanced voice quality may be related to aerodynamic and physiological adjustments within the vocal system as a result of training. Although the effects of training in singers have been examined through measurements of the vocal system, as well as through trained and untrained comparisons on cortical activation (51), these two domains have not been related. We examined the effects of training while performing at low (30% VC), intermediate (50% VC), and high (80% VC) LVLs on physiological voice measures and cortical activation patterns in vocal students. A semester of professional voice training had no significant effect on cortical activation patterns in the superficial cortex within the premotor (pM), auditory (A1), or somatosensory (S1) regions. Training did, however, significantly improve voice onset coordination, although variations in task performance over the three LVLs continued to have the same effects on voice after training. Performing the experimental production task at low and high LVLs elicited significant differences between cortical activation in pM, A1, and S1, but there were no differences between hemispheres (side), while at intermediate LVL (50% VC) there were no significant differences between locations. The relationship between LVL, task performance, and location may provide insight into what are the most cortically demanding LVLs at which singers perform.

The results of the physiological measurements of voice onset coordination revealed that LVL had an effect on intraoral pressure and integrated airflow. Further, both training, LVL, and their interaction have a significant effect on AMCI. No effects or interactions were discovered for VOT.

Voice onset time (VOT)

Previous research into training effects on VOT in female singers found that level of training had no significant effect on VOT of /p/ plosives (95). Our results are in agreement with these findings as we also found no significant differences in VOT after training ($p = .77$). The researchers concluded VOT may not be sensitive enough to detect minute articulatory and phonatory change due to training and our findings are in agreement with that conclusion.

We also found that LVL had no effect on VOT and only accounted for approximately 10% of the variability, a medium effect size ($p = .056$, $\eta^2 = .10$). This result is divergent from previous research showing that VOT decreases as LVL decreases in speech (30). Hoit et al. (30) determined that there was a significant association between LVL (measured as %VC) and VOT, characterized by a general tendency that, as LVL decreased, VOT decreased within 5 male subjects. We found that, though there may be a tendency for VOT to decrease as LVL decreases, the actual differences in VOT at high and low LVLs were not significant.

During normal speech, we rarely expire more than 10-20%VC, staying mostly in the 40-60%VC range for most utterances (7, 8). However, it is not entirely uncommon to initiate speech at 70-80%VC when speaking loudly, but speakers usually compensate by ending loudly spoken utterances at a higher %VC (~50%VC, range 50-70/80%VC). Conversely, rarely do speakers continue an utterance past their resting expiratory level (~30-40%VC). Speakers tend to stay in the 40-60%VC range because at extreme high and low LVLs, the forces of respiratory elastic recoil of the thorax (an entirely passive force) can result in subglottal pressures as high as 40 cm H₂O and as low as -40 cm H₂O

(12). Singers, however, routinely use breath group volumes between 30-50% of total VC, initiating phrasing at 70-80% VC, and often end phrasing below resting expiratory level at 25-35% VC (25). Female singers have been shown to use larger breath group volumes than males (25). Singers prefer to initiate vocalization below 80% VC and usually end phrasing at or slightly above resting expiratory level (25).

A possible source of the disagreement between our results and previous VOT and LVL results could be that we sampled VOT at specific LVLs (30, and 80% VC) to represent the typical initial and terminal LVLs that singers use, not the rare extremes above 80% VC and below 30% VC (9, 11, 24, 25). Hoit et al., (1993) had subjects speak the production task throughout 5-95% VC representing both the normal ranges and the most extreme. Perhaps, if we had measured out further to the extremes of VC a difference may have been detected, but the measures would have provided no practically significant results to singers and speakers. As it was, LVL accounted for only 10% of the variability in VOT ($\eta^2 = .1$), while training did not account for any of the variability in VOT.

Airflow modulation to contact initiation

One of the primary focuses of voice training is the voice onset during the initial vowel onset (28). Vocal students in the current study were trained extensively on coordinating initial onsets of vowels (vocal attack). We used plosive onsets and therefore could not measure the vocal attack time of vowel onsets, which consists of the difference between periodicity of the acoustic signal and the onset of vocal fold contact (90). We, therefore, derived AMCI as a measure similar to vocal attack time, but calculated after plosive release and used the modulation of the first derivative of the airflow signal, rather than the acoustic signal, to calculate the time interval. We wanted to see if extensive

behavioral training on vowel onsets would carry over to a similar process in plosive onsets. AMCI were significantly different at low and high LVLs prior to voice training (30-80% VC, $p = .006$), indicating AMCI significantly increased as LVL increased (Fig. 6B). However, after training, AMCI at 80% VC reduced significantly resulting in no differences between conditions. The effects of training accounted for 17% ($\eta^2 = .17$) of the variability in AMCI, while LVL accounted for 29% ($\eta^2 = .29$). Overall 52% of the variability in AMCI was accounted for in the study (interaction effect $\eta^2 = .07$).

The reduction in AMCI time after training was accompanied by a reduction in the standard deviation at all LVLs (reduced from an average $SD = 5.192$ to 2.697 post-training) as well as the distribution of values at each LVL indicating that singers were showing greater control of articulatory and respiratory variables affecting voice production after training (Fig. 6B). As previously discussed, McCrea and Morris (1997) determined that VOT was not precise enough to determine minute adjustments singers make. AMCI has the potential to be a measure that has the precision to determine the effects of very small changes singers make in the vocal system.

Intraoral pressure

Intraoral pressure was measured in the oral cavity behind the closed lips during the plosive. Our measurements of intraoral pressure were well within the range of normal subglottal pressure measurements in female speakers and singers (18, 29, 34, 96). Therefore, we will relate our findings for intraoral pressure with what others have found related to subglottal pressure.

We found that LVL had a significant effect on intraoral pressure which significantly increased as LVL increased from 30-80% VC. As our subjects had received

some level of choral and private voice training prior to the study, they were not “untrained,” but the results of intraoral pressure are consistent with other studies examining untrained singers (19, 29). Our subjects did not reduce the effect of LVL on intraoral pressure after training, which is incongruent with a previous study into LVL in trained singers which found no significant differences at low and high LVL (97).

Thomasson (25) inferred that the stable intraoral pressure in singers was due to the focus in highly trained singers on loudness control. Changes in loudness are significantly linked to changes in intraoral pressure (98). Thomasson investigated professional opera singers with 20+ years of training. The differences in our results may be that our singers (freshman vocal performance students with 3.1 years of training), after training in a university program, still more closely resembled untrained singers than professional singers. In our study training accounted for 0% of the variability in intraoral pressure (LVL accounted for 36%), which shows that training had no statistically significant effect, as well as no practically significant effect.

Integrated Airflow

Similar to intraoral pressure, airflow measures such as peak-to-peak flow have been shown to increase significantly with increased LVL (19, 29), as well as with increased loudness (34). As we were interested in exploring the effects of LVL and training on initial voice onset while examining temporal characteristics of the onset, we measured how much air volume was expelled during the plosive burst and not the airflow during voicing (peak to peak flow). The integrated airflow was the area under the airflow curve from airflow onset at plosive release to the onset of modulation of airflow in ml

(Fig. 3). We found that, much like peak-to-peak flow, integrated airflow significantly increased with increased LVL between 30 and 80% VC (Fig. 6D).

At high LVLs, the expansion of the chest wall has been shown to displace the larynx in a downward motion through tracheal pull (18, 29, 97, 99). This downward displacement in the vertical laryngeal position, along with increased pressures exerted on the glottis by elastic recoil forces of the thorax, are thought to be the main factors that affect speakers' and singers' ability to control flow and pressure through the glottis during phonation resulting in greater flow at high LVLs (18, 29, 97, 99). As evidenced in our results, integrated airflow and intraoral pressure were greater at high LVL before training, accompanied by increased AMCI. After training, however, AMCI significantly decreased, but intraoral pressure and integrated airflow did not. This occurrence seems to point to AMCI as an independent measure of vocal control on a very precise scale that can indicate laryngeal control overcoming pressure and flow effects.

Training and task effects on cortical behaviors

We saw two peaks, positive-positive or negative-positive in the hemodynamic response for all results (e.g. Fig. 5 and Fig. 9). This is perplexing because in previous research examining hemodynamic responses in speech, a 2 peak signal has not been reported in fNIRS. The lack of previous research showing a hemodynamic response like ours creates challenges when interpreting the results.

From the go signal to voicing took on average (3.4 s, range 2.72-4.34) with 30% VC taking the longest to reach target LVL and start singing, while 80% VC took the shortest time. Subjects usually had to inspire above the target, and then exhale into the target range. Their breathing took longer to reach the target for expiratory onset to begin

voicing. The time from the go signal to voice onset was highly variable between and within subjects. For that reason the go signal did not align with the cortical response. Therefore, we used onset of expiration to align the trials which reduced the signal-to-noise error in the hemodynamic response.

As the subject purposely controlled breathing, especially forced expiration at 50 and 30% VC, the HDR to the expiration would have been recorded with the fNIRS in our areas of interest. As expiration at high LVLs is an entirely passive event, even in speech and singing, we would expect not to see as much activation at 80% VC, and we do not (Fig. 9). Therefore, we believe that peak 1 is most likely related to expiratory onset.

Another issue arises when we examine the 80% VC condition. If peak 1 was related only to expiratory onset and controlled expiration, then at 80% VC we would see a flat signal until the rise for the second peak, which was the case except when there was a movement artifact as indicated where the HbO and HbR signals mirrored each other in the left A1 region at 80% VC and the left pM region at 80% VC (Fig. 9). Peak 2 also creates a challenge. It is occurring later than we would expect for it to be related to voice onset and was not modulated by task performance, suggesting that it was not related to voice initiation. Further study separating expiration from voice initiation, voice offset, and go signal offset will be needed to tease apart which event may be related to peak 2.

The results of the fNIRS measurements of pM, A1, and S1 showed that training had no significant effect on cortical activation patterns for peak 1 ($p = .919$) or peak 2 ($p = .28$). These findings are inconsistent with a previous cross-sectional study examining brain function using fMRI in non-singers, vocal students, and professional singers (51). They found that significant differences existed between vocal students and professional

singers with increased right hemisphere activation in overlapping S1/M1 (BA 3 and 4), as well as the parietal lobe. One reason we did not see a difference may be that our freshman vocal students in the current study, even after training, more resembled the untrained singers than vocal students or professional singers in Kleber et al. (51). The amount of training also points to this conclusion with our subjects reporting on average 3.2 years of private instruction, while the professional singers in Kleber et al.'s study reported an average of 20.2 years of professional training, and their vocal students were on average 7 years older ($M = 25.22$) than our vocal students ($M = 18.2$). In fact, none of our vocal students were as old as the youngest vocal student in the Kleber study; their vocal students had 6.8 more years of private instruction ($M = 9.7$) than our subjects ($M = 3.1$). Because of these large differences in age and experience, comparing the findings in our study to Kleber et al. (51) may not be valid as we examined a very different population of singers with experience somewhere between their vocal students and untrained subjects.

We found that differences between locations of activation significantly changed based on what LVL was being performed. At 80% VC, pM had significantly less activation than A1 and S1 while A1 also had significantly reduced activation from those of S1. S1 also had significantly greater activation at high LVL than pM at 30% VC which may suggest that sensory feedback was important at higher and lower LVLs. S1 in peak 2 had greater overall activation than pM, which is further evidence that sensory feedback is important at high and low LVLs.

We did observe a non-significant trend in the differences between right and left side ($p = .055$) showing that the left side overall may have had more activation than the right, but there was no interaction between side and training ($p = .197$) or location ($p =$

.796) or LVL ($p = .263$). The symmetrical distribution of activation in pM, S1, and A1 has been reported in singers (100-102). This is not in agreement with the finding that vocal students have increased right hemisphere activation (51).

Limitations

The testing of the LVL conditions was not counterbalanced to control for possible order effect. Future research on this topic, however, should counterbalance the %VC conditions to better control for order or fatigue effects which could have affected our measurements. We did not control intensity or change it; therefore, increases in intensity may have been caused by some of the voice measures, especially intraoral pressure and integrated airflow. Training was the only area of this study that was out of our control. We were assured by the voice faculty that the first semester training regimen is very similar across students with different levels of skill and previous training, but cannot be entirely confident that all students worked on very similar behaviors.

Conclusions

We found that cortical activation patterns were significantly affected by changes in LVL, but not by training. We confirmed previous research linking increased intraoral pressure and airflow with increased LVL. We also discovered that AMCI may be a useful tool for examining subtle changes in the vocal system of singers. Our findings are in disagreement with previous research on the relationship between VOT and LVL as no differences were found in VOT between high and low LVLs. In our examination of the cortical activation patterns associated with training and LVL, we did not see the differences between the two hemispheres that we would expect in singers based on the

literature. Differences in subject characteristics may have contributed as our teenage vocal students had less training than adult vocal students with professional voice training studied by Kleber et al. (51). Further study is needed over longer periods as untrained singers become experienced vocal students. We were not able to determine what the basis was for peak 2 as it only varied across S1 and pM, but not across side, LVLs or with training. Further study is needed to parse out behavioral events that may be related. Overall this research demonstrated that when respiratory and voice demands in young singers are greater on initiating voice at low LVLs, cortical activation is greater in all of the brain regions involved.

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Tables

Table 2

Talairach coordinates for regions of interest examined using fNIRS (51, 70, 86)

Regions		x	y	z
Left	Inferior Premotor area	-53	7	23
	Primary inferior Somatosensory Cortex (S1 - orofacial)	-57	-23	12
	Posterior superior temporal gyrus (Auditory)	-60	-34	16
Right	Inferior Premotor area	53	7	23
	Primary inferior Somatosensory Cortex (S1 - orofacial)	57	23	12
	Posterior superior temporal gyrus (Auditory)	64	34	12

Figure Legends

Figure 1.

Configuration for subject testing. A – Go signal light, B – biofeedback screen, C – pneumotachometer mask stand, D – pneumotachometer and pressure mask, E – EGG transducers, F – Inductobands for respiratory kinematics, G – fNIRS array, and H – fNIRS system and cart.

Figure 2.

Event-related design schematic. Example of screen subjects used during production task showing respiratory biofeedback (%VC) with target guidelines for target LVL acquisition and go signal. For this example (80%VC), go signal was turned on, subject inspired above “Target LVL” (Expiratory Onset), controlled expiration until in “Target LVL” range and then vocalized until signal was turned off.

Figure 3.

The method for calculating integrated airflow, intraoral pressure, VOT, and AMCI. Integrated airflow is the area under the curve from plosive release to airflow modulation. Intraoral pressure is the peak the of the pressure signal before plosive release. VOT is measured from the onset of airflow (denoting the point of plosive released) to the peak of the first derivative of the first glottal pulse indicating initial vocal fold contact (87). AMCI measured from the first periodic peak (+/- 1 SD) of the first derivative of the airflow signal (airflow modulation after plosive release) to the peak of the first derivative of the first glottal pulse (103).

Figure 4.

fNIRS optode placement. The letters are emitters and the numbers are detectors. The brain regions measured are the circles on the black lines between the emitters and detectors. C6 measures the dorsolateral premotor area, D5 – orofacial/laryngeal somatosensory area, and D4 – posterior superior temporal gyrus auditory area. The hemodynamic response traces give an example of where the activation is and how large the cortical response is for each location at a specific LVL target for the mean pre-training data at 50% VC.

Figure 5.

fNIRS output (HbO and HbR) for Right Auditory area at 50% VC pre-training condition in a averaged across all participants with ± 1 SE error bars. Baseline derived from an average of -5 s to -1 s for calculating Z-scores of change. The orange line is the go signal onset, the black line a 0 represents the expiratory onset, the green line represents the average onset of voicing (~2 s after expiratory onset), and the red line is voice offset. Peak 1 (P1) and peak 2 (P2) of the HbO signal are identified, along with P1 offset (O1). HbR inverse relationship HbO is present.

Figure 6.

Box plots for each of the four physiological voice measure by % VC for both training conditions. The significant effects of LVL can be seen in AMCI (B), intraoral pressure (C), and integrated airflow (D). The reduction of AMCI at 80% VC post-training is also evident in (B).

Figure 7.

Box plots of all locations combined by peak 1 by LVL for pre- and post-training conditions.

Figure 8.

Break down of combined peak 1 Z-scores by LVL for each of the 6 locations for pre- and post-training results which shows the significant interaction between location and LVL.

There is a clear depression of the signal that is likely do to motion artifact that can be seen in the Fig. 9 hemodynamic response for the left side.

Figure 9.

All traces of the mean hemodynamic responses in the left side for each location and LVL prior to training. The blue trace with +/- 1 SE error bars is the HbO and the green trace is the HbR deoxy signal. The peak 1 activation decreases from a large amount of activation at 30% VC (A, D, G) to much lower activation at 80% VC (C, F, I) is likely caused by added motion artifact from the large inhalations up to 80% VC. If looking vertically the locations can be compared as similar LVLs.

Figures

Figure 1.



Figure 2.

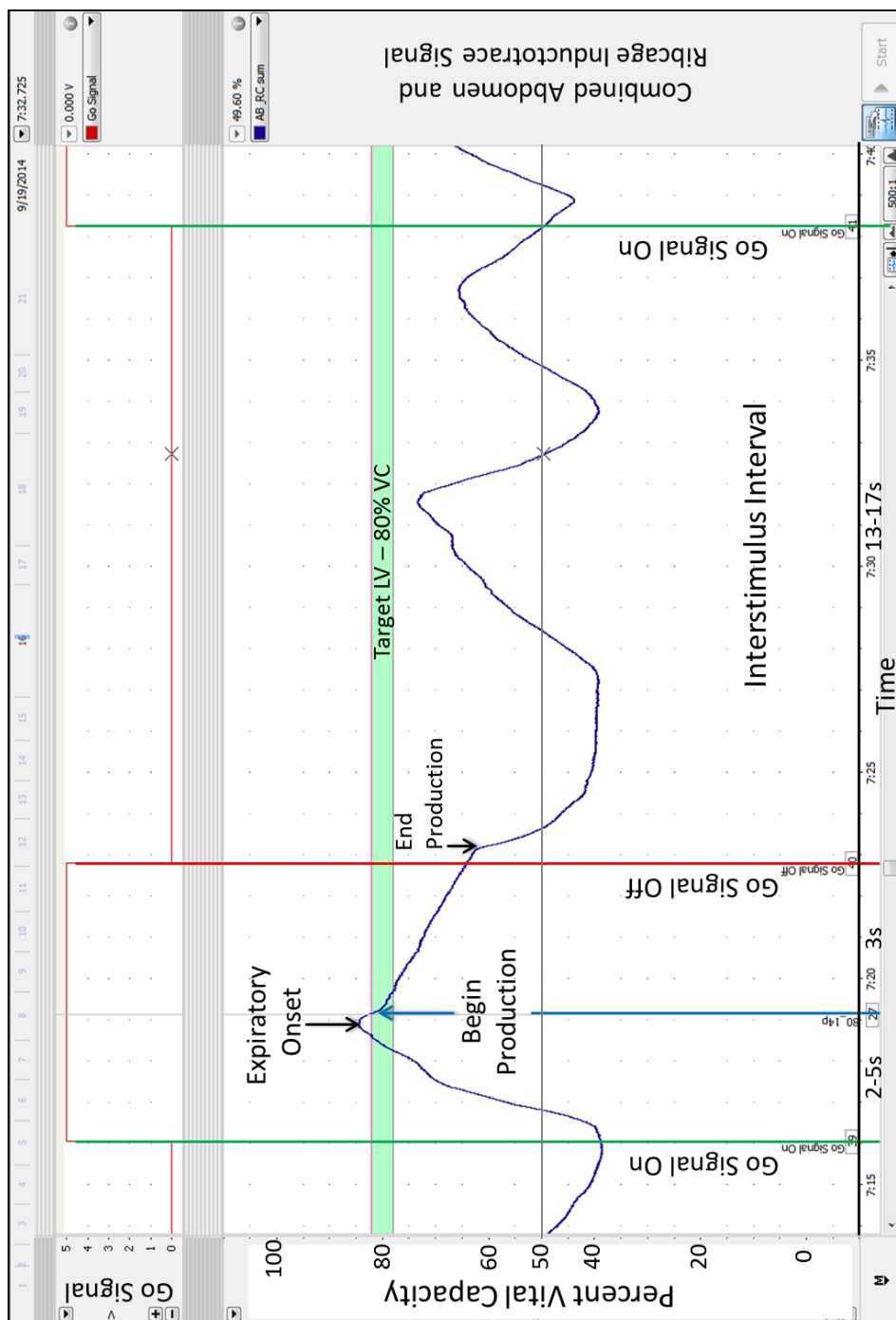


Figure 3.

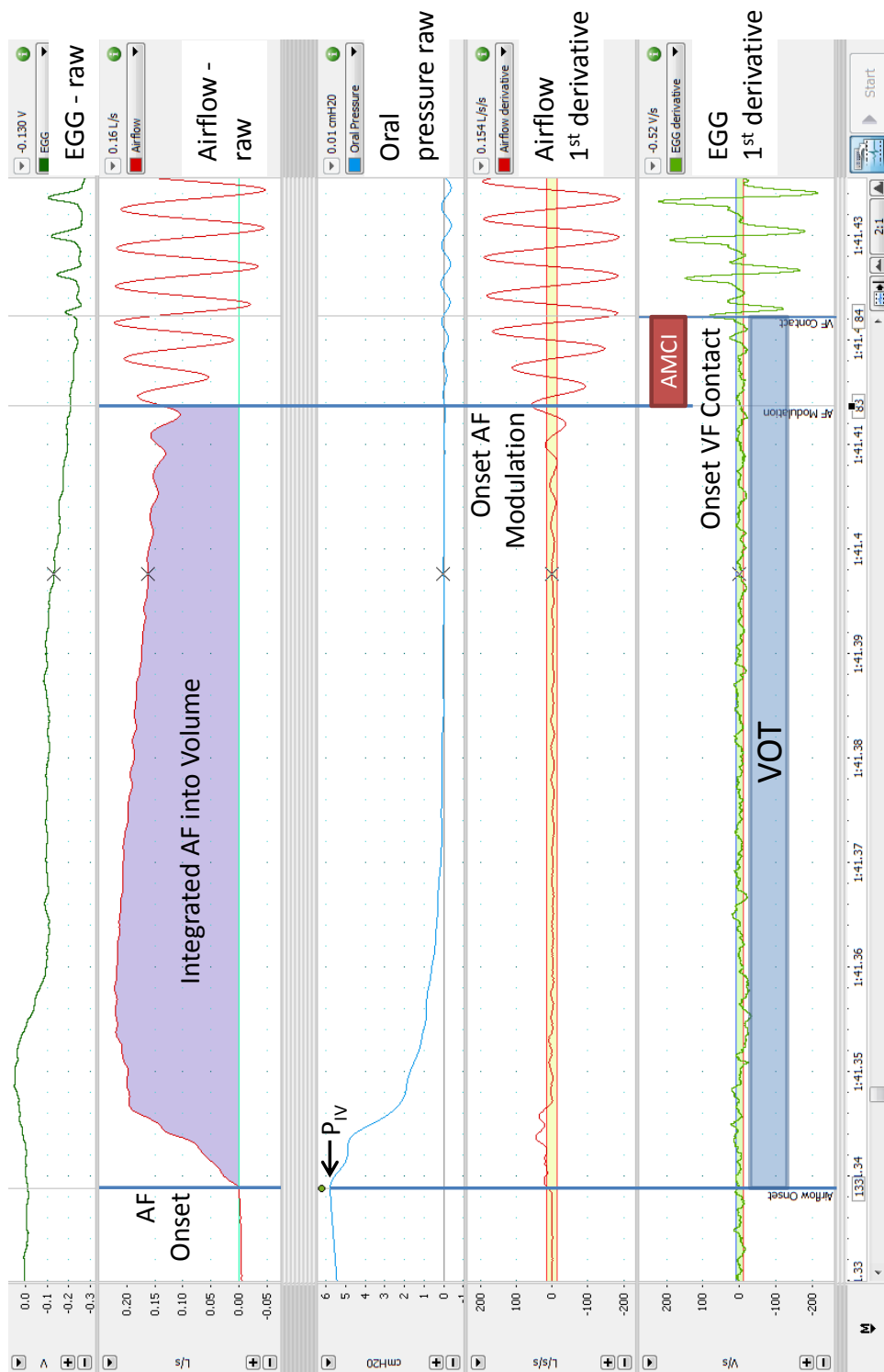


Figure 4.

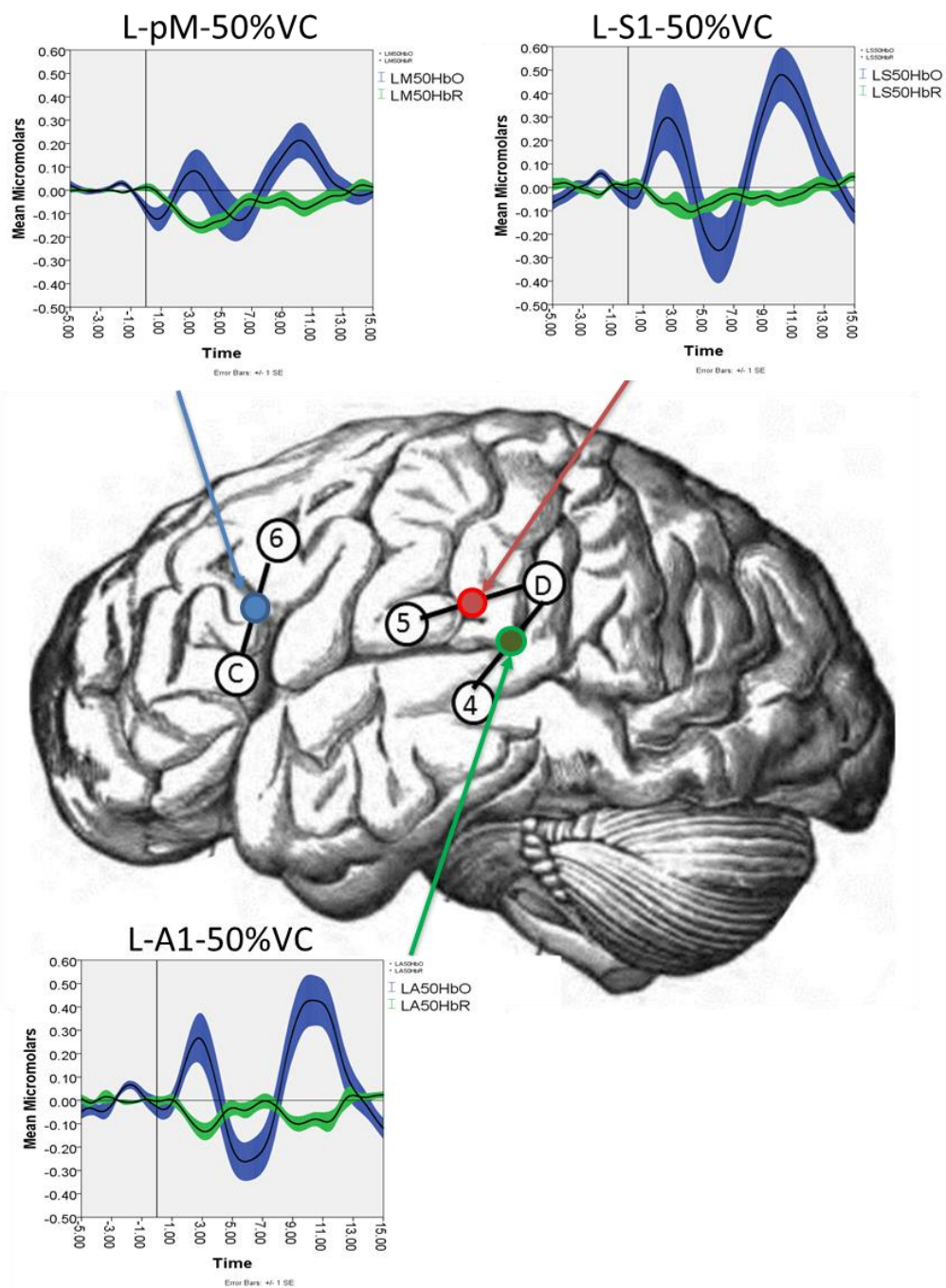


Figure 5.

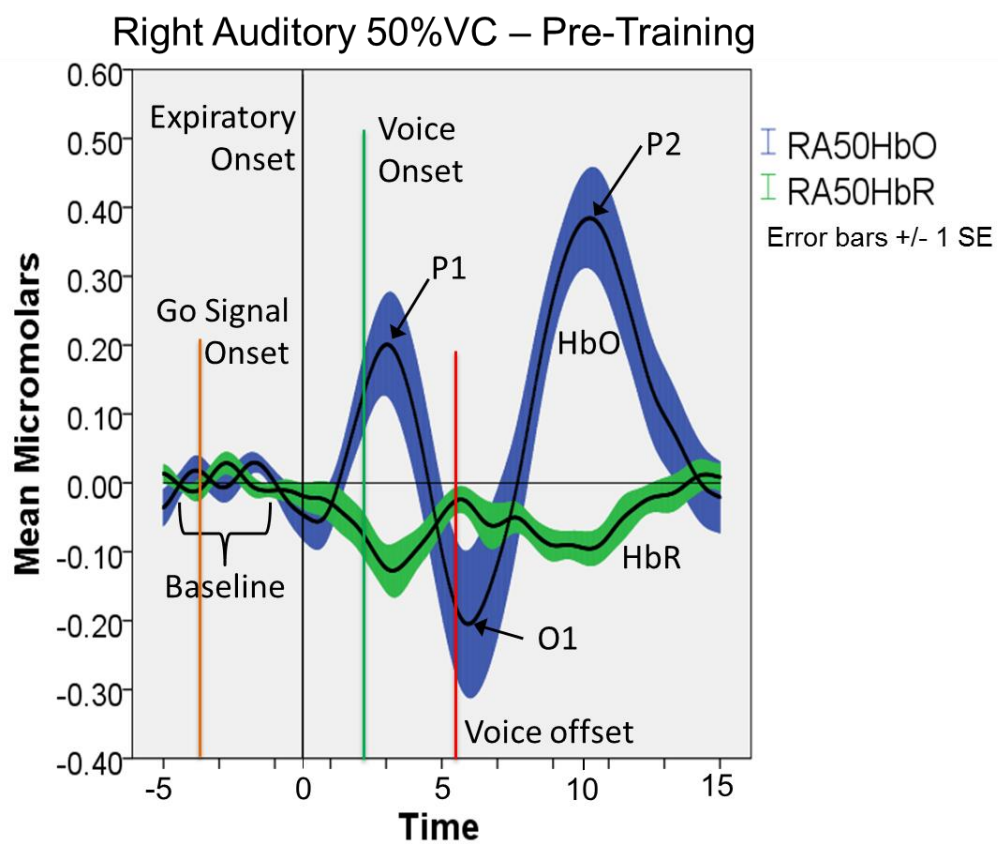


Figure 6.

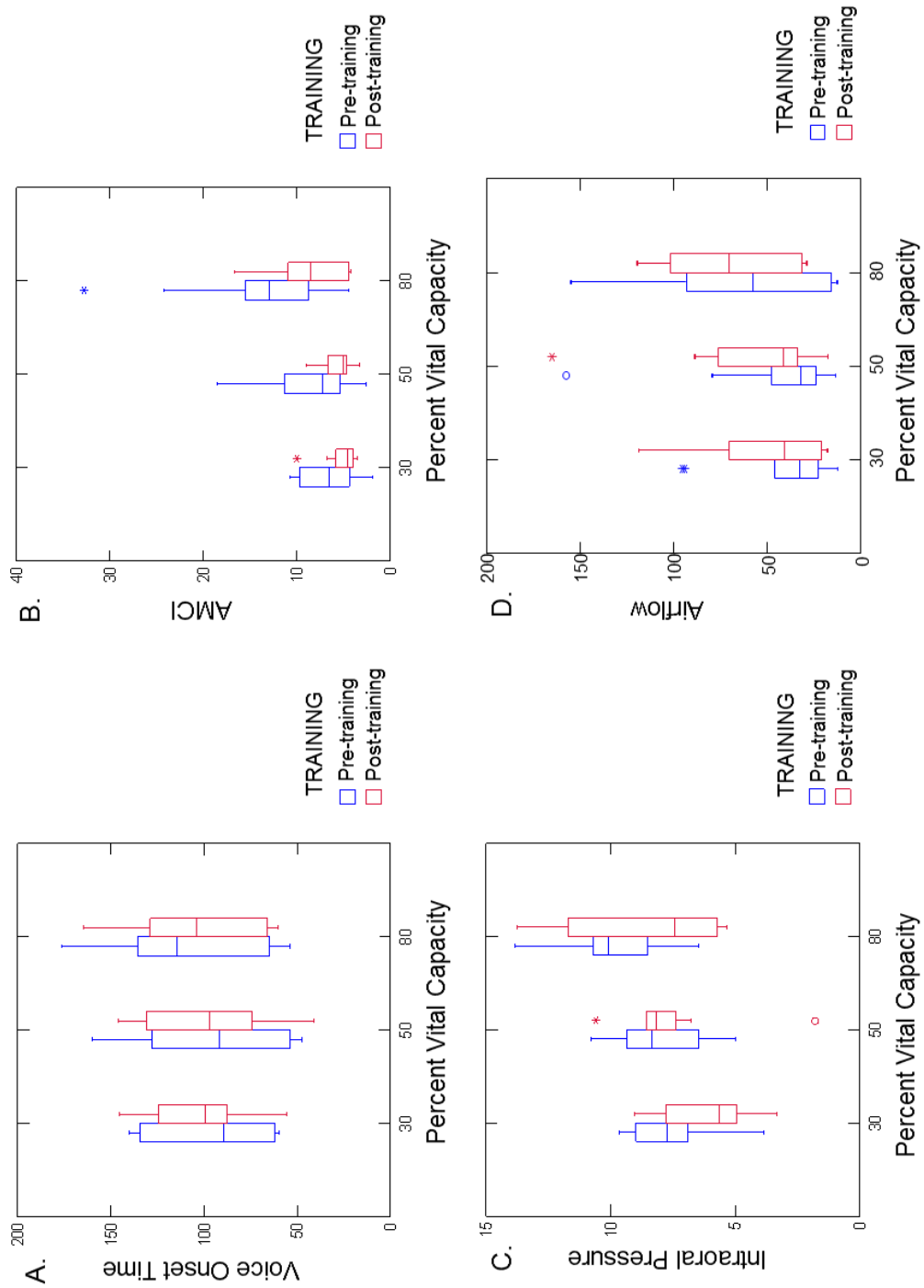


Figure 7.

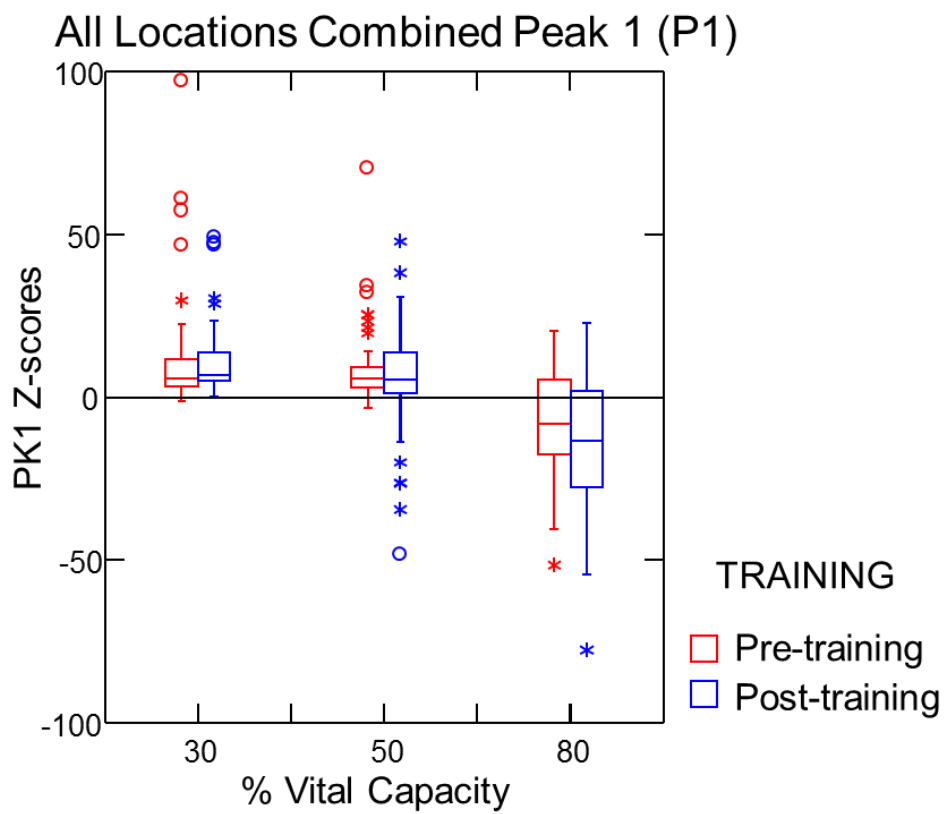


Figure 8.

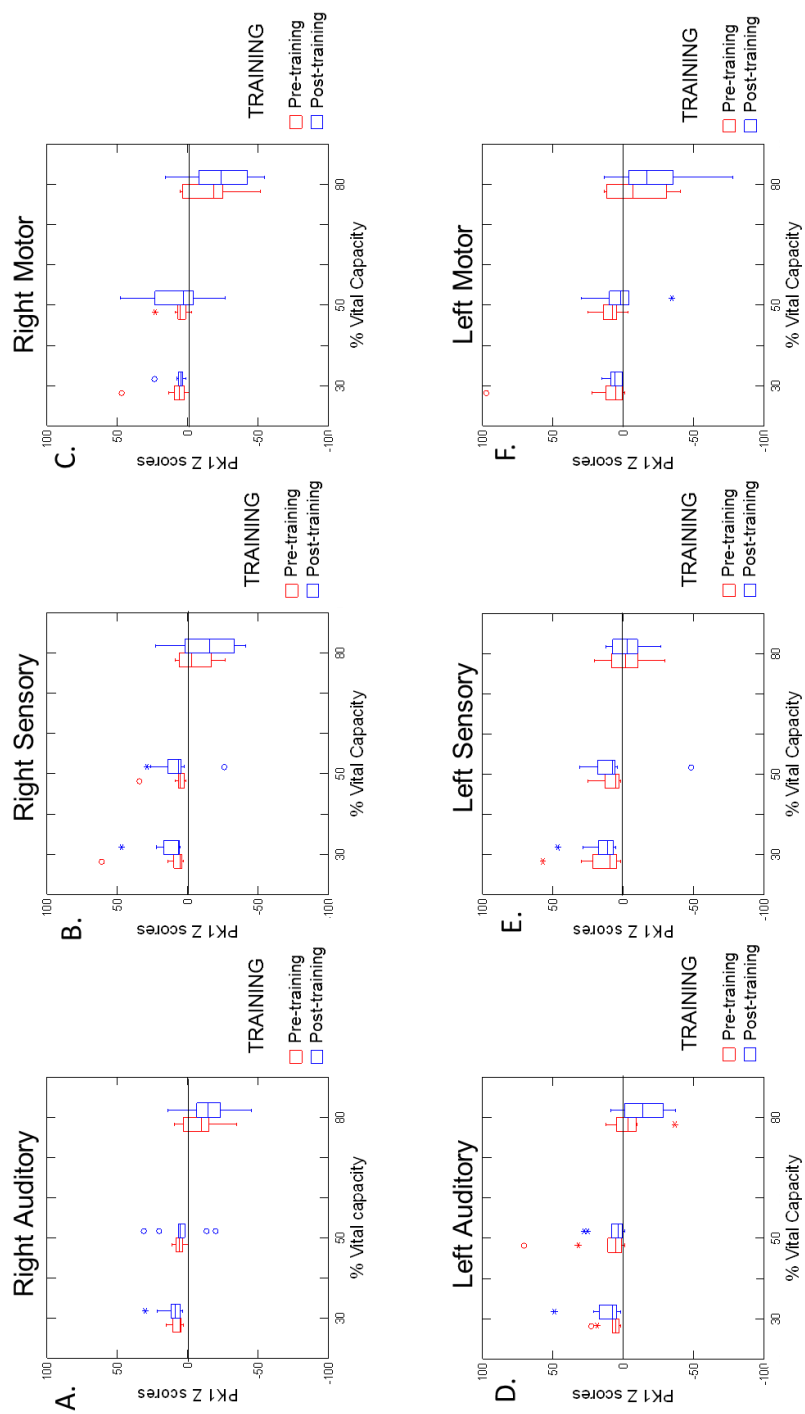
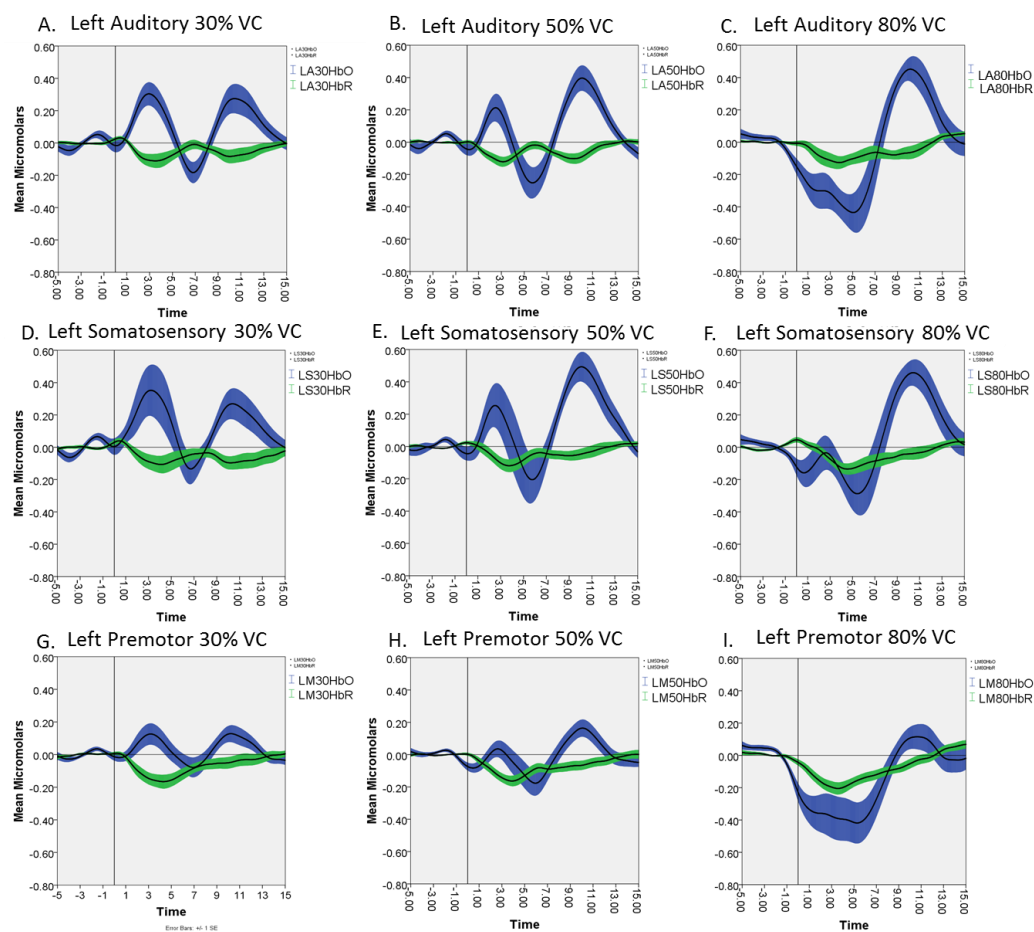


Figure 9.



IV. CONCLUSIONS

Two studies were conducted to examine the effects of LVLs and training on cortical activation patterns and physiological voice measures between non-singers and singers (manuscript 1), and also within vocal students (singers in the first study) before and after vocal training in a university program (manuscript 2). The first study found that as LVL increased from low to high, the integrated airflow and P_1 increased significantly in both non-singers and singers. Singers were the only group that showed a significant increase in AMCI with increased LVL, while the non-singers showed no change. The training effect in AMCI showed that training reduced the ability of singers to rapidly close the vocal folds after a plosive at high LVLs. VOT showed no significant effects or interactions between training and LVL.

Examination of fNIRS data revealed that training had no effect on cortical activation. These results are not in agreement with recent findings that vocal students show increased cortical activation in the S1, A1, and pM (Kleber et al., 2010). LVL was the only variable that had a significant effect on cortical activation. We observed two peaks in the hemodynamic response in both groups. Although further research is needed to determine the basis for peak 2 as it showed no change with task difficulty or performance. LVL was the only variable that had an effect on peak 1 cortical activation. Voicing at low LVL (30% VC) resulted in the greatest cortical activation while voicing at high LVLs (80% VC) showed significantly less cortical activation. Further research is needed to parse out what is causing the hemodynamic response related to these cortical events and their potential implications for the advancement of voice science and understanding of cortical control and coordination of voice and respiration.

In the second study we examined vocal students (singers from the first study) enrolled as freshman in a university vocal performance/education program prior to their first semester of training. Vocal students were tested before and after their first semester of vocal training to determine if training and LVL would have an effect on physiological voice measures and cortical activation patterns. There were no effects or interactions of training and LVL on VOT. These findings confirmed that VOT did not change with training and it did not change from low to high LVL. We found that, similar to the first study, intraoral pressure and integrated airflow were significantly affected by LVL, but not by training. AMCI revealed a within-subjects training effect in the singers and a between-subjects effect between singers before university training and untrained singers. There was a significant reduction in AMCI from pre- to post-training at the 80%VC condition. The university voice training increased glottal precision at high LVL and post-training vocal students could more rapidly close the vocal folds in a more consistent manner while still contending with significantly greater intraoral pressure and airflow. Given that singers had different amounts and types of private instruction before entering into university training. Their backgrounds may not be a good qualifier for group membership as “trained singers” and greater attention must be given to the level and extent of singing training. The findings indicate that AMCI may reveal itself to be a measure precise enough to show significant, though minute, details about possible articulatory, laryngeal, and or respiratory change in the system that the other temporal-acoustic measure (i.e. VOT) was unable to detect.

The results of the fNIRS data revealed that training did not have an effect on cortical activation patterns in the pM, A1, and S1 in vocal students. This was contrary to

a recent study on differences between different levels of training in singers (Kleber et al., 2010). Differences in the populations examined may have been the cause (our teenage vocal students with less training vs. adult vocal students with 3 times the professional voice training). However, we may have filled a gap between untrained singers and experienced vocal students.

The hemodynamic response post-training showed two peaks similar to the pre-training response and untrained singers in the first study. However, only peak 1 was related to differences in performance and task demands at different LVLs. Further investigation is needed to parse out behavioral components that are not related to changes in the first peak at high and low LVLs. Though we may not have the answers to those questions, the consistent results between and within different populations of singers and non-singers in response to differences in LVLs suggest that the results are meaningful.

We also determined that LVL, cortical location, and their interaction had a significant effect on peak 1 activation. The interaction between location and task revealed significantly decreased activation in pM compared to A1 and S1, which had the greatest activation at 80% VC, while the cortical activation in S1 at low LVL was again significantly greater than in the other locations. This suggests a role of sensory feedback when subjects were performing at low LVLs (e.g. 30% VC), which was the most difficult production task. Further research is needed to address this suggestion that sensory feedback plays a greater role in voice activation at low lung volume levels.

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