INTRINSIC LARYNGEAL MUSCLE RESPONSE TO A SPEECH PREPARATION STRESSOR: PERSONALITY AND AUTONOMIC PREDICTORS

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Widespread clinical wisdom holds that stress affects the voice, as stress also affects many somatic functions. Assuming the validity of this rather straightforward assertion, clearly, the final causal pathway in some stress-induced voice disorders must involve the intrinsic laryngeal muscles (ILMs), which are indeed targeted by behavioral treatments. Unfortunately, to date, no data have been reported that directly investigate the underlying assumption. Moreover, some causal models around links between stress and voice include upstream factors. Specifically, personality traits such as Stress Reactivity might increase one's susceptibility to these problems. In addition, the strength of the parasympathetic nervous (rest-and-digest) system response is implicated in the pathogenesis of voice disorders putatively involving ILM hyperfunction.

In the present study, 40 vocally healthy adult females were subjected to a stress-inducing speech preparation task. Measurements of heart rate, blood pressure, trapezius muscle (positive control site) activation, and anterior tibialis muscle (negative control site) activation were obtained before and during stressor exposure to confirm physiological stress response compared to baseline. Additionally, fine wire electromyography of the following ILMs was performed so that the activity of these muscles could be measured prior to and during the stressor. Findings were largely consistent with the hypothesis that the ILMs and trapezius significantly increase in activity during stress reactions compared to baseline, as does the anterior tibialis muscle. Personality measures uniquely predicted thyroarytenoid, upper trapezius, and anterior tibialis activity, whereas parasympathetic nervous system "tone" uniquely predicted the activity

of all muscles studied. Differences were observed in the latter predictor variable as a function of whether or not effects of respiration were accounted for in the variable's calculation.

The present study is the first to characterize ILM responses to psychological stress in vocally healthy participants, and further elucidates the contributing roles of trait Stress Reactivity and autonomic function in laryngeal muscle tension. This study helps to prepare a platform for future studies on individuals with common and somewhat poorly understood voice disorders often thought linked to stress, such as muscle tension dysphonia.

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PREFACE

Well, this has been a lot of fun! I couldn't have *won* a better committee. Dick and Jim—thank you for your availability, incisive feedback, and encouragement. Sue, your constant support and ready praise have buoyed me through many low points, surely more than you even realize. Connie, you are a total class act and a brilliant thinker, and I want to be like you when I grow up. Clark, I am endlessly honored that you found my work worth everything you invested into it—your precious time, your encouragement, your expertise. I am privileged to be your colleague and proud to be your friend. And Kittie, from the moment I saw you stylin' in your skinny jeans and talking about how a little more water shakra could energize the voice, I knew I was destined to be your progeny. I'm glad I tricked you into accepting me into your lab. There is too much to say, and too many thanks to offer, for this space. But I promise I will hold dear your mirror, forever and ever amen.

Wei, you had several chances to say "no" when I first asked you for help with this project years ago, and I hope you don't regret those missed opportunities. You have truly gone above and beyond on my behalf, and I fear I might never repay you. You are a talented scientist, a committed mentor, and a respected colleague. To my colleagues and friends on the research staff at Walter Reed circa 2007, you stoked the early flame that is now my ever-burning love of research. If I have the tiniest fraction of your intellect and skill as a scientist, I'll carry it proudly. Of course, Fancy Nancy, you're leading that parade. You are the stuff that mentoring dreams are made of, and I am doubly honored to also have earned your friendship over the years. Thank you. In my life, I will pay it forward in your honor.

Working in this lab has been a gift. I have learned a ton, laughed as much as the day is long, been cared for and protected and swept heartily into this surprisingly functional little family. Ryan, Nicole, Maria, Chaya, and Doug, thank you for taking me into the fold and setting a high bar. Drica and Catherine, I'd still be slogging through data collection and forgetting the ground cable if not for your dedication – thank you! Drica, I won't leave you; we can SBFF any time and I'll still help with maintaining the lab's tenuous feng shui. Amanda and Aaron…well…there just aren't words. We've got something really special in this little triumvirate. If anyone were paying me for this dissertation you'd surely both be entitled to a cut for all the listening/questioning/feedbacking you've both done. But then again, you'd both owe me a lot too, so I guess we're even. *Ziegillou*! And to my family of the flesh-and-blood variety, you all have so patiently waited for me to do whatever it is I've been doing. You believed in me, and chipped in, and were the constant cheering section in this game. Thank you.

Rabih, almost exactly a decade ago, I wondered if I was a fool for noting you in the preface to my master's thesis. We were really new—we weren't even a "we", actually—but apparently I excel in foresight where I fail in recollection. We're gonna keep steering our little ship, part reason and part passion, and maybe in another decade we'll finally dance to INXS like I know we can.

Finally, Aya, the work here represents my passion for puzzles, my curiosity, my desire to reveal the "truth" of things, and my endless fascination with the beautiful instrument and revealer of the Self that is the human voice. I won't wish to impart these things to you, because it's already pretty clear that you're an arrow sailing along your own course. But maybe someday, when you care to, you'll thumb through this and know a couple of things. First, maybe you'll know me just a little better, a little bit differently, for having skimmed this product of five years' work, or maybe just for reading about these people you might or might not have the honor of knowing. And second, I hope you know that in the beautiful and long-awaited spring of 2014, nothing on my horizon shone as brightly as you.

1.0 INTRODUCTION AND BACKGROUND

Stress affects the voice. However, the extent to which stress affects the intrinsic laryngeal muscles (ILMs), which are essential contributors to the "source" of the final vocal product, has scarcely been investigated. This issue is highly relevant to clinical and investigative voice specialists, as ILM hyperfunction is alleged to underlie a variety of common voice disorders, such as muscle tension dysphonia and vocal nodules (Altman, Atkinson, & Lazarus, 2005; Froeschels, 1952; Morrison, Nichol, & Rammage, 1986; Morrison & Rammage, 1993; Oates & Winkworth, 2008; Rubin, Blake, & Mathieson, 2007). Despite the patent lack of empirical evidence for ILM hyperfunction as a response to stress, somewhat theoretical proposals have been made to explain this putative phenomenon.

One model of particular interest is the Psychobiological Framework of Voice Disorders proposed by Dietrich and Verdolini (2008), which submits that laryngeal muscle tension arises from the concurrent and interactive engagement of the neuroendocrine, the autonomic nervous, and the somatic motor systems (see Figure 1-1). Influencing these systems' responses "upstream" is the individual's personality and how it uniquely responds to a given situation (i.e., the person-by-situation interaction). Others have proposed a potent influence of personality on voice within the context of voice disorders, such that introversion predisposes individuals to develop one type of disorder and extraversion predisposes individuals to develop a different type of voice disorder (e.g., Roy & Bless, 2000a). Dietrich and Verdolini's research supports the relevance of these broad personality traits to extrinsic laryngeal muscle response to stress (Dietrich & Verdolini Abbott, 2012). Dietrich and Verdolini Abbott (2008) advance that when psychological conditions align with somatic stress responses in a chronically unhealthy fashion, the resulting laryngeal environment is then ripe for the development of one specific voice disorder, muscle

tension dysphonia (described in upcoming section). However, since muscle tension dysphonia is not the only voice disorder proposed to be associated with ILM hyperfunction, as referenced previously, the present discussion will be focused more broadly on ILM hyperfunction rather than any one specific voice impairment that may be caused by it.

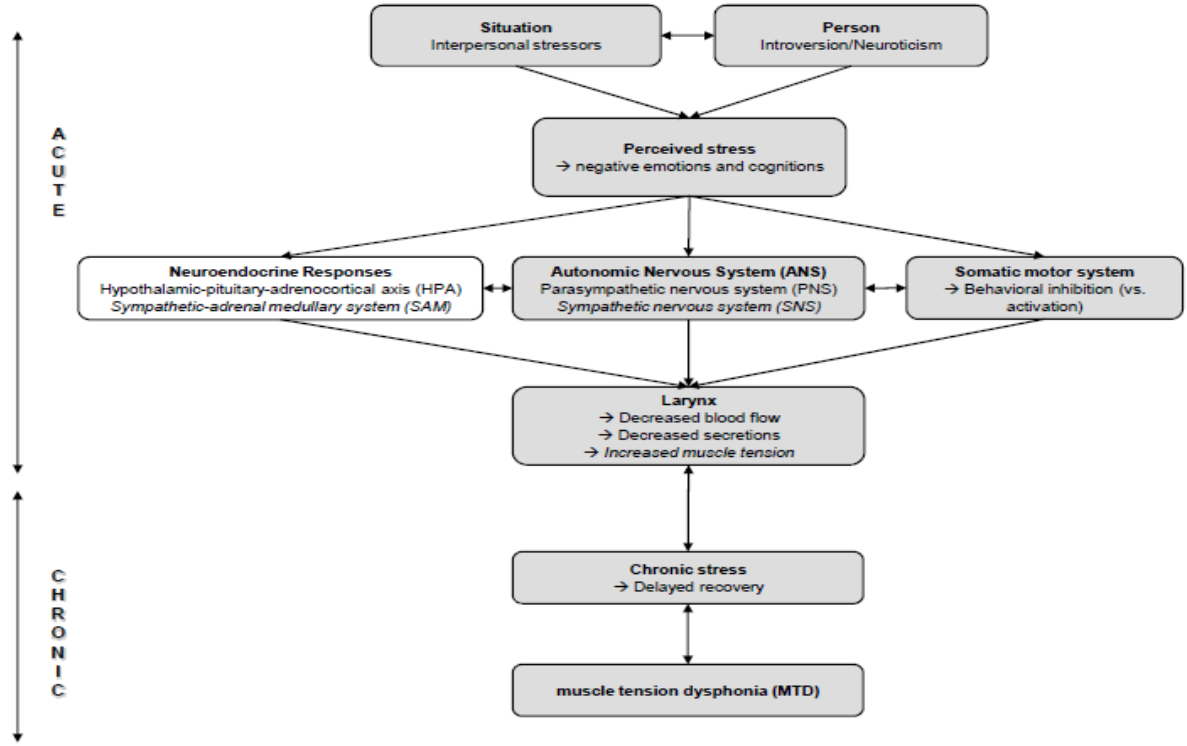


Figure 1-1. Psychobiological Framework of Voice Disorders (Dietrich & Verdolini, 2008).

The Psychobiological Framework of Voice Disorders is a reasonable approximation of the pathogenesis of muscle tension dysphonia, informed by both clinical and empirical understanding of the voice and MTD. At the broadest level, the present study seeks to investigate a few key aspects of the Psychobiological Framework of Voice Disorders. First, this study will examine the oft-held claim that the ILMs respond to stress with increased activation, and the actual nature of that response will be characterized at a general level. Second, one particular mediator of the presumed ILM stress response, the autonomic nervous system, will be investigated as a potential predictor of ILM activity in response to stress. Third, the "higher-level" element of personality will be investigated to determine whether trait stress reactivity is predictive of ILM activity in response to stress.

1.1 DEFINING TERMS

Stress. *Stress* is a broad term that refers to somatic or psychological "tension" (used ambiguously as a lay term) stemming from factors that disrupt one's equilibrium (Lovallo, 1997). More specifically, Cohen, Kessler and Underwood (1995) define stress as "a process in which environmental demands tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place persons at risk for disease." Conditions or experiences that cause stress, and that possibly tax an organism's ability to adapt, are referred to as *stressors*. Stressors can be physical, biological, or psychological, and may exist acutely or chronically (Dickerson & Kemeny, 2004). In the present document, the term *stress* refers specifically to *psychological stress* unless otherwise specified. The negative psychological response to a stressor is *distress*, which may be characterized by classic "fight" states such as anger, rage, and frenzy, or by classic "fear" states like anxiety, terror, and panic (Goldstein, 2001).

Anxiety. Another state that is commonly elicited or heightened during exposure to stressors is *anxiety. Anxiety* is considered an important "stress emotion" (Barlow, 1988; Endler & Kocovski, 2001; Lazarus, 1999). In all of its forms—state, trait, and psychopathological—it is rooted in the emotion of fear (Barlow, 1988; Barlow, 2002) and, at least in state forms, is closely related to autonomic nervous system activity (Friedman, 2007). Anxiety is related to constructs such as apprehension, avoidance, and panic, but is also riddled with terminological ambiguity; it has been used to refer to emotional states including boredom, doubt, mental conflict, disappointment, shyness, difficulty concentrating, etc (Barlow, 2002). Some have proposed that clear definitions be abandoned, and that anxiety be considered a non-specific, metaphoric, lay construct that simply refers to highly variable, individualized cognitive and somatic "points of reference" (e.g., how one feels during their own experience of anxiety) (Hallam, 1985)

Despite the semantic and definitional challenges associated with the construct, anxiety nevertheless is widely believed to exert potent influences on physical health and mental well-being. The working definition for anxiety in the present manuscript is the one proposed by Barlow (2002), who

characterizes anxiety as a "unique, coherent cognitive-affective structure within the defensive motivational system." His basic translation of "anxiety" is "that terrible event could happen again, and I might not be able to deal with it, but I've got to be ready to try." (Barlow argues that a better term may actually be *anxious apprehension* to emphasize the notion that the state of anxiety is "future-oriented" in that one is prepared to deal with future negative events. For simplicity, the present manuscript will use the more widely-used term *anxiety*.) Anxiety is characterized by perceptions of uncontrollability and unpredictability over possibly negative events. These two elements, predictability and especially controllability, are key elements of stressors that influence the magnitude of the perceived and physiological stress response (Dickerson & Kemeny, 2004). Anxiety is also characterized by hypervigilance to identify stressful events, and physiologic or somatic readiness to deal with the events. Like muscle tension dysphonia and largely other medically unexplained symptom complexes (e.g., irritable bowel syndrome), women experience anxiety disproportionately to men (Barlow, 2002). Other psychological conditions that are related to anxiety include generalized anxiety disorder, obsessive-compulsive disorder, panic disorder, and post-traumatic stress disorder (Friedman, 2007).

Muscle Tension Dysphonia. The Classification Manual of Voice Disorders defines the term *primary muscle tension dysphonia* (hereafter referred to as MTD) as follows: "dysphonia that arises from apparent excessive use or misuse and hyper- or hypofunctional voice patterns in the absence of current organic vocal fold pathology, psychogenic, or neurologic etiology." (Verdolini, Rosen, & Branski, 2006) MTD is the voice disorder most consistently discussed in the context of laryngeal muscular hyperfunction, as these hyperfunctional behaviors are a purported hallmark and diagnostic linchpin of the disorder. Most of the research cited herein pertaining to individuals with voice disorders involves a cohort of participants with MTD.

Laryngeal Muscular Hyperfunction. Laryngeal hyperfunction, or "hypertonicity" of the intrinsic and/or extrinsic laryngeal muscles, is thought to contribute to the development of not only MTD, but also vocal fold edema, general "vocal fold thickening", vocal fold nodules, polyps, and contact ulcers (Hillman, Holmberg, Perkell, Walsh, & Vaughan, 1989). For this reason, some investigators have argued

that a substantial proportion of patients with so-called "organic" voice disorders (e.g., vocal fold nodules, polyps, cysts) are simply presenting at late stages of impairment that is ostensibly due, at least in part, to an underlying laryngeal hyperfunction akin to the laryngeal hyperfunction underlying "functional" disorders such as MTD (Millar, Deary, Wilson, & MacKenzie, 1999). This stance informs the terminology in the present document. Rather than focus on the pathogenesis of disorders such as MTD, *per se*, which is muddied by debate of whether it is actually *caused* or simply *characterized* by laryngeal muscle hyperfunction, the onset of the laryngeal hyperfunction itself is of interest. Thus, the present study might be theoretically relevant to the broad array of voice impairments involving laryngeal muscular hyperfunction.

1.2 RELATIONSHIP OF VOICE TO STRESS AND ANXIETY

Most speakers can probably attest that stress and anxiety can alter voice. We can easily perceive stress or anxiety in a loved one, and learn to appreciate their nuanced vocal manifestations of such states. Even in strangers, listeners can perceive anxiety from the nonverbal aspects of speech alone (Laukka et al., 2008). As schematized in Figure 1-2, the vocal source and filter are modified by the intrinsic and extrinsic laryngeal muscles as well as by supraglottic structures. This schematic pertains to the *perceived stress→larynx→muscle tension dysphonia* relationship (mediated by the chronicity of stress) included in Dietrich and Verdolini's Psychobiological Framework of Voice Disorders (see Figure 1-1). The implicit assumption in that model is that voice is actually altered by the stress-induced changes to the larynx, and in the context of chronic stress, pathology characterized by chronic dysphonia may result (i.e., *perceived stress→larynx→voice changes→muscle tension dysphonia*). Despite the fact that the ILMs are not the only contributors to voice quality, they are of particular interest herein. Thus, the role of the extrinsic laryngeal muscles will be addressed to a lesser extent than the ILMs, and the role of the supraglottal structures in modifying voice is beyond the scope of this document. A stressor's impact on these major

parts of the phonatory system collectively affects the final product, voice (Scherer, 1995). Evidence of the effect of stress on voice is seen in the acoustic signal of the voice. The acoustically rich vocal signal serves as a sensitive gauge of emotional state, and many researchers have tried to decompose this signal to identify the parameters that index emotion.

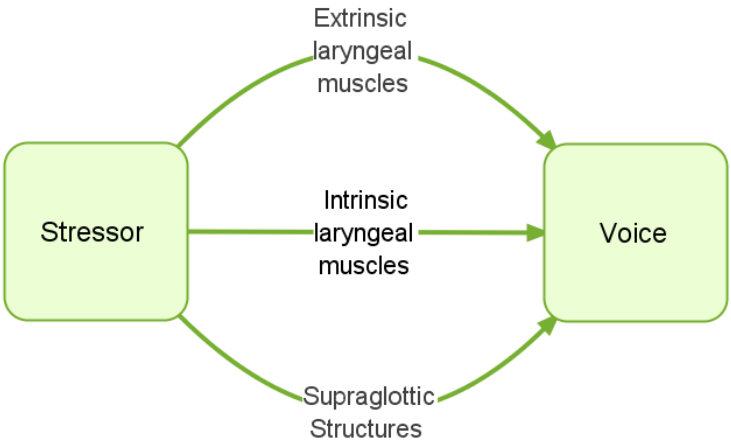


Figure 1-2. Conceptual schematic: stressor effect on voice.

Acute stress and anxiety can be acoustically characterized (Juslin &Laukka, 2003; Scherer, 1986) more accurately than other negative emotional states (e.g., anger, disgust, etc, which have been areas of great interest in voice research). Specifically, during stressed states, vocal acoustics may be characterized by specific patterns of vocal frequency (Dietrich & Verdolini Abbott, 2012; Van Lierde, Van Heule, De Ley, Mertens, & Claeys, 2009), intensity (Dietrich & Verdolini Abbott, 2012), and high-frequency spectral energy components (Laukka et al., 2008). However, the directionality of these voice changes are not homogenous, and acoustic measures generally exhibit exceptionally high inter-subject variability, a point which will be expanded upon shortly. Giddens, Barron, Byrd-Craven, et al. have recently put forth an elegant and thorough review of vocal indices of stress (2013). For the present discussion, select recent studies (within the total body of literature on voice outcomes of stress/anxiety/emotion induction) are summarized in **Table 1-1**. These studies all involved examination of the effects of stress or anxiety on

voice characteristics in healthy normals (i.e., not psychologically disordered or voice impaired), and as previously mentioned, reflect a high degree of inter-subject variability. For instance, these studies lack consensus regarding whether stress leads to increases (Mendoza & Carballo, 1998; Wittels, Johannes, Enne, Kirsch, & Gunga, 2002) or decreases (Dietrich & Verdolini Abbott, 2012; Van Lierde et al., 2009) in mean fundamental frequency (F0) during speech. A related point is that none of these studies controlled for intensity, which is closely coupled to F0. This point is relevant because the studies involved field or laboratory conditions (see, for example, Wittels et al. [2002] vs. van Lierde et al. [2009]). Each of these settings are likely to require drastically different speech intensity, which may weigh heavily on the studies' findings. One interesting finding was greater perceived overall severity [of dysphonia], roughness, breathiness, and strain (van Lierde et al., 2009) during a high-stress/anxiety task in vocally healthy females. These induced vocal quality characteristics are, of course, quite consistent with those seen in the population of patients with hyperfunctional voice disorders (Morrison & Rammage, 1993). Another relevant observation is reported by Mendoza et al., who show that measurable vocal differences (with respect to change from baseline) are maintained even after the stress-inducing condition ends. Although the vocal effects of stress may last beyond cessation of the stressor, it seems that the voice changes may be lesser with each subsequent stress exposure. Wittels et al. (2002) showed a stepwise decline in F0 with second and third exposures to the experimental stressor (subsequent to a significant spike in F0 with the first stressor exposure), which they take to be indicative of coping; despite the stepwise F0 decrease, F0 remained elevated relative to baseline for all three stressor exposures.

It is apparent from these studies that stress and anxiety impact vocal production, but not necessarily reliably. Although acoustic stress responses range widely across individuals, they are relatively more stable within individuals (Hecker, Stevens, von Bismarck, & Williams, 1968). Related to the relative consistency of vocal stress responses within individuals, one confounding element in psychological stress-inducing voice studies relates to participants' goals in the context of day-to-day

social evaluative threat. No known study has investigated this particular issue[1], but a logical argument is as follows. Anecdotally, it is safe to say that it is often preferable in social situations to suppress acoustic signs of stress and anxiety, and most speakers have likely developed their own strategies for "saving face". These speakers probably know what works best for them in this regard, and thus tend to employ the strategies that serve them well. Moreover, because most individuals have their own definition of when it is "safe" to let their voices belie emotional state (e.g., it may be "safer" to vocally express stress in front of one's mother compared to a stranger), studies seeking to understand vocal sequellae of stressor exposure are most certainly measuring two things: the direct impact of stress on the vocal signal, plus participants' overlying "filter" on that vocal signal based on how they wish to communicate their stress responses in the social evaluative setting. Some speakers are more adept at modulating visceral vocal responses via the exertion of volitional control.

On the whole, with respect to exactly *how* the voice is affected by stress and anxiety, the recent studies presented in this section are inconsistent and inconclusive. Nevertheless, *that* the voice is impacted by exposure to stressors is clear. Discrepancies in the literature cited are consistent with the larger body of literature pertaining to the manifestation of stress and anxiety in the vocal signal. The ability to identify stress and anxiety from the voice signal remains an eagerly pursued yet imperfect science. This is certainly due, at least in part, to methodological differences (of which there are many) that triggered unequal emotional state responses across study participants. Varying acoustic measurement approaches also contribute to the lack of consonance across studies. For future studies in this vein, the progression of acoustic analysis methods should be a boon, as newer methods (e.g., cepstral/spectral analysis) allow for acoustic analysis of running speech, which is an improvement over past methods.

[1] Giddens et al. (2013) summarize research in the area of vocal acoustics during lie detection and in the face of performance anxiety. While neither scenario is representative of "day to day" stress, findings generally support the logical argument that follows herein.

Table 1-1. Voice Parameters Secondary to Stress- or Anxiety-Inducing Conditions

Author(s)	N (# female)	Experimental Group	Experimental Condition(s)	Elicited State	Findings as a function of experimental intervention *(all findings significant unless stated otherwise)*
Mendoza & Carballo (1998)	82 (unknown)	Normals	All subjects underwent: 1. Baseline (alphabet recitation and sustained vowel) 2. Reverse alphabet spelling 3. Reading tongue twister with delayed auditory feedback (DAF) 4. Tongue twister without DAF	Anxiety	Anxiety-inducing conditions 2, 3, and 4 associated with: ▪ Mean F0 increase ▪ Short-term perturbation of F0 frequency and amplitude decrease ▪ High-frequency spectral noise (2800-5800 Hz) decrease relative to low-frequency harmonic energy (70-4500 Hz) ▪ Harmonic energy in 1600-4500 Hz range increases relative to that in 70-1600 Hz range ▪ No change in F0 range or F0 standard deviation
Wittels et al. (2002)	26 (0)	Austrian soldiers in voluntary special forces training	Emotionally and physically stressful military task involving negotiation of natural, dangerous obstacles – performed three times; compared to no-stress baseline	Emotional stress	Increase in stress induced: ▪ Significant increase in *mode* F0 at first task as compared to baseline, then significant stepwise decline during second and third repetitions of task
Johannes et al. (2007)	11 (0)	Austrian soldiers in voluntary special forces training	All subjects underwent: 1. Cognitive task with induced time pressure and increasing difficulty 2. Cognitive task with increasing difficulty but no time pressure 3. Two non-mental physical load tasks	Elicited emotional state not explicit ("mental load")	Increase in mental load induced: ▪ Significant increase in *mode* F0 at first task as compared to rest tasks, specifically for those tasks with a time pressure

Table 1-1 (Continued)

Van Lierde (2009)	54 (54)	Normals	Public speaking with: ▪ High-stress ▪ *or* no-stress	Anxiety	High-stress condition induces: ▪ Lower maximum phonation time ▪ Lower mean F0 ▪ Lower maximum F0, minimum F0, and maximum intensity ▪ Lower dysphonia severity index ▪ No difference in jitter, shimmer, frequency tremor response instability, or amplitude tremor response instability ▪ Greater perceived *Grade* (i.e., overall severity), *Roughness*, *Breathiness*, *Strain*
Giddens et al. (2010)	12 (6)	Normals	Cold pressor task	Stress	▪ Increase in subglottal pressure ▪ No change in mean F0, jitter, shimmer, maximum airflow declination rate, voice onset time, speaking rate
Dietrich et al. (2012)	54 (54)	Normals, grouped as (1) Introverts or (2) Extraverts with and without neuroticism	Public speaking task of Trier Social Stress Test	Stress	Stress phase of task induces: ▪ Lower mean F0 ▪ Lower mean vocal intensity

1.3 VOICE DISORDERS AND LARYNGEAL MUSCLE ACTIVITY

Given the evident aforementioned relationship between stressor exposure and voice changes, perhaps it is unsurprising that the voice disorders classically considered "functional" (i.e., as opposed to "organic") have long been linked to how individuals respond to stress and anxiety (Freidl, Friedrich, & Egger, 1990; Gerritsma, 1991; House & Andrews, 1988, 1987; Roy et al., 1997; Roy, Bless, & Heisey, 2000a; van Mersbergen, Patrick, & Glaze, 2008). After all, it is apparent that the vocal signal is vulnerable to stressor exposure, and the effects of stressors on the voice are mediated substantially by the activity of laryngeal musculature. Little is empirically known about volitional suppression of stressor effects on the vocal signal, although anyone who has felt a lump in the throat while resisting the urge to cry would probably attest to the muscular involvement in such an act. Thus, it is unsurprising that voice disorders principally characterized by laryngeal muscular hyperfunction (e.g., muscle tension dysphonia) have historically arisen from a perspective that the dysphonia is symbolic of psychological discord (e.g., the "conflict over speaking out" theory presented by House & Andrews, 1988). Perhaps it is this perspective that has driven investigation of laryngeal muscle tension via electromyography (EMG) in different voice-disordered cohorts and as a function of stressor exposure. Surface EMG (SEMG) biofeedback has been successfully utilized to decrease laryngeal tension and thus improve vocal quality (Stemple, Weiler, Whitehead, & Komray, 1980), which lends credence to the notion that elevated levels of perilaryngeal muscle tension contribute to certain voice disorders.

A handful of studies have examined the EMG characteristics of patients who have "hyperfunctional" voice disorders such as muscle tension dysphonia. Since intramuscular laryngeal EMG is technically challenging to perform and quite invasive, most studies have used surface SEMG to measure the electrical activity of facial and laryngeal muscles. Relatively elevated levels of laryngeal muscle "tension" in patient cohorts have been reported in several studies utilizing SEMG (Hocevar-Boltezar, Janko, & Zargi, 1998; Lowell, Kelley, Colton, Smith, & Portnoy, 2012; Redenbaugh & Reich,

1989; Stager et al., 2001; Stager, Bielamowicz, Regnell, Gupta, & Barkmeier, 2000; Stemple et al., 1980; Van Houtte, Claeys, D'haeseleer, Wuyts, & Van Lierde, 2011).

Redenbaugh and Reich (1989) compared absolute and relative SEMG signals in normal (n=7, five women) and "vocally hyperfunctional" (n=7, five women) speakers. In terms of symptoms, patients deemed "hyperfunctional" exhibited hard glottal attack and harsh voice quality, and complained of vocal fatigue, muscular tightness in the anterior neck area, and throat pain with prolonged talking. Four of these patients had a history of vocal fold lesions. On the other hand, normal participants were judged to be so based on perceived normal voice quality by the first author, and a negative reported history of acute or chronic voice impairments. To study these subjects' laryngeal muscle tension, SEMG electrodes were placed over the thyrohyoid membrane and signals were recorded during tidal breathing, two isometric maneuvers against resistance, prolonged vowel production, and sentence production. The voice-disordered speakers exhibited significantly higher SEMG values at rest than the normal speakers, although maximal and half-maximal EMG values were not different across groups during other non-speech activities. Hyperfunctional speakers also demonstrated significantly higher SEMG values during vowel and sentence productions. However, after normalizing SEMG activity during these conditions to the rest condition, no statistically significant findings remained. Nevertheless, the authors boldly proposed that their findings "most likely reflect…elevated EMG activity associated with abrupt phonatory initiations, excessively stiff vocal folds, high collision forces following vocal fold adduction, and high medial compressive forces during vocal fold closure." (page 72). It is important to note that SEMG data cannot be extended to infer intrinsic laryngeal muscle activity (Zealear et al., 2005). Despite the interpretive leap by Redenbaugh and Reich, it is on the basis of their conclusions that SEMG biofeedback has since been used (with reported success) to decrease the amount of laryngeal tension (presumably including that of the intrinsic laryngeal musculature) and thus improve vocal quality, in patients with hyperfunctional voice disorders (Sime & Healey, 1993).

Next, Hocevar-Boltezar (1998) sought to describe the SEMG characteristics of the lower face and anterior neck in patients with MTD (n=11) and a vocally normal cohort (n=5). Patients in this study were

classified according to the MTD types outlined by Morrison and Rammage (1993). SEMG electrodes were placed on the following sites (and muscle groups): upper and lower lip (orbicularis oris); chin (mentalis, quadrangularis labii mandibularis); beneath the chin (biventer-venter mandibularis, mylohyoid, platysma); at the mid-point of the submandibular triangle (mylohyoid, stylohyoideus, biventer-venter mastoideus, platysma); over the thyrohyoid membrane (thyrohyoid, omohyoid, sternohyoid, platysma); over the sternocleidomastoid (sternocleidomastoid, platysma); just beneath the thyroid cartilage (cricothyroid, sternohyoid, platysma); and over the muscles in the lower one-third of the neck (sternothyroid, sternohyoid, platysma). Mean SEMG activity at each electrode set was collected over three trials of vocalic phonation at modal pitch and loudness (determined by the subject, thus not controlled). Subjects with MTD and normal exhibited comparable SEMG signal magnitude at rest. Also similar across groups was the general tendency for SEMG activity to increase about 20-300 ms prior to acoustic onset, and for that activity to increase by at least two- to three-fold in amplitude. All normal participants and five of the MTD patients had SEMG signals that returned to baseline after this initial burst of peri-phonatory muscle activity. However, in six of the eleven MTD patients, the spike in SEMG activity just prior to phonatory onset was 6-8 times higher than rest, and remained higher throughout phonation (i.e., they did not follow the pattern of spike and return to baseline that was observed in normals and other five MTD subjects). This finding was highlighted as the most interesting result of this study. However, another compelling finding is that the summative muscle activity for each muscle group in this study is approximately equal between cohorts during an at-rest, non-speech condition. This observation is in contrast to the findings reported by Redenbaugh and Reich (1989). An additional observation of interest is that in the MTD group, inexplicably statistically significant asymmetries in response were observed for a variety of muscle groups.

More recently, van Houtte and colleagues reported their findings of a study that compared perilaryngeal tension between patients with and without MTD using SEMG (Van Houtte, Claeys, D'haeseleer, Wuyts, & Van Lierde, 2013). None of three examined muscle sites exhibited differences as a function of vocal status (MTD versus normal). Contrary to the aforementioned studies, van Houtte et al.

concluded that SEMG is an inappropriate tool for differentiation of MTD and normal voice. This corroborates the negative findings reported by another group of investigators who found no differences between normal speakers and those with vocal nodules, the latter of which putatively exhibits vocal hyperfunction and thus increased laryngeal muscle tension (Stepp et al., 2010, 2011).

As argued by van Houtte et al., the use of SEMG data to infer activity of the intrinsic laryngeal muscles is not prudent. This argument is empirically supported by investigators who utilized neuromuscular blockade in canines to determine which intrinsic laryngeal muscles contribute to surface EMG signals (Zealear et al., 2005). These researchers concluded that the TA muscle is a primary contributor to the SEMG signal whereas the PCA is a non-contributor. Thus, conclusions of studies using SEMG must be accepted with caution with respect to the actions of the intrinsic laryngeal muscles. While findings conflict in some ways, the long-held clinical stance that patients with "hyperfunctional" voice disorders actually exhibit more tension is supported in some cohorts. However, it is unclear whether laryngeal muscular hyperfunction is actually causal to the voice impairment or merely a symptom of it. Figure 1-3 merges the relationship of laryngeal muscle activity and voice disorders with aspects of the schematic displayed in Figure 1-2, to illustrate the common clinical concept of how response to stressors at the level of the larynx is thought related to the pathogenesis of voice disorders.

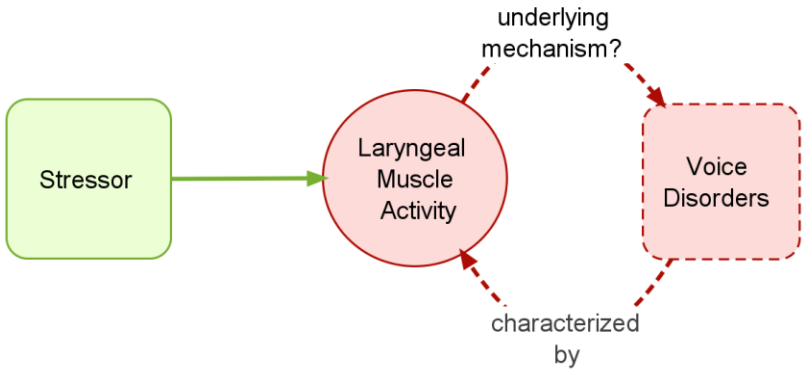


Figure 1-3. Conceptual schematic: relationship between stress, laryngeal muscle activity and voice disorders.

2.0 MUSCULAR HYPERFUNCTION AND STRESSOR EXPOSURE

Two key points were presented in the foregoing section: (1) the voice responds to stress, presumably in large part via the effects of the laryngeal muscles, and (2) the voice disorders commonly thought related to how individuals experience and respond to stressors are characterized by increased laryngeal muscle tension. Very little research has investigated the claim that the *intrinsic* laryngeal muscles respond to stressor exposure or characterized that response. Indeed, it is not a simple feat to measure ILM activity directly. The present section will examine muscular hyperfunction as a function of stressor exposure, using the notoriously stress-reactive trapezius muscle as a potential model for how the somatic nervous system might play out in the larynx during stressor exposure. In addition, the relationship between the larynx and the respiratory system will be discussed in the context of stressor exposure.

2.1 SKELETAL MUSCLE RESPONSE TO STRESS

The somatic nervous system is not considered a stress response pathway *per se*, although stress, anxiety, panic, and other negative states may certainly manifest in the skeletal muscles. Catecholamines released in response to sympathetic-adrenal-medullary activation (e.g., adrenaline and noradrenaline) exhibit coordinated actions with the autonomic nervous system to exert regulatory effects on the skeletal muscle. More specifically, sympathetic nervous system activity supports and mediates motor functions by increasing or maintaining stability of arterial blood pressure when the working muscles' blood demands increase. Peripheral nerves contain sympathetic fibers that innervate the vasculature of skin and skeletal

muscles, supply sweat glands, and convey afferent information from nociceptors. Muscle function and motor control can be influenced by SNS modulation of the local muscle blood flow, muscle contractile properties, muscle spindle afferents' proprioceptive activity, and even (in pathological states) nociceptors in the skin and muscles (Goldstein, 2001).

Increased skeletal muscle activation in response to psychological stressors is thought to play a major role in the pathogenesis of musculoskeletal disorders. In acute psychological stress conditions, blood pressure and heart rate are often associated significantly with muscle activity, especially in the trapezius muscle (e.g., Krantz, Forsman, & Lundberg, 2004; Lundberg et al., 1994). The trapezius muscle is a commonly studied "stress reactive" muscle, and myalgia (i.e., muscle pain) of the trapezius muscle is a common musculoskeletal disorder that implicates muscular hyperfunction in its pathogenesis. Like certain types of voice disorders, chronic neck and shoulder impairments seem to be related to personality traits such as neuroticism and trait anxiety (Lundberg et al., 1994).

2.1.1 Effects of stress on the trapezius muscles

Generally speaking, the impact of chronic stress on the trapezius muscles is the sensation of pain, which is often precipitated by fatigue and "discoordination". Multiple studies have demonstrated that the trapezius muscle is especially responsive to emotional, cognitive, psychological and physical stress (Hallman, Lindberg, Arnetz, & Lyskov, 2011; Hazlett, McLeod, & Hoehn-Saric, 1994; Krantz et al., 2004; Larsson, Alund, Cai, & Oberg, 1994; U Lundberg et al., 1994; Pluess, Conrad, & Wilhelm, 2009; Sjörs, Larsson, Dahlman, Falkmer, & Gerdle, 2009; Willmann & Bolmont, 2012; Willmann, Langlet, Hainaut, & Bolmont, 2012). The stress reactivity of the trapezius can be seen in healthy normal individuals. Lundberg et al (2002) engaged healthy normal individuals in physical stressors (cold pressor task) and psychological stressors (mental arithmetic and Stroop color-word interference tasks). They found that psychological stressors significantly increased the EMG activity of the trapezius muscle. Nilsen et al. (2007) implemented a different mental stressor (a reaction time task) in healthy normals and

also found a significant increase in trapezius EMG activity. In that normal cohort the muscle's recovery was rapid and complete after the stressor ended.

Repeated or prolonged exposure to emotional, cognitive, psychological and physical stressors are considered strong risk factors for the development of chronic neck and shoulder complaints (Lundberg, 2002; Westgaard, 1999). One proposed explanation for the pathogenesis of trapezius myalgia is that mental stress keeps low threshold (small type I) motor units activated nonstop for long periods of time, which in turn causes disturbed metabolism, degenerative processes, and pain (Lundberg, 2002). According to Henneman's size principle, in mixed-composition muscles, small Type I motor units are always activated before others, thus they are recruited for all motor tasks involving a particular muscle (Henneman, Somjen, & Carpenter, 1965). This pattern of activation results in these motor units being chronically overworked with no time for rest, thus they are dubbed "Cinderella fibers" (Hagg, 1991). The general idea of the "Cinderella Hypothesis" is that SNS activity on type I fibers fatigues them, thus worsening their performance over time (Hagg, 1991). This effect has been observed to a greater degree in jaw and trapezius muscles as compared to limb muscles (Passatore & Roatta, 2007). Also in alignment with the Cinderella hypothesis, Passatore and Roatta speculate that stress might actually facilitate the onset of chronic pain states, regardless of their origin (e.g., whiplash versus workplace musculoskeletal disorders), citing altered physiological states that, if maintained "long enough", may yield permanent changes in a system's function (2006). Thus, in addition to volitional behaviors that are driven by psychological stress, the SNS may exert direct effects on the skeletal muscles as well.

Unfortunately, specific information is sparse regarding the direct effects of long-term stress on the trapezius muscles. Because of the lack of studies directly examining chronic stress as an explicit *cause* of trapezius dysfunction or pain, the preponderance of studies specific to stress-induced effects on the trapezius muscle relate to chronic trapezius (or, more generally, neck and shoulder) pain, and often involve a study cohort of individuals who occupationally engage in repetitive physical tasks (e.g.,Voerman, Vollenbroek-Hutten, & Hermens, 2007). Clear differences between healthy individuals and those with neck and shoulder complaints are often not observed. Multiple investigators have shown

that in these two cohorts, muscle activation patterns are generally the same (Larsson et al., 1994; Westgaard, 1999). Other investigators fail to identify differences in average values of muscle activity amplitude, yet report increased *variability* of amplitude for patient groups compared to controls (e.g., Voerman, Vollenbroek-Hutten, & Hermens, 2007).

Interestingly, it seems that even in tasks where one trapezius muscles involved in the experimental task, bilateral trapezius responses may occur. Voerman et al. (2007) sought to examine the behavior of the dominant trapezius muscle during a unilateral task (mouse-clicking during a color-word interference stressor). They found that patients exhibited greater variability in trapezius muscle activation compared to healthy controls, and moreover, also exhibited activation of the contralateral muscle, despite it not being required for the task. Since such an activation of the non-dominant contralateral muscle is not functional (i.e., it serves no purpose in the experimental task), Voerman et al. hypothesized that the observed contralateral muscle response might be secondary to overflow from afferents in ipsilateral muscles to contralateral motoneurons. This explanation was attributed to the pain-spasm-pain model presented by Johansson and Sojka (1991). The model holds that muscle tone increases reflexively due to pain, and this increase in tone is accomplished by means of positive feedback loops in the gamma-motor system. Muscle activity increases result from cell membrane damage, the production of irritating substances and increased nociceptive activity which results in the perception of pain, increased activity of gamma muscle spindles, and in turn, increased muscle tone or tension.

Another interesting observation about the trapezius muscle is that compared to other skeletal muscles, it appears to lack an ability to adapt to repeated stressors. Willmann and Bolmont (2012) recently described this phenomenon, examining EMG signals from multiple sites obtained from a cohort of healthy adult males. During initial and repeated exposure to a moderate cognitive stressor (Stroop color-word interference test), EMG activity was collected from the following sites: flexor pollicis brevis, biceps brachii, triceps brachii, trapezius, gastrocnemius, and soleus muscles. All of the muscles except the trapezius muscle exhibited significantly lower EMG activity during the second task exposure. This indicated that the trapezius, which is a highly stress-responsive muscle, failed to adapt whereas other

muscles were capable of adaptation in the face of ongoing or repeated stress. This finding further supported the Cinderella Hypothesis by showing that psychological stress can maintain activity of low-threshold motor units in the trapezius, and that prolongation of this muscle activity could lead to damage to muscle fibers.

Taken together, these findings indicate that robust trapezius muscle activation is part of a normal response to a broad array of stressors. When chronic, such activation of the trapezius muscle leads to hyperfunction that may be related to pain and fatigue. Clear differences between healthy normals and individuals suffering from trapezius myalgia are often evasive.

2.1.2 "Stresspiration"

One critical difference between the trapezius muscle and the intrinsic laryngeal muscles in the context of skeletal muscle responses to stress is that the former has a substantially less complex relationship with respiration than the latter. To further understand that relationship, a brief discussion of how the respiratory system responds to stress is in order here, since the larynx serves a principal role in valving for the lungs. Far more research has been conducted regarding the respiratory response to stress than the laryngeal response to stress. Presumably, the larynx reacts commensurately with the needs of the lungs in times of stress, and in times of extreme respiratory demand it is fair to say that the laryngeal response involves a complementary increase of muscular activity that is well above normal baseline. While we cannot draw conclusions regarding laryngeal muscular hyperfunction *as a direct function of* the body's pulmonary needs, when considering the laryngeal response to stress, it is critical to consider this particular interaction.

Breathing impacts physiological regulation via interactions with or entrainment of respiratory oscillations to oscillations within other systems (e.g., heart rate, blood pressure, lymphatic system, digestive system, brain waves, and cellular metabolism), and its profound role in maintaining homeostatic levels of oxygen, carbon dioxide and pH (Courtney, 2009). In fact, breathing can be volitionally

manipulated to facilitate entrainment of other oscillations and to increase physiological regulation (Courtney, 2009). For instance, slowing respiration rate to between four and six breaths per minute (0.06-0.1 Hz) leads to synchronization of blood pressure, heart rate, and the autonomic nervous system within this frequency range, and these entrained oscillations are amplified due to resonance effects across the systems (Courtney, 2009).

Changing respiratory patterns can be due to volitional pulmonary control, limbic respiratory influences, direct afferent input to the respiratory complex, brainstem arousal, and metabolic changes (Shea, 1996). Interestingly, voluntary activation of the facial muscles leads to decreased respiratory resistance (i.e., dilation of the airways), and a principal mechanism for this change is thought to be vagal (i.e., parasympathetic) withdrawal (Ritz, 2004). Not only does breathing patterning and timing change with chronic emotional and psychological stress, but respiratory drive and the metabolic appropriateness of the respiratory response may also be affected (Courtney, 2009). The fact that level of trait anxiety influences behavioral breathing independent of metabolic demands is consistent with limbic modulation of respiratory drive (Masaoka et al., 2001).

Increased respiratory drive is a fundamental sympathetic nervous system survival function meant to prepare the body for fight and flight. Homeostasis is maintained through the calming functions of the parasympathetic nervous system, which facilitates a slow, relaxed and diaphragmatic/abdominal breathing pattern. Pronounced breathing irregularity is commonly observed in patients with anxiety and hyperventilation disorders (Boiten, Frijda, & Wientjes, 1994). Consciously controlling respiration can actually minimize subjective distress and facilitate the system's return to a physiological state of rest, influencing the brain and the autonomic nervous system to synchronise central and autonomic systems, and generally foster a state of psycho-physiological coherence (Boiten et al., 1994; Courtney, 2009). For this reason, breathing has been employed as a therapeutic tool in stress and anxiety disorders.

Not only can respiration impact affect, but respiration is, in turn, deeply affected by negative states (e.g., stress, anxiety, panic, and negative emotions). Hyperarousal elicited by mental and emotional stress adds to "allostatic load" (a term that refers to the cumulative effects of stress on the body, McEwen

2002), and impacts the body's capacity to maintain stability and respond adequately to change. Irregular and disordered breathing is a common clinical feature of patients with panic and anxiety disorders, with specific effects such as hypertonicity of the diaphragm causing it to be flattened and immobile (Courtney, 2009). Interestingly, the opposite is also true – voluntary or involuntary hyperventilation is shown to be critical in the *development of* clinical anxiety and panic disorder symptoms (Zvolensky & Eifert, 2001). Hyperventilation triggers a variety of physiological effects that in turn produce symptoms of anxiety or panic, and influence the way an individual perceives and responds to a stressor (Zvolensky & Eifert, 2001).

During conditions of psychological stress a variety of respiratory parameters exhibit change (e.g., increases in respiratory rate, resistance, minute volume, alterations in tidal volume, and decreases in blood and alveolar CO_2 levels) as compared to baseline (Bass & Gardner, 1985; Kreibig, Wilhelm, Roth, & Gross, 2007). More specifically, increased respiratory rate and decreased $pC0_2$ is a common pattern of response to certain stressors (Boiten et al., 1994). Mental stressors such as mental arithmetic yields shallow breathing patterns with zero or negligible change in respiratory rate, although as arousal levels increase, perhaps as a function of increasing task difficulty, respiratory rate elevates commensurately (Boiten et al., 1994). Overall, respiratory changes during experimental mental effort tasks apparently follow a pattern of rapid, shallow breathing, consistent with what is observed during "tense affects", during anxious anticipation of a noxious stimulus, and in high-anxiety patients (Boiten et al., 1994).

Emotions can profoundly influence respiration, and specific emotional and stress states are associated with different breathing patterns (Bloch, Lemeignan, & Aguilera, 1991; Boiten et al., 1994; Dampney, Horiuchi, & McDowall, 2008; Masaoka & Homma, 1997; Ritz, 2004). For instance, respiration rate typically increases during fearful emotional states and decreases during sad states, and the opposite pattern is observed for tidal volume (i.e., tidal volume decreases with fear and increases with sadness) (Kreibig et al., 2007). Anger causes increased respiratory rate and tidal volume (Boiten et al., 1994). Excited affects triggered by a stressor are typically associated with fast deep breathing, whereas tense anticipatory affects are associated with rapid shallow breathing (Boiten et al., 1994). Interestingly, it

seems that suppression of emotions (or expressing them inadequately) is more important in triggering asthma attacks than the type of emotion experienced (Ritz, 2004). A bidirectional relationship exists between breathing and emotion, as breathing falls under voluntary and involuntary control via intertwining feedback mechanisms involving autonomic networks, brainstem nuclei, limbic and cortical structures, and the neuroendocrine system (Ley, 1999). For instance, it is due to the impact of the parasympathetic nervous system that during brief and prolonged affective stimulation, narrowing of the airways is observed in both healthy and asthmatic adults (Ritz, 2004).

In addition to breathing patterns distinguishing affective states (e.g., fear, sadness, and neutral), they also may differentiate diagnostic groups (e.g., neurotics versus normals). Respiratory variables have even been utilized as diagnostic parameters (Kreibig et al., 2007; Shea, 1996). Respiration patterns may also be associated with personality and emotional orientations (Shea, 1996). For instance, individuals with high state anxiety tend to exhibit increases in respiratory rate during a psychological stressor, even when maintenance of respiratory rate would better serve their metabolic and homeostatic needs (Masaoka & Homma, 1997). It also seems that distinct respiratory responses are associated with arousal level (high versus low) above and beyond affect (positive versus negative) (Gomez, Zimmermann, Guttormsen-Schär, & Danuser, 2005).

2.1.3 Intrinsic laryngeal muscle activity and stressor exposure

As discussed in Section 1.2, underlying stress-induced acoustic changes in the voice are dynamic modifications to functions of respiratory, intrinsic laryngeal, extrinsic laryngeal, and the supralaryngeal muscles. Perhaps the least studied of the aforementioned muscle groups is the intrinsic laryngeal muscles (ILMs), the activity of which is most accurately captured using invasive methods. The ILMs are of particular interest in many contexts because they provide the source for much of the vocal signal generation (see Figure 2-1). Although some inferences can be made based on acoustic data, very little is directly known about the activity of the ILMs themselves during stress responses in individuals with

normal, healthy voices. This is especially problematic because understanding of certain voice disorders, including those without any clear organic basis, often hinges on the clinical observation of putative ILM activity that is deemed "aberrant", "dysfunctional", "imbalanced", and/or "hyperfunctional" (Aronson, 1980; Hillman et al., 1989; Morrison & Rammage, 1993; Roy et al., 1997; Roy, 2003).

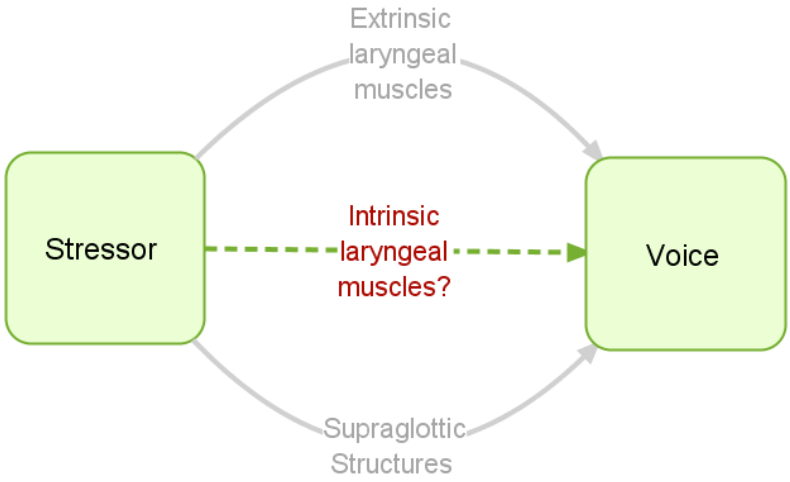


Figure 2-1. Conceptual schematic: stressor effect on voice via intrinsic laryngeal muscles.

To *directly* examine the effect of stress, anxiety, or other negative states on the larynx itself, investigators would need to somehow measure or observe a laryngeal response (e.g., laryngoscopically, electromyographically, electroglottographically) that can be directly attributed to a state of stress (or anxiety or fear). Even examining and describing laryngeal behaviors (e.g., anteroposterior squeezing or supraglottic hyperfunction) during a stressor may provide good insight, and yet regrettably the research is lacking in this respect (Dietrich, 2008).

One such study did assess the functional laryngeal response to experimentally-induced activation of the sympathetic nervous system (SNS). A preliminary study that was designed and executed by the candidate examined the response of the ILMs to a classic *physiologic* stressor, the cold pressor task, in eight healthy subjects (Helou, Wang, Ashmore, Rosen, & Abbott, 2013). The cold pressor task involves plunging the hand into ice-cold water, which triggers nociceptors and yields a robust, primarily

sympathetic response characterized by cutaneous vasoconstriction, dilation of blood vessels in muscle tissue, and elevated heart rate and arterial pressure (Lovallo, 1975). Helou et al. (2013) utilized hook wire electrodes to measure electrical activity of several intrinsic laryngeal muscles (abductors and adductors). Participants remained at rest during the cold pressor task and no vocalization or speech was involved, so as not to confound results with the muscular activity required for phonation.

Surface EMG of the trapezius muscle and ILM values, as well as cardiovascular measures of heart rate and blood pressure were used to characterize participants' response to cold pressor exposure as compared to at-rest baseline measures. Cardiovascular response to the cold pressor task was confirmed online for all subjects via heart rate (HR) or blood pressure (systolic, SBP and diastolic, DBP) measures. Post-hoc Bonferroni analysis confirmed statistically significant increases in HR ($p=.027$), SBP ($p<.001$), and DBP ($p<.001$) during the CP task as compared to baseline. Concurrent increases in muscle activity were generally observed in the trapezius, bilateral thyroarytenoid/lateral cricoarytenoid (TA/LCA) complex, bilateral cricothyroid (CT) muscles, and right posterior cricoarytenoid (PCA—left PCA was not examined for logistical reasons described shortly).

Figure 2-2 illustrates ILM recordings from three muscles in one subject across conditions. In this figure, (A) rectified and smoothed ILM traces (top row) are shown for the PCA muscle before (baseline, left), during (cold pressor, middle) and after (recovery, right) cold pressor administration. Values are relative to the mean level of the baseline. The first 60 seconds of each task are shown. Gray rectangles indicate sections that are expanded below each trace. The expanded traces (bottom row) show the intact (not smoothed or rectified) ILM signal for 10 seconds. Section (B) is the same as A, for the LTA muscle. Section (C) is the same as A and B, for the LCT muscle. All traces were recorded concurrently.

Three individuals repeated the cold pressor task, showing the same pattern of laryngeal response as observed in their first exposure. Thus, responses appeared replicable within subjects in this sample. In addition, results for repeated baseline measures, obtained after the cessation of the cold pressor task, were compared to pre- cold pressor baseline. In general, ILM activation remained high after the cold pressor condition was completed, and were elevated from baseline even after cardiovascular response had fully

attenuated. This finding is in alignment with those of other voice researchers. For instance, in their study on voice effects of different stressful cognitive tasks, Mendoza et al. (1998) found that statistically significant vocal (i.e., acoustic) differences were maintained even after the stressful episode was over.

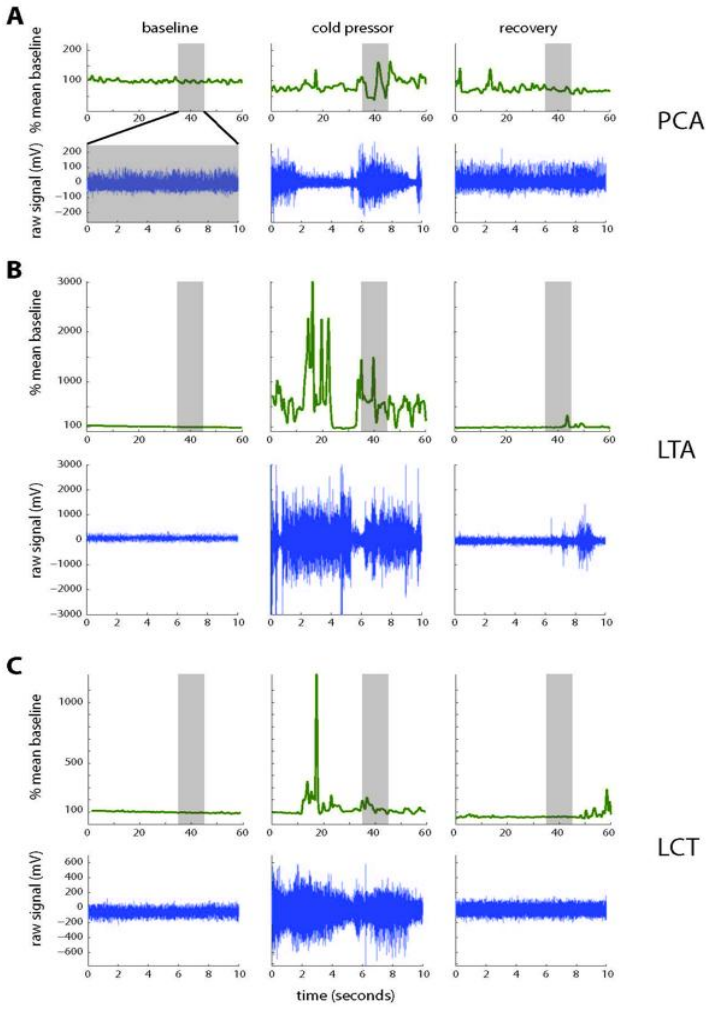


Figure 2-2. ILM_{EMG} data from preliminary study.

Overall, results of this preliminary study were consistent with the suggestion that human laryngeal muscles exhibit an elevated level of activation concurrent with ANS activation triggered by a cold pressor task. Although the study involved a small sample size (n=8), results were compelling in that they showed a consistent inter- and intra-subject response of increased muscular activity in the face of sympathetic nervous system stimulation. This finding was provocative because it suggested that the

larynx might demonstrate a "functional autonomic response" of sorts. This could be due to the vital respiratory valving role of the larynx, which responds "on behalf of" the autonomically-driven lungs. Interestingly, both abductor and adductor muscles were co-activated during the cold pressor task, lending credence to the notion that stress and anxiety trigger increased muscle activity in the larynx. The implications for hyperfunctional voice disorders were clear, as the data were relevant to stress-induced laryngeal hyperfunction and 'inefficient' patterns of laryngeal muscle activity.

3.0 VOICE AND PERSONALITY

3.1 PERSONALITY MEASURES OF INTEREST

Following the through line of Dietrich and Verdolini's Psychobiological Framework of Voice Disorders, the present section examines the proposed relationship between personality (represented in the framework by the *person-by-situation interaction*) and how it is thought to impact the voice by way of the laryngeal muscles. To this point, three specific personality measures are especially germane to our present understanding of personality and voice.

First, Gray's neuropsychological model of the nervous system (Gray, 1987) is comprised of three broad factors. Those factors are a Behavioral Activation System (BAS), a Behavioral Inhibition System (BIS), and a Nonspecific Arousal System (NAS). Gray proposed that the BAS is motivated by conditioned reward and non-punishment signals, and that it promotes "appetitive" goal-driven *approach* behavior, escape, and active avoidance (i.e., response activation). Opposingly, the BIS is motivated by conditioned signals of non-reward/punishment, novelty or threat, and innate fear responses, and that it promotes behavioral *inhibition*, critical inspection of the environment, and passive avoidance (i.e., response suppression). Finally, the NAS generally is equated with neuroticism, which is a personality trait associated with a low tolerance of arousal and a propensity to worry and display negative affect. The NAS becomes proportionally activated with both BAS and BIS, respectively reflecting and reinforcing approach or avoidance behaviors. The BAS and BIS are thought to have strong relationships with neural structures in the septohippocampal system, and the NAS is allegedly linked to phasic autonomic changes.

One major draw of Gray's theory is in the proposed predictability of behavioral response to anxiety-relevant cues as a function of personality traits.

Another commonly employed personality questionnaire is the *Eysenck Personality Questionnaire* (Eysenck & Eysenck, 1994). This measure assesses three independent personality traits: *Introversion/Extraversion, Neuroticism*, and *Psychoticism*. People who score highest on the *extraversion* dimension tend to be outgoing and chatty, are likely to seek external stimulation, and are high on "positive affect", or feeling good. Conversely, *introverts* tend to feel over-aroused and thus seek alone time to feel well-balanced. The second of Eysenck's domains, *neuroticism,* which refers to how well individuals care able to inhibit or control emotional responses, how easily they become upset or nervous, and how likely they are to experience negative affect (e.g., depression or anxiety) in the face of minor stressors. The final dimension, *psychoticism*, which is associated with predisposition to have a psychotic "break" from reality and to exercise aggression. Behaviors consistent with psychoticism include non-conformism, recklessness, hostility, impulsivity, and anger. Each of these domains are proposed to be related to biology as follows: extraversion is related to cortical arousal; neuroticism is related to the activity of the sympathetic nervous system, and Psychoticism is related to testosterone levels.

A third battery of questions measuring aspects of personality is the *Multidimensional Personality Questionnaire—Brief Form* (*MPQ-BF*, utilized in the present study) (Patrick, Curtin, & Tellegen, 2002). The *MPQ-BF* is a 155-item questionnaire comprised of three higher-order traits that map onto constructs of emotion and temperament that are thought to have clear psychobiological correlates. It was preceded by a lengthier questionnaire (*MPQ,*Tellegen, 1985) The three top-order traits of the *MPQ-BF* are as follows: *Positive Emotionality* (PEM), *Negative Emotionality* (NEM), and *Constraint* (CON). PEM and NEM are temperamental in nature, in that they index tendencies or dispositions toward positive and negative emotions (a psychological perspective), and are thus conceptually related to the central nervous motivational systems subserving behaviors of appetitive-approach and defensive-withdrawal (a neurobiological perspective) (Patrick et al., 2002; Tellegen, 1985, 1985). CON generally represents traits related to impulsivity versus behavioral restraint and is conceptually related to Gray's *behavioral*

inhibition system. Two primary second-order trait scales of the PEM and NEM are *Wellbeing* and *Stress Reaction*, respectively, which are dispositional correlates to *positive emotionality* and *negative emotionality* (Patrick et al., 2002; Tellegen, 1985; Watson & Tellegen, 1985)[2].

3.2 PERSONALITY AND VOICE

Roy and Bless proposed a seminal theory that would spawn hypothesis-generation regarding how personality might be involved in the pathogenesis of muscle tension dysphonia and vocal fold lesions (Roy, Bless, & Heisey, 2000b; Roy & Bless, 2000a, 2000b). This theory, today referred to as the Trait Theory of Voice Disorders, draws from both Eysencks' and Gray's theories relating to the intertwinement of mind (i.e., personality) and body (i.e., biology). The Trait Theory of Voice Disorders holds that personality is a predisposing factor that influences how individuals respond—emotionally, cognitively, and *vocal* behaviorally—to environmental cues. These trait-specific responses are thought to be conditioned and therefore predictable within adult individuals.

To test the Trait Theory of Voice Disorders, Roy and Bless mapped the key elements of Gray's model—BAS, BIS, NAS—onto extraversion, introversion, and neuroticism, respectively (Roy & Bless, 2000a, 2000b). Examining individuals with muscle tension dysphonia through the lens of the Trait Theory of Voice Disorders, it was observed that people with MTD tend to be BIS-dominant neurotic introverts who are particularly responsive to threat, punishment and new situations (Roy & Bless, 2000a, 2000b). Roy and Bless proposed that these traits may make these individuals more likely to experience anxiety and motor behavior inhibition, which may lead to elevated laryngeal muscle tension. More specifically, in comparison to healthy controls, patients with MTD demonstrated increased "neurotic

[2] Other scales are contained within the PEM and NEM dimensions, but full examination of these other trait aspects is not germane to the present discussion.

triad" (hypochondriasis-depression-hysteria), paranoia, psychasthenia, schizophrenia, introversion, trait anxiety, negative emotionality (especially Stress Reaction as measured by the *MPQ*), and constraint (Roy et al., 1997; Roy & Bless, 2000a, 2000b). On the other hand, people who are extraverted tend towards *behavioral* [and thus, vocal] *activation*, putting them at risk for the development of vocal fold lesions.

Findings of other investigators generally support the Trait Theory of Voice Disorders. Van Mersbergen, Patrick, and Glaze (2008) reported that patients with MTD and individuals with high levels of social anxiety (but no voice impairment) scored similarly in terms of trait Stress Reaction (as measured by that subscale of the *MPQ-BF*), and both groups displayed numerically higher stress reactivity than healthy controls. Their study might have been insufficiently powered to detect statistical differences between these three groups. They also showed that in emotional contexts, individuals with MTD exhibited diminished levels of tonic activation of muscles involved in behavioral expression (zygomaticus major and corrugator muscles, which should reflect positive and negative mood, respectively), as compared to the groups of healthy controls and individuals with high anxiety. This evidence of reduced expressive behaviors was seen despite clear cardiovascular evidence of a subjective emotional experience. Moreover, during emotion-inducing mental imagery, it was shown that behavioral inhibition of *speech* muscles (submental complex and thyrohyoid muscles) occured in patients with MTD to a greater degree than to those individuals in the other two experimental groups. Thus, these findings were interpreted as being consistent with behavioral inhibition (i.e., Gray's BIS) in MTD.

Also in support of the Trait Theory of Voice Disorders, Dietrich and Verdolini (2012) reported that compared to extraverts, introverts exhibited greater infrahyoid muscle[3] activity during exposure to a psychosocial stressor, and both of these parameters—infrahyoid activity and introversion—were significantly associated with diminished voice-related quality of life as measured by a self-report index. Dietrich and Verdolini proposed that the observed pattern of muscle activity may be one manifestation of response suppression or behavioral inhibition. It should be noted, though, that the interaction reported by

[3] The infrahyoid muscles serve to depress the larynx and hyoid bone during speech and swallowing.

Dietrich and Verdolini Abbott (2012) was significant only after controlling for levels of *neuroticism*. *Neuroticism*—which is generally comparable to the more current term *negative emotionality*—is a term used to describe a person who often experiences anxiety, mood shifts, depression, overreactivity to emotional stimuli, and difficulty recovering from emotional stimuli (Patrick et al., 2002; Suls & Martin, 2005). People who are high in neuroticism are often high in anxiety as well (Zelenski & Larsen, 1999). They also tend to be emotionally hyperreactive, tend to selectively process and elaborate information that has (or is perceived to have) negative content, are likely to view situations as threatening, often ruminate and recover more slowly from negative affect and threat, and demonstrate a lack of habituation to an experienced stressor which may reflect insufficient coping strategies (Suls & Martin, 2005). The aforementioned tendencies comprise a cycle of reinforcement and amplification of negative affect/neuroticism (Suls & Martin, 2005). Relatively elevated levels of neuroticism or negative emotionality (and sub-factors of these constructs) are commonly observed in and thought to hold a causal role in certain voice disorders, such as primary muscle tension dysphonia and vocal fold nodules (Freidl et al., 1990; Gerritsma, 1991; House & Andrews, 1987; Kinzl, Biebl, & Rauchegger, 1988; Pfau, 1975; Roy et al., 1997, 2000b).

Both neuroticism and negative emotionality are broad constructs that are comprised of several facets such as *anxiety* and *stress reactivity*. Of particular interest in the proposed study, *stress reactivity* is a core affective facet of *negative emotionality* that is closely linked to questionnaire measures of anxiety (Patrick et al., 2002). Thus, in general, individuals with high scores on a stress reactivity measure are relatively more likely to feel easily upset, anxious, worried, tense, vulnerable, *et cetera,* than others. Conversely, those with low stress reaction scores are generally more likely to recover quickly from upsetting experiences, can put worries and fears aside, and do not tend to feel especially vulnerable (Patrick et al., 2002). Van Mersbergen, Patrick, and Glaze (2008) demonstrated that individuals with MTD and those with high trait social anxiety (but no voice disorder) scored commensurately high on trait Stress Reaction (as measured by a subscale of the *MPQ-BF*), and both groups had higher trait stress reactivity than healthy normals. Moreover, the same investigators found that in emotional contexts, the

participants with voice disorders demonstrated attenuated levels of tonic activation in muscles of behavioral expression (e.g., submental complex, facial muscles), yet exhibited autonomic (i.e., physiologic) evidence of a subjective emotional experience (van Mersbergen et al., 2008). This finding supported the idea presented by Roy and colleagues that individuals with MTD exhibit behavioral inhibition.

Also germane to the proposed study, it has recently been shown that motor cortical control supporting speech and voice production varies as a function of trait stress reactivity (low vs. high) in normal adults (Dietrich, Andreatta, Jiang, Joshi, & Stemple, 2012). Specifically, using an fMRI paradigm, investigators revealed that individuals with high stress reaction scores exhibited elevated prefrontal and limbic activity during sentence reading, compared to others with low stress reaction scores. This apparent elevation of arousal and appraisal during a simple reading task was thought to influence voice sensorimotor control in participants without voice disorders. These findings broadly endorsed the Trait Theory of Voice Disorders, but also provided more discrete information regarding the central control of voice in humans.

As highlighted earlier, this body of research is pertinent to Dietrich and Verdolini Abbott's psychobiological framework of voice disorders (2008). Perceptions of and responses to stressors are idiosyncratic and variable across individuals. Stated differently, a *person-by-situation interaction* exists that impacts one's response to a given stressor. When individual traits are poorly matched to a situation (e.g., a shy person being called upon to lead a group), the stress response is greater than when traits are well-matched to a situation (e.g., an outgoing person being called upon to lead a group) (Cohen & Hamrick, 2003). While responses across individuals may vary greatly, individual responses *within tasks* are quite reliable over time (Cohen & Hamrick, 2003). This person-by-situation interaction is situated at the topmost level of the psychobiological framework.

Overall, the Trait Theory of Voice Disorders seems to be supported empirically. However, thus far the theory has examined only gross aspects of personality, setting aside finer parsing of broader constructs such as negative emotionality. One expectation is that examining elements of personality such

as *stress reactivity* might clarify more discrete aspects of personality in voice than have been previously described. These finer-grained traits might be related to the development of certain voice disorders by way of increased laryngeal muscular hyperfunction. The present study seeks to address this gap by investigating trait *stress reactivity* (as measured by the *MPQ-BF*) not in the context of any particular voice disorder, but in the context of ILM activity, which is generally accepted to play a major role in the development and clinical presentation of many voice disorders. Figure 3-1 schematizes this relationship, building on the same conceptual schematic presented in Figure 1-3.

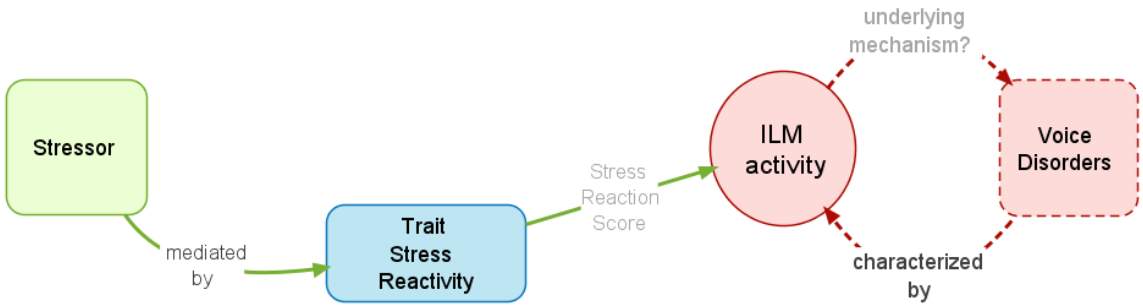


Figure 3-1. Conceptual schematic: trait stress reactivity mediating laryngeal response to stressor.

4.0 THE AUTONOMIC NERVOUS SYSTEM

In parallel to addressing the relationship between trait stress reactivity and ILM response, this study also targets a second gap that has been scarcely addressed in the empirical literature. That gap regards the biological pathways that may mediate the relation between stress reactivity and laryngeal response. Although suggestions have been made about the mediating role of the autonomic nervous system (ANS), to date, data have been patently lacking in the literature. Speculations about this role are reasonable. Somatic stress responses in general are heavily mediated by the autonomic nervous system (Goldstein, 2001). In people with stress and anxiety disorders, the ANS balance is often disrupted. Although the personality-stress interaction is known to be mediated by the autonomic nervous system, a clear empirical link to voice disorders remains outstanding. Despite copious references to the impact of the ANS on voice (Brantigan, Brantigan, & Joseph, 1982; Demmink-Geertman & Dejonckere, 2008, 2010; Demmink-Geertman & Dejonckere, 2002; Gates et al., 1985; House & Andrews, 1987; James, Griffith, Pearson, & Newbury, 1977; Scherer, 1986), and the wide acceptance of this concept, few data are available about ANS mediation of stress responses in the larynx.

Further background is as follows. As previously noted, autonomic health is profoundly linked to both anxiety stress (Goldstein, 2001). The ANS is comprised of the parasympathetic and sympathetic nervous systems (PNS and SNS, respectively), which are correspondingly known in colloquial terms as the "rest and digest" and "fight-or-flight" systems. The activity of each system has been deeply studied by way of cardiovascular responses, which lend themselves to non-invasive observation and are profoundly influenced by both branches of the ANS. In psychologically healthy individuals, PNS

dominance over the SNS is maintained in the heart during rest conditions. That is, the heart comes under tonic inhibitory control by the PNS (Goldstein, 2001). Individuals with purportedly low PNS "tone" are more likely to suffer from recent stress, depression, anxiety, low self esteem, and similar conditions (e.g., Davis, Montgomery, & Wilson, 2002; Delaney & Brodie, 2000; Jönsson, 2007; Licht, de Geus, van Dyck, & Penninx, 2009; Martens, Greenberg, & Allen, 2008). PNS "tone" is also referred to in the literature as "vagal tone" or "cardiac vagal control". The latter term will be used herein; it is thus named because the vagus nerve exerts parasympathetic efferent cardiac effects on the sinoatrial node.

Cardiac vagal control is conceptualized as the strength of the inhibitory "brake" of the parasympathetic vagus nerve over the excitatory effects of the SNS. Vagal control is effectively indexed by *respiratory sinus arrhythmia* (RSA), which is extracted from the electrocardiographic signal and reflects rhythmic fluctuations of vagal effects on the heart (Grossman & Taylor, 2007). This particular measure will be described further in Section 4.4.

Robust data demonstrate that ANS functions are influenced by affective traits. Specifically, healthy RSA responses are attenuated (i.e., calming PNS effects are weaker than SNS fight/flight effects) by relatively high expression of trait negative affect, which corresponds roughly with the personality factor of neuroticism/anxiety, and its subfactors, e.g., trait stress reactivity (how reactive one is to stressors). Important for the present discussion, data suggest that these psychological factors are strongly implicated in hyperfunctional voice disorders (Roy et al., 2000a; Roy, Bless, & Heisey, 2000b; van Mersbergen et al., 2008). Moreover, as previously described, recent research has suggested that motor cortical control of speech and voice is significantly modulated by a person's stress reactivity as measured via standardized personality questionnaire (Dietrich et al., 2012). However, to date, vagal control has not been examined in the context of the laryngeal musculature's reactivity to a stressor. Thus, the relationship between vagal control and ILM response remains speculative. Building on the conceptual schematic last presented in Figure 3-1, Figure 4-1 illustrates the two proposed elements—cardiac vagal control and trait Stress Reactivity—as mediators of the intrinsic laryngeal response to stressor exposure.

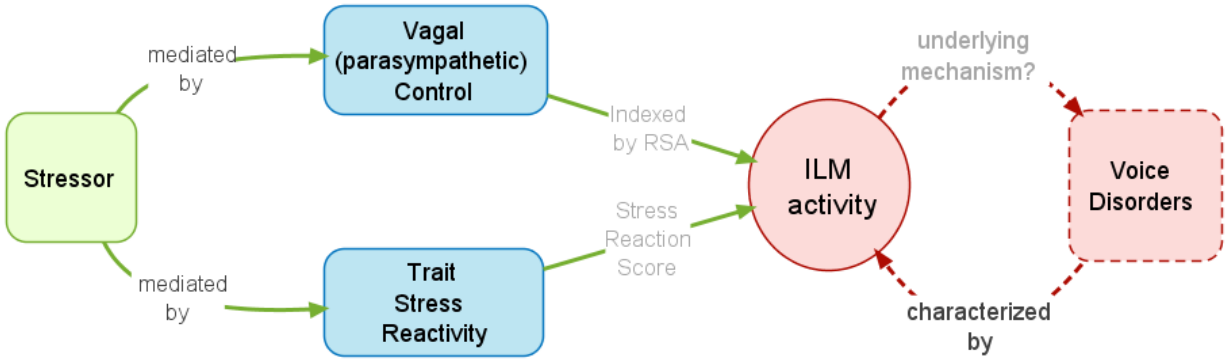


Figure 4-1. Conceptual schematic: vagal control impacting laryngeal response to stressor.

4.1 BASIC CONCEPTS RELATING TO STRESS RESPONSES

Before embarking on descriptions of the autonomic nervous system during stress, a brief discussion of homeostasis and allostasis is warranted. This information will help to set the stage for an understanding of how stress is understood in terms of its general effects on an organism. This section also presents alternative perspectives to the outdated "stress is bad" view, in that variability and rapid acclimation will be seen as signals of a healthy stress response. This section is largely constructed based on selected review works (Logan & Barksdale, 2008; McEwen, 2007), unless otherwise stated.

Homeostasis refers to the regulation that allows an organism's internal environment to maintain a relatively steady state (Cannon 1932). However, internal states and physiologic responses (e.g., temperature, blood pressure, hormones) are constantly changing to account for and respond to stressors. *Allostasis* is a conceptual extension of homeostasis; it represents the process of adaptation that complex physiological systems undergo in the face of physical, psychosocial and environmental challenges. Allostasis is recognized as an active process of regulation that constantly assesses and adapts to physiological needs. Mediators synthesized by the immune system, the endocrine system, and the autonomic nervous system contribute to allostasis (McEwen, 2002). Thus, one key difference between

allostasis and homeostasis is that the former takes into account normal fluctuations in a non-static biological system, whereas the latter implies a reduction of variability and maintenance of constancy in the system. The concept of allostasis leads one to interpret variability as a positive indicator that the internal environment is capable of adapting to challenges in order to support body functions. Thus, in contrast to viewing physiologic lability as reflective of pathology, patterned variability may actually be viewed as reflective of overall viability and strength of a system. The concept of *heterostasis* incorporates this stance, as it refers to the maintenance of stability in one physiological variable by adjustment of another (Friedman, 2007).

The normal allostatic response involves an initial stressor-triggered response, which is then sustained for some appropriate period of time and then terminated. When normal allostatic processes fatigue, cease, or fail to disengage, the physiological systems are unable to adapt. This state is referred to as *allostatic load*. McEwen (2002) identified four patterns of response to environmental challenges, each of which is related to a different type of allostatic load. **Error! Reference source not found.** is an llustration of these response patterns presented by McEwen (2002)[4]. The top panel in **Error! Reference source not found.** shows the normal allostatic response, which is characterized by a robust response to stressor exposure that is sustained for some appropriate amount of time, and then ceases. The four alternative response patterns that may lead to allostatic load are as follows: (1) repetitive "hits" from multiple new stressors; (2) lack of adaptation or habituation to the same stressors repeated over time; (3) prolonged response due to delayed shut down; (i.e., physiologic systems remain at elevated levels of activation, without recovery); and (4) inadequate adaptation response leads to compensatory hyperactivity elsewhere in the body. These allostatic responses may individually or collectively result in chronic illness. As an example, the inability of the trapezius muscle to adapt to repeated stressor exposure

[4] Note: From "Sex, stress and the hippocampus: allostasis, allostatic load and the aging process" by McEwen, 2002, *Neurobiology of Aging*. Copyright 2002 by Elsevier. Reprinted with permission.

(discussed in Section 2.1.1) pertains to the second pattern of response. Dietrich and Verdolini (2007) proposed that inadequate recovery plays a key role in creating chronic laryngeal tension related to voice pathologies, which is an example of the third response pattern in McEwen's model.

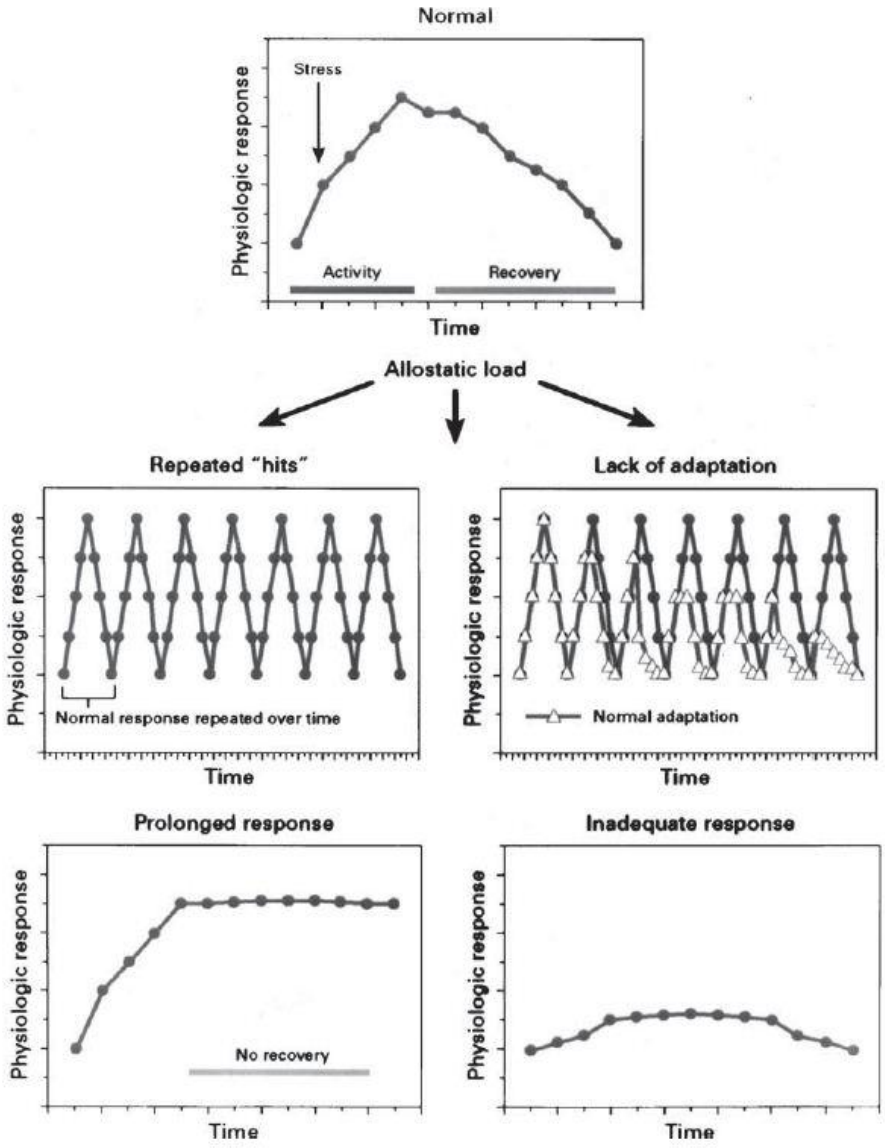


Figure 4-2. Allostatic Load Response Patterns (McEwen, 2002).

Allostatic load is primarily considered to result from the effects of the sympathetic nervous system, the HPA axis, and immune systems, each of which will be discussed in greater detail in section

4.3. In terms of physiologic parameters typically used to measure allostatic load, research primarily examines hormones (glucocorticoids such as cortisol) and catecholamines (adrenaline and noradrenaline), all of which mediate cardiovascular function. Although the cardiovascular system is the primary effector that has been studied in the context of allostatic load, the individual characteristics that modulate perception of stressors are widely varied. Allostatic changes in response to stressor exposure can be normal, and the capacity of an organism to maintain normal allostatic response patterns—and generally, to minimize "wear and tear"—in the face of stress is called *resilience* or *adaptive capacity* (McEwen, 2002). After all, allostasis is a brain-driven process. Stress is perceived centrally, and it is within the brain that behavioral and physiologic responses to perceived stress are generated. The hippocampus is a critical area for cataloguing former events and interpreting new events, thus it serves to regulate the principal stress mediators for a given allostatic state (Sapolsky, 2003). Moreover, the brain is a target organ for stress. The hippocampus, amygdala, and prefrontal cortex are structurally remodeled by a history of exposure to stressful stimuli (McEwen, 2002; Sapolsky, 2003). The finding that these areas are plastic and are remodeled by stress is also consistent with the observation that behavioral and clinical interventions (especially when they occur on a timely basis, and early on in the course of one's development) can increase one's resilience.

Overall, high levels of adaptive variability represent healthy physiology. Complex variability is ubiquitous in nature. This notion is foundational to the field of non-linear dynamics and has been broadly applied in biobehavioral contexts. As an example, the study of anxiety and complex variability in cardiovascular regulation has resulted in the understanding that pathological states are typically characterized by minimal variability and high predictability in variables such as heart rate (Friedman, 2007).

4.2 THE ANS-VOICE LINK

The voice literature is replete with references to the impact of the ANS on voice, typically in the context of stress or other "negative" states (e.g., high anxiety, depression, *et cetera*). Unfortunately, these references are commonly made without evidential support or details regarding the mechanisms by which the ANS impacts phonation. Scherer (1986) provides a general theoretical overview of the autonomic and somatic nervous systems' effects on voice production. He largely attributes vocal changes to the elevation of tension and "mode of functioning" in the muscles of the chest, throat, and head; these changes are alleged to be effected by the somatic nervous system, which is mediated in part by the ANS. He states that the sympathetic and parasympathetic nervous systems are aroused differentially across emotions, and that the autonomic nervous system affects voice production primarily via changes in mucus secretion and salivation (which would, in turn, modify the resonance characteristics of the vocal tract), and in respiration. However, it is important to note that Scherer's given example for the respiratory impact of the ANS on voice is based on changes in subglottal pressure, which can, of course, be mediated largely by laryngeal mechanisms while holding respiratory parameters constant (and vice versa). He states that, "The ANS is indirectly involved [in voice changes] because cardiovascular processes directly contribute to muscle tonus and activity." Taken together, these statements insinuate that activation of the ANS causes shunting of blood specifically to the peri- and intra-laryngeal region. Scherer supposes that the tonic co-activation of muscles involved in vocalization (both agonists and antagonists) are not under voluntary control and may be generally elevated, whereas the phasic effects, "even if held for a considerable amount of time, are voluntary and may often represent attempts of the organism to control expressive behavior." This overview of autonomic and somatic nervous system involvement in vocalization sounds strikingly similar to theories surrounding the general mechanisms of voice disorders involving laryngeal muscle hyperfunction. It is important to note that with few exceptions, this overview is presented largely as theory and opinion, and is not accompanied by empirical findings.

Additional support for an ANS-voice link can be found in studies that use beta-adrenergic blockade to combat vocal symptoms of stage fright. Blocking the effects of the sympathetic nervous system during a public performance is one clinical approach to managing performance anxiety, although research findings are divided; general performance quality may improve, decline, or stay the same with the use of beta-blockade (Brantigan et al., 1982; Gates et al., 1985; James et al., 1977), and to some degree these effects are dose-dependent (Gates et al., 1985).

These studies based performance assessments on perceptual ratings provided by expert musicians, and only one study to date has examined acoustic and aerodynamic parameters of voice after beta blockade (although not in a performance setting). Giddens, Barron, Clark, and Warde (2010) report on a double-blind, prospective, within-subjects trial in which the effects of cold pressor exposure were examined (first, with no pharmacologic intervention, and then again with propranolol, a beta-adrenergic blocker, or placebo) on a series of vocal parameters: mean fundamental frequency (F0), voice onset time (VOT), speaking rate, jitter, shimmer, maximum airflow declination rate, and subglottal pressure. Part of the value of this study lies in that by including two cold pressor exposures (with and without pharmacologic experimental intervention) it explores a causal model of ANS effects on voice. Specifically, the cold pressor task is considered to trigger strong activation of the sympathetic nervous system. Interestingly, none of the measures reflecting voice, itself, changed significantly after cold pressor exposure as compared to baseline; the only measure to change was subglottal pressure, which exhibits a statistically significant increase from baseline to cold pressor in the female participants (but not in the male participants). Moreover, the only parameter to significantly change as a function of propranolol administration was jitter, which significantly increased in the propranolol group during the cold pressor task; that is, blocking the effects of the SNS leads to decrease in phonational frequency stability[5].

[5] It should be noted that although this finding is statistically significant, it may not be clinically meaningful. The values reported for males and females fall generally within the range of normal jitter ratio values presented for

Finally, a small body of literature documents connections between voice production and autonomic responsivity in a clinical population. Demmink-Geertman and Dejonckere showed that females with nonorganic habitual dysphonia complain of significantly more subjective autonomic symptoms and complaints than healthy controls (2002). Such symptoms (both related and unrelated to voice) were significantly alleviated following behavioral voice therapy in subjects studies, and the non-voice related symptoms were reduced to a level comparable to those of healthy normal controls. The same findings could not be confirmed in the male cohort (n=18), although it was a substantially smaller sample than the female cohort (n=65) and thus may not have been sufficiently powered to detect an effect. Further, those researchers demonstrated efficacy from voice therapy that involved counseling on the management of chronic negative emotions, inhibitions, anxieties, emotional impulsiveness, fears, self-defeating actions or reactions, and even physical pain when it is judged to be related to emotional stress (L Demmink-Geertman & Dejonckere, 2008, 2010).

Taking these reports together, it must be acknowledged that the ANS-voice link is empirically tenuous, albeit theoretically compelling. In a recent review of stress effects on the voice, Giddens et al (2013) hypothesize that increases in heart rate and bronchodilation due to SNS activation would in turn cause increases in speaking fundamental frequency, subglottal pressure, vocal jitter and shimmer, maximum airflow declination rate, voice onset time, vocal intensity, and rate of speech. Nonetheless, as previously suggested, it is certainly possible that the involuntary effects of the ANS can be overridden by the volitional influence of the speaker's own psychological processes.

several studies by Baken & Orlikoff (2000, pgs. 204 and 209). Changes in jitter values may be attributable to fluctuations in vocal intensity, which was neither collected nor controlled for in this study.

4.3 ANS-MEDIATED CARDIOVASCULAR EFFECTS OF STRESS

Stressful episodes—and their subsequent stress responses—that persist over time may induce acute stress responses that are maladaptive (Selye, 1956) and ultimately lead to chronic cardiovascular impairment (Cohen et al., 2012; Lovallo, 1997). Negative states and traits can have powerful effects over the long term. Individuals who chronically worry to a high degree tend to exhibit blunted vagal cardiac control (as indexed by respiratory sinus arrhythmia, described subsequently) at rest (Delgado et al., 2009), and both depression and anxiety are linked to the development of coronary artery disease (Sheps & Sheffield, 2001). In fact, up to one-fifth of individuals with ischemic heart disease may concurrently have major depression (Sheps & Sheffield, 2001). Chronic stress can elevate the risk of coronary heart disease, increased blood pressure, atherosclerosis, and myocardial infarction (Goldstein, 2001) (one marker of this risk is depressed RSA, which exhibits an inverse relationship to cardiovascular morbidity and increased risk for death secondary to cardiac pathology (review in Fuller, 1992).

The extent to which one responds to stress is one contributing factor to the pathogenesis of cardiovascular disease. One measure of stress reactivity that incorporates both heart rate and blood pressure is *cardiovascular reactivity* (CVR), which refers to "an individual's propensity to experience cardiovascular reactions of greater or lesser magnitude, in relation to those of other persons, when encountering behavioral stimuli experienced as engaging, challenging, or aversive." (Manuck, 1994) The *reactivity hypothesis*, which serves as the framework underlying the measurement of CVR, holds that when cardiovascular reactivity to psychological stressors is exaggerated or prolonged, the development of cardiovascular disease is promoted (Obrist, 1981). This reactivity hypothesis is conceptually similar to the model of allostatic load submitted by McEwen (2002). Heightened CVR is linked to the development of hypertension, atherosclerosis, myocardial infarction, elevated left ventricular mass, and mortality secondary to coronary heart disease (Goldstein, 2001; Lovallo, 1997). Exaggerated blood pressure reactivity in the face of psychological stress is related to a constellation of specific cardiovascular

impairments (e.g., atherosclerosis, increased coronary artery calcification), and is even associated with functional neural activation in a specific set of brain systems, which may represent a neural phenotype characterizing people who are predisposed to high cardiovascular reactivity (Gianaros, Jennings, Sheu, Derbyshire, & Matthews, 2007).

Long-term effects of stress are brought about by repeated or continuous activation of the acute stress response (Goldstein, 2001). For logistical reasons, the acute stress response is more extensively represented in prospective research compared to chronic stress responses. In the next paragraphs, acute laboratory stressors (as opposed to naturally-occurring stressors) will be discussed, as the preponderance of prospective, controlled literature involves experimental stressors. Acute and chronic cardiovascular responses may be very similar, and one of the assumptions of the reactivity hypothesis is that response to acute laboratory stressors is reflective of one's real-life cardiovascular reactivity to stress (Obrist, 1981). Acute laboratory stressors and chronic stressors (e.g., anxiety, depression) may share pathophysiologic mechanisms in terms of predisposition to risk, and may actually be additive (Lovallo, 1997; Obrist, 1981; Sheps & Sheffield, 2001). Key outcomes of interest in investigations of cardiovascular reactivity include the magnitude of reactivity, the duration of a stress response before cardiovascular parameters return to baseline levels, and an individual's adaptation across repeated exposures to stressors.

Short-term cardiovascular effects of stress are vulnerable to the influence of several mediating factors. For instance, dramatic individual differences in stress response may be observed, perhaps due to physiological idiosyncracies and/or psychological factors (e.g., task engagement behaviors, self-challenge tendencies). To this point, several distinct physiological patterns of reaction to mental and psychological stressors seem to exist (Allen, Boquet, & Shelley, 1991; Kasprowicz, Manuck, Malkoff, & Krantz, 1990; Liu, Iwanaga, Shimomura, & Katsuura, 2007). For instance, some individuals are primarily "cardiac reactors", others are predominantly "vascular reactors", and some people are "mixed reactors" (Allen et al., 1991; Kasprowicz et al., 1990; Liu et al., 2007).

Similarly, physiological differences are often observed as a function of task type (Allen et al., 1991; Goldstein, 2001; Hurwitz et al., 1993; Liu et al., 2007). Cluster analyses of cardiovascular measures obtained by Allen et al. (1991) from subjects during mental arithmetic, reaction time, and cold pressor tasks revealed four to five distinct patterns of cardiovascular task responses. Although the response patterns showed some consistency across tasks, a substantial proportion of individuals did show variable responsivity patterns as a function of task. Taking all cardiovascular findings together, four response patterns were observed in the reaction time task: (1) very strong beta-adrenergic pattern; (2) moderate beta-adrenergic pattern; (3) mild alpha-adrenergic pattern; and (4) "non-reactor" pattern (although a small beta-adrenergic response may have been seen). Four response patterns were also observed for the mental arithmetic task: (1) strong beta-adrenergic pattern; (2) mixed pattern - mild beta-adrenergic activation with significant parasympathetic withdrawal; (3) mild alpha-adrenergic pattern; and (4) mild beta-adrenergic pattern, overall "non-reactor" pattern. Finally, five response patterns were observed in the cold pressor task: (1) large beta-adrenergic activation with parasympathetic withdrawal; (2) alpha-adrenergic pattern with slight beta-adrenergic response; (3) strong alpha-adrenergic pattern; (4) alpha-adrenergic activation with concurrent parasympathetic withdrawal; and (5) "non-reactor" pattern. Others have also reported differential patterns of cardiovascular regulation as a function of task. Preparation of a speech that will be evaluated tends to raise blood pressure by elevating cardiac output via increased heart rate and contractility, whereas a mirror tracing task elevates blood pressure by raising systemic vascular resistance (Hurwitz et al., 1993). In a different study, mental stress tasks triggered at least four patterns of circulatory response (in a fashion generally consonant with findings of Allen et al.), although on the whole, mental stress tasks tend to trigger an increase in total peripheral resistance (Liu et al., 2007).

The tendency of specific stressors to elicit myocardial over vascular responses (or vice versa) is evidence of *situational stereotypy*. Schneiderman, Ironson, and Siegel (2005) provide an evolutionary interpretation of situational stereotypical behaviors, which is summarized as follows. They propose that

public speaking and mental arithmetic tasks involve active coping strategies (i.e., the participant must do something) and are associated with myocardial responses[6]. Other stressors such as the cold pressor task or viewing a disturbing movie involve more vigilant or passive coping strategies and do not require movement, and are associated with vascular responses[7]. When considered from an evolutionary perspective, the cardiac responses are most consistent with the "fight-or-flight" response, as they facilitate active coping by shunting blood to the periphery (i.e., the skeletal muscles). On the other hand, vascular hemodynamic responses occur in the face of a stressor where action must be suppressed, and in which skeletal muscle inhibition and vigilance are more appropriate. The vascular response is considered adaptive, as it shunts blood away from the periphery and toward the internal organs in order to minimize blood loss in the event of a physical wound.

4.4 RESPIRATORY SINUS ARRHYTHMIA AS AN INDEX OF CARDIAC VAGAL TONE

Acute anxiety and stress are often accompanied by a cardiac autonomic imbalance in the direction of depressed cardiac vagal (i.e., parasympathetic) control (Friedman, 2007; Sheps & Sheffield, 2001). As previously noted, *cardiac vagal control* is conceptualized as the strength of the inhibitory "brake" of the parasympathetic vagus nerve over the excitatory effects of the SNS on the heart. In healthy individuals, parasympathetic dominance over sympathetic influences is maintained in the heart during rest conditions; that is, the heart is under tonic inhibitory control by the PNS (Thayer & Sternberg, 2006). Individuals with purportedly low parasympathetic or vagal tone are more likely to suffer from recent stress,

[6] Also distinguished as beta-adrenergic response pattern, which is characterized by a relatively high sympathetic component, as indexed by heart rate increase and elevated contraction of skeletal muscles. Myocardial responses are characterized by increased cardiac output and decreased total peripheral resistance.

[7] Also distinguished as alpha-adrenergic response pattern, which is characterized by a relatively high vascular resistance component, as indexed by arterial constriction and greater contraction of smooth muscles. Vascular responses are characterized by increased total peripheral resistance and decreased cardiac output. Mixed responses involve increases in both cardiac output and total peripheral resistance.

depression, anxiety, low self esteem, or other such disorders (e.g., Davis, Montgomery, & Wilson, 2002; Delaney & Brodie, 2000; Jonsson, 2007; Licht, de Geus, van Dyck, & Penninx, 2009; Martens, Greenberg, & Allen, 2008).

Unlike stress reactivity, ANS function cannot be assessed using self-report instruments. The electrocardiographic signal contains information thought related to ANS function. Specifically, *respiratory sinus arrhythmia* (RSA) is extracted from the ECG signal and thought to be principally modulated by vagal outflow to the heart. It reflects rhythmic increases and decreases of efferent cardiac vagal effects on the sinoatrial node (Grossman & Taylor, 2007). Vagal efference is inhibitory, thus vagal outflow to the heart results in slowing of heart rate via decreased firing of the sinoatrial node. This deceleration is greatest during expiration, whereas vagal withdrawal during inspiration accelerates heart rate (Berntson et al., 1997). Also known as high-frequency heart rate variability, RSA represents the high-frequency variation in the beat-to-beat cardiac rhythm, and is measured by calculating the time between R spikes (i.e., the R-R interval, also referred to as inter-beat interval) on an electrocardiograph trace. This is illustrated by the red arrows overlaid on the heart rate signal in Figure 4-3. The interval oscillations occurring in the ~0.15-0.40 Hz frequency band are referred to as the high-frequency power band (hence the alternate term high-frequency heart rate variability, referred to herein as RSA). Other rhythms also occur. Relevant to the present study, a low frequency band can be measured that spans ~0.05-0.15 Hz and is thought to be a closer representation of sympathetic outflow. However, these relationships are neither independent nor static; the high frequency band can be impacted by sympathetic activity, and likewise, the low frequency band can be influenced by vagal activity. (Berntson et al., 1997; Grossman, Karemaker, & Wieling, 1991)

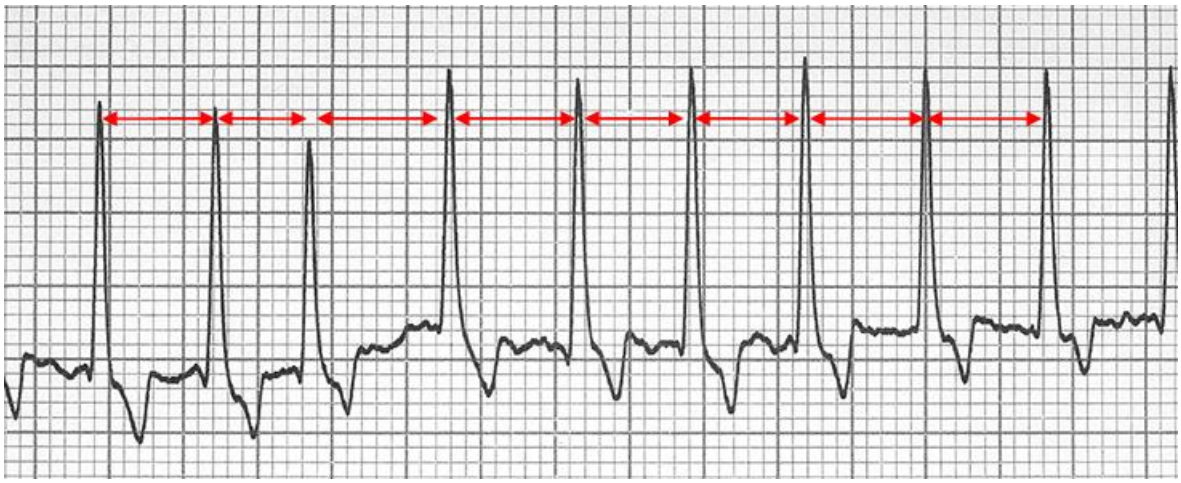


Figure 4-3. Electrocardiographic signal with Heart Rate Variability illustrated.

Women with high trait anxiety exhibit chronically lower RSA amplitudes at rest (and higher heart rate) than women with low trait anxiety (Fuller, 1992). Worry, which is a state very closely associated with anxiety, is also marked by cardiac vagal depression. RSA is also lower (i.e., cardiac sympathetic response is increased and vagal control is diminished) during panic, recollection of stressful events, exposure to traumatic stimuli, and perception of previous emotional stress (Friedman, 2007). These observations of diminished RSA are from human studies that corroborate analogous outcomes in several animal models (Goldstein, 2001). The preponderance of findings suggests that a broad array of stressful states trigger cardiac vagal withdrawal, as do laboratory stressors such as mental arithmetic and shock avoidance. Moreover, anxiety disorders (e.g., panic disorder, PTSD, generalized anxiety disorder, specific phobias, childhood anxiety disorders) are generally linked to low RSA. Friedman (2007) provides an excellent summary of multiple studies' major findings on anxiety disorders and RSA.

The cardiovascular system is exceptionally vulnerable to perturbations from external stimuli—for instance, mechanical, acoustic, thermal, or gravitational stimulation—that can influence the sympathetic-parasympathetic interaction (Bernardi, Porta, Gabutti, Spicuzza, & Sleight, 2001). Breathing, which can also be profoundly influenced by these factors, as well as other factors related to emotion and mood

(Bloch et al., 1991; Boiten et al., 1994), is one of the most confounding "external modulators" of cardiovascular variability (Spyer & Gourine, 2009). The respiratory network is situated near the cardiac vagal pre-ganglionic and pre-sympathetic neuronal circuits in the ventrolateral areas of the medulla, and cardiorespiratory integration is observed even within individual brainstem neurons (Spyer & Gourine, 2009). The rhythms of respiration and cardiovascular activity are synergistically regulated to maximize efficiency (i.e., adequate ventilation-perfusion matching within the lungs) of respiratory gas exchange (Spyer & Gourine, 2009). The frequency and depth of rhythm of the respiratory pacemaker cells, located in the brainstem, are largely controlled by central and peripheral chemoreflexes, but are also greatly influenced by factors such as stress, exercise, temperature, voluntary control, and the activity of the autonomic nervous system (Bernardi et al., 2001).

Substantial changes in breathing patterns may be triggered by varying types and degrees of stress, varying types and degrees of mental or cognitive demand, and changes in conditions related to speech production (Bernardi et al., 2000, 2001; Spyer, 2009). These effects are easily observed in healthy individuals, and other respiratory phenomena related to compensation may be observed in patients with cardiovascular disorders, such as heart disease (Bernardi et al., 2001). In fact, some researchers have theorized that respiratory sinus arrhythmia (RSA) serves to conserve cardiac and respiratory energy by minimizing unnecessary heartbeats during expiration, and also by minimizing ineffective breaths during waning phases of perfusion (the delivery of blood to a capillary bed) (Hayano & Yasuma, 2003). These investigators propose that RSA is an "intrinsic resting function" of the cardiopulmonary system, and reflects cardiorespiratory interaction.

Corollary to the discussion of RSA's reflection of pulmonary influences and of special relevance to the present research program, measures of RSA are confounded by voluntary and involuntary changes in respiration rate and tidal volume, both during mental tasks and under steady-state conditions[8]

[8] i.e., conditions during which both autonomic tone and metabolic activity are mostly constant.

(Grossman & Taylor, 2007). Specifically, respiratory rate and depth significantly influence measures of RSA, and therefore must be considered experimentally. Under steady-state conditions, RSA magnitude is inversely related to rate of respiration, and directly related to tidal volume (Grossman & Taylor, 2007). Stated differently, quick shallow breathing will decrease RSA magnitude, whereas slow and deep breathing will exaggerate RSA magnitude. It is important to note that the effects of respiration rate and tidal volume are at times independent and at other times interactive. For instance, large RSA elevations will be elicited by increasing tidal volume at relatively slow respiration rates, whereas comparable increases in tidal volume at more rapid respiration rates will result in less exaggerated RSA increases (Berntson et al., 1997; Grossman & Taylor, 2007).

Respiratory effects on RSA magnitude are problematic for assessment of cardiac vagal control under two key conditions: (1) when respiratory rate or tidal volume differ considerably between conditions or groups, and (2) when RSA, respiratory variables and cardiac vagal control do not covary with each other systematically (Beda, Jandre, Phillips, Giannella-Neto, & Simpson, 2007; Grossman, Karemaker, & Wieling, 1991; Grossman & Taylor, 2007). The latter issue is critically important because RSA magnitude is far more closely related to fluctuations in respiratory parameters, specifically rate of respiration and to a lesser degree tidal volume, than to changes in actual cardiac vagal control (Grossman & Taylor, 2007).

It might be argued that respiration should therefore be controlled in order to meaningfully interpret vagal control of heart rate via RSA, absent the "confounding" effects of respiration. On the other hand, one may not wish to control for respiratory effects if those effects are relevant to the psychological focus of the investigation. Several solutions have been proposed to control for or exclude respiratory effects on RSA (Berntson et al., 1997; Egizio, Eddy, Robinson, & Jennings, 2011; Grossman & Taylor, 2007). Discussion of these methods is beyond the scope of the present document. It is critical that investigative endeavors involving RSA or other cardiovascular parameters take respiratory effects into consideration. For cases in which respiratory correction is deemed prudent given the underlying

theory and concepts of a research question, an efficient and inexpensive within-subjects correction procedure is available to estimate respiratory contributions, and to provide a respiratory-corrected index of RSA (Egizio et al., 2011).

4.5 SEX-SPECIFIC CARDIOVASCULAR RESPONSES TO STRESS

Sex effects on cardiovascular responsivity are also widely reported. The literature regarding sex differences in cardiovascular response to stressors is vast, and thorough discussion is beyond the scope of this manuscript[9]. Some general findings are presented here. First, in general, females tend to exhibit less dramatic fight responses than males, and perhaps contrary to intuition, may also exhibit inhibited flight behavior (Taylor et al., 2000). Kajante and Phillips (2006) provide an excellent review of studies examining sex differences in ANS response (heart rate and blood pressure) to acute psychosocial stress in adults. Results widely vary, likely due in large part to methodological differences. However, it seems that during stressors, females tend to have greater myocardial reactivity, whereas males demonstrate greater vascular reactivity (Girdler, Turner, Sherwood, & Light, 1990; Kajantie & Phillips, 2006; Liu et al., 2007; Taylor et al., 2000). In addition, a pharmacological autonomic blockade study showed differences in the relative influence of sympathetic versus parasympathetic outflow over vascular regulation in men and women (Evans et al., 2001). In at-rest states, females exhibit higher RSA components, which is indicative of a predominance of parasympathetic activity, whereas males evidence a relative predominance of sympathetic activity[10]. Females also exhibit greater cyclical variability in

[9] Detailed discussion of sex differences would require more thorough discussion of a proposed alternative to the SNS-mediated "fight or flight" stress response, the "tend and befriend" response. This response capitalizes on social interactions, which involves befriending and nurturing, in order to promote safety and diminish distress when a threat is faced (Taylor et al., 2000). This stress regulatory system would necessary involve different physiological mechanisms than are classically associated with SNS activity.

[10] In women, peripheral vascular ANS activity had a β-adrenergic component, and in men it had a muscarinic component. Both components seem to be important in moderating the impact of tonic vasoconstriction.

stress responses due to the reproductive cycle, rendering their data sometimes conflicting or challenging to interpret. Females in the luteal phase (as opposed to the follicular phase) of their monthly cycle produce a stress response that more closely approximates that of males (Duchesne, Tessera, Dedovic, Engert, & Pruessner, 2012; Kirschbaum, Pirke, & Hellhammer, 1993).

5.0 RESEARCH QUESTIONS, HYPOTHESES, RELATED CONSIDERATIONS

5.1 RESEARCH QUESTIONS 1 & 2

To summarize the foregoing chapters, the combined effects of trait stress reactivity (specifically, high values) and cardiac vagal control (specifically, low values) may be highly disruptive to multiple somatic functions, and are thought to generally manifest in the form of elevated muscular activity. Unfortunately, despite claims about such causal mechanisms in hyperfunctional voice disorders, at least two substantial gaps are noted in the literature about such questions. First, quite astonishingly, with the exception of a single study preliminary to the current one (Helou, Wang, Ashmore, Rosen, & Verdolini Abbott, in press), no data are available about whether an acute stressor actually manifests in the larynx as a function of stress reaction and ANS balance at all. Second, assuming such responses may occur, the role of stress reactivity and autonomic function—or any other mechanisms for that matter—have not been empirically investigated in the area of voice disorders.

At the broadest level, the present study sought to examine two gaps in the literature via three broad research questions (RQs). Accordingly, the study's first aim was to address whether an ILM response actually occurs in the face of a psychological stressor, specifically a speech preparation task (RQ1). Data to this effect would be the first such data in the literature, and the subsequent characterization of resultant ILM responses (i.e., magnitude, pattern, direction of response, timing) would be highly informative. The study's second aim was to examine two possible mediating mechanisms for such a response, if it were to be seen: trait stress reactivity (psychological variable) and cardiac vagal

control (physiological variable) (RQs 2a and 2b). A third aim was to examine the extent to which these two potential mechanisms involved in a laryngeal stress response might be related, if indeed they were found (RQ2c). A final aim was to examine the potential contribution of subvocalization to ILM stress responses (RQ3).

This study was innovative in that it sought, for the first time, to characterize the response of ILMs to a psychological stressor using intramuscular EMG of the intrinsic laryngeal muscles (ILM)[11]. In addition, it aimed to predict the ILM response as a function of a psychometrically-derived psychological stress reaction score and RSA, both of which are in theory highly relevant to hyperfunctional ILM activity. The stressor used in the study was a speech preparation task based on the Trier Social Stress Test, which is a widely used and well-vetted experimental protocol to induce moderate psychosocial stress and yield significant changes in a series of cardiovascular parameters as well as increases in subjective stress ratings (Kirschbaum et al., 1993). This stressor is psychological in nature, and is designed to have good ecological validity. Stress reaction scores obtained from the *Multidimensional Personality Questionnaire – Brief Form* (*MPQ-BF*) (Patrick et al., 2002) served as one independent variable in the present study. Thus, the study examined the role that stress reactivity may play in ILM responses to stressor exposure.

In addition, the second independent variable, RSA, was utilized in the present study as an operationalized proxy for cardiac vagal control. Stated differently, RSA is thought to index the strength of the parasympathetic nervous system. The conceptual dependent variable was activity of the laryngeal muscles as well as positive and negative control muscles (upper trapezius and anterior tibialis, respectively). Such activity was represented using *magnitude of muscular response* and *resolution*

[11] Electrodes were inserted into the right posterior cricoarytenoid (PCA), bilateral thyroarytenoid/lateral cricoarytenoid (TA/LCA) complex, and bilateral cricothyroid (CT). Thus, three muscle groups of interest were sampled on five individual EMG channels. Because the TA/LCA and CT muscles were sampled bilaterally to protect against loss of data in the event of electrode displacement, the muscle displaying the *greatest* change from baseline in absolute value was included for analysis in the regression model. Hence, data from three muscles were included in statistical analyses for each subject: PCA, one TA/LCA complex (left or right), and CT (left or right).

latency. *Magnitude of response* refers to the magnitude of the change in activation from one time point (baseline) to another (the speech preparation stressor). *Resolution latency* refers to the time required for an individual's muscle activity to return to *Baseline Rest* values after exposure to the stressor. These two dependent variables draw from Obrist's Reactivity Hypothesis (see Section 4.3) and McEwen's allostatic response pattern of "prolonged response" (see Section 4.1), respectively. More detailed information regarding the collection and calculation of independent and dependent variables is presented in Chapter 7. Figure 5-1 provides a schematic of the first two research questions, which are detailed below and summarized as follows: *Does a speech preparation stressor impact ILM activity, and if so, is that response mediated by cardiac vagal control, or trait stress reactivity, or both? Are these two potential mediators interrelated?* The aforementioned variables will be investigated to address several specific research questions. Specific questions and hypotheses were as follows:

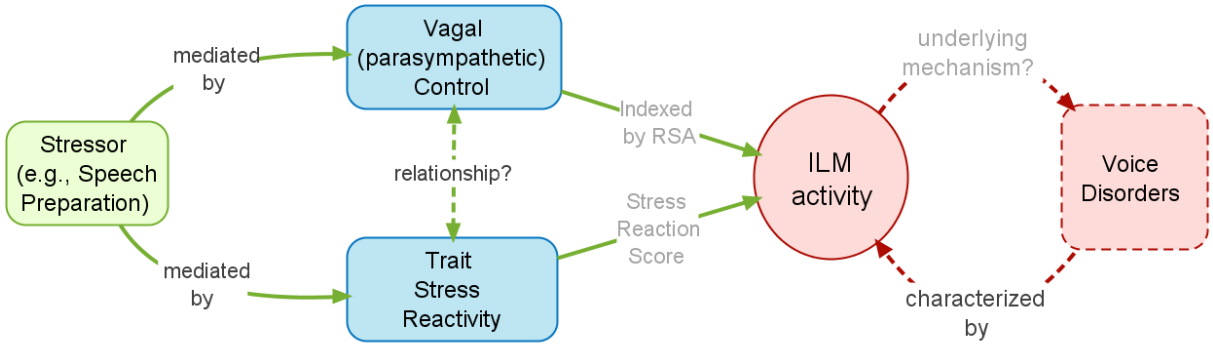


Figure 5-1. Research questions 1 & 2 schematic.

RQ1: Does human ILM activation [DV] change in response to a psychological stressor (i.e., speech preparation, IV), compared to a baseline condition? The hypothesis was that all ILMs and the positive control site (upper trapezius) would exhibit significant increases in activity during stressor exposure compared to baseline, and that the negative control site (anterior tibialis) would exhibit no change in activity.

RQ2a: Do *stress reaction scores* [IV1] predict [DV1] *magnitude of response* to the stressor [DV1] and *resolution latency* [DV2] following stressor exposure? The hypothesis was that higher values of trait stress reactivity, which is strongly related to neuroticism and anxiety, would predict greater magnitude of EMG activity and longer resolution latency, for each of the ILMs and the upper trapezius muscle, but not for the anterior tibialis muscle.

RQ2b: Does *respiratory sinus arrhythmia* [IV2] predict [DV1] *magnitude of response* [DV1] to the stressor and *resolution latency* [DV2] following stressor exposure? The hypothesis was that lower values of RSA, which indexes vagal control over the cardiovascular system during a stressor exposure, would predict greater EMG activity and longer resolution latency than higher RSA values, for each of the ILMs and the upper trapezius muscle, but not for the anterior tibialis muscle.

RQ2c: Are *stress reaction scores* [IV1] and *respiratory sinus arrhythmia* [IV2] significantly related to each other? The hypothesis was that the psychological measure (stress reaction score) would exhibit a weak negative correlation with the physiological measure (respiratory sinus arrhythmia).

5.2 RESEARCH QUESTION 3: A COMPETING HYPOTHESIS

As just discussed, one principal goal of the present study was to address whether an ILM response occurs during a psychological stress-inducing task. The logical assumption was that the resultant ILM activation may be a "stress response" of sorts. However, toward the aim of ultimately proposing a causal model for ILM activation during times of stress, other competing theories needed to be addressed. One competing theory that was considered as an alternative explanation for anticipated results from the study was related to the phenomenon known as *subvocalization*. Some describe subvocalization as low-grade activity of

speech muscles during silent reading and verbal thinking (Aarons, 1971), whereas more recently, the definition has evolved to refer to control processes that mediate a phonological representation of verbal material (most often written material in the context of reading) (Bosshardt, 1990). The more current definition of subvocalization, which is likened and related to Baddeley's articulatory loop concept (Bosshardt, 1990), was thought not to be highly relevant to the proposed study for a few reasons. First, subvocalization has been most widely studied in the context of reading, as it seems to facilitate reading proficiency and improve comprehension and recall of material (Aarons, 1971; Bosshardt, 1990; Laffey & Kelly, 1982). The proposed study did not involve an explicit reading task during the stressor. In addition, most research conducted on subvocalization has addressed it as a mediating reading strategy used within special populations, such as people who stutter or have dyslexia, or in children within the broader context of language and reading skill development, rather than in a normal adult population. The proposed study did not seek to examine individuals who have specific difficulties with speech or reading, and thus the healthy cohort in the present study was not be expected to exhibit stark subvocalization behaviors, during reading or otherwise. However, the lack of evidence for subvocalization at the level of the ILMs during reading or other linguistically-focused tasks might be merely a function of the fact that no one has sought to investigate—or has reported—the phenomenon.

Revisiting the earlier, more physiologic definition presented by Edfeldt (1960) Aarons (1971), which defined subvocalization (also called during that time "silent speech") as the presence of low-grade speech muscle activity/movement during reading or other forms of mental activity, other reasons arose for excluding subvocalization as a major theoretical (and thus, methodological) concern in the present study. Using surface electromyographic methods, Edfelt tested the hypothesis that reading an easy text results in less subvocalization than does the reading of a difficult text, and results supported that hypothesis. If a concern exists that even in the absence of reading, but in the presence of other demanding mental activity—for example, the speech preparation task used in this study, which was likely to be perceived as more "difficult" than a similar task expressly designed to be less stressful—the same finding would hold

true, then it was deemed reasonable to question whether subvocalization (i.e., versus ANS effects) might account for some of the ILM response observed in the present study. Thus, if subvocalization contributed to the ILM response in a stressful task by virtue of its linguistic underpinnings, the question remained regarding whether that phenomenon is either (a) a *meaningful component* of the *coordinated ILM stress response*, or (b) a separate and confounding variable. As discussed earlier, this study was not designed to interpret the degree to which the autonomic versus the somatic nervous systems contribute to the ILM stress response, but rather to identify whether a marker of ANS function (RSA) can predict ILM response.

Finally, one element of the preliminary study conducted by this author included two conditions designed to elucidate whether individuals subvocalized during supposedly non-stressful tasks with and without overt linguistic underpinnings (unpublished laboratory data). Participants' ILM activity was measured in 30 second periods during which participants (a) read an easy passage "under their breath" and then (b) counted backwards from 100 by 1 "under their breath." Participants were observed by the investigator during the tasks, but no effort was made to induce stress (via time pressure, reward/punishment, performance or accuracy judgment, *et cetera*) and participants' heart rate and blood pressure (physiologic markers of stress) remained steady and commensurate with baseline during the task. Contrary to what might be expected if subvocalization were strongly in play, the reading condition did not elicit greater ILM activity than the counting condition. In fact, no clear picture emerged with regard to ILM activity during either of these tasks. Participants' ILM activity non-uniformly increased, decreased or remained the same compared to baseline, with no apparent pattern within muscle groups, within subjects, or across subjects. Based on these findings, if subvocalization was involved in the ILM response, its effects appear to be random rather than systematic.

Taking the aforementioned points together, controlling for subvocalization in the present study did not appear to be vital based on empirical evidence. The fact remained that the extant literature contains no evidence that the ILMs are involved in subvocalization at all, and that this topic arose not because any others have proposed it as a competing theory for ILM activity in the face of a stressor, but

because logically it should be duly considered, as it has been. Nevertheless, the present study's design was sufficiently flexible to further explore this issue without compromising the fundamental goals established in the above discussion (see Research Questions 1, 2a, 2b, and 2c). Thus, the present study also explored the potential influence of subvocalization on the ILMs by examining ILM activity during a "true" at-rest baseline (*Baseline Rest*), as well as during a non-stressor, nonverbal task that involved linguistic processing (*Baseline Subvoc*). If any difference were found between these two tasks, it would be expected that the activity observed during the *Baseline Subvoc* would be greater than during the *Baseline Rest* task, and not the opposite. Thus, the *Baseline Subvoc* task served as the baseline against which the experimental stress condition (speech preparation task) was compared. Thus, to control for any confounding or contributing effects of subvocalization in the present study, the *Baseline Subvoc* task was be treated as the baseline measure to which data during experimental stressor exposure was compared, for the purposes of calculating the dependent variable *magnitude of change* (description forthcoming). This *a priori* decision might have yielded more conservative effect sizes and reduce the likelihood of statistically significant findings, but should sufficiently address any concern of subvocalization as a confounding/contributing variable. In the event that there were no statistically significant differences between the two baseline tasks, then the issue was moot and either task sufficed as a baseline.

The third, exploratory, research question related to subvocalization is schematized in Figure 2-2.

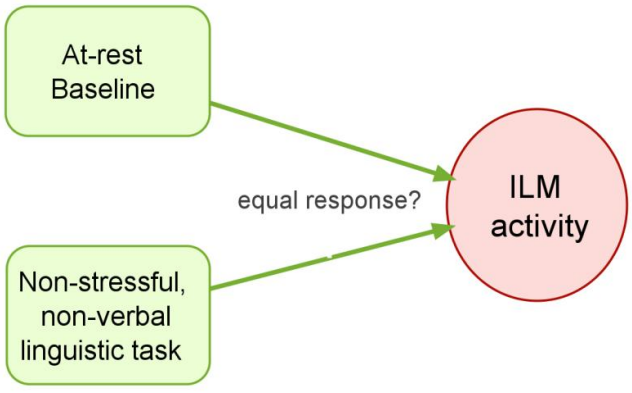


Figure 5-2. Research question 3 schematic.

RQ3: Does ILM activity differ during a non-stressful, nonverbal linguistic task as compared to "true" at-rest baseline requiring no linguistic processing? The hypothesis was that a statistically significant increase in muscle activity would be observed from the *Baseline Rest* to the *Baseline Subvoc* condition.

5.3 THEORETICAL CONSIDERATIONS AND CONCERNS

5.3.1 Roles of independent variables in theoretical model

Before proceeding with the description of experimental methods, several specific theoretical considerations and concerns should be addressed. First, the proposed roles of each IV in the larger model should be clarified. Autonomic function has been proposed as one possible mechanism for ILM response, by this author and others (e.g., Dietrich & Verdolini Abbott, 2012; Scherer, 1986). The proposed study will not definitively reveal whether observed ILM responses are due to ANS influences (i.e. versus effects of the somatic nervous system), although if ILM responses occur *in the absence of* anticipated [ANS-mediated] cardiovascular responses, this may weaken the argument for ANS involvement in ILM responses.

On the other hand, the other IV (trait stress reactivity) is not proposed as a mechanism *per se*. That is, given the overarching goal of better understanding mind-voice pathways in humans, trait stress reactivity represents one facet of the "mind" component, rather than an actual physical pathway mediating the mind-voice relationship. Instead, this IV is included in the proposed study because it represents a finer element of a broad personality construct—negative emotionality, broadly *vis-à-vis* introversion—that has been empirically shown to be germane to the development of primary muscle tension dysphonia

(Roy & Bless, 2000). The Trait Theory of Voice Disorder has examined personality at the top level, so to speak, parsing it into three broad domains: neuroticism, introversion and extraversion. Negative emotionality (comparable in certain ways to both introversion and neuroticism) seems to be causal in certain types of voice disorders such as primary muscle tension dysphonia, but almost all investigators in the field of psychology appreciate multiple subfactors of negative affect (for instance, Social Closeness, Alienation, Aggression). It is unlikely that all of these subfactors are equally relevant to the voice and disorders of the voice, and several investigators have proposed a special role of trait stress reactivity in so-called "functional" voice disorders. Thus, to help endorse and potentially move forward the Trait Theory of Voice Disorders, this particular element of personality will be examined for its mediating role in ILM response to a psychological stressor.

5.3.2 Alternative hypotheses and mechanisms of ILM response

Alternative mechanisms and explanations for ILM responses to the stressor in this study should be mentioned. Subvocalization was presented as a major competing or confounding factor for interpretation of findings from the present study. As described in Section 5.2, the potential contribution of subvocalization was be explored in the present study in an attempt to parse out its differential contribution to the ILM stress response.

Also previously noted, the ANS is the one potential mechanism theoretically touched upon in this study. However, Dietrich (2008) proposed that the neuroendocrine system may also play a role in laryngeal muscle hyperfunction via the mediating effects of the hypothalamic-pituitary-adrenomedullary (HPA) axis. It is unlikely that the HPA axis would be responsible for any effect on the ILMs in this study, as the HPA axis exerts its effects on the body quite slowly. Coordinated HPA responses are typically measured 20-60 minutes after the onset of stressor exposure (Herman et al., 2003), whereas in the proposed study, the ILM response was measured during a 3-minute stressor exposure and for 10

minutes following the exposure, which would not likely be sufficient time for the HPA response to be observed. Moreover, with regard to the fact that the present study used EMG-derived variables as outcome measures, there is no justifiable reason why the HPA axis might be responsible for ILM activity. The HPA axis seems to exert its effects via neuromodulating proteins throughout the laryngeal mucosa and epithelial lining (Hisa et al., 1999), and none of the extant literature details if or how the HPA axis directly impacts laryngeal muscle activity; presumably, it does not. It seems more likely that the HPA axis' role in laryngeal hyperfunction occurs with prolonged and recurrent exposure to and/or perception of stress, but not in situations such as those used in this study. Thus, potential involvement of the HPA axis in mediating results in the present study can be set aside with reasonable confidence.

5.3.3 Independence and collinearity of variables

A third concern of interest regarded the potential dependence of RQ2a and 2b on RQ1. That is, if no changes were observed in ILM activity during the stressor, it would have been impractical to explore the subsequent questions relating to whether ILM response can be predicted by trait stress reactivity and RSA. This concern was assuaged given several points of fact. Based on the preliminary study by Helou et al. (2013), which was described in greater detail in Section 2.1.3, it appeared that it would be extremely challenging to get zero ILM response to the stressor. As described previously, although they could not be easily interpreted, even the non-stressful task in the preliminary study yielded *some* significant findings. Furthermore, in that study, all five muscles were included in the analyses, whereas the present methods involved elimination of "redundant" muscles (i.e., those sampled bilaterally and exhibiting negative change or the least magnitude of change), thus increasing the likelihood that muscles exhibiting significant increases were the focus of analysis. In the preliminary study, the effect of the stressor on the ILMs was clear and compelling, even without eliminating the least or (non-) responsive muscles from analysis. Assuming the psychological stressor in the proposed study was as effective at inducing stress

(as measured via cardiovascular responses) as the physiological stressor in the preliminary study—and there was no reason to expect otherwise as the present study involved a widely used stress-inducing protocol—we expected the ILM response to be at least existent, if not especially provocative.

A fourth and final consideration was the degree to which the two primary IVs, trait stress reactivity and RSA, were related. This issue is generally relevant to the overall theoretical design of the study, and was specifically addressed in RQ2c. Whereas some studies lent support to the notion that certain personality traits (e.g., high trait hostility, high anxiety, depression) and RSA may be significantly correlated, a comparable number of other studies have not found support for such a relationship (see, for example, Beauchaine, 2001; Heponiemi, Keltikangas-Järvinen, Kettunen, Puttonen, & Ravaja, 2004; Keltikangas-Järvinen, Kettunen, Ravaja, & Näätänen, 1999; Thayer, Friedman, & Borkovec, 1996). A review of existing literature indicated that the relationship between the two specific independent variables of interest in the present study (trait stress reactivity and RSA) had not yet been investigated. Because both variables were expected to relate to the response to the speech preparation task, it was anticipated that a relationship would be detected, specifically in the negative direction (i.e., as stress reactivity scores increase, RSA measures decrease). Because the two variables were quite different in nature—one is a self-reported trait psychological measure and the other is a physiological measure—it was expected that any observed relationship would be quite weak.

6.0 METHODS

6.1 PARTICIPANTS

Healthy females between the ages of 18 and 30 years were recruited from the Pittsburgh metropolitan region. Power was calculated based on findings in the candidate's preliminary study (L. Helou et al., in press). Applying α=.05 and an anticipated moderate-to-large effect size for multiple regression (f^2 = .27), a sample size of 40 participants was required to achieve 80% power for research questions #2a and #2b, which are of particular empirical and theoretical focus (power was not calculated based on RQ1, as this question will principally be addressed using descriptive statistics and within-subjects analyses via interrupted time series analysis, which results in a separate *p* value for each participant.

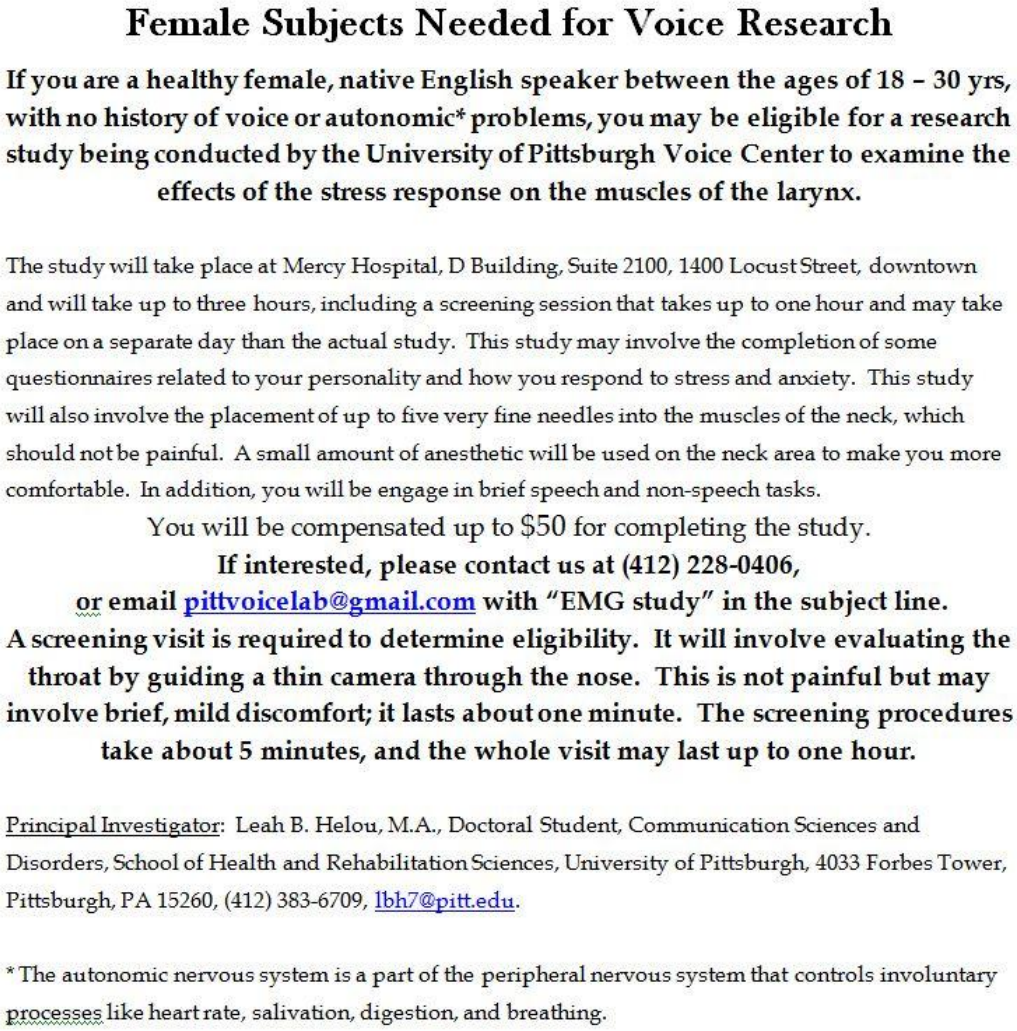
6.2 INCLUSION AND EXCLUSION CRITERIA

Exclusionary criteria by self-report was as follows: below 18 or above 30 years of age; frequent or high level of comfort with public speaking; pregnant; current lower or upper respiratory illness or seasonal allergies with respiratory manifestation; known allergy to local anesthetic medications such as Lidocaine®; history of: voice disorders; difficulty breathing or known respiratory disorders (e.g., obstructive lung diseases such as asthma or chronic obstructive pulmonary disease, restrictive lung disease); neck or throat surgery (e.g., thyroidectomy, parathyroidectomy, anterior cervical disc fusion,

tracheostomy, or other structurally invasive procedures); autonomic dysfunction or dysautonomia (e.g., postural orthostatic tachycardia syndrome, inappropriate sinus tachycardia, vasovagal syncope, neurocardiogenic syncope, orthostatic hypertension or hypotension); clinically diagnosed or suspected psychological disorders (e.g., depression, panic disorders, anxiety); asthma; blood clotting or coagulation disorders. Participants reported their height and weight, and those with body mass index at or above 31 (i.e., obese individuals) were excluded from participation because (1) obesity may impact respiration and (2) excessive fatty tissue in the neck may make it difficult to identify landmarks for hook wire electrode placement. Exclusionary criteria by clinical assessment during face-to-face screening included intolerance of laryngeal palpation and manipulation, and abnormal laryngeal structure or physiology as judged by a specialty laryngologist based on laryngoscopic imaging.

6.3 RECRUITMENT

Participants were recruited for this study using IRB-approved publicly posted flyers (Figure 6-1). The flyer was posted electronically via Craigslist (www.craigslist.com) as well as via the more traditional means of hanging the flyer on campus in businesses and University of Pittsburgh buildings.



Female Subjects Needed for Voice Research

If you are a healthy female, native English speaker between the ages of 18 – 30 yrs, with no history of voice or autonomic* problems, you may be eligible for a research study being conducted by the University of Pittsburgh Voice Center to examine the effects of the stress response on the muscles of the larynx.

The study will take place at Mercy Hospital, D Building, Suite 2100, 1400 Locust Street, downtown and will take up to three hours, including a screening session that takes up to one hour and may take place on a separate day than the actual study. This study may involve the completion of some questionnaires related to your personality and how you respond to stress and anxiety. This study will also involve the placement of up to five very fine needles into the muscles of the neck, which should not be painful. A small amount of anesthetic will be used on the neck area to make you more comfortable. In addition, you will be engage in brief speech and non-speech tasks.

You will be compensated up to $50 for completing the study.

If interested, please contact us at (412) 228-0406,
or email pittvoicelab@gmail.com with "EMG study" in the subject line.
A screening visit is required to determine eligibility. It will involve evaluating the throat by guiding a thin camera through the nose. This is not painful but may involve brief, mild discomfort; it lasts about one minute. The screening procedures take about 5 minutes, and the whole visit may last up to one hour.

Principal Investigator: Leah B. Helou, M.A., Doctoral Student, Communication Sciences and Disorders, School of Health and Rehabilitation Sciences, University of Pittsburgh, 4033 Forbes Tower, Pittsburgh, PA 15260, (412) 383-6709, lbh7@pitt.edu.

* The autonomic nervous system is a part of the peripheral nervous system that controls involuntary processes like heart rate, salivation, digestion, and breathing.

Figure 6-1. IRB-approved advertisement for experiment.

6.4 EXPERIMENTAL DESIGN

All research questions (RQs) were investigated using a single within-subject experimental design with multiple subjects. RQ1 was addressed by describing the direction, magnitude, and time course of ILM activity during stressor exposure compared to baseline and recovery phases. For RQ2a and RQ2b, respective independent variables (IVs) were **[IV1] Trait Stress Reaction score (SRscore)** derived from

the *Multidimensional Personality Questionnaire (MPQ),* and **[IV2] the change value of respiratory-corrected Respiratory Sinus Arrhythmia from True Baseline to the SPT (hereafter referred to as $\mathbf{RSA_{CORR\_DIFF}}$)**, a continuous variable derived from the heart rate. For both RQ2 and RQ3, dependent variables (DVs) were: **(DV1) magnitude of change**, as derived from the raw EMG waveform for TA/LCA, CT, and PCA muscles and the two control muscles, upper trapezius and anterior tibialis; and **(DV2) resolution latency**, which was defined as the time required for the activity in the same muscles to return to baseline following the experimental stressor. More detailed information regarding IV and DV calculation is included in Section 7.3 (Data Reduction).

6.5 EQUIPMENT

To screen for normal laryngeal anatomy and physiology, a flexible diagnostic nasendoscope (Olympus medical, Center Valley, PA) was used. In addition, an ambulatory blood and heart rate monitor (Omron Digital Blood Pressure Monitor, HEM 907-XL) was used to monitor heart rate and blood pressure online during the screening and experimental phases. This device provides readings every ~30 seconds.

Approximately 300 bipolar hook wire electrodes were constructed in-house (5 per participant x 40 participants + 100 extra electrode sets). For construction, custom-ordered bifilar 0.002-in diameter nylon-insulated annealed stainless steel wire (California Fine Wire Company, Grover Beach, CA) was wrapped around a custom template that allowed two 7 mm "windows" of exposure at each end. Wire was gently but securely wrapped around this template, and the wire was then stripped of insulation at each endpoint using an optic beam IE500 Laser Engraver (IEHK Technology Co. Ltd., Hong Kong). Figure 6-2 shows the wrapped and de-insulated wires.

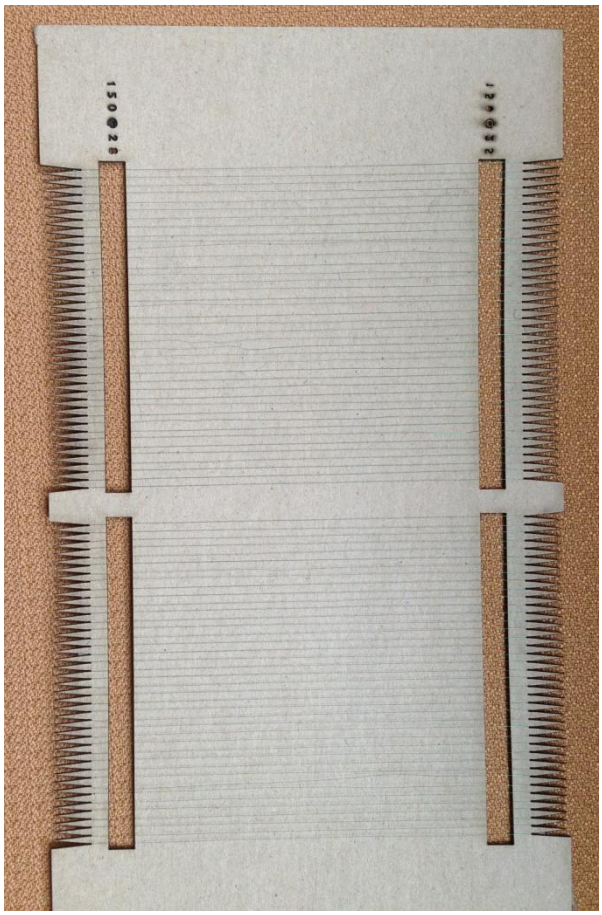


Figure 6-2. Wire template for electrode construction.

The wire was then cut off of the template one piece at a time, so that each cut wire was approximately 5" in length and was de-insulated at both ends. One end (the connector end) was cut so that the 7mm portion of de-insulated wire comprised that end, and the opposite tip (the hooked end) was folded over a thin piece of metal to create barbs of 1.1 and 1.6 mm, with only 1 mm of de-insulated wire remaining at that tip. This method of offsetting the de-insulated portions of each wire was implemented to prevent the occurrence of a short circuit. The bifilar wire was threaded through the lumen of a 1.5-in. 27 gauge hypodermic needle. Electrode sets were then packaged in groups of ten and gas sterilized. Figure 6-3 shows a hook-wire electrode constructed according to these methods. During data collection, the long de-

insulated section was used to couple each wire to the data acquisition system using a micrograbber, whereas the barbed tip end of the wires were implanted in the ILM of interest.

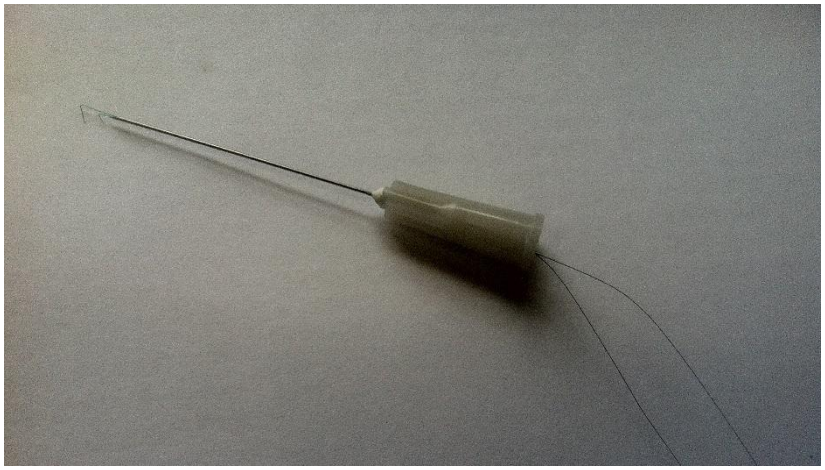


Figure 6-3. Completed hook-wire electrode.

Custom electrical wires were constructed for connecting the hook-wire electrodes to the equipment described subsequently. 1.5mm safety lead wires (Rochester Electro-Medical, Inc., Tampa, FL) were soldered to Micro 1-1/8" Smooth Clips (RadioShack®, Model 270-373; see Figure 6-4) and insulated at the point of connection. The clips were then covered in standard electrical heat-shrink tubing, which provided insulation while allowing for manipulation of the clips to position the hook-wire electrodes inside.

Figure 6-4. Micrograbber used for connecting electrodes.

The connector end of the hook-wire electrodes were coupled to a clinical multimedia EMG system (TECA Synergy 4.3, Oxford Instruments, UK) to facilitate electrode placement. Then, prior to data collection/recording periods, the hook-wire electrodes were coupled to two bridged 16-channel g.USB Biosignal Amplifiers and A/D Converters (Guger Technologies, Schiedelberg, Austria), where they remained for the duration of the experiment. To collect surface EMG and ECG data, 20 mm bipolar Ag/AgCl surface electrodes (Grass Technologies, Astro-Med, Inc., Warwick, RI) were used. Two Piezo Crystal respiratory effort transducers (Grass Technologies, Astro-Med, Inc., Warwick, RI) were used to measure respiratory rate and chest wall movement (representative of relative depth of breathing). The surface EMG and ECG electrodes and the respiratory effort transducers were also coupled to the g.USB amplifiers for data collection/recording.

A laptop computer (Dell Latitude E6420) with a 32-bit Vista Home Basic SP2 Operating System with 2.7 GHz Intel® Core processor, 4. 0 GB memory, and 250 GB 7200rpm hard drive was used for data acquisition. This laptop is equipped with BCI 2000 (Albany, NY, USA), which was the data acquisition software used in this experiment. EZ Air Plus (Biofeedback Federation of Europe) was used to provide biofeedback during the paced breathing tasks.

EMG data analysis was performed on a 64-bit desktop computer with a 3GHz Intel® Core™ 2 Duo Processor. This machine was equipped with Matlab 7.8.0 r2009a (MathWorks, Inc., Natick MA, USA) software programs, which was used for data analysis. Cardiovascular data analysis was performed on a desktop computer (HP Z210 Workstation with an Intel Core 3.4 GHz processor), which was equipped with MindWare 3.0.21 (Mindware Technologies LTD, Gahanna, OH, USA).

7.0 PROCEDURES

The study flowchart is presented in Figure 7-1. Items in bold correspond with specific stages or details of the screening and experimental sessions.

7.1 SCREENING PROCEDURES

Initial screening. As in the protocol in our IRB-approved preliminary study, individuals who responded to community flyers were directed to a secure **web screening** (*Stage I*, Figure 7-1) link via which they provided information relevant to the exclusionary criteria (details in Section 6.2). This web screening form is included in Appendix B. Within 24 hours of completion of the web screening, individuals were (a) notified of ineligibility, or (b) notified of eligibility and contacted to schedule a live screening.

Live screening. Eligible participants were invited to attend a live screening at the University of Pittsburgh Voice Center (*Stage II*, Figure 7-1). Prior to engaging in any screening procedures, inclusion/exclusion criteria was confirmed and informed consent was obtained. The consent form is presented in Appendix C. The full nature of the experimental conditions was partially disguised for reasons outlined subsequently. At the time of informed consent, participants were told that the following tasks would be involved in the experiment:

1. While you are lying back in an exam chair, we will place a blood pressure cuff on your arm, and non-invasive surface electrodes on your shoulder, chest, torso, and leg that will measure electrical

and movement activity of your body.

2. We will measure your blood pressure and heart rate for two minutes. We will also measure the other muscle activity via the non-invasive surface electrodes.
3. We will ask you to breathe at four different rates (i.e., a specific number of breaths per minute), for about two minutes per rate. You will get a short break between each condition, and the whole task will take about 15 minutes.
4. An Ear-Nose-Throat doctor (ENT) will place up to five fine wires (called fine wire electrodes) into your vocal muscles by guiding them through a thin needle into the neck area. The ENT may inject a small amount of lidocaine into your neck to make placement of the fine wire electrodes more comfortable for you. This will take 10-20 minutes.
5. After the fine wire electrodes have been placed, the investigator may verbally guide you through a relaxation task that should help you to relax. This will take 1-5 minutes.
6. While you are resting, we will record the electrical activity of your vocal muscles via the fine wire electrodes. You will not have to do anything during this period of time, which will last about two minutes.
7. Next, we will ask you to engage in a speech task for a few minutes.
8. Finally, you will rest for 15 minutes while we measure the electrical activity of your vocal muscles via the fine wire electrodes.

Next, **laryngeal examination** was performed using flexible endoscopy, for which the participant was positioned upright and provided local anesthetic (e.g. Cetacaine®) in both nasal passages in accordance with standard clinical care. The candidate has experience performing over ~750 flexible laryngoscopic exams since 2006, and is credentialed to perform flexible laryngoscopy for research purposes in the state of PA. The following tasks were performed under halogen light: sustained /i/ at comfortable pitch; high and low pitches achieved by glissando (register boundaries will be crossed) and sustained at each pitch extreme; sustained /i/ at quiet, comfortable and high loudness; rapidly alternating

nasal sniff and /i/ sound for five repetitions of each; the all-voiced sentence "We were wearing yellow ones." Laryngeal eligibility determination was based on the absence of structural and dynamic abnormalities. Normal laryngeal appearance was defined as no visible lesions, normal arytenoid dynamics on ab/adduction, normal vocal fold vibration during phonation, normal vocal fold shortening and lengthening with pitch changes, expected phonatory glottic closure, and the absence of apparent excessive laryngeal muscle hyperfunction.

Next, **laryngeal manipulation** was performed to ensure the individual's tolerance of the laryngeal rotation required to access the posterior cricoarytenoid muscle. Participants expressing discomfort or pain with laryngeal manipulation were deemed ineligible to participate. Also, participants with poorly identifiable landmarks were deemed ineligible to participate to help minimize discomfort and difficulty during fine wire electrode placement.

Finally, participants were directed back to the clinic's waiting area where they completed the paper-and-pencil ***Multidimensional Personality Questionnaire – Brief Form*** (*MPQ-BF*) in its entirety, according to the test instructions (Patrick et al., 2002).

7.2 EXPERIMENTAL PROCEDURES

7.2.1 Planned deception

The experimental task involved two principal elements of planned deception. Essentially, during the experiment, participants were led to believe that they would be expected to deliver a speech to a small group of judges, and they were not told about this stress-inducing task during the informed consent process. In reality, participants did not deliver a speech, although they did *prepare* a speech; this element of the planned deception will be discussed shortly. Participants were recruited and engaged in the study

under the belief that the goal of the study was to simply examine how the muscles that produce voice respond "during speech and non-speech tasks". The information that participants would be exposed to a stress-inducing speech preparation task was withheld at the time of informed consent. This helped to minimize anticipatory stress during the experimental session, and increased the likelihood that participants scoring throughout the full range of trait stress reactivity (IV1) were recruited into the study.

During the experimental session, to induce stress, participants were led to believe that they would deliver a speech (details in section 7.2.1). In reality, speech may confound the ILM_{EMG} data, and we did not wish to examine ILM functions during voice production *per se*. Rather, we were interested in the *activity of the ILMs* during a *moderately stressful non-speech task*. The expectation of having to deliver a public speech is stressful in and of itself (Kirschbaum et al., 1993), and to capitalize on this anticipation effect, it was deemed necessary to have participants believe that they would indeed prepare and deliver a speech to an audience. It was during this three-minute *speech preparation task* that the activity of the ILMs was recorded for subsequent analysis. Once the speech preparation task was completed, the participant was verbally debriefed in full (see script in Appendix D). This element of planned deception was not expected to cause harm or pain, or to cause greater stress than the participant was anticipating. On the contrary, it was expected that participants would be relieved to forego the actual speech delivery element of the experiment, and this was generally observed to be the case. More information regarding this task is contained subsequently in the section titled *Speech preparation task*.

7.2.2 Experimental Day

Scripted instructions for all experimental tasks are provided in Appendix D. The experiment took place in a quiet clinical procedure room at the UPMC Voice Center, and lasted from 90 to 120 minutes. All unnecessary electronic equipment and lights were turned off and unplugged (if possible) to minimize ambient electronic noise, and ambient temperature was maintained at 73.88°F on average. Room

temperature fluctuations within single experimental sessions were 0.43°F on average, and ranged from 0° to 2.80° (SD=0.45°). Upon arrival, participants were reclined at a ~120° angle in an exam chair, where they remained for the duration of the study, except during placement of fine wire electrodes (*stage V,* Figure 7-1) when they were reclined at ~170° angle. Participants were then fitted with the following equipment (see Equipment for each): (1) left arm cuff for intra-experimental measurement of average heart rate (HR), arterial systolic and diastolic blood pressure (SBP and DBP respectively) using an Omron Digital Monitor; (2) surface electromyography (SEMG) electrode positioned on the upper portion of the left upper trapezius muscle as a positive control site, hereafter referred to as $SEMG_{TPZ}$; (3) SEMG electrode positioned on the left anterior tibialis as a negative control site, hereafter referred to as $SEMG_{TIB}$; (4) surface electrocardiographic (ECG) electrodes to capture non-summated continuous HR for calculating RSA; (5) respiratory band to later inform the respiratory-corrected RSA index according to published methods (Egizio et al., 2011); and (6) ground and reference electrodes on the right olecranon (bony protrusion of the elbow) and the right earlobe, respectively.

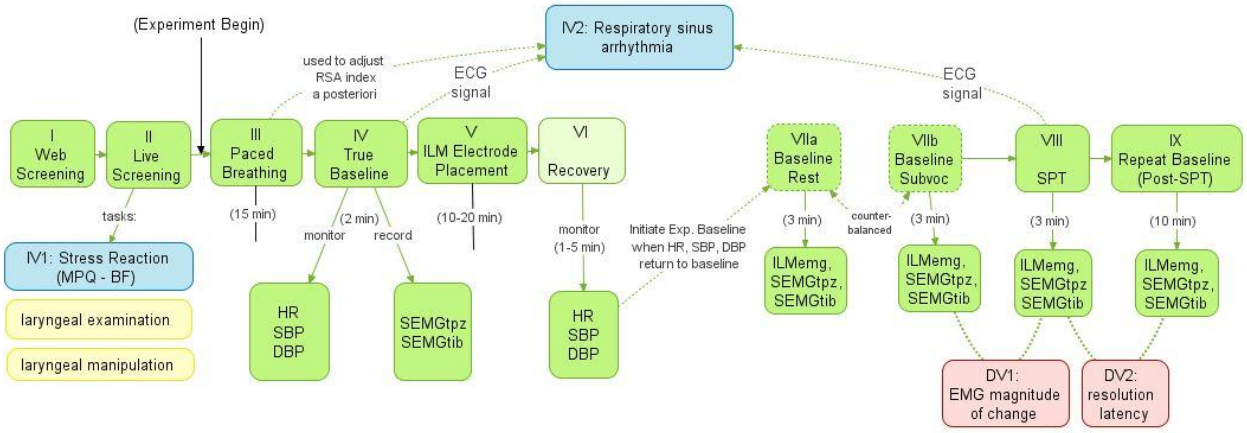


Figure 7-1. Study Flowchart.

Immediately following the fitting of this equipment, participants were presented with item 1 of Appendix E[12], which allowed them to quickly (in a matter of seconds) judge and rate the degree of stress and anxiety that they felt during the task just completed. Specifically, participants were instructed to mark a 100 mm visual analog scale at the area corresponding with their perceived stress and anxiety, where the leftmost point of the line (i.e., rating of zero) represented "not stressed/anxious at all" and the rightmost point of the line (i.e., rating of 100) represented "more stressed/anxious than ever." Participants were asked to rate their self-perceived stress and anxiety levels in the same manner immediately after conclusion of every subsequent Stage represented in the experimental flowchart, excluding Stage VI (Recovery) because it was essentially the same as the subsequent task into which it flowed (ILM Baseline, Stage VII) without announcement by the investigator. Participants' ratings served as supplemental (to the cardiovascular measures) information for *a posteriori* validation that participants' states were modified as expected across tasks, but will not be included in any analyses.

Next, participants underwent a paced breathing task (*stage III*, Figure 7-1) according to published methods (Egizio et al., 2011). This procedure was implemented for the purpose of later controlling for the confounding effects of respiratory rate and tidal volume on RSA values. A respiratory band measured pressure changes due to expansion and contraction of the thoracic cavity with breathing. The respiratory band was situated somewhere between ribs 5-8, at the point where maximal expansion was observed during inhalation. After a brief (~1 minute) practice trial, participants were paced across four breathing conditions (8, 10.5, 13, and 18 breaths/min) for 2 minutes per condition, using audiovisual cues provided by a computer software program (EZ Air Plus 1.0, 2009, Biofeedback Foundation of Europe). Breaks of approximately two minutes were given between each set of breathing conditions. ECG and respiratory

[12] Appendix E was developed as a supplemental measure (i.e., not as a key outcome variable) for the purposes of this study based on similar models developed and presented by other investigators (e.g., Willmann, Langlet, Hainaut, & Bolmont, 2012). Alternative standardized measures such as the 20- item state anxiety subscale of the State-Trait Anxiety Inventory (Spielberger, Gorusch, Lushene, Vagg, & Jacobs, 1983) or the 60-item Positive and Negative Affect Schedule (Watson & Clark, 1994) would be too lengthy to incorporate after each stage of the study.

band-derived signals were simultaneously recorded for later analysis. Immediately following this task, participants were presented with item 2 of Appendix E, which required them to rate the degree of stress and anxiety that they felt during the task just completed.

Next, the participant remained at rest for 120 sec while attending to emotionally neutral video stimuli (Van Emden, 2011), to establish baseline values (*stage IV*, Figure 7-1) as follows: (1) ECG and SEMG signals were continuously recorded and stored for later analysis, as described shortly; and (2) HR, SBP, and DBP values were automatically calculated and manually recorded by a research assistant using the Omron Digital Monitor (see Equipment) every ~30 seconds during all tasks so that the experimenter could monitor cardiovascular return-to-baseline throughout the study. Immediately following this task, participants were presented with item 3 of Appendix E, which required them to rate the degree of stress and anxiety that they felt during the task just completed.

Next, in preparation for ILM_{EMG} electrode placement (*stage V in* Figure 7-1), a board-certified laryngologist administered a superficial injection of 1-2 cc of 1% lidocaine subcutaneously over the cricothyroid membrane. Then, using audiovisual guidance via a clinical EMG system (TECA Synergy 4.3), hook-wire electrodes (see Equipment) were inserted into the right posterior cricoarytenoid (PCA), bilateral thyroarytenoid/lateral cricoarytenoid (TA/LCA) complex, and bilateral cricothyroid (CT)—thus, three muscle groups of interest, and five individual EMG channels—according to previously published methods (Munin, Rosen, & Zullo, 2003). Laryngeal rotation (~15-20°) was required for PCA electrode placement. After one electrode is seated in a PCA muscle, rotating the larynx again to place a contralateral electrode would risk dislocation of the first electrode and may cause pain. Thus, PCA electrodes were placed unilaterally for all participants, and to accommodate the physician's handedness, on the right side. Accuracy of electrode placement was verified online, visually and auditorily, using three consecutive sniff (PCA), valsalva (TA/LCA), and pitch glide (CT) tasks for each muscle group.

It should be noted that different bellies exist in the laryngeal muscles of interest, and there was no way of knowing exactly in which belly the electrode was situated. For instance, the PCA muscle has a

lateral vertically-directed belly that is thought to be the principal abducting bundle of the PCA, and a medial fan-shaped belly that is thought to stabilize and fix the arytenoids (Zemlin, 1998). Although it is possible that an electrode was situated in the medial belly, it is highly unlikely since (a) it is deeper to the surface of the neck than the lateral belly, and (b) placement of the PCA electrode was deemed accurate based on sniff tasks, which would activate the lateral vertical bundle of the PCA to a far greater degree than the medial bundle. It is perhaps more likely that placement of electrodes within the TA/LCA complex varied within and across participants. The TA and LCA muscles are in close physical proximity and serve the same functions, thus it was not possible to confirm exactly in which muscle, or which place in the muscle, an electrode was situated (Zemlin, 1998). Thus, discrepant findings in the present study with respect to this muscle complex could be due to unappreciated electrode placement differences. Likewise, with regard to the CT muscle, two bellies exist—the pars recta and pars oblique—thus contributing to the same potential confound as may occur in the TA/LCA complex (Zemlin, 1998).

Once electrode placement was verified for each muscle, the micrograbbers coupling the electrodes to the clinical EMG system were removed, the hook wire electrodes proximally secured to the subject's neck with tape, and the distal end of the wires reattached to the micrograbbers which were then coupled to two 16-channel g.USB Biosignal Amplifiers (see Equipment). Figure 7-2 shows a participant with all electrodes placed according to the methods just stated. Immediately following this task, participants were presented with item 4 of Appendix E, which requires them to judge the degree of stress and anxiety that they felt during the task just completed.

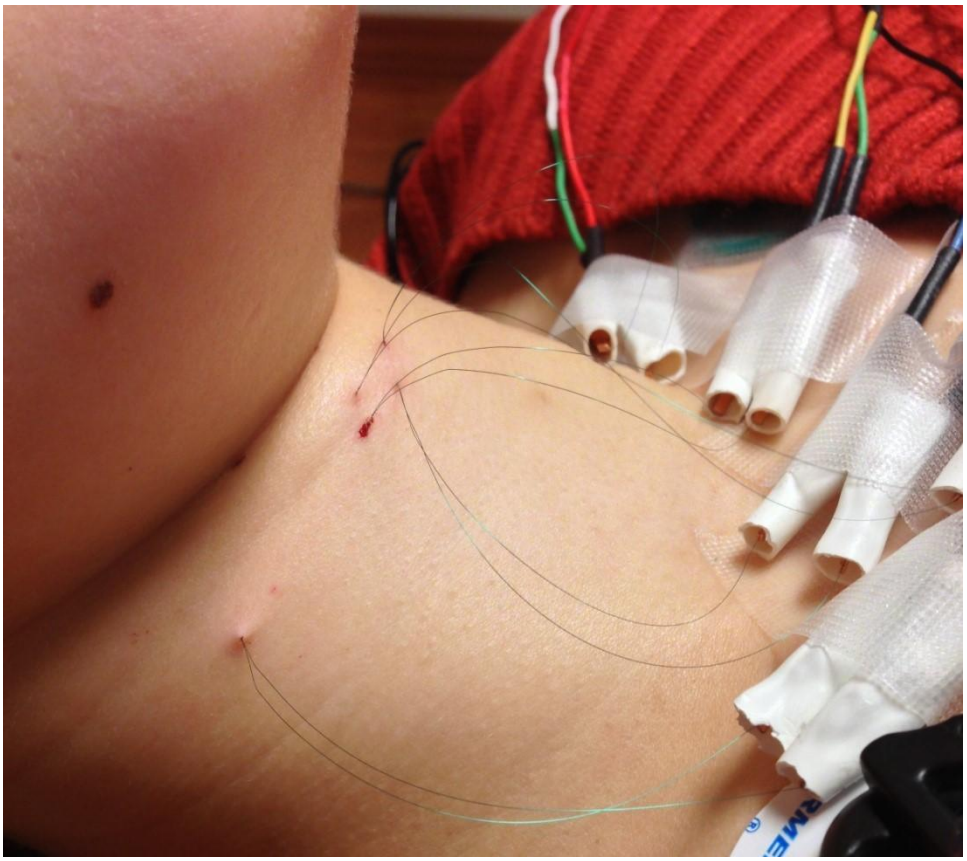


Figure 7-2. Photo of fine wire electrodes in the intrinsic laryngeal muscles.

Verification tasks were repeated and recorded at that time to allow *a posteriori* determination that the electrode uncoupling and recoupling procedures did not disrupt electrode placement (see section titled 'Verifying Electrode Placement'). Verification of placement of the control sites were performed similarly—once during initial placement and then again during the recording epoch following ILM verification tasks—for the control sites, using shoulder elevation (upper trapezius) and foot dorsiflexion (anterior tibialis) tasks. All ECG, ILM_{EMG} and SEMG signals were digitized using a sampling rate of 9600 samples/s/channel using the g.USBamp Biosignal Amplifiers and BCI 2000 acquisition software (see Equipment), and no online filtering was applied during data acquisition. Data were saved to laptop (see Equipment). Following placement of ILM electrodes and placement verification procedures, HR and

BP were monitored during recovery from ILM electrode placement (*stage VI*, Figure 7-1) until values returned to baseline per standard research protocol (Christenfeld, Glynn, & Gerin, 2000).

When return to baseline was verified (HR, SBP, DBP), two experimental baseline (post-ILM placement) tasks were completed. The participants engaged in a "true" at-rest baseline task, during which the participant remained at rest while observing emotionally neutral audio-visual stimuli (e.g., Van Emden, 2011) for three minutes. This task is hereafter referred to as *Baseline Rest* (*stage VIIa*, Figure 7-1). Halfway through the three-minute task period, participants were verbally encouraged by the investigator to maintain attention to the video stimulus. In addition, participants engaged in a non-stressful, non-verbal baseline task during which they completed a task requiring linguistic processing, hereafter referred to as *Baseline Subvoc* (stage VIIb). This task was designed to be as parallel as possible to the experimental stressor (speech preparation task), without actually causing stress or risking attenuation of the stress response triggered by the experimental stressor (e.g., due to practice effects). For this task, participants were prompted to "imagine a small group of people with whom you are very comfortable and at ease, and to imagine that you are talking with these people about your dream job." Participants were presented with short, bulleted, written prompts to imagine themselves describing (1) what their dream job entails, (2) the "who, what, where, and when" of this dream job, and (3) what they will accomplish in this dream job. Halfway through the three-minute task period, participants were verbally encouraged by the investigator to maintain attention to/engagement in the imaginative task, but otherwise the investigator did not directly observe or address the participant, in an attempt to avoid stress induction. The script for this task is available in Appendix D. The order of these two tasks—*Baseline Rest* and *Baseline Subvoc*—was counterbalanced such that the first 20 participants engaged in the *Baseline Rest* task first, and the second 20 participants engaged in the *Baseline Subvoc* task first. Activity was sampled across all recording channels for the duration of both baseline tasks. Immediately following each of these baseline tasks, participants were presented with items 5 and 6 of Appendix E, which required them to judge the degree of stress and anxiety that they felt during the task just completed.

Next, the speech preparation task (*SPT*; *stage VIII*, Figure 7-1) commenced, lasting for three minutes while ECG, ILM_{EMG} and SEMG data were continuously sampled. The next section provides details regarding the *SPT*. Immediately following this task, participants were presented with item 7 of Appendix E, which required them to judge the degree of stress and anxiety that they felt during the task just completed. After the *SPT*, the participant were informed of the planned deception related to the *SPT* (i.e., debriefed), and instructed to relax for the next 15 minutes before ceasing the experiment. Repeat baseline data from ECG, ILM_{EMG} and SEMG channels (*stage IX*, Figure 7-1) were collected while the participant rested for 10 minutes. As during the baseline conditions, participants were provided emotionally neutral audio-visual stimuli and were instructed to attend to them (e.g., Van Emden, 2011). Participants were instructed to rest, relax, and remain as motionless as possible during this phase. Immediately following this task, participants were presented with item 8 of Appendix E, which required them to judge the degree of stress and anxiety that they felt during the task just completed.

Finally, all participants ended the experimental session with three repeated trials each of sniff, valsalva, and pitch glides for final verification of accurate placement of ILM_{EMG} electrodes. Electrodes substantially compromised or lost during the experiment were identified based on these tasks and excluded from analysis, as described next. All experimental equipment was removed from the participant's body, thus concluding the experimental session. Participants completed a reimbursement form and were then guided out of the experimental room.

7.2.3 Speech preparation task

The SPT was generally modeled on the Trier Social Stress Test (TSST), which is a well-established experimental protocol to induce moderate psychosocial stress that yields significant increases in cardiovascular parameters and subjective stress ratings (Kirschbaum et al., 1993). During the speech preparation portion of the TSST, heart rate responses (beats per minute) spike significantly. The SPT in

the proposed study was similar to the TSST in the following ways: participants were observed by confederates of both sexes; participants were [made to believe they were being] video recorded during and that the videos would be subsequently viewed by people specially trained to monitor nonverbal behavior; participants were given time to outline their talks and were not allowed to speak during the preparation phase. The SPT in the proposed study differs from the TSST in the following ways: participants were [supposedly] observed directly during the preparation phase, rather than alone in a separate room; no actual speech presentation phase was involved; the speech preparation phase lasted three rather than ten minutes; no pencil or paper was provided for participants to outline their talk; the confederates were presented to the participants via supposed web-based video streaming rather than live.

As just described, subjects were led to expect a videotaped speaking task before a web-based audience of four confederates, and were given three minutes to prepare for the speech. During the SPT, pre-recorded videos were shown to subjects, who as noted were led to believe the videos were actual people interacting in real time from their own office. The confederates were sex-balanced because the effects of panel sex composition in the TSST is known to influence the physiological stress responses in women (Duchesne et al., 2012). In the videos, two confederates (sex-balanced) adopted attentive but neutral expressions for the duration of the task (Kirschbaum et al., 1993). An additional two confederates (sex-balanced) intermittently expressed somewhat more non-accepting and critical facial expressions (i.e., non-smiling, impatient, "stone-faced") during the task (Gruenewald, Kemeny, Aziz, & Fahey, 2004).

Confederates were positionally counterbalanced by sex as well (see screen shot,

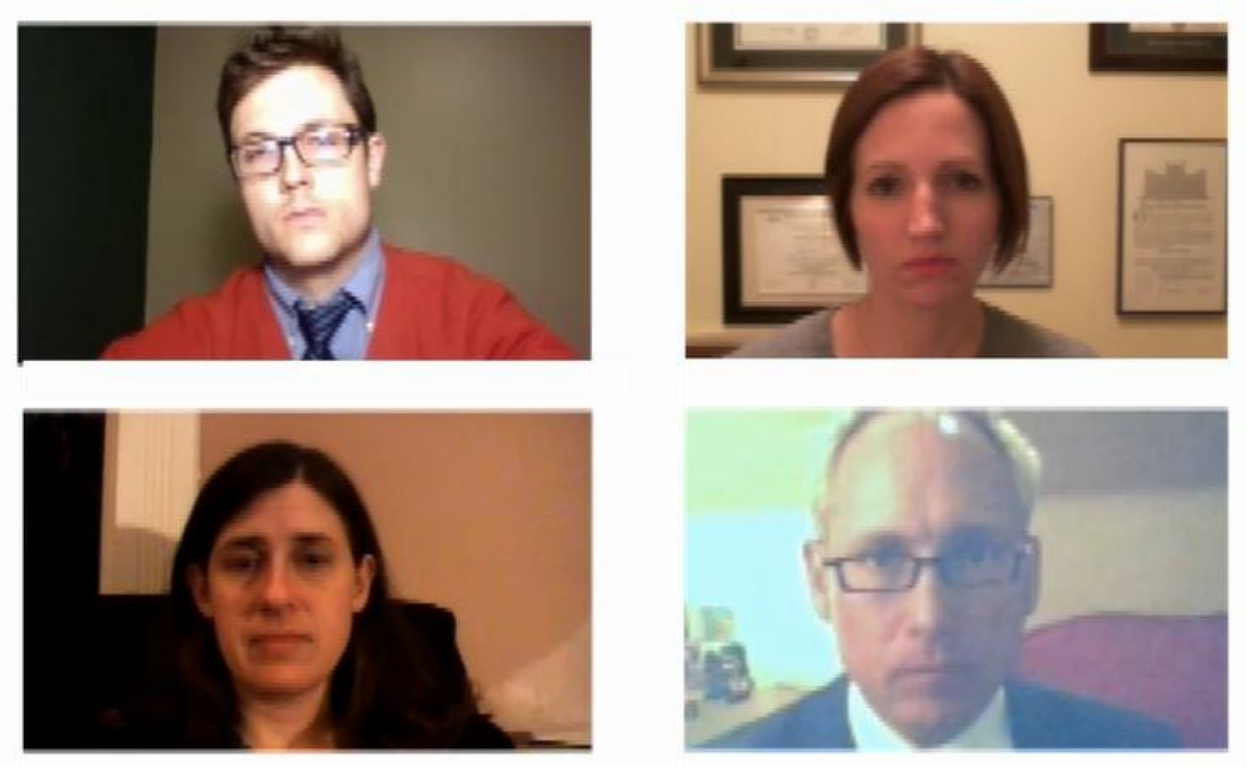


Figure 7-3). Prior to use in this experiment, the video was shown to other members of the general public for verification that confederates' expressions were conveyed in the intended manner.

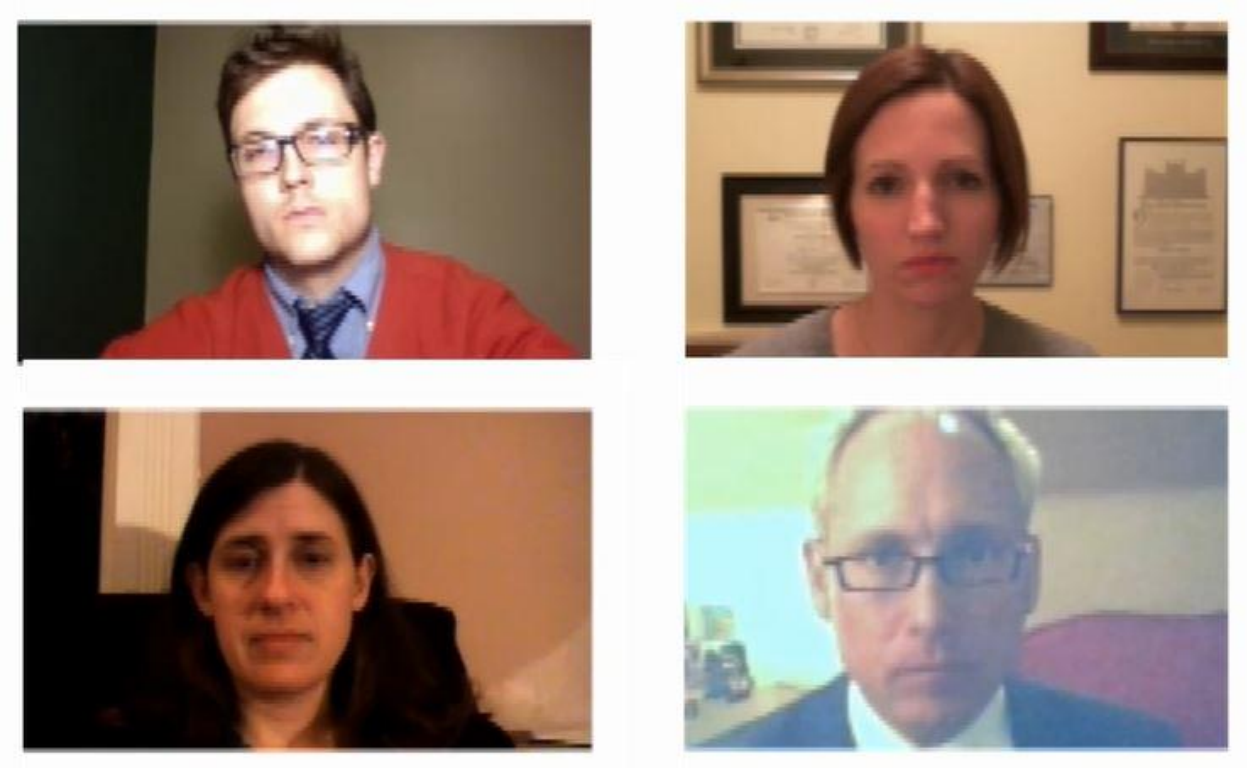


Figure 7-3. Screenshot of confederates for speech preparation task.

Participants were told that these confederates were professionals who specialize in nonverbal communication, and that all of these professionals would be evaluating participants' speech performance and ability to communicate ideas successfully in a social situation. Two or three investigators were also present in the room during the SPT. One investigator played the role of videographer and trained the camera directly on the participant while standing directly next to her, and remaining investigators sat quietly to the side of the participant, maintaining a neutral expression throughout the task. Table 1 further clarifies the differences between what participants were led to expect and what actually occurred before, during, and after the SPT.

Table 7-1. Elements of Deception and Reality During Public Speech Preparation Task

Deception	Reality
Participants were led to expect that they must deliver an impromptu video-recorded speech to **four professionals** (two male, two female) who would be **observing via web-based streaming video**. Participants were told that they must deliver the speech while looking directly into the camera, and can therefore practice looking at it during preparation if they want.	The professionals were not live-streamed; rather, a **pre-recorded video of four confederates** was presented to the participants via laptop computer. The professionals directly "observed" the participants throughout the task.
The expectation was set that **participants would be video recorded** during the preparation task, and that the video may be selected for later review by a class of undergraduates learning to score the participants' behaviors in the same way as the four professionals were scoring the participants' behaviors.	An investigator trained a video camera on the participant, but **participants were not recorded**, and no element of the participants' data or likeness was presented subsequently to undergraduates.
Participants were asked to prepare a speech as a component of a job interview. They were [allegedly] expected to **talk continuously for five minutes** while being observed by the four professionals. Participants were presented with a list of three items they must address during their speech: 1) Present three of your best and worst characteristics. 2) Use math to make a case for how much this job is worth to you (i.e., how much you expect to be paid). Factor in how much you have spent to date on education, travel and living expenses, and any other relevant financial details. 3) Describe your goals for the future in the form of a "five-year plan".	As stated above, ultimately **participants did not deliver a speech**.

7.3 DATA REDUCTION

7.3.1 File preparation

Data reduction and analysis described herein was performed using Matlab 7.8.0. (R009a). A 10 Hz high-pass filter was applied to all EMG channels in order to remove drift and offset. Notch filters of 60, 120,

and 180 Hz were applied to all data channels, and all data channels were full-wave rectified for analysis. For RSA_{CORR_DIFF} processing, *True Baseline* and *SPT* data files were (1) duplicated; (2) filtered as above and downsampled to 1000 Hz; (3) modified so that only the ECG and respiratory band channels remained; and (4) converted to two-channel (ECG and respiratory band) comma delimited text files.

7.3.2 Obtaining IV values

The first IV, the **Stress Reaction subscore (SRscore) of the *MPQ-BF*** (Tellegen, 1995), was calculated for each participant according to test instructions and using a custom SPSS script provided by the authors. Research permissions for using this measure in the proposed study were obtained from the University of Minnesota Press and are presented in Appendix F.

For the second IV, respiratory-corrected values of **respiratory sinus arrhythmia (RSA_{CORR_DIFF}), t**he two-channel files calculated for the *True Baseline* and *SPT* epochs were loaded into MindWare, which automatically marked each QRS peak in the ECG waveform and calculated output variables. ECG data were inspected to ensure that the software had correctly marked each QRS peak, and any errors were corrected manually. Respiratory band data were also inspected to ensure that the program's automatic calculation matched the number of breaths reflected in the waveform. Full reports with the software's standard output were saved to Excel file for each participant.

Corrections were then applied for the confounding influences of respiratory rate and depth (tidal volume), according to previously utilized methods (Egizio et al., 2011; Jain et al., 2011). In short, the spectral power of the 0.15-0.40 Hz frequency band (hereafter referred to just as RSA) using Fast Fourier Transform on inter-beat intervals was obtained from the MindWare output. To correct for the effects of respiration, (1) average respiratory cycle length in seconds was calculated for each task by dividing the total time of the task (~3 minutes) by the respiration rate during that period; and (2) separate within-subject regressions were calculated regressing RSA on average respiratory rate (Egizio et al., 2011); (3)

the regression line of RSA on respiratory variables was then utilized to estimate the task-related changes in RSA that were systematically lower or higher than anticipated values (i.e., respectively reflecting cardiac vagal decline or augmentation) (Grossman & Taylor, 2007). A somewhat arbitrary R^2 value of .70 was set, and all participants' regression equations with R^2 of .70 or higher were not inspected further.

7.3.3 Obtaining DV values

To obtain values for each DV, the following data reduction procedures were performed for signals from each ILM and the two control sites. First, the magnitude of ILM/trapezius/tibialis activity change (from baseline) was calculated. Recall that two baseline epochs were obtained in this study—*Baseline Rest* and *Baseline Subvoc*—and that for the primary research question(s) relating to the predictive roles of trait stress reactivity and RSA, the *Baseline Subvoc* epoch served as the baseline to which the experimental condition was compared (see Section 1.1.4). The *Baseline Subvoc value* was represented by calculating the mean of the entire *Baseline Subvoc* 120-sec task epoch. The *EMG value during SPT* was calculated in the same fashion. Values representing **magnitude of ILM/trapezius/tibialis activity change** were recorded as the absolute difference from *Baseline Subvoc* to *SPT* for EMG values. These values were used as the dependent variable for regression models. However, the effect sizes for these magnitudes of change were also calculated and used to represent magnitude of change for across-participants comparisons (e.g., analyses of variance). When pooling data across all participants, effect sizes are more appropriate and meaningful than absolute difference values.

Because the TA/LCA and CT muscles were sampled bilaterally to protect against loss of data in the event of electrode displacement, the muscle displaying the greatest change from baseline in absolute value was included for analysis in the regression model. Hence, data from three ILMs were included in separate statistical analyses for each subject: PCA, one TA/LCA complex (left or right), and CT (left or

right). In addition, only data from these three muscles were included in the resolution latency analysis, described next.

In essence, **resolution latency** was represented by time required for the Repeat Baseline signal to "be the same as" the *Baseline Rest condition*, for the ILMs and the two control sites. To calculate resolution latency, within-subjects analysis of amounts and amplitudes of muscle activation per unit/time via interrupted time-series analysis (ITSA) were performed using the ARIMA model 2 in Matlab. ITSA is intended to identify whether an event (e.g., *Repeat Baseline* task) is associated with the time-series pattern present in observations prior to the event (e.g., *Baseline Rest*). ITSA essentially estimates the amount of autocorrelated data in each set of data, subtracts the autocorrelated data from the raw data, and performs a *t*-test on the remaining non-autocorrelated data (Crosbie, 1993). ITSA was performed to compare the entire *Baseline Rest* signal to 30-sec rolling windows of *Repeat Baseline* data. Rolling windows shifted in 5-sec increments. The time point at which *three subsequent* windows of Repeat Baseline data exhibited a non-statistically significant value as compared to the *Baseline Rest* condition (i.e., ILM/trapezius/tibialis activity returns to baseline) was deemed the resolution latency. All DV values were obtained using custom Matlab scripts.

7.4 BLINDED VERIFICATION OF ELECTRODE PLACEMENT

EMG waveforms corresponding with pre- and post-experimental laryngeal electrode placement verification tasks (PCA, sniff; TA/LCA, valsalva; CT, pitch glide) were visually assessed *a posteriori* by the first author. Pre- and post-experimental verification files were individually prepared and assessed as follows: (1) a screen shot of raw EMG waveforms during each respective verification task of interest was obtained; (2) the screen shot was pasted into a PowerPoint document; (3) once all images were thus prepared and saved, the first author visually reviewed screenshots. All channels were inspected, and the

first author documented which channel(s) exhibited clearest activity representing repetitions of sniff, valsalva, or pitch glide. If no clear muscle activity was evident near the period of time that the keystroke was recorded, data from that channel were excluded from all analyses. Figure 7-4 shows the voluntary contraction tasks by channel for one participant. "R" indicates that the participant was engaging in the "sniff" task; "T" corresponds to the valsalva task; "I" corresponds to the sustained /i/ task; and "Y" corresponds to the pitch glide task. In this particular subject's voluntary contraction trials, all channels were judged to have viable signals, except for the LCT channel which was omitted from analysis.

The same level of methodological rigor was not necessary for the $SEMG_{TPZ}$ and $SEMG_{TIB}$ channels, as accurate electrode placement is substantially easier to verify during the experiment compared to intrinsic laryngeal muscle electrode placement. The SEMG signals of such large muscle groups were also much easier to monitor and troubleshoot online during data collection, whereas the ILM activity could be readily monitored during data collection and thus must be verified *a posteriori* according to the above methods. $SEMG_{TPZ}$ and $SEMG_{TIB}$ waveforms were reviewed cursorily and all exhibited clear activation during the appropriate/corresponding voluntary contraction task.

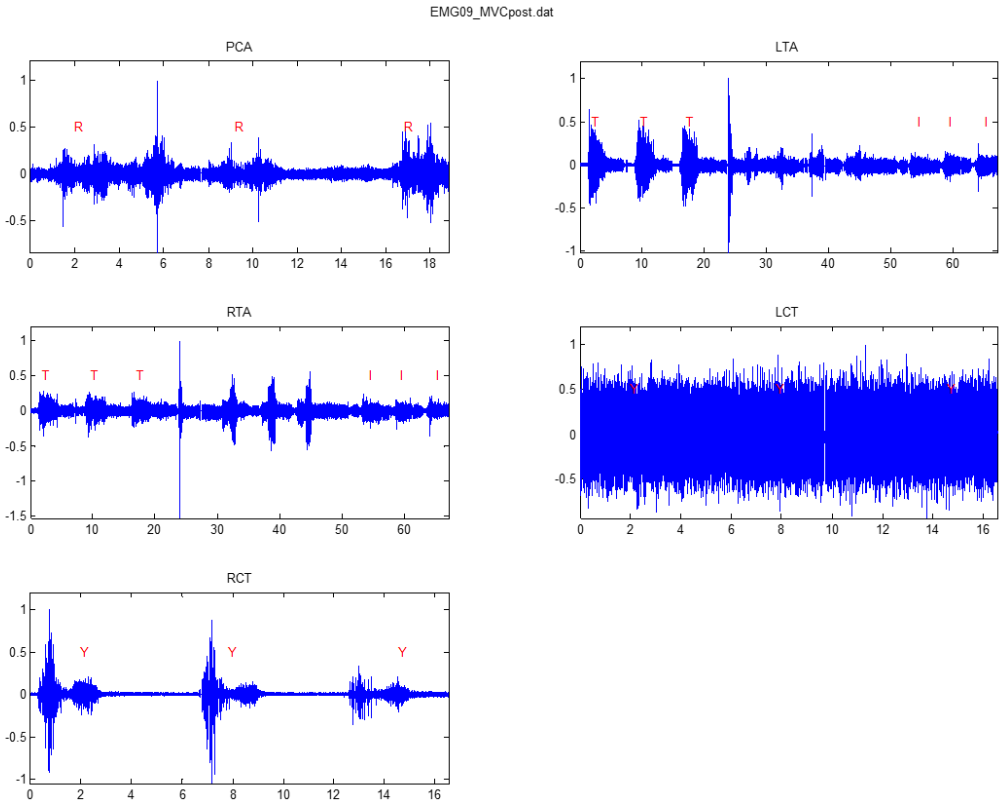


Figure 7-4. EMG tracing of ILM activity during voluntary contraction tasks relevant to each muscle. Representative of current data set.

7.5 STATISTICAL ANALYSES

Data were exported from Matlab and statistical analyses was performed using SPSS 21.0. The RQs—presented in Section 5.0 in order of theoretical and conceptual importance—are herein presented in order of statistical examination. The RQs, hypotheses, and basic statistical approach are contained in Table 7-2 for reference.

Table 7-2. Summary of Research Questions

RQ	Research Question	Hypothesis	General statistical approach
1	Do human ILMs change activation level during a psychological stressor (i.e., speech preparation)?	All ILMs and the positive control site (upper trapezius) will exhibit significant increases in activity during stressor exposure compared to the Baseline Subvoc condition, and the negative control site (anterior tibialis) will exhibit no change in activity	• Descriptive statistics • ITSA
2a	Do [IV1] *stress reaction scores* predict [DV1] *magnitude of response* to the stressor and [DV2] *resolution latency* following stressor exposure?	Higher values of trait stress reactivity, which is strongly related to neuroticism and anxiety, will predict greater magnitude of EMG activity and longer resolution latency during the *SPT* as compared to *Baseline Subvoc*, for each of the three ILMs and the upper trapezius muscle, but not for the anterior tibialis muscle	• Simultaneous multiple regression (i.e., one equation with two DVs and two IVs) will be performed for [DV1] *magnitude of ILM activity change* and [DV2] *resolution latency* predicted by [IV1, RQ2a] *stress reaction score on the MPQ-BF* and [IV2, RQ2b] *RSA, respiratory sinus arrhythmia.*
2b	Does [IV2] *respiratory sinus arrhythmia* predict [DV1] *magnitude of response* to the stressor and [DV2] *resolution latency* following stressor exposure?	Lower values of RSA_{CORR_DIFF}, which indexes vagal control over the cardiovascular system during a stressor, will predict greater EMG activity and longer resolution latency during the *SPT* as compared to *Baseline Subvoc*, for each of the three ILMs and the upper trapezius muscle, but not for the anterior tibialis muscle.	
2c	Are [IV1] *stress reaction scores* and [IV2] *respiratory sinus arrhythmia* significantly related to each other?	The psychological measure (stress reaction score) will exhibit a weak negative correlation with the physiological measure (respiratory sinus arrhythmia).	• Correlational analyses • Assess for presence of quadratic and curvilinear relationships
3	Does ILM activity differ during a non-stressful, nonverbal linguistic task as compared to at-rest baseline requiring no linguistic processing?	A statistically significant x-fold increase will be observed from an at-rest baseline (*Baseline Rest*) to the "subvocalization" condition (*Baseline Subvoc*)	• Descriptive statistics • ITSA

General descriptive statistics and—when appropriate—repeated measures analyses of variance were calculated for participant demographics, trait stress reactivity data, cardiovascular values by experimental

stage, and perceived stress and anxiety by experimental stage. Magnitude of change was calculated as specified previously, and either effect sizes or absolute differences were used in descriptive statistics, analyses of variance, and regression analyses.

The second dependent variable, Resolution Latency, was calculated as planned and found to have extremely poor distribution and thus inappropriate to include as a variable in regression analyses. Specifically, for all muscles in the preponderance of participants, activity had returned to baseline by the beginning of the 10-minute BLrpt task. In fact, for any given muscle including the positive and negative control muscles, no more than one participant took longer than the first 30 seconds of the BLrpt task to return to her baseline level of muscle activity. Visual inspection of waveforms during each task confirmed that the ITSA output was accurate. Figure 7-5 shows an example of a "typical" participant's (EMG60[13], TA muscle) waveforms comparing *True Baseline* (red signal) and [the first three minutes of] the *Repeat Baseline* (blue signal) epochs. It is clear that both signals are comparable in terms of average amplitude and relative size and number of spikes throughout the sample. From the end of the *SPT* stressor to the beginning of the BLrpt task, approximately three to five minutes passed while the participant was debriefed by the investigator, the second set of voluntary contraction tasks was performed, and the staging for the *Baseline Repeat* task was prepared. Presumably it was during this period, when no physiological recording was conducted, that muscles resumed their baseline activity levels. Because of the poor distribution of this dependent variable, Resolution Latency was not included in the regression analyses described herein.

[13] EMG60 demonstrated statistically significant increases in activity of all muscles during the *SPT* compared to the *Baseline Subvoc* task.

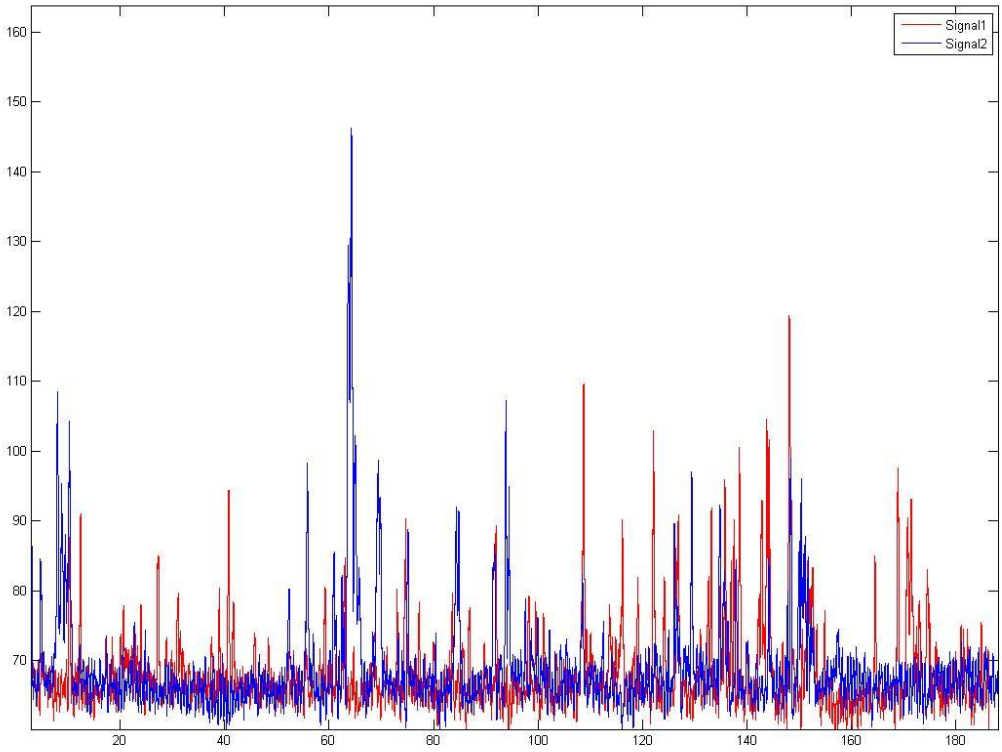


Figure 7-5. Representative data sample of true baseline and repeat baseline signals.

RQ1 and RQ3 were distinctly separate theoretical questions—respectively regarding whether the ILM activity differed as a function of psychological stress or linguistic underpinnings of the task—that were analyzed quite similarly, thus the statistical approach for both RQs will be described once, as follows. To generally characterize muscle activity across experimental tasks (a goal related to RQ1 and RQ3), activity of the ILMs and the two control sites were explored and analyzed during the following three conditions: *Baseline Rest*, *Baseline Subvoc* and *SPT*. Descriptive statistics (average, SD, minimum, maximum, effect size) for the **magnitude of change** from *Baseline Subvoc* and *SPT* (RQ1), as well as from *Baseline Rest* to *Baseline Subvoc* (RQ3) were calculated.

Next, to directly address both RQ1 and RQ3, individual ITSAs[14] were performed comparing: (1) *Baseline Rest* epoch to the *Baseline Subvoc* epoch and (2) and *Baseline Subvoc* epoch to the *SPT* epoch. These repeated ITSA analyses yielded a *p* value for each muscle (each of the ILMs, the upper trapezius, and the anterior tibialis muscle), for each participant. Then, to estimate the strength of the muscle response to a stressor, effect sizes were calculated for individual muscles for each participant, and descriptive statistics of the effect sizes (average, SD, minimum, maximum) were obtained. Conceptually, this approach is similar to a meta-analysis, and provides information regarding the magnitude of the total effect size of the phenomenon of interest (muscular response to the speech preparation stressor, RQ1), and to what extent this effect may be explained by behaviors related to subvocalization (RQ3).

Next, to address RQ2c, the Pearson product-moment correlation coefficient was obtained to measure the relationship between **[IV1]** *stress reaction score* and **[IV2]** RSA_{CORR_DIFF}, since the chance of type I error in subsequent regression models might be inflated if the relationship between the two IVs is strong. This analysis provided information regarding the strength of linear dependence between these two variables. The possible presence of quadratic and curvilinear relations was also assessed.

Finally, to address RQ2a and RQ2b, simultaneous multiple regression was performed for **[DV1]** *magnitude of ILM activity change* predicted by **[IV1, RQ2a]** *stress reaction score on the MPQ-BF* and **[IV2, RQ2b]** RSA_{CORR_DIFF}, *respiratory sinus arrhythmia*, for each of the three ILMs, the upper trapezius, and the anterior tibialis. Simultaneous multiple regression assumes that the variables are not highly correlated, and was appropriate because the variables could not be ordered by importance based on current theory, as would be required for hierarchical regression, for instance. In the event that both variables were highly correlated, one would have been eliminated and/or separate simple linear regressions may have been performed to regress the DVs on the IVs individually. The limitation of separate regression equations is that the unique contribution of one IV above and beyond the other

[14] The ITSA procedure is presented in Section 7.3.3.

remains unknown. If both IVs were highly correlated, it may have been most practical to perform hierarchical regression in which IV1 (stress reaction score) was entered first and IV2 (RSA_{CORR_DIFF}) was entered second. This decision would not have been theoretically driven, but rather a practical decision based on the fact that the paper-and-pencil stress reaction score is more simple to obtain and analyze than RSA. The data were screened for assumptions and influential cases. Regression diagnostics included examination of studentized deleted residuals, dfbeta, leverage, and Cook's distance. Outliers and influential cases were deleted and sensitivity analysis was performed.

8.0 RESULTS

8.1 PARTICIPANTS

Seventy-eight potential participants completed the web-based screening survey, and 50 potential participants attended the face-to-face screening. Forty participants satisfied all of the inclusion criteria and participated in the study. Of those 40 participants, one could not tolerate the placement of the fine wire electrodes, and was dismissed from the study. Data from two participants were corrupted for unknown reasons and hence could not be analyzed. Thus, complete data sets for 37 individuals are presented herein. Table 8-1 gives racial/ethnic information for this cohort.

Table 8-1. Number of Participants in Each Racial/Ethnic Category

Hispanic	Non-Hispanic	White/Caucasian	Black/African American	Asian
1	36	28	5	4

Table 8-2 shows age and body mass index values for all participants whose data were included in analysis. One participant exceeding the BMI criterion was included in the study; during the screening she was just below the Obese Class I threshold, but by the time she participated in the experiment her weight had increased. This difference was not realized or confirmed by the investigators until after the participant had completed the experiment.

Table 8-2. Descriptive Values for Age, Height, Weight and Body Mass Index

Anthropometric Indices		
	M (SD)	Range (min-max)
Age (years)	23.09 (3.1)	19-30
Height (inches)	64.78 (2.59)	60.5-71.0
Weight (pounds)	139.19 (23.43)	99-185
Body Mass Index (kg/m^2)	23.27 (3.34)	18.6-31.75

BMI Classification †		
	Cut-off points/ranges	*n* (% of total sample)
Underweight	<18.50	0
Normal	18.50-24.99	24 (67%)
Overweight	25.00-29.99	11 (31%)
Obese Class I	30.00-34.99	1 (3%)
Obese Class II	35.00-39.99	0
Obese Class III	≥40.00	0

† According to World Health Organization classification criteria (2000).

8.2 VIABLE MUSCLES/CHANNELS FOR ANALYSIS

As described in Section 7.4 (Blinded Verification of Electrode Placement), raw EMG waveforms recorded during voluntary contraction tasks were reviewed *a posteriori* to confirm that the signals stood out against the noise of that channel (e.g., all channels except LCT in Figure 7-4). This step preceded all

descriptive and statistical analyses. Channels that did not exhibit clear increases in muscle activity in the time frame that the voluntary contraction task was performed were excluded from further analysis (e.g., LCT channel in Figure 7-4). Table 8-3 provides information regarding which channels/muscles were deemed viable based on voluntary contraction signals collected intermittently during the experiment. Cells in gray and having a (–) sign are indicative of "lost" channels at each time point in the study, whereas cells with white background and a (+) sign are indicative of viable channels.

Table 8-3. Viable Muscles/Channels at Each Voluntary Contraction Task

	At time of placement					Pre					Post					Final				
Subj ID	PCA	LTA	RTA	LCT	RCT	PCA	LTA	RTA	LCT	RCT	PCA	LTA	RTA	LCT	RCT	PCA	LTA	RTA	LCT	RCT
EMG01	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG02	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG03	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
EMG04	+	+	+	+	+	-	+	-	+	+	-	+	-	+	+	-	+	-	+	+
EMG09	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+
EMG10	+	+	+	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
EMG11	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
EMG12	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+
EMG17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
EMG19	+	+	+	+	+	-	+	+	-	+	-	+	+	-	-	-	+	+	-	+
EMG20	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
EMG22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG24	+	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	-
EMG26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
EMG28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG29	+	+	+	+	+	+	-	+	-	+	+	-	+	-	+	+	-	+	-	+
EMG34	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG35	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
EMG31	+	+	-	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-
EMG32	-	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	-
EMG36	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
EMG39	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG48	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	-	-	-	-
EMG49	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG50	-	+	+	+	+	-	+	+	-	+	-	+	+	+	+	-	+	+	+	+
EMG52	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+
EMG55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+

Table 8-3 (Continued)

EMG57	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+
EMG60	+	+	-	+	-	+	+	-	+	-	+	+	-	+	-	+	+	-	+	-
EMG66	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+
EMG71	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	-
EMG72	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG74	-	+	+	+	+	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-
EMG75	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-
EMG76	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG78	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
# missing	4	0	2	3	8	11	2	6	8	10	12	3	7	9	10	14	3	9	7	10
# obtained	33	37	35	34	29	26	35	31	29	27	25	35	30	30	27	23	34	28	30	27
% obtained	89%	100%	95%	92%	78%	70%	95%	84%	78%	73%	68%	95%	81%	78%	73%	62%	92%	76%	81%	73%

8.3 GENERAL DESCRIPTIVE FINDINGS

Room temperature was recorded six times from the beginning to the end of the experimental session, at the following points: *True Baseline* (Stage IV in Figure 7-1), *Recovery* (Stage VI), *Baseline Rest*, *Baseline Subvoc*, *SPT*, and *Repeat Baseline*. Room temperature averaged 73.88 degrees for all experimental sessions. For all participants, room temperature within the experimental session varied from 0-2.80°F.

8.3.1 Trait Stress Reactivity Data

The first independent variable, Trait Stress Reactivity score (hereafter referred to as SRscore) was calculated according to the methods employed by (Patrick et al., 2002) using an SPSS script provided by the first author, Dr. Patrick. Values for this cohort (n=37) are presented in Table 8-4.

Table 8-4. Trait Stress Reactivity Scores and Norms

	Min	Max	Mean	SD
Raw Scores - Current Sample	**.00**	**11**	**3.65**	**3.25**
Raw Scores - Female Norms*	.00	12	6.15	3.54
Raw Scores – Young Cohort Norms**	.00	12	5.73	3.45
T-scores - Current Sample	**33.71**	**65.57**	**44.01**	**9.40**
T-Scores - Female Norms*	33.71	68.46	51.53	10.26
T-Scores– Young Cohort Norms**	33.71	68.46	50.32	9.98

*compared to normative sample of women, N=675, aged 18-70

**compared to normative sample of men and women, N=765, aged 18-40

8.3.2 RSA_{CORR_DIFF} calculation

Raw ECG data were saved, prepared and reduced according to the methods outlined in Section 7.3.2. Of the 36 participants who engaged in all tasks from which RSA values were to be derived, RSA data from only n=31 were usable. Two participants' data became corrupted toward the end of the experimental session including during the SPT task (one lost respiratory band trace, the other lost ECG trace), and three participants' ECG data were corrupt for all tasks. Table 8-5 gives high-frequency heart rate variability (i.e., RSA) change values *without* correction for the effects of respiration on the heart rate signal (RSA_{RAW_DIFF}). Also provided are the traditionally-reported values of low-frequency power, which ostensibly index sympathetic engagement, the natural log values (ln) for both low- and high-frequency power, and the ratio of low- and high-frequency power.

Recall that the original plan was to calculate a respiratory-corrected RSA value (RSA_{CORR_DIFF}) by first dividing observed RSA_{RAW} values by respiratory amplitude before regressing those values on respiratory rate according to the methods of Egizio et al (2011). Unfortunately, the respiratory amplitude data representing tidal volume were deemed invalid. This problem became apparent during initial exploration and organization of data, when the investigator noted that respiratory amplitude values were exceptionally high during the 8 and 10.5 breaths/min conditions of the paced breathing tasks, and exceptionally low during the baseline and stressor epochs. This finding was observed for about one-third of participants. Further exploration of the data revealed that the anticipated relationship was not observed for tidal volume and raw RSA (direct relationship) and tidal volume and respiration rate (inverse relationship). The data could have been corrupted due to slipping or loosening of the respiratory band within the session, although this does not seem likely. More reasonable explanations for the error in data might be that participants were insufficiently trained for the paced breathing task. Participants should have matched specific breathing rates without engaging in overly exaggerated abdominal excursions

associated with hyperventilatory behaviors. Breathing behaviors were not explicitly trained, although the investigators did guide participants to avoid such behaviors if they were observed during the session. Alternatively, perhaps participants were not given sufficient time to rest between paced breathing conditions. Approximately two minutes passed before moving on to the next breathing rate, and participants were always queried if they felt as though their breathing was "back to normal" before

Table 8-5. Heart Rate Variability Descriptive Data

	Mean	**SD**	**Min**	**Max**
True Baseline				
Inter-beat interval (ms)	959.76	142.93	683.22	1251.64
High Frequency power (RSA_{RAW}, ms^2)	1974.45	2010.45	34.51	9235.80
Low Frequency power (ms^2)	1095.78	1502.02	52.36	6675.41
Low-High Frequency Ratio	0.83	1.31	0.06	7.72
ln High Frequency power (ln ms^2)	3.05	0.54	1.54	3.97
ln Low Frequency power (ln ms^2)	2.74	0.53	1.72	3.82
Speech Preparation Task				
Inter-beat interval (ms)	725.28	145.40	485.15	975.38
High Frequency power ($RSA_{RAW,}$ ms^2)	567.10	613.42	7.95	2976.17
Low Frequency power (ms^2)	462.89	386.36	25.93	1541.86
Low-High Frequency Ratio	1.20	0.90	0.35	3.79
ln High Frequency power (ln ms^2)	2.51	0.54	0.90	3.47
ln Low Frequency power (ln ms^2)	2.49	0.45	1.41	3.19

moving on to the next paced breathing task. However, participants should not be expected to clearly or reliably perceive "normal" cardiovascular status, and preceding paced breathing conditions might still have impacted those that followed.

Because the respiratory amplitude data could not be used, RSA_{CORR_DIFF} was calculated differently than outlined in section 7.3.2. Instead of including chest wall movement/respiratory amplitude values as a proxy for tidal volume, the RSA_{RAW} values obtained during the four paced breathing conditions were regressed on each respective respiratory rate (8, 10.5, 13, and 18 breaths/min).[15] Analyses then proceeded as planned. Individual within-subjects regressions were performed for all subjects, and an arbitrary R^2 value of .70 was set so that regression equations with R^2 of .70 or higher were not inspected further. Eight participants had R^2 values less than .70. Scatterplots showing the relationship between RSA and respiratory rate across the paced breathing tasks were inspected to determine whether data should be used or not. As desired, all RSA values clearly decreased as respiratory rate increased, and thus no data were omitted from these eight participants. These scatterplots and the corresponding R^2 values are given in Appendix G.

The remaining complete ECG and respiratory rate data sets (n=24) had R^2 values ranging from .701 to .974 (M=.847, SD=.088). For all 32 participants with complete data sets, the *True Baseline* and *SPT* respiratory rate was entered into each participant's regression equation in order to obtain predicted values of RSA at both time points. To correct for the effects of respiration on RSA, these predicted RSA values for *True Baseline* and *SPT* were subtracted from the measured RSA values for each respective task. It is this value, RSA_{CORR_DIFF}, which served as one independent variable in subsequent regression analyses and will be described in greater detail below.

[15] This is not an unprecedented approach. Others have corrected for effects of respiration on RSA in the same fashion (e.g., Overbeek, van Boxtel, & Westerink, 2014), and some consider respiratory rate a substantially more important variable to control than tidal volume in RSA research (Berntson et al., 1997). This issue will be discussed in greater detail in the Discussion section.

Table 8-6 provides respiratory corrected values of RSA (RSA_{CORR}) for the three-minute *True Baseline* epoch, the three minute *SPT* epoch, and the calculated difference between those two experimental epochs (RSA_{CORR_DIFF}). Because the RSA_{CORR_DIFF} values can be either positive or negative, those values could not be pooled for calculation of descriptive statistics; therefore, data for those participants with positive RSA_{CORR_DIFF} values are presented separately from those with negative RSA_{CORR_DIFF} values. Positive RSA_{CORR_DIFF} values during *True Baseline* or the *SPT* indicate that the observed and uncorrected RSA value (RSA_{RAW}) for that epoch was greater than the predicted RSA value (RSA_{PRED}) for the same epoch, whereas negative values indicate that RSA_{RAW} was less than RSA_{PRED}. Regarding the change from baseline values, positive changes reflect increased vagal outflow from baseline to stressor as estimated by RSA_{CORR_DIFF} values, whereas negative changes reflect diminished vagal outflow.

Table 8-6. Respiratory-Corrected RSA (RSA_{CORR_DIFF}) Values

	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
True Baseline Task RSA_{CORR_DIFF} (ms^2)	Positive Values (n=12)				Negative Values (n=20)			
RSA_{RAW} - RSA_{PRED}	1255.4	1591.0	52.1	4531.6	-3469.0	3178.3	-9906.8	-28.6
Speech Preparation Task RSA_{CORR_DIFF} (ms^2)	Positive Values (n=18)				Negative Values (n=14)			
RSA_{RAW} - RSA_{PRED}	2054.7	1907.8	56.3	6069.1	-2566.6	2274.2	-7721.0	-222.0
Change from Baseline (ms^2)	Positive Values (n=20)				Negative Values (n=12)			
SPT RSA_{CORR} – *True Baseline* RSA_{CORR}	3644.3	2786.9	304.3	12132.1	-1546.7	1594.3	-4766.2	-0.2

To further explore the relationship between RSA_{RAW}, RSA_{PRED}, RSA_{CORR} and RSA_{CORR_DIFF} values, Spearman's rho was calculated for each relationship. Data are presented in a correlation matrix in Table 8-7. Interestingly, RSA_{RAW} values were not significantly correlated with any of the other RSA variables.

Table 8-7. Correlation Matrix of RSA Variables

Variable	1	2	3	4	5	6
1. RSA_{CORR_DIFF}	–					
2. RSA_{RAW} during *True Baseline*	-.106	–				
3. RSA_{RAW} during *SPT*	.196	.477**	–			
4. RSA_{PRED} during *True Baseline*	.287	.696**	.243	–		
5. RSA_{PRED} during *SPT*	-.444*	.353*	.168	.418*	–	
6. RSA_{CORR} during *True Baseline*	-.514**	-.116	-.144	-.726**	-.379*	–
7. RSA_{CORR} during *SPT*	.443*	-.378*	-.093	-.484**	-.988**	.410*

* correlation is significant at the 0.05 level (2-tailed).
** correlation is significant at the 0.01 level (2-tailed).

8.3.3 Respiration Rate from *True Baseline* to *SPT*

On the whole, respiration rate increased from baseline to the stressor condition. Table 8-8 provides descriptive data for the participants' respiratory rate (breaths/min) during the baseline epoch, the SPT epoch, and as a difference score from the baseline to the stressor epoch. On average, respiratory rate increased by five breaths/minute, although some participants breathed up to ten breaths less per minute during the stressor as compared to baseline, and for other participants a far more dramatic increase in

respiration rate was observed compared to the average. Individual respiratory rate data are reported later, in section 8.4.1.

Table 8-8. Respiration Rate Values (breaths per minute)

	Mean	**SD**	**Min**	**Max**
Respiration Rate: *True Baseline* Task	13.25	3.92	5.95	21.27
Respiration Rate: *Speech Preparation Task*	18.59	5.69	5.84	28.72
Difference in Respiratory Rate: *SPT* values Minus *True Baseline* values	5.52	6.16	-10.05	18.72

8.3.4 Heart Rate and Blood Pressure by Experimental Stage

Cardiovascular data were analyzed using a repeated measures analysis of variance using a within-subjects factor of Study Stage (*True Baseline*, *Recovery*, *Baseline Rest*, *Baseline Subvoc*, *SPT*, and *Baseline Repeat*). Mauchly's test indicated that the assumption of sphericity had been violated for systolic blood pressure $X^2(2)=55.247$, $p<0.001$, diastolic blood pressure $X^2(2)=53.133$, $p<0.001$, and heart rate $X^2(2)=185.082$, $p<0.001$, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε = .593, .670 and .307, respectively). A statistically significant main effect of Study Stage was observed for each dependent variable: systolic blood pressure, $F(2.967,106.81)=77.82$, $p<0.001$, $\eta_p^2=0.684$, diastolic blood pressure, $F(3.35,120.65)=54.35$, $p<0.001$, $\eta_p^2=0.602$, and heart rate $F(1.54,55.29)=99.32$, $p<0.001$, $\eta_p^2=0.734$. Table 8-9 provides mean (SD) values for Stress and Anxiety at each stage in the experiment.

Table 8-9. Mean (SD) Cardiovascular Values by Stage of Experiment

	IV True Baseline	VI Recovery	VIIa Baseline Rest	VIIb Baseline Subvoc	**VIII SPT**	IX Repeat Baseline
Heart Rate	63.82	64.99	65.01	66.31	**86.07**	66.07
	(9.61)	(9.61)	(8.46)	(9.11)	**(17.45)**	(8.94)
Systolic Blood Pressure	104.32	106.44	104.45	105.69	**118.02**	104.07
	(7.52)	(7.88)	(7.48)	(8.12)	**(10.26)**	(8.163)
Diastolic Blood Pressure	63.56	65.80	62.26	64.33	**73.01**	60.77
	(6.40)	(6.86)	(7.59)	(6.88)	**(8.99)**	(7.52)

Post hoc pair wise comparisons using an LSD test showed that all three cardiovascular variables—systolic and diastolic blood pressure, and heart rate—were significantly higher during the speech preparation stressor (Stage VIII) compared the other experimental stages. In addition, both systolic and diastolic blood pressure were significantly different during the *True Baseline, Baseline Recovery* from fine wire electrode placement (Stage VI), and *Baseline Rest* conditions, such that *True Baseline<Recovery>Baseline Rest*. Additional significant differences were observed for diastolic blood pressure as follows: *Recovery > Baseline Repeat* and *Baseline Subvoc > Repeat Baseline*.

8.3.5 Perceived Stress and Anxiety by Experimental Stage

Self-reported Stress and Anxiety data were analyzed using a repeated measures analysis of variance using a within-subjects factor of Study Stage (*Equipment Setup, Paced Breathing, True Baseline, ILM Electrode Placement*, *Baseline Rest*, *Baseline Subvoc*, *SPT*, and *Repeat Baseline*). Mauchly's test indicated that the assumption of sphericity had been violated for both Stress $X^2(2)=136.6$, $p<0.001$, and Anxiety $X^2(2)=128.4$, $p<0.001$, therefore degrees of freedom were corrected using Greenhouse-Geisser

estimates of sphericity (ε = .561 and .516, respectively). A statistically significant main effect of Study Stage was observed for self-reported Stress, $F(3.9,149.2)=42.829$, $p<0.001$, $\eta_p^2=0.529$ as well as for self-reported Anxiety, $F(3.6,137)=51.031$, $p<0.001$, $\eta_p^2=0.573$. Table 8-10 provides mean (SD) values for Stress and Anxiety at each stage in the experiment.

Table 8-10. Mean (SD) Self-Reported Stress and Anxiety Values by Stage of Experiment

	Setup	III Paced Breathing	IV True Baseline	**V Electrode Placement**	VIIa Baseline Rest	VIIb Baseline Subvoc	**VIII SPT**	IX Repeat Baseline
Stress	8	11	4	**39**	10	10	**39**	8
	(8)	(12)	(6)	**(25)**	(15)	(10)	**(22)**	(12)
Anxiety	10	11	5	**45**	10	10	**41**	11
	(11)	(11)	(7)	**(26)**	(15)	(11)	**(24)**	(19)

Post hoc pair wise comparisons using an LSD test showed that self-reported Stress was significantly higher during two stages—*ILM electrode placement* (Stage V) and *SPT* (Stage VIII)—compared to all other experimental stages, which are as follows: *Equipment Setup*, *Paced Breathing* (Stage III), *True Baseline* (Stage IV), *Baseline Rest* (Stage VIIa), *Baseline Subvoc* (Stage VIIb), and *Repeat Baseline* (Stage IX). In addition, participants reported significantly more stress during the *Paced Breathing* task as compared to the *True Baseline* that immediately followed that task. Finally, participants self-reported significantly more stress during the *True Baseline* as compared to the *Baseline Subvoc* condition; neither of those conditions differed significantly from the *Baseline Rest* condition.

The same *post hoc* pair wise comparisons were also examined for the construct of Anxiety. The findings mirrored those of self-reported Stress in that *ILM Electrode Placement* (Stage V) and the *SPT* (Stage VIII) elicited significantly higher Anxiety than all other stages of the experiment. Both the

Equipment Setup and the *Paced Breathing* stages elicited significantly higher levels of self-reported Anxiety than did the *True Baseline* stage.

8.4 PRIMARY OUTCOMES

8.4.1 RQ1: Magnitude of Change in Muscles of Interest from Baseline to Stressor

Recall that signals from the TA/LCA complex and the CT muscles were obtained bilaterally whenever possible; this redundancy was meant to minimize the total number of lost channels. However, fine wire EMG signals are highly vulnerable to noise contamination, especially since, in this study, the signals of interest occur *in the absence of* overt muscle activity or use (i.e., at rest). These tiny signals, measured in microvolts, must be able to arise from the ambient noise of the muscle activity and body. Thus, to ensure that analysis included only the cleanest and most robust signals, when both sides were deemed viable, their ITSA values were examined and the side with the smaller signal was omitted from analysis in regression equations. This method resulted in only three intrinsic laryngeal muscles—unilateral PCA, TA/LCA, and CT—being included in regression analyses. Thus, the first step toward selecting signals for regression analysis was to examine the number of viable channels that exhibited statistically significant changes from *Baseline Subvoc* during the *SPT*. Table 8-11 provides this information.

Table 8-11. Number (%) of Viable Channels with Significant Increase from *Baseline Subvoc* to *SPT*

	PCA	LTA	RTA	LCT	RCT	TPZ	TIB
# sig	16/25	21/35	18/30	15/30	9/27	24/37	23/37
	(72%)	(64%)	(59%)	(50%)	(33%)	(65%)	(59%)

Next, effect sizes were calculated and examined for each muscle with viable bilateral signals, and the side with the smaller effect size was omitted from the data set. Note that in the very few cases where the change from *Baseline Subvoc* to *SPT* was negative (i.e., muscles *decreased* in activity), those values were omitted even if the effect size was larger than in the positive-going contralateral muscle. Table 8-12 provides information about how many signals included for further analysis were obtained from the right and left sides.

Table 8-12. Total Number of Viable Channels by Side

	PCA*	**TA/LCA**		**CT**		**TPZ***	**TIB***
Side	Right	Right	Left	Right	Left	Left	Left
	25	17	20	24	10	37	37
Total	**25**	**37**		**34**		**37**	**37**

* Sampled *only* unilaterally

Table 8-13 provides data representing the number of viable signals that were included in regression analyses, described subsequently. That is, after specifying the viable left versus right channels for the TA/LCA and CT muscles, those data were merged into one dataset, with the resulting sample size for each muscle being specified in this table. The number of statistically significant changes from *Baseline Subvoc* is also specified as a percentage of total viable muscles. Further, this table indicates in which direction the change occurred (i.e., greater or lesser activation from *Baseline Subvoc* to *SPT*).

Table 8-13. Viable Unilateral Channels with Significant Change from *Baseline Subvoc* to *SPT*

	PCA	TA/LCA	CT	TPZ	TIB
Statistically Significant /Total Viable Muscles (%)	18/25 (72%)	26/37 (70%)	15/34 (44%)	24/37 (65%)	23/37 (62%)
Number increasing in activation (vs decreasing)	18/18	26/26	15/15	24/24	22/23

Finally, to further characterize the change in muscle activity from *Baseline Subvoc* to *SPT* in the present cohort, effect sizes were examined as a function of statistical significance (not significant versus significant based on ITSA). Table 8-14 provides the descriptive statistics (mean, minimum, maximum, and standard deviation of effect sizes) for all the muscles listed as viable in

Table 8-13. These data are further separated within that table by statistical significance versus non-significance. Note that the effect size values were calculated as follows, according to Cohen (1992):

$$\frac{\bar{x}\ \text{activity during}\ Baseline\ Subvoc - \bar{x}\ \text{activity during}\ SPT}{\bar{x}(\sigma\ \text{activity during}\ Baseline\ Subvoc\ \text{and}\ SPT)}$$

Thus, negative values reflect an *increase* in activity of the muscle from baseline to stressor, whereas positive values reflect a *decrease* in activation of the muscle.

Table 8-14. Effect Sizes for All Viable Muscles Included in Regression Analyses

	PCA	TA/LCA	CT	TPZ	TIB
Effect Sizes for All Muscles					
Mean	-3.06	-3.82	-3.28	-0.79	-1.89
Min/Max	-11.9/0.66	-18.0/1.64	-34.0/1.05	-3.19/-0.001	-13.37/0.03
SD	3.42	4.72	5.91	0.84	3.03
Effect Sizes for Muscles with Non-Significant Changes					
Mean	-0.39	-0.13	-1.13	-0.32	-0.40
Min/Max	-1.6/0.66	-1.5/1.6	-4.9/1.05	-1.2/-0.001	-1.0/-0.004
SD	0.70	0.79	1.28	0.36	0.31
Effect Sizes for Muscles with Statistically Significant Changes					
Mean	-4.10	-5.54	-6.01	-1.04	-2.68
Min/Max	-11.9/-0.03	-18/-0.1	-34.0/-0.27	-3.2/-0.04	-13.4/0.03
SD	3.51	4.85	8.13	0.92	3.61

Another way of examining the same data is by examining the fold-change from *Baseline Subvoc* to *SPT*. Figure 8-1 shows the average fold changes for all participants by muscle, with the following outliers omitted: PCA—EMG26; TA—EMG48; anterior tibialis—EMG32 and EMG48. Fold change was calculated as follows:

$$\frac{\bar{x}\ \text{activity during}\ SPT\ -\ \bar{x}\ \text{activity during}\ Baseline\ Subvoc}{\bar{x}\ \text{activity during}\ Baseline\ Subvoc}$$

The magnitudes of the fold-increases generally corroborate the effect sizes, particularly inasmuch as the laryngeal muscles exhibit greater magnitude of increase than the controls.

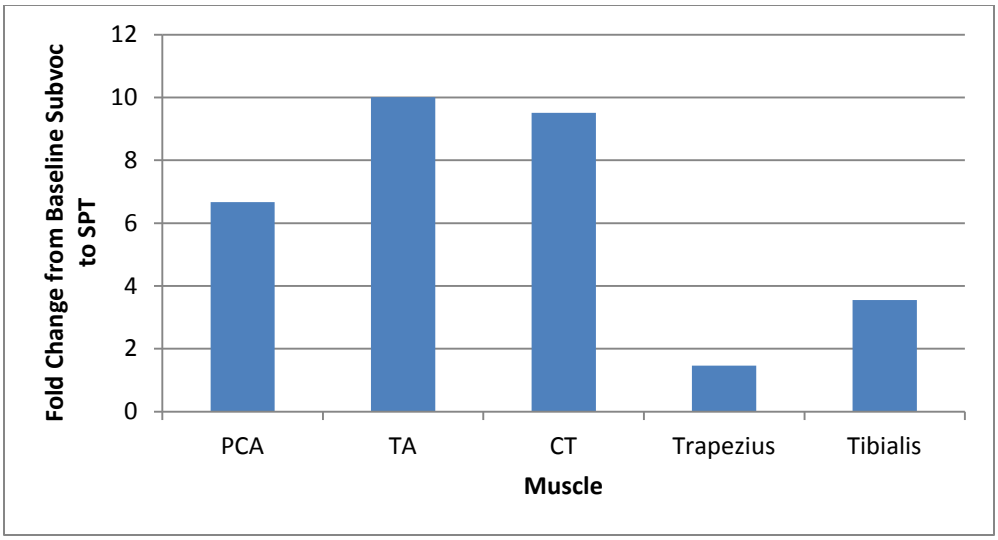


Figure 8-1. Fold change in muscle activity from *Baseline Subvoc* to *SPT*.

Finally, Table 8-15 provides the following information by subject: statistical significance of muscle activity change from *BLsubvoc* to *SPT* (based on ITSA calculations); change in heart rate (beats per minute, HRΔ) from *BLsubvoc* to *SPT*; change in systolic and diastolic blood pressure (mmHg, SBPΔ and DBPΔ, respectively) from *BLsubvoc* to *SPT*; change in respiratory rate (breaths/min) from *True Baseline* to *SPT*; and SRscore derived from the *MPQ-BF*. For each muscle, statistically significant and non-significant ITSA findings are represented by "SIG" and "NO" respectively, and are color coded to assist with review. All difference (Δ) scores were calculated by subtracting the *BLsubvoc* values from the *SPT* values. Missing data are represented by "---".

Table 8-15. Muscle, Cardiovascular, Respiratory and Stress Reactivity Data by Subject

SUBJID	PCA	TA	CT	TPZ	TIB	HRΔ	SBPΔ	DBPΔ	RRΔ*	SRscore
EMG01	NO	NO	NO	SIG	NO	11.92	10.50	3.92	-1.44	1
EMG02	SIG	SIG	NO	SIG	SIG	24.93	15.00	12.93	5.10	1
EMG03	---	SIG	SIG	SIG	SIG	14.27	18.60	10.40	3.78	7
EMG04	---	NO	SIG	SIG	SIG	26.25	18.95	13.00	4.16	0
EMG09	SIG	NO	NO	SIG	SIG	24.83	13.17	-0.58	0.19	0
EMG10	---	NO	NO	NO	NO	30.65	21.95	16.45	---	2
EMG11	---	SIG	SIG	NO	NO	19.27	15.47	14.40	3.00	7
EMG12	SIG	SIG	SIG	SIG	SIG	24.80	12.00	4.20	9.33	3
EMG17	SIG	SIG	NO	SIG	SIG	6.58	12.33	3.83	5.79	9
EMG18	SIG	SIG	SIG	SIG	SIG	48.67	14.73	18.53	6.35	0
EMG19	---	NO	SIG	NO	NO	7.67	12.33	9.58	3.73	5
EMG20	NO	NO	NO	NO	NO	45.33	27.27	1.13	---	4
EMG22	NO	SIG	NO	SIG	NO	16.33	10.40	14.67	-3.81	7
EMG24	---	NO	NO	NO	NO	17.20	6.80	7.07	15.62	5
EMG26	SIG	SIG	NO	NO	NO	22.92	6.50	12.42	2.91	6
EMG28	SIG	SIG	SIG	SIG	SIG	22.87	14.53	10.87	3.67	0
EMG29	SIG	SIG	SIG	SIG	SIG	47.67	20.80	14.20	3.43	10
EMG31	---	NO	NO	SIG	NO	16.25	21.17	15.17	0.27	2
EMG32	---	SIG	NO	SIG	SIG	13.87	8.53	7.33	---	2
EMG34	SIG	SIG	SIG	SIG	SIG	7.50	-0.67	7.75	10.05	2
EMG35	---	SIG	NO	NO	NO	11.67	8.33	1.67	4.29	2
EMG36	SIG	SIG	NO	NO	SIG	8.87	10.13	11.40	8.23	4
EMG39	SIG	SIG	NO	NO	SIG	23.87	15.87	8.73	---	5
EMG48	SIG	SIG	---	SIG	SIG	28.93	22.73	14.20	14.61	11
EMG49	SIG	SIG	SIG	SIG	SIG	20.25	4.92	7.92	9.92	11
EMG50	---	SIG	SIG	SIG	SIG	8.20	7.33	4.20	4.56	5
EMG52	NO	NO	NO	NO	NO	22.40	20.55	15.90	16.48	2
EMG55	SIG	SIG	NO	SIG	SIG	13.25	6.58	4.42	18.72	0
EMG58	NO	NO	NO	NO	NO	18.75	23.58	25.00	10.27	0
EMG60	SIG	SIG	SIG	SIG	SIG	37.95	8.20	3.85	-5.84	2
EMG66	SIG	SIG	NO	SIG	SIG	31.00	20.93	15.80	8.00	3
EMG71	---	SIG	SIG	SIG	SIG	7.27	2.13	3.73	5.39	5
EMG72	SIG	SIG	NO	SIG	SIG	39.95	13.85	2.55	10.45	3
EMG74	---	SIG	---	NO	NO	36.40	8.33	2.27	3.85	0
EMG75	SIG	SIG	---	SIG	SIG	16.50	5.75	5.75	2.99	2
EMG76	SIG	SIG	SIG	SIG	SIG	14.27	29.67	21.80	6.69	0
EMG78	NO	NO	SIG	NO	NO	34.60	17.53	3.27	11.59	7

8.4.2 RQ2a and 2b: Predictive Variables

Five separate multiple regressions were run to predict the magnitude of change in the three intrinsic laryngeal muscles and two control muscles of interest, by the independent variables RSA_{CORR_DIFF} and SRscore. Values for the following participants were found to include outliers and were thus removed from the analyses: EMG52 and EMG49 for the three intrinsic laryngeal muscles, EMG26 for the PCA, EMG48 for the TA, EMG02 and EMG12 for the CT, EMG29 for the upper trapezius muscle, and EMG04 for the anterior tibialis muscle. Regressions were then re-run and results are given below.

In addition, the same multiple regressions were re-run using the "raw", uncorrected RSA values calculated as a difference score from *True Baseline* to the *SPT* (RSA_{RAW_DIFF}). Values for the following participants were found to include outliers and were thus removed from the analyses: EMG12 and EMG49 for all muscles, EMG24 for all intrinsic laryngeal muscles, EMG02 for the CT, EMG29 for the upper trapezius muscle, and EMG04 for the anterior tibialis muscle. Regressions were then re-run and results are given below.

Recall that magnitude of change in muscle activity (the dependent variable) was calculated by subtracting the muscle activity during the stressor from the muscle activity during the baseline epoch. Using a hypothetical example, if baseline muscle activity was 5 μV and stressor muscle activity was 20 μV, the magnitude of change would equal -15 μV. Thus, when interpreting the regression equations it is important to bear in mind that negative values of the DV reflect an *increase* in muscle activity from baseline to stressor, whereas positive DV values reflect a *decrease*.

8.4.2.1 Prediction of PCA Activity

A multiple regression was run to predict magnitude of change in the PCA by RSA_{CORR_DIFF} and SRscore. The assumptions of independence of observations, linearity, homoscedasticity, unusual points and normality of residuals were met. The overall model statistically significantly predicted PCA activity,

$F(2,16)=5.081$, $p=.020$, $R^2=.388$. RSA_{CORR_DIFF} added statistically significantly to the prediction, $p=.016$, but Trait Stress Reactivity did not, $p=.170$. Regression coefficients and errors can be found in Table 8-16.

An additional multiple regression was run for the same variables, but with RSA_{RAW_DIFF} as the first IV. All assumptions were met. The model did not statistically significantly predict PCA activity, $F(2, 17)=1.373$, $p=.280$, $R^2=.139$. Regression coefficients and standard errors can be found in Table 8-16.

8.4.2.2 Prediction of TA activity

A multiple regression was run to predict magnitude of change in the TA by RSA_{CORR_DIFF} and SRscore. The assumptions of independence of observations, linearity, homoscedasticity, unusual points and normality of residuals were met. The overall model statistically significantly predicted TA activity, $F(2,26)=4.838$, $p=.016$, $R^2=.271$. RSA_{CORR_DIFF} added statistically significantly to the prediction, $p=.044$, as did Trait Stress Reactivity, $p=.024$. Regression coefficients and standard errors can be found in Table 8-16.

An additional multiple regression was run for the same variables, but with RSA_{RAW_DIFF} as the first IV. All assumptions were met. The model did not statistically significantly predict TA activity, $F(2,26)=.142$, $p=.868$, $R^2=-.011$. Regression coefficients and standard errors can be found in Table 8-16.

8.4.2.3 Prediction of CT activity

A multiple regression was run to predict magnitude of change in the CT by RSA_{CORR_DIFF} and SRscore. The assumptions of linearity, independence of errors, homoscedasticity, unusual points and normality of residuals were met. The overall model statistically significantly predicted CT activity, $F(2,22)=4.614$, $p=.021$, $R^2=.296$. RSA_{CORR_DIFF} added statistically significantly to the prediction, $p=.007$, but Trait Stress Reactivity did not, $p=.606$. An additional multiple regression was run for the same variables, but with

Table 8-16. Summary of Multiple Regression Analyses

Variable	*B*	SE_B	β		*B*	SE_B	β
Posterior Cricoarytenoid							
Intercept	-100.54	143.49		Intercept	-262.02	221.49	
RSA_{CORR_DIFF}	-.083	.031	**-.529***	RSA_{RAW_DIFF}	-.030	.137	-.050
SRscore	-40.27	28.02	-.282	SRscore	-66.12	39.99	-.380
Thyroarytenoid/Lateral Cricoarytenoid Complex							
Intercept	-864.01	243.75		Intercept	-856.01	333.54	
RSA_{CORR_DIFF}	-.116	.055	**-.355***	RSA_{RAW_DIFF}	-.044	.185	-.046
SRscore	125.83	52.443	**.402***	SRscore	28.51	61.46	.091
Cricothyroid							
Intercept	-192.44	151.03		Intercept	-400.28	169.81	
RSA_{CORR_DIFF}	-.100	.034	**-.530***	RSA_{RAW_DIFF}	-.125	.097	-.265
SRscore	-15.81	30.18	-.094	SRscore	-33.19	34.46	-.198
Upper Trapezius (positive control)							
Intercept	-2.63	2.22		Intercept	-5.37	2.46	
RSA_{CORR_DIFF}	-.001	.000	**-.377***	RSA_{RAW_DIFF}	-.001	.001	-.252
SRscore	-1.23	.431	**-.446***	SRscore	-1.48	.514	**-.477***
Anterior Tibialis (negative control)							
Intercept	-12.62	5.29		Intercept	-18.85	5.13	
RSA_{CORR_DIFF}	-.002	.001	**-.347***	RSA_{RAW_DIFF}	-.003	.002	-.313
SRscore	-2.39	.958	**-.407***	SRscore	-3.40	.99	**-.536***

Note. *B* = unstandardized regression coefficient; SE_B = Standard error of the coefficient; β = standardized coefficient. ***p* < .05 for the full model *and* for the variable.

RSA_{RAW_DIFF} as the first IV. All assumptions were met. This model did not statistically significantly predict CT activity, $F(2,22)=1.123$, $p=.343$, $R^2=.093$. Regression coefficients and standard errors can be found in Table 8-16.

8.4.2.4 Prediction of upper trapezius muscle activity

A multiple regression was run to predict magnitude of change in the upper trapezius muscle by RSA_{CORR_DIFF} and SRscore. The assumptions of independence of observations, linearity, independence of errors, homoscedasticity, unusual points and normality of residuals were met. The model statistically significantly predicted upper trapezius muscle activity, $F(2,28)=6.542$, $p=.005$, $R^2=.318$. Both RSA_{CORR_DIFF} and SRscore contributed significantly to the overall prediction, $p<.023$ and $p<.008$, respectively.

An additional multiple regression was run for the same variables, but with RSA_{RAW_DIFF} as the first IV. All assumptions were met. The model statistically significantly predicted upper trapezius muscle activity, $F(2,26)=5.217$, $p=.012$, $R^2=.286$. RSA_{RAW} did not contribute significantly to the overall prediction, $p=.140$ but SRscore did, $p=.008$. Regression coefficients and standard errors can be found in Table 8-16.

8.4.2.5 Prediction of anterior tibialis muscle activity

A multiple regression was run to predict magnitude of change in the anterior tibialis muscle by RSA_{CORR_DIFF} and SRscore. The assumptions of independence of observations, linearity, independence of errors, homoscedasticity, unusual points and normality of residuals were met. The overall model statistically significantly predicted tibialis activity, $F(2,28)=4.945$, $p=.014$, $R^2=.261$. Both RSA_{CORR_DIFF} and SRscore contributed significantly to the overall prediction, $p<.042$ and $p<.019$, respectively.

An additional multiple regression was run for the same variables, but with RSA_{RAW_DIFF} as the first IV. All assumptions were met. The model statistically significantly predicted anterior tibialis muscle

activity, $F(2,26)=7.730$, $p=.002$, $R^2=.373$. RSA_{RAW} did not contribute significantly to the overall prediction, $p=.054$, but SRscore did, $p=.002$. Regression coefficients and standard errors can be found in Table 8-16.

8.4.3 RQ2c: Intercorrelation of Independent Variables

As stated above, the assumption of multicollinearity was met for all regression analyses. The relationship between RSA_{CORR_DIFF} and SRscore was not found to be statistically significant, $p=.408$. The relationship between RSA_{RAW_DIFF} and SRscore was also assessed and found to be non-significant, $p=.210$.

8.4.4 RQ3: Potential Contributions of Subvocalization

ITSA was performed on all viable muscles to compare activity during *Baseline Rest* versus *Baseline Subvoc*. Table 8-17 provides the percentage of all viable channels exhibiting statistically significant change in activation from *Baseline Rest* to *Baseline Subvoc*, and in which direction the change occurred (i.e., greater or lesser activation from *Baseline Rest* to *Baseline Subvoc*). Note that all five intrinsic laryngeal muscles were included.

Table 8-17. Percentage of Viable Channels with Significant Change from *BLrest* to *Baseline Subvoc*

	PCA	LTA	RTA	LCT	RCT	TPZ	TIB
Statistically Significant /Total Viable Muscles (%)	6/22 (27%)	2/32 (6%)	2/28 (7%)	4/28 (14%)	2/26 (8%)	3/35 (9%)	1/36 (3%)
Number increasing (versus decreasing) in activation / total significant	3/6	1/2	2/2	0/4	1/2	2/3	1/1

Table 8-18 provides the effect size for these changes, organized by those with and without statistically significant changes from *Baseline Rest* to *Baseline Subvoc*.

Table 8-18. Effect Sizes for All Viable Muscles from *BLrest* to *Baseline Subvoc*

	PCA	LTA	RTA	LCT	RCT	TPZ	TIB
Effect Sizes for Muscles with Non-Significant Changes							
Mean	0.0001	-0.01	0.34	0.27	0.03	-0.02	0.02
Min	-0.20	-0.91	-0.09	0.08	-0.60	-0.28	-0.51
Max	0.60	0.98	0.49	0.44	1.12	0.17	0.95
SD	0.18	0.31	0.11	0.11	0.41	0.07	0.20
Effect Sizes for Muscles with Statistically Significant Changes							
Mean	0.054	0.110	0.006	0.01	0.06	0.02	-0.16
Min	-1.53	-0.13	0.0001	0.00004	-0.21	-0.16	n/a
Max	1.85	0.35	0.01	0.03	0.33	0.22	
SD	1.10	0.34	0.01	0.01	0.38	0.19	n/a

9.0 DISCUSSION

9.1 PRIMARY OUTCOMES

9.1.1 SRscores and success of *SPT* in eliciting stress response

The present experiment was successful in terms of recruiting and including participants whose trait stress reactivity scores spanned the normal expected range. The distribution of trait stress reactivity scores was moderately skewed toward lower (i.e., less stress reactive) scores, reflecting an understandable self-selection bias in the present sample, since people high in trait stress reaction are may be less likely to sign up for an invasive study involving needles in the neck.

Cardiovascular measures reflected the stress response that was desired and anticipated during the *SPT*. All participants responded to the *SPT* task with a statistically significant increase in heart rate compared to values collected during their *True Baseline*. The average magnitude of the change in heart rate—about 22 beats per minute—was just slightly lower than the 26 beat per minute increase observed by Kirschbaum, Pirke and Hellhammer (1993) in their laboratory investigation of the Trier Social Stress Test (TSST), on which the stressor in the present study was based. The fact that the methods in the present study approximated nearly the same magnitude of response as the original TSST is impressive, since the present study involved no actual delivery of a speech, which was the element of the TSST that involved the fastest heart rate across participants. Furthermore, participants are typically instructed to stand as part of the TSST, which would also increase the cardiovascular response to the task, yet

participants in the present study remained in a semi-reclined position for the duration of the experiment. Nevertheless, blood pressure measures also reflected the desired physiological stress response. With the exception of two cases in which a negligible increase was observed in SBP (n=1) and DBP (n=1), each of these cardiovascular measures also increased from baseline during the stressor. It should be noted that in the two cardiovascular "non-responders", a significant increase was observed in the laryngeal muscles from baseline to stressor. For the entire cohort, SBP increased about 12 mmHg from *True Baseline* to *SPT*, and DBP increased about 10 mmHg. The magnitude of these cardiovascular changes was medium-to-large based on interpretation of effect sizes according to Cohen (1992). Overall, the cardiovascular responses observed were consistent with characteristic stress responses such as increased force and pace of cardiac contraction and skeletal muscle vasodilation (Herd, 1991).

In addition to these cardiovascular indicators that the *SPT* was successful in eliciting a stress response, participants confirmed feeling Stress and Anxiety during the *SPT*. All but two participants reported an increase in self-perceived Stress and Anxiety as measured by an undifferentiated visual analog scale, and as a group these increases were found to be statistically significant. Interestingly, despite reporting less Stress and Anxiety during the *SPT* compared to *Baseline Rest*, these two participants did exhibit clinically significant increases in heart rate (16 and 25 beats per minute), systolic blood pressure (21 and 12 mmHg), and diastolic blood pressure (15 and 4 mmHg) that are consistent with physiologic stress responses. In fact, the perceived Stress and Anxiety during the *SPT* was, on average, about as much as was experienced during the insertion of needles in the neck. According to Cohen's interpretation of effect sizes (Cohen, 1992), a medium effect size was observed for both variables.

9.1.2 RQ1 – Characterizing the muscular response to *SPT*

As anticipated, the *SPT* task elicited statistically significant changes in muscle activity (compared to *Baseline Subvoc*) for the three intrinsic laryngeal muscles tested (posterior cricoarytenoid,

thyroarytenoid/lateral cricoarytenoid muscle complex, and cricothyroid) as well as for the positive and negative control muscles (upper trapezius and anterior tibialis, respectively). The overwhelming majority of these changes involved increased muscle activity (as opposed to decreased muscle activity) from baseline to stressor. Approximately two-thirds of subjects exhibited increased "responsiveness" in the PCA, TA, upper trapezius, and anterior tibialis muscles, whereas less than half of participants (44%) showed increased muscle activity in the cricothyroid. Results are discussed in greater detail below. Although a good deal of variability in muscle response was qualitatively observed upon inspection of the waveforms, a common pattern of response was increased baseline level of activation as well as increased "spikes" of activation. Figure 9-1 shows waveforms for one participant's TA muscle activity during the three-minute *Baseline Subvoc* period (blue waveform) as compared to the three-minute *SPT* (red waveform).

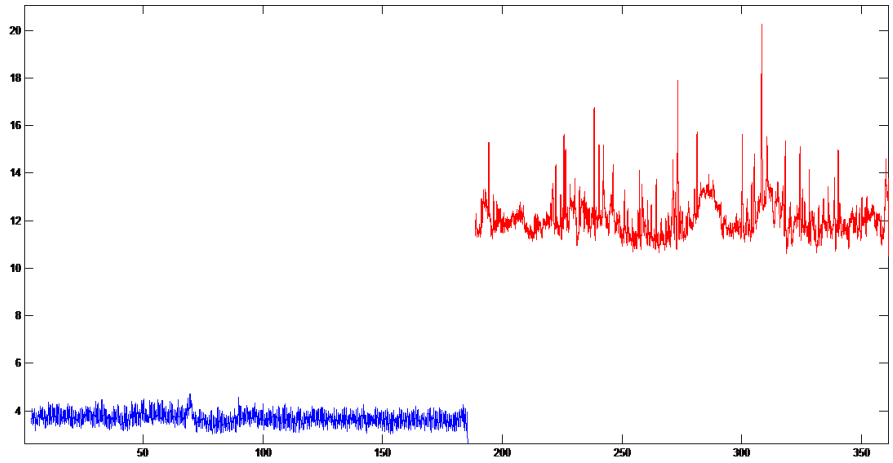


Figure 9-1. Example of change in muscle activity from baseline to stressor.

9.1.2.1 Magnitude of change in laryngeal muscles

Activity of the PCA and TA muscles increased significantly from *Baseline Subvoc* to *SPT* in two-thirds of the participants studied. Moreover, these two muscles were largely congruent in their responses. That is,

if the PCA activity increased, so did the TA activity, and if the PCA was non-responsive to the stressor then the TA tended to also be non-responsive. The CT, on the other hand, was non-responsive in about half of participants, and its activity was only congruent with the activity of the other two muscles about half of the time.

One tempting explanation for the increased activity of these laryngeal muscles during the *SPT* is that these muscles were simply responding to accommodate the increased respiratory rate observed during the stressor. After all, on average from baseline to stressor, the respiratory rate increased by five breaths per minute. However, simply considering the average change in respiratory rate may not be the most fruitful approach to understanding the respiratory behaviors associated with the *SPT* in this study. While the *average* increase was five breaths/min, some participants' respiratory rate increased more than ten breaths/min from baseline to *SPT*. Hillel (2001) reported increased activity of the PCA, TA and CT muscles during "fast breathing" as compared to "slow breathing". These findings are described subsequently, and included for reference in Table 9-1.

Table 9-1. Intrinsic Laryngeal Muscle Activity During Breathing (Hillel, 2001)

	SLOW BREATHING		**FAST BREATHING**	
Muscle	**Inspiration**	**Expiration**	**Inspiration**	**Expiration**
PCA	8/8	8/8	2/8**	----
TA	9/10	----	----	----
CT	9/9	4/9*	3/8†	----

*Still active, much less vigorous than during inspiration; ** "mild" activity; †"very mild activity"; "not active" indicated by "----"

In sum, Hillel found that during "slow breathing" in a cohort of normal participants, mild activity was observed in the PCA of 2/8 subjects, and "very mild" activity was observed in 3/8 subjects' CT muscles. This muscle activity was observed only during inspiration, and no muscle activity was observed during expiration in slow breathing. The TA muscle was not active at all during slow breathing. During "fast" breathing, the PCA was active during both inspiration and expiration for all subjects (n=8), the TA was active during inspiration in 9/10 subjects and silent during expiration for all subjects, and the CT was active during inspiration in all subjects (n=9) and less vigorous but still active during expiration in 4/9 subjects (Hillel, 2001).

An important limitation prevents us from interpreting these findings relative to the findings in the present study. Unfortunately, the actual rate of breathing in these "slow" and "fast" conditions was not provided by Hillel. Three images provided as figures in that manuscript included a scale marker for the x-axis of the EMG signals (see two of them in Figure 9-2, as examples). Using the scale markers in these images to calculate breathing rate, it appears that the "slow" breathing occurred at ~17 breaths/min and "fast" breathing occurred at ~48 breaths/min. Neither the average nor the range of respiratory rate for these conditions was given. Importantly, no one in the present study breathed as fast as 48 breaths/min. In fact, the fastest breathing rate during the *SPT* was about 20 breaths less than Hillel's apparent "fast" rate, at 28.72 breaths/min.

If the changes in laryngeal muscle activity in the present study were *solely* due to their role in valving for ventilatory purposes, it would stand to reason that those breathing within the "slow" range during the *SPT* might not exhibit laryngeal muscle activation, as reported by Hillel. Recall that in Hillel's study, the "slow breathing" condition was associated with very little muscle activation at all, even in the PCA, and this judgment was made by visual assessment of the EMG waveform. In fact, by Hillel's apparent criteria, "slow breathing" was very common during the *SPT* in the present study, as 14/32 of our participants breathed at ≤18 breaths/min during this condition. Nonetheless, these particular participants generally exhibited statistically significant increases in activity of the PCA (7/10), TA (11/14), and CT

(7/11) muscles. In fact, the laryngeal muscles studied globally increased in activity even for the participants whose respiratory rate *decreased* during *SPT* compared to baseline. Moreover, for all of our participants, if the laryngeal muscle activities' increases were a direct function of respiratory rate, then Hillel's findings would lead us to expect *more* overall involvement of the CT muscle across participants. Hillel observed increased engagement of the CT muscle during both inspiration *and* expiration from slow to fast breathing, whereas none of the TA muscles were engaged during expiration in either task. However, the TA muscle was more active in more participants during the *SPT* as compared to the CT muscle. Thus, it appears that the increase of laryngeal muscle activity in this study cannot justifiably be attributed solely to changes in respiration rate.

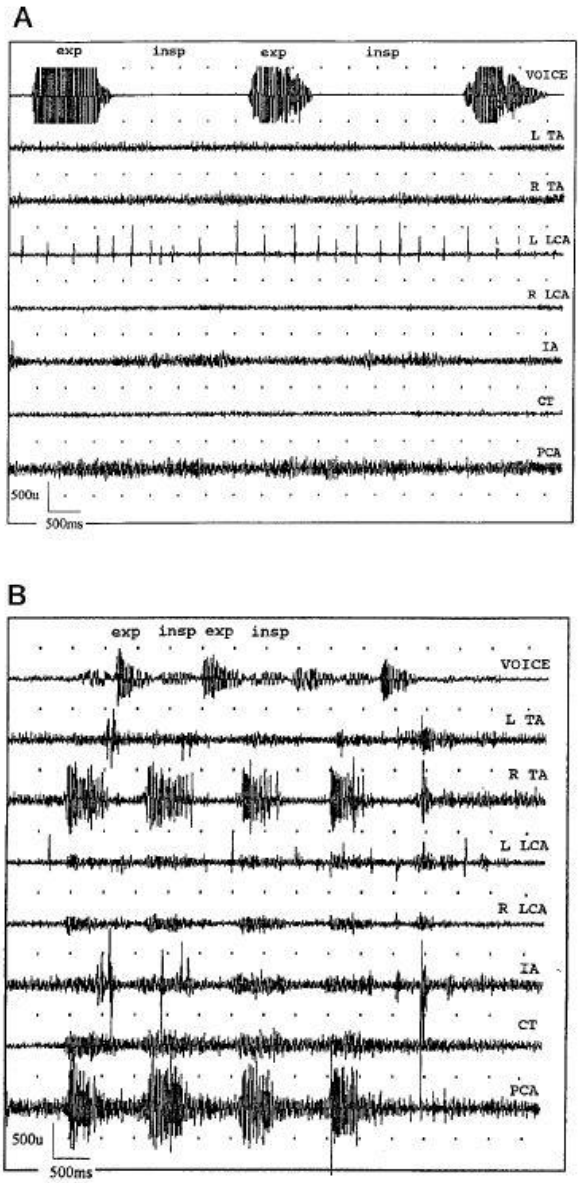


Fig. 18. (**A**) Eight-channel recording of slow breathing in subject 6. Note the absence of significant recruitment for any muscles. (**B**) Eight-channel recording of fast breathing in the same subject. Note the brisk activity of the PCA and CT during inspiration. Also note the increased activity of the adductors during inspiration.

Figure 9-2. EMG waveforms during "slow" and "fast" breathing.[16]

[16] Note: From "The Study of Laryngeal Muscle Activity in Normal Human Subjects and in Patients With Laryngeal Dystonia Using Multiple Fine-Wire Electromyography" by Hillel, 2001, *Laryngoscope*. Copyright 2001 by John Wiley and Sons. Reprinted with permission.

9.1.2.2 Laryngeal muscle response and respiratory observations

Building on the stance presented above that the laryngeal muscle activity changes observed from baseline to stressor do not appear to be cleanly linked to respiration rate changes, it should be noted that exploratory correlational analyses of respiratory rate and magnitude of change in muscle activity failed to show statistically significant relationships, for all muscles. If the role of the laryngeal muscles in pulmonary valving is to be considered a causal reason for their increased activity from baseline to stressor, it is critical that analysis include more than simply examining average changes in respiratory rate.

Indeed, not all participants had a marked increase in respiratory rate during the stressor as compared to baseline; five participants even exhibited *diminished* respiratory rate. Breath-holding was one respiratory behavior observed during the *SPT*. As an example of this observation, Figure 9-3 shows the respiratory band signal for one participant as a green tracing (the red tracing is the electrocardiographic signal). Ignoring the fine oscillations within the respiratory band signal which reflect signal contamination by the heartbeat, it is evident that this particular subject held her breath for several seconds on end during the stressor. Specifically, note the apneic periods from approximately 11-18 seconds and 24-31 seconds on the y-axis.

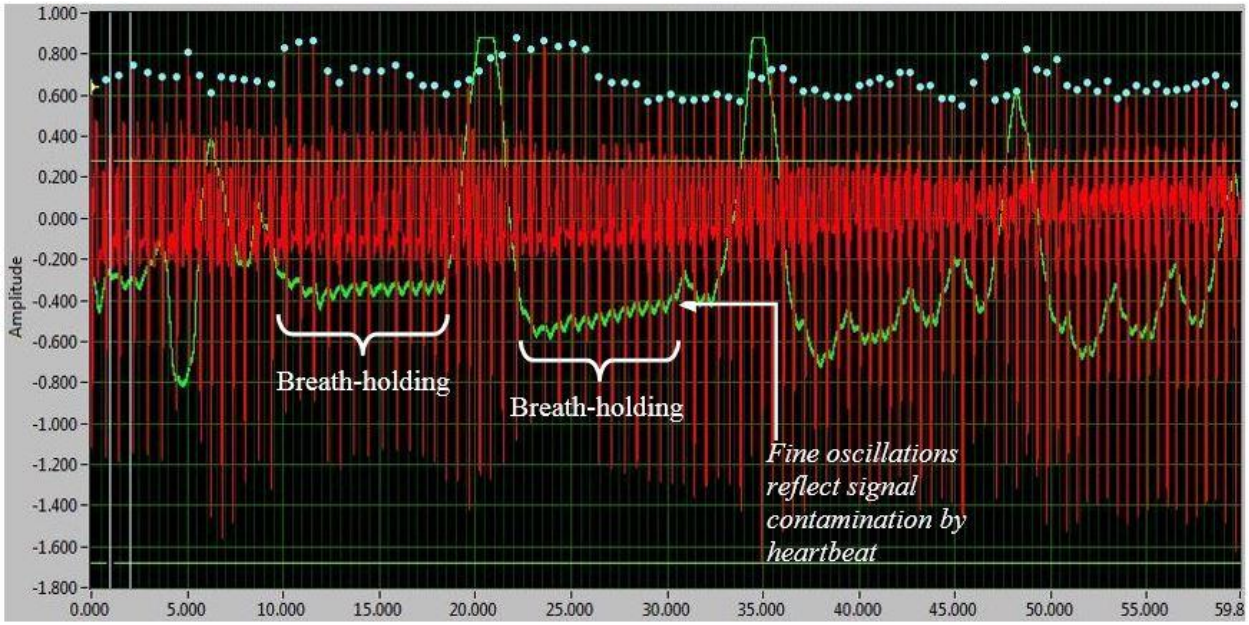


Figure 9-3. Breath-holding during *SPT*.

Another behavior commonly observed during the *SPT* was sighing, which typically refers to large, intermittent inhalations against a general backdrop of shallow breathing. Figure 9-4 illustrates two examples of sighing during the *SPT* epoch, each from a different participant. Note that in the lower image, brief periods of near breath-holding follow each sigh. It is plausible that the statistically significant changes in laryngeal muscle activity were driven by some of these more nuanced respiratory behaviors—for example, TA activity increase in association with periods of breath-holding, and PCA activity increase with each sigh—even if the respiratory rate *per se* is not driving the differences in laryngeal muscle activity. Further and more detailed investigation of the data collected in this study is warranted.

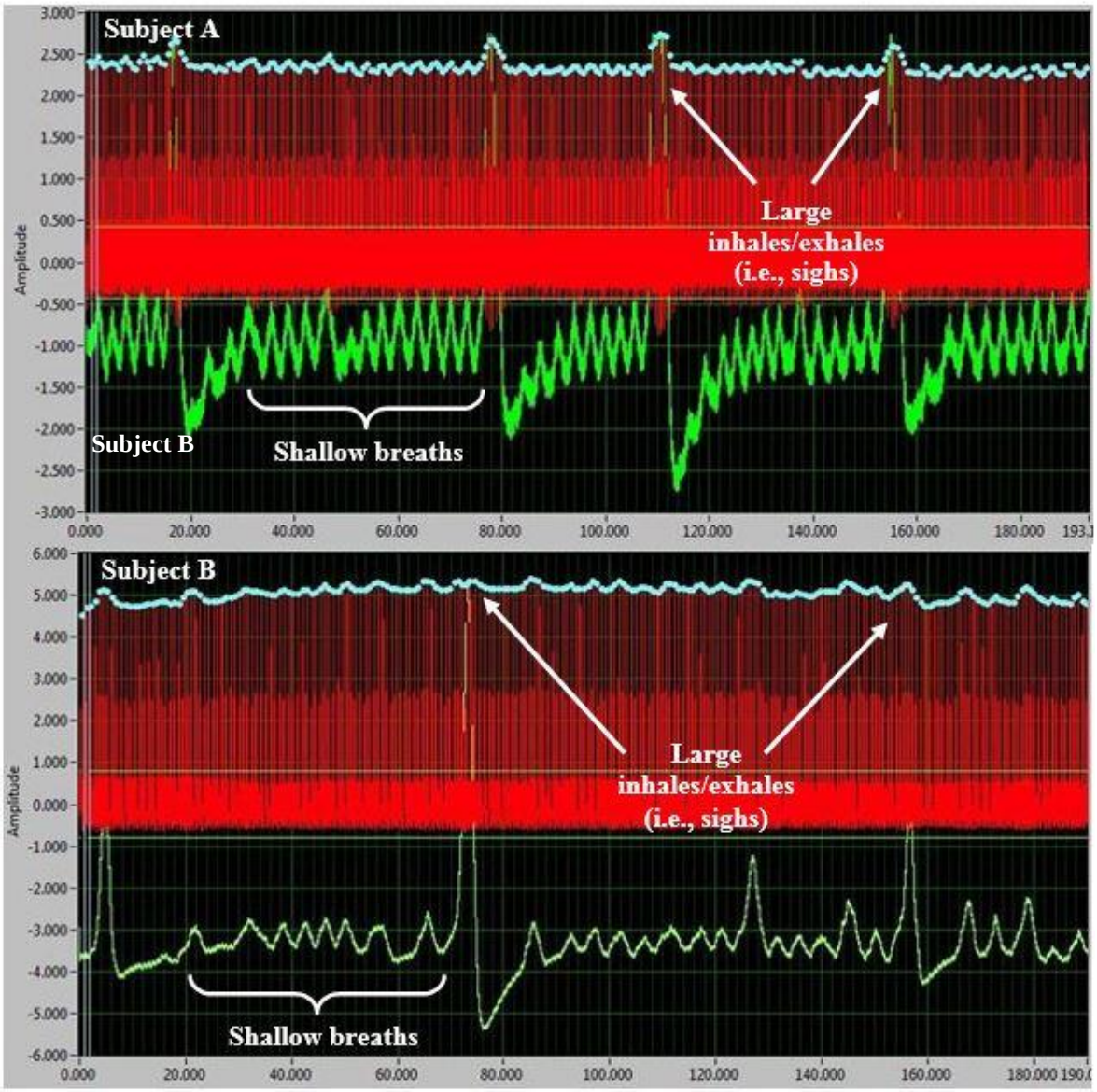


Figure 9-4. Sighing during *SPT*.

9.1.2.3 Recovery latency in laryngeal muscles

Regardless of the magnitude of the stress response in the laryngeal muscles studied, it appears that it does not last longer than a few minutes, at least in a healthy cohort such as the one in the present study. As explained in section 7.5, nearly all muscles studied for all participants had returned to their approximate

Baseline Rest values by the time the Repeat Baseline task commenced. Preliminary data suggested that in most subjects, laryngeal muscle activity following exposure to a physical stressor (the cold pressor task) remained elevated for at least three minutes after the stressor ceased (Helou et al., 2013). This elevation in laryngeal muscle activity remained even after heart rate had returned to normal. It cannot be ruled out that a recovery latency of 1-5 minutes did not actually occur in the present study, as in our preliminary study (Helou et al., 2013). However, it is a limitation of the present study that laryngeal muscle activity was not recorded during the several minutes of debriefing related to the *SPT* (Appendix D), which would have allowed observation of how and when muscle activity returned to baseline.

Nonetheless, given the fact that the women in this study were healthy normals with no apparent history of voice disorders or psychological disorders, it is to be expected that they recover quickly and fully following cessation of the stressor. Even though these participants spanned a fairly normal range of trait stress reactivity and "vagal tone" (i.e. people with both low and high values of each were included), perhaps the most extreme values were insufficiently extreme to effect recovery time in a negative fashion. That is, we might reasonably speculate that some threshold of disorder must be met before the person's ability to recover from the stressor is compromised. The resolution latency measures may have been less homogeneous if measured in a cohort of voice-disordered patients (e.g., patients with muscle tension dysphonia), as observed in studies comparing patients with chronic trapezius myalgia to controls (Sjörs et al., 2009).

Another consideration is that the nature of the experimental conditions prohibited thorough observation of the hypothesized response. We predicted that at least some people would have difficulty recovering from the stressor, as evidenced by taking a relatively long time to return to baseline values after the *SPT* was over (i.e., having high resolution latency values). While we designed the experiment to involve ecologically valid stressors, the act of debriefing may have facilitated more rapid recovery than what would have been observed without debriefing. After all, in "real world" settings where social stressors occur, it is rare that the person experiencing the stress is ever given a clear "green light" to relax

by some authority on the stressor itself. Instead, the person must decide independently when the threat of a stressor is over, and thus that she no longer needs to remain vigilant to the possibility of recurrence of that stressor. In this study, though, participants were promised by the investigator that there were no more needles, no more surprises, no more challenging tasks, and it was our clear intention to completely assuage any fear of additional threat. This important difference between real-world social stressors and our experimental stressor may have impacted participants' ability to return to pre-stressor baseline rapidly and completely.

9.1.2.4 Control muscles' response to *SPT*

The activity of both the positive control muscle (upper trapezius) and the negative control muscle (anterior tibialis) generally increased from *Baseline Subvoc* to *SPT*. The increased activity of the trapezius muscle was anticipated as it is a well-known "stress responder"(Stephenson & Maluf, 2010; Westgaard, 1999), but the increased anterior tibialis activity was somewhat more surprising. Not only was the total number of "responders" in the present cohort comparable for the two muscles, but the anterior tibialis muscle activity had a greater effect size than that of the upper trapezius, despite the fact that the upper trapezius muscle has a lower standard deviation which would help to boost the effect size. The anterior tibialis was specifically selected as a negative control point because it is not "classically" considered a stress responsive muscle, it is not functionally related to any of the tasks used in the present study, and because others have successfully used it as a negative control in methodologically and theoretically similar research paradigms (Dietrich, 2008).

Nevertheless, it is well within reason to anticipate skeletal muscle responses to stress throughout the body. Anderson and colleagues demonstrated evidence of increased sympathetic efferent nerve activity to the leg during mental stress, and that the sympathetic outflow to the leg was substantially greater than that to the arm, in which sympathetic outflow peaked *after* the stressor (Anderson, Wallin, & Mark, 1987). Especially germane to our findings in the anterior tibialis muscle, some investigators have

found that in anxious subjects, muscles high in activity at baseline remained high and exhibited relatively little change during psychological stress, whereas muscles that were low in activity at baseline increased to a greater extent during the stressor (Balshan, 1962; Hoehn-Saric, McLeod, & Zimmerli, 1989). This response was in accordance with the Law of Initial Values (Wilder, 1962) and has been observed in other physiological domains beyond EMG (e.g., see Hoehn-Saric & McLeod, 2000). Since the EMG values in the present study were not normalized to maximal contraction, we cannot investigate further to determine if a similar phenomenon existed in our data set.

A few additional explanations for the unanticipated findings regarding anterior tibialis activity are possible. The most likely reason regards the role of posture in each muscle's response. In the study by Dietrich that used the anterior tibialis muscle as a negative control site, participants sat in a wheelchair with their feet positioned in foot rests throughout the experiment. The downward bearing of participants' leg weight and gravity might have served to neutralize and minimize anterior tibialis muscle activity. Conversely, in the present study, participants' legs were elevated and supported by the extended exam chair, so that the legs were generally horizontal to the floor with a slight bend in the knee allowed for participant comfort. Relative to Dietrich's study conditions, the present study allowed for more free movement of the leg. It should also be noted that apart from systematic reminders by the investigator to "keep your body still and quiet" (these reminders occurred regularly at the beginning and mid-point of each baseline and stressor condition), participants were not specifically asked or advised to keep their legs still.

Also related to the proposal that posture may have contributed to the discrepant findings between upper trapezius and anterior tibialis activity, the reclined position of the subjects may have attenuated trapezius response to stress. For the present experiment, participants were generally reclined at a 120~140º seated angle. Other investigators have shown that the response of the upper trapezius muscle to physical and mental stress is decreased by lying in the supine position (McCann, Wootten, Kadaba, & Bigliani, 1993; Rubini, Paoli, & Parmagnani, 2012), elbow support and/or arm suspension (Schüldt,

1988), and neck and head support (e.g., Yoo, Lee, Jung, & Yang, 2011). Another study that aimed to assess the ability of cervical traction to treat trapezius myalgia conceded inconclusive findings, since once the patients complaining of shoulder pain were put in the supine position to receive the treatment, the electrical activity of the trapezius was "completely silenced" and thus could not be improved upon with treatment (Jette, Falkel, & Trombly, 1985).

Finally, the methods for analyzing anterior tibialis response in the present study differed from those employed by Dietrich. As is common practice with EMG methods, Dietrich normalized each participant's anterior tibialis EMG signal based on their voluntary contraction of the same muscle, thus resulting in a dependent variable that represented that muscle's activity during the stressor as a percentage of its maximum activity. We, on the other hand, used ITSA to compare the entire baseline epoch (e.g., *Baseline Subvoc*) to the *SPT* epoch, thus deriving significance values for each individual and negating the need for normalization procedures. These variations in analysis approach may limit comparability across studies.

As with the laryngeal muscles, the resolution latency of the two control muscles indicated that their recovery was apparently rapid and complete. Nilsen et al. (2007) implemented a reaction time task in healthy participants and found statistically significant increase in trapezius muscle activity, although the effect did not last once the task ceased. Relatively rapid recovery of the trapezius muscle following a stressor was also observed in a group of controls compared to a cohort with chronic trapezius myalgia (Sjörs et al., 2009). These findings are consistent with our proposal that in a healthy cohort, resolution latency may show little variation.

9.1.3 RQ2 – Predicting the laryngeal response

The two sets of independent variables—SRscore and RSA_{CORR_DIFF}, and SRscore and RSA_{RAW_DIFF}—were not correlated, thus the variables were included together in individual multiple regression models for each

muscle of interest. Consistent with RSA literature (Berntson et al., 1997; Grossman & Taylor, 2007; Ritz, 2009), the regression models were substantially impacted by whether the RSA value was corrected (RSA_{CORR_DIFF}) or uncorrected (RSA_{RAW_DIFF}).

9.1.3.1 SRscore and RSA_{CORR_DIFF} values predicting magnitude of change

RSA_{CORR_DIFF} as a predictor of ILM activity. In this model—SRscore and RSA_{CORR_DIFF} predicting the magnitude of change in muscle activity from baseline to stressor—the independent variable RSA_{CORR_DIFF} significantly predicted the activity of all the muscles examined. Specifically, higher values of RSA_{CORR_DIFF} were associated with increases in muscle activity from *Baseline Subvoc* to *SPT*. The direction of this predictive relationship was counter to our original hypothesis that increases in muscle activity would be observed in those individuals with relatively large *decreases* in RSA_{CORR_DIFF}. Recall that increases in RSA ostensibly reflect increased vagal tone/strength, whereas decreases reflect withdrawal of the parasympathetic nervous system. In section 4.1, it was noted that the long-held, classic conceptualization of homeostasis in the face of physiological stress and "fight-or-flight" responses, such as was originally proposed by Cannon (1939), were outdated. However, our hypothesis regarding RSA_{CORR_DIFF} (that increases in this variable reflecting SNS activation would predict greater ILM responses to stress) nevertheless reflects our having fallen into a similar logical trap. The idea that increases in laryngeal muscle tension are "bad" and that they are principally triggered by sympathetic nervous system activity is one linchpin of the proposed pathophysiology in certain voice disorders (e.g., Dietrich & Verdolini Abbott, 2008). However, the present study's results suggest otherwise, in that they indicate that the characteristic laryngeal response in healthy, non-voice disordered women is one of dramatic yet fast- resolving muscular activity increases that may be related to parasympathetic/vagal outflow to the heart. This finding is consistent with the concept of healthy allostatic resilience proposed by McEwen (2002) and discussed in section 4.1. That is, participants in this study generally exhibited physiological flexibility, as is expected in healthy normal individuals. They demonstrated robust

cardiovascular, state, and somatic muscle responses to a potent stressor, and then returned to baseline values once the perceived threat was removed.

One implication for these results relates to the visual observation of "laryngeal hyperfunction" during clinical diagnosis of disorders such as muscle tension dysphonia. For many patients, flexible nasolaryngoscopy and perhaps even the clinical environment might trigger stress, thus the "laryngeal hyperfunction" that can be appreciated on physical exam might partially be due to acute stress. Alternatively or additionally, it may be highly valuable for patients with suspected muscle tension dysphonia to undergo some stress provocation protocol under direct laryngoscopy, to observe the laryngeal muscle response to induced stress. Analagous practices are commonplace in asthma and allergy clinics, and the [cardiovascular] reactivity hypothesis described by Obrist (1981, see section 4.3) also relates to classifying and predicting disease via reactivity measures. After all, the present study confirmed that during psychological stress, antagonistic laryngeal muscles exhibit statistically significant increases in activity, but this study did not actually elucidate the functional result—glottal dilation, constriction, or maintenance—of these muscular changes.

Moreover, RSA experts caution investigators against assuming that what is true for cardiac parasympathetic control is also true for other systems in the body, as it is known that fractioning of responses occurs across organ systems (Grossman & Taylor, 2007; Ritz, 2009). Regarding the airway, solid evidence exists that vagal excitation is associated with smooth muscle constriction and subsequent narrowing of the airway (see review by Ritz, 2004). It appears that the present study is the first to examine RSA changes associated with the intrinsic laryngeal muscles, which play a critical airway valving role. Unfortunately, it remains unknown to what extent the larynx is entrained to the needs of the lungs, and to what extent it "acts on its own behalf". Combining that particular gap in knowledge with the aforementioned fact that the present study did not examine the glottic response to the stressor exposure, the airway status (i.e., dilated versus constricted) of these participants cannot be described empirically. It stands to reason that the larynx would act in concert with the airway, at least as long as the

goals of each system were the same. For instance, during exercise the sympathetic nervous system's outflow peaks, resulting in simultaneous bronchodilation via the smooth muscles of the airway and glottal dilation via the intrinsic laryngeal muscles. However, once goals of each system are considered—for instance, increased intensity of phonation (laryngeal goal) during high respiratory drive (pulmonary goal)—myriad degrees of freedom across the lungs and larynx are introduced, even ignoring interactive supraglottal effects at the level of the articulators and resonators. Germane to the present discussion, laryngeal muscle activity specifically as a function of increased vagal outflow to the airway remains wholly unknown.

SRscore as a predictor of ILM activity. Also in this model, SRscore significantly predicted the activity of the upper trapezius and anterior tibialis muscles, such that for every unit increase in SRscore, a greater magnitude of change in muscle activity was observed from *Baseline Subvoc* to *SPT*. This directionality was consistent with our hypothesis that the higher the SRscore, the greater the activity of the muscles would be. According to the creators of the *MPQ-BF* (Patrick et al., 2002), high scorers on this subscale tend to be tense and nervous, often feel worried, anxious, and emotionally volatile, and feel vulnerable. On the other hand, low scorers are able to quickly recover from upsetting situations, rarely feel emotional turmoil, and can easily set fears and worries aside.

SRscore also significantly predicted the activity of the TA muscle, but the direction of the relationship unexpectedly differed from that of SRscore and the control muscles. For every unit increase in SRscore, an *increase* in TA muscle activity was expected from baseline to stressor. However, the opposite was observed in that SRscore increases predicted a *decrease* in TA muscle activity. Assuming this finding is not spurious, two possible explanations for it are offered. First, the high stress reactivity/low muscle activation relationship is aligned with others' empirical observations of behavioral inhibition as it pertains to voice disorders (Dietrich & Verdolini Abbott, 2012; van Mersbergen et al., 2008). Those studies found that individuals with muscle tension dysphonia scored higher on stress reactivity as measured by the *MPQ-BF*, and also exhibited blunted facial and extralaryngeal muscle

responses to emotion induction. Although the present study involved participants with unimpaired voices and did not utilize an emotion-induction paradigm, its observations are consistent with the notion of behavioral inhibition being intertwined with high levels of stress reactivity.

Alternatively, a very logical yet purely speculative interpretation is as follows. If those individuals with relatively higher trait stress reactivity were to evidence a greater sympathetic nervous system stress response, as might reasonably be anticipated, then one would expect measurable bronchodilation to occur (e.g., Ritz, 2004). Had that sequence of events occurred in the present cohort during the stressor, the resultant diminished activity of the TA might be an instance of bronchosomatic coupling in that the TA "gets out of the way" to further support bronchodilation via the smooth muscles of the airway. Further investigation is warranted regarding this hypothesis.

The prediction of SRscore on the PCA and CT muscles was not statistically significant. Although it is possible that the SRscore indeed has no predictive value in the context of these muscles, it is also possible that the regression tests for the laryngeal muscles were insufficiently powered or the muscle responses were of insufficient magnitude to detect a predictive effect of SRscore on those particular muscles. To this point, the magnitude of change in the TA muscle (which was significantly predicted by SRscore) was the greatest of all muscles examined in this study. Moreover, if SRscore is actually associated with behavioral inhibition during stress as postulated, then it stands to reason that the regression models would be insufficiently powered to reveal this relationship, because so few participants in this study actually exhibited diminished muscle activity during the stressor as compared to baseline.

On the whole, this model matched the original hypotheses fairly well. Of course, it remains unknown how well the RSA_{CORR_DIFF} variable would predict the activity of the laryngeal muscles if it had included the element of tidal volume; this limitation is discussed further in section 9.2.1.

9.1.3.2 SRscore and RSA_{RAW_DIFF} values predicting magnitude of change

RSA_{RAW_DIFF} as a predictor of ILM activity. In the second model—SRscore and RSA_{RAW_DIFF} predicting the magnitude of change in muscle activity from baseline to stressor—RSA_{RAW_DIFF} did not significantly predict the activity of any of the muscles in this model. The effects of respiration may actually obscure the relationship between vagal outflow and muscle activity, even in the muscles with the greatest responses from baseline to stressor. It is important to bear in mind that the signals measured via EMG were miniscule relative to the far more robust heart rate and respiratory signals; for instance, the activity of the intrinsic laryngeal muscles during rest would be measured in tens of microvolts. Respiration can heavily influence RSA within individuals. In fact, even when heart rate remains relatively stable across paced breathing tasks ranging from 8 to 18 breaths/min, up to 55% of RSA variance is due to rate and depth of respiration (rate>depth) (Grossman et al., 1991; Ritz, Thöns, & Dahme, 2001; Ritz, 2009). Thus, it should not be surprising that respiratory effects on RSA might obscure other relationships.

Ritz (2009) reports the concordance within studies that examined both RSA_{RAW} and RSA_{CORR}. Of the ten studies reviewed, eight showed different findings as a function of which RSA variable was utilized, and most of these differences were in the direction of RSA_{CORR} revealing a finding that was otherwise obscured by using RSA_{RAW}. Although the experimental paradigms differ from that employed in the present study—emotion induction, erotic imagery, facial expressions, and forehead cooling versus stress induction—it is obvious that RSA_{CORR} and RSA_{RAW} are two completely different variables rather than slightly adjusted versions of each other. In the present study, the latter measure was an ineffective predictor of muscle activity for any of the sites investigated. Furthermore, in the present study, RSA_{CORR} underwent further calculation in order to reflect changes from one time point to another, resulting in the final measure of $RSA_{CORR\_DIFF.}$ This approach has not been widely used and is likely to further broaden the divide between raw and respiratory-corrected measures.

SRscore as a predictor of ILM activity. In this model, SRscore significantly predicted the activity of both the upper trapezius and anterior tibialis muscles such that increases in SRscore were associated with greater magnitudes of change in muscle activity from baseline to stressor. SRscore did not predict the activity of the laryngeal muscles. We did hypothesize that the upper trapezius muscle activity would be predicted by SRscore, but the significant prediction of anterior tibialis activity was not originally anticipated. Potential explanations regarding the response of the control muscles were delineated earlier (see section 9.1.2.4) and are relevant here as well.

It is possible that no relationship exists between the independent variables and the magnitude of change in the laryngeal muscles, although there may be other explanations as well. It could be that the participants in this normal cohort were essentially "too normal" to observe the anticipated relationship. Also, despite the seemingly large effect sizes for the intrinsic laryngeal muscles, it may be that without the correction for the effects of respiration on RSA, the predictive value of RSA on those variables could not be realized.

9.1.4 RQ2c – Relationship between independent variables

A weak negative correlation between RSA_{CORR_DIFF} and SRscore was predicted, but the two independent variables did not exhibit any statistically significant relationship. Thus, although the two measures can be conceptually linked, they appear to measure fully different phenomena. RSA_{CORR_DIFF} was predictive of increases in ILM activity in the face of stress, whereas SRscore predicted apparent inhibition of TA activity during stress. Thus, these variables provided different information about ILM response in this cohort.

9.1.5 RQ3 – Laryngeal muscle activity due to subvocalization

In the present cohort, the phenomenon of subvocalization at a laryngeal level was generally not observed. Even for the few muscles that did exhibit statistically significant increases from the *Baseline Res*t to the *Baseline Subvoc* condition, the direction of change was not consistent and the average effect sizes were dramatically smaller than those observed for the change from *Baseline Subvoc* to *SPT*. Moreover, the changes were distributed across the participants rather than all being observed in a few participants (i.e., no participant seemed to demonstrate subvocalization consistently in all laryngeal muscles). As in the *SPT* condition, the *Baseline Subvoc* task was designed so that participants had to engage in a nonverbal/nonvocal linguistic task, and were instructed to "address" three specific things as they imagined speaking to an audience. However, the *Baseline Subvoc* task did not include stressful elements such as an emphasized time pressure, videorecording of the participant, mental arithmetic, and other elements of the *SPT* meant to induce stress.

Based on these findings, it seems safe to conclude that the changes from *Baseline Subvoc* to *SPT* are representative of a "stress response" rather than a response related to working memory and/or the linguistic nature of the task. Thus, future studies needn't be concerned about the phenomenon of laryngeal subvocalization as a confounding variable in laryngeal stress responses where linguistic tasks are involved.

9.2 CONSIDERATIONS AND LIMITATIONS

9.2.1 Effects of respiration on RSA measures

The present study failed to include tidal volume estimations in its respiratory-corrected RSA values (RSA_{CORR_DIFF}), which might be considered a substantial limitation with regard to determining how autonomic changes impact the muscles of interest. Nevertheless, some correction for respiration is better than none at all, and while it is widely accepted that correcting RSA values with both rate and volume measures is a more complete approach than using just one of those measures, respiratory rate apparently trumps tidal volume in terms of its power to impact RSA values (Berntson et al., 1997; Ritz, 2009). In addition, the idiosyncratic respiratory behaviors of some participants (such as those whose data are pictured in section 9.1.2.2) should be expected to substantially impact RSA measures and thus would not likely be well-represented by average values of respiratory rate or volume. The behaviors observed during the stressor in some individuals, such as breath holding and sighing, seem to be important idiosyncratic responses to the stressor that should heavily influence RSA (Grossman & Taylor, 2007), and yet cannot be captured or appreciated by that measure. Thus, RSA-related findings in the present study should be interpreted with prudence and attention to the possibility of outstanding respiratory confounds.

The differences in the two regression models (which were each performed across all five muscles) as a function of whether the RSA change scores were corrected (RSA_{CORR_DIFF}) or not (RSA_{RAW_DIFF}) are not necessarily surprising. Accounting for the influence of respiration on RSA sometimes obscures effects, and other times reveals or enhances effects (Ritz, 2009). It is, however, somewhat surprising that RSA_{RAW_DIFF} did not predict the activity of the PCA and TA muscles, but RSA_{CORR_DIFF} did. Given the fact that the PCA and TA are both key muscles in respiratory valving, on the surface, it stands to reason that the model *not correcting for* respiration would better predict the activity of these muscles than the model *accounting for* respiration. However, if Hillel's findings as described in

section 9.1.2.1 apply to the present study, then the participants' pulmonary needs alone were likely insufficient to trigger substantial increases in laryngeal EMG activity. Indeed, correlations of difference scores in respiration rate and muscle activity were all observed to be statistically non-significant. Thus, if the PCA and TA muscle activity were not directly related to the elements of respiration corrected for in the RSA_{RAW_DIFF} variable, then it makes sense that the RSA_{RAW_DIFF} values did not better predict the activity of these muscles than the RSA_{CORR_DIFF} values.

9.2.2 Response specificity

The topics of idiosyncratic stress response patterns and situational stereotypy have been discussed previously herein (see section 4.3). The present study employed only one stressor in an attempt to characterize the laryngeal muscle response. Other studies evoking responses to stressors have suggested that *situational stereotypy* is common (Schneiderman et al., 2005). *Situational stereotypy* refers to the tendency of certain stressors to yield myocardial over vascular responses (or vice versa). For instance, when participants are expected to do something in the stressor, such as in a public speaking task, they must employ active coping strategies and thus myocardial responses are observed to a greater degree than vascular responses. But when the stressor requires vigilance or passive coping strategies and do not require movement (e.g., cold pressor task, watching emotionally laden videos), vascular responses are observed well beyond myocardial responses. The present study did not allow for comparison of laryngeal responses across a variety of stressors (e.g., physical stressors like cold pressor; non-linguistic stressors like mirror tracing tasks).

In fact, it is not immediately clear if the present study would have uniformly elicited active versus passive responses to the stressor, differences in which have been implicated in discordant findings of asthma studies (Lehrer et al., 1996). On one hand, during the speech preparation task in this study, not only was no bodily movement required, but the task included explicit instructions for the participants to

"keep your body still and quiet". On the surface, these methods would seem to be consistent with a passive approach, in which case muscular responses might even have been blunted compared to what might be expected in an overt speech task. More physically or socially active tasks—for instance, having participants stand up during the *SPT*, or actually having participants deliver their prepared speech—would likely yield proportionally larger muscular responses from baseline to stressor. Several considerations guide this hypothesis: (1) physical activity and speech are associated with increased respiratory demand, which might influence somatic motor responses in general and laryngeal responses in particular; and (2) hearing one's own voice during a speech delivery may add a layer of vulnerability to the subject's perceived experience, either because her voice belies the underlying somatic stress response or because vocalization involves one additional layer of potential social threat.

On the other hand, perhaps the task did not yield passive coping strategies by all participants. Although participants were involved in a no-movement, no-voice task, they were under high pressure to prepare a speech that they thought would be imminently delivered. Further, they were under the impression that their performance during this non-verbal task was being judged by experts in nonverbal behavior. These conditions might have triggered more active coping mechanisms. A final consideration is that some individuals might have had to actively suppress their desire to use speech during the speech preparation stressor. Ritz cites conclusions by Rees (1980, in Ritz 2004) that more important in the onset of asthma attacks than the *type of emotion experienced* was whether or not the emotions were *suppressed or inadequately expressed*. If an analogous phenomenon holds true for the upper airway (i.e. larynx) as the lower airway, then suppression of speech (and by definition, then, *voice* as well) during a speech preparation task would be expected to trigger increased activation of the laryngeal muscles (presumably, the adductors). Of course, this is highly speculative and several key discrepancies should be emphasized: the present study involved non-asthmatic individuals and induced stress/anxiety rather than emotional states. However, it is probably safe to say that in the present study, active and passive forces were at play

in a non-homogeneous fashion across participants. Future studies should attempt to measure or control for active and passive coping mechanisms.

9.2.3 Stress responses in the absence of speech

The above proposal that active speech tasks would exhibit even greater muscular responses during the stressor as compared to baseline should be further considered. If—like the trapezius muscle—the intrinsic laryngeal muscles are influenced by posture and speech, it stands to reason that they would show even greater changes during a stressor involving speech in an upright position. It may be that the act of speech or vocalization actually triggers a greater centrally-driven (i.e., psychological) ANS reaction. In terms of mechanics, speech production may trigger increased metabolic demand that increases autonomic reactivity. Further, social elements associated with response vocalization, like performance anxiety or fear of judgment, might also elevate autonomic reactivity.

9.2.4 Sympathetic nervous system activation and contribution to muscular response

Evidence of sympathetic activation during this study's stress-inducing condition was seen in significantly increased heart rate and blood pressure measures. However, further investigation is warranted to more fully characterize the sympathetic response in these participants with respect to the muscular response. Correlational analyses were not conducted between heart rate, blood pressure, and muscle activation change because key assumptions for Pearson's (normality) and Spearman's (monotonic relationship) were violated. Thus, it remains unclear if what seems to be a "laryngeal stress response" is indeed directly related to sympathetic outflow. Information contained in the electrocardiogram, like pre-ejection period or the T-wave, might prove valuable to this end.

However, this very line of thought may be flawed. Berntson, Cacciopio, and Quigley (1991) emphasize that autonomic control is not a continuum extending from parasympathetic to sympathetic outflow. Rather, in the face of stress, a number of modes of autonomic engagement might be seen. For instance, it is possible for both parasympathetic and sympathetic outflow to increase during times of stress. Adding to this consideration the argument by Ritz et al. (2004, 2006, 2009) that parasympathetic outflow to the airway might actually be quite different than to the heart, it is entirely possible that RSA, which indexes *cardiac* vagal outflow, is a poor proxy for *airway* vagal control, thus rendering interpretations of RSA within the context of airway stress responses fallacious. Perhaps the classically-anticipated *withdrawal* of parasympathetic outflow to the heart during stress is actually reciprocally complemented by *increased* parasympathetic outflow to the airway, including to the upper airway comprised, in part, by the laryngeal muscles. The characteristics and boundaries of the autonomic-cardiac-airway relationship remain to be seen. However, the main point here is that the importance and relevance of sympathetic outflow in laryngeal muscle response perhaps should not be assumed. It may very well be that laryngeal stress responses stem from parasympathetic influences—whether withdrawal or engagement—above and beyond sympathetic influences.

9.2.5 Neuroendocrine considerations

The HPA axis' effects were not directly addressed in the present study because it was thought to be unlikely that the HPA axis would be responsible for any effect on the ILMs in this study, since the HPA axis exerts its effects on the body quite slowly. Coordinated HPA responses are typically measured 20-60 minutes after the onset of the stressor (Herman et al., 2003), whereas in the proposed study, the muscular response was measured during a three-minute stressor and for 10 minutes following the stressor, which would not likely be sufficient time for the HPA response to be observed.

Moreover, with regard to the fact that the present study sought to use EMG-derived variables as outcome measures, there is no obvious reason why the HPA axis might influence ILM activity. The HPA axis exerts its effects via neuromodulating proteins throughout the laryngeal mucosa and epithelial lining (Hisa et al., 1999), and none of the extant literature details if or how the HPA axis directly impacts laryngeal muscle activity; presumably, it does not. It seems more likely that the HPA axis' role in laryngeal hyperfunction occurs with prolonged and recurrent exposure to and/or perception of stress, but not in situations such as those utilized in this study. Thus, potential involvement of the HPA axis in mediating results in the present study was set aside.

However, in the spirit of rigorously exploring alternative explanations and confounding variables, a few considerations should be noted. First, participants participated in this study in morning, afternoon, and evening sessions. Cortisol levels follow a diurnal cycle (e.g., they peak in the morning and dip in the early afternoon), and might impact one's response to a stressor. Although we did record time-of-day for each participant's session, this study was insufficiently powered to covary for time of day in statistical analyses. In addition, the present study did not control or covary for menstrual phase, which may reasonably impact stress responses (Duchesne et al., 2012; Kirschbaum et al., 1993). It is possible that some proportion of the participants' stress response was mediated by naturally fluctuating cortisol levels.

A second consideration is that although individual tasks occurred across very short time spans (as mentioned above), stressful elements of the experiment that occurred early on may have impacted stress responses later in the experimental session. For instance, from the beginning of fine wire electrode placement to the time when the stressor occurred, 20-30 minutes had easily passed. Peaks in cortisol production triggered by the stress of having needles in the neck might have arisen around the same time of the stressor, potentially mediating the participants' response to the stressor.

The HPA stress response is profoundly intertwined with social interactions, and a variety of disruption to social relationships and the "social self" will elevate HPA axis activity (Bosch et al., 2009; Dickerson & Kemeny, 2004; Levine, 2000). Conversely, HPA axis markers like cortisol can influence

responses to stressors. For instance, abnormally increased levels of cortisol are associated with socially submissive behavior and social avoidance, whereas higher levels of testosterone are associated with socially dominant behavior (Roelofs, Bakvis, Hermans, van Pelt, & van Honk, 2007). Dickerson & Kemeny (2004) argue that the HPA system may be activated and cortisol released any time that social esteem, respect, or acceptance are threatened. In humans, these threats seem to be analogous to social status threats observed in numerous studies of animals. Thus, a situation involving 'social evaluation' and threats to the social self will elicit a significant increase in cortisol. In fact, the greater then number of forms of social evaluation present, the greater the cortisol response. Furthermore, individuals with characteristics that render them especially sensitive to social evaluation, such as children with poor social competence skills, will exhibit exaggerated cortisol responses to acute stressors. Another example of this is that a stressor such as color-word interference test will not elicit significant changes in HPA activation if performed in a one-on-one setting, but if that task is conducted in the presence of an evaluative audience, a significant cortisol increase is observed (Schommer, Hellhammer, & Kirschbaum, 2003). Given the intertwined nature of the HPA axis and the ANS response to social stressors, future studies with comparable paradigms will be strengthened by controlling for time of day, measuring cortisol levels, and employing other methods that help to account for the influence of the HPA axis over the dependent variables.

9.3 SYNTHESIS

The present study was the first in the extant literature to describe the functional response to psychological stress in a healthy female cohort. Generally speaking, these findings corroborated those of other studies that reflect a global increase in somatic muscle activity during psychological stress. Specific to the larynx/upper airway, it was obvious that the activity of the antagonistic abductor and adductor/tensor

muscles were concurrently increased during psychological stress. About two-thirds of all participants exhibited statistically significant increases in the activity of the posterior cricoarytenoid, thyroarytenoid/lateral cricothyroid muscle complex, upper trapezius muscle, and anterior tibialis muscle. Less than half of all participants exhibited statistically significant increases in the activity of the cricothyroid muscle.

Based on these findings, one overarching conclusion is that increased activity of the intrinsic laryngeal muscles during stress is a normal and adaptive finding. In healthy normal females, the observed spike in muscle activity is substantial but brief, fully resolving within minutes of removal of the stressor. Moreover, it appears that this stress effect is modulated to some extent by the parasympathetic nervous system. The relationship between increased vagal outflow and increased skeletal muscle activity might be explained in the context of bronchosomatic coupling (Obrist, 1981; Ritz, 2004). Although the effects appear to be widespread in the body—they were observed in the laryngeal muscles as well as the upper trapezius and anterior tibialis muscles—the magnitude of the stress response was greatest in the laryngeal muscles, despite the fact that their electrical signals were undoubtedly the smallest and most difficult to fully capture.

In addition, the present study confirms that the phenomenon of "laryngeal subvocalization" is not contributing a meaningful amount of muscle activity during a speech preparation task. This potential competing explanation for laryngeal responses can be reasonably set aside for future studies.

The Trait Theory of Voice Disorders (Roy et al., 2000b; Roy & Bless, 2000a) was neither supported nor refuted by results from this study, as trait Stress Reactivity scores were not clearly associated with intrinsic laryngeal muscle activity. However, from a qualitative/anecdotal perspective, at least some participants exhibited patterns of response during the stressor that were consistent with the Behavioral Inhibition described by Roy et al., as well as by Van Mersbergen and colleagues (2008). These responses included (1) attenuated laryngeal adductor muscle (TA) response to stress induction predicted by increases in trait stress reactivity, and (2) diminished or unchanged levels of muscle

activation despite substantial increases in cardiovascular and/or self-reported stress or anxiety. Of course, clearly eliciting evidence in support of the Trait Theory of Voice Disorders would be more effectively done by examining a voice-disordered cohort, so the tepid findings related to trait Stress Reactivity in the present study are not entirely unanticipated.

Although this study's findings do not support strong conclusions regarding the Trait Theory of Voice Disorders, they may help to further guide other existing frameworks by which we try to explain certain voice disorders such as muscle tension dysphonia. One thought is that future studies aiming to identify a cohort of "laryngeal stress responders" might seek individuals with high RSA_{CORR_DIFF} values, since it seems that vagal outflow is a good predictor of increased laryngeal muscle activity, and high trait stress reactivity as measured by the *MPQ-BF*, since that trait appears to be associated with laryngeal muscle inhibition. In addition, our findings indicate that Dietrich and Verdolini's psychobiological framework of voice disorders (Figure 1-1) should be expanded in several ways. Note that while this study did not test a cohort of patients with muscle tension dysphonia, as is the final node in Dietrich and Verdolini's schematic, we did seek to further understand laryngeal muscular hyperfunction, which is a putative key element of that particular voice disorder. The remainder of this section will propose changes to the psychobiological framework of voice disorders that are based on this study's findings and rationale

from the existing literature. A modified framework is presented in

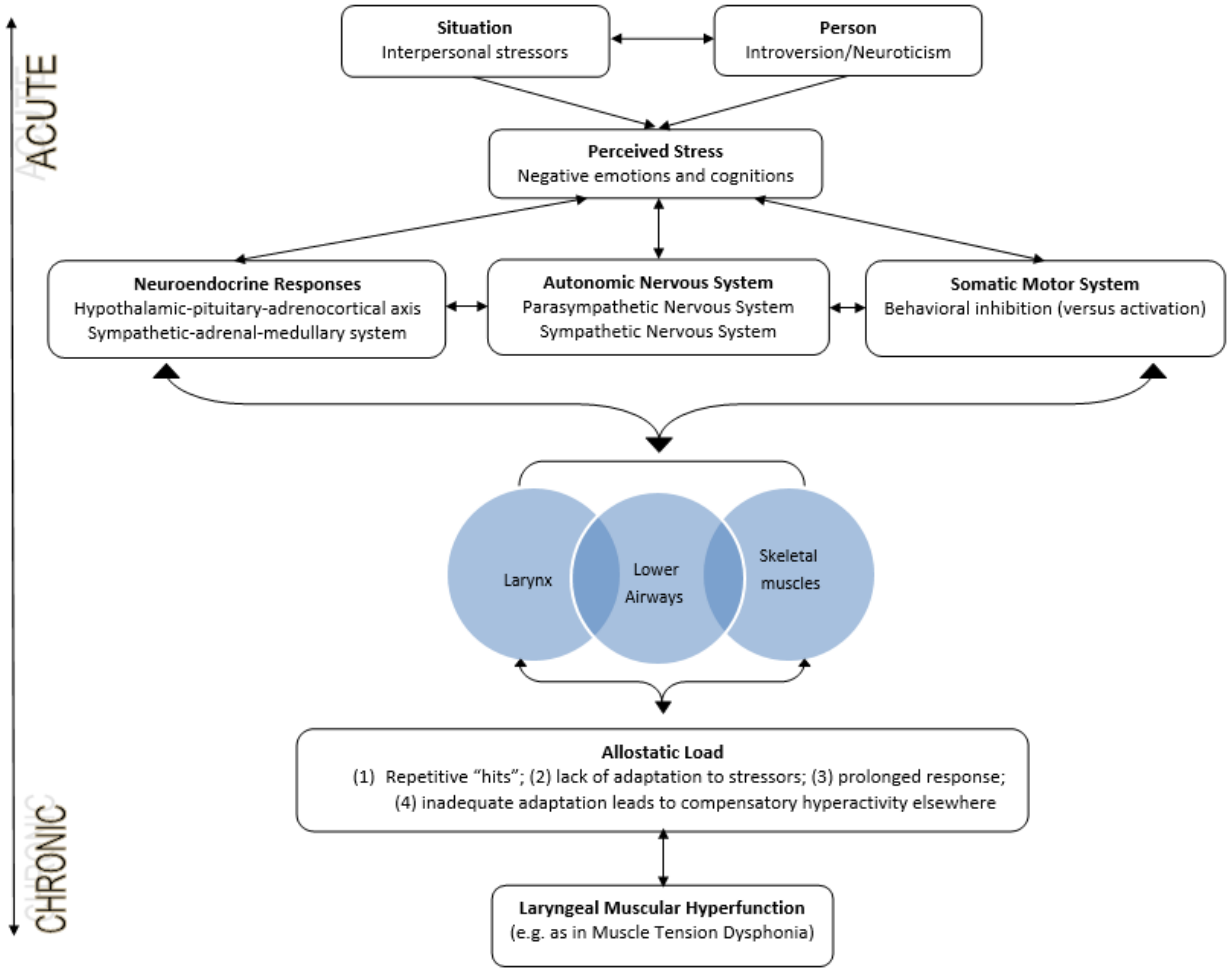


Figure 9-5.

First, it seems clear that the psychobiological framework of voice disorders should **explicitly include the trachea and conducting airways as an effector organ of stress**. A clear respiratory response was observed during the stressor in this study, and this pulmonary feedback may be a secondary driver of the laryngeal muscle response (i.e., above and beyond the "primary" laryngeal response). It remains unclear to what extent the larynx acts "on its own behalf" and to what extent its actions are entrained to the needs of the respiratory and ventilator system. However, it is indisputable that a respiratory response to the stressor employed in this study existed. Based on the structural and functional association of the larynx to the lower airways, the latter should be considered in a psychobiological

framework of voice disorders. Exclusion of the respiratory system ignores the possibility of imbalances across systems and instead fosters the probably erroneous idea that disorders such as muscle tension dysphonia are due to unidimensional (i.e., laryngeal) disruption. The conclusions of the present study point to bronchosomatic coupling, but given the interrelationships between cardiac, parasympathetic, respiratory, and phonatory phenomena, nothing precludes intermittent supremacy of the larynx in this complex equation (e.g., *laryngobronchial* coupling?). To that point, inclusion of the respiratory system would dramatically expand the dialogue surrounding the psychobiological underpinnings of voice disorders, allowing us to test formal theories relating to respiratory imbalances (e.g., hyperventilation theory, suffocation false alarm theory described, for instance, by Blechert, Michael, Grossman, Lajtman, & Wilhelm, 2007).

A second way to modify the existing psychobiological framework for voice disorders is to ***equally* investigate and consider the role of the sympathetic and parasympathetic nervous systems**. Others with interest in voice and stress have emphasized a sympathetic role (e.g., Dietrich & Verdolini Abbott, 2008; Giddens, Barron, Byrd-Craven, Clark, & Winter, 2013), but vagal (i.e., parasympathetic) outflow to the lungs has clear, predictable, and dramatic effects. The present study endorses the notion of a relationship between RSA (as a proxy for cardiac vagal control) and the laryngeal and control muscles'

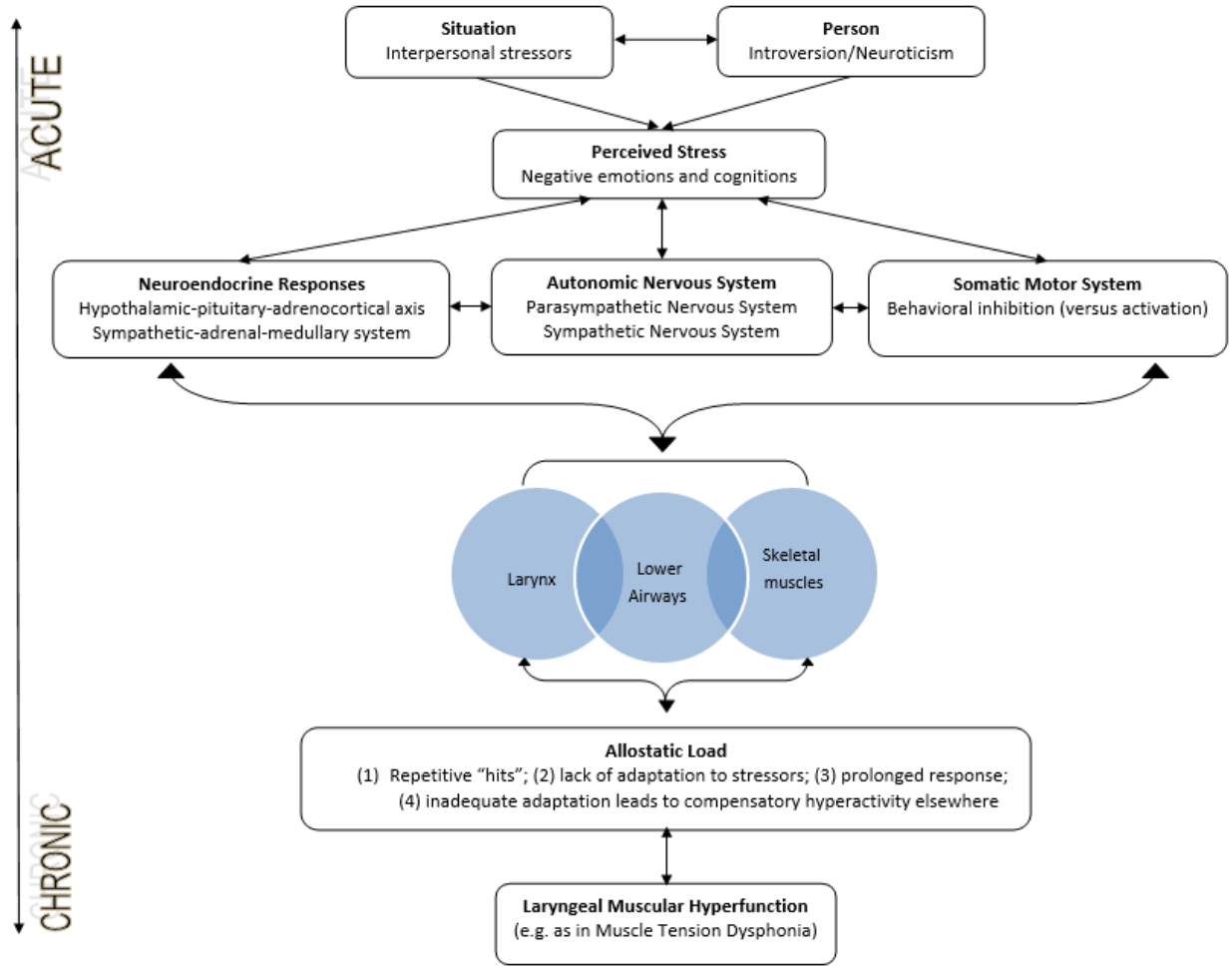


Figure 9-5. Revised Psychobiological Framework of Voice Disorders.

responses to psychological stress. Regression models using respiratory corrected and uncorrected values of RSA (RSA_{CORR_DIFF} and RSA_{RAW_DIFF}, respectively) provided disparate findings regarding how well muscle activity was predicted by a putative measure of vagal (i.e., parasympathetic) tone, although other investigators provide assurance that such dissociations are normal when dealing with RSA (Grossman & Taylor, 2007; Ritz, 2009). However, since the ability of RSA to accurately index actual individual differences in cardiac vagal tone is known to be highly variable (Grossman & Taylor, 2007), because sympathetic activity can modulate RSA (Grossman & Taylor, 2007), and because we did not strictly adhere to best practice measures for respiratory correction methods, conclusions regarding autonomic

tone in this study should be heavily tempered. Nevertheless, this study's findings can allow us to cautiously conclude that increases in RSA_{CORR_DIFF} —which should, in theory, more accurately estimate actual cardiac vagal control than RSA_{RAW_DIFF} —from baseline to stressor are associated with increases in activity of the PCA, TA and trapezius muscles. Thus, the hypothesis that some autonomic role exists in laryngeal muscle response is tentatively endorsed, but the response may be more strongly related to parasympathetic than sympathetic effects.

With regard to the explicit inclusion of the PNS in the current framework, Giddens et al. also recently suggested that it should be considered in the context of voice and stress (2013). They cited the Polyvagal Theory proposed by Porges, which emphasizes the common pathway of the PNS and those muscles of social communication (i.e., the vagus nerve) (Porges, 1995; Porges & Lewis, 2009; Porges, 2007). The Polyvagal Theory does hold that immobilization behaviors would be anticipated in the face of stress, as would be airway constriction (discussed herein), pupillary constriction (not measured in this study), and slowed heart rate (opposite of what was observed in this study). The Polyvagal Theory in the context of the present discussion should be further considered.

A third way to modify the psychobiological framework is to **represent afferent and feedback information by making the relationship between the stress systems and the effector organs bidirectional**. That is, in the original framework, the stress systems (neuroendocrine, autonomic and somatic systems) were shown to have bearance on the effector organ (larynx), but not the other way around. With the lungs and the larynx represented in the schematic, the afferent innervations and feedback loops associated with each organ should be represented by making those relationships bidirectional. By the same token, since it is known that those stress systems might further influence the perception of stress, bidirectional relationships higher up on the schematic are also in order.

A fourth suggestion for modifying the psychobiological framework is to **replace the somewhat simplistic notion of "chronic stress" with the more contemporary concept of "allostatic load"**. Allostatic load is resultant of the effects of the autonomic nervous system, the HPA axis, and the immune

systems. McEwen (2002) proposes four types of allostatic load, as outlined in section 4.1. The normal allostatic response might be dramatic (e.g., large laryngeal muscle activity in response to stress), and it might be sustained for some amount of time before it is terminated. When these normal allostatic processes endure too much "wear and tear"—that is, they fatigue, cease, or fail to disengage—the relevant physiological systems can no longer exhibit resilience to additional or ongoing stress. The concept of allostatic load subsumes that of "chronic stress", but better represents the complex variability that is ubiquitous in nature, and provides more detailed language at the point of that one "node" in the framework.

An additional benefit of discussing laryngeal muscle hyperfunction secondary to stress using the conceptual scaffold of allostatic load can be proposed. The Allostatic Load Model presented by McEwen (1998) dovetails nicely with the Cinderella Hypothesis discussed in section 2.1.1. Both models are related to voluntary muscle disorders and ultimately implicate the lack of rest and recovery as a risk factor for the development of musculoskeletal disorders. Although the trapezius was not as responsive as anticipated in the present study (see section 9.1.2.4 for proposed explanation), it is a muscle highly vulnerable to stress. In addition, the hallmarks of muscle tension dysphonia are similar to those associated with chronic trapezius myalgia (e.g., increased muscular activation, muscular fatigue, dyscoordination, pain).

One final proposition that might further enrich the psychobiological framework is to **incorporate risk factors for laryngeal reactions to stress at the top level of the model**, alongside the person-by-situation interaction. This suggestion is not informed by the present study (and is thus not incorporated

into

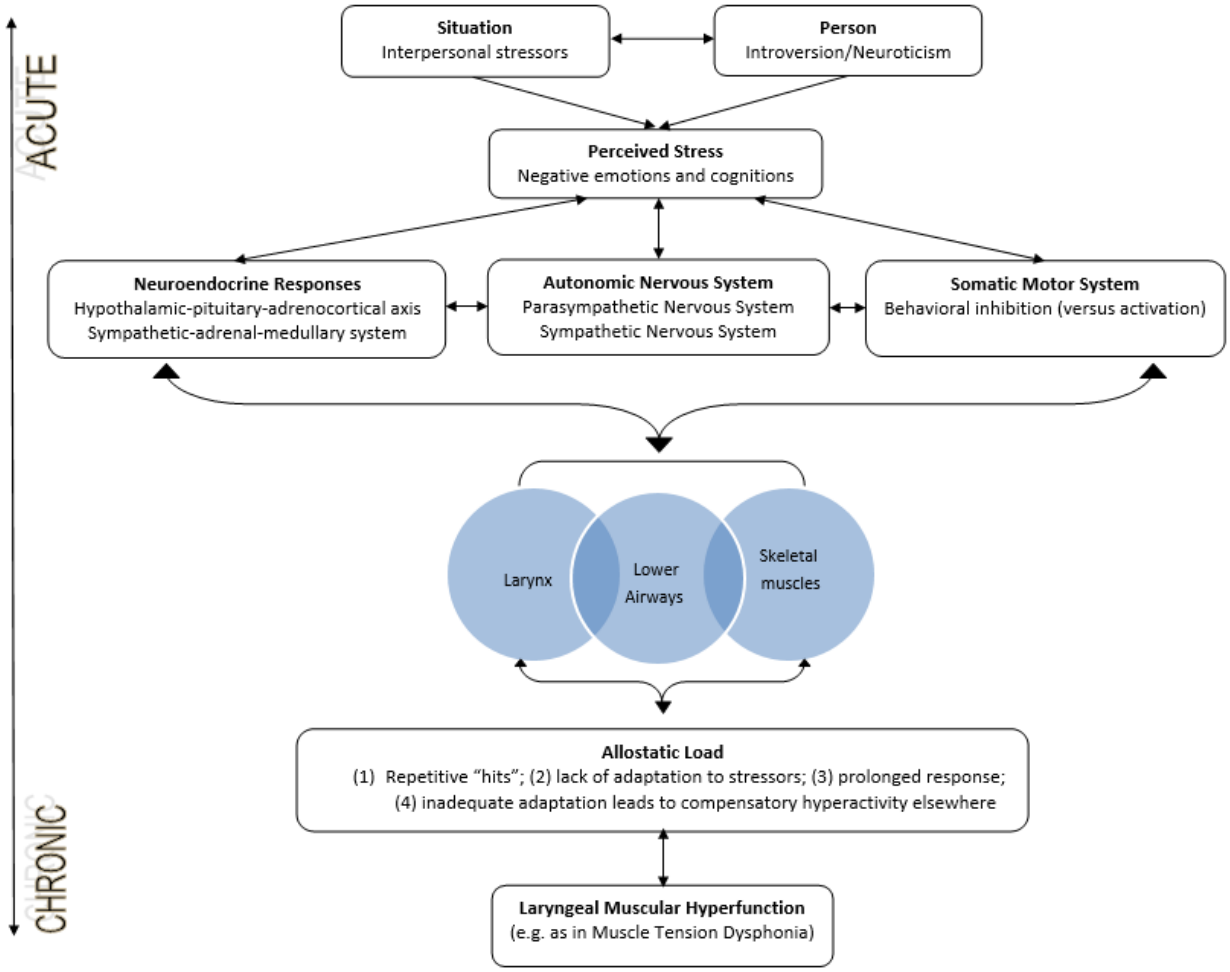


Figure 9-5), but by common sense and clinical experience. The role of environmental and behavioral risk factors in the pathogenesis of hyperfunctional voice disorders is explicit in current patient care models, as evidenced by the standard application of indirect therapy techniques early in the course of voice therapy (Colton, Casper, & Leonard, 2011). Indirect therapy techniques include reducing the amount of talking, reducing vocal loudness, identifying and minimizing vocal misuse, and manipulating the environment among other approaches (Colton et al., 2011). The developers of the Psychobiological Framework of Voice Disorders have noted this issue, but to date, the framework itself has not been adjusted to accommodate the change. Dietrich and Verdolini write that "Introverted individuals appear to possess a unique vulnerability for laryngeal reactions in response to stress. However, this vulnerability

may contribute to a voice disorder only in interaction with other risk factors such as occupational voice use, work stress, an overtaxing personal situation, or poor physical wellbeing. In other words, a person's dispositional vocal behavior must first become counterproductive or dysfunctional in social or professional contexts to manifest as a voice disorder." (2012, pp 984) Since the goal of the psychobiological framework of voice disorders is to reflect the stress-related pathogenesis of voice impairment, it stands to reason that this "make-or-break" factor should be represented alongside the other top-level predisposing elements, Person and Situation.

10.0 FUTURE DIRECTIONS

Several areas for improvement upon the present study's paradigm have already been suggested in the foregoing discussion. Future studies should examine the laryngeal response to a stressor in the context of phonation, and more specifically, speech. This addition would add another layer of ecological validity as well as stress induction, as described previously herein. The further characterization of laryngeal responses to stress in a male cohort and a voice-disordered cohort would be valuable as well. Within a female sample, seeking to parse out the effects of the reproductive cycle on the laryngeal response to stress could also be valuable. Additional short- and long-term possibilities for investigation are outlined below.

10.1.1 Further characterization of muscle activity

Further exploration is warranted to characterize these participants' laryngeal muscle responses in much finer detail. For instance, when examining PCA and TA muscle responses in time, do they actually increase in an on/on fashion (i.e., both increase concurrently) or an on/off fashion (i.e., one turns on while the other turns off, then vice-versa)? Another way to consider these muscles' activity is to explore the question presented by Fridlund et al (1982; 1986) of general tension (i.e., mean increase in tonus) versus agitation (i.e., intermittent, large spikes in activity against a relatively low-activity background). They argued that it is not greater *tension* that characterizes the skeletal muscle response during high-anxiety

tasks, but rather greater *agitation*. Fridlund and colleagues proposed that an elevated general tension factor during anxiety provocation would corroborate inhibitive or immobilizing behaviors, whereas they believed that the higher muscular agitation observed in high-anxiety participants serves to activate, rather than to immobilize or defend. On the other hand, Scherer proposed that the muscles involved in vocalization would be tonically co-activated in the face of a stressor, and that this general elevation of muscle activity was involuntary (Scherer, 1986). He further speculated that the phasic effects, "even if held for a considerable amount of time, are voluntary and may often represent attempts of the organism to control expressive behavior." Neither Fridlund et al. nor Scherer clearly defined the time courses of "tonic" versus "phasic" effects, and it is important to note that they sought to investigate entirely different—yet highly related—constructs (i.e., anxiety and stress, respectively) in non-comparable research paradigms.

Generally, review of waveforms for these muscles across baseline and stressor tasks revealed commensurately elevated ambient/baseline muscle activity in antagonistic muscles, and evidence of phasic on/off modulation was not observed (e.g., as shown in Figure 9-1). However, visual inspection of these waveforms typically compared the envelope of muscular activity in three-minute epochs, perhaps obscuring details that might be appreciated upon closer inspection. Systematic investigation of this question could further clarify and characterize the nature of intrinsic laryngeal muscles' "stress responses".

10.1.2 Personality inventory, perceived stress, and muscle activity

The data obtained from the *Multidimensional Personality Questionnaire – Brief Form* should be explored further to determine whether other factors of this measure seem related to the muscle activity observations in this study. For instance, investigation of broader factors such as Negative Emotionality might lend support to the Trait Theory of Voice Disorders. Another possible trait to explore is that of Social Potency

(another subscale of the *MPQ-BF*), since others have reported that social dominance and persuasive tendencies might pertain to voice sensorimotor control (Dietrich et al., 2012). In addition, descriptive and exploratory investigation of perceived stress and anxiety scales compared to magnitude change of muscle activity might also provide more systematic evidence of Behavioral Inhibition or Behavioral Activation, two key elements of the Trait Theory of Voice Disorders.

10.1.3 Final product: laryngeal protection, muscle oxygenation, or maintenance?

The present study was important in that it clarified that both the abductor and adductor muscles are simultaneously active in many individuals during psychological stress. However, since we did not also visualize the larynx during the stressor, it remains unknown what the "final product" of that concurrent activation was. Stated differently, if both the PCA and TA increased in activity during the stressor, which was a common observation in this study, then which muscles "won"? Some functional response of the larynx to stress must exist, and it is not known whether this response would be seen consistently across subjects, or if people diverged in terms of their reactions to this stressor. This notion is related to the earlier discussion about response specificity (section 9.2.2).

Logically, three options exist. First, the combined forces of these antagonistic muscles may result in glottic closure (i.e., the adductors would win), perhaps to protect the lungs, to build intrathoracic pressure in order to exert pressure against the outside world, to build subglottal pressure in order to sound a call, and/or to facilitate the silence that comes with "holding one's breath" in a state of vigilance. The second option would be that the glottis would open (i.e., the PCA would win) in order to oxygenate the muscles in preparation for fight or flight. The third and final option would be that no change in glottal aperture would be observed, perhaps as evidence of something along the lines of "laryngeal homeostasis".

Future studies might follow a comparable paradigm, replacing or supplementing laryngeal EMG with laryngoscopy. Such an addition would provide more insight into the "functional" response of the

larynx to psychological stress. Most voice clinicians would likely hypothesize an increase in observed "muscle tension" with stress as evidenced by medial compression of the vocal folds on laryngoscopic evaluation (option one).

10.1.4 Further exploration of RSA and electrocardiogram

It would be valuable to re-analyze the present study's RSA data using additional and alternative methods. Specifically, Grossman (2007) summarizes a RSA analysis approach wherein the residuals of RSA during each experimental condition are calculated from within-subject regressions. RSA analysis described for the present study used the paced breathing conditions to calculate RSA_{PRED}, and did not utilize other epochs (*Baseline Rest, Baseline Subvoc, Repeat Baseline*). Including these additional epochs, as well as the *True Baseline* and *SPT* epochs in the respiratory correction procedure would add over 20 minutes of recorded data, potentially improving upon the accuracy of the respiratory correction procedure. In addition, using the residuals for each epoch as the actual value for the independent variable of RSA in the group's regression equations might also improve upon the methodology, since the additional step of calculating a difference score would no longer be necessary.

In addition, repeated-measures analyses of variance of the relevant experimental epochs should be conducted to describe how RSA changed over time in the present study and determine statistical significance of those changes. It might then be most prudent to only focus interpreations on those epochs which are deemed significant in post-hoc analyses.

Another way to explore the data obtained in the present study is to observe the level of entrainment across electromyographic and electrocardiographic signals. For instance, discharge to the heart by vagal efferents is principally blocked during inhalation and active during exhalation (Grossman & Taylor, 2007). To further understand the relationship between RSA and the upper airway (i.e., the larynx), it may be valuable to more explicitly examine the outflow of laryngeal muscle activity and its

relationship with the ECG signal during specific phases of the respiratory cycle. This information could help to inform a relatively large body of literature concerned with the "respiratory gate" concept, which holds that respiration is the gatekeeper to autonomic responsiveness (Eckberg, 2003; Lopes & Palmer, 1978).

10.1.5 Investigating lung-larynx coupling and divergence

Relatively little is known about the relationship between lung and laryngeal function. To what extent is each entrained or coupled to the other? What goals—phonatory, physiologic, cognitive—will cause the natural, healthy balance between the two systems to be disrupted? What conditions will disrupt the balance of these systems in a healthy normal individual, acutely and chronically? Presumably some threshold exists at which point the duration or dose of laryngeal hyperfunction secondary to stress becomes pathological in nature, and better understanding of how and when that occurs is critical.

10.1.6 Understanding laryngeal hyperfunctional voice disorders in broader context

Various aspects of voice disorders characterized by laryngeal hyperfunction (e.g., muscle tension dysphonia) render them roughly analogous to other medically unexplained symptom complexes. For instance, irritable bowel syndrome, fibromyalgia, and chronic migraines are all medically unexplained symptom complexes characterized by a stark female-to-male preponderance, clear connections to stress and anxiety, and associated autonomic complaints and "imbalance". These disorders are also often diagnosed by exclusion of other "organic" pathology. Further studies branching far beyond the field of communication science and disorders should seek to understand so-called "functional" voice disorders within the frameworks of these other symptom constellations, and building upon a robust body of literature pertaining to somatic stress responses, personality traits impacting stress response and recovery,

and other such areas. An approach that is more inclusive of such work will allow voice investigators to "stand on the shoulders of giants" as they continue to develop the theoretical framework for mind-voice connections and disorders of the voice related to such.

Appendix A: **Acronyms Key**

ANS – autonomic nervous system

BP – blood pressure

DBP – diastolic blood pressure

ECG – electrocardiograph

EMG – electromyography

HR – heart rate

ILM – intrinsic laryngeal muscle

MTD – muscle tension dysphonia

PNS – parasympathetic nervous system

RQ – research question

RSA – respiratory sinus arrhythmia

- RSA_{CORR} – RSA corrected for effects of respiration
- RSA_{CORR_DIFF} – RSA_{CORR} change score from *True Baseline* to *SPT*
- RSA_{RAW} – RSA not corrected for effects of respiration
- RSA_{RAW_DIFF} – RSA_{RAW} change score from *True Baseline* to *SPT*
- RSA_{PRED} – RSA value predicted by paced breathing (via within-subjects regressions)

SBP – systolic blood pressure

SEMG – surface electromyography

SNS – sympathetic nervous system

SPT – speech preparation task

TSST – Trier Social Stress Test

Intrinsic laryngeal muscles (ILMs):

CT – cricothyroid

LCT – left cricothyroid

LTA – left thyroarytenoid/lateral cricoarytenoid complex

PCA – posterior cricoarytenoid

RCT – right cricothyroid

RTA – right thyroarytenoid/lateral cricoarytenoid complex

TA – thyroarytenoid/lateral cricoarytenoid complex

Appendix B: **Web Screening Form**

Intrinsic laryngeal muscle stress response: personality and autonomic predictors

Hello!

You have received this form because you have expressed interest in participating in a research study being conducted by the University of Pittsburgh. This research is being conducted to help us determine how the muscles that help us produce voice are responsive during speaking and non-speaking tasks. We are looking specifically for females who meet our eligibility requirements. If you are eligible to participate in this study, you will be asked to participate in a face-to-face screening that may last up to one hour, and a separate visit that may last up to two hours.

WHAT WILL BE INVOLVED
This research study will take place at the University of Pittsburgh Voice Center at Mercy hospital, and your participation will last up to three hours, including some screening procedures that will take place on a separate day. This is an invasive research study, so if just hearing about it makes you very anxious, it's probably not the best study for you. You will be asked to complete some questionnaires that are related to personality. To measure the activity of the laryngeal (i.e., voice-producing) muscles, we will place very fine wires in the neck. Surprisingly, the discomfort associated with this is typically pretty minimal, but to make this as comfortable for you as possible we will inject a small amount of anesthetic before placing the fine wires. You will also be fitted with some non-invasive equipment that measures your blood pressure and heart rate. We may also ask you to engage in a speech task.

WHAT THIS SURVEY IS FOR
To determine if you are eligible to attend an in-person screening, we need to ask you a series of questions concerning demographic, medical, and psychological information about yourself. This survey will take just a few minutes. After you have completed the survey, a member of our staff will contact you to schedule an in-person screening, which may last up to one hour. We will aim to contact you within 48 hours of you clicking "Submit" at the end of the survey.

YOUR PRIVACY
By clicking "Continue" below and providing your personal information, you are allowing our research team to have access to the information that you choose to enter in this survey. Your information will be sent to a password-protected spreadsheet, where we will review it and contact you regarding your eligibility. If you are not eligible to participate in this study, we will delete any information that has been collected. Please note that there is a possibility that the web connection may not be 100% secure. To help protect your information, please ensure that the web address in your browser begins with "https".

To continue with the survey, please press "Continue."

If you have any questions regarding this study or this survey, please contact us at pittvoicelab@pitt.edu, or call (412)228-0406.

Study Title: Intrinsic laryngeal muscle stress response: personality and autonomic predictors
University of Pittsburgh IRB #: PRO12110063
Principal Investigator: Leah B. Helou, M.A.

Continue »

Intrinsic laryngeal muscle stress response: personality and autonomic predictors

Contact information

For us to contact you, we need to know how you prefer to be contacted. Please fill out your preferred contact information below.

First name

Last name

Phone number

Email address

« Back Continue »

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Report Abuse - Terms of Service - Additional Terms

Are you male or female? *

- Male
- Female

« Back Continue »

Are you between the ages of 18 and 30? *

- Yes
- No

« Back Continue »

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Report Abuse - Terms of Service - Additional Terms

Are you pregnant? *

- (•) No
- ( ) Yes

« Back | Continue »

Have you ever had surgery on your neck or thoat?

If you have had minor surgery on your neck or throat, e.g. mole removal, then answer "No"

- (•) No
- ( ) Yes (e.g. thyroidectomy, parathyroidectomy, anterior cervical disc fusion, tracheostomy, or other structurally intrusive procedures)

« Back | Continue »

Have you ever been diagnosed with, or do you suspect that you might have a voice disorder? *

- (•) No
- ( ) Yes
- ( ) Not sure

Have you ever been diagnosed with, or do you suspect that you might have autonomic dysfunction? *

Autonomic dysfunction is also called "dysautonomia". Examples of these types of disorders are postural orthostatic tachycardia syndrome (a condition associated with a large increase in heart rate when you stand up), inappropriate sinus tachycardia (fast heart rate), vasovagal syncope (repeated episodes of fainting in response to certain triggers), neurocardiogenic syncope (episodes of lightheadedness, fuzzy thinking, hot flashes, and other symptoms, which may result in fainting), and orthostatic hypertension or hypotension (increased or decreased blood pressure when you transition from sitting to standing).

- (•) No
- ( ) Yes
- ( ) Not Sure

« Back | Continue »

Have you ever been diagnosed with, or do you suspect that you might have depression, panic disorders, anxiety disorders, or other psychological disorders? *

- (•) No
- ( ) Yes
- ( ) Not Sure

« Back | Continue »

Have you ever been diagnosed with, or do you suspect that you might have a respiratory disorder or asthma? *

- (•) No
- ( ) Yes
- ( ) Not Sure

« Back Continue »

Have you ever been diagnosed with, or do you suspect that you might have hemophelia or other blood clotting/ coagulation disorders? *

- (•) No
- ( ) Yes
- ( ) Not Sure

« Back Continue »

Are you allergic to any anesthesia, especially Lidocaine? *

- (•) No
- ( ) Yes
- ( ) Not Sure

« Back Continue »

Please enter your height in inches. *
(4' = 48"; 5' = 60"; 6' = 72")

Please enter your weight in pounds. *

« Back Continue »

Preliminary scheduling

The questions on this page are not required, but your responses might help to streamline our scheduling process and minimize the amount of communications needed in the future. If you have an extra minute, please consider your availability and indicate when you might be able to attend face-to-face screening sessions.

If eligible, please indicate on which of the dates below you would be willing and able to participate in our research study, which will last ~1.25 hours. (Click all of the dates you could potentially participate in the study)

- [ ] Monday, August 12
- [ ] Friday, August 16

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Intrinsic laryngeal muscle stress response: personality and autonomic predictors

Thank you for your time!

We appreciate you taking the time to fill out this survey. Someone from the University of Pittsburgh Voice Physiology & Motor Learning Lab will contact you by phone or email within 48 hours to let you know if you are eligible to attend an in-person screening. If you are eligible for the face-to-face screening, you will be asked to come to the UPMC Voice Center at Mercy hospital. We will look at your larynx from the inside to make sure that you don't have any abnormalities that would make you ineligible for this study. This involves guiding a thin camera through your nose in order to look at the larynx while you say some sounds. It feels a little odd and is even a little uncomfortable for some people, but it only takes a minute. We will also feel and manipulate your neck to make sure that you are comfortable with the manipulations that will be done as part of the study.

If you wish to contact us, please email pittvoicelab@gmail.com, or call (412)228-0406. Thank you!

« Back Submit

Never submit passwords through Google Forms.

Intrinsic laryngeal muscle stress response: personality and autonomic predictors

Thank you for your time (Ineligible)

You have answered one or more questions in a way that indicates you are not eligible to participate in this study. If you have questions, please feel free to email pittvoicelab@gmail.com.

« Back Continue »

Appendix C: **Consent Form**

(begins on next page)

University of Pittsburgh

School of Health and Rehabilitation Sciences
Department of Communication Science and Disorders

4033 Forbes Tower
Pittsburgh, Pennsylvania 15260
412-383-6540
Fax: 412-383-6555

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

Title: Intrinsic laryngeal muscle stress response: personality and autonomic predictors

PRINCIPAL INVESTIGATOR:

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Email: rosenca@upmc.edu

This study is supported by departmental funds associated with the Department of Communication Science and Disorders at the University of Pittsburgh.

Why is this research being done?

Evidence exists that certain types of voice disorders are linked to psychological factors, such as personality. Unfortunately, relatively little experimental research has been done to clarify this relationship. This research is being conducted to help us determine how the muscles that help us

produce voice are responsive during speaking and non-speaking tasks. The muscles that we are interested in are located in the neck. A measurement called an electromyography (EMG) using thin wires measures the electrical activity of the voice muscles or how a muscle is responding to certain conditions.

Who is being asked to take part in this research study?

People invited to participate in this study must be healthy females between 18-30 years of age. Participants will be ineligible if they have a known current pregnancy, or if they suspect they may currently be pregnant. Participants may not have any of the following: known allergy to anesthesia, particularly Lidocaine; history of voice disorders; history of neck or throat surgery (e.g., thyroidectomy, parathyroidectomy, anterior cervical disc fusion, tracheostomy, or other structurally invasive procedures); history of clinically diagnosed or suspected psychological disorders including depression, eating disorders, or anxiety and panic disorders; history of asthma or respiratory disorders (e.g., obstructive lung diseases such as asthma or chronic obstructive pulmonary disease, restrictive lung disease); history of blood clotting or coagulating disorders such as hemophilia; current upper respiratory illness or seasonal allergies that affect the respiratory system. Obese individuals (i.e., body mass index > 30) may not participate in this study. Also, participants may not have a history of autonomic dysfunction, or dysautonomia. Autonomic dysfunction usually affects different parts of the body and is not the same in everyone. People with autonomic dysfunction may have excessive thirst, excessive fatigue or tiredness, very fast or slow heart rate, feelings of panic or anxiety, or a number of other symptoms. Examples of autonomic disorders include postural orthostatic tachycardia syndrome (a condition associated with a large increase in heart rate when you stand up), inappropriate sinus tachycardia (fast heart rate), vasovagal syncope (repeated episodes of fainting in response to certain triggers), neurocardiogenic syncope (episodes of lightheadedness, fuzzy thinking, hot flashes, and other symptoms, which may result in fainting), and orthostatic hypertension or hypotension (increased or decreased blood pressure when you transition from sitting to standing). Up to 40 individuals will participate in this study.

What procedures will be performed for screening purposes?

We need to look at your throat from the inside to make sure that you don't have any abnormalities that would make you ineligible for this study. This will take place at the University of Pittsburgh Voice Center at Mercy Hospital. This procedure involves guiding a thin camera through your nose in order to look at the voice box while you say some sounds. It feels a little odd and is uncomfortable for some people, but it only takes about one minute. To make it more comfortable for you, we may spray a numbing agent into your nose before passing the camera. This numbing spray does not taste good, and its effects will last for about twenty minutes.

At the same time as this screening procedure, we will feel and manipulate (move) your neck to ensure that we can identify important anatomical landmarks (parts of your neck that are easy to see and feel), and that you will be able to comfortably tolerate manipulations of certain parts of

your neck without difficulty. Also, a medical history will be obtained in order to assure eligibility. We will review the questions that you answered in the phone or web screening to ensure that you are eligible (e.g., not pregnant, no history of asthma, etc).
If you are deemed ineligible based on these screening procedures, you will not be able to participate in this study. The screening procedures will be completed in about fifteen minutes, and the whole screening visit may last up to one hour. You are permitted to stop the screening procedure at any time and withdraw from the study. It is possible that these screening procedures will be conducted on the same day as the experimental procedures, but it is more likely that the two procedures will occur on separate days. We may ask you to complete some questionnaires as part of the experiment, and you may choose to complete these on the same day as the screening procedures if that works best with your schedule.

What procedures will be performed for research purposes?

If you are eligible and decide to take part in this research study, you will undergo the following procedures. All procedures will take place at the University of Pittsburgh Voice Center at Mercy Hospital. These procedures may take up to two hours to complete. You are permitted to stop the research procedures at any time and withdraw from the study.

Completion of Questionnaires: You will be asked to fill out questionnaires that ask questions about how you physically and mentally feel when you are under stress and/or aspects of your personality. These typically take ~30 minutes to complete.

Experimental Procedures, in order of occurrence:

1. While you are lying back in an exam chair, we will place a blood pressure cuff on your arm, and non-invasive surface electrodes (sticky patches) on your shoulder, chest, torso, and leg that will measure electrical and movement activity of your body.
2. We will measure your blood pressure and heart rate for two minutes. We will also measure the other muscle activity via the non-invasive surface electrodes.
3. We will ask you to breathe at four different rates (i.e., a specific number of breaths per minute), for about two minutes per rate. You will get a short break between each condition, and the whole task will take about 15 minutes.
4. An Ear-Nose-Throat doctor (ENT physician) will place up to five fine wires (called fine wire electrodes) into your vocal muscles by guiding them through a thin needle into the neck area. The ENT physician may inject a small amount of lidocaine (anesthetic) into your neck to make placement of the fine wire electrodes more comfortable for you. This will take 10-20 minutes.
5. After the fine wire electrodes have been placed, the investigator will verbally guide you through a relaxation task that should help you to relax. This will take 1-5 minutes.
6. While you are resting, we will record the electrical activity of your vocal muscles via the fine wire electrodes. You will not have to do anything during this period of time, which will last about two minutes.
7. Next, we will ask you to engage in a speech task for a few minutes.
8. Finally, you will rest for 15 minutes while we measure the electrical activity of your vocal muscles via the fine wire electrodes.

What are the possible risks, side effects, and discomforts of this research study?

The possible risks of this research study may be due to the EMG procedures or the experimental task. As with any experimental procedure, there may be adverse events or side effects that are currently unknown and certain of these unknown risks could be permanent, severe, or life threatening. A physician and emergency equipment will be readily available should you experience any adverse reactions from these tasks.

Risks associated with the screening procedures:
The camera that is guided through the nose to examine the inside of the throat is small enough that most individuals feel no discomfort during the exam. However, if you have especially small nasal passages, swollen or inflamed nasal passages, or structural abnormalities (e.g., deviated nasal septum, nasal spurs), you may experience discomfort or even pain during this procedure, or you may have a nose bleed. In the event that you experience discomfort, you should let us know, because we can examine your throat using a different camera that goes through the mouth instead of the nose. To minimize your discomfort, we may spray a small amount of numbing agent (lidocaine) into your nose before passing the camera, we will lubricate the camera with a small amount of gel, and we will try to complete the examination in less than one minute. There is also a very rare chance that the presence of the camera in your nose might lead to something called vasovagal syncope, which could make you feel lightheaded and dizzy. If this happens, the camera will be quickly and gently removed from your nose, and you will rest in a reclined position until you feel better (usually a minute or two). There are no long-term risks associated with the screening procedures.

Risks associated with lidocaine use:
Some people may be allergic to lidocaine, which is a very commonly used numbing agent. In addition to the lidocaine that may be sprayed into the nose for the screening procedures, we will be injecting a very small amount of lidocaine just beneath the skin of the neck at the time of the study. It is relatively uncommon, but minor allergic responses to lidocaine may include burning, itching, or redness of the area where lidocaine was used. Responses most typically occur immediately, but they may also be delayed, showing up a day or so after the lidocaine was applied.

Risks of the EMG procedure:

1. This EMG procedure is a common, standard clinical procedure for people who have certain voice problems. It may cause a tickling sensation in your neck, mild physical discomfort, or even pain. To access one of your vocal muscles with the EMG electrode, the physician will physically rotate your larynx, which may be uncomfortable. While you may experience a stinging "needle sensation" while the lidocaine is being administered, and mild discomfort during EMG electrode placement, it is not expected that the EMG electrode placement will be painful. Most people get used to having the EMG electrodes in their neck within a minute of placement.

2. Infrequent adverse events: A small bruise or redness may arise at the site of electrode placement. In addition, tenderness and soreness of the neck may occur in the 1-2 days following the procedure.

What are possible benefits from taking part in this study?

You will receive no direct benefit from taking part in this research study. This study will contribute to the body of knowledge associated with people who have certain types of voice disorders.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

Neither you, nor your insurance provider, will be charged for the costs of any of the procedures performed for the purpose of this research study. We will not request any information regarding your health insurance.

Will I be paid if I take part in this research study?

You will be paid $50 for your participation in this study. If you begin but are unable to complete the full experiment because of discomfort, pain, or greater-than-expected levels of anxiety, you may be compensated $10 for your time.

Who will pay if I am injured as a result of taking part in this study?

If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

Who will know about my participation in this research study?

Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results.

Will this research study involve the use or disclosure of my identifiable medical information?

No. Apart from the medical history questions that have already been asked as part of the screening procedures for this study, we do not collect identifiable medical information from you or from your medical records as part of this research study.

Who will have access to identifiable information related to my participation in this research study?

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?

The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to your participation in this research study for a minimum of seven years after final reporting or publication of a project.

Is my participation in this research study voluntary?

Your participation in this research study is completely voluntary. Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh.

May I withdraw, at a future date, my consent for participation in this research study?

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. Any identifiable research or medical information recorded for, or resulting from, your participation in this research study prior to the time that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh.

If I agree to take part in this research study, can I be removed from the study without my consent?

It is possible that you may be removed from the research study by the researchers if you appear to be having a stronger-than-expected negative response to any of the procedures. If you are withdrawn from participation in this research study prior to completion of the experimental tasks, you will be compensated $10.

****
****

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations that have occurred during my participation.

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____	_____	_____
Participant's Signature	Printed Name of Participant	Date

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation.

Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____	_____
Printed Name of Person Obtaining Consent	Role in Research Study
_____	_____
Signature of Person Obtaining Consent	Date

Appendix D: **Debriefing Script**

Placement of Electrodes and Respiratory Band

"We are going to place several electrodes on your skin that will measure your heart rate and your muscle activity. They will be on your shoulder, ribs, chest, and leg. We might put a little gel on the electrode to help make the signal strong; it will wipe off easily when we are finished with the study. Also, we will put an elastic band around your torso, which will give us some information about your breathing. Please just try to lie here and relax while we get these items in place." *[Place electrodes and respiratory band.]* "Thank you, everything is in place. Because there are so many wires here, we are going to ask you to stay still—but as relaxed as possible, not stiff or rigid—for most of the experiment. If you need to make any big adjustments to how you are positioned, please let one of us know as we might need to help you do it without pulling on the equipment."

***Stage III* – Paced Breathing**

"For this task, we will ask you to breathe at several different rates (speeds) for two minutes each rate. You will listen to this tone [*play tone for participant to hear*] and inhale when you hear it, and exhale when it is silent. As best as you are able, you'll want to match the rhythm of your breathing to this tone. Let's try the first one."

[Play 8 Hz pacer tone, guide participant as needed to ensure that breathing rate matches tone rate. Begin recording when participant is able to demonstrate the task successfully. At the end of the first two-minute recorded pacing period:] "Great job. Now just lie here and bring your breathing back to your own natural rate for a couple of minutes."

[Allow two minutes of rest, then introduce the remaining three pacing tones in order (10.5, 13, 18 Hz) with a two minute rest between each condition, and ending with a two-minute rest.]

***Stage IV* – Baseline Values**

"Nice job. We are finished with the breathing tasks, but we will keep that elastic band on your torso for the rest of the study. Are you comfortable?" [*Allow participant to make physical adjustments if needed.]* "For the next few minutes, we want you to just lie here and try to remain still and relaxed." [*Draw participant's attention to laptop computer with video of neutral emotional stimuli.]* "Try to just watch this video and allow your attention to be on it for the next few minutes. Please refrain from talking, coughing, clearing your throat, and otherwise using your voice." [*Begin recording when participant is settled and still.]*

***Stage V* – ILM Electrode Placement**

"Next, we are ready to place the fine wire electrodes in your neck muscles. Dr. Rosen will start by putting some lidocaine, a numbing medicine, under the skin of your neck with a small needle. That might burn just a little at first. Then, he will walk you through the placement of the electrodes. It's normal to be a little nervous, so if you need a little break just let us know. I'll stay right here." [*Begin electrode placement*.]

Voluntary Contraction$_{PRE}$ tasks:

1) Sniff three times (PCA) *one per second, investigator will pace with her fingers*
2) Valsalva three times (TA/LCA) *hold for one second, rest for one second; investigator will pace with her fingers*
3) Glide from a low note to a high note three times (CT)
4) Sustained "eee" in normal speaking voice (TA/LCA)
5) Flex "toes to your nose" (anterior tibialis)
6) Raise straightened left arm 90º in front of body, meet investigator's hand and give resistance against it (upper trapezius)

***Stage VI* – Recovery**

"You did an excellent job, and now we are all done with needles. Hopefully you're not really noticing those little wires now, or you'll stop feeling them soon. If anything, you might feel a little bit of pressure from time to time, but you shouldn't feel pain. Let me know if you're uncomfortable. Let's take a couple of minutes to just relax after having those electrodes placed. You can focus your attention on this video again, and just lie here silently and try to relax your body and your mind." [*Monitor heart rate and blood pressure, move to Stage VII when both are near baseline, or after five minutes. If needed after three minutes have passed, provide brief guidance to encourage relaxation (e.g., "try to slow your breathing and relax your feet…relax your hands and arms…relax your jaw, et cetera) according to standard clinical relaxation protocol.]*

***Stage VIIa* –Baseline Rest**

"You're doing fine. Just continue to lie here quietly while keeping your body still and focusing on the video. This task will last for about three minutes." [*Record all channels for three minutes. After the first and second minute of the task, quietly say, "Keep focusing on the video while keeping your body still and quiet."*]

***Stage VIIb* –Baseline Subvoc**

*"*Nice job. For this next task, you're going to imagine that you're talking with a small group of 3 or 4 people with whom you feel very comfortable and at ease. You will not be actually using your voice or talking, but rather, you'll be imagining that you are telling these people about your dream job. Here is a short list of things you might want to imagine talking about [*Investigator draws participant's attention to list of prompts, which is taped to the side of the laptop for the duration of the task*]: what your dream job entails, the *who, what, where,* and *when* details of your dream job, and what you hope you will accomplish in your dream job. Remember, your goal is to imagine that you're talking for the full three minutes, but you will ultimately keep your body still and quiet, and refrain from talking. Ready to get started?" [*Record all channels for three minutes. After the first and second minute of the task, quietly*

say, "Keep imagining that you are talking about your dream job, while keeping your body still and quiet."]

Stage VIII **– Speech Preparation Task (SPT)**

Investigator #1 preps participant for task: "Okay, we're ready now to begin preparing for your speech task, which you'll do here, in the same position you've been in for the other tasks. For this task, you will have three minutes to prepare a speech, which you'll deliver to four professionals. [Investigator #2's name] is getting in contact with your audience now, as they'll be observing you via Skype." [*Investigator #2 begins preparing laptop and pre-recorded file of professionals in the background while participant is prepped for the task*.] "Two of these professionals specialize in non-verbal behavior, meaning the things you communicate without talking. They will be taking notes about your non-verbal behavior while you prepare your speech. All of the professionals will score your speech performance, and your ability to communicate your ideas successfully in a social situation. We have a few things you'll need to be sure you cover in your speech, and they'll check to make sure you address each point. Any questions about what I just said?" [*Allow participant to ask questions.*]

"Your speech will consist of trying to successfully convince your 'interviewers' that you are the best candidate for a competitive job as an assistant to a powerful executive. The job pays very well, $35/hour. You'll have to convince the 'interviewers' of your good communication skills, ability to think on your feet, and forward-thinking nature by addressing each of these points. [*Investigator #1 presents the poster with the three items of discussion listed, which are taped to the side of the laptop for the duration of the task, and reads the questions to the participant in order.*] Do you have any questions?"

"While you are preparing your speech, you are to remain here and lie still, and you may not talk at all. You'll have to do all of your speech preparation in your head. [Investigator #3's name] will be videotaping you while you are preparing and giving your speech, and because we are interested in nonverbal and verbal communication, your video will be shown to an undergraduate course so that they

can score your behaviors in the same way as the four professionals. Do you have any questions?" [*Allow participant to ask questions.*]

"Okay, let's begin. I think that [Investigator #2's name] has connected with your audience, and they are ready to go. [*Investigator #2 positions the laptop to face the participant for the first time, and all four professionals are presented on the screen.*] They are not allowed to talk to you, as I also mentioned, once we get started, you are also not allowed to use your voice or talk. Ready to go? Here's your timer, let's get started." [*Set timer for three minutes and begin task.*]

AFTER SPT – Subject debriefing*, to be conducted immediately after conclusion of the SPT (Stage VIII of study flowchart) and prior to initiation of the Repeat Baseline Phase (Stage IX of study flowchart):*

"Thank you for your attention to this public speech preparation task, you did a great job. At this point, we will NOT ask you to deliver a speech to the four professionals who have been observing you. As you may know, scientific methods sometimes involve withholding complete information about the research until after certain parts of the study are completed.

"One thing that is stressful for a lot of people is the thought of giving a public speech, and we have been interested in observing how your voice muscles respond to the stress associated with preparing for a public speech. Of course, for that to be stressful, you have to actually *believe* that you will be giving a speech. However, as you have noticed, we have asked you to remain silent throughout this experiment so far, because we are actually interested in what your voice muscles are doing *in the absence of* voice use. So having you follow through with the speech would violate our desire to observe your voice muscles at rest.

"So we led you to believe that you would give a speech, and that part of your payment would depend on your performance, in hopes that this would trigger some amount of stress. In fact, you were not being video recorded, and my colleagues who were observing you did not score your performance.

Do you understand why keeping this information from you–that you would NOT be giving a public speech—was deemed necessary for this study? [*Allow participant to ask questions for clarification.*]

"At this point, I have no more surprises for you. Now that I have explained this, are you okay to continue with the rest of the experiment? We would just like for you to lie here and rest, without talking, for the next 15 minutes." [*Allow participant to respond in the negative or affirmative that she is willing to complete the experiment.*]

[*At this point, providing the participant has indicated her consent, continue with the experiment.*]

Note: Notice that this disclosure does not include an explanation that the four professionals who were "observing" were pre-recorded. If participants specifically ask about this detail, we will fully disclose that the seemingly live observers were pre-recorded. However, in the event that participants disclose the element of deception to other participants, it may still be expected that mere observation could still trigger the desired stress response (although it would likely be attenuated).

Voluntary Contraction$_{POST}$ tasks: sniff, valsalva, pitch glide, sustained "eee", toe flex, arm raise (all as in Voluntary Contraction $_{PRE}$)

***Stage IX* – Repeat Baseline (Post-SPT)**

"Continue to keep your body still, refrain from using your voice or talking, and let your attention stay mostly on this video. You will sit like this for the next 15 minutes, and then we will be finished."

Voluntary Contraction$_{FINAL}$ tasks: sniff, valsalva, pitch glide, sustained "eee", toe flex, arm raise (all as in **Voluntary Contraction**$_{PRE}$)

Fine Wire Electrode Removal: "I need to gently pull these wires in order to remove them – you will feel a little tug." [*Remove the wires and surface electrodes, clean skin, etc.*]

Appendix E: **Self-Perceived Stress and Anxiety (Visual Analog Scale)**

Instructions verbally provided by investigator: *"I would like for you to rate the amount of stress and anxiety that you feel on the lines below. This portion of the line [point to leftmost boundary] represents "no stress at all" or "no anxiety at all." This portion of the line [point to rightmost boundary] represents "more stress or anxiety than I have ever experienced." After each task, I'll ask you to rate how much stress and anxiety you felt during that task, and you can just mark the point on the line that represents how much stress/anxiety you felt."*

- CONTINUED ON NEXT PAGE -

Item 1 - After equipment placement

How did you feel when we were placing all of this equipment?

STRESS: Not stressed at all _____ More stressed than ever before

ANXIETY: Not anxious at all _____ More anxious than ever before

Item 2 - After Stage III

How did you feel when you were doing those breathing tasks?

STRESS: Not stressed at all _____ More stressed than ever before

ANXIETY: Not anxious at all _____ More anxious than ever before

Item 3 - After Stage IV

How did you feel when you were resting here quietly?

STRESS: Not stressed at all _____ More stressed than ever before

ANXIETY: Not anxious at all _____ More anxious than ever before

Item 4 - After Stage V

How did you feel when we were placing the electrodes?

STRESS: Not stressed at all _____ More stressed than ever before

ANXIETY: Not anxious at all _____ More anxious than ever before

Item 5 - After Stage VIIb

How did you feel when you were imagining yourself talking about your dream job?

STRESS: Not stressed at all _____ More stressed than ever before

ANXIETY: Not anxious at all _____ More anxious than ever before

Item 6 - After Stage VIIa

How did you feel when you were resting here quietly?

STRESS: Not stressed at all _____ More stressed than ever before

ANXIETY: Not anxious at all _____ More anxious than ever before

Item 7 - After Stage VIII

How did you feel when you were preparing for your speech?

STRESS: Not stressed at all _____ More stressed than ever before

ANXIETY: Not anxious at all _____ More anxious than ever before

Item 8 - After Stage IX

How did you feel when you were resting here quietly?

STRESS: Not stressed at all _____ More stressed than ever before

ANXIETY: Not anxious at all _____ More anxious than ever before

Appendix F: **Permission for Research Use of *MPQ-BF***

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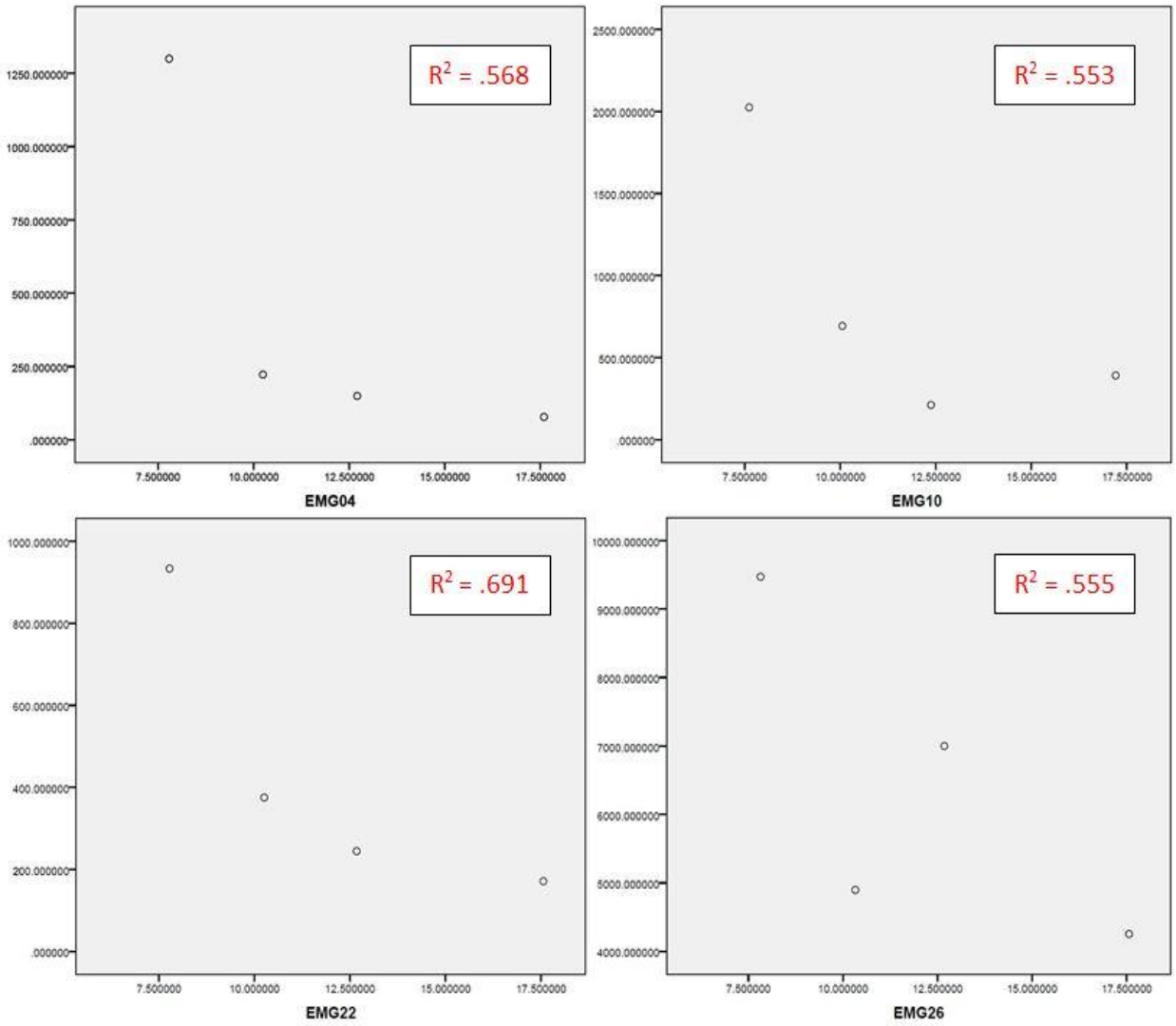
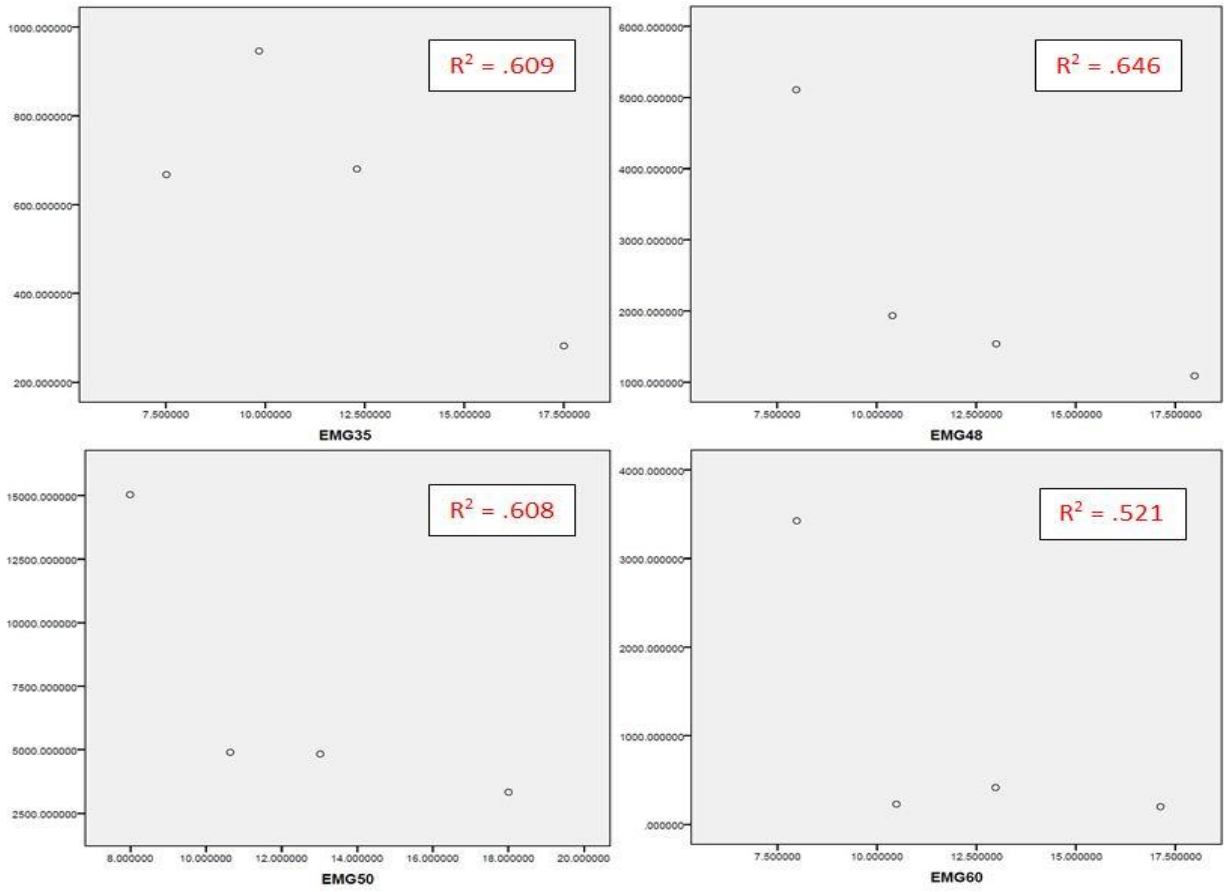
Appendix G: **Paced Breathing Scatterplots for Subjects with R^2 Scores <.70**

Y-axis represents high-frequency variability of the heart rate signal (i.e., RSA value). X-axis represents respiratory rate.




R² = .609
EMG35
R² = .646
EMG48
R² = .608
EMG50
R² = .521
EMG60

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