THE INFLUENCE OF STRESS ON THE VOICE

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ABSTRACT

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Although stress has been frequently attributed to voice disorder development and progression, little work has been done to determine the role of activation of the two major stress systems [the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis (HPA)] on changes in voice production parameters.

Nineteen healthy female participants (median age: 18; range: 18 to 23) were subjected to the Trier Social Stress Test protocol. Voice production parameters (average airflow, estimated subglottal pressure, laryngeal airflow resistance, open quotient from the EGG signal, speaking fundamental frequency, and percent of syllables produced in vocal fry) were measured at seven measurement time points (2 before the stressor, 1 after an anticipatory period, and 4 after the stressor). Participants rated their levels of stress and nine emotions and provided saliva samples at each measurement time. Salivary cortisol and salivary alpha-amylase were measured from the saliva samples.

Ten of the 19 participants experienced a minimum 2.5 nmol/l increase in salivary cortisol levels from before the stressor to after the stressor, indicating that they had HPA axis activation. There were no significant changes in aerodynamic or electroglottographic measures over the seven measurement time points. There was a significant increase in speaking fundamental frequency before the stressor and a reduction in fundamental frequency after the stressor. Estimated subglottal pressure and laryngeal airflow resistance measures were significantly higher in participants who did not experience an HPA axis response.

The findings of the current study further support the body of literature that has reported mainly individual changes in voice production parameters following stress. However, the addition of salivary cortisol measures in the present study revealed the novel finding that there are consistent voice production differences between participants who experience HPA axis activation and those who do not. The higher estimated subglottal pressure and laryngeal airflow resistance measures in the group of participants who did not experience HPA activation overlap with pressures and resistances reported for those with voice disorders. Based on this, it is postulated that HPA axis response may be related, either through behavioral or physical adaptations, or personality factors, to the development of voice disorders.

This dissertation is dedicated to

“Papa” Copenhagen & “Bah Humbug” Frazer

and

my husband, Martin.

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Martin: I love you! You know what you did and you know what I won't say again even if I mean it more now!

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ABBREVIATIONS

ACTH	Adrenocorticotropic hormone; Corticotropin
ANOVA	Analysis of variance
AVP	Vasopressin; Arginine vasopressin
CRH	Corticotrophin-releasing hormone
CT	Cricothyroid muscle
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram
ENT	Ear, nose, and throat doctor
HF	High frequency components of the electrocardiogram
HPA	Hypothalamic-pituitary-adrenal; Hypothalamus-pituitary-adrenal
HRV	Heart rate variability
ILP	Nucleus intermediolateralis pars principalis
LCA	Lateral cricoarytenoid muscle
LF	Low frequency components of the electrocardiogram
MyHC	Myosin heavy chain
PCA	Posterior cricoarytenoid muscle
SAM	Sympatho-adrenal medullary system
SNS	Sympathetic nervous system
TA	Thyroarytenoid muscle
TSST	Trier Social Stress Test
VAMS	Visual Analog Mood Scales ™

CHAPTER I: INTRODUCTION

Some people may develop a voice disorder due to "environmental stress and interpersonal conflict" and others may not (Aronson, 1990, p. 121). Early work has suggested that there may be a relationship between a specific type of interpersonal conflict, called "a conflict over speaking out," and the development of functional voice disorders (Baker, 2010; House & Andrews, 1988). A "conflict over speaking out" often occurs after life events such as infidelity or violence against the individual (Baker, 2010; House & Andrews, 1988). Nevertheless, this theory is incomplete because there are many people who experience such a conflict who do not develop voice problems, who develop different problems, and who do not develop any problems at all (Aronson, 1990; Deary & Miller, 2011).

Some factor or factors must be different between the people who experience stress and do develop a voice problem, and people who experience stress and do not develop a voice problem. People who develop voice problems may have vocal vulnerabilities. Indeed, organs with a high risk for problems may experience more behavioral changes during stress (Lovallo, 2016). In addition, recent work has contended that people with voice problems may have differences in their stress level and how they react to stress (Gassull, Casanova, Botey, & Amador, 2010; Holmqvist, Santtila, Lindström, Sala, & Simberg, 2013; van Mersbergen, Patrick, & Glaze, 2008).

Stress reaction

Definitions of stress must include the stress stimuli, the objective and subjective processing of the stressor, and the response to stress (Levine, 2005). A "conflict over speaking out" is a stress stimulus that will result in a psychological stress reaction. A psychological stress

reaction occurs when the stress stimulus is in the external environment. The processing of the stress stimulus results in a top-down activation pattern, where the brain sends projections to the body (Lovallo, 2016). The response to stress may result in a change in how the body works in the short- and long-term because of what it means to the individual (Kollack-Walker, Day, & Akil, 2000; Lovallo, 2016). The goal of the stress response is to return the body to homeostasis.

There are two stress response systems of the body: (1) the sympathetic nervous system (SNS), including the sympatho-adrenal medullary system (SAM), and (2) the hypothalamic-pituitary-adrenal axis (HPA). The SNS is activated by a challenging situation that one believes that he or she can put effort forward to master (Kudielka, Hellhammer, & Kirschbaum, 2007). The HPA axis is activated in response to a stressor that may be perceived as uncontrollable or gives one a sense of helplessness (Kudielka et al., 2007) and proves to be a threat to a goal to which the individual has committed (Dickerson & Kemeny, 2004). The largest activation of the HPA axis occurs when the stressor includes a social-evaluative component (e.g., a group evaluation) and the stressor is perceived as uncontrollable by the person processing the stressor (Dickerson & Kemeny, 2004).

When the SNS is activated, norepinephrine is released from neurons to act on target tissues. When the sympathetic nervous system is activated, the SAM system is also activated, and epinephrine and to a lesser extent norepinephrine are released as hormones into circulation. In addition, salivary alpha-amylase, a protein in the saliva, increases in the saliva when the autonomic nervous system is activated (reviewed in Nater & Rohleder, 2009). When the HPA axis is activated, levels of the hormone cortisol increase. Stress hormones play a primary role in mediating the body's response to a stressor in an effort to return the body to homeostasis.

Stress and voice

According to Holmqvist et al. (2013), people who indicate that they feel nervous or tense when they must speak and feel generally strained or exhausted (not strained or exhausted in relation to speaking) report more frequently occurring voice symptoms. When people feel nervous or tense when they must speak, they may feel an inability to control consequences or a sense of helplessness, and a sense of being judged by others (social evaluation).

All of the aforementioned factors lead to increased activation of the hypothalamic-pituitary-adrenal axis (HPA axis) (Dickerson & Kemeny, 2004; Kudielka et al., 2007). The HPA axis regulates glucocorticoid hormone synthesis and release (e.g., cortisol creation and release into circulation). Thus, it may be expected that people who have nervous and tense feelings (emotions) when they speak may have higher cortisol levels because of increased HPA axis activation (Holmqvist et al., 2013). This may lead to changes in the voice. Indeed, people with higher cortisol levels measured at one point in time have more self-reported voice symptoms (Holmqvist, Santtila, Johansson, Westberg, von der Pahlen, & Simberg, 2015; Holmqvist-Jämsén, Johansson, Santtila, Westberg, von der Pahlen, & Simberg, 2017). In addition, people who experience a great increase in cortisol levels have a higher fundamental frequency than before experiencing the stressor (Pisanski, Nowak, & Sorokowski, 2016).

When examining the influence of activating the sympathetic nervous system alone on the voice, Giddens, Barron, Clark, and Warde (2010) and Alvear, Barón-López, Alguacil, and Dawid-Milner (2013) did not find any significant changes in vocal acoustics, but Plien (2014) did note an increase in fundamental frequency. Studies of the influence of HPA axis activation on the voice have reported contradictory acoustic changes; some studies have noted an increase in fundamental frequency (Johannes et al., 2007; Rothkrantz, Wiggers, van Wees, & van Vark,

2004; Tse, Wong, Whitehill, Ma, & Masters, 2014) and one other has found a decrease (Dietrich, 2008). Although these studies and others provided measures to suggest an increase in activation of the SNS, such as heart rate and blood pressure measurements, these studies do not provide a biological measure to support that the participants are experiencing changes in the HPA axis activity. Consequently, the differences in the acoustic findings may be because the participants are not experiencing equal activation of the HPA axis by the laboratory procedures (Pisanski et al., 2016). It has thus been suggested that biological measures of stress system (or systems) under investigation should be collected to provide information on the amount of stress the person is experiencing (Pisanski et al., 2016). In addition, unfortunately the current literature does not lead to a greater understanding of the underlying mechanistic changes caused by stress that may be revealed from measuring vocal aerodynamic parameters (Dietrich, 2008).

Research questions

The proposed research is a study of acoustic and aerodynamic voice changes in relation to reactivity to an acute, social-evaluative (psychological) stressor. By taking biological measures of stress (salivary cortisol and salivary alpha-amylase), the research questions can address reactive changes in these measures as they relate to vocal parameters. The present study asks the following research questions:

1. How does an acute, social-evaluative stressor change subglottal pressure estimates, average airflow, electroglottographic open quotient, speaking fundamental frequency, and the percent of syllables produced in vocal fry during a reading task?
2. Are subglottal pressure estimates, average airflow, electroglottographic open quotient, speaking fundamental frequency, and the percent of syllables produced in vocal fry

during a reading task different, or do the measures change differently, from before an acute, social-evaluative stressor to after the stressor in those who experience HPA axis activation (a 2.5 nmol/liter increase in salivary cortisol at some point after the stressor) and those who do not experience HPA axis activation?

3. Do individual changes from before a stressor to after a stressor in the following voice parameters reflect more than intra-subject variations: subglottal pressure estimates, average airflow, electroglottographic open quotient, speaking fundamental frequency, and the percent of syllables produced in vocal fry?
4. Are changes from before a stressor to after a stressor in the following voice parameters related to changes in emotional state: subglottal pressure estimates, average airflow, electroglottographic open quotient, speaking fundamental frequency, and the percent of syllables produced in vocal fry?

In addition, these areas will be addressed in an exploratory manner:

1. if there are other ways to divide the participants who experience an HPA axis response that further explain the voice changes experienced or the differences in voice parameters; and
2. the relationship between salivary cortisol and subglottal pressure estimates, average airflow, electroglottographic open quotient, speaking fundamental frequency, and the percent of syllables produced in vocal fry during a reading task.

CHAPTER II: REVIEW OF THE LITERATURE

Biological systems related to stress reaction

Understanding a person's response to stress is complicated because it involves a simultaneous series of biological chain reactions and behavioral changes to cope with or defend the body from stress (APA, 2017). It is an important observation that the goal of a stress response is to return the body to homeostasis. When a psychological stressor is acknowledged by the senses, it is relayed to the primary cortical projection areas of the appropriate sensory modality by the thalamus, processed by the prefrontal cortex and the limbic system, and then sent to the hypothalamus via ascending somatosensory pathways and visceral pathways (Herman et al., 2003; Lovallo, 2016; Sawchenko et al., 1996). The hypothalamus acts like a relay center for the stress response of the following systems: (1) the autonomic nervous system, (2) the neuroendocrine stress systems, and (3) the neural mechanisms of motivation and emotions (Horn & Swanson, 2013; Steckler, 2005).

The autonomic nervous system. The autonomic nervous system, which may also be called the autonomic motor system, is divided into three divisions: (1) the sympathetic nervous system; (2) the parasympathetic nervous system; and (3) the enteric nervous system (Horn & Swanson, 2013). The sympathetic nervous system has been thought to control the *fight-or-flight* response and the parasympathetic nervous system has been thought to control the *rest-and-digest* response, which involves controlling heart rate and respiration. The enteric nervous system is involved in the control of the gastroesophageal function.

The paraventricular nucleus of the hypothalamus is connected to the locus coeruleus (Samuels & Szabadi, 2008a). The locus coeruleus is a collection of norepinephrine-containing nuclei located in the posterior portion of the pons by the fourth ventricle (pontomesencephalic

junction) (Benarroch, 2009; Samuels & Szabadi, 2008a). The locus coeruleus directly projects neurons to the sympathetic and parasympathetic preganglionic neurons to increase activation of the sympathetic nervous system and decrease the activation of the parasympathetic nervous system when activated (Samuels & Szabadi, 2008b).

Sympathetic nervous system activation results in the release of norepinephrine from the postganglionic sympathetic nerve fibers (Roatta & Farina, 2010). In addition, this leads to activation of the sympatho-adrenal medullary system (discussed below) that releases epinephrine as a hormone into circulation. The sympathetic nervous system mediators (epinephrine and norepinephrine) increase and respond within a matter of seconds and then quickly return to baseline within a few minutes (Linden, Earle, Gerin, & Christenfeld, 1997). The increase in activation of the sympathetic nervous system leads to changes in the body that quickly prepare the body for greater energy expenditure (Lovallo, 2016).

The neuroendocrine stress systems. There are two major neuroendocrine stress systems: the sympatho-adrenal medullary system (SAM system) and the hypothalamic-pituitary-adrenal axis (HPA axis). The SAM system becomes activated when the parasympathetic nervous system becomes inhibited, which leads to lack of inhibition of the sympathetic nervous system (Kaltsas & Churousos, 2007; Ziegler, 2012). When the stressor is severe (Ziegler, 2012) or is determined to be uncontrollable (Dickerson & Kemeny, 2004; Henry, 1992; Ziegler, 2012), the HPA axis also becomes activated because negative feedback to brain areas such as the hippocampus is overridden (Kudielka et al., 2007). Activation of these neuroendocrine stress systems results in increased glucocorticoid and catecholamine secretion, which along with other products of the stress system leads the adaptive response of the organism to stress (Kaltsas &

Chrousos, 2007). The two neuroendocrine stress systems interact in both the brain and the body (Kvetňanský et al., 1995).

Sympatho-adrenal medullary system (SAM). The SAM system has also been referred to as the adrenal-medullary catecholamine system (Henry, 1992) and the sympathetic-adrenal medullary response (Frankenhaeuser, Lundberg, & Forsman, 1980). The SAM system is the connection of the sympathetic nervous system to the endocrine system. The SAM system is activated when the sympathetic nervous system is activated (Chrousos & Gold, 1992).

When a stressor occurs, projections from the paraventricular nucleus of the hypothalamus (Hosoya, Sugiura, Okado, Loewy, & Kohno, 1991) travel down the spinal cord and exit from the nucleus intermediolateralis pars principalis (ILP) at T7-T10 (less prominently from T2-L1) (Hamill, Shapiro, & Vizzard, 2012). The nerves pass through the sympathetic chain branching into the greater splanchnic nerve (Hamill et al., 2012). Both branches travel through the celiac ganglion but do not synapse, as this is a preganglionic pathway. The preganglionic nerves synapse with cells of the adrenal medulla (called chromaffin cells) using acetylcholine as their primary neurotransmitter. When the acetylcholine attaches to receptors, epinephrine and to a lesser extent norepinephrine are released from their stores in the adrenal medulla into circulation (Dimsdale, 1987). The body is not able to differentiate catecholamines that were secreted into the bloodstream from the adrenal medulla by the sympatho-adrenal medullary system and those that are released as neurotransmitters, making the response of the body during flight-or-fight due to the combination of the sympathetic nervous system and the sympatho-adrenal medullary system outputs (Goodman, 2009).

Hypothalamic-pituitary-adrenal axis (HPA). The regulation of glucocorticoid hormone secretion is dependent on the connection between the hypothalamus, the pituitary gland

(especially the anterior pituitary gland), and the adrenal cortex. This connection is coined the hypothalamic-pituitary-adrenal axis (sometimes the hypothalamic-pituitary-adrenocortical axis) and is abbreviated the HPA axis.

Briefly, the parvocellular cells of the paraventricular nucleus of the hypothalamus secrete corticotropin-releasing hormone (CRH) and vasopressin (AVP). CRH and AVP are secreted from the parvocellular cells that travel through the median eminence and are secreted into the hypothalamo-hypophyseal portal system to the anterior lobe of the pituitary gland[1]. Together, CRH and AVP act synergistically to increase the output of the anterior pituitary gland, which releases corticotropin, also called adrenocorticotropic hormone (ACTH) (Gillies, Linton, & Lowry, 1982; Rivier & Vale, 1983)[2]. ACTH acts on cells in the zona fasciculata and zona reticularis layers of the adrenal cortex to stimulate the creation and release of cortisol (Goodman, 2009).

Steroid hormones, such as cortisol, are released into circulation immediately after they are produced, unlike other hormones (Borer, 2013; Goodman, 2009). Steroid hormones are able to enter the blood-stream via diffusion through the plasma membrane down the concentration gradient. This process is possible because steroid hormones are lipid-soluble (Vining, McGinley, & Symons, 1983). The steroid hormones travel in the blood bound to proteins, specifically transcortin, also called corticosteroid binding globulin, or albumin (Sandberg & Slaunwhite, 1971). The biologically active steroids are called free steroids, and are steroids that are not traveling through the blood bound to proteins (Baxter, 1976).

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[1] During stress, GABA serves to inhibit the activity of CRH cells (Kovacs, Miklos, & Bali, 2004).

[2] During a restraint stress in rats, β-endorphin is partially involved in the stimulation of the HPA axis. β-endorphin uses opiate receptors to stimulate the release of ACTH. CRH is involved in mediating the increase of ACTH secretion caused by β-endorphin (Yamauchi, Shibasaki, Wakabayashi, & Demura, 1997).

As cortisol can easily pass through the blood brain barrier due to its low molecular weight and lipid solubility, it is able to regulate its own production. Through a negative feedback loop, cortisol, along with neuronal signals, are sent to the hypothalamus where by interacting with glucocorticoid receptors, it inhibits corticotrophin releasing neurons, thus inhibiting the secretion of CRH and AVP (de Kloet, Joëls, & Holsboer, 2005; Herman et al., 2003). Cortisol also travels to the anterior pituitary gland where it inhibits the corticotropes and inhibits the amount of ACTH secreted.

Glucocorticoids are secreted in pulses throughout the day (Kaltas & Chrousos, 2007). At basal levels, the products of the HPA axis are involved in regulating the circadian rhythms, such as the sleep-wake cycle and the food intake cycle (Lemaire, Piazza, & Le Moal, 2005). During stress, the frequency of the secretory pulses increases to increase the amount of CRH and AVP secreted (Kaltas & Chrousos, 2007). This results in an increase in ACTH and cortisol, the primary hormonal products of the HPA axis.

The neural mechanisms of motivation and emotion. One of the many functions of the hypothalamus and other limbic structures is control of emotion and motivational behaviors (Wilkinson & Brown, 2015). Rottenberg (2005) defines emotions as "quick-moving reactions that occur when organisms encounter meaningful stimuli that call for adaptive responses (p. 167)." Activation of the autonomic nervous system, changes in hormone levels, and changes in specific neurotransmitters, are related to different emotional conditions (Fowles, 2009). Specifically, an emotional reaction can occur based on an internal or external trigger and involve changes in how one feels, one's behavior, and one's physiology (Péron, Dondaine, Le Jeune, Grandjean, & Vérin, 2012; Rottenberg, 2005). Hicks' (1980) definition of stress states indicates

that stress must have specific emotions that occur with it. These specific emotions are likely anxiety, fear, and anger (Hollien, 1980).

Lovallo (2016) also defines anxiety, fear, and anger as the three main emotions, or psychosocial factors, that motivate the stress response. Anxiety is problems regulating fear (Hyman & Cohen, 2013). Anxiety expectancy can occur in situations in which fear is anticipated because the individual has learned that the situation brings about fear (Reiss, Peterson, Gursky, & McNally, 1986; Tovote, Fadok, & Lüthi, 2015). Anxiety sensitivity occurs when the individual believes that experiencing anxiety will be embarrassing or cause illness or more anxiety (Reiss et al., 1986). A stress response may or may not be accompanied by anxiety (Endler, 1997). Fear is a sense of arousal when a specific threat is present and anger is considered an urge to act outwardly in a destructive manner (Lovallo, 2016).

Interaction of the biological stress systems and motivation. The motivation to change behavior during stressful situations occurs partially due to the anxiety, fear, and anger emotions, and partially due to the influence of the autonomic nervous system and the neuroendocrine systems. Henry (1992) found that simple challenging situations lead to an increase in norepinephrine, released through the action of the sympathetic nervous system. If the anxiety associated with the stress activity increases and a sense of loss of control occurs, in addition to the increase in epinephrine relative to norepinephrine, ACTH and cortisol levels increase. The amount of norepinephrine compared to epinephrine is most likely determined by the amount of glucocorticoids present (Goodman, 2009). Phenylethanolamine-N-methyltransferase must be induced by glucocorticoids to change norepinephrine into epinephrine in the cytosol of the chromaffin cells of the adrenal medulla. However, the amount of glucocorticoid present seems to

be related to anxiety and the sense of control the person experienced during the task, both motivational factors.

In another study, Gruenewald, Kemeny, Aziz, and Fahey (2004) found cortisol levels increased more in participants who experienced greater amounts of shame and greater losses of social self-esteem following a social-evaluative stressor. Besides shame and social self-esteem, the authors examined other motivational factors, such as anxiety and performance self-esteem. These variables were not significantly different in participants who experienced a social-evaluative stressor (significant increase in cortisol) and those who experienced a stressor with no social-evaluation (no significant increase in cortisol).

Taken together, the studies by Henry (1992) and Gruenewald et al. (2004) point to the need to account for psychosocial factors when studying the stress response. Van Eck, Berkhof, Nicolson, and Sulon (1996), however, advocate for caution when interpreting the influence of psychosocial factors on cortisol levels due to the relationship not only among the psychosocial variables, but also the intercorrelations in the tools used to measure them.

Functional voice disorders: Stress, anxiety, and personality

Some researchers and clinicians have associated the development of functional voice disorders to psychosocial stress factors (reviewed in Roy & Bless, 2000). However, the influence of the autonomic nervous system and the neuroendocrine systems has not been given much attention. A functional voice disorder is a dysphonia that is not the result of a change in the vocal fold tissue (organic voice disorder) or neural innervation (neurologic voice disorder) (Roy, 2003). These changes may be the result of misuse of the laryngeal musculature (e.g., hyperadductive or hypoadductive glottal shaping) or due to psychogenic reasons (Roy, 2003). It

should be noted that some authors (e.g., Dietrich, 2008; Dietrich, Abbott, Gartner-Schmidt, & Rosen, 2008; Dietrich, Andreatta, Jang, Joshi, & Stemple, 2012) consistently use the diagnostic phrase "muscle tension dysphonia" in place of functional voice disorders.

"Conflict over speaking out." The development of functional voice disorders has been attributed to a "conflict over speaking out." House and Andrews (1988) define a conflict over speaking out as a situation in which the person has a "strong commitment" and a conflict related to that commitment in which the person feels the need to speak out to cope with the situation, but does not speak out because speaking out may make the conflict worse. The conflict has been attributed to motivational and emotional factors such as fear and shame (Aronson, 1990). Fifty-four percent of females with an ENT diagnosis of functional dysphonia experienced a conflict over speaking out in the last year or were experiencing a "conflict over speaking out" at the onset of their voice problem or the interview, compared to 16% of control females (House & Andrews, 1988). Baker (2010) also found that patients with functional dysphonia were significantly more likely than non-voice disordered controls and those with organic voice problems to have a "conflict over speaking out" before the development of dysphonia. Baker (2010) emphasizes that women with functional voice disorders were also significantly more likely to be unable to affect change even if they were to speak out regarding their conflict. People with a "conflict over speaking out" are thought to develop a voice disorder because they turn these emotional symptoms into physical symptoms (Baker, 2010). Deary and Miller (2011) and Aronson (1990) caution that people with other types of disorder but without voice disorders often have emotional issues, such as a "conflict over speaking out."

House and Andrews (1987; 1988) and Baker (2010) use the Life Events and Difficulties Schedule (LEDS) with the addition of the "conflict over speaking out" category to categorize

stressful events. This measure bases a stress score on an objective judge's rating of how traumatic each individual event is. Through a semi-structured interview, the interviewee develops a narrative of the emotional impact of events on the person that the judges later score. There is evidence that people attribute stressful feelings to the wrong stressor (Keating, 1979), particularly if the stressor occurred long ago (Roy & Bless, 2000). This may influence what the participants talk about in the interviews with researchers when the LEDS is used.

Stressful life events and voice disorders. Other measures assign a score to specific life events that have been experienced (e.g., death of a spouse receives the highest score and minor violation of the law receives the lowest score) as reported by the participant. Using the Social Readjustment Rating Scale, Guimarães and Abberton (2005) found that dysphonic speakers had significantly higher levels of stress than normal speakers (117.8 ± 88.9 and 87.5 ± 63.9, respectively). In the Social Readjustment Rating Scale, points are assigned to events using standards (Holmes & Rahe, 1967). Scores under 150, however, indicate no life crises (Holmes & Rahe, 1967). The Schedule of Recent Experiences (Holmes & Rahe, 1967) has also been given to patients with bilateral vocal fold nodules, hyperfunctional voice disorders, and healthy controls (Goldman, Hargrave, Hillman, Holmberg, & Gress, 1996). People with hyperfunctional voice disorders had significantly higher ratings of stressful events than healthy controls. Interestingly, these participants did not note significantly more voice use compared to the other groups. These measures should be interpreted with caution because they do not take into account the effects of the stressful event on the individual (Cohen, Kamarck, Mermelstein, 1983).

The Perceived Stress Questionnaire (Cohen et al., 1983) asks the participant to rate how stressful different events in his or her life were over the course of the last month. It presents an appraisal of stress that represents more recent stressors than the aforementioned scales. Dietrich

et al. (2008) compared patients with muscle tension dysphonia, benign vocal fold lesions, paradoxical vocal fold movement disorder, and glottal insufficiency with norms of the Perceived Stress Scale-10 and the Hospital Anxiety and Depression Scale. They found that for patients with a variety of voice disorders, only 25% of the patients reported high stress (measured using the Perceived Stress Scale-10), 36.9% reported high anxiety, and 31.2% reported a high depression score (both measured using the Hospital Anxiety and Depression Scale) compared to norms of the respective scales. A higher percentage of patients with muscle tension dysphonia reported stress and depression than those patients with vocal fold lesions.

Anxiety disorders and voice disorders. Brodnitz (1962) suggested that personality and emotional problems may lead to voice disorders which may lead to psychological problems and personality changes. The relationship between anxiety disorders and voice disorders is difficult to fully understand because it is nearly impossible to know if the anxiety disorder developed because of the voice disorder, if the voice disorder developed because of the anxiety disorder, or if the two disorders developed in spite of each other (e.g., Mirza, Ruiz, Baum, Staab, 2003).

Seventeen percent of patients in the House and Andrews (1987) cohort of people with a functional voice disorder that was related to a "conflict over speaking out" met the criteria of being diagnosed with an anxiety disorder of any type using the DSM-III, and 20% of patients in Willinger, Völkl-Kernstock, and Aschauer's (2005) study met the criteria of being diagnosed with an anxiety disorder of any type using the DSM-IV. Mirza et al. (2003) found that 29.4% of patients with functional dysphonia had a major psychiatric illness measured by the Brief Symptom Inventory. The prevalence of psychiatric disorders in patients with functional dysphonia is lower than the prevalence of psychiatric disorders in patients with vocal fold paralysis, cancer, non-cardiac chest pain, and cirrhosis (Mirza et al., 2003). Teachers without

voice disorders have been found to have lower levels of psychological distress than teachers with voice disorders, as measured by the Rand 36-Item Short Form Health Survey (Nerrière, Vercambre, Gilbert, and Kovess-Masféty, 2009). However, it should be noted that there was only a four point difference in the mental health score between those with voice disorders and those without (Nerrière et al., 2009).

Trait theory of voice disorders. Roy and Bless (2000) have associated the development of functional voice disorders with the personality traits of neurotic introverts. Neuroticism and introversion are two of the Big Five personality dimensions. Neuroticism has been found to lead to instability and stress proneness when one deals with anxiety, insecurity in the self, and depression (Judge, Higgins, Thoresen, & Barrick, 1999). Introversion, the opposite of extroversion, is associated with individuals who are potentially considered unsociable because they tend to be quiet, passive, and careful (Roy & Bless, 2000). In this theory, neuroticism exacerbates introversion tendencies to result in more anxiety and distress and thus activation of the behavioral inhibition system (Roy & Bless, 2000). Mirza et al. (2003) suggest that the low rates of anxiety disorders in people with functional dysphonia supports the idea that a personality difference may be more related to the development of functional voice disorders than if the patient has an anxiety disorder.

Biological stress reactivity and voice changes

As previously mentioned, the current literature seems to be missing information about differences in underlying activity of the biological stress systems that may explain some of the differences between those who develop a functional voice disorder and those who do not. Specifically, there appears to be lack of information regarding the examination of the influence

of differences in stress reactivity related to those who do and do not have a voice disorder. However, several studies suggest that differences in stress systems should be considered in the development of a functional voice disorder. People with a perception of voice problems measured as a score of nine or above on the Voice Handicap Index (VHI) apparently have higher levels of stress reactivity measured using the Stress Reactivity Index (Gassull et al., 2010). The Stress Reactivity Index asks participants to mark the various ways that they react to stress and allows researchers to calculate a stress reactivity score. Holmqvist et al. (2013) found that people who feel nervous or tense in situations where they are required to speak had more frequently occurring vocal symptoms. The authors suggested that these results may reflect an acute stress reaction with a social evaluative component in people with vocal symptoms. Indeed, Holmqvist et al. (2015) found women, not men, who more frequently had more vocal symptoms had higher salivary cortisol levels. Nichol, Morrison, and Rammage (1993) noted that extra tension in the larynx may be due to "overactivity of autonomic and voluntary nervous systems in individuals who are unduly aroused and anxious" (p. 644). The studies by Holmqvist et al. (2013; 2015) and Nichol et al. (1993) indicate that the sympathetic nervous system, parasympathetic nervous system, and the hypothalamic pituitary adrenal axis may be implicated in changes in the voice that may lead to the development of a functional voice disorder.

Sympathetic nervous system activation and voice changes. When the sympathetic nervous system is activated, and thus the parasympathetic nervous system is generally inhibited, the sympatho-adrenal medullary system also becomes activated. The change in tone (background activity level) of the sympathetic nervous system results in changes in the body that include an increase in glycogenolysis, increased blood flow in the heart, decreased blood flow in other areas to reduce heat loss, dilated pupils, relaxed bladder, reduction in secretions and peristalsis in the

intestines, and an increase in protein in the saliva (alpha-amylase) (Garrett, 1999; Goodman, 2009; Lovallo, 2016; Roatta & Farina, 2010; Ziegler, 2012). In active skeletal muscles, the reduction in blood flow due to sympathetic nervous system activation may result in a reduced muscle force (Hirvonen & Sonnenschein, 1962). The skeletal muscles also experience a release of lactate and pyruvate (Goodman, 2009).

In addition, the increase in epinephrine, released from the adrenal medulla has an influence on skeletal muscles. There is a movement of heat away from contracting muscles due to increased levels of epinephrine (Roatta & Farina, 2010). Some of the influence of epinephrine on skeletal muscles seems to depend on the fiber type of the skeletal muscles. In animal and computer models, Type I and Type II muscle fibers (slow and fast twitch, respectively) experience positive inotropism (an increase in the strength of muscular contraction) due to the interaction of epinephrine with the ryanodine receptors resulting in an increase in the amount of calcium released from the sarcoplasmic reticulum (Roatta & Farina, 2010). Type IIX muscle fibers (glycolytic muscles fibers) may experience an increase in muscle force during maximal contraction (but not low-intensity contractions) under conditions of sympathetic stimulation that is related to impaired vasoconstriction and increased arterial pressure (Thomas, Hansen, & Victor, 1994). In Type I muscle fibers (slow twitch), there is also an increased relaxation rate that may reduce the strength of the muscle contraction due to an increase in calcium reuptake into the sarcoplasmic reticulum (Roatta & Farina, 2010). This reduced muscle strength is potentially caused by two mechanisms: a shortened twitch duration and a decrease in the force amplitude of a single fiber twitch (Roatta & Farina, 2010).

When epinephrine was injected into the isolated vocalis muscle removed from three canines with normal larynxes, there was an increase in the amplitude of the evoked action

potentials and an increase in the duration of the evoked action potentials (Ueda, Ohyama, Harvey, Mogi, & Ogura, 1972). This effect was not seen when isoproterenol was injected (Ueda et al., 1972). In the canine, the medial portion of the TA (the vocalis muscle) is made up of mostly fibers that coexpress myosin heavy chain IIB (MyHC-IIB) and MyHC-IID and other fibers that coexpress MyHC-IIA and MyHC-IID (Bergrin, Bicer, Lucas, & Reiser, 2006). These are all considered to be fast-twitch fibers. The observations made by Ueda et al. (1972) of the vocalis muscle may suggest an increase of the strength of the muscle contraction by the increase in calcium release from the sarcoplasmic reticulum (Andersson et al., 2012; Roatta & Farina, 2010). However, Roatta & Farina (2013) argue that the increase in muscle force due to stress has never been observed physiologically, so it is important to consider that the observations made by Ueda et al. (1972) were made in an isolated muscle under non-physiological hormonal conditions.

In humans, in general the laryngeal muscles are made up of a combination of slow-twitch (slow-β) and Type IIA and Type IIX fast-twitch muscle fibers, with the fast-twitch muscle fibers making up a greater percentage of the muscle fibers (Shiotani, Westra, & Flint, 1999; Wu, Crumley, Armstrong, & Caiozzo, 2000). Mascarello, Toniolo, Cancellara, Reggiani, and Maccatrozzo (2016) also identified very low levels of Type IIB fast-twitch muscle fibers in human intrinsic laryngeal muscles. The vocalis muscle is made up of primarily slow-twitch muscle fibers, while the muscularis (more lateral) portion of the thyroarytenoid muscle is made up of more fast-twitch muscle fibers (Sanders, 2014). The lateral belly of the posterior cricoarytenoid muscle (PCA) has an equal distribution of Type I and Type IIA muscle fiber types, while the medial or horizontal bundle of the PCA has mostly Type I muscle fibers and a higher percentage of oxidative muscle fibers (Asanau et al., 2011). In the

interarytenoid muscles, the presence of both extrafusal fibers (contracting muscle units) and intrafusal fibers (muscle spindles) is noted, unlike in the PCA and TA that lack muscle spindles (Tellis, Thekdi, Rosen, & Sciote, 2004). The extrafusal fibers of the interarytenoid muscles contain Type I, Type IIA, and Type IIX (Tellis et al., 2004). The lateral cricoarytenoid (LCA) muscle has more fast-twitch muscle fibers (Sanders, 2014), as does the cricothyroid muscle (Wu et al., 2000). Overall, the laryngeal muscles are unique in that individual muscles contain both Type I and Type II fibers and some contain hybrid fibers (Helou, Wang, Ashmore, Rosen, & Abbott, 2013).

Cold pressor test. One way that is commonly used to activate the sympathetic nervous system (and inhibit the parasympathetic nervous system) is to use the cold pressor test. During the cold pressor test, participants place their hand or foot in ice water for one or two minutes. The pain and temperature nerves send signals to the cortex to process the feelings and to the tectum to trigger a reflex (Lovallo, 1975). The processing of the signal in the cortex, subcortex, limbic system, and periphery leads to activation of the sympathetic nervous system to increase blood pressure and cardiac output (Lovallo, 1975). The increase in heart rate is likely due to increased activation of the sympathetic nervous system and not withdrawal of the parasympathetic nervous system activity since people treated with propranolol (a beta-adrenergic blocker) do not experience an increase in heart rate (Victor, Leimbach, Seals, Wallin, & Mark, 1987). The sympathetic activity to the heart following the cold pressor test also seems to be different than the sympathetic activity to the skeletal muscles following the cold pressor test (Victor et al., 1987). The heart rate is expected to peak 30 seconds into the task and skeletal muscle activity is expected to peak during the second minute of the cold pressor task (Victor et al., 1987). The cold pressor test has only been found to produce small or no increases in cortisol

levels (al'Absi, Petersen, & Wittmers, 2002; Duncko, Cornwell, Cui, Merikangas, & Grillon, 2007).

Intrinsic laryngeal muscle activity following sympathetic activation. To test how an increase in sympathetic nervous system activation influences the intrinsic laryngeal muscles in vivo, measurement of the activity was made of the PCA, TA-LCA combination (left and right), and CT (left and right) during the cold pressor test without speech. Helou et al. (2013) found that although most subjects had a significant increase in intrinsic laryngeal muscle activity, some subjects experienced no change in activity in some muscles, and some subjects experienced a significant decrease in some muscles. Helou et al. (2013) suggest that the reduction in muscle activity arises from problems with electrode placement relative to the muscle fiber types. This may be the case as the decreases in muscle activity were only noted in the PCA and TA-LCA combination, which contain a mix of type I and type II fibers (Asanau et al., 2011; Sanders, 2014), unlike the CT muscle, which is made up of mostly type II muscle fibers (Wu et al., 2000).

While it is important to note that Helou et al.'s (2013) study only measured activity in resting muscles, if the findings extend to active muscles there may be important implications. The CT muscle controls the passive tension of the vocal folds and the medial TA muscle (vocalis) controls the active tension of the vocal folds. When the CT contracts and the medial TA does not, the resulting increase in vocal fold length results in an increase in passive tension of the mucosa and the vocalis muscle. Under these conditions, when the mucosa only is in vibration, there will likely be an increase in fundamental frequency. When a greater amount of the vocal fold (mucosa plus vocalis muscle) is in vibration, the resulting fundamental frequency will depend on the amount of tissue in motion and the effective tension of the combined mucosa and vocalis muscle (R. Scherer, 2014). If there is a significant increase in subglottal pressure, there

may be an increase in fundamental frequency due to the increase excursion of the vocal fold tissue adding to the passive tension of the system and the inclusion of more of the vocalis muscle (and its contraction level). However, if the medial TA does contract along with contraction of the CT under stress, that is, if the medial TA contracts less and the CT contracts more when stress is created, there should be a notable increase in fundamental frequency that may be accompanied with the extra CT muscle contraction.

Interestingly, however, studies suggest that the CT and medial TA may not contract more, or if they do, they have countering effects, during stressful situations that activate the sympathetic nervous system. Alvear et al. (2013) did not find an increase in fundamental frequency despite increases in blood pressure variables but not heart rate. Giddens et al. (2010) similarly found no change in fundamental frequency, voice onset time, speaking rate, jitter, shimmer, maximum flow declination rate, and subglottal pressure, with significant increases in systolic and diastolic blood pressure but not heart rate. Despite finding no vocal acoustic changes, understanding the authors' ideas about what should have changed may lead to more understanding of the influence of non-muscular changes of the sympathetic nervous system on the voice.

Heart rate and vocal perturbations. Giddens et al. (2010) and Alvear et al. (2013) based one hypothesis for voice change on the idea that heart rate is related to perturbation measures, namely jitter and shimmer. Orlikoff and Baken (1989) found that around 0.5% to 20% of the jitter in a prolonged vowel can be attributed to the heart beat cycle (both diastolic and systolic). The percentage of jitter that can be attributed to the heart beat cycle decreased as fundamental frequency increased. Similarly, 5% to 22% (mean 11.8%) of the shimmer can be attributed to the heart beat cycle (Orlikoff, 1990). The percentage of shimmer that can be attributed to the heart

beat cycle decreased as fundamental frequency and sound pressure level increased. Giddens et al. (2010) predicted that an increase in heart rate would result in an increase in jitter and shimmer measures. However, neither Giddens et al. (2010) nor Alvear et al. (2013) found a significant increase in heart rate following the cold pressor test and both measured both heart rate and fundamental frequency after the heart rate was expected to peak. In addition, because these heart rate dependent changes in jitter and shimmer are dependent on fundamental frequency, females may not be expected to have changes in jitter and shimmer due to the increased heart rate.

Bronchodilation and vocal aerodynamics. Other hypothesizes of Giddens et al. (2010) were based on the bronchodilation that occurs when the sympathetic nervous system is activated. In the respiratory system, the bronchial smooth muscles relax allowing more oxygen into the system (Roatta & Farina, 2010). The goal of bronchodilation is to increase airflow to the lungs. Bronchodilation in the human airway is accomplished by non-adrenergic non-cholinergic nerves using nitric oxide as a neurotransmitter, not sympathetic nerves using norepinephrine as a neurotransmitter (Barnes, 2012). Although there is a lack of sympathetic nerves in the lower airway, sympathetic tone (background activity level) in the lower airways is controlled by epinephrine in circulation from the adrenal medulla and nearby sympathetic nerves (Barnes, 2012; Wright, Rodriguez, & Cohen, 1998). Bronchodilation is quantified by measuring the volume of air exhaled during the first second of forced exhalation (abbreviated FEV1).

The authors suggest that bronchodilation would lead to an increase in transglottal airflow, which would lead to an increase in the speed of the vocal folds coming together, which would result in an increase in fundamental frequency and maximum flow declination rate (MFDR) (Giddens et al., 2010). The authors also suspect that bronchodilation will also lead to an increase in voice onset time, as voice onset time has been found to increase when the lung volume is

higher (Hoit, Solomon, & Hixon, 1993). Giddens et al. (2010) noted a significant increase in subglottal pressure in female participants after the cold pressor test. However, the increase was less than 1 cm H_2O. This suggests that despite the option of increased glottal airflow, participants may have controlled the amount of air used.

Basal sympathetic tone. Helou et al. (2013) note that while three of the eight female participants in their study did not experience an increase in heart rate due to the cold pressor test, all but one subject experienced an increase in activity of the positive control muscle (the trapezius) suggesting that changes in skeletal muscle activity can occur in the absence of cardiovascular activity. This may be due to the differential effects of sympathetic outflow to the heart and skeletal muscles (Victor et al., 1987). Giddens et al. (2010) suggest, however, that the lack of increase in heart rate in their study is due to variations in baseline sympathetic tone which may account for the lack of significant results in their investigation.

The relationship between sympathetic tone and parasympathetic tone can be monitored using normalized heart rate variability measures (HRV) made from electrocardiogram (ECG) recordings. Specifically and most simply, low frequency components (LF; 0.04 to 0.15 Hz) that have been normalized to both low and high frequency components indicate that the control is primarily sympathetic and high frequency components (HF; 0.15 to 0.4 Hz) indicate that the control is primarily parasympathetic (Camm et al., 1996). The HF components are mostly dependent on vagal activity (Camm et al., 1996). Some authors argue that the LF components are due to the combination of sympathetic and vagal activity (Camm et al., 1996). At rest, vagal tone and thus high frequency components in the spectral analysis are more prominent (Camm et al., 1996; Levy, 1971). The reaction to stress would be changed based on if a person has stronger

parasympathetic control compared to sympathetic control during stress (Wright et al., 1998) or if a person has too much baseline sympathetic tone (Giddens et al., 2010).

The innervation of the larynx is provided by the vagus nerve (10th cranial nerve). The vagus divides into the recurrent laryngeal nerve (superior and inferior branches) and the superior laryngeal nerve. Both nerves perform sensory, motor, and autonomic functions (Malmgren, Lyon, & Gacek, 1976; Uno & Hisa, 2016a; Uno & Hisa, 2016b; Yoshida, Tanaka, Hirano, & Nakashima, 2000). The external branch of the superior laryngeal nerve provides sympathetic nerve fibers to the CT muscle, while the remaining intrinsic laryngeal muscles receive sympathetic innervation from the internal branch of the superior laryngeal nerve and the recurrent laryngeal nerve (Bando, Toyoda, & Hisa, 2016). There is also sympathetic innervation of the blood vessels and mucosa (Hisa et al., 1999; Uno & Hisa, 2016a). Neurons from the dorsal motor nucleus of the vagus nerve project via the internal branch of the superior laryngeal nerve to parasympathetic ganglia in the larynx (Uno & Hisa, 2016b). As the larynx receives motor input from both the sympathetic and parasympathetic nervous systems, a change in sympathetic tone may have ramifications on voice production.

To summarize, the evidence suggests that there will be limited change in voice production and voice quality following sympathetic nervous system activation. There may be several reasons for the limited change in voice parameters secondary to sympathetic nervous system activation. (1) First, the speed of sympathetic nervous system activation and return to primary parasympathetic nervous system control may prevent measurable voice changes. The sympathetic nervous system responds to a stressor within a matter of seconds and returns to baseline control within a few minutes (Linden et al., 1997). For Gidden et al.'s (2010) study where voice measures were taken in the first two minutes after the cold pressor, this may explain

the lack of findings, but Alvear et al. (2013) made their fundamental frequency measures *during* the cold pressor test and also found no significant differences. While neither Gidden et al. nor Alvear et al. noted an increase in heart rate following the stressor, Helou et al. (2013) and Plein (2014) measured an increase in heart rate in their participants following the cold pressor test. Respectively, these studies reported an increase in intrinsic laryngeal muscle activity for several minutes after the stressor ended and the heart rate returned to baseline, and an increase in fundamental frequency. Together this may suggest that activation of the sympathetic nervous system that results in a measured increase in heart rate may co-occur with the necessary physiological changes to result in measurable changes in the voice. (2) Second, this limited change may be due to control over the voice exerted by the speaker under stressful situations (K. Scherer, 1986). A reduction in skeletal muscle activity (such as the decrease in PCA and LCA-TA muscle activity), for example, may be due to defensive or mobilization activity (Grassi, Turri, Vailati, Dell'Oro, & Mancia, 1999). (3) Third, the limited change in voice production may be because the use of the cold pressor test may not lead to whole body sympathetic reactions. Lovallo (1975) indicates that the vasoconstriction induced by the cold pressor test is localized to the skin of the immersed limb despite the increase in heart rate and blood pressure. In another task that is thought to activate the sympathetic nervous system via bottom-up activation (Lovallo, 2016), a physical load task (i.e., pedaling on an ergometer until exhausted and unable to continue), male participants all experienced a significant increase in voice pitch when the pedaling effort was submaximal and maximal (Johannes et al., 2007). Vaic and Friedrich (1982) as cited by Johannes et al. (2007) also found an increase in pitch when the participants were subjected to an unidentified physical stressor. It may be that the influence of stress on the voice

only occurs when there is sympathetic involvement of more of the body or when another stress system is activated.

Suspected HPA axis activation and voice changes. The majority of studies that examine the influence of stress on the voice are difficult to interpret because they do not include biological markers of the HPA axis to ensure that the participants are indeed experiencing a response. Some contain perceptual ratings of stress that should be analyzed with caution as those who had a biological stress response to an acute social-evaluative stressor had no differences in subjective ratings of stress from those who did not have a biological stress response, according to a study by Schommer, Hellhammer, and Kirschbaum (2003) and another study by Balodis, Wynne-Edwards, and Olmstead (2010). Other studies contain measures that indicate sympathetic nervous system activity, such as heart rate (e.g., Tse et al., 2014) and blood pressure measures (e.g., Dietrich, 2008).

To best organize the current literature related to stress and vocal acoustics, only studies that use tasks that meet the criteria for HPA axis activation as reviewed by Dickerson and Kemeny (2004) will be included. Tasks that are uncontrollable and contain a social-evaluative threat are those that elicit the largest cortisol response (Dickerson & Kemeny, 2004). Reliable cortisol responses also are associated with other tasks, such as cognitive tasks, public speaking or verbal interaction tasks, and public speaking and cognitive tasks (Dickerson & Kemeny, 2004).

Although the history of studying the influence of stress on the voice is based on studies of voice changes in airplane pilots in stressful situations (e.g., Kuroda, Fujiwara, Okamura, & Utsuki, 1976), more natural stressors (such as measuring call center operators voice changes during blackouts or pilots voices during flight) may not lead to a greater understanding of the influence of stress on the voice and will not be examined here (Kirchhubel, Howard, & Stedmon,

2011). In addition, studies that enlist actors to mime or mimic a stressed voice (e.g., studies by K. Scherer, 1995) are important. However, as it is unlikely that cortisol increases in these acting situations, they are not included in the present review.

Again, it must be emphasized that the studies included in the following sections did not measure cortisol to ensure HPA axis activation but did involve tasks that *may* have activated the HPA axis.

Cognitive task. Mendoza and Carballo (1998) created cognitive tasks, including spelling the Spanish alphabet forward (baseline condition) and backward and reading a tongue twister with and without delayed auditory feedback in an attempt to determine the effects of psychological stress from the effects of cognitive workload. The participants completed these tasks under a high stress condition (speed and accuracy were required to prevent a reduction in their overall course grade) and a low stress condition (no instruction, no impact on their grade, and practice reciting the alphabet backwards was allowed). The dependent variables were all collected from the Multi Dimensional Voice Program (MDVP), the program for the Computerized Speech Labs created by Kay Elemetrics. The researchers found no differences in any of the acoustic variables in the high stress condition and the low stress condition.

Hecker, Stevens, von Bismark, and Williams (1968) investigated the impact of a cognitive stressor in which the participant added together six numbers in an ever-decreasing amount of time. The acoustic analysis was completed on one of five phrases comparing a stressed phrase value with an unstressed phrase value. The researchers found few consistent changes across people during the stressed production but found many consistent changes within the speech of a stressed individual. The authors note that the changes due to stress were mostly in

the glottal pulses (rate, amplitude, contour, shape, regularity, initiation of the glottal pulses) and in duration and precision of articulatory targets.

Rothkrantz et al. (2004) found when analyzing changes in the speech variables across each of the five minutes of the Stroop task (color and word naming task), with the first minute representing a low or no stress condition, fundamental frequency increased some from the normal condition and jitter decreased from the normal condition. No statistical tests were performed to determine the strength of the changes. The results of this study should be interpreted with caution, however, because the color words of the Stroop task (blue, red, green, yellow, and brown) were analyzed as the speech sample and did not appear equally across each minute of the Stroop task. Each vowel has a slightly different average fundamental frequency (Peterson & Barney, 1952). In another study that utilized the Stroop task, Plein (2014) found fundamental frequency increased in instances when the time to response increased in the more difficult incongruent tasks (the color of the ink does not match the color word presented) compared to the congruent tasks (the color of the ink does match the color word presented), suggesting that in instances of "cognitive conflict" the fundamental frequency may increase (p. 15).

Vaic and Friedrich (1982) as cited by Johannes et al. (2007) did not find an increase in fundamental frequency when male participants solved a "mental concentration task" (p. 268). Johannes et al. (2007) in their own study, found a significant increase in pitch during mental stressors with and without time constraints. Griffin and Williams (1987) found that fundamental frequency increased as task complexity increased in psychomotor and dichotic listening tasks. The authors also found word duration to significantly decrease. Brenner and Ship (1988) found the same results as Griffin and Williams (1987) in a tracking task of increasing difficulty.

Public speaking or verbal interaction task. Van Lierde, van Heule, De Ley, Mertens, & Claeys (2009) noted a decrease in fundamental frequency and the following variables in healthy females on an oral contraceptive participating in a public reading task with a social evaluative element: DSI (a measure of voice quality), maximum phonation time, the highest intensity of the voice range profile, the lowest frequency of the voice range profile, and the highest frequency on the voice range profile. The grade and breathiness of the GRBAS increased during the stressed condition. However, during the second reading task, the state anxiety of the participants decreased, suggesting that the participants did not perceive the task to be stressful. It is also important to note that the data were collected 10 minutes before the reading task. This suggests that the results of the study represent the influence of the anticipatory period before or an anticipatory anxiety period, without the influence of a present stressor on voice parameters.

In a modified version of the Trier Social Stress Test (TSST) that only included a public speaking component and no mental arithmetic, Tse et al. (2014) found an increase in both fundamental frequency and the standard deviation of fundamental frequency along with an increase in heart rate and a modest increase in self-rating of stress. Using a similar stress protocol, Dietrich (2008) found a decrease in fundamental frequency during the stressed condition compared to the baseline and recovery speech samples. Dietrich (2008) further noted the presence of more vocal fry during the stressed condition. She also noted a decrease in intensity (dB) along with an increase in perceived vocal effort during the stressed condition. All participants had an increase in systolic blood pressure, a measure indicating the increase in blood flow that is expected in the heart following sympathetic nervous system activity (Goodman, 2009).

To summarize, the literature presents great variation in whether or not, and how much, measures of acoustic variables change as a function of a stress stimuli that may activate both the HPA axis and the sympathetic nervous system. This may be because there apparently are no specific voice changes associated with stress, because the studies in this area have not determined strong associations between voicing variables under conditions of stress that *should* activate the HPA axis and the SNS. In addition, it is often thought that reactions to stress are idiosyncratic (Hecker et al., 1968; Lovallo, 1975). However, in the only study to this author's knowledge that examines changes in cortisol levels relative to fundamental frequency, Pisanski et al. (2016) found that mean and minimum fundamental frequency increased from the un-stressed to stressed measurement point, and that free cortisol levels positively predicted mean fundamental frequency change under stress. This suggests that in many of these studies, participants may not be experiencing an increase in activity of the HPA axis as a result of the supposed stressor. The participants in some studies indeed may not even be experiencing an increase in sympathetic nervous system activation as a result of the stressor.

Cortisol responses in other communication disorders

Aphasia. People with aphasia following a left-hemisphere stroke have been found to have no cortisol response following the TSST, despite rating their perceived stress as higher following the TSST (Laures-Gore, Heim, & Hsu, 2007). In a longitudinal study comparing people with left hemisphere strokes and aphasia and those with a right hemisphere stroke, there were no significant differences in afternoon salivary cortisol levels in these groups, nor was there a difference in salivary cortisol based on aphasia severity (Laures-Gore, 2012). People with aphasia have also been found to have an absent cortisol awakening response (Laures-Gore,

Buchanan, & Cahana-Amitay, 2017). Many people with aphasia have been found to have low hair cortisol levels (a month-by-month marker of stress), although there were no correlations between hair cortisol levels and measures of depression and anxiety from a modified version of the Perceived Stress Scale (Smith & Hunting Pompon, 2017). It is proposed that these differences in HPA axis response to stress may be due to dysregulation of the HPA axis due to the brain changes related to the stroke or habituation to stressors (Laures-Gore, Heim, & Hsu, 2007). From a brief review of the literature, it appears that the brain changes and not the aphasia leads to a lack of cortisol response. This is supported by work in those with traumatic brain injury. Twenty-one percent of patients one year (SD: 8 months) following a traumatic brain injury were found to have anterior pituitary dysfunction, with 4% having secondary hypocortisolism (Berg et al., 2010).

People who stutter. Unlike the lack of response seen in people with aphasia (secondary to a stroke), people who stutter may have an elevated cortisol response when the stressor is natural. Gordon Blood and colleagues have studied the cortisol response in people who stutter. People who stutter and age, education, and sex matched people who do not stutter presented with no differences in salivary cortisol levels from before to after a five minute oral mental arithmetic stressor (adding, subtracting, multiplying, and dividing multi-digit numbers; Blood, Blood, Frederick, Wertz, and Simpson, 1997). However, when the participants were divided into "High Communication Apprehensive" and "Low Communication Apprehensive" groups based on scores on the Personal Report of Communication Apprehension, all people (people who stutter and people who do not stutter) in the "High Communication Apprehensive" group had significantly higher salivary cortisol after the stressor than people in the "Low Communicative Apprehensive" group. When divided further, the people who stutter in the "High Communication

Apprehensive" group had the greatest percent change in salivary cortisol following the stressor. In another study, Blood, Blood, Bennett, Simpson, and Susman (1994) used a natural stressor, such as a final examination, a speech in a class, or a "day where everything was going wrong." Under a more natural stressor, people who stutter were found to have significantly higher cortisol levels than people who do not stutter on a stressful day. These studies suggest that participants with more apprehension about communicating will have higher salivary cortisol levels following the TSST, but that a more natural stressor may result in more differentiation of people with communication disorders based on cortisol.

HPA axis responders and non-responders

The main purpose of the increase in glucocorticoid levels during stress is hypothesized to be in returning the body to homeostasis and helping the body adapt to stress by ending the stress response of the HPA axis (Fulford & Harbuz, 2005; Herman et al., 2003). The proposed positive feedback loop between the HPA axis and the locus coeruleus (Chrousos & Gold, 1992; Kaltas & Chrousos, 2007) will lead to activation of the HPA axis when the locus coeruleus is activated because of the inputs of the locus coeruleus to the paraventricular nucleus of the hypothalamus (Samuels & Szabadi, 2008a). However, this co-activation does not seem to be true in all cases. Understanding when the HPA axis is not activated and thus not helping to end the stress response is important because it may help to understand the changes in the voice as a result of stress.

The HPA axis has been noted to be activated in cases of a severe stressor, an uncontrollable stressor, a perceived sense of helplessness, a threat to a committed goal, or when there is a social-evaluative component to the stressor (Dickerson & Kemeny, 2004; Henry, 1992; Kudielka et al., 2007; Ziegler, 2012). Dickerson and Kemeny (2004) admit that all stressors are

not equally effective at eliciting a cortisol response. This is suspected to be the reason for the inconsistencies in the current literature regarding the influence of stress on the voice (Pisanski et al., 2016). Indeed, even the use of the same stressor in different people does not elicit a uniform cortisol response. Using the Trier Social Stress Test (TSST), a public speaking and mental arithmetic task with social-evaluation, seventy to eighty percent of participants have a two to threefold increase in cortisol levels (Kudielka et al., 2007). The other twenty to thirty percent of people did not experience a significant increase in cortisol.

In general, hyporeactivity of the HPA axis occurs after a long period of hyperreactivity of the HPA axis in which the person has not had time or ability to recover from the stressor(s) (Heim, Ehlert, & Hellhammer, 2000). For example, people with stress-related neuropsychiatric disorders, such as major depression and post-traumatic stress disorder, have difficultly regulating their reaction to stress due to a decrease in glucocorticoid secretion or a reduced sensitivity of the receptors to glucocorticoids (Raison & Miller, 2003). As this prevents these patients from regulating their stress response, these factors may lead to stress related changes in behavior and a suppression of an acquired immunity response (Raison & Miller, 2003). Clearly, a stress reaction can occur without a psychological disorder (Cohen & Williamson, 1988).

Hyporeactivity of the HPA axis may also be due to variations in basal or baseline cortisol levels. Elevated cortisol levels at baseline, aside from the expected elevation in the morning due to waking, are indicative of problems recovering from stress (Lemaire et al., 2005). Baseline cortisol levels may be high due to chronic stress (Buckingham & Hodges, 1974; Gray & Munson, 1951; Hodges & Sadow, 1967; Stark, Acs, & Szalay, 1969) and factors such as sex, age, race, education level, diet, hormonal factors, medications, and clinical disorders (reviewed in Kudielka et al., 2007 and Kudielka, Hellhammer, & Wüst, 2009; Dowd et al., 2011). One

study suggests that 35% of the variability in cortisol response to stress can be explained by "anticipatory cognitive appraisal" of the situation (Gaab, Rohleder, Nater, & Ehlert, 2005). These factors can be controlled for in the experimental design. Other factors, such as genetic factors, life experience factors, and social support factors, to name a few, are more difficult to control for and do contribute to the variability in HPA axis response (Kudielka et al., 2009).

In a study comparing people who had a salivary free cortisol response of at least 2.5 nmol/liter over their baseline levels and those who did not, the people who had the expected cortisol response (73.9% of participants) had higher values for salivary free cortisol, total plasma cortisol, ACTH, and epinephrine than did the non-responders (Schommer et al., 2003). The higher levels of ACTH and cortisol in the responders suggest that the HPA axis was indeed not activated in the non-responders, as ACTH is one of the first measurable products of the HPA axis. There were no differences in sex, age, body mass index (BMI), norepinephrine, heart rate reactivity, or subjective ratings of stress between responders and non-responders (Schommer et al., 2003). The latter two similarities (heart rate reactivity and subjective ratings of stress) indicate that the non-responders did perceive the stressor to be a threat to them. It is important to note that in this study, the female participants were all in the luteal phase of their menstrual cycle and did not use oral contraception.

The study by Pisanski et al. (2016) appears to be the only study that has examined changes in cortisol levels relative to fundamental frequency. The researchers measured cortisol and fundamental frequency two weeks before and just prior to an oral examination[3]. Using this

[3] In this two week time period, the 34 female participants used in this study may be in a different stage of their menstrual cycle. If a female has a cycle length of 28 days, the follicular phase begins with the first day of flow and ends when ovulation begins (around day 14) and the luteal phase occurs from ovulation until the menstrual flow begins again (Hastrup & Light, 1984). The fundamental frequency of a spontaneous speech sample has been found to be highest days before ovulation and lowest during ovulation (Fischer et al., 2011). In addition, the standard deviation of the fundamental frequency of a spontaneous speech sample has been found to be significantly higher in the days before ovulation (Fischer et al., 2011).

natural stressor, the authors found that not all participants experienced a significant increase in cortisol levels from the baseline measure to the stressful event. In their participants, from the unstressed condition to the stressed condition, mean and minimum fundamental frequency increased, but maximum fundamental frequency and the standard deviation of fundamental frequency did not change. However, the average increase in fundamental frequency across all participants (even those with no significant increase in cortisol) was only 1.9 Hz for the read speech and 4.9 Hz for the spontaneous speech. In both spontaneous ($r_s = 0.46$) and read ($r_s = 0.45$) speech, free cortisol levels positively predicted mean fundamental frequency under stress. The researchers found cortisol levels accounted for 20% of fundamental frequency variation in the stressful situation. The authors importantly note that measuring the influence of stress on the voice should be done only on people who are confirmed to be stressed (Pisanski et al., 2016). Ironically, Pisanski et al. (2016) completed all analyses of the influence of stress on the fundamental frequency on all participants, despite indicating that some participants had no increase in cortisol levels from baseline to stressor.

Hypothesized influence of acute stress on the voice

Abitbol and Abitbol (2000) state that "the voice is changed by the hypothalamus-pituitary axis," but do not indicate how this change occurs (p. 317). Creating hypotheses about changes in voice production and voice quality is clearly complicated. The changes depend on whether the sympathetic nervous system is activated with or without HPA axis activation. Also, it may be that top-down versus bottom-up activation of the sympathetic nervous system influences how much of the system is activated and thus the potential changes in the voice. In addition, changes based on experiences and motivation need to be considered (Kemeny, 2003).

Since psychological stressors are more common than physical stressors, understanding the influence of top-down activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis is necessary. In top-down activation, control centers in the brain (i.e., evaluation by the limbic system), not changes in metabolic demand, activate peripheral stress changes, such as increases in heart rate (Lovallo, 2016). The cold pressor test and physical load tasks are thought to activate the sympathetic nervous system in a bottom-up manner; the body has increased metabolic need, and thus there is an increase in metabolic energy mobilization.

The Trier Social Stress Test (TSST) (Kirschbaum, Pirke, & Hellhammer, 1993) is a commonly used psychosocial stressor that leads to top-down activation of the sympathetic nervous system and activation of the hypothalamic-pituitary-adrenal axis in seventy to eighty percent of participants (Kudielka et al., 2007). The test involves a public speaking task in front of an audience of three people, for which the participant has around ten minutes to prepare, and a mental arithmetic task. Because the TSST includes uncontrollability and a social-evaluative component, it generally leads to significant increases in HPA axis activity (Dickerson & Kemeny, 2004; Kudielka et al., 2007). In addition, based on the history of the participant, the task may involve emotions, such as anxiety, fear, or anger.

In a study by Dietrich et al. (2012), people with higher stress reactivity scores (determined using the Multidimensional Personality Questionnaire-Brief Form) had greater activity in the primary somatosensory cortex, the dorsolateral prefrontal cortex, and the periaqueductal gray while orally reading a sentence, than those with lower stress reactivity scores. The results of the study suggest that stress reactivity, personality, and emotions can lead to increased activation of prefrontal and limbic areas (brain areas that are involved in the top-down stress response) and differences in sensorimotor control for the production of voice. It is

important to note that personality factors are not associated with differential HPA axis response in the first exposure to a stressor, but may result in differential response in later exposures to the same stressor (Kirschbaum, Bartussek, & Strasburger, 1992a; Van Eck et al., 1996).

In a study of the changes in extralaryngeal muscle activity due to a modified version of the TSST with no mental arithmetic, Dietrich (2008) found no effect of stress on the activity of the submental muscle and infrahyoid muscle using surface EMG. However, in another study using a modified TSST with only the speech preparation phase (anticipatory phase), Helou (2014) found that two-thirds of the participants experienced an increase of activity in the PCA and TA-LCA muscles, and less than half experienced an increase in CT muscle activity. These measures of the intrinsic laryngeal muscles were made during non-speaking tasks. These results are in contrast to the more consistent increase in activation of the CT muscle and less consistent increases in other intrinsic laryngeal muscles found when the sympathetic nervous system alone was activated (Helou et al., 2013). Thus, one of two profiles are expected in the participants who experience activation of both the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis:

Profile 1. An increase in TA muscle activity and CT muscle activity will increase the active and passive tension of the vocal fold. Acoustically, this may be measured as a small increase in fundamental frequency as found by Pisanski et al. (2016). Pisanski et al. (2016) found vocal pitch increased mostly in participants who had a doubling of their baseline cortisol under stress. This indicates that a dramatic increase in activation of the HPA axis may be needed to increase fundamental frequency.

Profile 2. An increase in TA muscle activity without an increase in CT muscle activity, which was noted by Helou (2014), will increase the active tension of the vocal fold and increase

the thickness of the vocal fold (McGlone & Shipp, 1971). This may result in more instances of vocal fry, as anecdotally noted by Dietrich (2008). Aerodynamically, this may result in greater laryngeal airflow resistance (McGlone & Shipp, 1971). It is expected that this profile will be seen in people who have a significant increase in SNS and HPA axis activity but less than a doubling of their baseline cortisol levels under stress.

A third profile is presented for those who do not experience any increase in hypothalamic-pituitary-adrenal axis activity:

Profile 3. There is little expected change in vocal acoustics, aerodynamics, and voice quality.

CHAPTER III: METHODS

Participants

Recordings were made from nineteen female participants. The participants were aged 18 to 23 (mean: 18.89; standard deviation: 1.45; median: 18). All females were selected for the present study because females are more likely to experience and report voice problems (Roy et al., 2004; Russell, Oates, & Greenwood, 1998; Smith, Kirchner, Taylor, Hoffman, & Lemke, 1998) and are more likely than males to have severe voice problems (Roy et al., 2004). In cases of Cushing's syndrome, where there is excessive cortisol secretion due to increased ACTH production, male voices are thought to be unaffected while female voices may experience weakness and problems achieving high notes (Abitbol & Abitbol, 2003).

One participant (F4) reported a diagnosis of laryngeal myasthenia (ICD-9 478.79: other diseases of the larynx) by an ENT 2 years prior to the research project. Laryngeal myasthenia is diagnosed when there is dysphonia that is the result of fatigue of the intrinsic laryngeal muscles in patients who are otherwise healthy (Stemple, 1993) and was diagnosed in 7.1% of patients in the study by Coyle, Weinrich, and Stemple (2001). Laryngeal myasthenia is often diagnosed when there is an anterior glottal gap during phonation (Donahue, 2012; Stemple, 1993). Multiple studies categorize laryngeal myasthenia as a functional voice disorder because there are no visible changes to the tissue (Stemple, 1993) and it has also been called "laryngeal tension-fatigue syndrome" (Koufman & Blalock, 1988). F4 reported she did not receive treatment of this issue.

Two participants (F11 and F15) reported that their voice on the day of the study was not representative of their normal voice. Additionally, two participants reported that their voice feels tired or fatigued at the end of the day (F4 and F11). The reason for the inclusion of these

questions was twofold. First, patients with dysphonia have been found to be more fatigued in general than normal controls (O'Hara, Miller, Carding, Wilson, & Deary, 2011). Second, the ratio of LF/HF on an ECG, which represents the balance between the sympathetic nervous system and parasympathetic nervous system, has been found to be lower in those with higher ratings of fatigue (Park et al., 2011). This may suggest that there is a relationship between stress reactivity, fatigue, and voice changes that will be investigated in an exploratory way in the present study.

Males experience a greater free cortisol and salivary cortisol response to the TSST than females (Kirschbaum, Wüst, & Hellhammer, 1992b). However, when controlling for sex hormones (testosterone, estradiol, and progesterone), there is no difference in reaction to the TSST in males and females (Juster et al., 2016). Females have higher baseline cortisol levels likely because estrogen is thought to increase cortisol secretion (Silva, 1999). It was expected that the female participants would experience a 50 to 150 percent increase in salivary cortisol levels from baseline (Kudielka et al., 2009).

Older adults experience a larger cortisol response to the Trier Social Stress Test than younger adults, although they do not report higher stress levels (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). Because of this mismatch between self-reported stress levels and cortisol response in older adults, only younger adults were selected for the present study. In addition, there are commonly reported voice changes associated with menopause due to the increase in androgens (male sex hormones such as testosterone) (Abitbol & Abitbol, 2003).

All of the participants used no birth control method or a non-hormonal birth control method (reported by 3 participants), with the expectation then of a reasonable cortisol response. Women using oral contraception that contains estrogen have a reduced or absent increase in free

cortisol after the TSST despite an increase in heart rate and subjective ratings of stress (Kirschbaum, Pirke, & Hellhammer, 1995). Kirschbaum, Kudielka, Gaab, Schommer, and Hellhammer (1999) also found this reduction in free cortisol levels in women on oral contraception. This muted response is likely because women taking estrogen-containing oral contraception have elevated plasma free cortisol at baseline (Meulenberg, Ross, Swinkels, & Benraad, 1987) resulting in a smaller increase in free cortisol levels after waking (Pruessner et al., 1997). In addition, estrogen has been found to bind to corticotrophin releasing hormone cells in the hypothalamus, reducing the activity of the HPA axis and reducing the negative feedback of the HPA axis by cortisol (Silva, 1999). Despite this increase, it has been found that salivary cortisol still accurately reflects unbound cortisol levels but not total plasma cortisol levels in women taking oral contraception (Kudielka et al., 2009; Meulenberg et al., 1987; Šimůnková et al., 2008; Vining et al., 1983).

All participants provided signed consent to participate in the present study which was approved by the BGSU IRB (Appendix A).

Health questionnaire and baseline stress measurement. All participants completed an extensive health questionnaire (Appendix B) that included many exclusionary criteria. Women who were currently or recently pregnant were excluded from the present study because cortisol levels are elevated throughout the day in women who are pregnant (Vining et al., 1983), likely due to the increased free cortisol levels (Kudielka et al., 2009). Women who were currently breastfeeding were excluded because they have a lower free cortisol response to the TSST while actually feeding their baby (Heinrichs et al., 2001). People who smoke or use nicotine or other tobacco products were excluded from the present study because smokers have higher ACTH and cortisol levels at baseline which probably would lead to a reduced HPA axis response to the

TSST (Kirschbaum, Strasburger, & Langkrär, 1993; Kirschbaum, Scherer, & Strasburger, 1994) and female smokers specifically have lower levels of salivary alpha-amylase than female non-smokers (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). In addition, there are changes to the laryngeal mucosa associated with smoking (e.g., Ueha et al., 2017). Participants with gingivitis were excluded to ensure that the salivary concentrations of alpha-amylase and cortisol were not impacted by trace amounts of contamination in the saliva. The health questionnaire also ensured the exclusion of participants with a medical history of any of the following items: autoimmune diseases, diabetes, asthma or other breathing problems, hypertension, regularly taking medications that may impact hormone levels or stress levels (e.g., steroids), and recently using a hormone product (steroid or otherwise). Atopic patients (those with asthma or allergic rhinitis, for example) have been found to have a reduced cortisol response and reduced alpha-amylase activity following a psychosocial stressor (Hlavacova, Solarikova, Marko, Brezina, & Jezova, 2017). Participants with professional voice and speech training were also excluded because they may have more control over the expression of emotions under stress. One participant (F4) reported less than a year of professional voice training during high school.

In addition, participants completed the Perceived Stress Scale (PSS) (Cohen et al., 1983) (Appendix C). The PSS is a test with well-accepted psychometric properties (reviewed in Lee, 2012) that asked the participant to rate how stressful different events in her life were over the course of the last month. The score, which ranges from 0 to 56, will serve as a baseline measure of how much appraised stress the participant experienced in her life. Cohen and Janicki-Deverts (2012) found that the mean for females (all ages) increased over a 26-year period from a mean of 13.68 (SD: 6.57) in 1983 to a mean of 16.14 (SD: 7.56) in 2009. When only participants younger than 25 years of age were included (all sexes included), a similar increase was found

from 1983 to 2009 (1983 mean: 14.54; SD: 5.95; 2009 mean: 16.78; SD: 6.86). In these surveys, women had higher stress than men and younger people had higher stress than older people. PSS scores are presented in Table 1.

The participants also completed a modified version of the Voice Handicap Index-10 (Appendix D), with a possible range of scores between a low of 0 and a high of 40. The questionnaire consisted of the first ten questions from the original 30 item Voice Handicap Index (VHI; Jacobson, Johnson, Grywalski, Silbergleit, Jacobson, Benninger, & Newman, 1997), but not questions that make up the official 10 question VHI-10 (Rosen, Lee, Osborne, Zullo, & Murry, 2004). Three of the ten questions on the modified version of the VHI-10 used in the present study are on the VHI-10. A modified VHI-10 was used mistakenly in the present study, making a comparison to normed scores on the VHI-10 not possible. Modified VHI-10 scores are presented in Table 1.

Table 1. Scores on the Perceived Stress Scale (PSS) and modified version of the Voice-Handicap Index-10 (mVHI-10) for each participant.

HPA Axis Response Group	**PSS**	**mVHI-10**	**No HPA Axis Response Group**	**PSS**	**mVHI-10**
F1	22	11	F3	25	8
F2	15	5	F4	24	6
F5	17	5	F6	17	6
F8	31	10	F7	25	6
F10	26	2	F9	34	1
F11	22	9	F12	38	13
F13	28	2	F15	15	5
F14	29	7	F16	16	5
F17	25	1	F19	13	7
F18	8	6			
Average	***22.3***	***5.8***	***Average***	***23***	***6.33***
SD	***7.15***	***3.5***	***SD***	***8.69***	***3.16***
Average All	***22.63***	***6.05***			
SD All	***7.7***	***3.26***			

Session scheduling

Menstrual cycle. When scheduling, participants were asked to provide the date their last period began and the date their last period ended. From this information the researcher attempted to schedule sessions in or near the luteal phase of the menstrual cycle. During the luteal phase of their cycle, progesterone is at its highest level of the entire menstrual cycle. Estrogen levels are lower than during ovulation but higher than the follicular phase. It was attempted to schedule sessions outside times of menstruation and ovulation to avoid hormone related swelling of the vocal fold tissue (Abitbol, Abitbol, & Abitbol, 1999; Davis & Davis, 1993; Milbrath & Solomon, 2003), the intrinsic laryngeal muscle hypotension reported in some (Chernobelsky, 1998), and the acoustic changes that result (Fischer et al., 2011). Voice changes, including vocal fatigue, hoarseness, and lower efficiency of the vocal mechanism, have also been reported at the end of the luteal phase of the menstrual cycle and are associated with premenstrual hormonal changes (Anderson, Anderson, & Sataloff, 2005). In addition, the salivary cortisol response to the TSST of females in the luteal phase of their cycle is similar to that of males (Kirschbaum et al., 1999). Females in the follicular phase of their menstrual cycle have increased basal salivary alpha-amylase activity and higher salivary alpha-amylase activity following a stressor than females in the luteal phase of their menstrual cycle (Abrão, Leal, & Falcão, 2014; Hlavacova et al., 2017).

Despite the attempt to schedule participants during the early part of the luteal phase of the menstrual cycle, all of the participants were not in the luteal phase of the cycle based on a self-report of the dates of the last cycle and the expected start date of the next menses by the participant, although all participants reported regular menstrual cycles. Mean length of cycle of the participants in the present study was 29.58 days (SD: 3.88), with 89.5% of participants reporting cycle lengths between 22 and 36 days. These lengths are comparable to other reports of

females with regular menstrual cycles (Chiazze, Brayer, Macisco, Parker, & Duffy, 1968; Fehring, Schneider, & Raviele, 2006). As the length of individual cycles presented with normal variation, ovulation was considered to occur 14 days prior to the next menses (day 1 of the next expected cycle) (Beckmann, Ling, Herber, Laube, Smith, & Barzansky, 1998) and ovulation was set to last 6 days (Wilcox, Weinberg, & Baird, 1995), although it should be noted that there is variability in the length of time of the follicular phase among females with regular menstrual cycles (Fehring, Schneider, & Raviele, 2006). Based on ovulation occurring 14 days prior to day 1 of the next menstrual cycle and lasting 6 days, the position in the cycle (follicular, ovulation, and luteal) was determined for each participant and is presented in Figure 1. Three participants were in the non-ovulating portion of the follicular phase of the menstrual cycle, with two reporting current menstruation (participants F13 and F19). Ten participants were likely ovulating and six participants were likely in the desired luteal phase of the menstrual cycle.

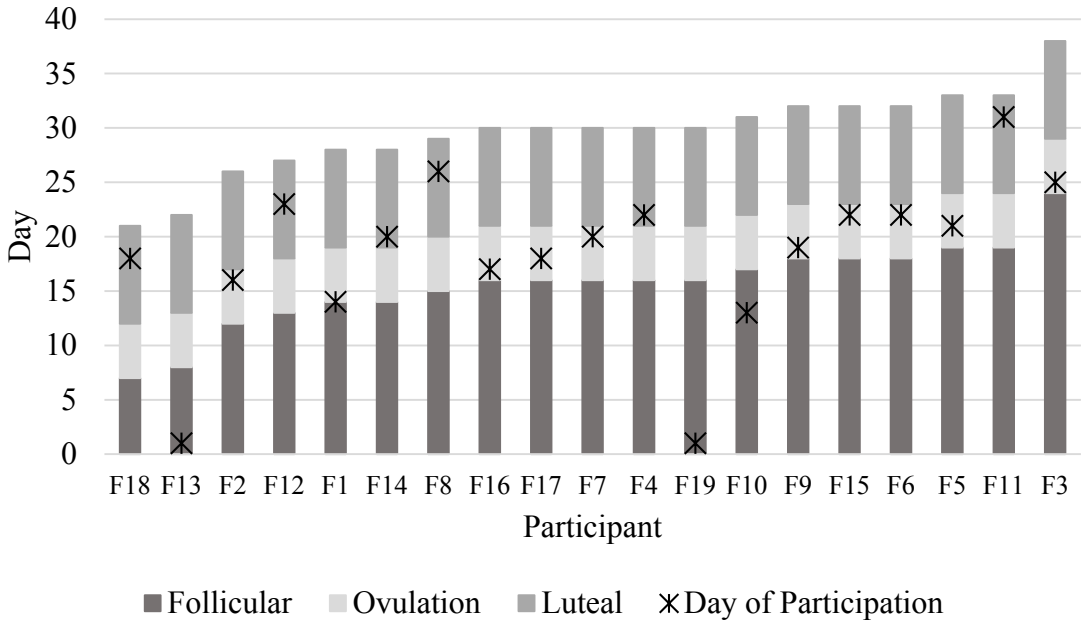

Figure 1. Predicted menstrual cycle phase for participants. Length of menstrual cycle and each of the phases (follicular, ovulation, and luteal) for each participant based on participant self-report of previous menstrual cycle and self-reported date of next menstruation. The day the participant was recorded for the present study is marked with an asterisk.

Time of day. Kirschbaum and Hellhammer (1989) recommend conducting experiments using cortisol at times throughout the day when no unstimulated cortisol changes should occur, namely, 800 to 900 hour, 1100 to 1200 hour, 1500 to 1600 hour, and 2000 to 2200 hour. Sessions were scheduled between 1500 and 1600 hour (3:00 pm and 4:00 pm) to avoid the cortisol response to waking up in the morning (Kudielka & Kirschbaum, 2003) and the meal-related increase in cortisol secretion (Kirschbaum et al., 1992a). Table 2 presents the start and end time of each session.

To ensure that there was no effect of waking on cortisol levels, participants were told to wake up a minimum of 3 hours before the session began. Wake-up times are reported in Table 2. Free cortisol levels have been found to increase by 50% to 70% within the first 30 minutes of waking, an increase that is independent of factors such as amount of sleep and time of awakening (Pruessner et al., 1997). The cortisol levels after waking may still be 34% higher than expected one hour after waking (Wüst et al., 2000). While some studies suggest that premenopausal women only (not men or postmenopausal women) had elevated cortisol levels more than 60 minutes after waking (Pruessner et al., 1997), others found no differences in morning free cortisol levels between men and premenopausal women (Kudielka & Kirschbaum, 2003; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999).

Participants were told to avoid consuming caffeine three hours before participating as caffeine has been found to combine with the effects of stress and act on the HPA axis (al'Absi et al., 1998; Lovallo, al'Absi, Blick, Whitsett, & Wilson, 1996). In lieu of standardizing glucose levels before the start of the study as suggested by Kudielka et al. (2007), participants were told to refrain from eating one hour before the start of the study.

Table 2. Self-reported wake up time, time of arrival for research session, and time of departure from research session for each participant. "~" indicates approximate time.

Participant	Time of Waking	Start Time of Recording Session	End Time of Recording Session
F1	8:30 AM	3:08 PM	5:33 PM
F2	9:00 AM	3:07 PM	5:23 PM
F3	11:00 AM	3:04 PM	~5:19 PM
F4	9:45 AM	3:02 PM	5:08 PM
F5	8:00 AM	3:12 PM	5:25 PM
F6	7:20 AM	3:05 PM	5:12 PM
F7	10:30 AM	2:59 PM	5:09 PM
F8	8:30 AM	2:51 PM	5:03 PM
F9	10:00 AM	3:04 PM	5:15 PM
F10	9:00 AM	3:00 PM	5:09 PM
F11	8:00 AM	2:44 PM	5:06 PM
F12	6:30 AM	2:58 PM	~5:17 PM
F13	10:30 AM	2:58 PM	~5:06 PM
F14	10:00 AM	2:50 PM	4:53 PM
F15	10:45 AM	3:02 PM	5:11 PM
F16	6:50 AM	3:01 PM	5:20 PM
F17	7:45 AM	3:01 PM	5:21 PM
F18	6:07 AM	2:35 PM	4:52 PM
F19	7:00 AM	3:02 PM	5:12 PM

Protocol

The full experimental protocol can be found in Appendix E and briefly listed in the following paragraph. (1) Following the completion of the initial health questionnaire, the recording equipment was explained to the participant. The participant practiced producing smooth, even /pa/ syllable repetitions on one breath. The participant was given verbal feedback to guide her productions to a plateau shaped oral pressure /p/ occasion with the lips closed long enough to ensure air pressure equilibration within the airways. (2) Initial voice recordings, stress marker measurements, and ratings of stress and emotion were completed. (3) The participant rested in a quiet room (the research lab) for 10 minutes with no sleeping, phone use, TV watching, reading, etc. This served to reduce the influence of the previous activities on stress

levels (Kudielka et al., 2007). (4) Basal un-stressed voice recordings, stress marker measurements, and ratings of stress and emotion were made. (5) The participant then completed the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) protocol to induce psychosocial stress. The TSST involved an anticipatory period in which the participant was given just under ten minutes to prepare a job interview speech that must last for five minutes. (6) Voice recordings, stress marker measurements, and ratings of stress and emotion were made. (7) The participant was then taken to a conference room (down the hall from the recording laboratory where the aforementioned procedures took place) where there were two or three trained "committee members" who then "evaluated" the performance of the participant while she gave her speech. That is, the committee was seen by the participant as individuals judging what she was saying and her behavior during her talk. After five minutes of speaking or being questioned if the speech did not last for five minutes, the participant was asked to subtract 13 from the number 6233 out loud, continuing to repeatedly subtract 13 from each of the new numbers, again in front of the evaluation committee. Together these tasks are thought to induce activation of the HPA axis because they are considered relatively uncontrollable and because they involve a social-evaluative threat (Dickerson & Kemeny, 2004). (8) Following the TSST the participant returned to the voice laboratory where voice recordings, stress marker measurements, and ratings of stress and emotion were made. (9) The participant is debriefed about the nature of the stressor using the script presented in Appendix E. (10) The participant rested for three, 10 minute periods. Voice recordings, stress marker measurements, and ratings of stress and emotion were made every ten minutes.

Participants were not monitored by members of the research team during each of the 10 minute rest periods. All rest periods by all participants took place at the same desk in the same

room. As it has been suggested that a rest time of 30 to 40 minutes will help to reduce the influence of previous events on salivary cortisol (Kudielka, Hellhammer, & Kirschbaum, 2007), what the participant did during the last 2 rest periods is less important than what they did during the pre-stress rest period and the first post-stress rest period. Anecdotally, many participants reported that the rest periods were difficult because it was difficult not to do anything.

Measurements and recordings

Rating of stress. At each measurement point, participants rated their current stress level using a 100 mm line (visual-analog scale). This is presumed to be a rating of psychological stress. It is important to collect information about psychological stress because of the inconsistencies between the psychological stress response system and the physiological stress response systems. Specifically, the psychological stress response is activated quickly and changes rapidly while the cortisol response changes slowly and does not peak until fifteen to twenty minutes after the stressor begins (Kudielka et al., 2009). Schlotz et al. (2008) found subjective ratings of arousal (a marker of psychological stress) were elevated before cortisol levels were elevated. Interestingly, the authors also found that high cortisol levels were related to lower anxiety levels five to ten minutes later (Schlotz et al., 2008).

Rating of emotion. Self-reported ratings of emotion were made by participants using the Visual Analog Mood Scales ™ (VAMS ™) (Nyenhuis, Yamamoto, Stern, Luchetta, & Arruda, 1997). Although the test is called the Visual Analog Mood Scale, the VAMS protocol asked participants to rate emotional states. As previously discussed, emotions are quick reactions related to a stimulus. In contrast, moods are slow-moving states that are not necessarily related to a stimulus or situation (Rottenberg, 2005). Moods do not change behavior and physiology like

emotions do (Rottenberg, 2005). The directions of the VAMS tell the participants to rate "how they are feeling right now." This is reflective of an emotional state that will change over the course of the experimental procedure due to exposure and recovery from a stressor.

The VAMS asks the participants to rate how afraid, confused, sad, angry, energetic, tired, happy, and tense they feel, using a 100 mm vertical line. Zero represents that the participant does not feel that particular emotion and one hundred represents the maximum the participant could feel that emotion. T-scores for each raw score for each emotion were determined from a normative table based on the sex and age of the participants. The T-scores represent linear transformed raw scores with a mean of 50 and a standard deviation of 10 from a sample population (Stern, 1997). A 95% confidence interval for scores, due to the expected lower test-retest reliability that occurs when measuring states, is plus or minus 10*T*, suggesting that a change of 20*T* is a reliable indicator of a change in emotional state (Stern, 1997). As stress reactivity is related to psychosocial factors, it is important to account for the participant's self-report of emotional state (Gruenewald et al., 2004; Henry, 1992).

Stress markers. Cortisol and alpha-amylase were measured from saliva samples. These markers represent activity of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system activity, respectively.

As the catecholamine norepinephrine can take up to an hour to transfer from the blood to saliva, another salivary marker of the SNS system was needed (Kennedy, Dillon, Mills, & Ziegler, 2001). The salivary glands change their secretion based on if the primary stimulation is from the parasympathetic nervous system or the sympathetic nervous system (Iversen, Iversen, & Saper, 2000). If the sympathetic dominates, saliva is viscous with a higher amylase content (Iversen et al., 2000). If the parasympathetic dominates, saliva is more watery (Iversen et al.,

2000). Salivary alpha-amylase has been used as a substitute for non-invasively measuring activity of the sympathetic nervous system (e.g., Nater & Rohleder, 2009). Salivary alpha-amylase has been found to increase in activity in response to both psychological and physical stressors (Nater & Rohleder, 2009). Salivary alpha-amylase does not show the same pattern of change as salivary cortisol, suggesting that it does not reflect changes in the HPA axis (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996). Salivary alpha-amylase peaks immediately after the cessation of the stressor and returns to basal levels 20 minutes after the stressor (Maruyama et al., 2012). Salivary alpha-amylase is independent of flow rate of the saliva (Rohleder, Wolf, Maldonado, & Kirschbaum, 2006).

Salivary cortisol is highly correlated with free (unbound) cortisol in the plasma and in serum. There is a non-linear relationship between salivary cortisol and total cortisol. At lower levels of total cortisol, salivary cortisol is 1-2% of total cortisol while at higher levels of total cortisol, salivary cortisol is 8-9% of total cortisol (Hellhammer, Wüst, & Kudielka, 2009). When the TSST is administered, however, total cortisol is not at high enough levels to result in a non-linear relationship between total cortisol and salivary cortisol (Hellhammer et al., 2009) because it is considered a "moderate psychosocial" stressor (Kudielka et al., 2007). Since salivary cortisol enters the saliva through an intracellular route, the concentration of cortisol in the saliva is not dependent on saliva flow rate (Vining et al., 1983). A participant was considered to have a salivary cortisol response if their salivary cortisol increased 2.5 nmol/l above the measure of cortisol taken in that individual after the rest period (the second measure in the present study). This criterion was suggested by Weitzman et al. (1971).

Saliva samples were collected using the SalivaBio Oral Swab (Salimetrics, State College, PA). The synthetic swab improves volume of saliva collected. The swab was placed under the

tongue of the participant for between 1 to 2 minutes, until approximately 1 to 2 mL of saliva was collected. Before saliva was collected the first time, participants rinsed their mouth out with water to ensure no food particles were present. Each participant was instructed to hold the swab under her tongue and not to move the swab around or chew on the swab. The soaked swab was placed in the designated 17 mm x 100 mm storage tube and the lid was snapped in place. The saliva samples were labeled and immediately placed in a -20 degree Celsius freezer.

Salivary samples were sent to a lab (University of Michigan Core Assay Facility) to be analyzed for cortisol concentration and alpha-amylase activity. The samples were placed into two aliquots, one for cortisol analysis and one for alpha-amylase analysis. Enzyme immunoassay (EIA) analysis was completed on the samples for cortisol analysis (using a Salimetrics Cortisol ELISA Kit) and an enzymatic method was used for the alpha-amylase assay (using a Salimetrics Alpha-Amylase Assay Kit). In the case of a specific type of EIA analysis, an ELISA analysis (enzyme-linked immunosorbent assay), the antigen (cortisol) was attached to a plate so it was no longer mobile. An antibody specifically for each antigen (one for cortisol) was added to the sample. The antibody bound with the antigen and excess materials that were not bound to the antibody were washed off the plate. Another enzyme was then added to make the reaction measurable, indicating how much antigen was in the sample. For the alpha-amylase assay, alpha-amylase acts on 2-chloro-p-nitrophenol linked to maltotriose to form 2-chloro-p-nitrophenol, which was measured using spectrophometery at a wavelength of 405 nm.

For cortisol, five kits were used to analyze all of the samples. The inter-assay coefficient of variance ranged from 6.13% at 2.700843 nmol/l (SD: 0.165452) to 2.68% at 27.07469 nmol/l (SD: 0.72663) and the intra-assay coefficient of variance was 5.48% across all samples. Salivary cortisol could not be determined for two samples (F14 recording 4 and F15 recording 7) because

not enough saliva was provided. For alpha-amylase, eleven kits were used to analyze all of the samples. The inter-assay coefficient of variance ranged from 8.42% at 22.534 U/mL (SD: 1.896955) to 5.18% at 233.889 U/mL (SD: 12.11375) and the intra-assay coefficient of variance was 2.16% across all samples.

Voice recordings. Aerodynamic recordings were made using the Glottal Enterprises aerodynamic system (MSIF-2 S/N 2049S). This system includes a clear face mask with holes that are covered by mesh wire. Oral air pressure recordings were made by using a thin tube that extended into the corner of the mouth with its tip placed just past the lips but not obstructed by the tongue; the tube was connected to a pressure transducer attached to the mask. Another pressure transducer for measurement of trans-mask air pressures that were calibrated to airflow through the mask was used for oral airflow recordings. This system was calibrated using constant flow and constant pressure techniques (calibrations are given in the Appendix F and G). An electroglottograph (Kay Elemetrics EGG, Model 6130) was used to obtain the EGG signal. A condenser microphone attached to a headset that was placed approximately 4 cm and 45 degrees to the left side of the mouth was used to record audio (C 420 III PP MicroMic from AKG Acoustics; frequency response 20-20,000 Hz).

A 16 bit DATAQ A/D converter system (Model DI-2108 Series) with Windaq Pro + software was used to digitize the simultaneous audio, pressure, flow, and EGG signals into computer files at 20,000 samples per second for each channel.

Acoustic and aerodynamic recordings consisted of repetitions of /pa/ seven to nine times on one breath in a comfortable speaking voice. Five or more sets of repetitions of /pa/ were produced at each recording point to ensure that five sets of /pa/ repetitions could be averaged at each measurement time point. The /pa/ syllable string was produced smoothly, at a comfortable

and constant effort level, and with relatively flat pressure plateaus indicating pressure equilibration throughout the entire airway during the lip occlusion (Frazer, 2014). Participants were monitored during the recordings to ensure that the mask was placed firmly against the participant's face to ensure that there were no leaks around the rim of the mask. In addition, the recordings were monitored on the computer screen by a researcher in real-time to ensure that there were no velar leaks or blocks in the oral pressure tube. The participants were briefly instructed on this procedure and participants were all given a chance to practice the recording technique before the first recording was made.

Following the /pa/ repetitions, the participant read the first paragraph of the Rainbow Passage (Fairbanks, 1960) without the aerodynamic mask on her face. Only the microphone and EGG signals were recorded during the paragraph reading.

During the TSST, a laptop microphone was used to record the speech, although analysis of these recordings are not presented in the present paper.

Analysis of the Rainbow Passage

Counting vocal fry. Recordings of the participant reading a brief paragraph were reviewed for the presence of vocal fry by members of the research team (RS and BP). The research team listened to the recordings through speakers on a laptop computer. Team member BP reviewed the files independently and marked syllables that presented with vocal fry auditorily. Together at a later time, BP and RS listened to the recordings together and arrived at a consensus regarding the presence or absence of vocal fry on each syllable. RS used both auditory and visual cues to guide his decision. Yuasa (2010) used a similar method of first listening to the samples and then visually confirming the presence or absence of "creaky voice" (which is the

term the author applies to common definitions of vocal fry) using both the waveform and the spectrogram. Vocal fry appears as irregular vertical striations in the spectrogram, although Yuasa (2010) notes that the waveform may not appear irregular in instances in which the spectrogram reveals widely spaced vertical striations.

Similar to descriptions of vocal fry reported in the literature (Keating, Garellek, & Kreiman, 2015), in the present study, several categories of vocal fry were included in the vocal fry count. The categories used in the present study include "prototypical" fry (Keating, Garellek, & Kreiman, 2015) (Figure 2), aperiodic fry (Figure 3), period double fry (Figure 4), and a new category called "onset fry". Onset fry is vocal fry that occurs on a single syllable word that begins with a vowel and can be divided into onset fry with a delay between glottal pulses and the start of the vowel proper (Figures 5 and 6) or onset fry without a delay, where vocal fry of any of the aforementioned categories occurs at the beginning of a word that starts with a vowel (Figure 7).

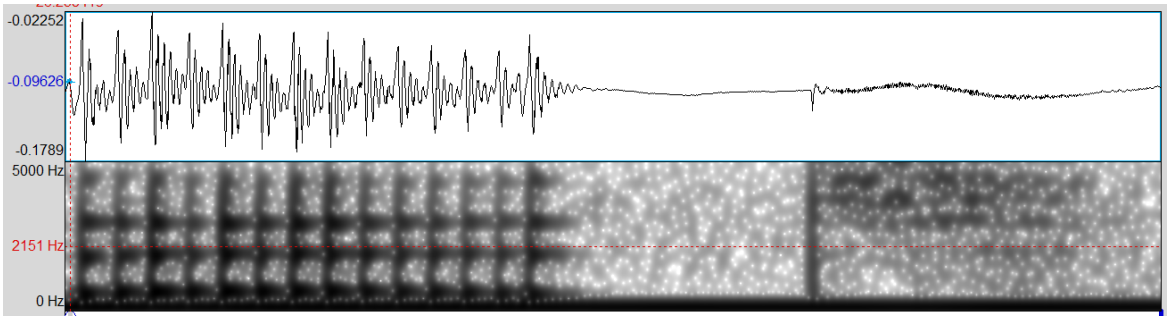

Figure 2. The word "it" produced with "prototypical" fry by participant F1 in Recording 1.

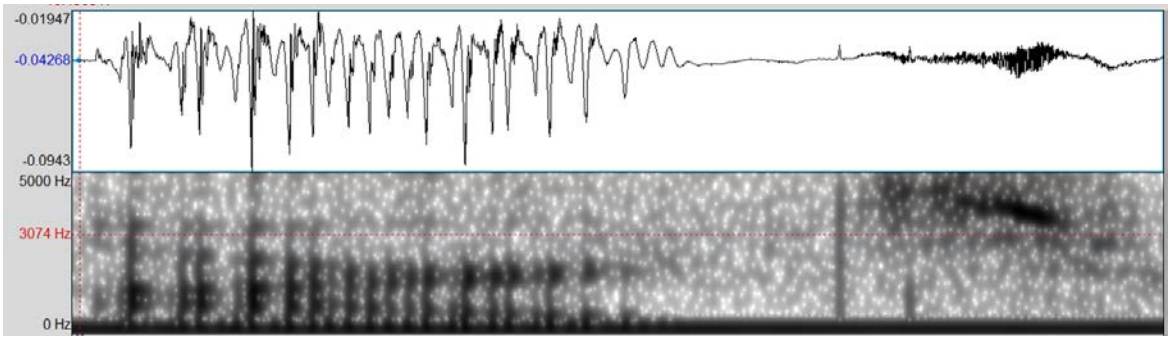

Figure 3. The word "arch" produced with aperiodic fry by participant F16 in Recording 5.

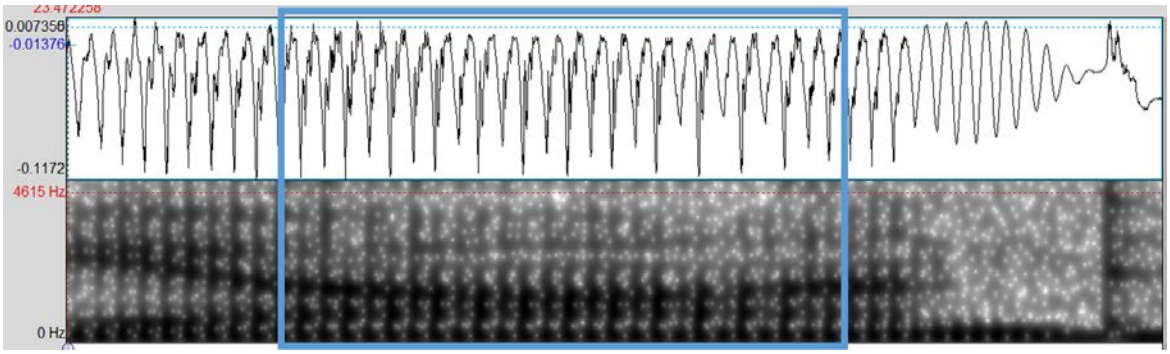

Figure 4. The syllable "yond" of the word "beyond" produced with period double fry by participant F16 in Recording 2. The portion of the syllable produced with period double fry is highlight by the box.

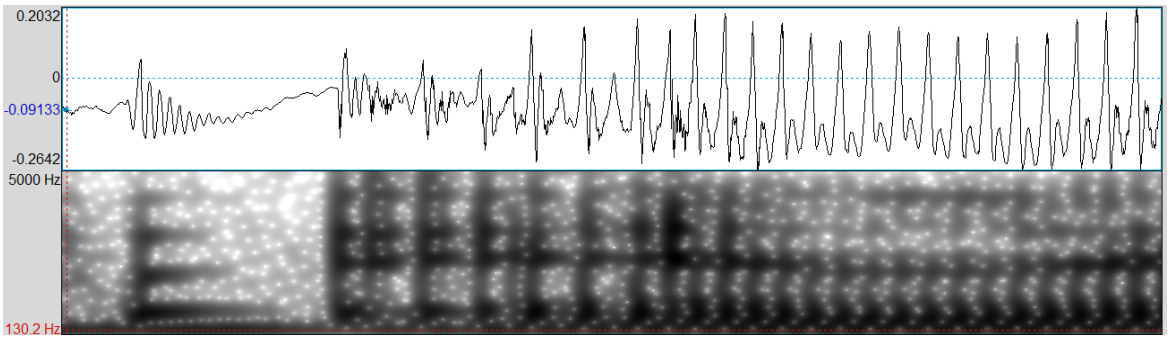

Figure 5. The word "and" produced with vocal fry at the beginning of the word by participant F3 in Recording 1. This production is characterized by a single glottal cycle followed by a delay before the next glottal cycle all of which are produced at a fundamental frequency lower than the participant's modal register. Note that the /d/ was not released at the end.

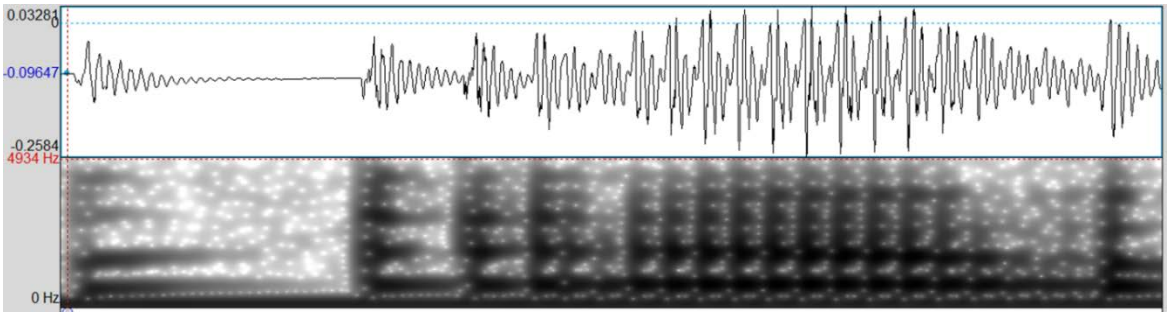

Figure 6. The word "at" produced with vocal fry at the beginning of the word by participant F1 in Recording 1. This production is characterized by a single glottal cycle followed by a prolonged delay before the next glottal cycle, all of which are produced at a fundamental frequency lower than the participant's modal register.

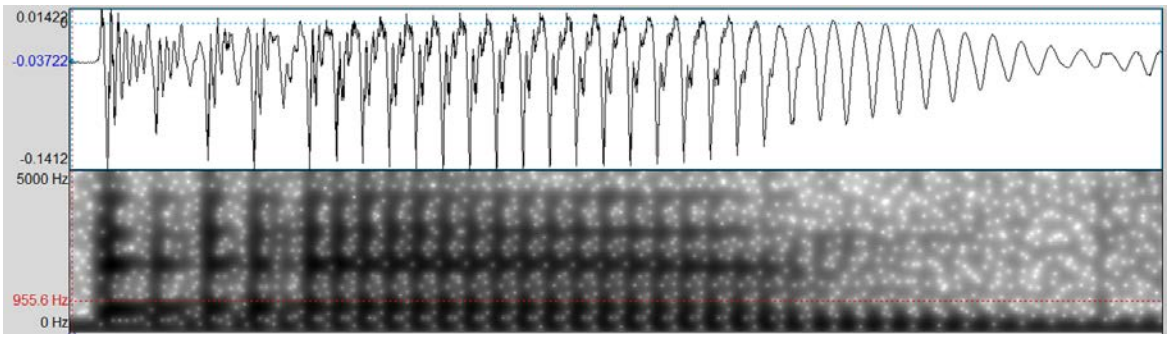

Figure 7. The word "ends" produced with vocal fry at the beginning of the word (normal vocal fry and two cycles of period double vocal fry produced at the onset of the word) by participant F16 in Recording 2.

Determining fundamental frequency. The mean and standard deviation of the fundamental frequency were determined from the second sentence of the Rainbow Passage (Fairbanks, 1960). The second sentence of the Rainbow Passage was used because the measure of speaking fundamental frequency for this paragraph is highly correlated with the speaking fundamental frequency of the entire first paragraph and the use of the second sentence avoids voice and speech changes often noted in the first and final sentences of the passage (Horii, 1975; Shipp, 1967). Praat Software (Version 6.0.14) was used to find the mean and standard deviation of the fundamental frequency for that sentence. The second sentence was extracted from the whole recording. The Praat-based pulse analysis was used to extract the fundamental frequency. The pulses were removed from the entire syllable of any syllable that had been marked as containing vocal fry. Pulses were also removed from locations that did not present with glottal pulses upon examination of the spectrogram. Errors by the participant in speaking the sentence were not removed from analysis unless the error met either of the above conditions. The remaining pulses were extracted to "PointProcess" and then to "PitchTier" with a maximum interval of 0.005 seconds in Praat. The mean and standard deviation were recorded based on the points and not the area under the curve. The number of points was also noted.

Analysis of /pa/ syllable repetitions

Each participant produced seven to nine /pa/ repetitions five or more times for each recording (number 1 through 7). The first and the last /pa/ repetition were not included for each set. For example, if a person produced seven /pa/ syllables on a breath group, six times during one recording, there were 30 syllables analyzed (7 syllables produced in a breath group, where the middle 5 were chosen, times the 6 sets produced, equaling 30 syllables analyzed) if all syllables were considered acceptable (see below). Whenever an average value is reported for a recording (the recordings are numbered 1 through 7 and are called measurement time points), the average value is the average of all syllables from all sets from that recording.

Determining aerodynamic measures. The oral airflow and oral air pressure signals were analyzed from the repeated /pa/ syllables using custom software called SIGPLOT. The first syllable and the last syllable were never used for analysis. The pressure signal was averaged using a moving average of 20 samples (10 samples to the left of the target sample and 10 samples to the right). The airflow signal was averaged similarly using either 200 samples or 3000 samples (the average airflow did not alter significantly for 200 samples average versus 3000 samples average). The amount of averaging of the flow resulted in a varying amount of peak to peak variation of the averaged flow signal (Table 3). The researcher chose points on the pressure and flow signals to derive an average airflow measure and an estimate of subglottal pressure during the vowel from the oral airflow and the oral air pressure signals, respectively. Only mostly rectangular or slightly sloped oral pressure /p/ occlusions were included in the analysis (Frazer, 2014). Estimates of subglottal pressure from oral air pressure with sloped /p/ occlusions have been reported to be less than 2% different from actual measures of subglottal pressure (Hertegård, Gauffin, & Lindestad, 1995). In addition, the raw airflow value during the /p/

occlusion was examined to ensure that the airflow during the /p/ occlusion was 0 cm^3/s, indicating that full lip occlusion occurred and equilibration of the system was likely.

Table 3. Number of averaging samples for the flow signal and the average peak to peak variation for all recordings of a given participant of the averaged flow signal (rounded to the nearest whole number)

Participant ID	Number of averaging points	Peak to peak variation (cm^3/s)
F1	3000	<1
F2	3000	<1
F3	3000	<1
F4	3000	<1
F5	200	2
F6	200	19
F7	200	15
F8	200	17
F9	200	25
F10	200	10
F11	200	12
F12	200	13
F13	200	23
F14	200	13
F15	200	55
F16	200	7
F17	200	14
F18	200	12
F19	3000	<1

When a syllable had an average airflow lower than 70 cm^3/s, the extracted oral air flow for that syllable was removed from further analysis (Table 4). A criterion of 70 cm^3/s was chosen because mean airflow values below 70 cm^3/s have not been seen in many studies including young female participants (Biever & Bless, 1989; Peppard, Bless, & Milenkovic, 1988; Rau & Beckett, 1984). Although a member of the research team monitored the mask positioning on the face of the participant, it is suspected that average flow values below 70 cm^3/s were the result of incomplete contact between the mask and the face. All analyzed syllables had an average airflow less than 70 cm^3/s for F3 recording number 2 (basal) and recording number 5 (post-stress 1) and

for F5 recordings 2 through 7 (all recordings except beginning) and were thus removed from analysis. For syllables in which the average airflow was removed, the estimated subglottal pressure was still included in the analysis, resulting in estimates of subglottal pressure for the total number of syllables [removed, (REM) + remaining (RMNG)] in Table 4. The exception is F19. F19 presented with many /p/ occlusions that were rounded, with raw oral airflow signals that did not return to 0 cm^3/s, suggesting that pressure equilibration did not occur for these syllables. The number of pressure syllables included in the analysis for F19 is noted at the bottom of Table 4.

Laryngeal airflow resistance [(kPa)/(L/s)] was calculated by taking a ratio of the estimated subglottal pressure and the airflow (Smitheran & Hixon, 1981). Each recording (1 through 7) yielded a measure of average subglottal pressure, average airflow, and average airflow resistance. Airflow resistance could only be calculated for syllables that had both pressure and flow values, and thus resistance was only calculated for the remaining (RMNG) syllables in Table 4 (with the exception of F19).

Calculating EGG open quotient. Open quotient was calculated from the derivative of the electroglottographic signal during the /a/ of the /pa/ repetitions. Open quotient was only calculated from sets of /pa/ repetitions that were included in the aerodynamic analysis. The first and the last syllables were excluded. The positive peak of the time derivative of the EGG signal was automatically taken as the first moment of glottal closure and the moment of glottal opening was chosen manually, because the minimum of the time derivative of the EGG signal is often not clear enough for the process to be automatic. The calculation of open quotient used in the present study can be found in Scherer, Vail, and Rockwell (1993) and Figure 8. For each vowel, if the standard deviation of the open quotient was greater than 0.1, the open quotient for that vowel

Table 4. Number of syllables removed (REM) due to average airflow below 70 cm^3/s and the number of syllables remaining (RMNG) for each recording that were analyzed for average oral airflow and estimated subglottal pressure.

	1		2		3		4		5		6		7	
	REM	RMNG	REM	RMNG	REM	RMNG	REM	RMNG	REM	RMNG	REM	RMNG	REM	RMNG
F1	0	30	0	30	0	36	0	25	0	30	0	29	0	30
F2	0	28	0	29	0	28	0	36	0	26	0	30	1	29
F3	2	25	39	**0**	29	11	32	4	27	**0**	33	2	39	3
F4	0	28	0	28	0	30	0	30	0	30	0	30	0	30
F5	26	3	24	**0**	20	**0**	19	**0**	22	**0**	22	**0**	26	**0**
F6	0	27	0	29	0	20	0	27	0	30	0	24	0	14
F7	0	23	0	26	3	23	8	14	0	26	0	26	0	28
F8	15	10	6	20	1	26	16	16	4	21	2	25	6	22
F9	7	17	0	21	0	20	0	16	0	22	0	23	0	21
F10	0	12	0	16	0	17	0	19	0	16	0	17	0	16
F11	0	20	0	27	0	28	0	22	0	7	0	11	0	17
F12	0	15	0	26	0	33	0	27	1	26	0	29	0	27
F13	0	25	0	26	0	20	0	23	0	22	0	17	0	18
F14	0	21	0	30	0	24	0	25	0	25	0	21	0	26
F15	1	30	1	33	0	29	0	34	0	18	0	33	0	28
F16	0	24	0	19	0	36	0	36	0	34	0	27	0	23
F17	0	8	0	16	0	14	0	21	0	28	0	29	0	36
F18	0	10	0	19	0	23	0	15	4	17	1	15	8	17
F19*	0	23	0	27	0	30	0	36	0	30	0	30	0	35

* F19 is the only participant for whom the total number of syllables analyzed (REM + RMNG) does not equal the number of syllables for which subglottal pressure was estimated from oral pressure. (Recording 1: 23 syllables were included in the pressure analysis; Recording 2: 3 syllables were included in the pressure analysis; Recordings 3-7: 0 syllables were included in the pressure analysis)

was removed from further analysis. Then, for each vowel, individual glottal cycles that presented with an open quotient plus or minus two standard deviations from the mean for that vowel were removed. Each recording (1 through 7) yielded a measure of the average EGG open quotient. An accurate EGG signal could not be obtained for F13, F15, F16, F5 recordings 3 (anticipatory), 5 (post-stress 1), 6 (post-stress 2), and 7 (post-stress 3), and F1 recording 7 (post-stress 3).

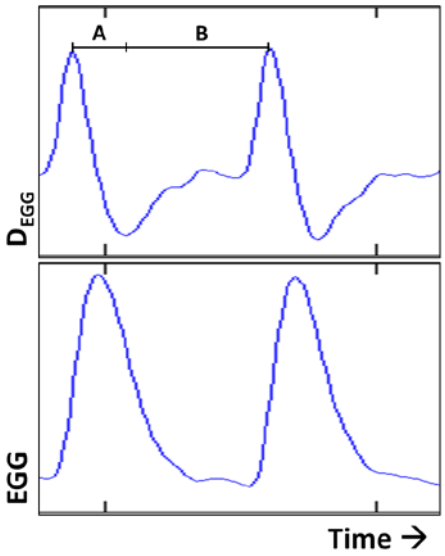

Figure 8. Derivative of the EGG signal (top panel) and smoothed EGG signal (smoothed at 5 points) (bottom panel). EGG open quotient was calculated as *EGG OQ = B / (A+B)*.

Analysis of salivary stress markers

To differentiate participants who experienced a cortisol increase from those who did not, the increase in cortisol was calculated as the basal cortisol measure (measure at time 2) minus the maximum cortisol value achieved (Hellhammer & Schubert, 2012). An increase of 2.5 nmol/l from time 2 to any time after the stressor (times 4, 5, 6, or 7) placed the participant in the HPA axis response group (Weitzman et al., 1971).

Reliability

Intra-rater and inter-rater reliability were both tested for each dependent variable. The main researcher (BP) and other researchers trained in the analyses reanalyzed the data from 2 participants (10.5% of the data) for the estimated subglottal pressure, average airflow, laryngeal airflow resistance, and speaking fundamental frequency and the data from 1 participant (5.25%) for the open quotient from the EGG signal. Pearson Product Movement Correlation was used for both intra- and inter-rater reliability checks. The Pearson Product Movement Correlation was significant for intra-rater reliability (average airflow: $r = .990$, $p < .01$; estimated subglottal pressure: $r = .999$, $p < .01$; laryngeal airflow resistance: $r = .995$, $p < .01$; open quotient: $r = .955$, $p < .01$; speaking fundamental frequency: $r = .999$, $p < .01$) and for inter-rater reliability (average airflow: $r = .949$, $p < .01$; estimated subglottal pressure: $r = .997$, $p < .01$; laryngeal airflow resistance: $r = .994$, $p < .01$; open quotient: $r = .940$, $p < .01$; speaking fundamental frequency: $r = .944$, $p < .01$). The mean absolute intra-rater percent difference was near or below 2% for all measures (average airflow: 1.33%; estimated subglottal pressure: 0.78%; laryngeal airflow resistance: 2.07%; open quotient: 1.08%; speaking fundamental frequency: 0.22%) and the mean absolute inter-rater percent difference was below 4% for all measures (average airflow: 2.33%; estimated subglottal pressure: 3.35%; laryngeal airflow resistance: 2.67%; open quotient: .86%; speaking fundamental frequency: 0.45%).

Statistical analysis

The voice parameters were average airflow, estimated subglottal pressure, laryngeal airflow resistance, EGG open quotient, fundamental frequency during reading, and percent of syllables produced in vocal fry during reading. To determine if there was an effect of stress on

those voice parameters following the acute, social-evaluative stressor, repeated measures analysis of variance (ANOVA) was conducted with measurement time point as the independent variable with 7 levels (beginning, basal, anticipatory, post-stress 0, post-stress 1, post-stress 2, post-stress 3) and the aforementioned variables as dependent variables in their own ANOVA model. Main effects were interpreted using a significance value of $p < .05$.

To examine if participants who experienced HPA axis activation following the acute, social-evaluative stressor had different values for the voice parameters at any or all measurement time points, or if the participants who experienced HPA axis activation had different patterns of change in the voice parameters over the measurement time points than those who did not experience HPA axis activation, a two-way repeated measures analysis of variance (ANOVA) was conducted for each dependent variable (average airflow, estimated subglottal pressure, laryngeal airflow resistance, EGG open quotient, fundamental frequency during reading, and percent of syllables produced in vocal fry during reading). Group was the between-subjects factor (2 levels: HPA axis activation, no HPA axis activation) and time was the within-subjects factor (7 levels). Participants who experienced a 2.5 nmol/l increase in cortisol at any measurement time point after the stressor (4, 5, 6, or 7) were considered to have HPA axis activation. Main effects and interaction effects were interpreted using a significance value of $p < .05$.

To determine changes in estimates of subglottal pressure, average airflow, electroglottographic open quotient, and speaking fundamental frequency during the reading task, the values of the aforementioned variables at time 2 were subtracted from the values at time 4. A percent change was calculated by dividing the difference between the dependent variable from time 2 to time 4 by the value at time 4 and multiplying by 100. The coefficient of variation was

calculated by dividing the mean value into the standard deviation and multiplying the result by 100 for each dependent variable for each participant at each measurement time point.

To determine the relationship between changes in emotions and changes in estimates of subglottal pressure, average airflow, electroglottographic open quotient, and speaking fundamental frequency during the reading task, a series of Chi-square tests was conducted using dummy coded emotion ratings ("1" if there was a change of 20*T* or more from time 2 to time 4 or "0" if there was not a change of at least 20*T* from time 2 to time 4) and dummy coded changes in the voice parameters ("1" if there was a change in the variable from time 2 to time 4 that exceeded the maximum coefficient of variation calculated in the previous step and "0" if there was no such change). The associations were interpreted using a significance value of $p < .05$.

CHAPTER IV: RESULTS

Salivary cortisol, salivary alpha-amylase, and self-rating of stress across the seven measurement time points

Figures 9, 10, and 11 present the change in salivary cortisol, salivary alpha-amylase, and self-rating of stress, respectively, for all participants over the 7 measurement time points. The results of a series of repeated measures ANOVAs to determine if the changes in salivary cortisol, salivary alpha-amylase, and self-rating of stress were significant following the stressor are presented in the following paragraphs.

A repeated measures ANOVA for logarithmically transformed salivary cortisol was conducted. Mauchly's test of sphericity was significant, indicating that the assumption of sphericity had been violated, $\chi^2(20) = .001$, $p < 0.001$. The results of the ANOVA were interpreted using a Greenhouse-Geisser correction ($\varepsilon = .292$) and revealed that salivary cortisol was statistically significantly different at different measurement time points, $F(1.750, 27.996) = 4.443$, $p = .025$, partial $\eta^2 = .217$. There was a statistically significant increase in salivary cortisol from measurement time point 3 (before the stressor; mean: 4.23 nmol/l; SEM: 0.59) to measurement time point 4 (just after the stressor; mean: 6.97 nmol/l; SEM: .86). It is likely that measurement time points 5 (mean: 8.52 nmol/l; SEM: 1.68) and 6 (mean: 7.12 nmol/l; SEM: 1.18) when salivary cortisol was expected to be most different were not significantly different from other measurement time points made before the stressor because the SEM was high.

A repeated measures ANOVA for salivary alpha-amylase was conducted. The assumption of normality was violated at measurement time point 2, $p < .001$, and measurement time point 4, $p = .036$. No transformations were made. The assumption of sphericity was also violated, $\chi^2(20) = .014$, $p < 0.001$ so a Greenhouse-Geisser correction was applied, $\varepsilon = .403$.

Salivary alpha-amylase was not significantly different at different measurement time points, $F(2.416, 43.479) = .880$, $p = .439$, partial $\eta^2 = .047$.

A repeated measures ANOVA for self-rating of stress was carried out. The assumption of normality was violated at measurement time point 1, $p = .012$, measurement time point 2, $p = .007$, measurement time point 4, $p = .049$, and measurement time point 7, $p = .002$. No transformations were made. Again the assumption of sphericity was violated, $\chi^2(20) = .004$, $p < 0.001$ and a Greenhouse-Geisser correction was applied, $\varepsilon = .364$. Self-rating of stress was significantly different at different measurement time points, $F(2.181, 39.263) = 21.669$, $p < .001$, partial $\eta^2 = .546$. Based on the result of post-hoc t-tests with alpha levels corrected using a Bonferonni-type adjustment, measurement time point 4 was significantly different (higher) than all other measurement time points ($p < .001$). In addition, measurement time point 7 was significantly different (lower) than measurement time point 3 ($p = .014$), 4, 5 ($p = .002$), and 6 ($p = .008$).

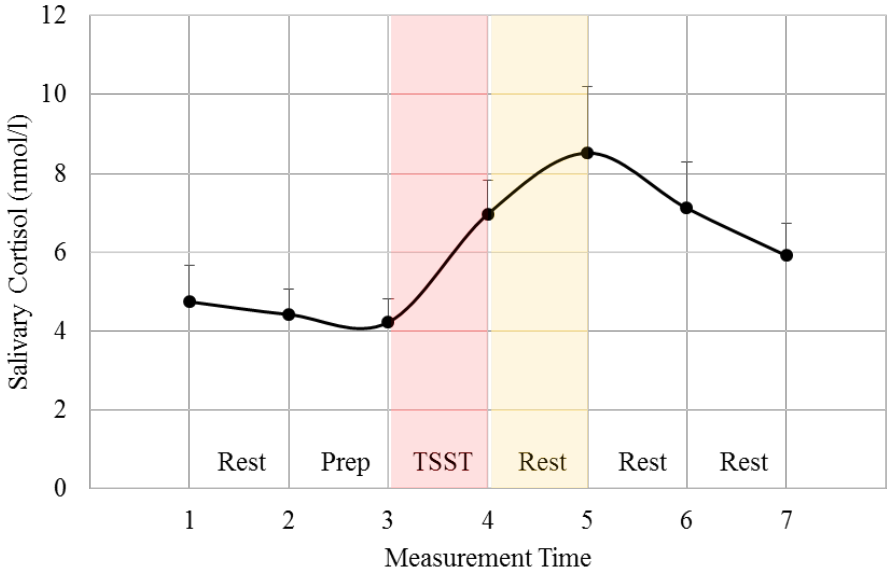

Figure 9. Mean salivary cortisol (nmol/l) and standard error for all participants across all 7 measurement time points. The stressor occurred between measurement time points 3 and 4.

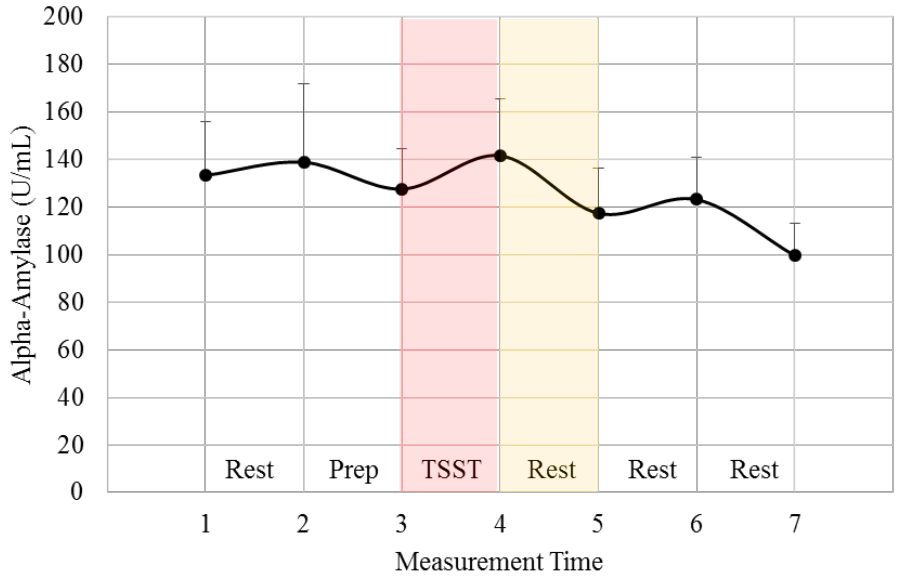

Figure 10. Mean salivary alpha-amylase (U/mL) and standard error for all participants across all 7 measurement time points. The stressor occurred between measurement time points 3 and 4.

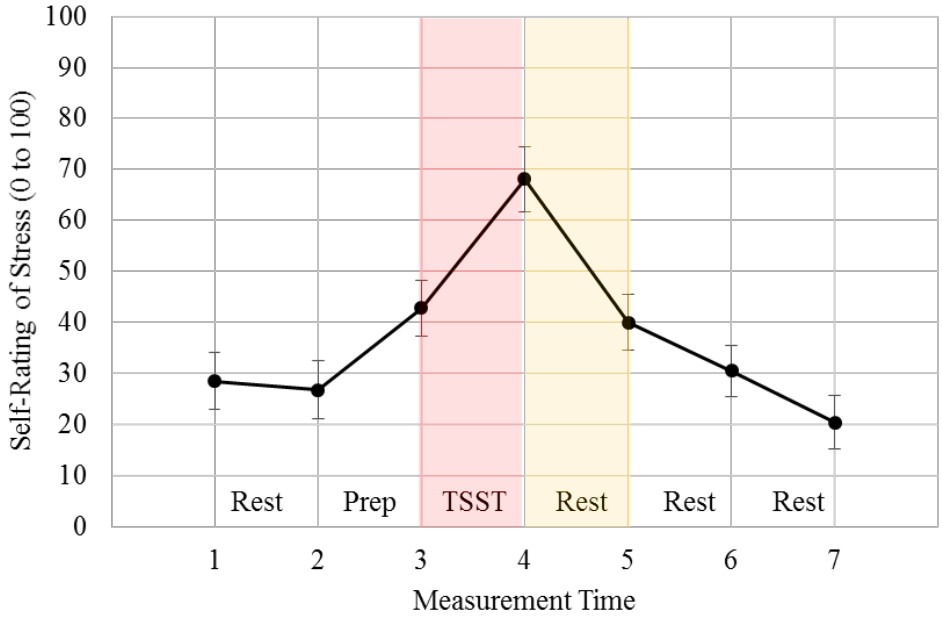

Figure 11. Mean self-rating of stress and standard error for all participants across all 7 measurement time points. The stressor occurred between measurement time points 3 and 4.

If participants had an increase in salivary cortisol from time 2 to time 4 greater than or equal to 2.5 nmol/l, they were considered to have HPA axis activation. If participants had an increase in salivary alpha-amylase of 10% from time 2 to time 4, they were considered to have

SNS activation. Table 5 lists the participants who experienced HPA axis activation only, SNS activation only, activation of neither system, and activation of both stress systems.

Table 5. List of participants organized by stress system activation following the TSST

Neither SNS nor HPA axis	Both SNS and HPA axis	SNS only	HPA axis only
F7	F2	F3	F1
F12	F8	F4	F5
F15	F10	F6	F14
	F11	F9	F17
	F13	F16	
	F18	F19	

Q1: Influence of an acute, social-evaluative stress task on voice parameters in all participants

Average airflow. Average airflow could not be determined for F3 recordings 2 and 5, F5 recordings 2 through 7, and F9 recording 1. Figure 12 shows the group means and standard error (error bars) for the average airflow at each measurement time point. A repeated-measures ANOVA was conducted to compare the effects of time (7 levels) on average airflow. Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(20) = 21.939$, $p = 0.359$, and the airflow values at all seven time points were normally distributed based on a Shapiro-Wilk test. There was a significant effect of time on average airflow from the /pa/ repetitions, $F(6, 90) = 2.598$, $p = .023$, partial $\eta^2 = .148$; however, no post-hoc t-tests using a Bonferroni correction were significant.

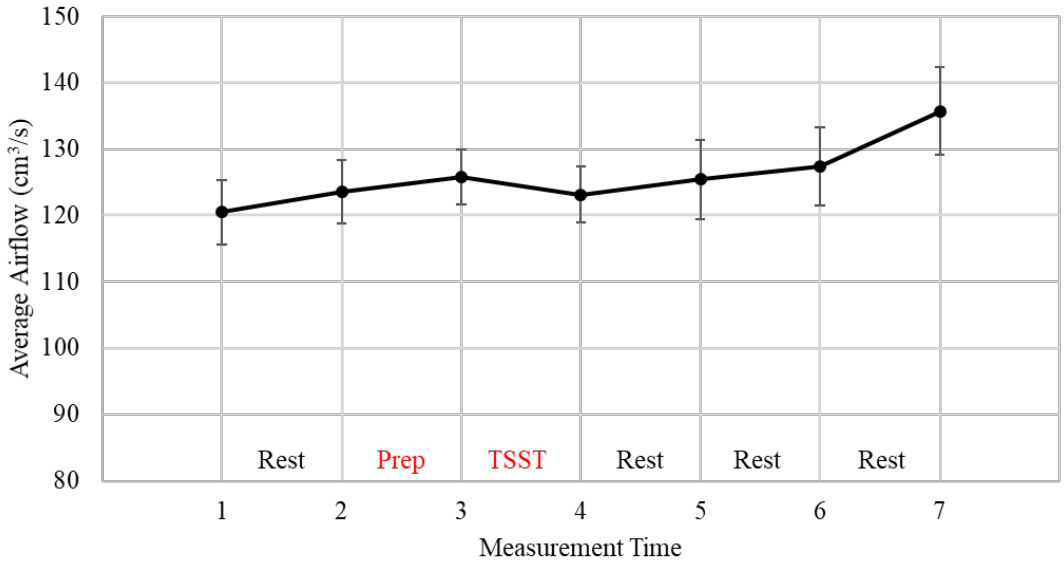

Figure 12. Mean average oral airflow (cm^3/s) over the measurement time points for the participants included in the analysis. Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Estimated subglottal pressure. Estimated subglottal pressure from oral pressure could not be completed for the last four recordings from F19 due to poor lip closure around the oral pressure tube. Figure 13 shows the group means and standard error (error bars) for the estimated subglottal pressure at each measurement time point. A repeated-measures ANOVA was conducted to compare the effects of time (7 levels) on estimated subglottal pressure. No outliers were identified. A Shapiro-Wilk test of normality revealed that estimates of subglottal pressure were normally distributed with the exception of subglottal pressure estimates at measurement time point 3, $SW = .887$, $df = 18$, $p = .035$. No adjustments were made to the estimated subglottal pressure values. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(20) = 45.74$, $p < 0.001$. The repeated-measures ANOVA with a Greenhouse-Geisser correction (to correct for the violation of sphericity; $\varepsilon = .504$) revealed no statistically significant differences in estimated subglottal pressure over the measurement time points, $F(3.024, 51.406) = .793$, $p = .504$, partial $\eta^2 = .045$.

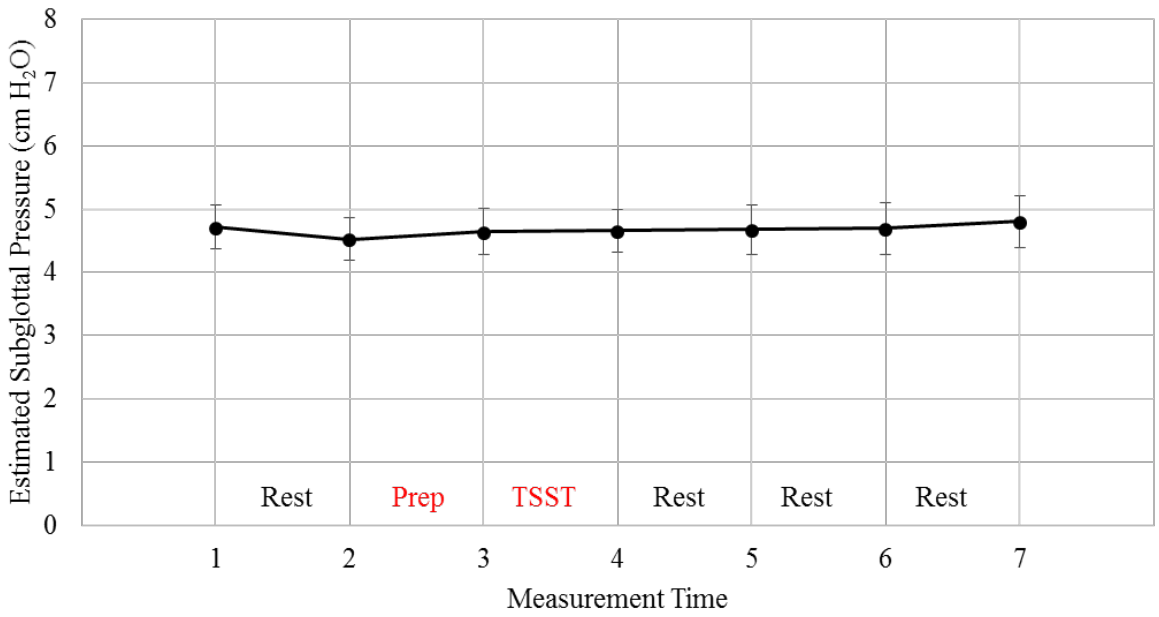

Figure 13. Mean average estimated subglottal pressure from the oral air pressure (cm H_2O) over the measurement time points (solid black circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Laryngeal airflow resistance. In all cases in which either or both estimated subglottal pressure and average airflow were not able to be determined, laryngeal airflow resistance could also not be determined. Figure 14 shows the group means and standard error (error bars) for the calculated airflow resistance at each measurement time point. A repeated-measures ANOVA was conducted to compare the effects of time (7 levels) on laryngeal airflow resistance. In a test for outliers, the airflow resistance values for F15 (measurement time points: 1, 2, 4, 5, and 7), F12 (measurement time points: 2, 3, 4, 5, and 7), and F7 (measurement time point: 4) were identified as outliers (greater than 1.5 times the interquartile range) and were expurgated from this analysis. After removing the outliers, the remaining airflow resistance values (n = 12) at all seven time points were normally distributed based on a Shapiro-Wilk test. A more liberal exclusionary criterion for outliers was used (1.5 times the interquartile range versus 3 times the interquartile range) to get a normal distribution. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(20) = 39.661$, $p = 0.008$. The repeated-measures ANOVA with a

Greenhouse-Geisser correction (to correct for the violation of sphericity) revealed no statistically significant differences in laryngeal airflow resistance over the measurement time points, $F(2.680, 29.480) = .957$, $p = .418$, partial $\eta^2 = .080$.

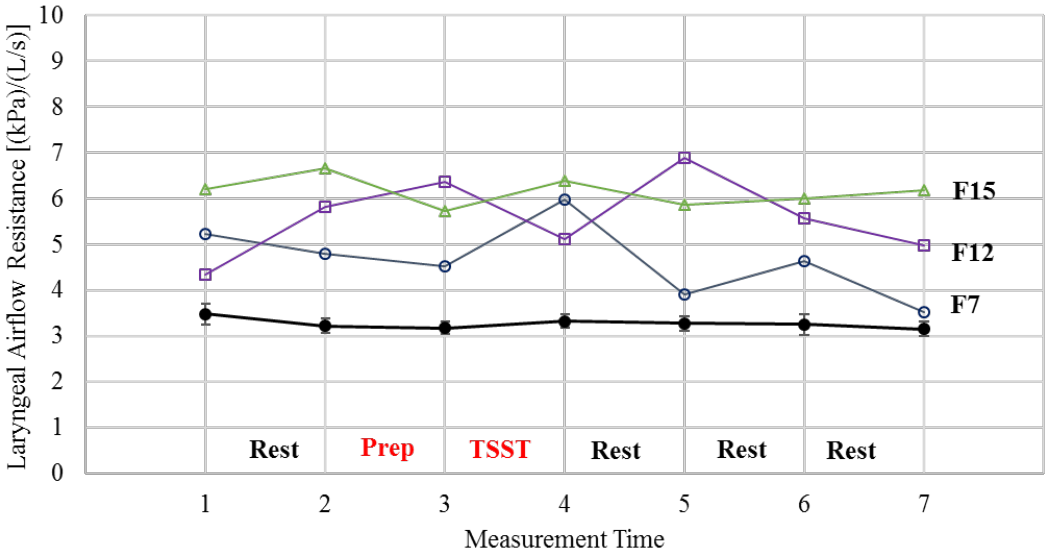

Figure 14. Mean laryngeal airflow resistance [(kPa)/(L/s)] over the measurement time points for participants included in the statistical analysis (solid black circles). Error bars represent standard error. The open symbols represent laryngeal airflow resistance for participants F7 (blue circles), F12 (purple squares), and F15 (green triangles). One or more values of laryngeal airflow resistance were identified as outliers and were removed from this analysis (see text). The stressor occurred between measurement time points 3 and 4.

Open quotient from the EGG signal. An accurate EGG open quotient could not be calculated for the following participants (recordings) due to a weak EGG signal: F1 (recording 7), F5 (recordings 3, 5, 6, and 7), F13 (all recordings), F15 (all recordings), and F16 (all recordings). Figure 12 shows the group mean and standard error (error bars) for the calculated open quotient from the EGG at each measurement time point. A repeated-measures ANOVA was conducted to compare the effects of time (7 levels) on open quotient. Values at all measurement time points were normally distributed based on a Shapiro-Wilk test except measurement time point 5, $p = .044$. No transformation was performed. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(20) = 36.964$, $p = 0.015$. The repeated-

measures ANOVA with a Greenhouse-Geisser correction (to correct for the violation of sphericity, ε = .516) revealed no statistically significant differences in the EGG open quotient over the measurement time points, $F(3.093, 40.211) = .834$, $p = .486$, partial $\eta^2 = .060$. Figure 15 presents the means across all seven measurement time points.

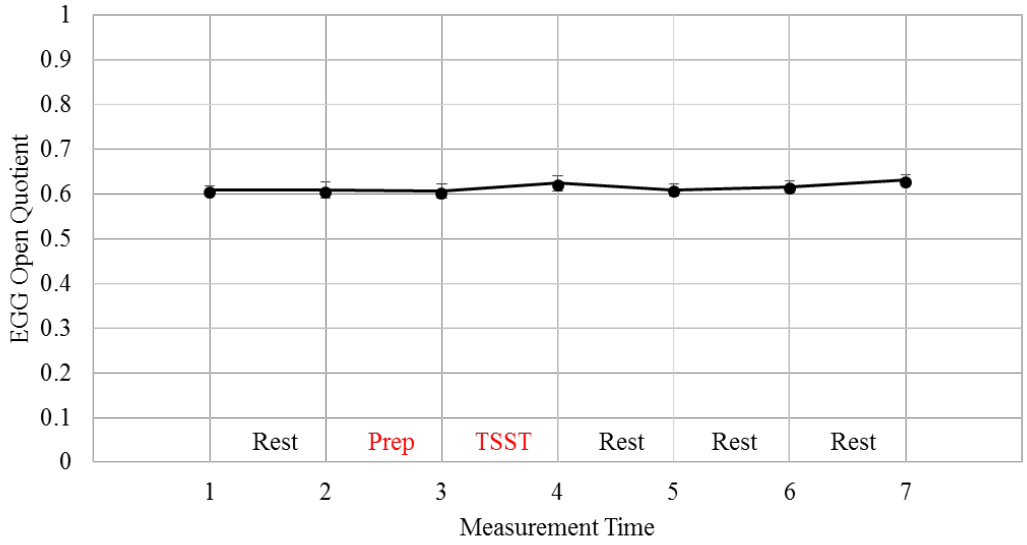

Figure 15. Mean open quotient calculated from the derivative of the EGG signal over the measurement time points (solid black circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Fundamental frequency from the second sentence of the Rainbow Passage. Figure 16 shows the group mean and the standard error at each measurement time point for the fundamental frequency of the second sentence of the Rainbow Passage ("The rainbow is a division of white light into many beautiful colors."). Any syllables produced in vocal fry were removed from the fundamental frequency analysis, so the mean fundamental frequency is a representation of modal register only. No values were identified as being more than three times the interquartile range so no outliers were removed. Frequency values (Hz) at all seven time points were normally distributed based on a Shapiro-Wilk test. Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(20) = 15.766$, $p = .738$. There was

a significant effect of time on speaking fundamental frequency, $F(6, 108) = 3.089$, $p = .008$, partial $\eta^2 = .146$. A series of post-hoc t-tests using a Bonferroni adjustment to the alpha level were conducted to determine which measurement time points were significantly different in speaking fundamental frequency. Measurement time points 1 and 2 (before the introduction of the stressor) were not significantly different, suggesting a stable baseline measurement of speaking fundamental frequency. Both measurement time points 1 and 2 differed significantly from measurement time points 3 (anticipating the stressor) and 4 (just after the stressor). Measurement time point 4 (just after the stressor) was significantly different from measurement time point 6 (around 20 minutes after the stressor). In addition, measurement time point 2 was significantly different from measurement time point 6 and measurement time points 6 and 7 were significantly different from each other. Table 6 presents the values (in semitones) for the significant pairwise comparisons. N.B.: The frequency differences between measurement time points are very small (a whole tone or major second is two semitones) and not likely to be perceptually different despite the statistically significant differences.

Table 6. Statistically significant differences in frequency between measurement time points and the difference in semitones.

Significantly different measurement time points	Difference in semitones
1 to 3	0.3103301
1 to 4	0.330259864
2 to 3	0.366154015
2 to 4	0.386083778
2 to 7	0.309753641
4 to 6	-0.297440995
6 to 7	0.221110858

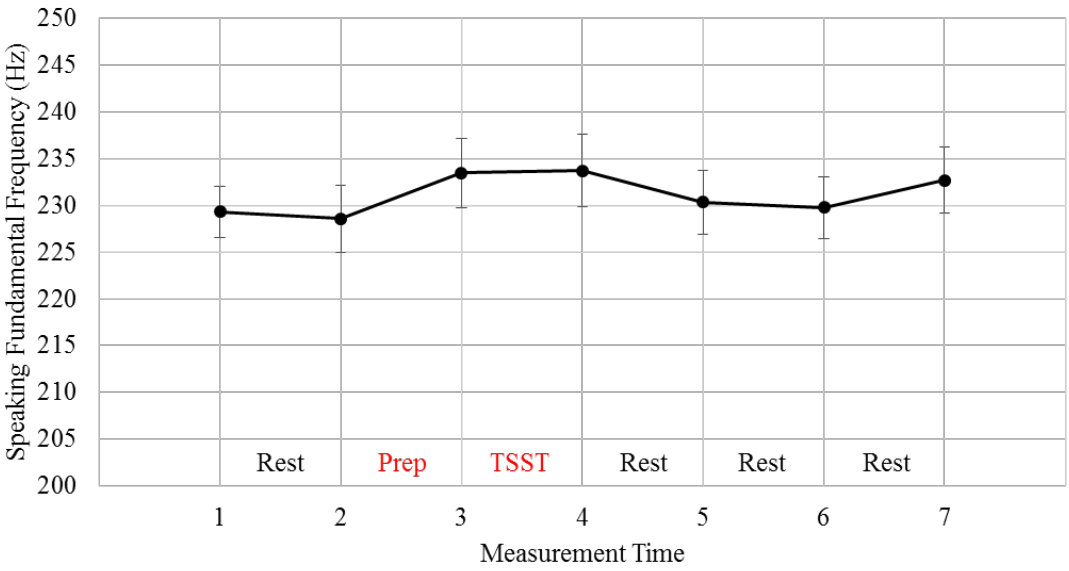

Figure 16. Mean speaking fundamental frequency from the second sentence of the Rainbow Passage over the measurement time points for participants included in the statistical analysis (solid black circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Percent of syllables produced in vocal fry. As the data are percentages, the data do not follow a normal distribution. As such, the data were arcsine transformed. An arcsine transformation was used instead of a logit transformation to prevent the inflation of differences at the ends of the scale (near 0 in the current case as over 45% of the data across all 7 measurement time points fall below 0.2). No values were identified as being more than three times the interquartile range so no outliers were removed. Following the arcsine transformation, the data were normally distributed based on a Shapiro-Wilk test. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(20) = 63.359$, $p < 0.001$. The repeated-measures ANOVA with a Greenhouse-Geisser correction (to correct for the violation of sphericity) revealed no statistically significant differences in arcsine transformed syllables produced with vocal fry over the measurement time points, $F(2.520, 45.352) = .898$, $p = .435$, partial $\eta^2 = .048$. Figure 17 shows the group mean and the standard error at each measurement time point for the percent of syllables produced in vocal fry in the Rainbow Passage.

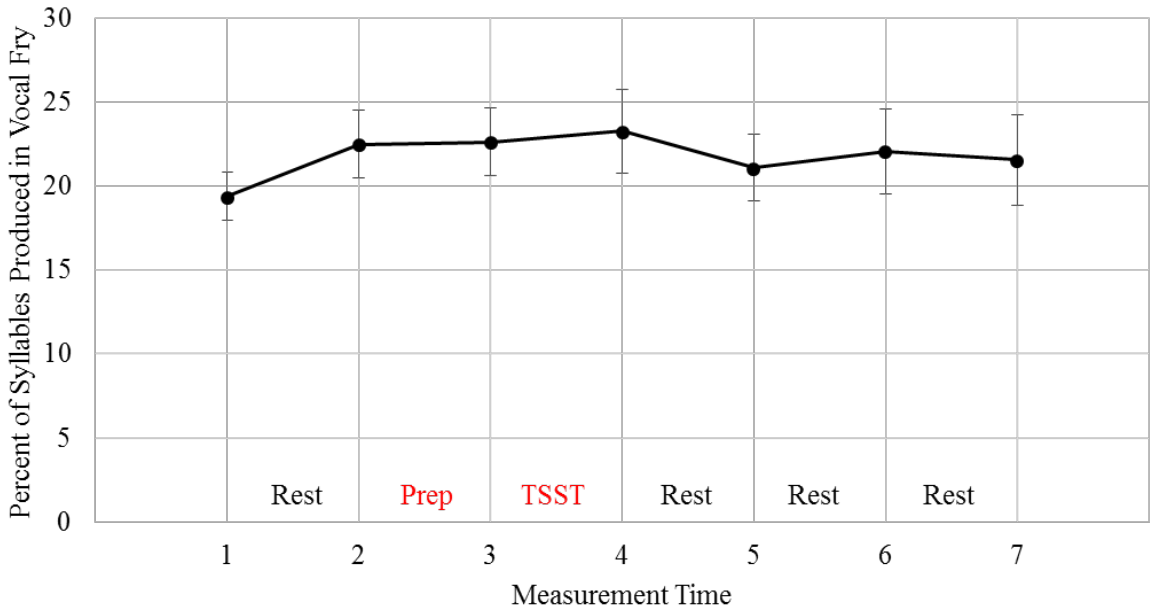

Figure 17. Mean percent of syllables produced in vocal fry from the Rainbow Passage over the measurement time points for participants included in the statistical analysis (solid black circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Q2: Differences in voice parameters of those who experienced HPA axis activation and those who did not, following an acute, social-evaluative stressor

Salivary cortisol could not be determined from two samples due to low saliva volume (F14, recording 4 and F15, recording 7). Salivary cortisol was significantly elevated from basal (second measurement time point) in response to the Trier Social Stress Test, $F(1, 36) = 10.31$, $p = .003$. However, only 10 of the 19 participants experienced a 2.5 nmol/l increase in salivary cortisol from basal (second measurement time point) to maximum (Figure 18) (Schommer et al., 2003). These 10 people are considered to have an HPA axis response in the writing that follows. The maximum occurred at recording time point 4 for n = 2 participants, recording time point 5 for n = 5 participants, recording time point 6 for n = 2 participants, and recording time point 7 for n = 1 participant.

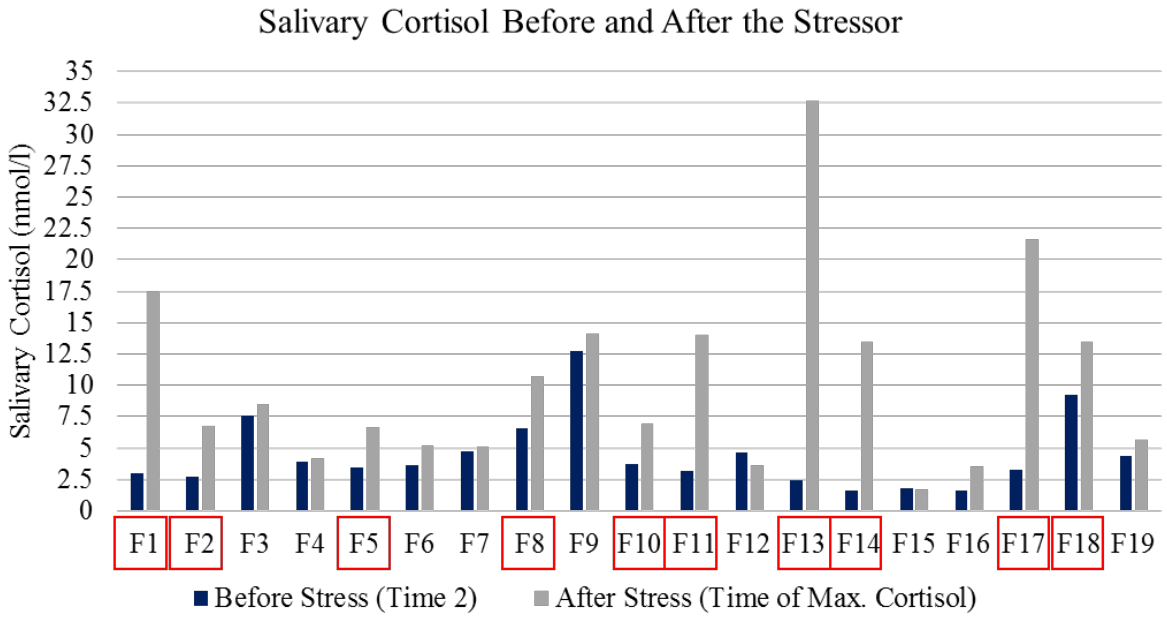

Figure 18. Salivary cortisol at measurement time 2 (before the stressor) and the measurement time point of maximum measured salivary cortisol (measurement time 4, 5, 6, or 7) for all participants. Participants who experienced at least a 2.5 nmol/l increase in salivary cortisol are indicated by a box around their participant number.

A series of independent t-tests were completed ($\alpha = .01$) to determine if the groups differed on any measured characteristic. There were no statistically significant differences in age, $t(17) = -.016$, $p = .987$, Perceived Stress Scale score, $t(17) = .193$, $p = .850$, and modified VHI-10 score, $t(17) = .348$, $p = .732$, between those who did experience an HPA axis response following the stressor and those who did not. The groups did not differ on suspected phase in the menstrual cycle, $t(17) = -.274$, $p = .788$. Additionally, there was no significant difference in salivary cortisol levels at time 2 (shown in Figure 18) between those who experienced an HPA axis response following the stressor and those who did not, $t(17) = .825$, $p = .421$. The descriptive statistics can be found in Table 7.

Table 7. Means and standard deviations for age, PSS score, modified VHI-10 score, and salivary cortisol levels at time 2 (nmol/l)

	HPA Response (n = 10)		No HPA Response (n = 9)	
	M	*SD*	*M*	*SD*
Age	18.90	1.729	18.89	1.167
PSS score	22.30	7.15	23.00	8.689
Modified VHI-10 score	5.80	3.49	6.33	3.162
Salivary cortisol at time 2 (nmol/l)	3.9121	2.25887	4.9899	3.37999

Average airflow. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 2 levels) and the within-subjects factor (time, 7 levels) on average airflow. There were no outliers and all data were normally distributed. Levene's test of homogeneity of variance revealed heterogeneity at measurement time point 6, $p = .048$. No transformations were performed. Box's test of equality of covariance matrices could not be run. Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction, $\chi^2(20) = 20.015$, $p = .475$. There was no statistically significant interaction between HPA axis response group and time on average airflow, $F(6, 84) = 1.161$, $p = .335$, partial $\eta^2 = .077$. The main effect of measurement time point showed a statistically significant difference in average airflow measures at the different time points, $F(6, 84) = 3.003$, $p = .010$, partial $\eta^2 = .177$. Pairwise comparisons revealed that measurement time point 7 had significantly higher average airflow values than measurement time point 1, $p = .043$, and measurement time point 5, $p = .041$. The main effect of HPA axis response group showed no statistically significant differences in average airflow measures between groups, $F(1, 14) = .156$, $p = .699$, partial $\eta^2 = .011$. Figure 19 presents the mean average airflow values for those who experienced an HPA axis response and those who did not.

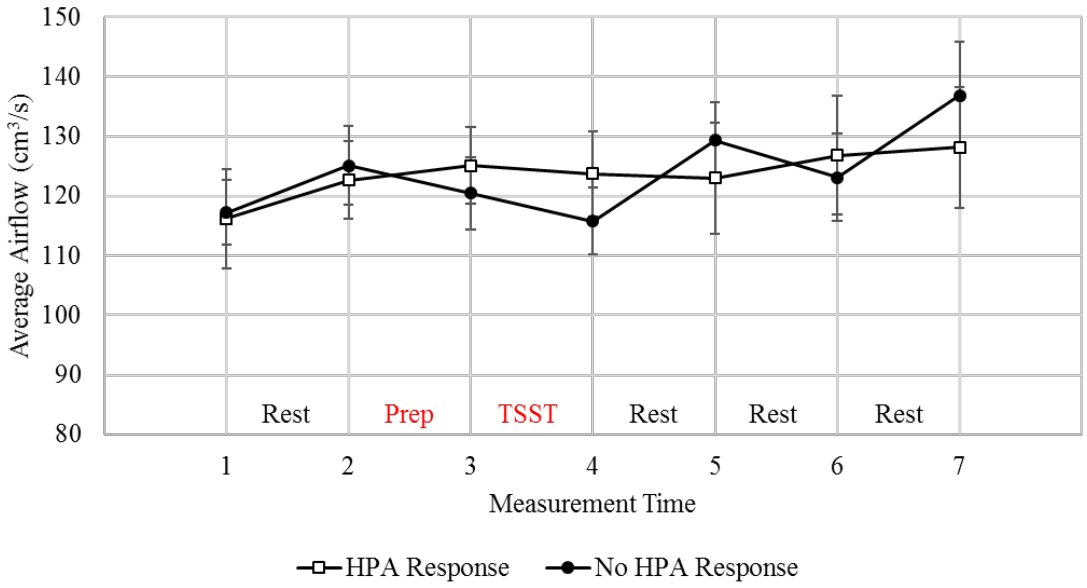

Figure 19. Mean average airflow (cm^3/s) over the measurement time points for the participants who experienced an HPA axis response (open squares) and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Estimated subglottal pressure. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 2 levels) and the within-subjects factor (time, 7 levels) on estimated subglottal pressure. The estimated subglottal pressure for F11 at measurement time point 7 was identified as an outlier (3 times the interquartile range), but was not removed. All data were normally distributed as assessed by the Shapiro-Wilk's test, except those for the HPA axis response group at measurement time point 3, $p = .029$. No transformations were performed. Levene's test of homogeneity of variance was not significant at any measurement time point, $p > .05$. There was homogeneity of covariances, as assessed by Box's test for quality of covariance matrices, $p = .090$. Mauchly's test of sphericity indicated that the assumption of sphericity was not met for the two-way interaction, $\chi^2(20) = 43.696$, $p = .002$, therefore the results were interpreted using a Greenhouse-Geisser correction ($\varepsilon = .497$). There was no statistically significant interaction between HPA axis response group and time on estimated subglottal pressure, $F(2.985, 47.757) = .227$, $p = .876$, partial $\eta^2 = .014$. The

main effect of measurement time point showed no statistically significant difference in estimated subglottal pressure measures at the different time points, $F(2.985, 47.757) = .725$, $p = .541$, partial $\eta^2 = .043$. The main effect of HPA axis response group showed a statistically significant differences in estimated subglottal pressure measures between groups, $F(1, 16) = 6.630$, $p = .020$, partial $\eta^2 = .293$. Figure 20 presents the estimated subglottal pressure for the HPA responders and the HPA non-responders.

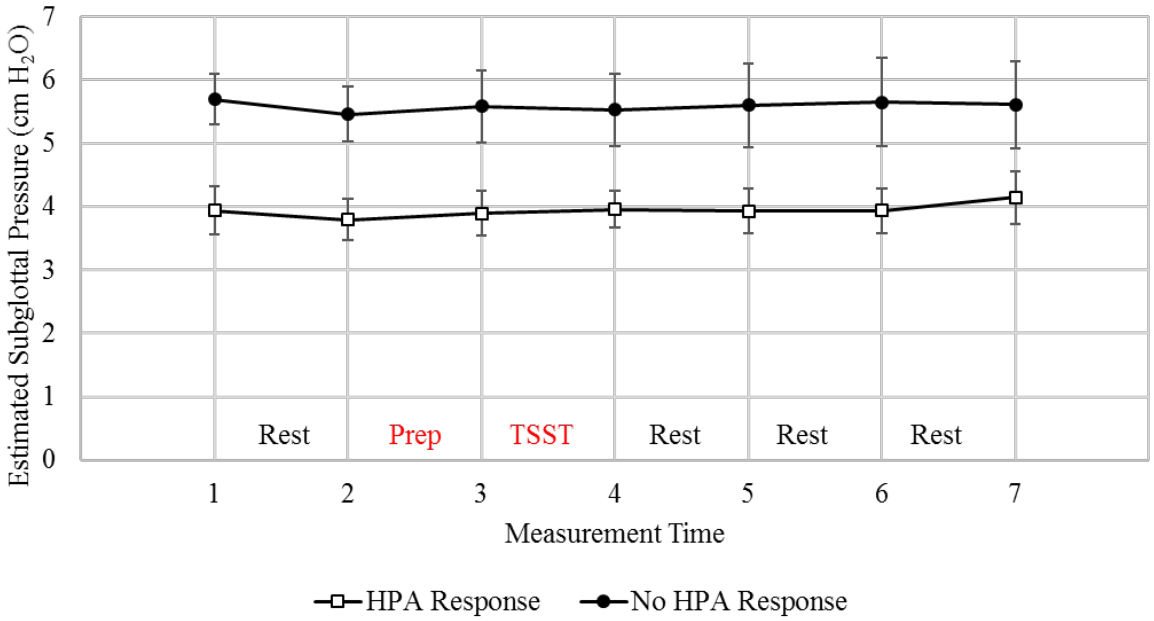

Figure 20. Estimated subglottal pressure (cm H_2O) over the measurement time points for the participants who experienced an HPA axis response (open squares) and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Laryngeal airflow resistance. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 2 levels) and the within-subjects factor (time, 7 levels) on laryngeal airflow resistance. The laryngeal airflow resistance for F12 at measurement time point 5 was identified as an outlier (3 times the interquartile range), but was not removed. All data were normally distributed as assessed by the Shapiro-Wilk's test, except those for the no HPA axis response group at measurement time point

7, p = .027. Levene's test of homogeneity of variance was significant at measurement time points 3, p = .048, and 4, p = .014. No transformations were performed. Box's test of equality of covariance matrices could not be run. Mauchly's test of sphericity indicated that the assumption of sphericity was not met for the two-way interaction, $\chi^2(20) = 34.576$, p = .028, therefore the results were interpreted using a Greenhouse-Geisser correction (ε = .518). There was no statistically significant interaction between HPA axis response group and time on laryngeal airflow resistance, $F(3.110, 37.317) = 1.919$, p = .141, partial η^2 = .138. The main effect of measurement time point showed no statistically significant difference in laryngeal airflow resistance measures at the different time points, $F(3.110, 37.317) = 1.629$, p = .198, partial η^2 = .120. The main effect of HPA axis response group showed a statistically significant differences in laryngeal airflow resistance measures between groups, $F(1, 12) = 5.363$, p = .039, partial η^2 = .309. Figure 21 presents the laryngeal airflow resistance for the HPA responders and the HPA non-responders.

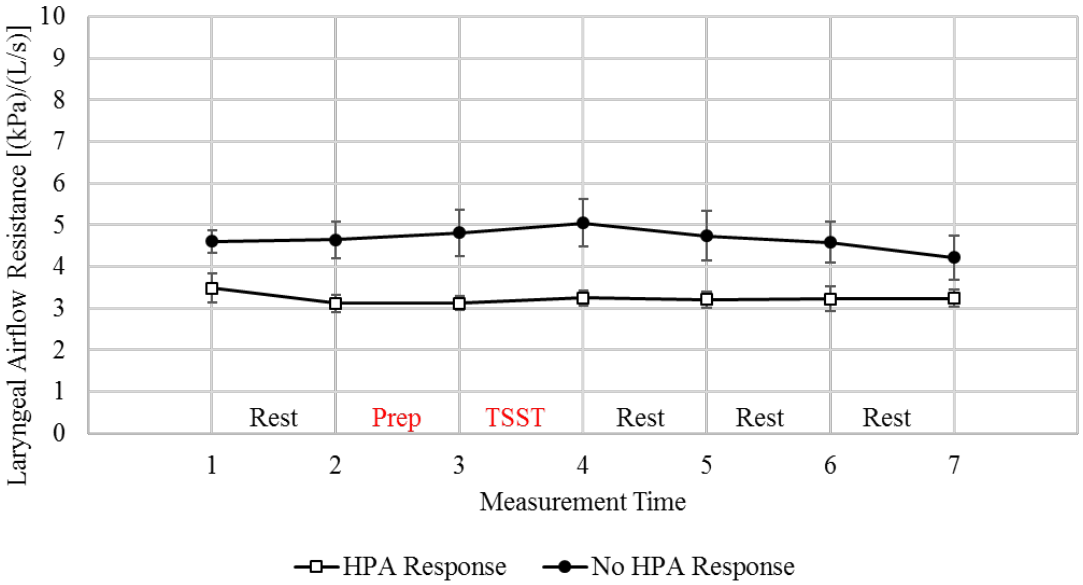

Figure 21. Mean laryngeal airflow resistance [(kPa)/(L/s)] (not transformed) over the measurement time points for the participants who experienced an HPA axis response (open squares) and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Open quotient from the EGG signal. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 2 levels) and the within-subjects factor (time, 7 levels) on open quotient from the EGG signal. No outliers were identified from examination of the boxplots and all data except those from the HPA axis response group at measurement time point 5 ($p = .030$) were normally distributed as assessed by the Shapiro-Wilk's test. Levene's test of homogeneity of variance was not significant at any measurement time points, $p = .05$. Box's test of equality of covariance matrices could not be run. Mauchly's test of sphericity indicated that the assumption of sphericity was not met for the two-way interaction, $\chi^2(20) = 37.343$, $p = .014$, therefore the results were interpreted using a Greenhouse-Geisser correction ($\varepsilon = .498$). There was no statistically significant interaction between HPA axis response group and time on open quotient from the EGG signal, $F(2.988, 35.853) = .345$, $p = .792$, partial $\eta^2 = .028$. The main effect of measurement time point showed no statistically significant difference in open quotient from the EGG signal measures at the different time points, $F(2.988, 35.853) = .792$, $p = .506$, partial $\eta^2 = .062$. The main effect of HPA axis response group showed no statistically significant differences in open quotient from the EGG signal measures between groups, $F(1, 12) = .093$, $p = .766$, partial $\eta^2 = .008$. Figure 22 presents the mean EGG open quotient for the HPA responders and the HPA non-responders.

Fundamental frequency from the second sentence of the Rainbow Passage. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 2 levels) and the within-subjects factor (time, 7 levels) on speaking fundamental frequency. Speaking fundamental frequency values for F9 at measurement time points 1 and 3 and F12 at measurement time point 3 were identified as outliers by examination of the boxplots, but were not removed. All data except those from the no HPA axis

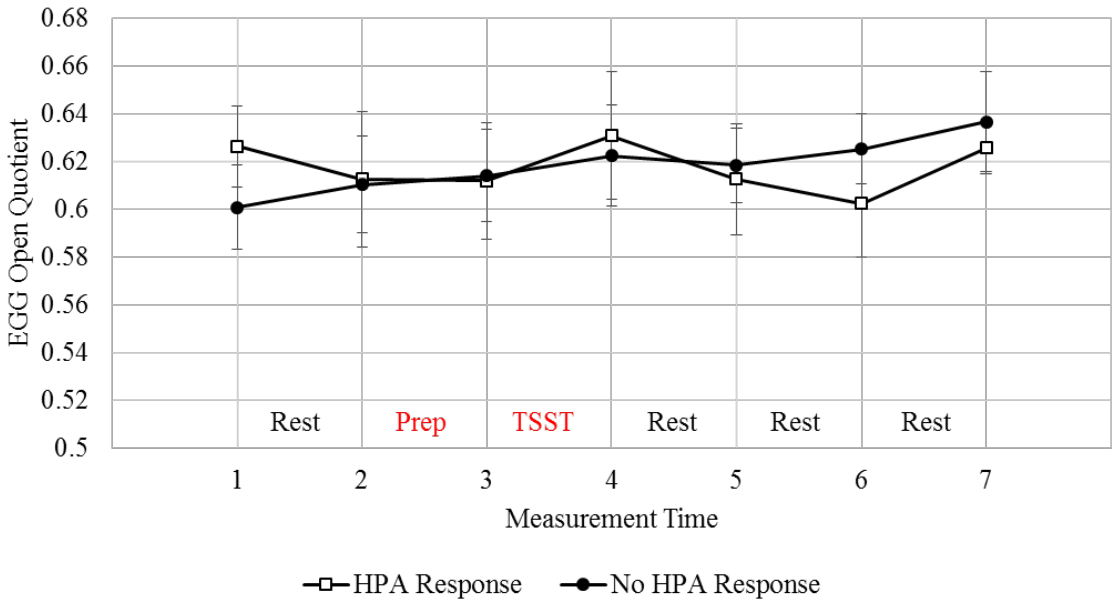

Figure 22. Mean open quotient of the EGG signal over the measurement time points for the participants who experienced an HPA axis response (open squares) and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

response group at measurement time point 1 (p = .013) were normally distributed as assessed by the Shapiro-Wilk's test. Levene's test of homogeneity of variance was significant at measurement time point 1 only, p = .022. There was not homogeneity of covariances, as assessed by Box's test for quality of covariance matrices, p = .004. Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction, $\chi^2(20) = 10.050$, p = .969. There was a statistically significant interaction between HPA axis response group and time on speaking fundamental frequency, $F(6, 102) = 2.795$, p = .015, partial η^2 = .141. To interpret the interaction effect, a series of univariate ANOVAs were conducted to determine if there was a simple main effect for group, which there was not. A series of repeated measure ANOVAs were run to determine if there was a simple main effect for time. There was a statistically significant effect of time on speaking fundamental frequency for the HPA axis response group, $F(6, 54) = 6.242$, $p > .001$, partial η^2 = .410. Pairwise comparisons revealed that speaking fundamental

frequency was significantly higher at measurement time point 3 than measurement time point 5, $p = .011$ (0.57 semitone), and was significantly higher at measurement time point 4 than measurement time point 6, $p = .044$ (0.52 semitone) for the HPA axis response group. Figure 23 presents the mean speaking fundamental frequency for the HPA responders and the HPA non-responders.

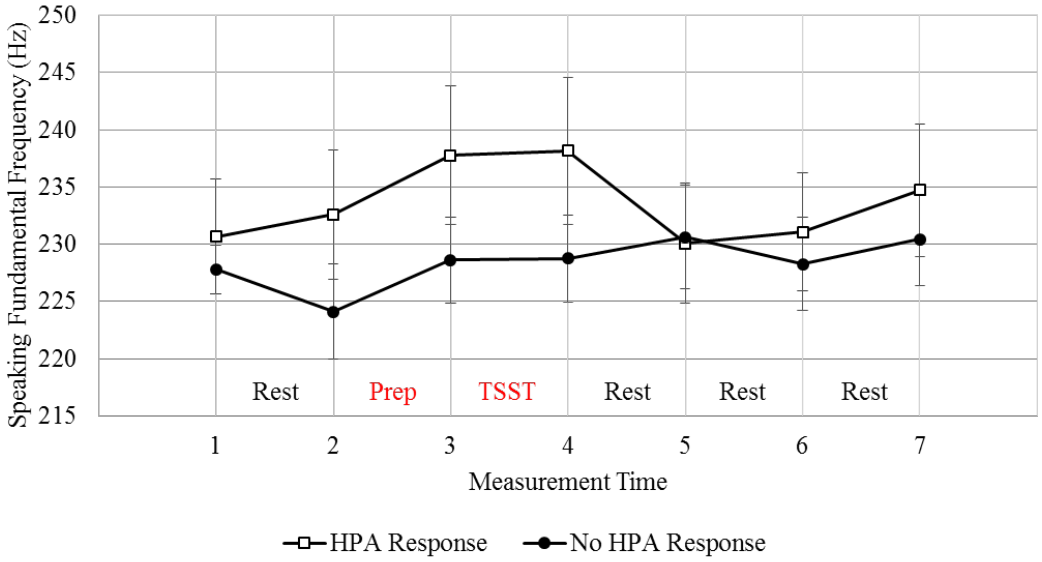

Figure 23. Mean speaking fundamental frequency over the measurement time points for the participants who experienced an HPA axis response (open squares) and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Percent of syllables produced in vocal fry. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 2 levels) and the within-subjects factor (time, 7 levels) on percent of syllables produced in vocal fry. The data were arcsine transformed for reasons stated previously. Shapiro-Wilk's test revealed that all data were normally distributed. The percent of syllables produced in vocal fry for participant F3 was identified as an outlier at measurement time point 7, but was not removed. Levene's test of homogeneity of variance was not significant at any measurement time, $p > .05$.

There was homogeneity of covariances, as assessed by Box's test for quality of covariance matrices, $p = .232$. Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, $\chi^2(20) = 61.498$, $p > .001$. Thus, the results were interpreted using a Greenhouse-Geisser correction ($\varepsilon = .410$). There was no statistically significant interaction between HPA axis response group and time on syllables produced in vocal fry, $F(2.459, 41.810) = 2.171$, $p = .117$, partial $\eta^2 = .113$. The main effect of measurement time point showed no statistically significant difference in syllables produced in vocal fry at the different time points, $F(2.459, 41.810) = 1.019$, $p = .383$, partial $\eta^2 = .057$. The main effect of HPA axis response group showed no statistically significant differences in syllables produced in vocal fry between groups, $F(1, 17) = .053$, $p = .821$, partial $\eta^2 = .003$.

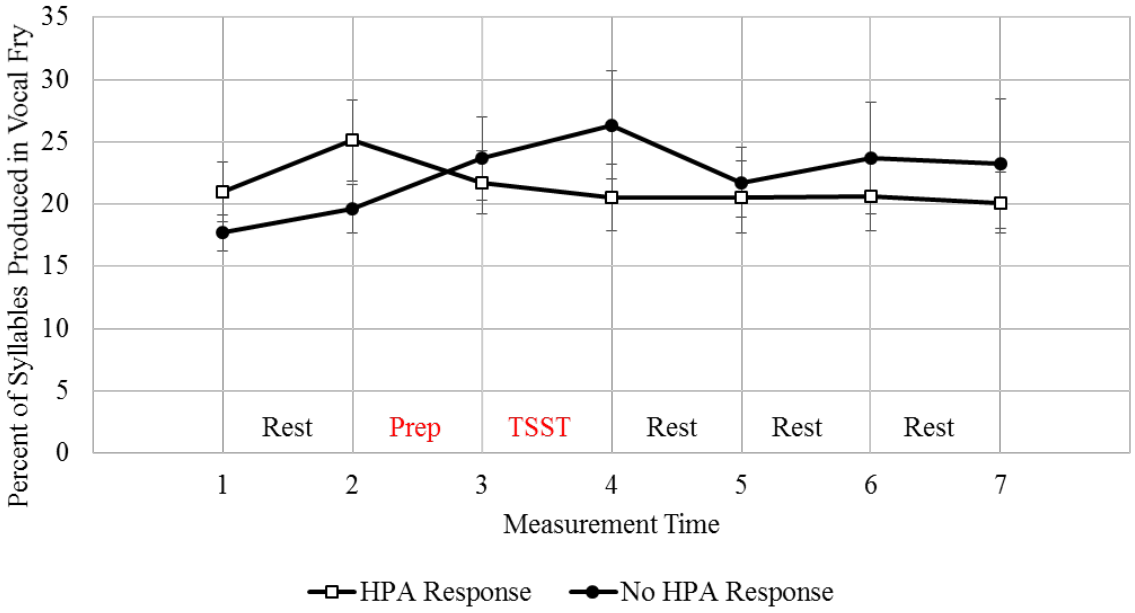

Figure 24. Percent of syllables produced in vocal fry while reading the Rainbow Passage over the measurement time points for the participants who experienced an HPA axis response (open squares) and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Q3: Individual changes from time 2 (before the stressor) to time 4 (after the stressor)

Intra-subject variability was calculated for each participant for each speech variable at each measurement time. As previously mentioned, each participant produced multiple syllable repetitions at each time. The mean at each time was divided into the standard deviation and the result was multiplied by 100 to get a coefficient of variation (percentage). This can be used as a measure of relative variability at each of the seven measurement time points. The coefficient of variation for each participant for average airflow, estimated subglottal pressure, laryngeal airflow resistance, and EGG open quotient in Table 8 represents the maximum coefficient of variation that occurred at one of the seven measurement time points. The average coefficient of variation across all participants for all seven recordings (or the inter-subject coefficient of variation) is 12.0% for average airflow, 8.1% for estimated subglottal pressure, 12.1% for laryngeal airflow resistance, and 10.4% for EGG open quotient. There was no difference in average coefficient of variation based on measurement time point for average airflow, $F(6, 90) = .943$, $p = .468$, partial $\eta^2 = .059$, estimated subglottal pressure, $F(2.753, 46.795) = 2.123$, $p = .115$, partial $\eta^2 = .111$, laryngeal airflow resistance, $F(6, 84) = 1.348$, $p = .245$, partial $\eta^2 = .088$, or EGG open quotient, $F(6, 78) = 1.558$, $p = .171$, partial $\eta^2 = .107$.

Next, the change in each measure from before the stressor to just after the stressor was calculated for each participant by subtracting measurement time point 2 from measurement time point 4 (Table 9). As the majority of these changes are very small, it is necessary to compare the changes to each participant's intra-subject variation. The values in Table 9 were divided by the value of the dependent variable at measurement time point 4 and the result was multiplied by 100 to give a percent difference from before stress to after stress. In Table 9, measures that have a percent difference greater than the individual's intra-subject variation are marked with an

asterisk. These changes may be due to more than just variation in production by the participant. Half (5 of 10) of the participants who experienced an HPA axis response had a change (3 had an increase and 2 had a decrease) in estimated subglottal pressure from time 2 to time 4 that was greater than their intra-subject variation. Only 2 of 9 participants who did not experience an HPA axis response to the acute, social-evaluative stressor had a change (1 increase and 1 decrease) in subglottal pressure that was greater than their intra-subject variation. Only 4 participants (1 from the HPA axis response group and 3 who experienced no HPA axis response to stress) had a change in airflow that was greater than their intra-subject variation and all but one change was a decrease in airflow. The 3 participants who had a change in laryngeal airflow resistance greater than their intra-subject variation were all from the group that did not experience HPA axis activation following the stressor. Only 1 participant had a change in EGG open quotient that was greater than her intra-subject variation and she also experienced an HPA axis response.

The coefficient of variation for speaking fundamental frequency does not represent intra-subject variation like the other measures. The coefficient of variation for speaking fundamental frequency is calculated by dividing the standard deviation of the measure of fundamental frequency (or the frequency intonation variations across the sentence due to prosody) by the mean speaking fundamental frequency from the second sentence of the Rainbow Passage. Thus, the coefficient of variation for fundamental frequency (Table 10, first column) represents the percent variation of frequency across the sentence. Again, a percent change in speaking fundamental frequency from before the stressor to after the stressor [100* (speaking fundamental frequency at time 4 – speaking fundamental frequency at time 2) / speaking fundamental frequency at time 4] (Table 10, second column presents just the difference between time 2 and time 4). There were no cases in which the percent change in speaking fundamental frequency

from time 2 to time 4 was greater than the maximum coefficient of variation for the fundamental frequency (the prosody variation).

Table 8. Maximum coefficient of variation (intra-subject variation) for average airflow, estimated subglottal pressure, laryngeal airflow resistance, and EGG open quotient for each participant (maximum relative variability for each measure for each person)

	Average airflow	Estimated subglottal pressure	Laryngeal airflow resistance	EGG open quotient
F1	17.3%	14.6%	17.8%	27%
F2	10.9%	8.6%	10.2%	10.9%
F3	19.6%	11.8%	20.2%	16.2%
F4	12.4%	13.9%	14.2%	15.7%
F5	NA	16.7%	NA	11.5%
F6	24.0%	24.3%	12.2%	15.1%
F7	26.3%	9.2%	22.6%	12.7%
F8	25.0%	13.1%	20.2%	20.7%
F9	17.5%	9.4%	16.7%	22.6%
F10	10.6%	9.4%	15.5%	5.8%
F11	20.2%	12.9%	19.4%	23.7%
F12	18.4%	12.7%	16.8%	14.5%
F13	10.9%	6.2%	13.5%	NA
F14	11.7%	9.0%	10.7%	18.3%
F15	20.1%	10.6%	20.2%	NA
F16	14.9%	7.2%	14.3%	NA
F17	12.0%	16.2%	17.5%	7.9%
F18	20.4%	10.1%	21.5%	16.6%
F19	12.6%	7.1%	7.8%	8.6%

Table 9. Differences in voice parameters for all participants from time point 2 to time point 4.

	Airflow (cm^3/s)	**Estimated Psub (cm H_2O)**	**Laryngeal airflow resistance [(kPa)/(L/s)]**	**EGG open quotient**
HPA axis response				
F1	9.291	.272	.034	.029632
F2	-4.15	.005	.13	.016649
F5	NA	.23	NA	-.002187
F8	-.34	.102	.149	.028672
F10	-2.17	-.07	-.01	.021518
F11	-1.85	.784*	.675	-.027939
F13	-2.89	-.54*	-.3	NA
F14	-28.7*	-.99*	-.07	.194444*
F17	15.47	.749*	.308	-.054557
F18	25.16	1.085*	.278	-.041601
Average	**1.09**	**.16**	**.13**	**.01829229**
SD	**15.03**	**.62**	**.28**	**.07308376**
No HPA axis response				
F3	NA	.115	NA	.008038
F4	-21.6*	.234	.686*	.004219
F6	10.31	-.35	-.5*	.071652
F7	-13.6	-.82*	1.188	.019509
F9	-23.8*	1.083*	2.356*	-.103958
F12	14.04	-.06	-.71	.062363
F15	7.963	.319	-.27	NA
F16	13.35	.33	-.19	NA
F19	-27.17*	NA	NA	.023061
Average	**-5.13**	**.11**	**.37**	**.0121263**
SD	**18.20**	**.56**	**1.11**	**.05743954**

Note. A positive number means the measure increased from time point 2 to time point 4. The "*" represents a measure that had a greater percent change than the participants maximum intra-subject variation. "NA" indicates participants for whom measures could not be made.

Table 10. Maximum coefficient of variation and change from before stress to after stress for speaking fundamental frequency

	Maximum Coefficient of Variation for f_o	**Time 4 – Time 2 Speaking f_o change (Hz)**
HPA axis response		
F1	19.6%	-.767865
F2	8.5%	13.08926
F5	7.1%	2.02911
F8	7.2%	-.182355
F10	7.8%	2.529294
F11	5.3%	-2.338087
F13	15.6%	7.683168
F14	8.5%	16.62734
F17	9.1%	2.535708
F18	18.6%	14.83282
Average	-	**5.60383935**
SD	-	**6.95896375**
No HPA axis response		
F3	9.4%	4.934493
F4	8.6%	6.852686
F6	13.2%	10.85021
F7	10.3%	-6.063934
F9	21.0%	1.119689
F12	8.4%	12.28263
F15	13.1%	-1.757446
F16	8.5%	5.719078
F19	16.9%	7.959942
Average	-	**4.65526091**
SD	-	**5.93756742**

Q4: Changes in voice parameters and the relation to ratings of emotions from time 2 to time 4

A series of Chi-Square tests was completed to determine if there were any significant associations between a change in any voice parameter from time 2 to time 4 and a change in rating of emotion of more than 20*T* from time 2 to time 4. Based on the results of the previous research question, airflow, estimated subglottal pressure, laryngeal airflow resistance, and open quotient were dummy coded to either a 1 (change greater than the intra-subject variability) or 0 (no change greater than the intra-subject variability). As no participant experienced a change in fundamental frequency greater than their maximum prosody variations, fundamental frequency was not included in this analysis. Similarly, each of the nine emotion ratings were dummy coded to 1 (change greater than 20*T* from time 2 to time 4) or 0 (change was not greater than 20*T* from time 2 to time 4). Table 11 presents the number of participants who had a 20*T* change (increase or decrease) in rating for each emotion. Only two participants experienced a decrease of 20*T* of an emotion: F12's rating of tired decreased and F15's rating of happy decreased. These are included in the count in Table 11. As no participant experienced a change of more than 20*T* in their rating of "energetic," no analyses were completed to determine the associations between the voice parameters and "energetic."

A series of Fisher's Exact tests were conducted between the voice parameters (airflow, estimated subglottal pressure, laryngeal airflow resistance, and open quotient) and the eight emotions. A Fisher's Exact test was used in place of a chi-square test for association because the expected count in the cross tabulation for the voice parameters by emotion tables did not exceed five observations (i.e., the samples size was too small).

Table 11. Number of participants experiencing a 20*T* increase in their rating of emotion from time 2 to time 4

Emotion	Number of participants out of 19 experiencing a change from time 2 to time 4
Afraid	4
Confused	3
Sad	3
Angry	3
Energetic	0
Tired	2
Happy	1
Tense	5

Note. One participant did not have a measure of "Tired" or "Happy" at measurement time point 4.

There were no statistically significant associations between airflow and the emotions of afraid, $p = .121$, confused, $p = 1.000$, angry, $p = .541$, tired, $p = .426$, happy, $p = 1.000$, and tense, $p = .538$. There was a statistically significant association between airflow and the emotion sad, $p = .006$. All 3 participants who rated sad higher at time 4 also had a change in their airflow measure from time 2 to time 4 that was greater than their intra-subject variability. Stated otherwise, 3 of the 4 participants who experienced a change in airflow from time 2 to time 4 that was greater than their intra-subject variability, rated sad higher at time 4.

There were no statistically significant associations between estimated subglottal pressure and the emotions of afraid, $p = .528$, confused, $p = .137$, sad, $p = .137$, angry, $p = .245$, tired, $p = 1.000$, happy, $p = 1.000$, and tense, $p = 1.000$.

There were no statistically significant associations between laryngeal airflow resistance and the emotions of afraid, $p = 1.000$, confused, $p = 1.000$, sad, $p = .242$, angry, $p = 1.000$, tired, $p = 1.000$, happy, $p = 1.000$, and tense, $p = .450$.

There were no statistically significant associations between open quotient and the emotions of afraid, $p = .188$, confused, $p = 1.000$, sad, $p = .188$, angry, $p = 1.000$, tired, $p = 1.000$, and tense, $p = .450$.

The analysis could not be completed for the emotion of "happy" because the only participant who experienced a 20*T* increase in her rating of "happy" did not have a measure of open quotient.

Exploratory analyses

Earlier responders and later responders. Within the cortisol responders (those who had a cortisol increase of at least 2.5 nmol/l from measurement time point 2 to measurement time point 4, 5, 6, or 7) there was a great deal of variability in the measurement time point at which participants had their peak cortisol level following the TSST (Figure 25). Engert, Efanov, Duchesne, Vogel, Corbo, and Pruessner (2013) have identified two distinct profiles of cortisol response following the TSST which the authors call anticipatory responders and reactive responders. In their study, anticipatory responders experienced a 2.5 nmol/l increase in salivary cortisol levels between 10 and 16 minutes after the onset of the anticipatory period in the TSST protocol. Reactive responders are those who experience a 2.5 nmol/l increase at least 18 minutes after the onset of the anticipatory period.

There is a difference in the timing of the anticipatory response between the present study and Engert et al. (2012) that should be briefly discussed. Engert et al. (2012) found that the cortisol was significantly elevated around 20 minutes after the onset of anticipation when taking saliva samples every 2 minutes throughout the study. Due to a limited number of salivary cortisol samples in the present study, the anticipatory elevation in salivary cortisol may not have been

and was not recognized until around 37 minutes after the onset of anticipation. In addition, in their study, Engert et al. (2012) did not see an increase in salivary cortisol in the reactive responders until around 16 minutes after the onset of the stressor, similar to when a change is seen in the reactive responders in the present study. In addition, Engert et al. (2012) only included male participants and it has been suggested that females do not have a significant increase in cortisol during the anticipatory period (Kirschbaum, Wüst, Faig, & Hellhammer, 1992c). This may mean that it is more appropriate to think of the anticipatory responders in the present study as "earlier responders" and the reactive responders as "later responders."

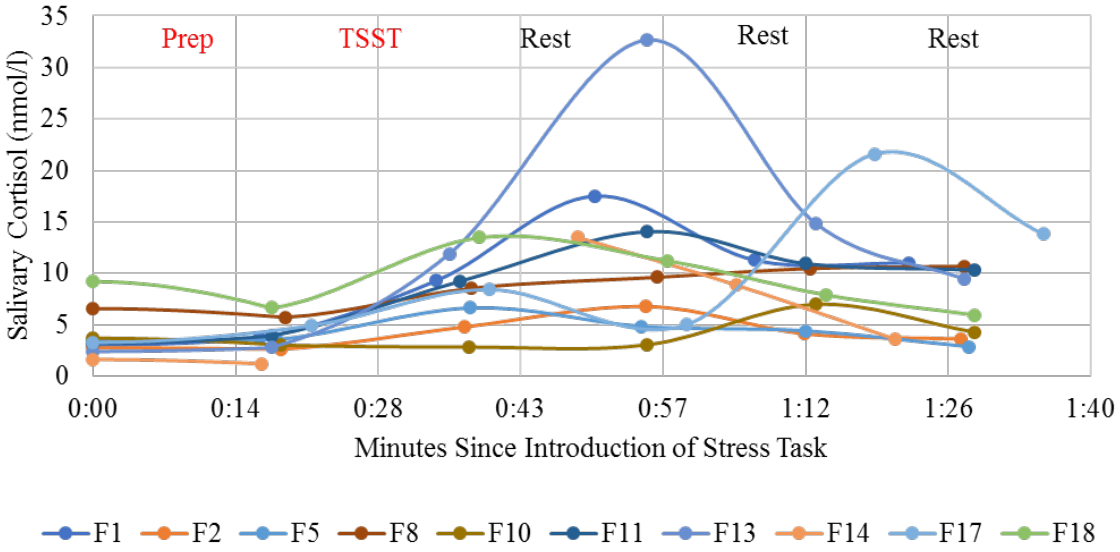

Figure 25. Salivary cortisol for the last 6 measurement time points (excludes first measurement) for the participants who experienced a 2.5 nmol/l increase in salivary cortisol from the first measurement time point (0:00) to measurement time point 4, 5, 6, or 7. Time is presented in minutes since the stress task was introduced to the participant.

In the present study, of the 10 participants who had at least a 2.5 nmol/l increase in salivary cortisol following the TSST, 6 had at least a 2.5 nmol/l increase in salivary cortisol at time point 4 (36 minutes, 57 seconds ± 1 minute, 47 seconds after onset of anticipatory period),

suggesting that these participants experienced an earlier response (Figure 26). A 3 x 7 ANOVA was conducted to determine if there were significant differences in cortisol levels in the 3 groups (earlier HPA responders, later HPA responders, and non-responders) over the 7 measurement time points. Cortisol was log transformed to allow for the assumptions of normality, $p = .475$, and homogeneity, $F(20, 110) = .484$, $p = .968$, to be met. There was a statistically significant interaction between measurement time point and HPA response group for salivary cortisol levels, $F(12,110) = 2.525$, $p = .006$, partial $\eta^2 = .216$. To interpret the interaction effect, a Bonferroni adjustment was made to the level at which statistical significance is declared so that significance occurs when $p < .017$. There was a statistically significant difference in mean salivary cortisol levels between times for those participants with an earlier HPA axis response, $F(6, 110) = 5.697$, $p < .001$, partial $\eta^2 = .237$. For those participants with an earlier HPA axis response, mean beginning (not shown in Figure 26) salivary cortisol levels were .497 nmol/l (95% CI, -.942 to -.051, $p = .016$) lower than post-stress 0, .573 nmol/l (95% CI, -1.018 to -.128, $p = .002$) lower than post-stress 1, and .539 nmol/l (95% CI, -.985, -.094, $p = .006$) lower than post-stress 2. In this same group, mean basal (time 0:00 in Figure 26) salivary cortisol levels were .494 nmol/l (95% CI, -.939 to -.049, $p = .017$) lower than post-stress 1 (time 0:56 in Figure 26). There was also a statistically significant difference in mean salivary cortisol levels between groups at the post-stress 1 measurement time point, $F(2, 110) = 5.636$, $p = .005$, partial $\eta^2 = .093$, and the post-stress 2 measurement time point, , $F(2, 110) = 8.392$, $p < .001$, partial $\eta^2 = .132$. Specifically, at post-stress 1 (time 0:56 in Figure 26) the no response group had mean salivary cortisol levels that were .435 nmol/l (95% CI, -.753 to -.117, $p = .004$) lower than the earlier HPA axis response group and at post-stress 2 (time 1:12 in Figure 26) the no response group had mean salivary

cortisol levels that were .518 nmol/l (95% CI, -.836 to -.200, $p < .001$) lower than the earlier HPA axis response group.

As the earlier HPA response group had significant changes in cortisol from before the stressor to after the stressor and the other groups did not, follow-up analyses were conducted to determine in the three groups varied on any of the voice parameters over time (i.e., the analysis for research question 2 was repeated with the 3 groups).

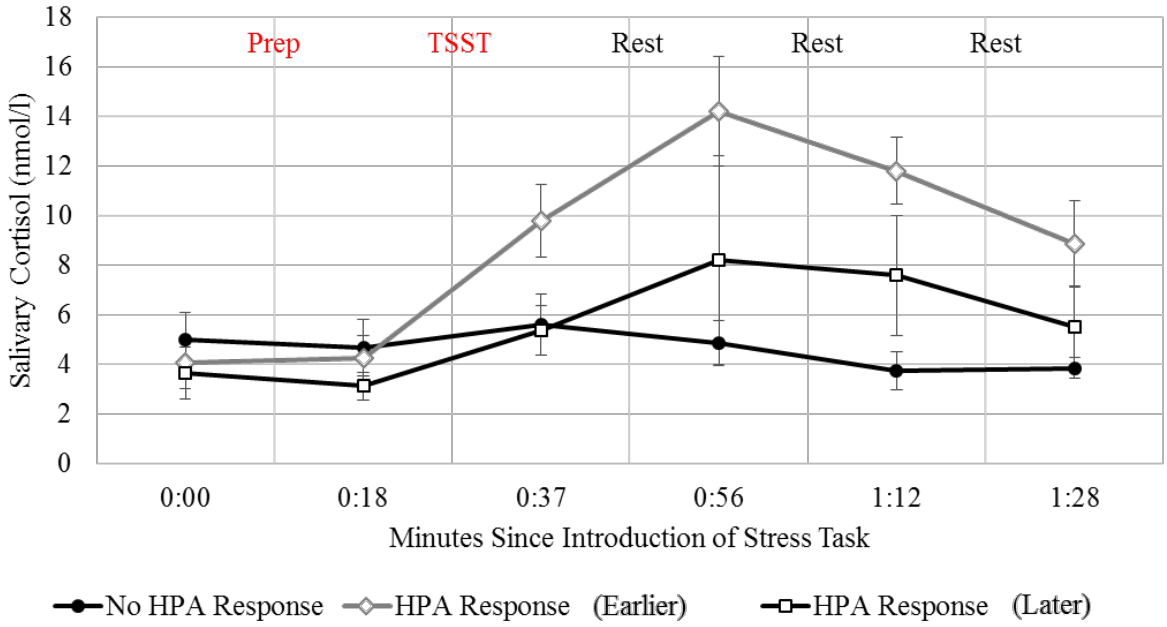

Figure 26. Mean salivary cortisol for the last 6 measurement time points (excludes first measurement) for the participants who had no HPA response (solid black circles), an HPA response characterized by an earlier response (unfilled grey diamonds), and an HPA response characterized by a later response (unfilled black squares). Error bars represent standard error. Time is presented in minutes since the stress task was introduced to the participant.

Average airflow. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 3 levels) and the within-subjects factor (time, 7 levels) on average airflow. Average airflow values for F16 at measurement time point 1 and F19 at measurement time point 2 were identified as outliers, but were not removed. Shapiro-Wilk's test revealed that all data were normally distributed. Levene's

test of homogeneity of variance was not significant at any measurement time, $p > .05$. Box's test of equality of covariance matrices could not be run. Mauchly's test of sphericity indicated that the assumption of sphericity was not violated for the two-way interaction, $\chi^2(20) = 25.729$, $p = .192$. There was no statistically significant interaction between HPA axis response group and time on average airflow, $F(12, 78) = 1.112$, $p = .363$, partial $\eta^2 = .146$. The main effect of measurement time point showed no statistically significant difference in average airflow at the different time points, $F(6, 78) = 1.797$, $p = .111$, partial $\eta^2 = .121$. The main effect of HPA axis response group showed no statistically significant differences in average airflow between groups, $F(1, 13) = 3.415$, $p = .064$, partial $\eta^2 = .344$. The group means and standard errors are shown in Figure 27.

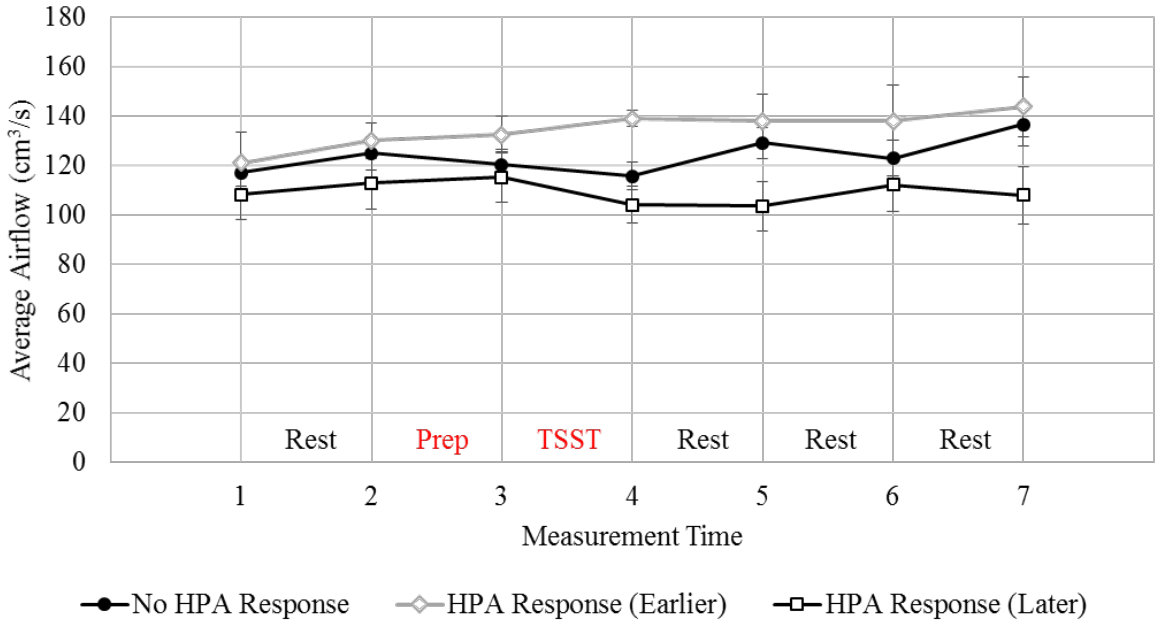

Figure 27. Average airflow (cm^3/s) over the measurement time points for the participants who experienced a later HPA axis response (open squares), those who experienced an earlier HPA axis response (open diamonds), and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Estimated subglottal pressure. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 3 levels) and the within-subjects factor (time, 7 levels) on estimated subglottal pressure. No outliers were identified after examining the boxplots. Shapiro-Wilk's test revealed that normal distribution could be assumed for all data except the earlier HPA axis response group at measurement time point 7, $p = .046$. Levene's test of homogeneity of variance was significant at measurement time point 4 only, $p = .040$. Box's test of equality of covariance matrices could not be run. Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, $\chi^2(20) = 35.903$, $p = .018$. A Greenhouse-Geisser correction was used to interpret the within-subjects effects ($\varepsilon = .530$). There was no statistically significant interaction between HPA axis response group and time on estimated subglottal pressure, $F(6.364, 47.730) = 1.138$, $p = .355$, partial $\eta^2 = .132$. The main effect of measurement time point showed no statistically significant difference in estimated subglottal pressure at the different time points, $F(3.182, 47.730) = .450$, $p = .729$, partial $\eta^2 = .029$. The main effect of HPA axis response group showed a statistically significant differences in estimated subglottal pressure between groups, $F(1, 15) = 3.783$, $p = .047$, partial $\eta^2 = .335$. However, t-tests using a Bonferroni correction to the alpha failed to reveal significant differences in estimated subglottal pressure between HPA axis response groups.

The group means and standard errors are graphed in Figure 28. As the means appear to be different at measurement time points and the main effect of HPA axis response group was significant in the two-way repeated measures ANOVA, a series of one-way ANOVAs were conducted to determine if estimated subglottal pressure differed between groups at any of the measurement time points. Estimated subglottal pressure was significantly different between

groups at measurement time point 1, $F(2, 16) = 5.532$, $p = .015$, measurement time point 2, $F(2, 16) = 4.611$, $p = .026$, and measurement time point 4, $F(2, 15) = 4.167$, $p = .036$. At measurement time point 2, no post-hoc t-test comparisons were significant, but at both measurement time points 1 ($p = .022$) and 4 ($p = .043$), the no HPA axis response group had significantly higher estimated subglottal pressure than the later HPA axis response group using a t-test with alpha levels corrected using a Bonferonni adjustment.

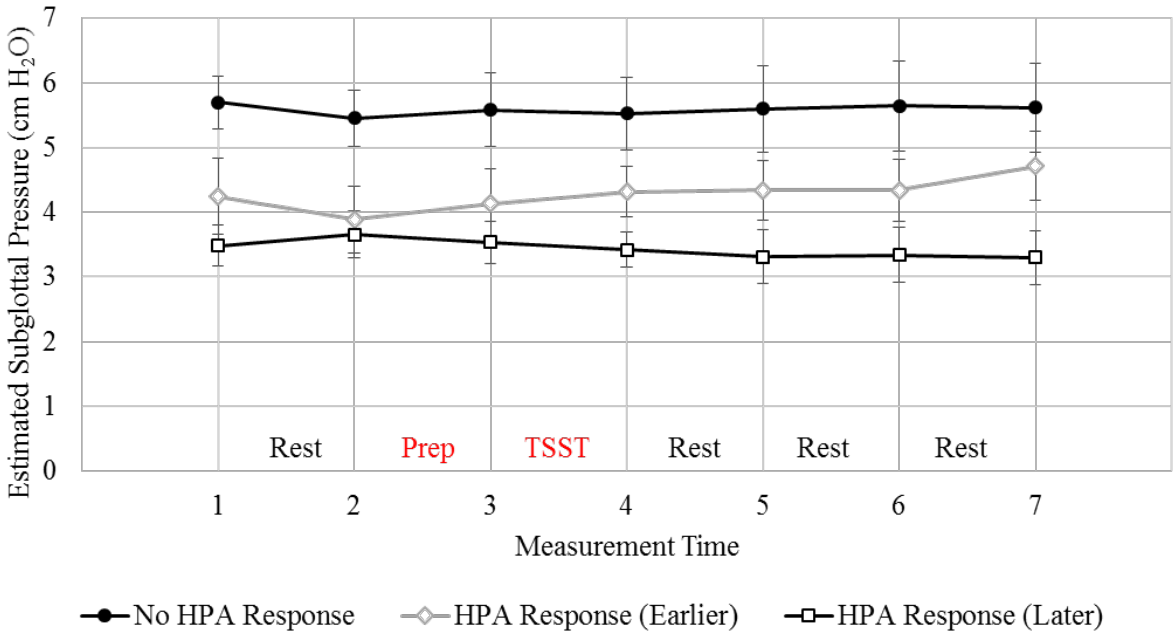

Figure 28. Estimated subglottal pressure (cm H_2O) over the measurement time points for the participants who experienced a later HPA axis response (open squares), those who experienced an earlier HPA axis response (open diamonds), and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Laryngeal airflow resistance. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 3 levels) and the within-subjects factor (time, 7 levels) on laryngeal airflow resistance. Examination of the box plots indicated that F6 at measurement time point 6 had a laryngeal airflow resistance value greater than 3 times the interquartile range. This value was not removed. Shapiro-Wilk's test

revealed that normal distribution could be assumed for all data except the no HPA axis response group at measurement time point 7, $p = .027$. Levene's test of homogeneity of variance was significant at measurement time points 1, $p = .048$, 4, $p = .024$, and 6, $p = .013$. Box's test of equality of covariance matrices could not be run. Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, $\chi^2(20) = 39.521$, $p = .008$. A Greenhouse-Geisser correction was used to interpret the within-subjects effects ($\varepsilon = .498$). There was no statistically significant interaction between HPA axis response group and time on laryngeal airflow resistance, $F(5.975, 32.861) = 1.282$, $p = .293$, partial $\eta^2 = .189$. The main effect of measurement time point showed no statistically significant difference in laryngeal airflow resistance at the different time points, $F(2.987, 32.861) = .836$, $p = .483$, partial $\eta^2 = .071$. The main effect of HPA axis response group showed no statistically significant differences in laryngeal airflow resistance between groups, $F(1, 11) = 2.505$, $p = .127$, partial $\eta^2 = .313$. The data are graphed in Figure 29.

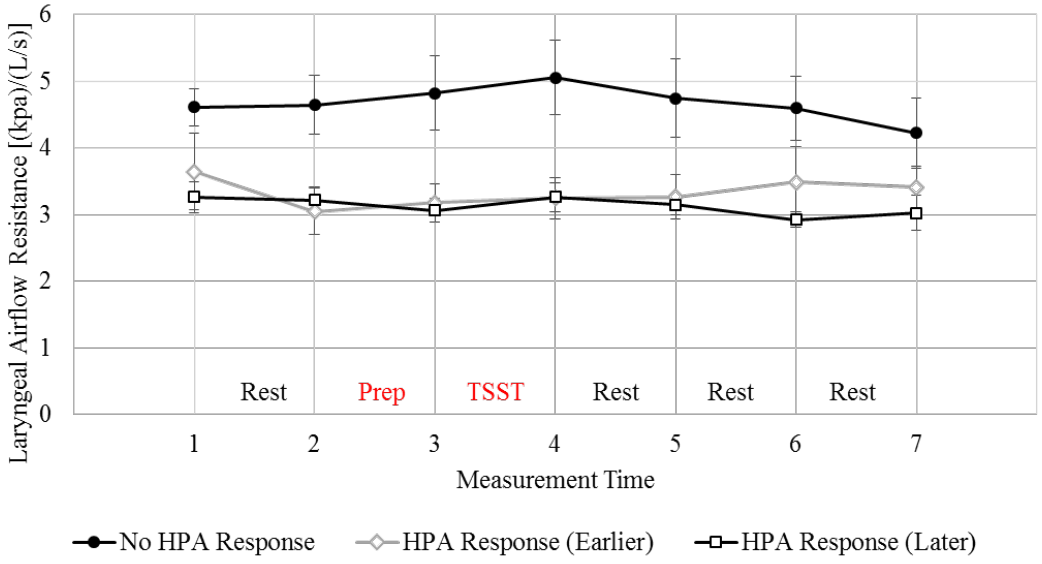

Figure 29. Laryngeal airflow resistance [(kPa)/(L/s)] over the measurement time points for the participants who experienced a later HPA axis response (open squares), those who experienced an early HPA axis response (open diamonds), and those who did not experience an HPA axis response (filled circles). Error bars represent standard error.

Open quotient from the EGG signal. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 3 levels) and the within-subjects factor (time, 7 levels) on open quotient. No outliers were identified from examination of the boxplots. Shapiro-Wilk's test revealed that normal distribution could be assumed for all data except the later HPA axis response group at measurement time point 6, $p = .023$. Levene's test of homogeneity of variance was significant at measurement time points 1, $p = .040$. Box's test of equality of covariance matrices could not be run. Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, $\chi^2(20) = 35.421$, $p = .024$. A Greenhouse-Geisser correction was used to interpret the within-subjects effects ($\varepsilon = .563$). There was no statistically significant interaction between HPA axis response group and time on open quotient, $F(6.754, 37.149) = 1.429$, $p = .225$, partial $\eta^2 = .206$. The main effect of measurement time point showed no statistically significant difference in open quotient at the different time points, $F(3.377, 6.754) = .697$, $p = .576$, partial $\eta^2 = .060$. The main effect of HPA axis response group showed no statistically significant differences in open quotient between groups, $F(1, 11) = .083$, $p = .921$, partial $\eta^2 = .015$. The data are graphed in Figure 30.

Speaking fundamental frequency. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 3 levels) and the within-subjects factor (time, 7 levels) on speaking fundamental frequency. Speaking fundamental frequency for F5 at measurement time point 1 and F5 and F6 at measurement time point 3 were identified as outliers. The outliers were removed. Shapiro-Wilk's test revealed that normal distribution could be assumed for all data except the no HPA axis response group at measurement time point 1, $p = .042$. Levene's test of homogeneity of variance

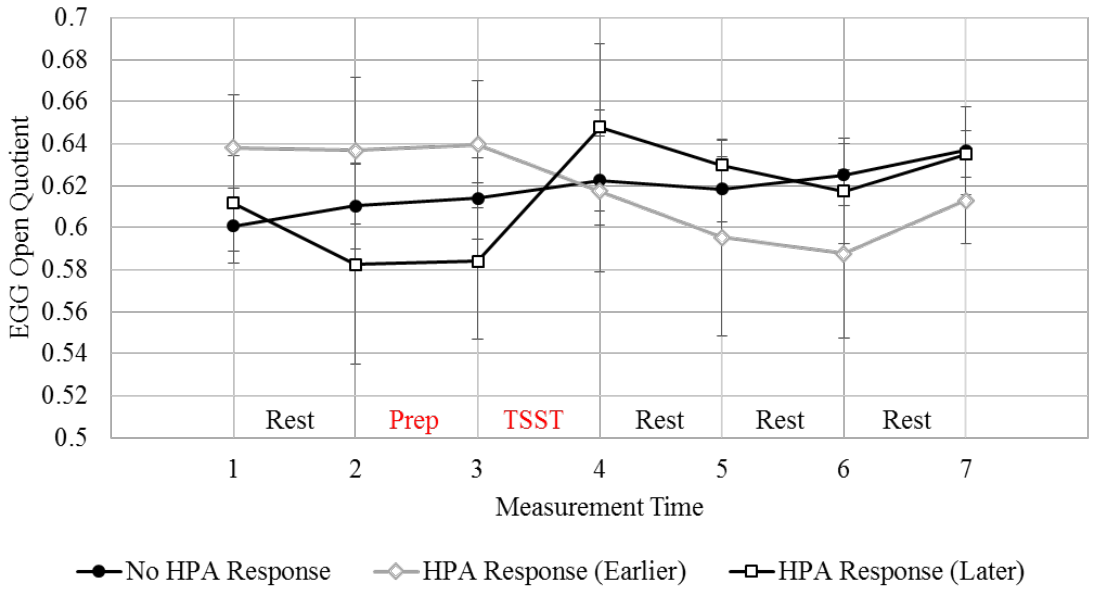

Figure 30. Open quotient from the EGG signal over the measurement time points for the participants who experienced a later HPA axis response (open squares), those who experienced an earlier HPA axis response (open diamonds), and those who did not experience an HPA axis response (filled circles). Error bars represent standard error.

was significant at measurement time point 1, $p = .029$. Box's test of equality of covariance matrices could not be run. Mauchly's test of sphericity indicated that the assumption of sphericity was not violated for the two-way interaction, $\chi^2(20) = 9.569$, $p = .977$. There was no statistically significant interaction between HPA axis response group and time on speaking fundamental frequency, $F(12, 84) = 1.094$, $p = .376$, partial $\eta^2 = .135$. The main effect of measurement time point showed a statistically significant difference in speaking fundamental frequency at the different time points, $F(6, 84) = 4.422$, $p = .001$, partial $\eta^2 = .240$. No post-hoc t-test was significant using a Bonferroni correction to the alpha. The main effect of HPA axis response group showed no statistically significant differences in speaking fundamental frequency between groups, $F(1, 14) = 1.005$, $p = .391$, partial $\eta^2 = .126$. The data are graphed in Figure 31.

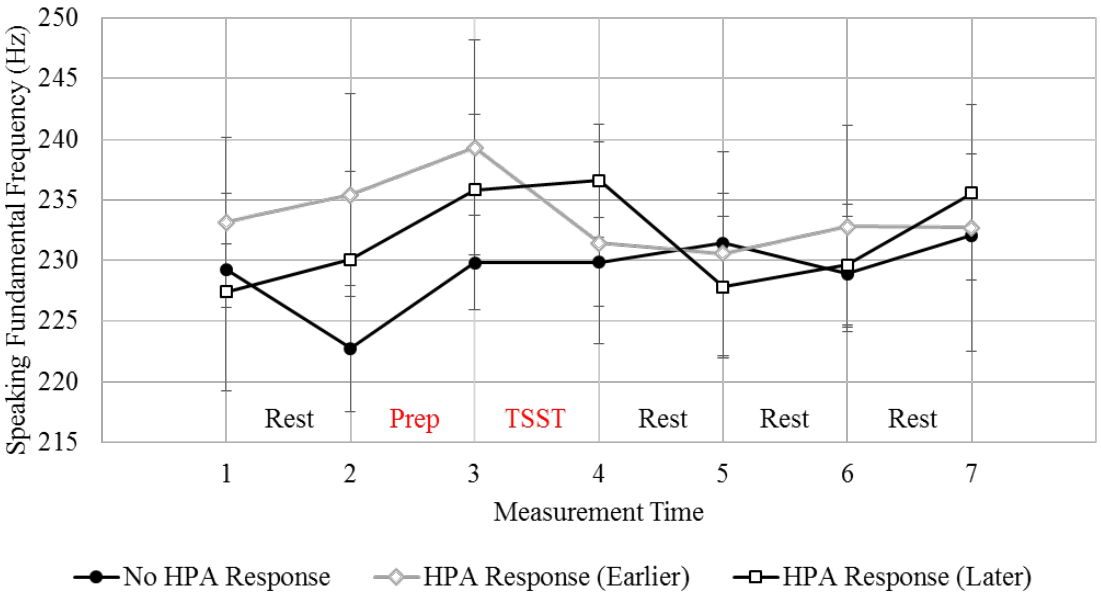

Figure 31. Speaking fundamental frequency (Hz) over the measurement time points for the participants who experienced a later HPA axis response (open squares), those who experienced an earlier HPA axis response (open diamonds), and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Percentage of syllables produced in vocal fry. A two-way repeated measures ANOVA was completed to determine if there is an interaction between the between-subjects factor (group, 3 levels) and the within-subjects factor (time, 7 levels) on the percentage of syllables produced in vocal fry. Arcsine transformed data were used due to issues with normal distribution. Percentage of syllables produced in vocal fry for participant F6 at measurement time point 7 was identified as an outlier but was not removed. Shapiro-Wilk's test revealed that normal distribution could not be assumed for the following two measurement time points for the later HPA axis response group: measurement time point 1, $p = .048$ and measurement time point 4, $p = .008$. The data were also not normally distributed for the no HPA axis response group at measurement time point 7, $p = .033$. No transformation improved normality. Levene's test of homogeneity of variance was not significant, $p > .05$. Box's test of equality of covariance matrices could not be run. Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the

two-way interaction, $\chi^2(20) = 68.912$, $p < .001$, and a Greenhouse-Geisser correction was used to adjust the degrees of freedom for the within-subject effects ($\varepsilon = .393$). There was no statistically significant interaction between HPA axis response group and time on percent of syllables produced in vocal fry, $F(4.711, 37.686) = 1.201$, $p = .328$, partial $\eta^2 = .130$. The main effect of measurement time point showed no statistically significant difference in percent of syllables produced in vocal fry at the different time points, $F(2.355, 37.686) = .718$, $p = .516$, partial $\eta^2 = .043$. The main effect of HPA axis response group showed no statistically significant differences in percent of syllables produced in vocal fry between groups, $F(1, 16) = 3.228$, $p = .066$, partial $\eta^2 = .288$. The data are graphed in Figure 32.

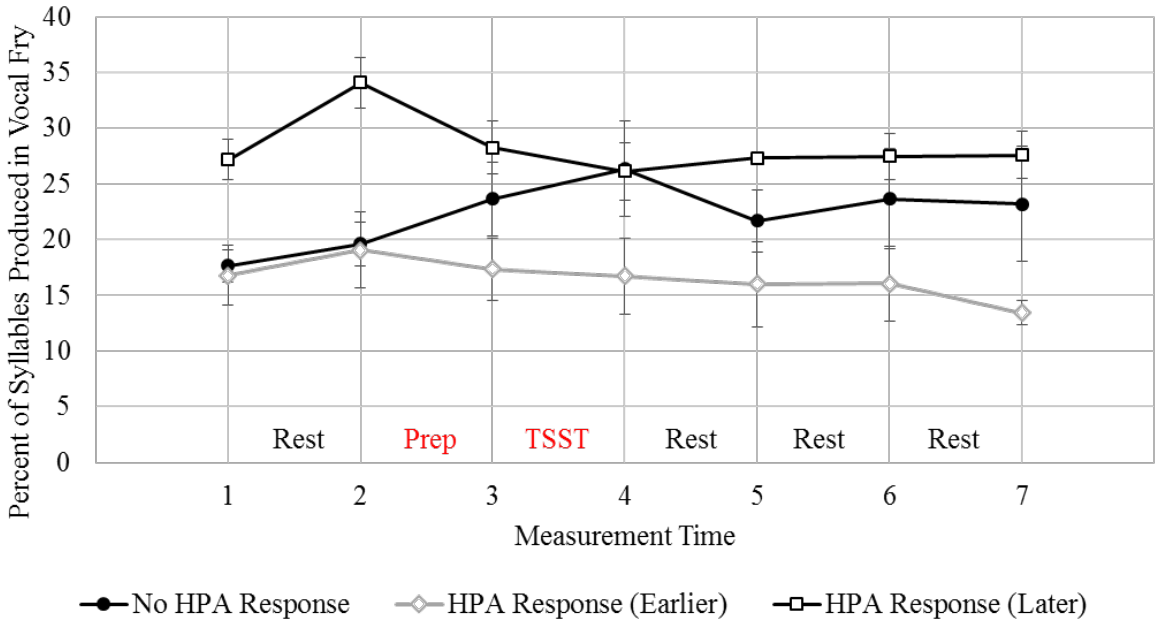

Figure 32. Percent of syllables produced in vocal fry during a reading of the Rainbow Passage over the measurement time points for the participants who experienced a later HPA axis response (open squares), those who experienced an earlier HPA axis response (open diamonds), and those who did not experience an HPA axis response (filled circles). Error bars represent standard error. The stressor occurred between measurement time points 3 and 4.

Correlations between salivary cortisol, salivary alpha-amylase, and voice production parameters. A series of Spearman correlations were conducted to determine if there was an

association between any of the voice parameters measured in the present study and salivary cortisol and salivary alpha amylase for all participants and the participants divided by HPA axis response. Prior to running the correlations, salivary cortisol values and salivary alpha-amylase values were winsorized to 3 standard deviations from the mean for the specific measurement time point (i.e., cortisol values that were greater than 3 standard deviations from the mean for a specific measurement time point were replaced with the value of 3 standard deviations from the mean). Table 12 presents how the values were winsorized and what values were winsorized.

Table 12. Salivary cortisol and salivary alpha-amylase values that were adjusted for the correlation analysis

	Measurement Time Point	Participant	Original Value	Winsorized Value
Cortisol	1	F9	19.4101 nmol/l	16.91888 nmol/l
	3	F9	12.91399 nmol/l	11.96168 nmol/l
	5	F13	32.68191 nmol/l	30.50454 nmol/l
Alpha-Amylase	1	F14	298.498 U/mL	296.6445 U/mL
	1	F15	384.672 U/mL	296.6445 U/mL
	2	F15	653.025 U/mL	429.411 U/mL
	3	F1	240.174 U/mL	223.6267 U/mL
	3	F18	240.502 U/mL	223.6267 U/mL
	4	F4	428.087 U/mL	312.244 U/mL
	4	F11	334.376 U/mL	312.244 U/mL
	5	F15	303.412 U/mL	250.2332 U/mL
	6	F14	347.155 U/mL	233.9099 U/mL
	7	F1	186.274 U/mL	176.0015 U/mL
	7	F11	232.966 U/mL	176.0015 U/mL
	7	F14	189.387 U/mL	176.0015 U/mL

Note. The winsorized value is 3 standard deviations from the mean for the specific measurement time point.

Table 13 presents the correlation coefficients. There was a weak positive association between salivary cortisol and speaking fundamental frequency (Figure 33) and a weak negative association between salivary cortisol and percent of syllables produced in vocal fry (Figure 34). In addition, there was a weak negative association between salivary alpha-amylase and open quotient from the derivative of the EGG signal (Figure 35). Next, a series of Spearman

correlations were conducted to determine if there was an association between any of the voice parameters and salivary cortisol and salivary alpha-amylase at any specific measurement time point (Table 14). Salivary cortisol was moderately associated with speaking fundamental frequency at measurement time point 2 (basal) and measurement time point 4 (post-stress 0) (Figure 36). In addition, salivary cortisol was moderately associated with laryngeal airflow resistance at measurement time point 3 (anticipatory).

Salivary alpha-amylase is not included in Table 14 for space reasons. There were two significant (at the .05 level) correlations between salivary alpha-amylase and open quotient: measurement time point 7 (post-stress 3), r_s = -.559, and between salivary alpha-amylase and average airflow at measurement time point 3 (anticipatory), r_s = .557.

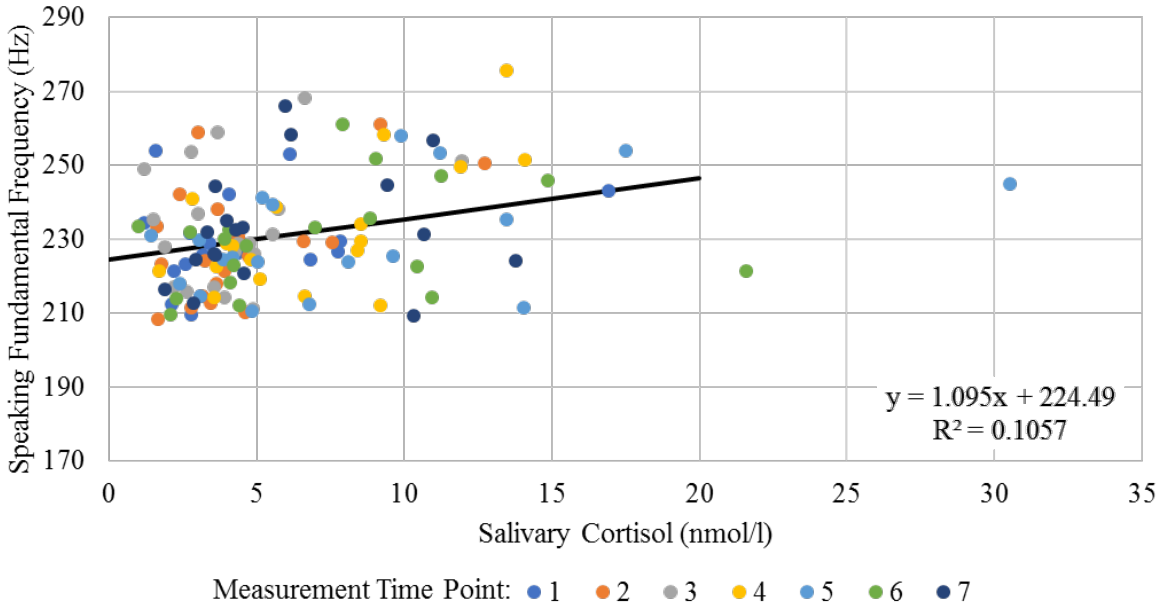

Figure 33. Comparison of salivary cortisol (nmol/l) and speaking fundamental frequency (Hz) for all participants. The trendline represents the equation for all participants. The individual colors represent each measurement time point. Each participant has one data point of each color.

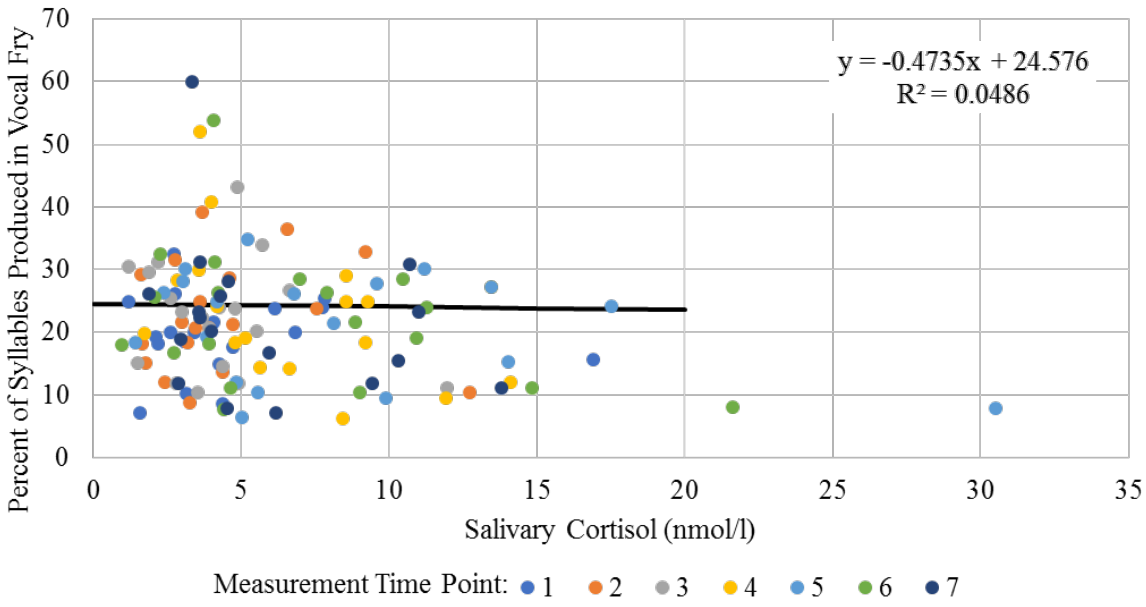

Figure 34. Comparison of salivary cortisol (nmol/l) and percent of syllables produced in vocal fry for all participants. The trendline represents the equation for all participants. The individual colors represent each measurement time point. Each participant has one data point of each color.

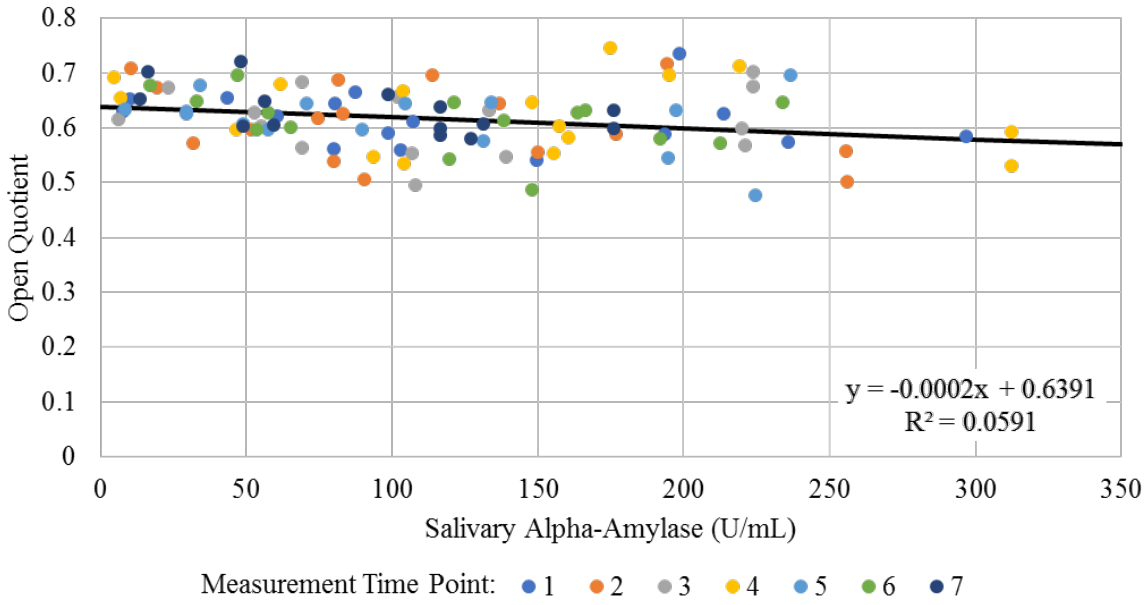

Figure 35. Comparison of salivary alpha-amylase (U/mL) and open quotient for all participants. The trendline represents the equation for all participants. The individual colors represent each measurement time point. Each participant who had a viable EGG signal has one data point of each color.

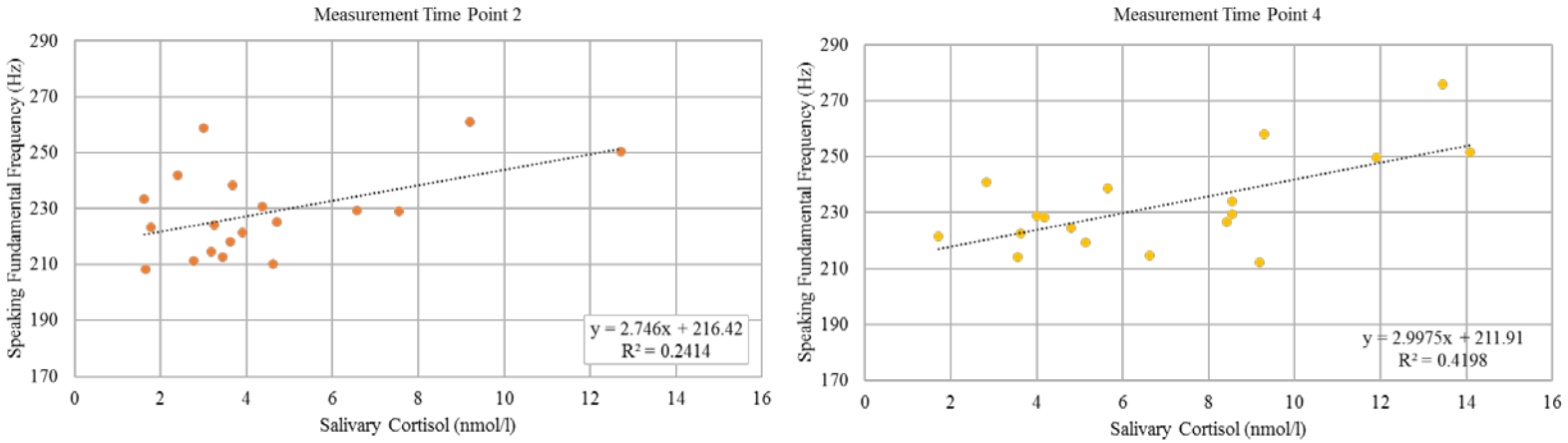

Figure 36. Comparison of salivary cortisol (nmol/l) and speaking fundamental frequency (Hz) for all participants. The individual panels represent the different measurement time points. Measurement time point 2 and measurement time point 4 have a moderate positive correlation.

Table 13. Correlations between voice production parameters and salivary cortisol and salivary alpha-amylase

	All Participants (n = 19)		No HPA Axis Response (n = 9)		Earlier HPA Axis Response (n = 6)		Later HPA Axis Response (n = 4)	
	Salivary Cortisol	Salivary Alpha-Amylase	Salivary Cortisol	Salivary Alpha-Amylase	Salivary Cortisol	Salivary Alpha-Amylase	Salivary Cortisol	Salivary Alpha-Amylase
Airflow	.151	.060	-.129	.051	.313	.026	-.351	.110
Estimated P_{sub}	.353	.066	.309*	.204	.234	.363*	-.189	.008
Laryngeal Airflow Resistance	-.036	-.015	.378**	.122	-.043	.374*	.321	-.219
Open Quotient	-.082	-.243*	-.135	-.491	-.068	-.235	-.092	.040
Speaking F_o	.325**	.160	.640**	.060	.173	.160	-.053	.204
% of Syllables Produced in Vocal Fry	-.223*	-.015	-.290*	-.162	-.022	.543**	-.041	-.437*

* p < .05 **p < .01

Table 14. Correlations between voice production parameters and salivary cortisol for all participants organized by measurement time point

	Beginning	Basal	Anticipatory	Post-Stress 0	Post-Stress 1	Post-Stress 2	Post-Stress 3
Airflow	-.294	-.132	-.317	.087	.395	.365	.009
Estimated P_{sub}	.401	.163	.337	.120	.023	-.147	.053
Laryngeal Airflow Resistance	.052	.234	.516*	.097	-.243	-.365	.014
Open Quotient	-.247	.130	.022	-.351	-.036	-.023	-.204
Speaking F_o	.287	.491*	.277	.648**	.446	.235	.175
% of Syllables Produced in Vocal Fry	-.011	.031	-.202	-.413	-.275	-.362	-.301

* p < .05 **p < .01

CHAPTER V: DISCUSSION

The purpose of this study was to better understand the influence of an acute psychosocial stressor on aerodynamic and acoustic voice parameters. Psychosocial stress has been implicated in many voice disorders, especially non-organic (functional, muscle tension dysphonia) voice disorders (e.g., Aronson, 1990; Roy & Leeper, 1993; Seifert & Killbrunner, 2005; Van Houtte, Van Lierde, & Claeys, 2011). In a retrospective chart review of 150 patients with muscle tension dysphonia (median age of 42.3 years old), 19% of patients had reported high stress levels (Altman, Atkinson, & Lazarus, 2005). In another investigation of patients with voice disorders, 25% of patients scored higher than norms on the Perceived Stress Scale-10 (Dietrich et al., 2008). In addition, teachers who were categorized as having an unhealthy voice based on self-reported voice symptoms were more likely to endorse experiencing psychosocial stressors (de Alvear, Martínez, Barón, & Hernández-Mendo, 2010).

The body's reaction to stress is controlled by two major stress systems: the sympatho-adrenal medullary system (SAM system; controlled by the sympathetic nervous system) and the hypothalamic-pituitary-adrenal axis (HPA axis). Activation of the former system leads to increased epinephrine, norepinephrine, and levels of free fatty acid, in addition to increases in muscle tension, blood pressure, and the amount of blood pumped by the heart per minute (Herd, 1984). Activation of the HPA axis leads to increased production of glucocorticoids (e.g., cortisol), increased levels of free fatty acids, and a decrease in immune functioning (Linden et al., 1997). As the HPA axis is more indicative of chronic stress, it may be more related to the development of diseases or disorders (Dienstbier, 1989).

In the current investigation, participants were subjected to the Trier Social Stress Test, a stress protocol that involves freely speaking about qualifications for a job and mental arithmetic

in front of an audience. At seven measurement time points throughout the study (two before the stressor was introduced, one after the stressor was introduced, and four after the stressor was completed), recordings of /pa:/ repetitions and the Rainbow Passage were made and saliva samples were collected. From the voice recordings, the dependent variables of average airflow, estimated subglottal pressure, laryngeal airflow resistance, open quotient from the EGG signal, speaking fundamental frequency, and percent of syllables produced in vocal fry were extracted. From the saliva samples, salivary cortisol levels and activity levels of salivary alpha-amylase were measured and used as indicators of hypothalamic-pituitary-adrenal axis activity and sympathetic nervous system activity, respectively.

Research question 1: The effect of an acute stressor on voice parameters

The results of the present study suggest that there is no effect of an acute, social-evaluative stressor on several voice parameters (average airflow, estimated subglottal pressure, open quotient calculated from the derivative of the EGG signal, and percent of syllables produced in vocal fry). The only variable that showed change over time that appears to be related to the stressor was speaking fundamental frequency.

The voice parameters measured in the present study are reasonable based on a review of the literature. All of the values of average airflow are within 2 standard deviations (70 cm^3/s) of the mean (190 cm^3/s) reported by Holmberg, Hillman, and Perkell (1988). 74.2% of the estimated subglottal pressures are within 2 standard deviations (1.4 cm H_2O) of the mean (6.3 cm H_2O) and 25% of the estimated subglottal pressures are below 2 standard deviations of the mean (below 3.5 cm H_2O) reported by Holmberg et al. (1988). The estimated subglottal pressure values found in the present study are similar to those reported for soft phonation (Konnai,

Scherer, Peplinski, & Ryan, 2017). The values of speaking fundamental frequency in the present study all fall within the range (192.2 to 275.4 Hz) reported by Stoicheff (1981) for female non-smokers between the ages of 20 and 29. The mean open quotient from the derivative of the EGG signal (0.618; SD: 0.056) for all participants in the present study is slightly higher than the mean values reported for young females by Winkler and Sendlmeier (2006) (0.5019; SD: 0.0564). A higher open quotient (or lower closed quotient) is associated with a more breathy voice quality (Peterson, Verdolini-Marston, Barkmeier, & Hoffman, 1994).

The lack of significant differences from before the stressor to after the stressor in the aerodynamic and electroglottographic measures is not completely unexpected. In electromyographic examination of selected external laryngeal muscles (infrahyoid and submental), Dietrich (2008) only found significant differences over time because some of the times involved speech tasks and some did not. The times that involved speech tasks had increased activity of both muscles compared to the times that did not involve speech. In the present study, the structure of the speech tasks may have also influenced the lack of significant changes over time. The aerodynamic measures were made from /pa:/ repetitions. In order to achieve oral pressure signals that can be used to estimate subglottal pressure, the participant must produce the syllable repetitions smoothly and evenly. The participants were trained at the beginning of the experimental protocol and received additional training throughout if their oral air pressure signals failed to reach a plateau during the lip occlusion. This task may have limited the variability in aerodynamic measures that may exist following stress.

Speaking fundamental frequency and psychosocial stress. Speaking fundamental frequency was the only dependent variable to significantly change as a result of stress. Mean speaking fundamental frequency increased significantly (0.386 semitones) from measurement

time point 2 to measurement time point 4. When divided into two groups (HPA axis responders and HPA axis non-responders) there was an interaction effect of time and group; the HPA axis group experienced a drop in fundamental frequency after the stressor that was significantly (measurement time point 3 to 5 and measurement time point 4 to 6).

The majority of the studies looking at the influence of stress on the voice have reported a significant change in fundamental frequency from before the stressor to after the stressor (Table 15). When examining the direction of change in fundamental frequency, three reported an increase in fundamental frequency and two reported a decrease in fundamental frequency. The differences in reports of fundamental frequency increase and fundamental frequency decrease under stress are likely due to individual variation. In the present study, 5 participants had a decrease in speaking fundamental frequency (average: -2.22 Hz; SD: 2.31) and 14 had an increase in speaking fundamental frequency (average: 7.79 Hz; SD: 5.05). Brenner Shipp, Doherty, and Morrissey (1985) reported that five out the seven participants in their study had an increase in fundamental frequency under stress and Pisanski et al. (2016) indicated large individual variations in fundamental frequency change under stress.

An effort has been undertaken to understand the discrepancies in fundamental frequency changes following stress. Pisanski et al. (2016) suggest that in laboratory studies the mean fundamental frequency increase is smaller than in natural settings and thus the intra-individual variation (i.e., the participants that have a decrease in fundamental frequency) was too great. In Dietrich (2008), both groups (introverts and extroverts) experienced a decrease in fundamental frequency; however, these groups had differences in ratings of vocal effort and infrahyoid muscle activity under stress suggesting these variables do not explain differences in fundamental frequency. Finally, Brenner et al. (1985) found although five out of the seven participants

experienced an increase in fundamental frequency under stress, six out of seven experienced a significant increase in cricothyroid muscle activity suggesting other factors besides CT muscle activity are needed to understand the change in fundamental frequency.

In the present study, HPA axis response could not be used to explain direction of change of speaking fundamental frequency in individuals; two participants were in the no HPA axis response group and three were in the HPA axis response group. As such, an exploratory analysis was completed to see if the individuals who had an increase in speaking fundamental frequency under stress and the individuals who had a decrease in speaking fundamental frequency under stress in the present study vary on any other demographic factor. Of note, because the interest is in the stress response, only those who had an HPA axis response were included in this analysis (n = 10). There were no significant differences in age, $p = .583$, phase in menstrual cycle, $p = .242$, Perceived Stress Scale score, $p = .463$, baseline[4] average airflow, $p = .877$, baseline estimates of subglottal pressure, $p = .700$, or baseline open quotient, $p = .580$. There was, however, a significant difference in modified VHI-10 scores, $p = .008$. The participants who had a decrease in fundamental frequency had a higher modified VHI-10 score (n = 3; average: 10.00; SD: 1.00) than those who had an increase in fundamental frequency (n = 10; average: 4.29; SD: 2.69) from before the stressor to after the stressor. A higher VHI-10 score is indicative of self-perceived voice problems (and not necessarily objective measure of voice functioning; e.g., Hsiung, Pai, & Wang, 2002). Although the sample size is small, the results of this exploratory analysis indicate that those who have HPA axis activation and a decrease in fundamental frequency following stress also may have self-perceived problems with their voice.

[4] Baseline (measurement time point 1) values were used to simplify the analysis as there were no significant changes in these measures over time.

Another note about the direction of change in fundamental frequency in the present study involves the change in fundamental frequency in the three groups: those with no HPA axis response, those with an earlier HPA axis response, and those with a later HPA axis response. There was a statistically significant influence of time when the participants were divided into these three groups, although post-hoc t-tests using a Bonferonni adjustment did not reveal any significant differences. There are some interesting trends in the means. For the no HPA axis response group, the speaking fundamental frequency is nearly the same at measurement time points 1, 3, and 4, suggesting that their fundamental frequency was not influenced by the stressor. However for the early HPA axes response group, speaking fundamental frequency increased somewhat until just after the anticipatory period and then decreased for the remaining measurement time points. For the later HPA axis response group, the speaking fundamental frequency increased until just after the anticipatory period, but stayed at that same level for the measurement just after the stressor, and then decreased. Despite large variability, this does seem to support the notion that cortisol explains some of the variability in fundamental frequency as suggested by Pisanski et al. (2016), especially since there were no significant changes in subglottal pressure over time in the present study.

In addition, similar to what was reported by Pisanski et al. (2016), cortisol and fundamental frequency were correlated. However, unlike Pisanski et al. (2016), in the present study, cortisol and speaking fundamental frequency were correlated for all measurement time points ($r_s = .325$), for just the basal measurement before stress ($r_s = .491$), and for measures made right after the stressor ($r_s = .648$). In the present study, when the participants were divided into the three groups based on HPA axis response, those who experienced no HPA axis response to the TSST were the only group to have a significant association between speaking fundamental

frequency and cortisol (r_s = .640). A previously made critique of the article by Pisanski et al. (2016) is that it seems the authors did not remove those who experienced no HPA axis response (as measured by a cortisol increase). It may be, as is the case in the present study, that the correlation between speaking fundamental frequency and cortisol is driven by those who do not experience an HPA axis response.

When examining the magnitude of change in fundamental frequency in the studies looking at the influence of stress on the voice, it is evident that the magnitudes of change are not large (Table 15). This is also the case in the present study, where the change in speaking fundamental frequency from before the stressor to after the stressor was less than the intra-individual variation. This suggests that although the changes may be significant, they may not be perceptual because it is less than the prosodic variations occurring over the course of the sentence.

Although the magnitude of change of speaking fundamental frequency was small in the present study, it may represent a notable change in physiology as it is a consistent finding throughout the literature related to stress and the voice. The string equation for passive tension, $fo = (1/2L)(T/\rho)^{0.5}$, where L is the length of the tissue in vibration, T is the passive tension of the tissue, and ρ is the density of the tissue, can be used to estimate how length of the tissue in vibration and passive tension change to create a small change in fundamental frequency (Titze, 1994, p. 200). When the change in fundamental frequency was 5 Hz, say from 220 Hz before the stressor to 225 Hz after the stressor (which is in the range of speaking fundamental frequencies in the present study), the change in length and tension could be represented by the equation, $(L_1/L_2)(T_2/T_1)^{0.5}=1.02$. The relationship can be expressed as $T_2 = 1.046(L_2/L_1)^2T_1$, to state that the tension of the vocal fold tissue in motion after the stressor is approximately 4.6% of the ratio

of the length of the vocal folds after the stressor to the length of vocal folds before the stressor, squared, multiplied by the tension before the stressor relative to the change in length of the vocal folds to the tension from before the stressor (Titze, 1994). Both changes in tension and length depend on the relationship between CT muscle and TA muscle contraction (Titze, 1994). It is also important to consider that the change in fundamental frequency (around 5 Hz) in the present study is most likely not due to a change in subglottal pressure as 1 cm H_2O change in subglottal pressure is needed for a 2 to 6 Hz change in fundamental frequency (Titze, 1989).

Research question 2: Differences in voice parameters between those with HPA axis activation following an acute stressor and those without

In the present study, only 10 of the 19 participants had an increase in salivary cortisol greater than 2.5 nmol/l. It was expected that more participants (up to 70%) would experience an HPA axis response following the stressor. Higher levels of cortisol before the stressor and higher basal cortisol levels tend to decrease or inhibit the physical response to stress (Buckingham & Hodges, 1974; Gray & Munson, 1951; Hodges & Sadow, 1967; Stark, Acs, & Szalay, 1969). This certainly appears to explain why participants F9 and F3, who both presented with high baseline measure of salivary cortisol, did not experience HPA axis response following the stressor. Although it has been suggested that there is no HPA axis response because the person does not interpret the stressor to be a threat to a committed goal (Lovallo, 2016), this does not seem to be the case in the present study as there were no differences in self-rated stress level at measurement time point 4 between those who experienced an HPA axis response (mean: 63.5, SD: 27.7) and those who did not (mean: 73.1, SD: 28.5). There appear to be no measures collected in the present study that explain why the remaining participants did not experience an

Table 15. Review of the changes in fundamental frequency from the stress literature

Study	Change in F_o	Speaking Task	Stress Task
Present Study: all participants (n = 19)	0.386 ST *	"The rainbow is a division of white light into many beautiful colors."	TSST
Present Study: HPA axis responders (n = 10)	0.402 ST	"The rainbow is a division of white light into many beautiful colors."	TSST
Present Study: HPA axis non-responders (n = 9)	0.360 ST	"The rainbow is a division of white light into many beautiful colors."	TSST
Tse et al. (2014): approximated from Figure 1 (n = 20)	3 ST *	"North Wind and the Sun" passage in Cantonese	Modified TSST
Dietrich (2008): extroversion group (n = 27)	-0.855 ST *	"we were away"	TSST
Dietrich (2008): introversion group (n = 27)	-0.70137 ST *	"we were away"	TSST
Van Lierde et al. (2009) (n = 54)	-0.513 ST *	Spontaneous speech in Dutch	Oral reading for 20 minutes
Mendoza & Carballo (1998) experimental instructions (n = 82)	1.115 ST *	/a/ vowel (native speakers of Spanish)	Tongue twister
Mendoza & Carballo (1998) experimental instructions (n = 82)	1.378 ST *	/a/ vowel (native speakers of Spanish)	Tongue twister with DAF
Mendoza & Carballo (1998) experimental instructions (n = 82)	1.234 ST *	/a/ vowel (native speakers of Spanish)	Recitation of alphabet backwards
Brenner et al. (1985) (n = 7)	0.624 ST *	Numbers 0-9	Seven digit repetition task
Pisanski et al. (2016) (n = 34)	0.149 ST *	First 3 sentences of the Rainbow Passage in Polish	Oral examination for a course
Pisanski et al. (2016) (n = 34)	0.351 ST *	Spontaneous speech in Polish	Oral examination for a course

Note. In the present study, syllables produced in vocal fry were removed from fundamental frequency analysis. From Dietrich (2008), Van Lierde et al. (2009), Mendoza and Carballo (1998), and Pisanski et al. (2016), Brenner et al. (1985) the change in fundamental frequency in semitones was calculated from the mean value from the stressed condition and the mean value from the unstressed condition. ST = semitones; * = F_o during the stressed condition was significantly different from the unstressed condition; DAF = delayed auditory feedback

increase in salivary cortisol. Engert et al. (2012) also had a lower number of participants who experienced an HPA axis response compared to what has been described in the literature. These authors attribute the lower number of responders to a familiarity with the experimental procedure (the TSST) due to how frequently it is used in research (Engert et al., 2012)[5]. In addition, this group may have been habituated to the stressor due to exposure to similar tasks in the recent past (Van Eck et al., 1996).

Changes in measures from before the stressor to after the stressor based on HPA axis response. The speaking fundamental frequency was the only variable that changed over time that appears to be related to the stressor; it was higher after the anticipatory phase and just after the stressor (measurement time points 3 and 4) for the HPA axis response group then after the stressor (measurement time points 5 and 6, respectively).

The lack of significant changes in the voice parameters for the groups from before the stressor to after the stressor suggest that the profiles presented in the Introduction do not hold. Profile 1, which proposed that those who had a doubling of cortisol would experience a greater increase in fundamental frequency, is not accurate. Those who experienced a doubling of cortisol from measurement time point 2 to measurement time point 4 had a 0.456 semitone (SD: 0.5215) change in speaking fundamental frequency and those who did not experience a doubling of cortisol had a 0.339 semitone (SD: 0.4552) change in speaking fundamental frequency. In addition, there were no changes in vocal fry or laryngeal airflow resistance over time (negating profile 2 which suggested that fundamental frequency and laryngeal airflow resistance would increase in those with SNS and HPA axis activity).

[5] After the experimental procedure in the present study, participants were asked not to discuss the TSST portion of the experiment with others.

Group differences in voice parameters based on HPA axis response. There were no significant group differences for measures of average airflow, open quotient, speaking fundamental frequency while reading, or percent of syllables produced in vocal fry while reading, but the groups did vary significantly on estimated subglottal pressure and laryngeal airflow resistance. The participants who had no HPA axis response to the TSST had significantly higher estimated subglottal pressure and significantly higher laryngeal airflow resistances than those who did have an HPA axis response to the stressor.

Figure 37 presents the estimated subglottal pressure graphed against average airflow for all participants. The majority of participants in both groups had pressure and flow values (and thus laryngeal airflow resistance values) that fell within the range of normal as reported by Zraick, Smith-Olinde, & Shotts (2012). Holmberg (1980) and Smitheran and Hixon (1981) also reported similar values. However, as seen in Figure 37, there is some overlap with the disordered population in the higher range of estimated subglottal pressure values (Netsell et al., 1991). These significantly higher pressure values are associated with the significantly higher laryngeal airflow resistances, which can be altered by changing pressure and adduction individually or by changing both measures. Higher laryngeal airflow resistances, as seen in those in the no HPA axis response group, often occur when a pressed voice is produced (Holmberg, 1980; Konnai et al., 2017).

The participant who had the highest estimated subglottal pressure values was F9, who was the participant with the highest baseline cortisol levels. The participant who had an HPA axis response and had estimated subglottal pressure values that were higher than others in the HPA axis response group was F13. This participant also had the largest cortisol response to stress.

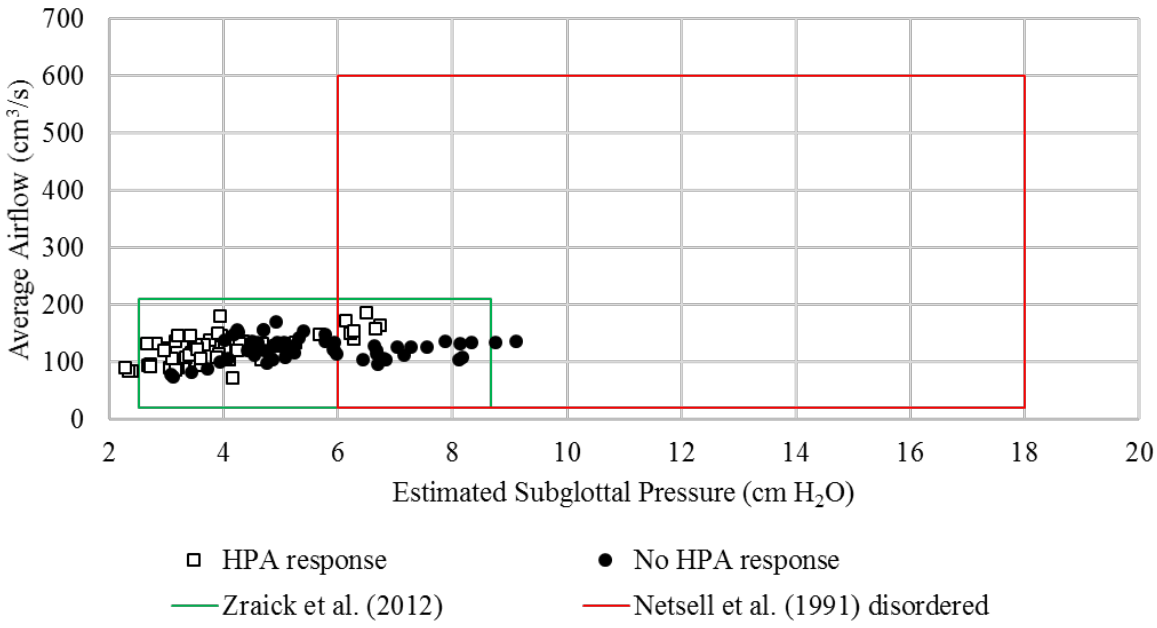

Figure 37. Estimated subglottal pressure and average airflow for participants with an HPA axis response (unfilled squares) and no HPA axis response (filled circles). The green box is the range of normal pressures and flows from Zraich et al. (2012) and the red box is the range of pressures and flows for participants with various voice disorders reported by Netsell et al. (1991) speaking at a normal pitch and normal loudness.

Based on work by Engert et al. (2013), the participants were divided into an earlier HPA axis response group if the cortisol was more than 2.5 nmol/l at measurement time point 4 compared to measurement time point 2 and a later HPA axis response group if the cortisol was more than 2.5 nmol/l at measurement time point 5, 6, or 7 (but not measurement time point 4) compared to measurement time point 2. Based on this, there remained nine participants in the no HPA axis response group, there were six participants in the earlier HPA axis response group, and there were four participants in the later HPA axis response group.

Dividing the participants into three groups (versus two groups) did not result in any new statistically significant results. When the participants were divided into three groups (those with no HPA axis response, those with a later HPA axis response, and those with an earlier HPA axis response), there was a main effect of group, but post-hoc t-tests using a Bonferonni adjustment to

the alpha level did not reveal any significant group differences. When reviewing the means, the HPA axis response group was found to have higher estimated subglottal pressure at measurement time points 1 and 4. When all measurement time points were averaged together, the participants who experienced no HPA axis response following the acute, psychosocial stressor had an average subglottal pressure of 5.59 cm H_2O (SEM: 0.205794). The participants who experienced an earlier HPA axis response (an increase in cortisol at measurement time point 4) had an average lower subglottal pressure of 4.283 cm H_2O (SEM: 0.180077), and the participants who experienced a later HPA axis response (an increase in cortisol at measurement time point 5, 6, or 7) had an average lowest subglottal pressure of 3.43 cm H_2O (SEM: 0.124983). As there is a significant effect of group on estimated subglottal pressure when participants are divided into two or three groups based on HPA axis response, it appears that some factor separates the groups.

As part of the normal physical and behavioral adaptations that occur when a stressor is present, the body tries to contain the stress response (Chrousos & Gold, 1992). Those without activation of the HPA axis and sympathetic nervous system following stress, despite the acknowledgement of the stressor (indicated by increased self-ratings of stress), may rely more heavily on behavioral adaptations to prevent changes in voice production under stressful circumstances. In the present study, the group who did not experience HPA axis response may have used higher lung pressures consistently to prevent changes in voice quality that are more apparent at lower lung pressure. For example, vocal perturbation measures have been found to increase as loudness decreased (Brockmann, Storck, Carding, & Drinnan, 2008; Brockmann-Bauser, Bohlender, & Mehta, 2018).

However, the potential behavioral compensation of using higher subglottal pressure to prevent voice quality changes may not be completely effective in this group. The percent of syllables produced in vocal fry was mildly and negatively correlated with salivary cortisol for all participants (r_s = -.223). When participants were divided into the three groups, the no HPA axis response group was the only group that had a significant association between salivary cortisol and percent of syllables produced in vocal fry (r_s = -.290). Although these correlations are weak, they may suggest that in those who do not have the expected stress system activation, voice quality (which may or may not be related to an increase in vocal fry depending on individual opinion) may be reduced.

Vocal fry and psychosocial stress. Dietrich (2008) anecdotally noted that vocal fry seemed to increase when the participants were under stress. In the present study, the number of syllables produced in vocal fry during the Rainbow Passage reading were counted and that count was divided by the total number of syllables produced (some participants did not read the passage completely or correctly and thus produced a different number of syllables). In the present study, the percent of syllables produced in vocal fry did not change significantly over time for all participants together or for the participants when they were divided into two or three groups based on HPA axis activation. When the participants were divided into HPA axis responders and HPA axis non-responders and when they were divided into earlier HPA axis responders, later HPA axis responders, and HPA axis non-responders, there was no group difference in mean percent syllables produced in vocal fry. Importantly, the group with the highest average percent syllables produced in vocal fry also had the lowest average airflow and the group with the lowest average percent syllables produced in vocal fry had the highest average airflow (Table 16).

Table 16. Percent of syllables produced in vocal fry, average airflow, and estimated subglottal pressure.

	Percent of Syllables Produced in Vocal Fry (SD)	Average Airflow (SD)	Estimated Subglottal Pressure (SD)
Later HPA axis response (n = 4)	28.3% (4.4)	109.43 cm^3/s (18.63)	3.43 cm H_2O (0.66)
No HPA axis response (n = 9)	22.26% (10.6)	123.99 cm^3/s (20.08)	5.59 cm H_2O (1.57)
Earlier HPA axis response (n = 6)	16.56% (7.15)	134.48 cm^3/s (22.81)	4.28 cm H_2O (1.17)

In the present study, the decision was made to report the results as percent of syllables produced in vocal fry because in multi-syllabic words, many times only one syllable was produced in vocal fry (e.g., in the word *horizon* only the *zon* was produced in vocal fry). However, as the literature presents the percent of words produced in vocal fry and the instances of vocal fry per minute, Table 28 presents the vocal fry data for the percent of words produced in vocal fry and the estimated vocal fry per minute. The vocal fry per minute is estimated from the reading of the first paragraph of the Rainbow Passage, which took participants an average of 29.42 seconds (SD: 3.04) to read.

Females aged 18 to 25 years of age have been reported to use 13.8 (SD: 7.1) instances of vocal fry per minute in spontaneous speech (Oliveira, Davidson, Holczer, Kaplan, & Paretzky, 2016). American females have also been estimated to use vocal fry on 12.4% of words (Yuasa, 2010). Five percent (SD: 2.79) of the Rainbow Passage was reported to be produced in vocal fry by Plexico and Sandage (2017). From Table 17 it is evident that the participants in the present study produced vocal fry more frequently than what has been reported by Yuasa (2010) and Oliveira et al. (2016). It is suspected that the higher percentage of vocal fry in the present study is due to low average airflow and estimated subglottal pressure used by participants in the

present study (N.B., the aerodynamic measures were taken from /pa:/ syllable repetitions and the vocal fry counts were from reading of the Rainbow Passage).

Table 17. Percent of words produced in vocal fry and the estimated vocal fry per minute with standard deviations

	Measurement Time Point:	1	2	3	4	5	6	7
All Participants (n = 19)	% of words produced in vocal fry	23.23	27.62	26.94	26.44	24.9	25.57	24.94
	Standard deviation	6.376	8.479	9.009	11.54	9.827	10.73	11.62
	Estimated vocal fry per minute	43.37	52.86	52.77	51.78	49.62	50.57	49.65
	Standard deviation	10.25	14.73	14.59	20.26	18.18	18.51	20.05
No HPA Axis Response (n = 9)	% of words produced in vocal fry	20.83	23.29	26.55	28.45	25.44	26.17	25.93
	Standard deviation	4.37	5.74	9.11	12.47	10.30	13.73	15.63
	Estimated vocal fry per minute	42.01	45.51	53.09	58.48	51.41	52.23	52.63
	Standard deviation	10.50	6.94	14.93	21.92	18.88	22.98	29.67
Earlier HPA Axis Response (n = 6)	% of words produced in vocal fry	20.18	26.06	21.65	19.32	19.10	20.07	18.39
	Standard deviation	5.14	8.80	6.49	10.98	10.67	7.95	4.98
	Estimated vocal fry per minute	38.66	51.40	43.73	38.16	38.27	40.73	36.87
	Standard deviation	8.79	16.80	11.73	20.00	20.29	15.51	8.93
Later HPA Axis Response (n = 4)	% of words produced in vocal fry	30.92	38.03	32.35	30.08	30.42	30.95	30.53
	Standard deviation	4.54	4.20	8.03	7.93	3.14	3.08	3.11
	Estimated vocal fry per minute	52.60	70.32	60.47	55.44	60.01	59.56	59.68
	Standard deviation	8.68	13.91	11.89	10.69	2.86	5.44	8.97

A possible personality influence. Although personality was not measured in the present study, attention has been given to it due to the trait theory of voice disorders. The trait theory of voice disorders proposes that those at risk for vocal fold lesions have a tendency to have behavioral impulsivity because they have the personality traits of extraversion and neuroticism working together and that those at risk for muscle tension dysphonia have a tendency to have

behavioral inhibition because they have the personality traits of introversion and neuroticism working together (Roy & Bless, 2000). Roy, Bless, and Heisey (2000b) have tested their theory and found that low-extroversion and high-neuroticism traits were found in 49% of patients with muscle tension dysphonia. The vocal nodules group had 43% or patients in the high-extroversion and low-neuroticism group and 32% of patients in the high-extroversion and high-neuroticism group (Roy et al., 2000). Both groups have also been described as stress reactive by the Multidimensional Personality Questionnaire (Roy, Bless, & Heisey, 2000a). Patients with vocal nodules have also been found to have lower scores on the "Harm Avoidance" section and higher scores on the "Novelty Seeking" section of the Temperament and Character Inventory (Mattei, Revis, & Giovanni, 2017). To summarize, the theory suggests that personality will result in predictable behavioral response in the following domains: emotional, cognitive, and vocal (Roy & Bless, 2000).

In terms of vocal aerodynamic measures, those with non-phonotraumatic vocal hyperfunction (which includes muscle tension dysphonia) had AC flows (the peak-to-peak amplitude of the glottal flow) that were 51 cm^3/s lower than controls and those with phonotraumatic vocal hyperfunction (that includes vocal nodules) had AC flows that were 91 cm^3/s higher than controls at conversational loudness (Espinoza, Zañartu, Van Stan, Mehta, & Hillman, 2017). Average airflow (Peppard, Bless, & Milenkovic, 1988) and estimated subglottal pressure (Espinoza et al., 2017) are also higher for those with vocal nodules compared to controls. People with vocal nodules have thus been reported to have lower laryngeal airflow resistance values (Smitheran & Hixon, 1981).

Limited work has been done to expressly examine the differences in cortisol reactivity between introverts and extraverts (supported by Netter, 2004). Netter (2004) combines evidence

to suggest that extraverts experience the opposite cortisol reaction and patterns from those with neuroticism. Neuroticism has been associated with higher baseline cortisol levels and a decrease in the response of cortisol to a stressor (Netter, 2004). Introverts may have similar reactions to those described for neuroticism (Netter, 2004). This suggests that introverts and those with a strong neurotic trait would be more likely to have no HPA axis response or a lower HPA axis response to a stressor because they begin with higher cortisol levels. However, Kirschbaum, Bartussek, and Strasburger (1992a) and Van Eck et al. (1996) have found personality only contributes to habituation to a stressor and not response to the first experience with the TSST. In addition, there is a body of work (reviewed in Stelmack, 1990) that suggests introverts have larger autonomic activation than extroverts.

It is hypothesized that those who have vocal nodules or muscle tension dysphonia are less likely to have a normal cortisol response because of the neurotic tendencies that may reduce the reaction to stress (Netter, 2004). An altered response of the HPA axis is often related to the development or advancement of disease states (McEwen, 1998). In the present study, those who experienced no HPA axis response due to the TSST did not have any demographic variable collected (e.g., age, phase in menstrual cycle, and waking time) that explained the lack of response and many variables that have been attributed to a lack of HPA axis response were controlled for (e.g., birth control, food intake prior to session, race). This may suggest the no response group was either habituated to the stressor or was a lower HPA axis response group due to a personality trait. Aerodynamically, this group had significantly higher estimates of subglottal pressure (5.59 ±1.57 cm H_2O) than the HPA axis response group (3.94 ±1.07 cm H_2O) and significantly higher laryngeal airflow resistance [4.66 ±1.34 kPa/(L/s) compared to 3.24 ±0.71 kPa/(L/s)]. These findings together may suggest that this group may fall into the neurotic

category, combining neurotic introverts (those with expected higher laryngeal airflow resistances) and neurotic extroverts (those with expected higher subglottal pressures because of a tendency towards loudness; K. Scherer, 1978).

Future research using a similar research protocol to the present study may also provide a measure of personality factors using the Eysenck Personality Questionnaire to categorize participants into the extroverted or introverted and neurotic groups in those with vocal nodules and muscle tension dysphonia. The cortisol measures would help to determine how and if these groups differ based on HPA axis reactivity. All participants should be asked how often they give public talks to determine the roll of habituation.

The missing increase in alpha-amylase. In the present study there was no statistically significant change in salivary alpha-amylase, a protein marker of the sympathetic nervous system, following the TSST protocol. Other studies have found a significant change in salivary alpha-amylase following the TSST protocol (e.g., Maruyama et al., 2012; Rohleder et al., 2004). The salivary alpha-amylase values noted by the previously mentioned studies varied greatly, with Maruyama et al. (2012)[6] presenting post-stress values between 40 and 60 kU/L and Rohleder et al. (2004) presenting post-stress values near 300 U/mL. In the present study, mean salivary alpha-amylase levels were between the values reported by Rohleder et al. (2004) and Maruyama et al. (2012). Taken together, this suggests that there needs to be greater understanding of the expected values of salivary alpha-amylase to better interpret the results of these studies.

[6] Maruyama et al. (2012) and the present study collected data around 3:00 PM, the time when Rohleder et al. (2004) reported the highest values of salivary alpha-amylase when measuring alpha-amylase over the course of a day to find a circadian rhythm for salivary alpha-amylase (between 200 U/mL and 250 U/mL) making the lower values reported by Maruyama et al. (2012) worthy of further investigation

To this author's knowledge, there is no threshold of change or consistently reported amount of change in salivary alpha-amylase that is a criterion to indicate an increase in sympathetic nervous system activity following the TSST. Some studies (e.g., Gordis, Granger, Susman, & Trickett, 2008; Kivlighan, Granger, Blair, & Family Life Project Investigators, 2005) have reported the percentage of participants who had a 10% increase in salivary alpha-amylase from before the stressor to after the stress. Ten percent is used because it is generally larger than the coefficients of variation of the enzymatic analysis, which is the case in the present study (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007). In the present study, 12 of the 19 (63.16%) participants experienced a more than 10% increase in salivary alpha-amylase levels from measurement time point 2 (basal) to measurement time point 4 (post-stress 0). Gordis et al. (2008) also found that 63.1% of their eighty-four 9 to 14 year-olds experienced a 10% increase in salivary alpha-amylase values following a social stress protocol.

The main proposed reason for not seeing a statistically significant increase in salivary alpha-amylase following the TSST is that the saliva samples were taken too long after the stress task so that some participants had already returned to baseline levels of alpha-amylase. The 7 participants who did not experience an increase in salivary alpha-amylase, saliva samples were collected an average of 17 minutes and 38 seconds (SD: 1 minutes and 24 seconds) and the 12 who did experience an increase in salivary alpha-amylase saliva samples were collected at an average of 18 minutes and 23 seconds (SD: 1 minute and 3 seconds) after the stressor began.

The decision was made to collect saliva samples after the speech tasks were completed to ensure that the maximum effect of stress was captured in the speech measures and to prevent the stimulated saliva from plugging the oral pressure tube. As such, saliva samples were collected at least 8 minutes after the participant returned from the TSST room in the present study. Salivary

alpha-amylase returns to baseline between 10 to 15 minutes after the stressor (Granger et al., 2007; Rohleder et al., 2004). Therefore, it is likely that in the present study salivary alpha-amylase was back near baseline for many of the participants when it was collected following the stressor.

Research question 3: Individual changes in voice parameters from before the stressor to after the stressor

To determine if an individual experienced a change in any of the voice parameters from before the stressor to after the stressor, the coefficient of variation was calculated for each participant for each variable for each measurement time point. The participant's maximum coefficient of variation was compared to the percent change experienced from before the stressor (measurement time point 2) to after the stressor (measurement time point 4) for each measure. If the percent change in the measure from before stress to after stress was greater than the maximum coefficient of variation, the person was considered to have a change in the measure due to stress.

Individual changes in average airflow. Four participants (F14, F4, F9, and F19) had changes in airflow from measurement time point 2 to measurement time point 4 that were greater than their maximum intra-individual variation. However, these changes were on average a decrease of 25.32 cm^3/s. Other work has indicated that airflow differences of 20 cm^3/s in females represent normal measurement variations (Higgins, Netsell, & Schulte, 1994). Therefore, the changes in airflow in the present study from before the stressor to after the stressor should not be interpreted as meaningful.

Individual changes in estimated subglottal pressure. Unlike the changes in average airflow, several studies suggest that the changes in estimated subglottal pressure likely represent real and meaningful changes. Seven participants had a change in subglottal pressure from before the stress to after the stress that was greater than their intra-subject variation. The absolute average change in subglottal pressure in the present study was .86 cm H_2O with absolute changes between 0.5 and 1.1 cm H_2O. Higgins et al. (1994) suggest that participants who have a subglottal pressure change of more than 15% may "lack respiratory control" (p. 42). Five participants in the present study had more than a 15% change in estimated subglottal pressure from measurement time point 2 to measurement time point 4 (F7, -16%; F11, +16%; F14, -30%; F17, +22%; F18, +23%). All except one (F7) of the participants who had more than a 15% change in estimated subglottal pressure also had HPA axis activation. F7 did not experience an increase in salivary cortisol or salivary alpha-amylase. Patients diagnosed with non-phonotraumatic vocal hyperfunction had estimated subglottal pressures that were 0.2 cm H_2O higher than matched controls at a comfortable loudness (2.32%) (Espinoza et al., 2017). When normalizing these values for sound pressure level, the difference was statistically significantly different.

As this is one of the first studies to examine aerodynamic changes related to stress, vocal intensity changes (non-linearly related to subglottal pressure) in other studies will be examined. Dietrich (2008) found an average decrease in intensity in participants with a large overlap between measurement time points. Hecker et al. (1968) found that intensity increased in one participant, decreased in three participants, and showed little or inconsistent changes in the remaining 6 participants. In the only other study to measure subglottal pressure before and after the cold pressor test, Giddens et al. (2010) found that females had a significantly higher

subglottal pressure before the stressor compared to after the stressor and that the difference was approximately 1 cm H_2O. It may be that, similar to other voice production measures, individuals experience idiosyncratic voice changes as a result of stress.

Due to the experimental design, it is possible that the changes in estimated subglottal pressure may be due to vocal warm-up, vocal rest, or vocal loading and not to stress at all. In the experimental procedure for the present study, participants were using their voices for very limited amounts of time in the hour (SD: 4 minutes) from arrival to the TSST. Phonation threshold pressure, the minimal amount of subglottal pressure needed to initiate and sustain phonation (Milbrath & Solomon, 2003) and estimated subglottal pressure (Vilkman et al., 1999) were not found to change following vocal rest of between 30 minutes and 45 minutes. In regards to vocal warm-up, Elliot, Sundberg, and Gramming (1993) found variability in phonation threshold pressure following vocal warm-up, although all participants had an effect of vocal warm-up on phonation threshold pressure. It is also important to note that although phonation threshold pressure has been found to increase following vocal loading tasks, it is unlikely that the short 10 minute duration of the stressor using modal pitch and normal loudness levels acted as a vocal loading task (Fujiki & Sivasankar, 2017). Future work is needed to differentiate if the effects are due to warm-up or stress.

Individual changes in laryngeal airflow resistance. Three participants (F4, F6, and F9) had a change in laryngeal airflow resistance that was greater than their coefficient of variation. All three of these participants had no HPA axis response and an SNS response (an increase in alpha-amylase activity levels) following the TSST. This is 50% of the participants who had no HPA axis response and an SNS response following the TSST. Unfortunately in the present study, laryngeal airflow resistance could not be calculated for two participants (F3 and F19) in the

aforementioned group. Future work is needed with a larger sample size to determine if there is a trend for those with only a sympathetic nervous system response and no HPA axis response to experience and increase in laryngeal airflow resistance.

There was no pattern of airflow and air pressure change common in these three participants. F4 decreased airflow and had a small increase in pressure. F6 had a small increase in airflow and a small decrease in pressure (neither of which was greater than her intra-subject variations). F9 had a decrease in airflow and a large increase in pressure, resulting in the largest change in laryngeal airflow resistance. It is interesting to note, that F9 had the highest rating of all participants on the Perceived Stress Scale, a measure of stress over the course of the previous month, but had no notable differences in other measures and that F4 is the only participant with a history of a voice disorder.

Helou et al. (2013) found that PCA and TA-LCA muscle activity increased in some participants and decreased in other participants during a non-speech task in all participants when an SNS nervous system stressor occurred. When examining their data further, it appears that TA (either left or right) muscle activity decreased only when there was no significant change in EKG. Cautiously, it can be suggested that the participants in the present study had an increase in TA muscle activity. If the participants also experienced an increase in PCA muscle activity, it is possible that too much antagonistic muscle activity led to a decrease in the efficiency of laryngeal behaviors and an increase in laryngeal airflow resistance.

Individual changes in the percent of syllables produced in vocal fry. In addition, although there was no significant influence of time on the percent of syllables produced in vocal fry, the no HPA axis response group did have a gradual increase in percent of syllables produced in vocal fry from measurement time point 1 (17.67%; SD: 4.34), measurement time point 2

(19.61%; SD: 5.88), measurement time point 3 (23.64%; SD: 9.99), and measurement time point 4 (26.34%; SD: 12.90), with a decrease after the stressor at measurement time point 5 (21.70%; SD: 8.43).

Even though a coefficient of variation of the percent of syllables produced in vocal fry could not be calculated because there was only one paragraph from which the percent of syllable produced in vocal fry was calculated, examination of individual changes in vocal fry from before the stressor (measurement time point 2) to after the stressor (measurement time point 4) was completed. The average difference in percent of syllables produced in vocal fry for all participants was .77% (SD: 8.88). Those with an HPA axis response appear to have a behavioral change that reduces the percent of syllables produced in vocal fry while those with no HPA axis response to stress appear to have a behavioral change that increase the percent of syllables produced in vocal fry from before the stressor to after the stressor.

Five of the nineteen participants had a greater than 10% change in percent of syllables produced in vocal fry from before the stressor to after the stressor. Of these participants, two had a decrease in the percent of syllables produced in vocal fry and three had an increase in the percent of syllable produced in vocal fry. The two participants who had the decrease in percent of syllables produced in vocal fry that was greater than 10% had a sympathetic nervous system response to the stressor and both had a later HPA axis response to the stressor (F2 and F10). They represent two of the three participants who had this response pattern. The other participant who had this response pattern (F8) had a decrease in percent of syllables produced in vocal fry of 7.5%. When examining all of the participants who experienced an HPA axis response (n = 10), all but two (F1 and F11) had a decrease in the percent of syllables produced in vocal fry from

before the stressor to after the stressor (mean: -4.61%; SD: 5.10; median: -4.07%; range: -13.16% to 3.2%).

The three participants (F6, F12, F16) who had an increase in the percent of syllables produced in vocal fry greater than 10% from before the stressor to after the stressor had no HPA axis response. Of the nine participants who experienced no HPA axis response, all but one (F7) had at least a small increase in the percent of syllables produced in vocal fry from before the stressor to after the stressor (mean: 6.74%; SD: 8.49; median: 3.39%; range: -2.21% to 23.3%).

Vocal fry is produced with glottal closed phases that are nearly double that of modal register, resulting in lower open quotients (Childers & Lee, 1991). Those participants who had no HPA axis response had the highest laryngeal airflow resistance across all measurement time points, which suggests greater adduction of the arytenoid cartilages, setting the vocal folds to a posture that would predispose them to the production of vocal fry. Because there were no significant changes in the aerodynamic measures over time, it seems unlikely that there was a behavioral change (e.g., change in tension of the intrinsic or extrinsic laryngeal muscles) that resulted in the increase in percent of syllables produced in vocal fry following the stressor for the no HPA axis response group. As such, the increase following the stressor may be due to a change (or lack of change) in the laryngeal mechanism that should be explored further in future research.

Research question 4: Emotional changes and voice parameters

It was predicted based on work by Hicks (1980) and Hollien (1980) that anxiety, fear, and anger would change more due to the stress response and be more related to voice parameters than the other emotions measured. It was also expected that those with higher baseline levels of cortisol would be less likely to have high ratings of emotions of anger and sadness, as

exogenously supplied cortisol has been found to reduce the emotional response to stress (Reuter, 2002).

In the present study, four participants experienced an increase in ratings of "afraid" from time 2 to time 4, three experienced an increase in ratings of "angry" from time 2 to time 4, and five experienced an increase in ratings of "tense" from time 2 to time 4. None of these increases were related to a change in any voice parameter. From Figure 38 it is evident that in this small samples size, there does not appear to be a relationship with the number of emotions that experienced a 20*T* change in rating from time 2 to time 4 and baseline (measurement time point 1) cortisol levels.

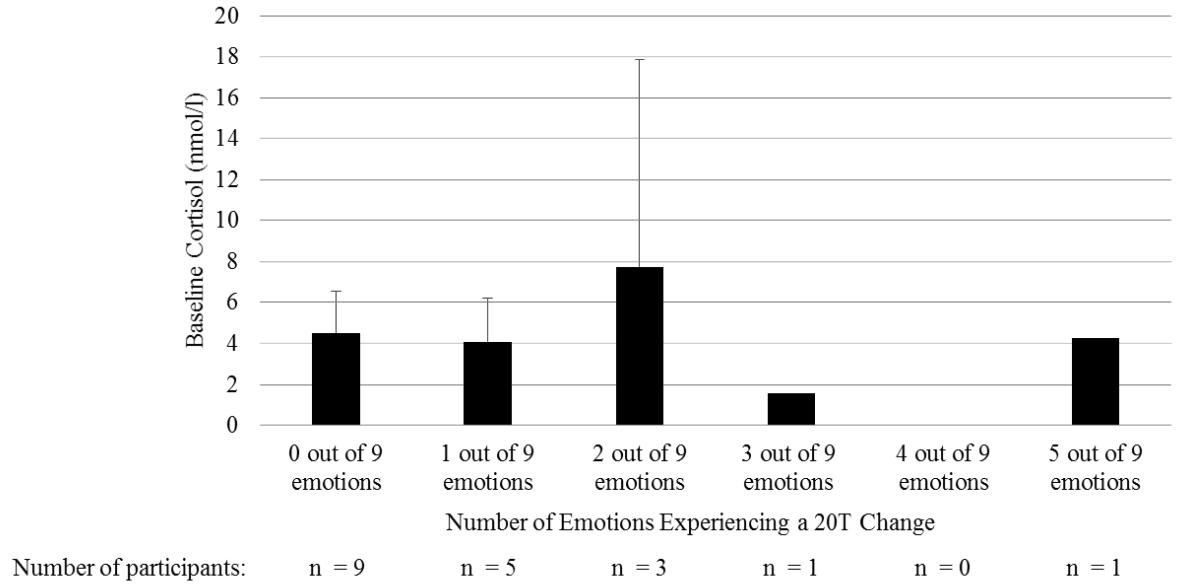

Figure 38. The average baseline cortisol (nmol/l) based on the number of emotions that experienced a 20*T* change in rating from time 2 to time 4. For example, nine participants had 0 out of 9 emotions that had a 20 *T* change in rating and their average baseline cortisol was 4.52 nmol/l.

The only emotion that was found to relate to a voice parameter was sadness. The three participants (F9, F14, and F19) who rated sad 20*T* higher at time 4 compared to time 2 also had a change in airflow that was greater than their intra-subject variability. All of these participants

had a decrease in flow. Although the change in average airflow in the present study is very small and would not result in a change in voice quality, less flow in the flow ranges used in the present study would most likely generally result in a slightly strained voice quality. This is similar to findings by Gobl and Chasaide (2003) who found that although synthesized breathy voice was somewhat related to sadness (as suggested by K. Scherer, 1986), a lax-creaky voice quality was more strongly associated with sadness.

A higher open quotient has also been associated with the emotions of sadness, surprise, and enthusiasm (Laukkanen, Vilkman, Alku, & Oksanen, 1996). F14 in the present study was the only participant to experience an increase in open quotient from the derivative of the EGG signal that was greater than the intra-subject variability. In addition, Laukkanen et al. (1996) found a decrease in estimated subglottal pressure associated with sadness. However, those in the present study who had higher ratings of sadness at time 4 compared to time 2 had either an increase in estimated subglottal pressure (F9) or a decrease in estimated subglottal pressure (F14) (F19 did not have pressure measures at measurement time points 3 through 7).

Many of the studies that have examines emotions and voice have used actors (e.g., K. Scherer, 1986) or synthesized voice (e.g., Gobl & Chasaide, 2003: Laukkanen et al., 1996). Further examination of the data from the present study may lead to a better understanding of voice changes associated with real emotions perceived by the participant.

Importance of measuring biomarkers

In the present study, activity of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system were measured by salivary markers cortisol and alpha-amylase, respectively. In addition, self-rating of stress was measured at each measurement time point. A correlation was

conducted to see if there was an association between self-rating of stress and salivary cortisol and salivary alpha-amylase. As salivary cortisol takes time to peak, the value of salivary cortisol from one time after the self-rating of stress was compared to the self-rating of stress (i.e., salivary cortisol from measurement time point 2 was compared to self-ratings of stress from measurement time point 1). There were no significant correlations between salivary cortisol and self-rating of stress and salivary alpha-amylase and self-rating of stress (Table 18). This suggests the need for biomarkers when measuring the stress reaction.

Table 18. Correlation coefficients for the association between salivary cortisol and self-rating of stress and salivary alpha-amylase and self-rating of stress

	Salivary Cortisol	Salivary Alpha-Amylase
Measurement Time Point 1 (Beginning)	.169	.298
Measurement Time Point 2 (Basal)	.181	.148
Measurement Time Point 3 (Anticipatory)	.312	-.185
Measurement Time Point 4 (Post-Stress 0)	.322	.028
Measurement Time Point 5 (Post-Stress 1)	.171	.096
Measurement Time Point 6 (Post-Stress 2)	.343	.307
Measurement Time Point 7 (Post-Stress 3	-	.327

Note. - correlation could not be completed because there were no salivary cortisol measures after this measurement time point

Limitations and future directions

In the present study, the aerodynamic measures were made during a syllable repetition task and the acoustic measures were made during a speaking task. The syllable repetition task required extensive training on the part of the participant to ensure that the repetitions allowed for accurate estimates of subglottal pressure from the oral pressure. These trained syllable repetition tasks may not represent the average airflows and the subglottal pressures used during speech. Future research could measure aerodynamic and electroglottographic measures during both syllable repetition tasks and speaking tasks to compare the results.

The budget for the present study limited the number of participants from whom saliva samples could be collected. The results of the present study suggest the continued need for research using salivary cortisol measures. Salivary alpha-amylase measures could be limited to before the stressor and only one measure taken immediately after the stressor to save money. Future research with a larger sample size is needed to determine if the results that differentiate the three groups (no HPA axis response, earlier HPA axis response, and later HPA axis response group) on voice measures are generalizable to a larger population.

Intensity (sound pressure level) was not measured in the present study. This added piece of information would have allowed the researcher to normalize the pressure and flow by sound pressure level. These measures normalized by sound pressure level take out the influence of loudness differences.

CHAPTER VI: CONCLUSIONS

In the present study, the influence of stress on the voice was examined by measuring average airflow, estimated subglottal pressure, the derived measure of laryngeal airflow resistance, electroglottographic open quotient, speaking fundamental frequency, and percent of syllables produced in vocal fry before and after a psychosocial stressor. Salivary cortisol and salivary alpha-amylase levels were measured as markers of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system, respectively. Because salivary alpha-amylase was measured too long after the end of the stressor, it did not show the expected increase in the majority of participants. Salivary cortisol was used to divide the participants into two groups. Participants who had a 2.5 nmol/l increase in salivary cortisol after the stressor (at measurement time points 4 through 7) were considered to have an HPA axis response (n = 10) and participants who did not have this increase were considered to have no HPA axis response (n = 9). Participants who had an HPA axis response were further divided into two groups: earlier HPA axis response group (at least a 2.5 nmol/l increase in salivary cortisol at measurement time point 4) (n = 6) and later HPA axis response group (at least a 2.5 nmol/l increase in salivary cortisol at measurement time point 5, 6, or 7 but not time point 4) (n = 4).

Several studies have examined the influence of sympathetic nervous system activation on voice parameters (e.g., Alvear et al., 2013; Giddens et al., 2010; Helou et al., 2013) and several studies have used HPA axis stressors to determine changes in vocal acoustic and intrinsic laryngeal muscle activity following stress (e.g., Dietrich, 2008; Hecker et al., 1968; Mendoza & Carballo, 1998; Tse et al., 2014; Van Lierde et al., 2009). In each of these studies, the changes in voice production variables, mainly fundamental frequency, were often inconsistent and small. In the only study to this author's knowledge that measured cortisol and a voice parameter

(fundamental frequency), Pisanski et al. (2016) found that cortisol levels explained approximately 20% of the variation in fundamental frequency when people where measured during the stressful situation.

The present study seems to further support the idea that people experiencing stress may only experience changes in some voice parameters (Hecker et al., 1968) and that the changes may be based on the individual. The only voice production variable to change significantly from before the stressor to after the stressor was speaking fundamental frequency. Speaking fundamental frequency increased by an average of 0.3 semitones for the whole participant pool (n = 19) from before the stressor to after the stressor, as was expected from the literature. No other voice production variable measured in the present study changed significantly from before the stressor to after the stressor.

Several individuals had changes in voice measures from before the stressor to after the stressor that were greater than their intra-subject variability. Estimated subglottal pressure changed in seven participants. The change (increase or decrease) in estimated subglottal pressure does not seem to be related to HPA axis response as some of the participants had an HPA axis response and some did not, but it is possible that the changes were related to the stressor. Three participants, all of whom had an increase in alpha-amylase only (no cortisol increase), had a change in laryngeal airflow resistance greater than their intra-subject variability from before the stressor to after the stressor. This suggests that the sympathetic nervous system may be more involved in changes in voice production following stress than the hypothalamic-pituitary adrenal axis or that the lack of HPA axis activation may serve to explain the differences.

There were significant differences between those who experienced an HPA axis response and those who did not on voice production variables. Those who experienced no HPA axis

response and those who did, had significantly different estimates of subglottal pressure, with the largest subglottal pressure attributed to the no HPA axis response group. Those who had no HPA axis response also had significantly higher laryngeal airflow resistance than the other group. In addition, the percent of syllables produced in vocal fry was highest for the later HPA axis response group, in the middle for the no HPA axis response group, and the lowest for the earlier HPA axis response group, although these changes were not significant.

Taken together these findings suggest that there is an underlying difference in voice production parameters overall, not just in response to the stressor, that is related to stress system activation. Translational research is warranted to determine if these differences (due to either physical adaptation or behavioral adaptation) put people who do not experience HPA axis response to stress at risk for voice problems. These findings also further support the need to attend to stress reactivity differences in the voice clinic.

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APPENDIX A: IRB APPROVAL LETTER

BGSU
BOWLING GREEN STATE UNIVERSITY
Office of Research Compliance

DATE:	March 22, 2017
TO:	Brittany Perrine
FROM:	Bowling Green State University Institutional Review Board
PROJECT TITLE:	[1016709-3] The Influence of Stress on the Voice
SUBMISSION TYPE:	Revision
ACTION:	APPROVED
APPROVAL DATE:	March 21, 2017
EXPIRATION DATE:	February 6, 2018
REVIEW TYPE:	Expedited Review
REVIEW CATEGORY:	Expedited review categories # 3 & 7

Thank you for your submission of Revision materials for this project. The Bowling Green State University Institutional Review Board has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a project design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

The final approved version of the consent document(s) is available as a published Board Document in the Review Details page. You must use the approved version of the consent document when obtaining consent from participants. Informed consent must continue throughout the project via a dialogue between the researcher and research participant. Federal regulations require that each participant receives a copy of the consent document.

Please note that you are responsible to conduct the study as approved by the IRB. If you seek to make any changes in your project activities or procedures, those modifications must be approved by this committee prior to initiation. Please use the modification request form for this procedure.

All UNANTICIPATED PROBLEMS involving risks to subjects or others and SERIOUS and UNEXPECTED adverse events must be reported promptly to this office. All NON-COMPLIANCE issues or COMPLAINTS regarding this project must also be reported promptly to this office.

This approval expires on February 6, 2018. You will receive a continuing review notice before your project expires. If you wish to continue your work after the expiration date, your documentation for continuing review must be received with sufficient time for review and continued approval before the expiration date.

Good luck with your work. If you have any questions, please contact the Office of Research Compliance at 419-372-7716 or orc@bgsu.edu. Please include your project title and reference number in all correspondence regarding this project.

This letter has been electronically signed in accordance with all applicable regulations, and a copy is retained within Bowling Green State University Institutional Review Board's records.

BGSU.
BOWLING GREEN STATE UNIVERSITY
Office of Research Compliance

DATE: June 1, 2017

TO: Brittany Perrine
FROM: Bowling Green State University Institutional Review Board

PROJECT TITLE: [1016709-4] The Influence of Stress on the Voice
SUBMISSION TYPE: Amendment/Modification

ACTION: APPROVED
APPROVAL DATE: June 1, 2017
EXPIRATION DATE: February 6, 2018
REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category # 7

Thank you for your submission of Amendment/Modification materials for this project. The Bowling Green State University Institutional Review Board has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a project design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

Modifications Approved:

CHANGE 1:

The approved 8.5" x 11" flyers will be posted in approved locations at businesses and churches in the Perrysburg and Bowling Green area. Permission will be obtained from the appropriate person at each business and church (via email or in person) prior to hanging the flyer.

CHANGE 2:

Past studies have examined the influence of emotions or feelings on voice changes. Specifically, Holmqvist et al. (2013) found that people who experience nervous and tense feelings (emotions) when they speak have higher cortisol levels. It was thus decided, that the addition of a mood rating scale to the present study would be an important addition. Updated consent document.

Participants will complete the Visual Analog Mood Scale at each time when they complete a self-rating of stress in the protocol (steps 2, 6, 10, 13, 15). The Visual Analog Mood Scale asks the participant to rate eight mood states (afraid, confused, sad, angry, energetic, tired, happy, and tense) on a 100 mm vertical line. The protocol is trademarked. Administration of the Visual Analog Mood Scale takes less than 5 minutes.

The final approved version of the consent document(s) is available as a published Board Document in the Review Details page. You must use the approved version of the consent document when obtaining consent from participants. Informed consent must continue throughout the project via a dialogue between the researcher and research participant. Federal regulations require that each participant receives a copy of the consent document.

Please note that you are responsible to conduct the study as approved by the IRB. If you seek to make any changes in your project activities or procedures, those modifications must be approved by this committee prior to initiation. Please use the modification request form for this procedure.

All UNANTICIPATED PROBLEMS involving risks to subjects or others and SERIOUS and UNEXPECTED adverse events must be reported promptly to this office. All NON-COMPLIANCE issues or COMPLAINTS regarding this project must also be reported promptly to this office.

This approval expires on February 6, 2018. You will receive a continuing review notice before your project expires. If you wish to continue your work after the expiration date, your documentation for continuing review must be received with sufficient time for review and continued approval before the expiration date.

Good luck with your work. If you have any questions, please contact the Office of Research Compliance at 419-372-7716 or orc@bgsu.edu. Please include your project title and reference number in all correspondence regarding this project.

This letter has been electronically signed in accordance with all applicable regulations, and a copy is retained within Bowling Green State University Institutional Review Board's records.

APPENDIX B: HEALTH QUESTIONNAIRE

Age: _____

What time did you wake up this morning? _____

Please place an "X" in the appropriate box.	Yes	No
Do you take or use a hormonal birth control product?		
Do you use a non-hormonal birth control method?		
Do you have a regular period?		
Are you currently menstruating?		
Are you expecting your period in the next 3 to 4 days?		
Did you have a period in the last 10 days?		
Are you pregnant?		
Have you been pregnant in the last 12 months?		
Are you currently breastfeeding?		
Do you have an autoimmune disease?		
Do you have diabetes?		
Do you have gingivitis?		
Do you have asthma or any other breathing problems?		
Do you have hypertension?		
Do you take any medications regularly?		
Have you used a hormone product (steroid or otherwise) in the last month?		
Do you smoke?		
Do you use other tobacco products?		
Do you use other nicotine products?		
Do you have an allergy to metals?		
Have you consumed any caffeine in the last 2 hours?		
Have you eaten anything in the last 1 hour?		
Did you wake from sleeping in the last 3 hours?		
Have you ever had professional voice or speech training?		
Are you in generally good health?		

Have you been diagnosed with a voice disorder in the last 12 months?		
What was the diagnosis?		
Did you receive therapy for this problem?		
Have you ever been diagnosed with a voice disorder?		
What was the diagnosis?		
When was the diagnosis?		
Did you receive therapy for this problem?		
Do you think you have had a problem with your voice in the last 12 months?		
Please briefly describe the problem.		
What did you do to alleviate this problem?		
Does your voice feel tired or fatigued during the day?		
Does your voice feel tired or fatigued at the end of the day?		
Does your voice feel tired or fatigued right now?		
Is your voice right now representative of your normal voice?		

APPENDIX C: PERCEIVED STRESS SCALE

Subject ID# _____

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate *how often* you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

	Never	Almost Never	Some-times	Fairly Often	Very Often
1. In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4. In the last month, how often have you dealt successfully with irritating life hassles?	0	1	2	3	4
5. In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?	0	1	2	3	4
6. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
7. In the last month, how often have you felt that things were going your way?	0	1	2	3	4
8. In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
9. In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
10. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
11. In the last month, how often have you been angered because of things that happened that were outside of your control?	0	1	2	3	4
12. In the last month, how often have you found yourself thinking about things that you have to accomplish?	0	1	2	3	4
13. In the last month, how often have you been able to control the way you spend your time?	0	1	2	3	4
14. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

(Cohen, et al., 1983)

APPENDIX D: MODIFIED VOICE HANDICAP INDEX-10

Instructions: These are statements that many people have used to describe their voices and effects of their voices on their lives. Circle the response that indicates how frequently you have the same experience.

0 = never 1 = almost never 2 = sometimes 3 = almost always 4 = always

1.	My voice makes it difficult for people to hear me.	0	1	2	3	4
2.	I run out of air when I talk.	0	1	2	3	4
3.	People have difficulty understanding me in a noisy room.	0	1	2	3	4
4.	The sound of my voice varies throughout the day.	0	1	2	3	4
5.	My family has difficulty hearing me when I call them throughout the house.	0	1	2	3	4
6.	I use the phone less often than I would like to.	0	1	2	3	4
7.	I'm tense when talking to others because of my voice.	0	1	2	3	4
8.	I tend to avoid groups of people because of my voice.	0	1	2	3	4
9.	People seem irritated with my voice.	0	1	2	3	4
10.	People ask, "What's wrong with your voice?"	0	1	2	3	4

APPENDIX E: EXPERIMENTAL PROCEDURES

(1) ~ 20-30 minutes
- Participant completes the following:
  - Informed consent
  - Health questionnaire
  - Cohen Perceived Stress Scale
  - Modified Voice Handicap Index-10
  - Explain and practice the recording procedures
    - Attach the EGG unit to the participant's neck. The EGG electrodes will be worn the length of the session.

Saliva collection, stress and emotion rating, acoustic and aerodynamic recording, voice quality recording (beginning)

(2) 10 minutes
- Rest period (no sleeping, phones, TV, reading, etc.)

Saliva collection, stress and emotion rating, acoustic and aerodynamic recording, voice quality recording (basal)

(3) 2-3 minutes
- Explain the directions for the Trier Social Stress Test following the script

(4) 10 minutes
- Allow the participant to prepare speech with paper and pen.

Saliva collection, stress and emotion rating, acoustic and aerodynamic recording, voice quality recording (anticipatory)

(5) Walk the participant to the room where the committee is seated (down the hall from the research lab)

(6) 5 minutes
- Speaking task

(7) 5 minutes
- Mental arithmetic task

Saliva collection, stress and emotion rating, acoustic and aerodynamic recording, voice quality recording (post-stress 0)

(8) 5 minutes
- Debriefing

(9) 30 minutes
- Recovery period (no sleeping, phones, TV, reading, etc.)
- Recording every 10 minutes

Saliva collection, stress and emotion rating, acoustic and aerodynamic recording, voice quality recording (post-stress 1, post-stress 2, post-stress 3)

Trier Social Stress Test Protocol

- Experimenter to participant: *"Imagine you have applied for a job as a lawyer [or whatever the participant's professional interest might be] and you were invited to present yourself before a committee which will evaluate you on the basis of your personal characteristics. Your task in this experiment is to convince the committee in a free speech that you are the best candidate for the vacant position. Following these instructions, you have about ten minutes to prepare for the speech. You will later step in front of this table, so that your voice can be recorded by a microphone. Please also note that you will be recorded by a video camera as well. We will record your speech for a subsequent voice frequency analysis to reveal any paraverbal signs of stress. The camera recording is used for later behavioral analysis. The members of the committee are trained in behavioral analysis and will take notes during your speech. It is important to make eye contact with the committee members. Following your speech, which is supposed to take five minutes, you will then be given a second task by the committee which will only be explained to you by the committee; that will also take about five minutes. Do you have any questions?"*
- The participant will take notes and prepare for the speech. The participant was given 10 minutes to prepare the speech. The participant was alone in the room where the recordings were taking place
- At the end of the 10 minutes, record the time and make recordings.
- After the recording, the participant was taken to a room where a committee of 3 were seated at the end of a camera. They each have a clipboard with paper, a pen, and a stopwatch. They are serious and do not interact with the participant. If the participant attempts to interact with the committee, the committee should tell the participant to direct all questions and comments to the experimenter. One of the committee members is the committee chair and will speak for the committee. The speaker was always a male in the present experiment.
- The experimenter places a laptop that is recording the participant on the table in front of the participant and leaves the room. There was a window in the door through which the experimenter watched the proceedings.
- Committee chair to participant: *"Please step behind the line, name your S-number and begin your speech."* At the same time, one committee member closes the cassette door on the video recorder.
- The speaker of the committee starts his stop watch when the participants begins speaking.
- All committee members made eye contact with the participant during the speech.
- If the participant stopped talking before the 5 minutes elapsed, the committee chair waited 20 seconds and said, *"You still have time, please continue..."*
- If the participant again stopped talking before the 5 minutes elapsed, the committee chair waited 10 seconds and then begin asking the following questions until 5 minutes elapsed:
    - *Why do you think that you are the best applicant for this position?*
    - *What other experiences have you had in this area?*
    - *What about your studies identifies a special aptitude and motivation for this position?*
    - *Where else do you plan to apply? Why?*
    - *What would you do, if your application here would not succeed?*

- If the participant began to talk about educational experiences in great detail, the committee chair interrupted the participant by saying: *"We believe that you know how to perform, but we would be more interested to find out why you were so involved in or drawn to this area."*
- After 5 minutes, the committee chair said: *"Thank you very much, that should be enough for now. We now want to ask you to work on a second task. This task has nothing to do with the job application. This one is about mental arithmetic. We ask you to count backwards to zero in 13-number steps, starting at 1687, and to do it as fast and correctly as possible. Should you miscalculate, you will be told so and you start again at 1687. Do you have any questions about this? ...Please begin, then."*
    - During this time, the committee members noted errors from an answer sheet. If the participants made an error, the committee chair said to the participants: *"Error. 1687."*
    - After 5 minutes the committee noted what number the subject made it to.
- The experimenter re-enters the room and walked the participant back to the research lab to make a recording. After recordings are made, the participant is debriefed using the following script:

Thank you very much for your participation in our research study. We are interested in if and how psychological stress influences how the voice is produced. You may know that stress has many effects on health and how the body works. There are many studies on this topic. However, there are not many studies of how the voice is produced as a result of stress and how the sound of the voice changes as a result of stress. In this study, the mask you wore allowed us to collect information about the airflow and the pressure you are using to produce your voice. The microphone information will allow us to examine how your voice changes due to the stressful situation. Although this was a study of people with normal voices, this type of study may someday help the field to better understand the relationship between voice disorders and stress.

During the session, we did record audio during your job speech. The audio will only be used by the research team and not the committee members for the purposes of understanding how the voice changes due to stress. The content of what you said will not be evaluated for any purpose. The committee members will not discuss you or the content of your speech in any context. The committee members notes from during your speech will be destroyed.

Again, thank you for your participation. If you have any further questions at this point or in the future, please do not hesitate to contact the Principal Investigator of this study, Brittany Perrine (phone 419-372-5531) or Dr. Ronald Scherer (phone 419-372-7189). You may contact the BGSU Institutional Review Board Administrator, Dr. Hillary Snyder (1-419-372-7716) to discuss problems, concerns, and questions.

If at any time you need high-quality, affordable psychological services, you may contact the BGSU Psychological Services Center. The center is located at 300 Psychology Building and can be reached at 419-372-2540. If you ever experience a psychological crisis, feel suicidal, or feel homicidal, call The Link at 419-352-1545 or 1-800-472-9411.

APPENDIX F: FLOW CALIBRATION

Calibration of the Glottal Enterprises aerodynamic flow mask (MSIF 2 S/N 2049S) was performed using a two-way sweeper that was set to push or pull air through a calibrated pneumotach (Rudolph Pneumotach 37888 Lot #980890) and through the Glottal Enterprises flow mask which was held flush against a mold (Figure A1, used with permission from Frazer, 2014). The voltage was varied in increments of approximately 1 volt with a range of -9 to 9 volts by adjusting the airflow from the sweeper using the line valve and the bleed valve to increase the voltage when pushing air and decrease the voltage when pulling air. The voltage on the pressure transducer (Validyne, MP45-16-871, S/N 119473, settings shown in Table A1) represents the amount of flow through the pneumotach and the voltage on Voltmeter 2 in Figure A1 represents the amount of pressure drop across the mask. The pressure drop across the mask is linearly related to the flow through the mask.

Each time a calibration is performed, a best fit line is used to reveal the relationship between the voltage obtained from the mask system and flow between -4,000 cc/s and +4,000 cc/s. The system was re-calibrated frequently during the course of the present study. For all flow values between approximately -4,000 cc/s and +4,000 cc/s, the conversions of voltage to flow used in the present study are presented in Table A2 and in Figures A2, A3, and A4.

Table A1. Validyne pressure transducer settings.

Parameter	Setting
Sensitivity Gain	15 m V/V
Filter	10 Hz
Suppressed Output	Off

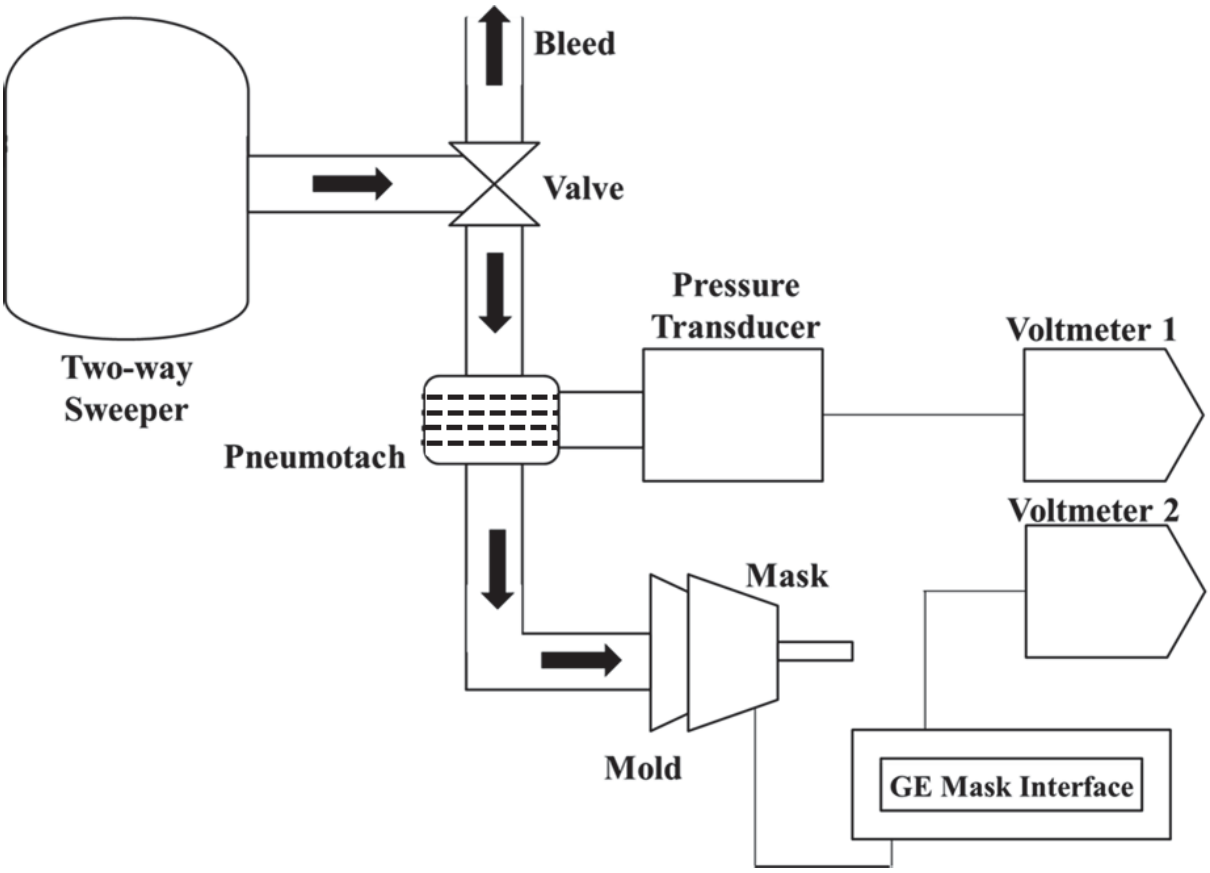

Figure A1. Arrangement of equipment for flow calibration of the Glottal Enterprise aerodynamic mask. The air was being pushed through the mask in this figure. In another calibration setup, the air was pulled through the mask. In that case, the direction of the airflow was opposite.

Table A2. Calibration equations for flow and to whom the equations were applied.

Date of Calibration	Equation	Mask Size	Participant(s)	Figure
8/4/2017	F = 657.2 * V	Large	F1, F3, F4, F5, F6	A2
8/4/2017	F = 838.19 * V	Small	F2, F7, F8, F9, F10, F12, F13, F14, F15, F16, F17, F18, F19	A3
9/29/2017	F = 650.71 * V	Large	F11	A4

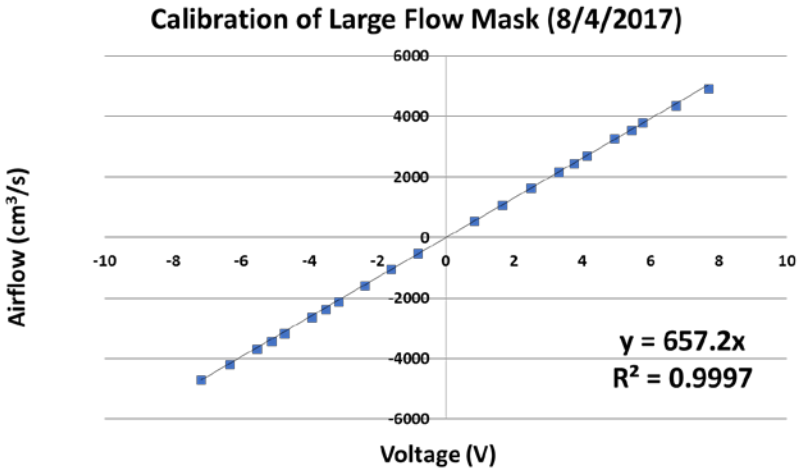

Figure A2. Voltage outputs compared to the flow for the Glottal Enterprise's large flow mask from 8/4/2017.

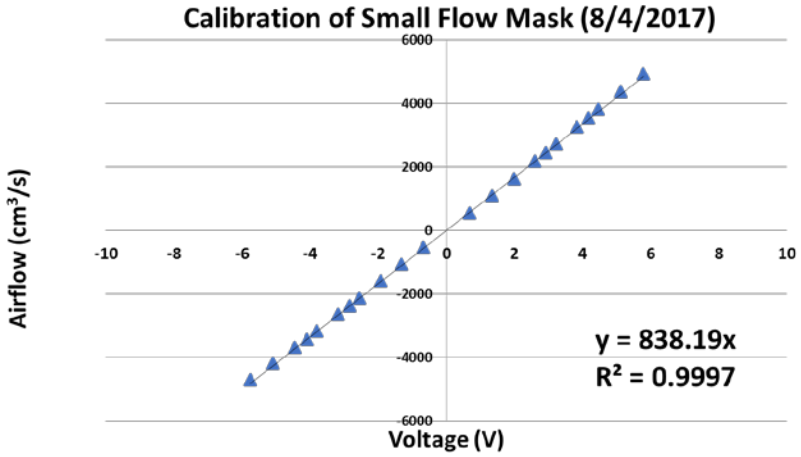

Figure A3. Voltage outputs compared to the flow for the Glottal Enterprise's small flow mask from 8/4/2017.

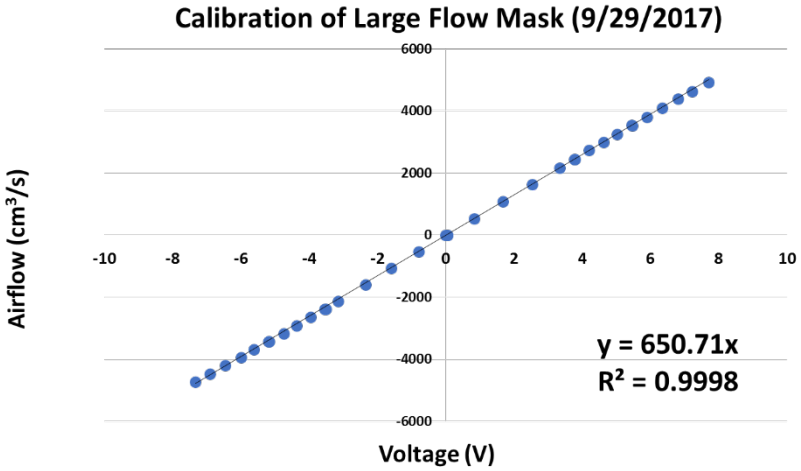

Figure A4. Voltage outputs compared to the flow for the Glottal Enterprise's large flow mask from 9/29/2017.

APPENDIX G: PRESSURE CALIBRATION

Calibration of the oral pressure transducer (PTL 116) was completed using a pressure source that created equal pressure in the transducer and u-tube manometer (Figure A3). An experimenter applied a positive or negative pressure to the pressure transducer and u-tube manometer. The flexible tubing was crimped to hold the pressure constant while measurements were taken. Simultaneous readings of the voltage on the voltmeter and the manometer were taken for each pressure. An equation comparing voltage and oral pressure was determined using a best fit line of the data. The equation used in the present study was created on August 4, 2017 and is *Pressure (cm H_2O) = 4.9345 * V*, where *V* is voltage. The calibration equation is graphed in Figure A4. The equation is valid for any voltage below 9 volts.

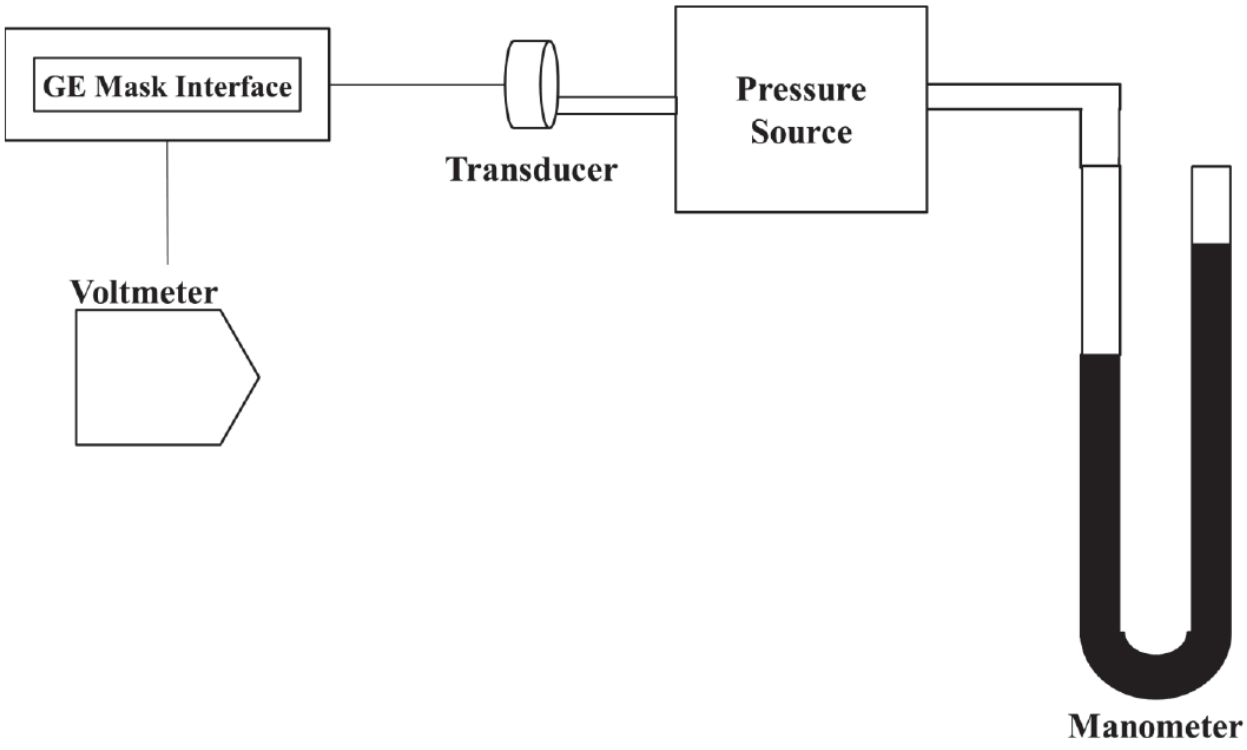

Figure A5. Arrangement of equipment for calibration of the oral pressure transducer of the Glottal Enterprises aerodynamic mask.

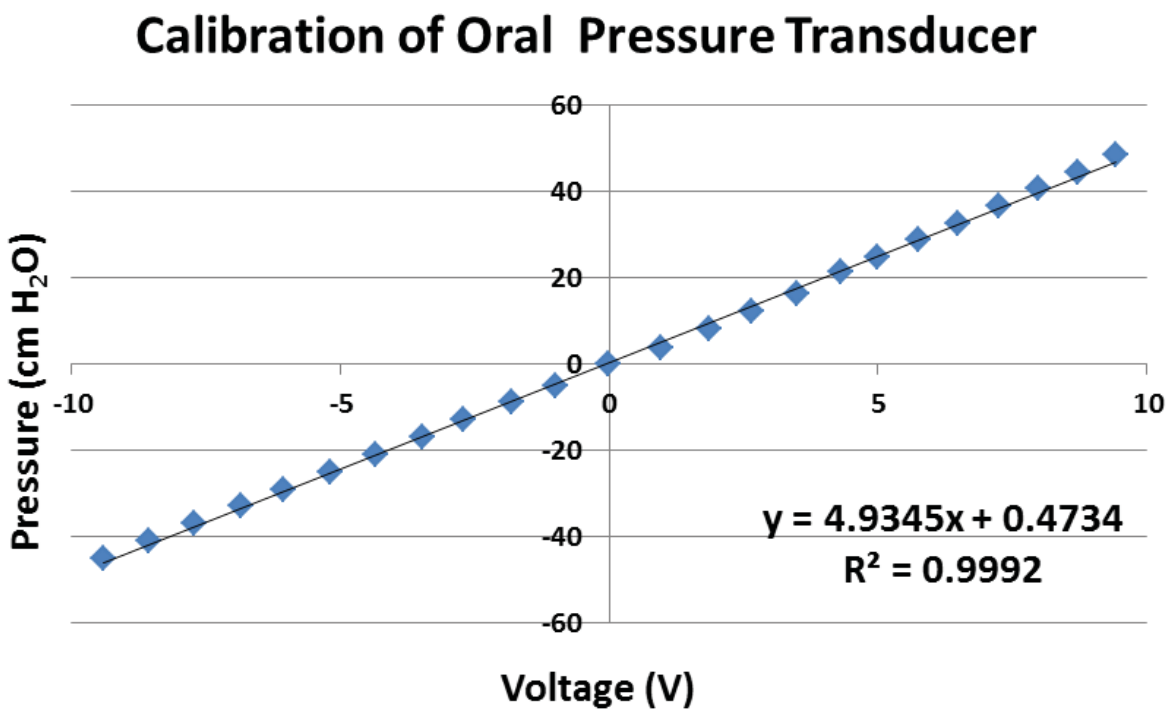

Figure A6. Voltage of pressure transducer compared to the pressure from the oral pressure transducer. The linear relationship is valid between -9 volts and +9 volts.