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Swallow and breathing coordination following suprahyoid muscle injury.

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SWALLOW AND BREATHING COORDINATION FOLLOWING SUPRAHYOID MUSCLE INJURY

By

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B.A.- Bellarmine University, Louisville, KY, May 2008

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A Thesis Approved on

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DEDICATION

To my wife, Kate, for all her support and patience.

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ABSTRACT

SWALLOW AND BREATHING COORDINATION FOLLOWING SUPRAHYOID MUSCLE INJURY

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Swallowing motility disorders (dysphagia) are a major complication following radiation treatment for head and neck cancer, affecting ~50% of those treated. One reason for this is that radiation causes muscle damage, provoking sensorimotor pathologies. Previous work has suggested that injury may cause discoordination between breathing and swallowing behaviors. We sought to determine if muscle injury provokes changes in this behavior. We hypothesized that acute suprahyoid muscle damage would alter cross-behavior excitability, causing destabilization of the respiratory-swallow pattern. Swallowing was evoked in anesthetized spontaneously breathing cats via injection of a 3cc bolus of water into the oropharyngeal cavity. A suprahyoid injury was induced unilaterally by applying a ~2mm cryoprobe to the belly of the mylohyoid muscle. Electromyography (EMG) activity (duration, amplitude) was measured concurrently in thyrohyoid, mylohyoid, thyropharyngeal, and diaphragm muscles. Swallow-breathing coordination (SBC) was measured by determining inspiration/expiration phase during the onset and offset of a swallowing event, based off the mylohyoid initiation and thyropharyngeal termination bursts, respectively. Results showed an injury-related effect in the mylohyoid muscle, as indicated by a significant decrease in the mean amplitude post-injury compared to pre-injury. During swallowing, the expiratory phase was found to predominate the respiratory cycle in both pre- and post-injury. No significant changes to the swallow-breathing coordination were found following injury. Results demonstrate that mylohyoid muscle injury does not appear to interfere with short-term changes in the respiratory-swallow pattern. Although deficits in muscle function were found immediately following injury, an extended time or more severe injury may be needed to adversely inhibit these behaviors. Thus, disrupting the stable coupling between respirations and swallowing, which has been found clinically, may not be apparent immediately after injury and require long-term changes in function.

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INTRODUCTION

Swallowing is a vital, complex, and stereotypic motor act involving multiple independent movements to transport food through the alimentary tract and protect the airway against aspiration (Jean, 1990; Miller, 2008). Swallowing impairments (dysphagia) can affect approximately 50% of patients being treated for head and neck cancer (HNC) (Garcia-Peris et al., 2007). Chemoradiation therapy, a primary treatment option for HNC, can lead to swallowing impairments, such as delayed laryngeal vestibular closure, reduced base of tongue motion, reduced laryngeal elevation, and delayed pharyngeal swallow (Eisbruch et al., 2002; C. L. Lazarus et al., 1996; Logemann et al., 2006). Dysphagia results in a higher likelihood of malnutrition, dehydration, prolonged tube feeding, weight loss, poor oral intake, and increases the risk of aspiration and pneumonia (McQuestion, Fitch, & Howell, 2011; Nguyen et al., 2006; Sura, Madhavan, Carnaby, & Crary, 2012). This can directly affect a patient's quality of life, leading to psychological and social disturbances, such as a patient's perception of self (body image), restructuring of caretaker roles, and the loss of social events associated with eating (McQuestion et al., 2011). Therefore, evidence based therapies are needed to improve dysphagia outcomes in these patients.

Swallowing impairments associated with HNC can be classified as "injury-induced dysphagia" to denote dysphagia resulting from damage to peripheral structures (e.g., mucosa, muscle, nerves) (Roden & Altman, 2013; Wolf, 1990). Patients with injury-induced dysphagia often present with reduced swallowing efficiency due to disturbances in muscle movement (Hutcheson et al., 2012; Silver, Dietrich, & Murphy, 2007; Smith, Kotz, Beitler, & Wadler, 2000). Other clinical examples of injury-induced dysphagia include: chronic obstructive pulmonary disease (COPD) (Kobayashi, Kubo, & Yanai, 2007; Shaker et al., 1992; Terada et al., 2010), gastroesophageal reflux disease (GERD) (Koufman, 1991), smoking (Dua, Bardan, Ren, Sui, & Shaker, 1998, 2002), and intubation injuries (Brodsky et al., 2014; El Solh, Okada, Bhat, & Pietrantoni, 2003; Stauffer, Olson, & Petty, 1981). This contrasts with dysphasia secondary to a neurogenic impairment, as seen in spinal cord injuries or a cerebrovascular accident (CVA) (Buchholz, 1994; Roden & Altman, 2013).

Clinical evidence has demonstrated that peripheral injuries not only directly affect swallowing function, but also motor coordination of other upper airway behaviors, such as respiration/breathing patterns (Brodsky et al., 2010). Most often, research has focused on radiation injuries in the mucosa and the epidermis, which causes pathological disruptions in cell depletion, inflammation, and hypoplasia. (Dorr & Hendry, 2001). Inflammation and injury reduces the pliability of normal tissue, impairing its movement. This can be seen through post-radiation inflammatory responses in the mucosa and connective tissue restraining nearby muscles, thereby reducing their mobility (Eisbruch et al., 2004). Radiation can also damage muscle fibers directly, resulting in muscle weakness and reduced range of motion. The pathological changes within muscles following radiation exposure are poorly understood, but evidence has shown that a single high dose of radiation can lead to permanent muscle contractures (Bergstrom, Blafield, & Salmi, 1962; Bergstrom & Salmi, 1962), microvascular necrosis, and type II myofiber atrophy (Khan, 1974). Muscle fibers are divided classically by three types based on physiological, histochemical, and biomechanical attributes: Type I slow twitch, Type IIa fast twitch, and Type IIb fast twitch (Herbison, Jaweed, & Ditunno, 1982). Type IIb fibers have high glycolytic properties relative to Type I and Type IIa fibers, which put these myofibers at increased risk for metabolic changes and oxidative stress associated with radiation exposure (Anderson & Neufer, 2006). As such, muscle injuries may be responsible for abnormal motility of the swallowing musculature, which has been presented clinically in patients with radiation-induced dysphagia (Smith et al., 2000). Clinically, radiation has been shown to limit range of motion (ROM) and strength based on impairments seen in movement of the base of tongue, pharyngeal constrictors, reduced laryngeal elevation, and reduced cricopharyngeal opening relative to their pre-treatment status (Pauloski, 2008; Pauloski et al., 2006).

Normal Swallowing and Respiratory Patterns

The normal swallowing process involves the oral, pharyngeal, and esophageal phases. In normal adult humans, the transition from the oral phase to the pharyngeal phase is marked by the point when the bolus head passes the ramus of the mandible (Logemann, 1997; Martin-Harris, Brodsky, Michel, Lee, & Walters, 2007; Tracy et al., 1989). Electromyography (EMG) studies have shown that the onset of the pharyngeal swallow is also correlated with increased burst of activity in the mylohyoid muscle. The pharyngeal phase is triggered and shaped by sensory afferents connected to several important swallowing muscles, including the glossopharyngeal nerve (Kitagawa, Shingai, Takahashi, & Yamada, 2002) the superior laryngeal nerve (SLN) of the vagus nerve, the maxillary branch of the trigeminal, and hypoglossal cranial nerves (Miller, 1972).

Triggering of the pharyngeal phase results in posterior movement of the base of tongue, velopharyngeal closure, peristaltic pharyngeal constriction, laryngeal elevation, glottal closure, and relaxation of the upper esophageal sphincter (Ardran & Kemp, 1951, 1956; Bosma, 1957; Doty & Bosma, 1956; Mandelstam & Lieber, 1970; Miller, 1982). Prior to initiation of the pharyngeal swallow, the larynx is elevated and glottis is closed, thus causing apnea. This allows food and liquid to travel safely through the pharynx to the esophagus (Brodsky et al., 2010).

Swallowing and Respiratory Patterns following HNC Treatment

Understanding the coordination of swallow-breathing patterns taking place following injury-induced dysphagia can help us determine the underlying problems associated with injury that leads to impairments in airway protection. Further, investigating these changes will help us to determine the onset and pattern of disease processes (Martin-Harris, 2008). Before and after a swallow, a period of inspiration (breath in) or expiration (breath out) can occur. Therefore, four possible respiratory patterns during swallowing have been documented across normal and dysphagia patients, including: expiration-expiration [E-E], expiration-inspiration [E-I], inspiration-expiration [I-E], and inspiration-inspiration [I-I]. With normal adults, the E-E pattern dominates during a swallow (Martin-Harris et al., 2005; Martin-Harris, Brodsky, Price, Michel, & Walters, 2003), meaning air flows out of the system before and after a swallow. Studies with anaesthetized (Doty & Bosma, 1956) or decerebrated (Dick, Oku, Romaniuk, & Cherniack, 1993) cats have reported that the E-E phase predominates during swallowing. During a swallowing event, the E-E pattern has several physiological advantages. These advantages include the adduction of the vocal folds (VFs) prior to the initiation of the pharyngeal phase of swallow and greater superior-anterior movement of the hyoid, providing increased protection of the airway against penetration and aspiration (Charbonneau, Lund, & McFarland, 2005; Martin-Harris et al., 2005; Martin, Logemann, Shaker, & Dodds, 1994). SBC in patients with dysphagia secondary to a CVA (Leslie, Drinnan, Ford, & Wilson, 2002), COPD (Shaker et al., 1992), and post-radiation treatment for HNC (Brodsky et al., 2010) have been characterized as exhibiting inspiratory dominant patterns ([I-E], [E-I], or [I-I]). These inspiratory patterns may cause food/liquid to be sucked into the airway, increasing the patient's risk for aspiration and penetration.

Muscle injury can lead to interruptions in motor movements required for adequate swallowing and respiratory drive. In one study by Brodsky et al. (2010), both pre-radiation and post-radiation oropharyngeal HNC patients demonstrated an inspiratory phase preference in their SBC relative to the control group. The following modulations in respiratory patterns before and after swallowing post-radiation were found (Control, Post-Radiation): E-E (72.5%, 36.6%), E-I (5%, 13.6%), I-E (20%, 50%), I-I (2.5%, 0%). The authors hypothesized that inspiratory patterns prior to or immediately after swallows lead to an increased risk for penetration and aspiration, as evidenced by higher Penetration-Aspiration Scale (PAS) scores in patients demonstrating such a pattern. Martin-Harris and colleagues (2005) hypothesized that the E-E pattern provides greater airway protection because expiration aids in expelling misdirected food and the VFs are in the paramedian position during expiration preventing entry into the lungs. This biomechanical advantage of E-E patterns may help explain the increased risk for aspiration following shifts in swallow-breathing coordination.

Mylohyoid

Pharyngeal swallowing is a complex process that involves the coordinated activation and inhibition of muscles that compose laryngeal and pharyngeal structures, while also involving the tongue and suprahyoid muscles. The mylohyoid muscle (MyHy) is a suprahyoid muscle activated during the pharyngeal phase of a swallow, and is part of the leading complex of sequential swallows. It is involved in a number of upper airway functions, including chewing (Moller, 1966), swallowing (Doty & Bosma, 1956; Spiro, Rendell, & Gay, 1994), respiration (Van de Graaff et al., 1984), and phonation (Faaborg-Andersen & Sonninen, 1960). The MyHy is located in the floor of the oral cavity between the geniohyoid dorsally and the anterior belly of the digastric ventrally. The MyHy is a thin, suprahyoid muscle that originates along the mylohyoid line of the mandible and inserts into the body of the hyoid bone. The mylohyoid nerve, drawn from the mandibular division of the trigeminal nerve (CN V), provides innervation for the MyHy. During pharyngeal swallowing, the MyHy elevates the hyolaryngeal complex. It has the greatest potential for superior hyoid displacement, which is necessary for adequate glottal closure and aids in distension of the upper esophageal sphincter (Pearson, Langmore, & Zumwalt, 2011). Airway closure prevents aspiration and penetration of food into the trachea during the pharyngeal phase of swallow, which can lead to airway inflammation and possible pneumonia.

MyHy activity serves as a reliable indicator for the triggering of reflexive swallows. Doty and Bosma (1956) studied swallowing motor patterns during feeding using EMG in intact or decerebrate animal models. Results demonstrated that contraction of the suprahyoid complex (i.e., MyHy and geniohyoid), which moves the hyoid anteriorly and superiorly, is a leading motor event during the pharyngeal swallowing phase. Later work by German et al (2009) confirmed these findings and further defined muscle activity involved by analyzing EMG and videofluoroscopy in intact or decerebrate pig models. Results demonstrated that suprahyoid activity occurred prior to stylohyoid and hyoglossus activity, which are involved in pulling the hyoid posteriorly and superiorly during sequential swallows. Other suprahyoid muscles, such as the anterior digastric, demonstrated significant lag relative to the MyHy, indicating the MyHy is a strong candidate for determining the initiation of reflexive swallows (Thexton, Crompton, & German, 2007). Furthermore, the MyHy's motor pattern has been shown to be tightly regulated with burst patterns during swallowing relative to other motor acts that recruit the MyHy (e.g., sucking, licking, and lapping). Analyzing fluctuating burst onsets of the MyHy can help distinguish pharyngeal swallows from sucking, licking, and lapping (Kaplan & Grill, 1989; Naganuma, Inoue, Yamamura, Hanada, & Yamada, 2001; van Eijden & Koolstra, 1998), as MyHy EMG activity shows bursts during these actions while the hyoglossus, stylohyoid, or thyrohyoid (ThHy) EMGs readings do not (German, Crompton, & Thexton, 2009). As such, numerous studies have used the onset of MyHy activity to denote the initiation of pharyngeal swallowing behavior (Holman et al., 2014; Holman et al., 2013; Kajii et al., 2002; Pitts et al., 2015; Thexton, Crompton, & German, 2012).

Thoracic Diaphragm

The diaphragm muscle is critical to upper airway function, and is actively involved in both swallowing and respiratory behaviors. The diaphragm is a thin unpaired muscle that separates the abdomen and thoracic cavities. During contraction, the diaphragm moves inferiorly and flattens out as the rib cage expands, bringing air into the lungs (inspiration) (Seikel, Drumright, & King, 2014). Activity of the diaphragm aids in inspiration by creating negative alveolar pressure relative to atmospheric pressure. Upon exhalation, the elastic properties of the lungs and cartilage recoil and allow the diaphragm to return to its more superior, relaxed position. Motor innervation to the diaphragm is primarily provided by the phrenic nerve, originating from C3-5 of the cervical spinal cord (Seikel et al., 2014). The diaphragm is active during inspiration and inhibited during expiration. Therefore, motor patterns of breathing can be determined by analyzing composition of EMG activities in the diaphragm during swallowing.

EMG signals from both the MyHy and diaphragm were used to determine which respiratory phase predominates after injury (Bonis et al., 2011; Doty & Bosma, 1956; German et al., 2009; Thexton et al., 2007). Bursts in the MyHy EMG signal denoted the beginning of the pharyngeal swallowing phase (Doty & Bosma, 1956; Thexton et al., 2007), which can be compared to phasic diaphragmatic activity, revealing information on the expiration (minimal to zero diaphragmatic signal) or inspiration (diaphragmatic signal spike) pattern during swallowing (Bonis et al., 2011).

When swallowing takes place, inspiratory muscles (e.g., diaphragm, parasternal muscles, and external intercostal) can induce a sudden, brief burst in activity known as a schluckatmung (German for "swallow-breath") (Ingelfinger, 1958). This reflexively induced inspiratory activity has been observed in felines (Gestreau, Milano, Bianchi, & Grelot, 1996; Hukuhara & Okada, 1956) and humans (Hardemark Cedborg et al., 2009; Wilson, Thach, Brouillette, & Abu-Osba, 1981). Schluckatmung activity can be distinguished from gradual onset and offset of the eupneic rhythmic breathing, which is normal breathing pattern. The schluckatmung is believed to aid in deglutition by producing increasing inspiratory drive, resulting in negative transdiaphragmatic pressure as determined by multiple studies based on manometric pressure patterns and videofluoroscopy data (Cerenko, McConnel, & Jackson, 1989; McConnel, 1988; McConnel, Cerenko, & Mendelsohn, 1988; McConnel, Guffin, & Cerenko, 1991; McConnel, Guffin, Cerenko, & Ko, 1992). This negative pressure likely helps propel the bolus through the alimentary tract during the transition from pharyngeal and esophageal phases of swallowing (McConnel et al., 1991).

Central Pattern Generators

The oral cavity, pharynx, and other anatomical structures involved in swallowing are shared by other bodily functions, such as breathing and coughing, and therefore require constant coordination. This coordination of stereotypic movements associated with breathing and swallowing are generated by central pattern generators (CPGs) located within the brainstem of the central nervous system (CNS) (Alheid & McCrimmon, 2008; Grillner, 2006; Jean, 1990). It is hypothesized that a common pool of interneurons may exists between the respiratory CPGs, located within the pons and medulla, and the swallowing CPGs, located within the dorsal and ventrolateral medulla, due to their relative proximity within the brainstem and similar musculature they control (Chiao, Larson, Yajima, Ko, & Kahrilas, 1994; Jean, 2001; Kessler, 1993; Oku, Tanaka, & Ezure, 1994). In essence, CPGs are composed of three systems: central and peripheral afferent inputs, efferent motor outputs, and a central system that programs motor patterns (Jean, 1990). The central system must remain highly flexible and plastic to adapt to feedback provided by the afferent system. For example, nociceptive sensory information obtained from mechanoreceptors, thermoreceptors, and chemoreceptors within the oral cavity and pharynx travels to the brainstem through sensory channels of the trigeminal, glossopharyngeal, and vagal cranial nerves (Doty, 1951). This afferent information helps modify swallowing and respiratory patterns at the central level, which further modulates motor output (Jean, 1990; Rubin, Shevtsova, Ermentrout, Smith, & Rybak, 2009; Sumi, 1964). These modifications have been demonstrated through studies into varying bolus consistencies (Hrycyshyn & Basmajian, 1972), swallows with water versus without water boluses (Dodds, Hogan, Reid, Stewart, & Arndorfer, 1973; Hollis & Castell, 1975), and direct evidence through microelectrode recordings of vagal sensory fibers (Andrew, 1956; Clerc & Mei, 1983a, 1983b; Falempin, Mei, & Rousseau, 1978; Falempin & Rousseau, 1983).

Evidence exists for the impact of peripheral afferents on central processes, such as through the activation of pain receptors known as nociceptors. Nociceptors can be activated at the periphery by noxious mechanical, thermal, or chemical stimuli in the environment (Bessou & Perl, 1969). Polymodal nociceptor neurons are present within the trigeminal sensory system, and within afferents pathways of the glossopharyngeal, and vagal cranial nerves (Nomura & Mizuno, 1982, 1983; Shigenaga et al., 1986; Sweazey & Bradley, 1986, 1989). These pathways transmit sensory information to the nucleus tractus solitarius in the brainstem (Nomura & Mizuno, 1982, 1983; Sweazey & Bradley, 1986), which can lead to modifications in motor output during swallows. The influence of nociceptors on swallowing central plasticity has been demonstrated through capsaicin studies, where activation of transient receptor potential cation channel subfamily V member 1 (TRPV1) has resulted in reduced swallow latency (Ebihara et al., 2005; Shin, Shutoh, Tonai, & Ogata, 2016), enhanced hyoid movement, and shortened laryngeal vestibule times (Rofes, Arreola, Martin, & Clave, 2013). Previous research also suggests that nociceptive input from musculoskeletal structures may impact the autonomic system (Lovick, 1993), evidenced by cardiovascular changes following acute muscular injuries (Grimm, Cunningham, & Burke, 2005). Prolonged nociceptor activation of the cranial nerve afferents in chronic injuries has also shown to influence the autonomic system. Research indicates that sustained nociceptive stimulation of myofascial trigger points has a centralizing effect by evidence of long lasting changes in prolonged pain sensitivity in rats (Woolf & Wall, 1986). A study by Dessem and Lovering suggests that enhanced central processing is maintained through nociceptor stimuli to the masseter (2011). Orofacial nociception via the trigeminal nerve, as through the masseter, has been shown to be conveyed through the rostral and caudal brain stem (Dessem, Moritani, & Ambalavanar, 2007). Further evidence of central nociceptive processes includes long term changes in nociceptive withdrawal reflexes (Biurrun Manresa, Morch, & Andersen, 2010), and evidence of cutaneous capsaicin increasing myofascial trigger point pressure sensitivity in the infraspinatus and gluteus medius muscles (Srbely, Dickey, Bent, Lee, & Lowerison, 2010). This evidence points to central processes being altered due to peripheral nociceptive stimuli.

Current Study

While the mechanical importance of the MyHy is well understood (van Eijden & Koolstra, 1998), our knowledge of its role in the swallow and respiratory pattern is less established. Little in-depth research has been conducted into how MyHy injuries may influence motor function in the upper airway. The medical relevance of a MyHy injury is of particular importance when looking at HNC, where resection of the floor of the mouth may extend to the MyHy (Hirano et al., 1992) or radiation treatments may cause inflammation and injury to this area (Kumar et al., 2014). A previous study by Pauloski and colleagues (1993) concluded that the mobility of structures needed for airway protection was impaired following floor of mouth resections, while Hirano and colleagues (1992) determined that extensive removal of the MyHy is associated with increased risk for aspiration. Further, radiation injuries to the suprahyoid complex are associated with poorer swallowing outcomes (Eisbruch et al., 2004; Kumar et al., 2014; Starmer et al., 2015). In theory, an insult to the MyHy's movement may result in changes to motor function and affect SBC. This paper explores what swallow and respiratory changes occur following thermal injuries to the MyHy in an anesthetized cat model.

Hypotheses

We develop five (5) hypotheses for this study. Our first hypothesis is a significant shift in respiratory phase preference during onset of swallowing will occur following acute injury to the mylohyoid from the expiratory phase to the inspiratory phase or expiratory-inspiratory transition. Our second hypothesis is a significant shift in respiratory phase preference during offset of swallowing will occur following acute injury to the mylohyoid from the expiratory phase to the inspiratory phase or expiratory-inspiratory transition. Third, significant changes in muscle activity amplitude will occur in the swallowing muscles following injury to the mylohyoid. Fourth, significant changes in muscle activity duration will occur in the swallowing muscles following injury to the mylohyoid. Lastly, a significant change will be seen in respiratory phase preference during swallowing onset between pre-injury and one to five minutes (1-5 min.) post-injury, and pre-injury to six to ten minutes (6-10 min.) post-injury.

CHAPTER 2

METHODS

Experimental design, protocols, and surgical procedures

Experiments were performed on six spontaneously breathing adult cats–one male and five female—with weights ranging from 2.66 to 4.62kg. Protocols were approved by the University of Louisville Institutional Animal Care and Use Committee (IACUC). Animals were initially anesthetized with inhalation of 4-5% sevofluane (U.S. Pharmacopeia, Rockville, MD) in an enclosed chamber and then weaned onto sodium pentobarbital (35 mg/Kg IV) via radial vein intravenous injection. Supplementary doses were given at 3 to 5 mg/Kg IV as needed by means of the femoral IV catheter. A cannula was placed in the femoral artery and vein to monitor blood pressure and administer IV fluids. A rectal temperature probe controlling an electric heated pad was used to maintain body temperature throughout the study. Arterial blood gas composition was maintained and monitored by drawing 1cc of blood from the femoral artery cannula every 60 to 90 minutes, totaling 8 to 22cc's of blood. Sodium bicarbonate (Sigma-Aldrich, St. Louis, MO) was subsequently administered through the femoral IV line when total blood pressure was found to be below the normal physiologic range. The trachea was cannulated by making a sagittal incision through the anterior portion of the skin, removing sternohyoid muscles, and bluntly dissecting to isolate the trachea.

Electromyography (EMG) data was recorded with bipolar insulated fine wire electrodes. Electrodes were inserted into the following muscles via 26-guage hypodermic needle to evaluate the swallow-breathing phase: MyHy, ThHy, thyropharyngeus (ThPh), and bilateral crural diaphragm (DiaCru). Specifically, the digastric muscle was bluntly dissected from the surface of the MyHy, and electrodes were placed in the left MyHy perpendicular to fiber course. The ThPh muscle was revealed by slightly rotating the larynx and pharynx counterclockwise, exposing the superior laryngeal nerve, which facilitated the lateral placement of the ThPh muscle electrodes into the intermediate belly of the muscle. The ThPh is a fan shaped muscle with the smallest portion attached to the thyroid cartilage. Electrodes were placed in the ventral, caudal portion of the muscle overlaying the thyroid cartilage within 5 mm of the rostral insertion of the muscle. ThHy muscle electrodes were inserted approximately 5 mm rostral to the attachment to the thyroid cartilage. Electrodes were also placed in the costal diaphragm, as well as crural regions of the diaphragm bilaterally. Electrode placement was confirmed via post-mortem visual inspection.

Pharyngeal swallowing was evoked by injecting tap water into the pharynx using a 3cc syringe with a 1-inch piece of PE-90 catheter tubing placed into the oropharynx. The water injections were performed by the same person within each animal and directed at the same anatomical location across trials at the junction of the oral and pharyngeal cavities. A minimum of one-minute was given between each stimulation trial. Three trials were tested for each condition: pre-injury and 1, 5, 10 and 15 minutes post-injury. EMG signals were recorded using a sampling frequency of 10 kHz. Raw signals were amplified using a Grass AC preamplifier (model P511, Warwick, RI) and monitored throughout the study. Spike2, version 8.10a software (Cambridge Electronic Design, UK) was used to verify, record, monitor, and analyze EMG activity. Electrode position was established prior to the start of the experiment by viewing activity patterns during breathing and swallow. Swallows that could not be differentiated from other behaviors (e.g., licking, cough, and aspiration reflex) were excluded from analysis. Coughing was denoted by large inspiratory and expiratory excursions in esophageal pressure (EP). Swallowing was defined as a ballistic-like burst of the ThHy, MyHy, and ThPh activity (German et al., 2009; Thexton et al., 2007; Thexton et al., 2009).

EMG Analyses

To remove the background signal, a Power Spectrum analysis of each channel was conducted to compare the signal background noise to target events prior to analysis. A high pass filter was then applied to the EMG channel based on the results of the Power Spectrum analysis. EMGs were rectified and smoothed with the time constant of 20 ms.

Amplitude and duration of EMG bursts from each muscle were measured from all swallow events. Amplitude values were normalized to maximum intensity for each individual animal, and reported as a percentage of maximum. Amplitudes of each muscle were calculated by averaging the intensity of the rectified EMG signal. Duration was calculated from time stamps obtained automatically by documenting the rising threshold, max value, and falling thresholds settings from each burst that exceeded the threshold marker.

Thermal Injury

All animals underwent unilateral cryoinjury to the right caudal region of the MyHy. A 2mm diameter cryoprobe (Cry-AC-3 B-800, Brymill Cryogenic Systems; UK) filled with liquid nitrogen was applied to MyHy for 40 seconds. The probe was then non-traumatically detached by allowing it to equilibrate to room temperature prior to removal to prevent tearing of the muscle. Location of injury was confirmed post-mortem, with gross tissue changes present in the MyHy.

Prior to our experiments, animals were used in an unrelated study by our group where they underwent a left C2 hemisection injury of the spinal cord. Current study protocol was started approximately 2 hours following the C2 injury. After inserting electrodes, animals were placed in prone position and the head was fixed to a stereotaxic apparatus for stabilization (Sciencelab.com, Inc., Houston, Texas). The C2 hemisection injury was performed by a researcher with over 10 years of surgical experience with the procedure. Extent of the injury was confirmed histologically post-mortem and animals were excluded if boundaries were found to be more extensive.

Swallow-Breathing Phase Determination

An assigned coding system was used for the respiratory phase in which the swallow occurred; inspiratory as “I”; swallows during the inspiratory-expiratory transition as “E1”; and swallows occurring during expiration as “E2”. Further information on phase classification is outlined in Table 1.

The initiation of swallows was determined as the onset of the MyHy burst signal and the termination of swallows was determined as the offset of the ThPh burst signal, and compared to the EMG signal of the crural diaphragm (Table 1, Figure 1). The inspiratory phase was defined as the period between the rising threshold and maximum value of the DiaCru signal, with the inspiratory-expiratory transition as the duration between the maximum value and falling threshold. Expiration was classified as the period between the DiaCru falling threshold and the following rising threshold during eupneic breathing. A protocol was created to analyze swallows that occurred during nonrhythmic, apneic diaphragmatic activity. This protocol was necessary to distinguish between diaphragmatic activity that resulted in eupneic inspirations, and could thus be labeled as an "inspiratory phase" and those where eupneic inspiration was absent. Because the laryngeal vestibule is technically closed when schluckatmung activity occurs during swallowing, any burst of activity resembling the schluckatmung was classified as inspiratory-expiratory transition or expiratory phase in this study. Other activities involving the suprahyoid musculature and diaphragm, such as licking, sucking, and abductor laryngospasm, were excluded from analysis. Normalization of eupneic breathing was established by analyzing diaphragmatic activity most proximal to the swallow (n) against three rhythmic diaphragmatic patterns prior to a swallowing event (n-3, n-2, n-1) and three rhythmic patterns following the swallow (n+1, n+2, n+3). In instances where the difference between swallow onset and DiaCru onset was greater than 1 second, the most proximal DiaCru signal prior to the swallow onset was classified as "n-1" and an "n" was not established, with the swallow onset occurring in E2. Signal data from the MyHy, ThHy, ThPh, and DiaCru was analyzed to determine whether a significant change in amplitude occurred relative to duration in pre- and post-injury conditions. DiaCru signal was taken in the muscle contralateral to C2 hemisection.

Rhythmic diaphragm activity was used to determine the pre-and post-swallow respiratory rate and duty cycle with each swallowing event. Each rhythmic respiratory cycle was defined as the period between the rising threshold in the initial DiaCru signal to the rising threshold in the following DiaCru burst. The respiratory rate (RR) was calculated before the presentation of the water bolus into the pharynx (n-3 and n-2) and after the final swallow following presentation of the bolus (n+2 and n+3). The duration of rhythmic cycles n-3 and n-2 were combined and divided by 120 seconds to determine the pre-swallow RR per minute, and the durations of rhythmic cycles n+2 and n+3 were combined and divided by 120 seconds to determine the post-swallow RR per minute.

Statistical analysis

A mean ± standard deviation was calculated based on all swallows conducted for each condition within each animal. Mean values for each animal were averaged for each condition tested. For statistical analysis, we performed a repeated ANOVA to test main effects of injury on the respiratory function, respiratory-swallow phase, and EMG amplitude and duration measures. A *p*-value of less or equal to 0.05 was considered statistically significant.

Table 1.

Respiratory Phases

Phase Name	Code	Description
Inspiratory	I	Period between rising threshold and maximum value of the diaphragmatic activity; air is drawn into lungs
Inspiratory-Expiratory	E1	Period between maximum value and falling threshold of diaphragmatic activity; air is evenly and gradually released from the lungs through recoil mechanisms
Expiratory	E2	Period between falling threshold and rising threshold of diaphragmatic activity; air actively released from lungs via contraction of abdominal and thoracic muscles

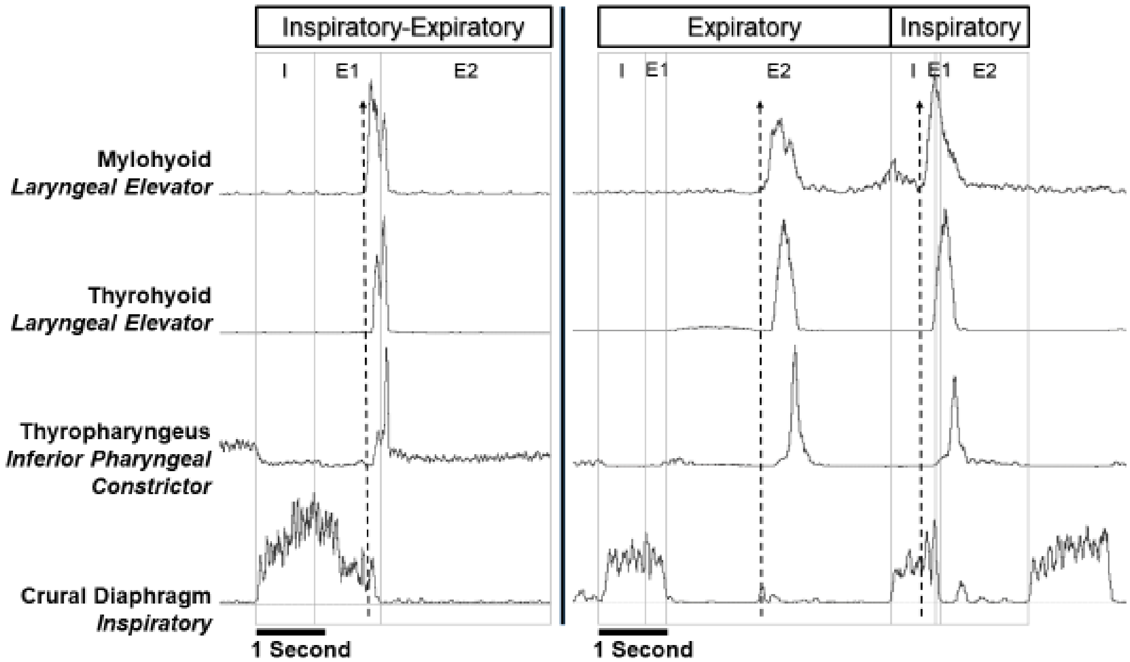


Figure 1.

Example of methods used to distinguish breathing and swallowing phases.

Vertical solid gray lines delineate between the different respiratory phases during swallowing onset: inspiratory phase (I), inspiratory-expiratory transition (E1), and expiratory phase (E2). Vertical dotted arrows delineate when swallowing onset occurs, as indicated by burst activity in the mylohyoid. Headers at the top of the figure indicate in which respiratory phase the swallow onset occurs. The vertical solid black line delineates between two separate events.

CHAPTER 3

RESULTS

Effect of Suprahyoid Injury on Swallow-Breathing Coordination

To determine if a shift in respiratory phase preference during swallowing occurred in response to a suprahyoid injury, we compared diaphragm EMG activity at onset MyHy activity and offset ThPh burst during swallowing. These activities represent the start and end of the pharyngeal swallowing pattern. The ratio of respiratory phases during the onset of swallowing is represented in Figure 2 and Table 2. The offset of swallowing is represented in Figure 3 and Table 3. Differences between the respiratory phases were statistically significant in pre-injury ($p < 0.001$) and post-injury ($p < 0.001$). No statistically significant changes ($p > 0.05$) in respiratory phase preference during the offset or onset of swallowing was observed. Swallowing onset occurring during the expiratory phase was predominated significantly in both pre-injury (67%) and post-injury (71.76%; $p < 0.001$); no statistically significant differences were found in swallowing onset between pre- and post-injury ($p = 0.52$). Following the injury, the ratio of swallow onsets occurring during the inspiratory phase decreased from 26.31% pre-injury to 13.76% post-injury, however no statistical differences were noted ($p = 0.16$). Inspiratory-expiratory transitions increased during the onset of swallowing from 6% pre-injury to 14% post-injury, but no statistically significant differences were found ($p = 0.13$). As for swallowing offset, the expiratory phase predominated throughout with minimal to no significant changes occurring after injury (94% pre-injury and 99% post-injury). Of note, swallow offset took place in the inspiratory phase in only one animal (36.36% I-Phase pre-injury, 4% I-Phase post injury), with the falling threshold of ThPh occurring after the onset of diaphragm in n+1. No significant differences were observed in duty cycle (p =0.47) nor for respiratory rate ($p = 0.67$), as shown in Table 4.

Effect of Suprahyoid Injury on EMG Amplitude and Duration

We determined the effect of injury on swallow-related muscle activity by analyzing the amplitude and duration of the EMG signal during swallowing pre- and post-injury in the following muscles: MyHy, ThHy, ThPh, and DiaCru. Results from EMG amplitude are presented in Figure 4 and Table 5 and duration is shown in Figure 6 and Table 8. No differences were found in the frequency of swallows pre- and post-injury. Following injury, MyHy amplitude decreased significantly during swallowing post-injury compared to swallows pre-injury ($p < 0.02$). No significant changes in ThHy (p=0.62), ThPh (p=0.38), or DiaCru (p=0.984) EMG amplitudes were found after injury compared to pre-injury. MyHy amplitude was significantly decreased during the first 5-minutes post-injury relative to pre-injury (p=0.02), but not in the ThHy (p=0.98), ThPh (p=0.71), or DiaCru (p =0.67), presented in Table 6. Figure 5 provides an example of decreased MyHy amplitude post-injury. During the 6 to 10-minutes post injury, MyHy amplitude was significantly decreased (p=0.01) relative to pre-injury, but the ThHy (p=0.94), ThPh (p =0.76), and DiaCru (p=0.83) were not, as shown in Table 7. No statistical differences were observed in signal duration of the MyHy (p=0.22), ThHy (p=0.28), ThPh (p=0.29), or DiaCru (p=0.92) pre- and post-injury.

Table 2.

Respiratory Phases During Onset of Swallowing

Phase	Pre-Injury	SD	Post-Injury	SD	p-value
Inspiratory	26.31%	±11.27	13.76%	±13.05	0.163
Inspiratory-Expiratory	6.28%	±11.51	14.48%	±5.14	0.125
Expiratory	67.41%	±15.99	71.76%	±13.16	0.517

Note: SD=Standard Deviation of Means between subjects (n=6)

Table 3.

Respiratory Phases During Offset of Swallowing

Phase	Pre-Injury	SD	Post-Injury	SD	p-value
Inspiratory	6.06%	±14.84	0.67%	±1.63	0.363
Inspiratory-Expiratory	0%	±0	0%	±0	0
Expiratory	93.94%	±14.84	99.33%	±1.63	0.363

Note: SD=Standard Deviation of Means between subjects (n=6)

Table 4.

Respiratory Rate and Duty Cycle

	Respiratory Rate	Duty Cycle
Pre-Injury	25.113 b/m	37%
SD	±8.867	±5.97
Post-Injury	24.928 b/m	36.17%
SD	±8.548	±7.83
p-value	0.678	0.474

Note: SD=Standard Deviation of Means between subjects (n=6)

b/m=Breaths per Minute

Table 5.

Muscle Activity Amplitude Pre-Injury and Post-Injury

Muscle	Pre-Injury	SD	Post-Injury	SD	p-p
MyHy	81.26%	±8.49	45.24%	±25.98	0.017
ThHy	72.46%	±11.51	76.50%	±10.13	0.62
ThPh	61.01%	±12.89	64.49%	±11.53	0.379
DiaCru	48.20%	±12.49	48.04%	±6.62	0.984

Note: SD= Standard Deviation of Means between subjects (n=6)
Bold values indicate statistical significance (ANOVA, $p < 0.05$)

Table 6.

Muscle Activity Amplitude Pre-Injury and First 5-Minutes Post-Injury

Muscle	Pre-Injury	SD	Post-Injury	SD	p-value
MyHy	81.09%	±9.48	40.97%	±24.97	0.022
ThHy	70.31%	±11.44	70.70%	±16.48	0.975
ThPh	61.24%	±14.40	63.41%	±17.03	0.379
DiaCru	51.23%	±11.23	45.75%	±16.73	0.674

Note: SD= Standard Deviation of Means between subjects (n=5)*
Bold values indicate statistical significance (ANOVA, $p < 0.05$)
*Data excluded from one subject due to low number of swallows in first 5-minutes

Table 7.

Muscle Activity Amplitude Pre-Injury and 6 to 10-Minutes Post-Injury

Muscle	Pre-Injury	SD	Post-Injury	SD	p-value
MyHy	81.26%	±8.49	42.81%	±24.48	0.011
ThHy	72.46%	±11.51	73.14%	±11.53	0.935
ThPh	61.01%	±12.89	62.38%	±12.73	0.76
DiaCru	48.20%	±12.49	49.76%	±5.02	0.833

Note: SD= Standard Deviation of Means between subjects (n=6)
Bold values indicate statistical significance (ANOVA, $p < 0.05$)

Table 8.

Muscle Activity Duration Pre-Injury and Post-Injury

Muscle	Pre-Injury	SD	Post-Injury	SD	p-value
MyHy	53.33%	±10.01	44.23%	±16.31	0.219
ThHy	32.83%	±6.46	35.17%	±8.13	0.276
ThPh	36.17%	±22.47	38.33%	±23.44	0.290
DiaCru	40.67%	±24.21	41.33%	±16.07	0.924

Note: SD=Standard Deviation

Figure 2. Ratio of respiratory phases during swallowing onset

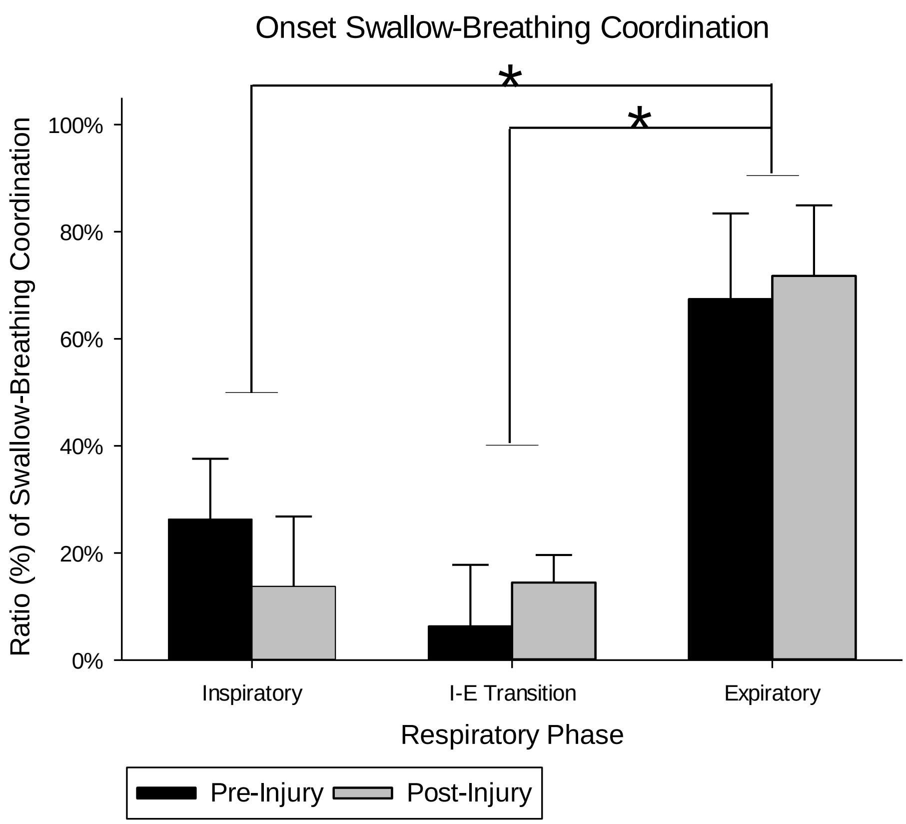


Figure 3. Ratio of respiratory phases during swallowing offset

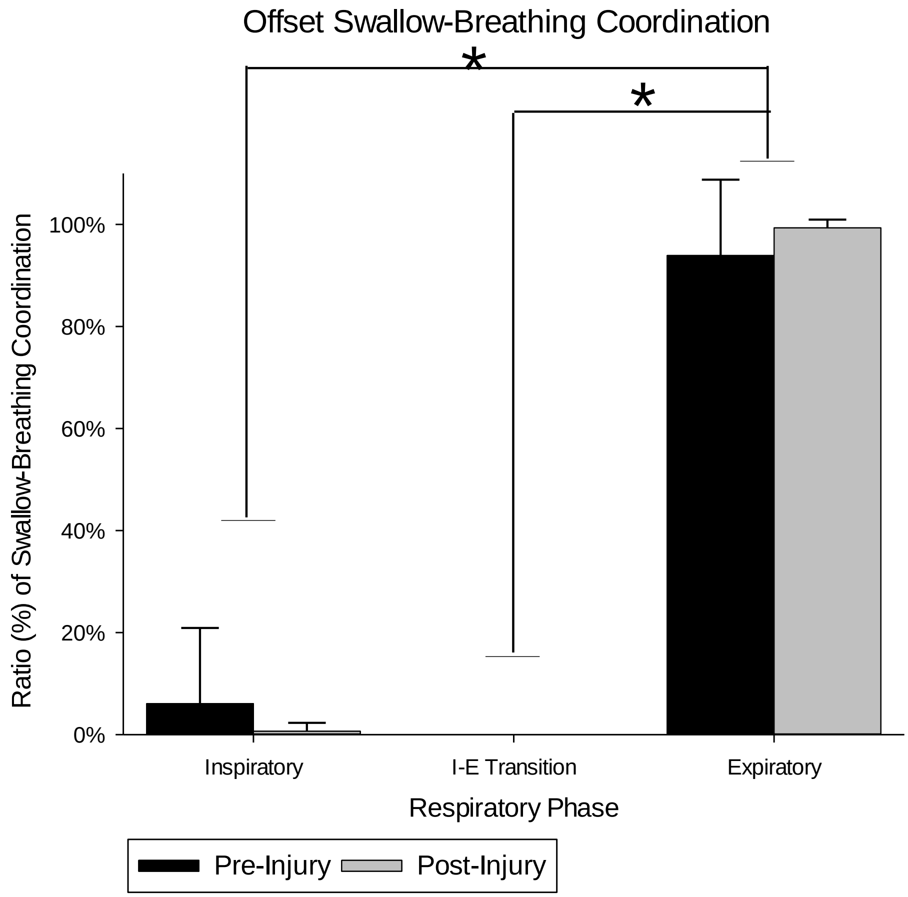


Figure 4. Muscle activity amplitude pre- and post-injury

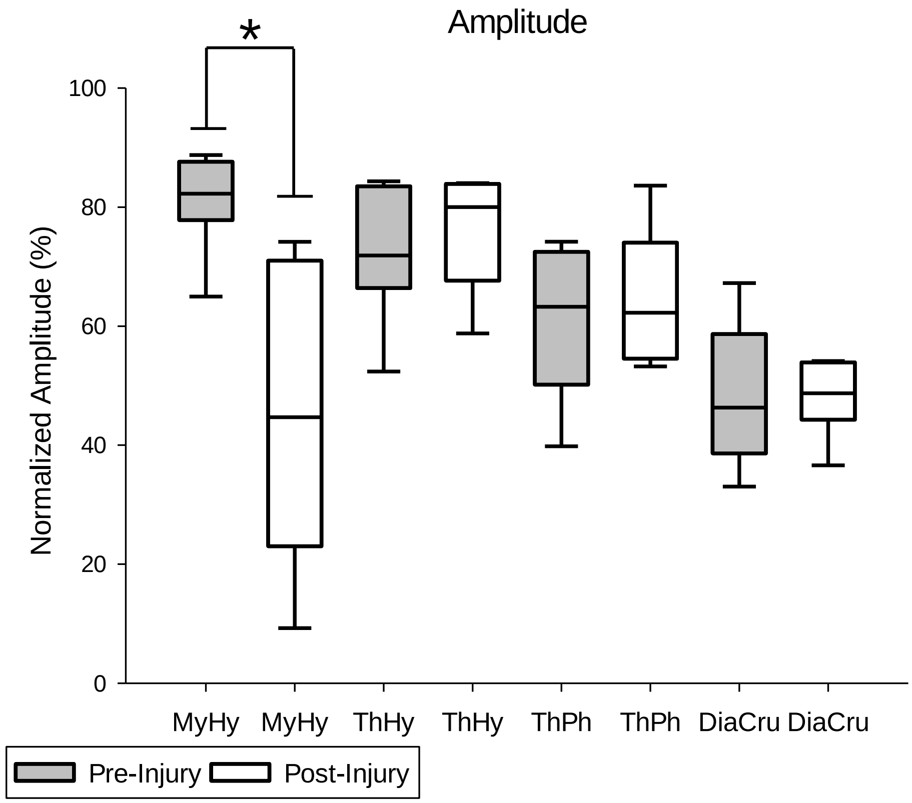


Figure 5. Examples of MyHy Amplitude Pre- and Post-Injury

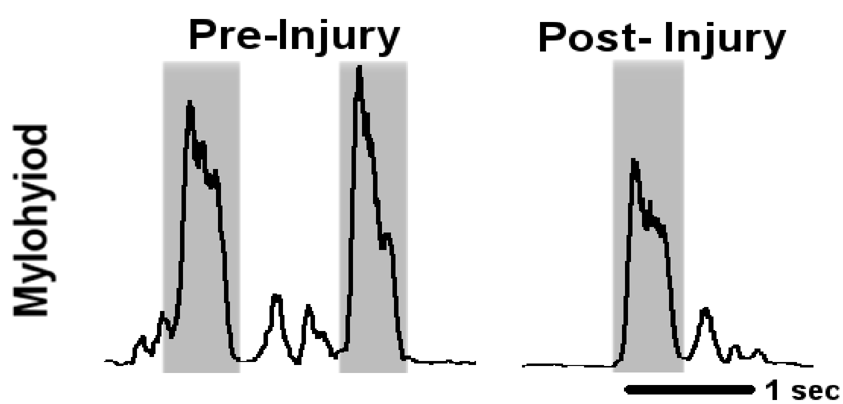
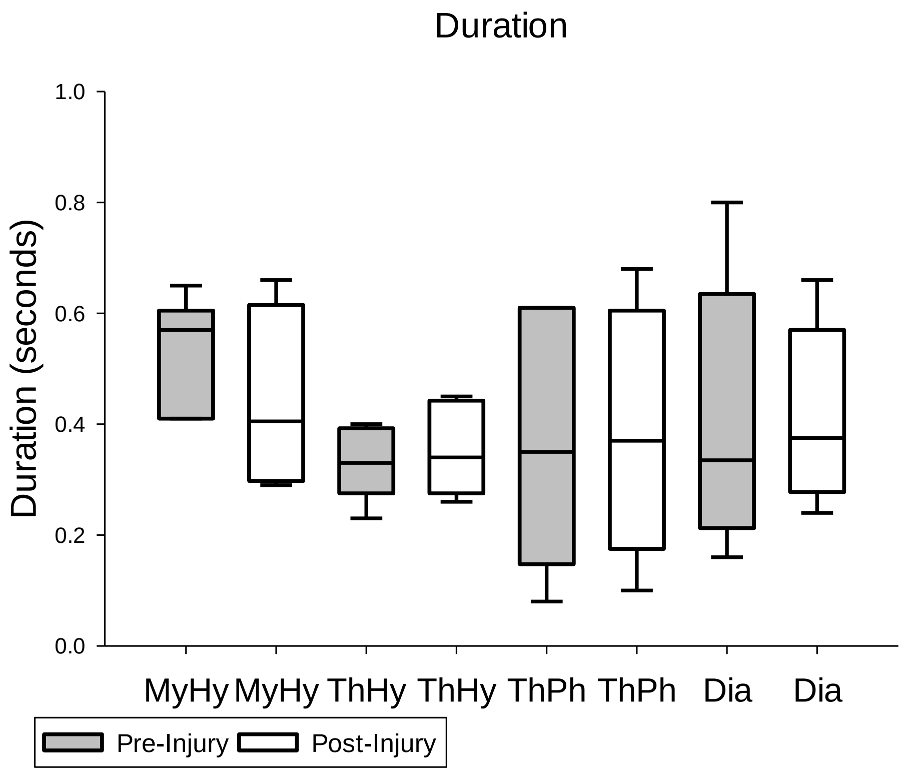


Figure 6. Muscle activity duration pre- and post-injury



CHAPTER 4

DISCUSSION

Pharyngeal swallowing disorders are a major consequence of radiation for the treatment of head and neck cancer. Clinically, it has been proposed that injuries to the swallowing muscles post radiation contribute to changes in swallowing function. As such, determining anatomical locations at greatest risk of causing pharyngeal dysphagia has been a major focus of research to date. The MyHy was identified as one such structure affected by radiation injury due to its role in airway protection and bolus propulsion (Eisbruch et al., 2004). However, it is not known to what extent the reflexive swallowing motor pattern changes when an injury occurs to submental muscles. Pharyngeal swallowing encompasses >10 muscles moving in synchrony, in the same order of activity and relative timing of activation. Previous work has shown that rhythmic activities such as suckling and swallowing can influence the temporal pattern of MyHy EMG activity during pharyngeal swallowing (German et al., 2009). This may be because of the extensive overlap between brainstem pathways and muscles involved in these coordinated activities. Therefore, changes in motor behavior during swallowing due to an injury may alter the coordination of respiration and swallowing patterns. Clinical work has corroborated this notion, as shifts in swallow-breathing coordination to inspiratory phase or inspiratory-transition phases have been found in HNC patients following radiotherapy (Brodsky et al., 2010). In our current study, using a localized cryoinjury to the MyHy, we found minimal short-term changes in the respiratory and swallow phase preferences following an acute injury to the suprahyoid musculature.

Although the suprahyoid injury depressed MyHy activity, our results did not support our hypothesis that an acute muscle injury would immediately disrupt swallow-breathing coordination. The expiratory phase is known to predominate during swallowing in both intact and decerebrate feline models (Dick et al., 1993; Doty & Bosma, 1956). However, our notion that we would observe short-term increases in inspiratory and inspiratory-expiratory transitions during swallowing onset in response to injury was refuted in our model, as the expiratory phase continued to prevail immediately post-injury. However, swallow-breathing phases appeared to fluctuate across animals. Swallowing occurring during the expiratory phase provides physiological advantages, such as airway protection and greater anterior-superior hyoid movement (Martin-Harris et al., 2005). It is possible that the short-time frame of our experiments was not an adequate time for developing swallow-breathing discoordination. Clinically, decoupling of swallow-breathing coordination was found in HNC patients 12 months post radiation (Brodsky et al., 2010). Thus, patients are in a more chronic phase of radiation injury when a greater predominance of swallows occurred during the inspiratory phase and inspiratory-expiratory transition, thereby increasing their risk of aspiration (Brodsky et al., 2010). Radiation injuries are associated with profound morphological changes to mucosa, epidermal tissues, and deep tissues that progressively worsen beyond the time frame of our current study. Aberrancies in MyHy activity may require increasing compensation from other swallowing muscles, especially during fatiguing or taxing behaviors (e.g., feeding). Further clinical work is needed to determine the onset of swallow-breathing decoupling to establish the temporal progression of the aberrant condition.

Our initial hypothesis that a suprahyoid injury may result in a shift in respiratory phase preference is based on our suspicion that such an injury may muddle sensory feedback or (de)sensitize afferent systems, which in turn would decouple the breathing and swallowing phases. Central neurons controlling breathing and swallowing are closely interrelated, which allow for coordination within the shared oral and pharyngeal structures during deglutition. This system allows for ad hoc alterations to motor outputs based on afferent feedback, including bolus consistency and volume. Previous work has further indicated that sensory stimulation with capsaicin, an inflammatory agent, can modulate motor outputs. Specifically, rodents demonstrated reductions in tongue muscle activity and delays in tongue movements post-capsaicin to Vagal C-fibers, which are needed to stabilize the upper airway (Adachi, Lowe, Tsuchiya, Ryan, & Fleetham, 1993). Furthermore, injection of capsaicin into the whisker pad, masseter, sternohyoid, digastric, and lingual muscles have shown to result in a decreased number of reflexive swallows in rodents (Tsujimura, Kitagawa, Ueda, & Iwata, 2009; Tsujimura, Kondo, et al., 2009; Tsujimura et al., 2011). These changes suggest that injury or inflammatory signaling at the periphery likely affect swallowing motor outputs.

Radiation Injury and Its Late Effects

The onset of dysphagia symptoms is an important consideration with radiation injuries, because of the unique temporal differences between onset of radiation damage. When accounting for their temporal progression, radiation injuries are often classified in the literature into two categories: early (<6 months) and delayed (>6 months). Early injuries typically manifest from inflammation due to irradiation (Dorr & Hendry, 2001). Delayed injuries arise due to abnormalities in wound healing process, leading to chronic inflammation and subsequent fibrosis. There is potential evidence for this progressive development of radiation injury-related dysphagia in the literature. Patients who have undergone radiotherapy following oropharyngeal cancer resections often present with declines in oropharyngeal swallow efficiency 3-months after resection surgery and further declines at 6- and 12-months, while patients who underwent resections without radiotherapy presented with considerable improvement (Pauloski et al., 1994). Further studies have shown significant decreases in the frequency of functional swallows (i.e., no aspiration and minimal residue) and in the portion of patients tolerating normal diets, at 3- and 12-months post-radiotherapy (Logemann et al., 2008). Histological studies of intrinsic laryngeal muscles have shown significant changes in fibril organization and reductions in the average area occupied by muscle fibers 7 to 15 months post-radiotherapy (Tedla et al., 2012). These late effects appear to coincide with the disruptions in swallow and breathing described previously (Brodsky et al., 2010). For our study, we induced an acute injury to the MyHy, but the predictability of acute injury progressing to late radiation effects and dysphagia remains a subject of debate. Predictive factors for late dysphagia risk may include specific lesion and treatment sites, with radiation injuries to the suprahyoid musculature being associated with poorer swallowing outcomes (Eisbruch et al., 2004; Kumar et al., 2014). Future studies are needed to investigate whether a correlation exists between late radiation injury progression and shifts in swallow-breathing coordination.

The use of local injury to a single muscle in our study may account for why swallow-breathing coordination was not significantly altered. Studies have shown that high doses of radiation to the geniohyoid/mylohyoid complex, suggestive of greater injury, are at higher risk for dysphagia. However, radiation injuries do not occur in isolation. Radiation often causes diffuse injuries, affecting multiple structures within a large area of the neck. While techniques such as intensity-modulated radiation therapy (IMRT) may help to minimize damage to surrounding tissues, HNC often requires more intense and aggressive approaches. Given the close proximity of swallowing structures associated with post-radiation dysphagia, multiple anatomical structures important in swallowing may be affected during radiotherapy. It is possible that an accumulation of injuries may occur in the swallowing muscles, thus leading to compounding effects on swallowing kinematics and the late decoupling of swallow-breathing coordination seen clinically. Therefore, it is possible that changes in swallow-breathing coordination are influenced by injury severity and temporal changes. Our current injury model only investigated the response to injury to the mylohyoid muscle alone. Further studies are needed to determine temporal changes in swallow-breathing coordination post-radiation injury, where injury to multiple muscles is likely.

Impact of Injury on the Mylohyoid

Previous work has shown that the suprahyoid muscular is an area at risk for post-radiotherapy dysphagia. A study by Kumar et al. demonstrated high dose radiation exposure to the floor of the mouth, including the MyHy, is associated with higher risk for aspiration (Kumar et al., 2014). Their group later released a study demonstrating radiation dose to the suprahyoid musculature is correlated with poorer bolus clearance and reduced laryngeal vestibular closure (Starmer et al., 2015). Our results showed a significant decline in MyHy amplitude during swallowing following injury to the MyHy, thus suggesting that MyHy's function in superior displacement of hyolaryngeal complex may be impaired (Ekberg & Sigurjonsson, 1982; Logemann et al., 1992; Pearson et al., 2011). However, caution should be exercised when analyzing EMG data to not equate amplitude for muscle force. Various factors, including muscle length, velocity, and activation/deactivation kinetics, can impact the how much force a recruited motor unit produces (Gabaldon, Nelson, & Roberts, 2008; Roberts & Gabaldon, 2008). Given MyHy's physiological importance and role in the initiation of the sequential swallowing, injury to the MyHy may also inhibit compensatory actions from other synergistic swallowing muscles (e.g., thyropharyngeal, thyrohyoid). Together, these changes may cause alterations in laryngeal and pharyngeal movement involved in swallowing. Thus, inhibitions in swallowing efficiency may help explain the late shift in swallow-breathing coordination seen clinically. For now, this remains unknown, as further research is needed on late effects of injury to the MyHy and its correlation to swallow-breathing coordination.

Limitations

We encountered limitations during our research that warrant further discussion. First, the primary study from which our data was collected was not designed with swallow-breathing coordination as its main objective. As such, timing of the introduction of the bolus relative to each respiratory phase was not controlled, which may have caused increased variability. It is unclear from our current study if there would have been any differences in swallow-breathing coordination had the bolus been introduced during the expiratory phase compared to the inspiratory phase or inspiration-expiration transition. Second, as mentioned in the methods, animals underwent C2 hemisection prior to starting our experiments. Although both our pre- and post- measures were treated equally, there is potential influence due to the increased respiratory drive elicited (Navarrete-Opazo, Vinit, & Mitchell, 2014; Vinit, Keomani, Deramaudt, Bonay, & Petitjean, 2016). C2 hemisection injures ipsilateral respiratory motor neurons, while sparing contralateral projections. Thus, descending respiratory signals from the brainstem are interrupted ipsilaterally to the hemisection site, which can alter the breathing pattern. A preliminary study conducted in our lab revealed that C2 hemisection also influences the excitability of upper airway muscles involved in swallowing. Thus, we would expect that if MyHy injury altered swallow-breathing coordination that a more pronounced change would be observed compared to a normal animal model (without C2 hemisection).

Lastly, studying diaphragmatic motor activity may not be sensitive enough to show changes in swallow-breathing coordination. Alternative methods for respiratory analysis may have provided potential advantages, such as: whole-body barometric plethysmograph, which allows for the calculation of tidal volume and minute ventilation, in additional to respiratory rate; bidirectional gas flow discriminator, which can detect airflow through the mouth and/or nose and determine whether inspiration or expiration are taking place through airflow directional readings. The use of these devices would have provided further objective confirmation of respiratory activity, as activation of the diaphragm is also associated with activities other than eupneic respirations (e.g., hiccups, schluckatmung, and cough). Airflow measures also allow for duration measurements of obligate pauses in respiration during swallowing, referred to clinically as apnea or respiratory pauses (Martin-Harris et al., 2005; Martin-Harris et al., 2003; Wang et al., 2015). Multiple studies have used plethysmography and/or airflow directional readings in combination with videofluoroscopic recordings to determine swallow-breathing patterns (Martin-Harris et al., 2005; Martin-Harris et al., 2003), permitting direct visual confirmation of swallowing versus indirect confirmation via EMG data. With this approach, airflow directional measures can be analyzed alongside simultaneously recorded videofluoroscopy data to determine which respiratory phase is associated with various swallowing events. (Martin-Harris et al., 2003). Plethysmography and/or airflow measures have also been used with surface submental EMGs to determine swallow-breathing coordination patterns (McFarland et al., 2016; Terzi et al., 2007; Wang et al., 2015).

Clinical Application

Our findings showed that acute injuries to the suprahyoid musculature inhibited MyHy activity, but did not alter respiratory phase preference during swallowing. Further research is needed to determine the long-term effects of injury on altering swallow-breathing coordination. This information can beneficial to the development of therapeutic approaches focused on preventing these pathological changes from occurring. For example, if fibrotic changes in muscle are determined to have a causative relationship with pathological swallow-breathing coordination shifts, then swallowing exercises targeting range of motion (ROM) may be indicated (e.g., oral and base of tongue exercises, (Veis, Logemann, & Colangelo, 2000), the Mendelsohn maneuver (Kahrilas, Logemann, Krugler, & Flanagan, 1991) (C. Lazarus, Logemann, & Gibbons, 1993), hyolaryngeal ROM exercises such as the Shaker (Shaker et al., 2002), and the Super-

Supraglottic Swallow (Logemann, Pauloski, Rademaker, & Colangelo, 1997)). A correlation with post-radiotherapy muscular atrophy may promote the use of prophylactic swallowing exercises to improve muscle strength and/or stamina. While these techniques have shown to be effective tools in improving swallowing outcomes in post-radiotherapy HNC patients, the underlying mechanisms to explain how this effect contributes to improved swallowing outcomes remains unknown.

Conclusion and Future Directions

Acute injury to the MyHy muscle depresses within muscle activity, which may impact swallowing kinematic and biomechanical function. However, no changes in swallowing and breathing coordination were found. These findings may suggest that temporal response and severity of damage following injury to swallow muscles may play a role in destabilizing the strong coupling between swallowing and breathing. Future research should include survival studies to determine whether phase preference shifts occur at points beyond the timeline of this study. Alternative methods of determining respiratory phase in conjunction with electromyography data may prove to be more sensitive at studying changes in breathing patterns.

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APPENDIX: ABBREVIATIONS

CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CPG	Central Pattern Generator
CVA	Cerebrovascular Accident
DARS	Dysphagia/Aspiration-Related Structures
DiaCru	Crural Diaphragm
E1	Inspiratory-Expiratory Transition
E2	Expiratory Phase
EMG	Electromyography
GERD	Gastroesophageal Reflux Disease
HNC	Head and Neck Cancer
HSP	Hypopharyngeal Suction Pump
I	Inspiratory Phase
IMRT	Intensity-Modulated Radiation Therapy
MyHy	Mylohyoid Muscle
PAS	Penetration-Aspiration Scale
ROM	Range of Motion
SBC	Swallow-Breathing Coordination
SW-IMRT	Swallowing Sparing Intensity Modulating Radiotherapy
ThHy	Thyrohyoid Muscle
ThPh	Thyropharyngeus
TRPV1	Transient Receptor Potential
VFs	Vocal Folds

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