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SWALLOW, BREATHING AND SURVIVAL: SEX-SPECIFIC EFFECTS OF
OPIOIDS

By

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B.A., English- University of Louisville, 2013

M.S., Communicative Disorders- University of Louisville, 2018

M.S., Physiology- University of Louisville, 2022

A Dissertation

Submitted to the Faculty of the
School of Medicine of the University of Louisville
in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy
in Physiology and Biophysics

Department of Physiology
University of Louisville
Louisville, Kentucky

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DEDICATION

To all the people who have played a role in my education:

I am so grateful.

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First, I must thank Dr. Pitts. You are the mentor I needed. I entered your lab with ten thumbs, and am leaving your lab with ten thumbs that can rise to any occasion. You changed my view of what's possible. Thank you so much.

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ABSTRACT

SWALLOW, BREATHING AND SURVIVAL: SEX-SPECIFIC EFFECTS OF OPIOIDS

Michael L. Frazure

May 23, 2023

This dissertation presents a series of studies examining mechanisms of deglutition and respiration, and how these vital processes are impacted by opioids. The experiments in Chapter Two investigated the role of the upper esophagus in airway protection through systematic activation of pharyngeal and esophageal mechanoreceptors in a cat electromyography model. Chapter Three compared effects of opioid administration on breathing and swallowing between male and female rats, and found that females are more susceptible to opioid-induced depression of breathing and swallow than males. Findings from Chapters Two and Three led to the development of a translational model of opioid-induced dysphagia using videofluoroscopy. Chapter Four demonstrated that opioid administration resulted in a significant decline in airway protection during swallow in freely feeding, unrestrained cats. This work has advanced knowledge of the regulation of the upper aerodigestive tract, and its dual roles in breathing and swallowing. An improved understanding of the neural control of deglutition will facilitate the development of effective treatments for dysphagia. This dissertation includes the first study to compare effects of opioids on pharyngeal swallow between sexes, and provides mechanistic and clinically-translatable insights into opioid-induced dysphagia

Elucidating the actions of opioids on the brainstem breathing and swallowing networks will aid the prevention and treatment of opioid-induced respiratory depression and dysphagia related complications such as aspiration pneumonia.

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CHAPTER 1

INTRODUCTION

Swallow and breathing are life sustaining behaviors that share neural circuitry enriched with opioid receptors (Blivis, Mentis, O'Donovan M, & Lev-Tov, 2007; Bolser & DeGennaro, 1994; George, Zastawny, Briones-Urbina, Cheng, Nguyen, Heiber, Kouvelas, Chan, & O'Dowd, 1994; Irnaten, Aicher, Wang, Venkatesan, Evans, Baxi, & Mendelowitz, 2003; Negus, 1942; Zhuang, Gao, Gao, & Xu, 2017). Depression of breathing following opioid administration has been well documented, but few investigators have studied specific effects of opioids on swallow function (Bateman, Saunders, & Levitt, 2023; Ramirez, Burgraff, Wei, Baertsch, Varga, Baghdoyan, Lydic, Morris, Bolser, & Levitt, 2021). A growing number of studies devoted to physiological sex differences indicate sex-specific modulation of swallow and breathing, and that females are more susceptible to opioid-induced respiratory depression than males (A. Huff, M. D. Reed, K. E. Iceman, D. R. Howland, & T. Pitts, 2020; Alyssa Huff, Mitchell D Reed, Kimberly E Iceman, Dena R Howland, & Teresa Pitts, 2020; Marchette, Carlson, Frye, Hastings, Vendruscolo, Mejias-Torres, Lewis, Hampson, Volkow, Vendruscolo, & Koob, 2023). This body of work will describe the mechanisms of deglutition and respiration, discuss how opioids impact these critical processes, and highlight the importance of physiological sex differences as a variable in biomedical research.

Swallow

Upper Aerodigestive Tract

Comprised of the mouth, nasal passages, larynx, pharynx, and proximal esophagus, the upper aerodigestive tract is a shared space for breathing and swallowing (Laitman & Reidenberg, 1997, 1993). Under normal conditions, breathing and swallowing are precisely regulated to prevent ingestion of air and inhalation of food and liquid (Horton, Segers, Nuding, O'Connor, Alencar, Davenport, Bolser, Pitts, Lindsey, Morris, & Gestreau, 2018). The aerodigestive tract has a rich neural supply, with afferent and efferent connections to several nerves, including the trigeminal (CN V), facial (CN VII), glossopharyngeal (IX), vagus (X), hypoglossal (XII), and cervical spinal nerves (Frazure, Brown, Greene, Iceman, & Pitts, 2021; Miyamaru, Kumai, Ito, & Yumoto, 2008; Pilarski, Leiter, & Fregosi, 2019; Sakamoto, 2013; Yamaoka, Furusawa, Fujimoto, Iguchi, & Kumai, 1992). Pathologies that compromise the sensory and/or motor innervation of the aerodigestive tract may impair the coordination and efficiency of breathing and swallowing.

Deglutition

Swallow is a mechanism of energy intake essential to life for many organisms, from protozoans to humans (Negus, 1942). In mammals, swallow occurs in three phases: Oral, pharyngeal, and esophageal (German, Crompton, & Thexton, 1998, 2009; Negus, 1942; Pitts & Iceman, 2023). The oral phase of swallow is sometimes further subdivided into 2 stages: Oral preparatory and oral (Logemann, 1995, 2007, 2008).

During the oral preparatory stage of swallow, food is brought to the mouth, manipulated by the oral tongue, masticated to a consistency that can be swallowed, and

formed into a bolus (Logemann, 2007). During the oral phase of swallow, a food or liquid bolus is transported backward toward the oropharynx by the tongue (Logemann, 1995). Lingual propulsion during the oral stage generates pressure such that when the bolus contacts the posterior pharyngeal wall, pharyngeal mechanoreceptors are activated (Negus, 1942).

Activation of pharyngeal mechanoreceptors elicits the pharyngeal swallow reflex (Jean, 2001b; Logemann, 2007). During the pharyngeal phase of swallow, coordinated laryngeal elevation, velopharyngeal closure, laryngeal closure, and pharyngeal constriction functionally seal the airway as food or liquid is propelled through the pharynx (Pitts, 2014). The upper esophageal sphincter is tonic at rest (Jean, 2001b; Logemann, 2007). During swallow, the upper esophageal sphincter (UES) opens to allow passage of a food or liquid bolus from the pharynx into the esophagus. UES opening is achieved through relaxation of the cricopharyngeus muscle, antero-superior excursion of the hyoid and larynx, and pressure generated by the oropharyngeal swallow (Jean, 2001b; Logemann, 2007).

Return of resting UES tone indicates the completion of pharyngeal swallow (Doty & Bosma, 1956). Rebound of UES tone forms a functional barrier between the airway and ingested material in the esophagus (Negus, 1942; Shaker, 1995; Shaker & Hogan, 2000). During the esophageal phase of swallowing, ingested material is propelled through the esophagus and into the stomach by peristaltic contractions (Jean, 2001b; Logemann, 2007).

The oral and pharyngeal phases of swallow are centrally driven by cranial nerves in all mammals (Doty, 1951; Doty & Bosma, 1956; Jean, 2001b; Negus, 1942; Pitts &

Iceman, 2023). There is species variability in the muscular composition and innervation of the esophagus. In species where the esophagus contains only striated muscle (e.g., rats), the esophageal phase of swallow is centrally driven by vagal and cervical spinal nerves. In species where the esophagus contains striated and smooth muscle (e.g., cats, humans), the proximal-striated portion of the esophagus is centrally driven, and the distal-smooth portion is controlled by the enteric nervous system (Frazure, Brown, Greene, Iceman, & Pitts, 2021; Lang, Medda, & Shaker, 2019).

Swallow Motor Pattern

Swallow has traditionally been described as an all-or-none reflex event (Doty, 1951; Kirchner, 1993; Laitman & Reidenberg, 1993; Negus, 1942). Classic electromyography studies established that the pharyngeal stage of deglutition is accomplished by stereotypic rostral-caudal activation of the aerodigestive musculature (Doty & Bosma, 1956; Thexton, Crompton, & German, 2007a). Allowing for minor variation in muscle structure/function, a temporal swallow motor pattern has been described in the rat, cat, dog, and pig that is characterized by sequential activation of muscles that elevate the hyolaryngeal complex, adduct the larynx, and constrict the pharynx (Doty, 1951; Doty & Bosma, 1956; German, Crompton, Gould, & Thexton, 2017; German, Crompton, & Thexton, 1998, 2009; Alyssa Huff, Mitchell D Reed, Kimberly E Iceman, Dena R Howland, & Teresa Pitts, 2020; T. Pitts, M. J. Rose, I. Poliacek, J. Condrey, P. W. Davenport, & D. C. Bolser, 2015; Spearman, Poliacek, Rose, Bolser, & Pitts, 2014; Thexton, Crompton, & German, 2007a).

The oral phase of swallow is under volitional control, and can be interrupted and resumed at any time (Jean, 2001b). In contrast, once initiated, the pharyngeal swallow is

an irreversible, reflex event (Doty, 1951; Doty & Bosma, 1956; Negus, 1942). Interestingly, while pharyngeal swallow has a stereotypic motor plan, it has been shown to adapt to peripheral inputs (Frazure, Brown, Greene, Iceman, & Pitts, 2021; Jean, 2001b; Spearman, Poliacek, Rose, Bolser, & Pitts, 2014). Videofluoroscopic and manometric swallowing studies in humans and animals have demonstrated that timing of swallow onset, and total swallow duration, vary based on bolus characteristics (e.g., viscosity, logemann) (German, Crompton, & Thexton, 1998; Kahrilas, Lin, Chen, & Logemann, 1996; Perlman, Schultz, & VanDaele, 1993; Pongpipatpaiboon, Inamoto, Aihara, Kagaya, Shibata, Mukaino, Saitoh, & Gonzalez-Fernandez, 2022). Surface electromyography (sEMG) studies in humans also demonstrate that force and duration of pharyngeal swallow can be modulated volitionally (Soyler, Serel Arslan, Demir, & Kiylioglu, 2023; Wheeler-Hegland, Rosenbek, & Sapienza, 2008). While pharyngeal swallow has a characteristic motor sequence, its intensity and duration adapt to accommodate demands placed on the system. The important point is that pharyngeal swallow is not a simple reflex; it is an irreversible, but nuanced and modifiable reflex behavior (Frazure, Brown, Greene, Iceman, & Pitts, 2021).

The esophageal component of deglutition is also involuntary, yet complex (Doty & Bosma, 1956; Jean, 2001b; Shaker & Hogan, 2000). Primary peristalsis is initiated following completion of the last pharyngeal swallow in a feeding bout, and propels ingested material toward the stomach (Lang, Medda, Jadcherla, & Shaker, 2016; Pitts & Iceman, 2023). When a bolus reaches the gastroesophageal junction, the lower esophageal sphincter (LES) is disinhibited, permitting food or liquid to enter the stomach (Doty & Bosma, 1956; Frazure, Brown, Greene, Iceman, & Pitts, 2021). Similar to the

UES, once a bolus has entered the stomach, there is rebound of LES tone to prevent reflux of gastric contents (Negus, 1942; Shaker & Hogan, 2000). The studies of Lang and Shaker have demonstrated that when the esophagus is stimulated, a number of reflexes may occur based on the location and nature of the stimulus, including: Secondary peristalsis, which functions to propel residual material in the esophagus toward the stomach; esophageal-evoked pharyngeal swallow, which functions to protect the airway during clearance of residual or refluxed material; and the esophagoglottal closure reflex, which closes the larynx following sudden, forceful distension of the esophagus, functions to protect the airway during large volume retrograde esophageal transit (e.g. regurgitation) (Lang, Medda, Jadcherla, & Shaker, 2016; Lang, Medda, & Shaker, 2019; Shaker, 1995; Shaker & Hogan, 2000).

Deglutition is both stable and flexible (German, Crompton, & Thexton, 1998; Jean, 2001b). The remarkable sensitivity of the aerodigestive mechanism is accomplished through central integration of information from several afferent beds in the larynx, pharynx and esophagus, which enables dynamic modulation of motor output based on peripheral conditions (Frazure, Brown, Greene, Iceman, & Pitts, 2021; Pitts & Iceman, 2023). This highly regulated process is patterned by the brainstem swallow pattern generator (Jean, 2001b).

Neural Control

In a classic review of brainstem control of swallowing, Jean described a medullary swallow pattern generator comprised of three components: Central and peripheral afferent inputs to the pattern generator; a network of interneurons that program the motor pattern; and efferent output to motor neuron pools (Jean, 2001b). This network

of control is distributed throughout the medulla and contains at least two key functional regions, the dorsal swallowing group and the ventral swallowing group (Hashim & Bieger, 1987; Jean, 2001b; Pitts & Iceman, 2023; T. Pitts, I. Poliacek, M. J. Rose, M. D. Reed, J. A. Condrey, H. W. Tsai, G. Zhou, P. W. Davenport, & D. C. Bolser, 2018).

The dorsal swallowing group includes the nucleus tractus solitarius (NTS) and nearby reticular formation (Jean, 2001b). Afferent fibers from oropharyngeal receptors, laryngeal receptors, thoracic receptors, pulmonary stretch receptors and esophageal stretch receptors converge in the solitary tract and converge in the NTS (Frazure, Brown, Greene, Iceman, & Pitts, 2021; Lang, Medda, Jadcherla, & Shaker, 2016; Miyamaru, Kumai, Ito, & Yumoto, 2008; Pitts & Iceman, 2023; Sakamoto, 2013; Yamaoka, Furusawa, Fujimoto, Iguchi, & Kumai, 1992). The dorsal swallowing group is the site of swallow programming, and the activity of these neurons closely mirror the swallow motor pattern (Jean, 2001b).

Interneurons transmit the swallow motor plan from the dorsal swallowing group to the ventral swallowing group, located above the nucleus ambiguus (NA) in the ventrolateral medulla (Pitts & Iceman, 2023). Premotor neurons in the ventral swallowing group have connections to motor neurons distributed throughout the brainstem (Jean, 2001b). The ventral swallowing group distributes the swallow command to the trigeminal, facial, glossopharyngeal, vagus, and cervical spinal motor nuclei, which drive muscle activity during swallow (T. E. Dick, Y. Oku, J. R. Romaniuk, & N. S. Cherniack, 1993; Jean, 2001b; Pitts & Iceman, 2023).

Swallowing Disorders

Dysphagia

Dysphagia, or impaired swallow function, is a common disorder with an estimated one million new diagnoses per year in the United States (McCarty & Chao, 2021; Patel, Krishnaswami, Steger, Conover, Vaezi, Ciucci, & Francis, 2018). Older adults, and individuals with history of neurological injury/disease, peripheral damage to swallowing muscles, and respiratory conditions (for example, chronic obstructive pulmonary disease (COPD) are at greatest risk for dysphagia. (Ghannouchi, Speyer, Doma, Cordier, & Verin, 2016; Jukic Peladic, Orlandoni, Dell'Aquila, Carrieri, Eusebi, Landi, Volpato, Zuliani, Lattanzio, & Cherubini, 2019; Lancaster, 2015; Maeshima, Osawa, Miyazaki, Seki, Miura, Tazawa, & Tanahashi, 2011; McCarty & Chao, 2021). Dysphagia related complications include aspiration pneumonia, asphyxiation, weight loss, malnourishment, and dehydration, and are associated with increased rates of mortality (Mandell & Niederman, 2019; McCarty & Chao, 2021).

Studies estimate more than 60,000 deaths from dysphagia per year in the United States, and have shown that hospitalized patients with a diagnosis of dysphagia are 1.7 times more likely to die in the hospital than patients without dysphagia (McCarty & Chao, 2021; Patel, Krishnaswami, Steger, Conover, Vaezi, Ciucci, & Francis, 2018). The financial burden of dysphagia is an estimated \$4-7 billion per year in the United States (McCarty & Chao, 2021; Patel, Krishnaswami, Steger, Conover, Vaezi, Ciucci, & Francis, 2018). Patients with dysphagia experience higher rates of hospitalization, length of stay, hospital readmission, and diminished quality of life (Attrill, White, Murray, Hammond, & Doeltgen, 2018; Jukic Peladic, Orlandoni, Dell'Aquila, Carrieri, Eusebi,

Landi, Volpato, Zuliani, Lattanzio, & Cherubini, 2019; Maeshima, Osawa, Miyazaki, Seki, Miura, Tazawa, & Tanahashi, 2011; McCarty & Chao, 2021; Patel, Krishnaswami, Steger, Conover, Vaezi, Ciucci, & Francis, 2018).

Dysphagia Assessment

Dysphagia is a challenging diagnosis for patients and clinicians. Individuals with positive screening or increased risk for dysphagia are typically referred to a speech-language pathologist for a clinical swallowing evaluation, which includes a comprehensive medical history, cranial nerve and oral mechanism examination, and food and liquid intake assessment (Rommel & Hamdy, 2016). A number of factors, including feeding independence and oral phase efficiency, impact decision making, but clinical indicators of aspiration (coughing, throat clearing, wet phonation) are often used as a primary outcome measure (Logemann, 2007).

The clinical swallow evaluation provides good insight into cognitive status and orofacial sensorimotor function, but is an unreliable measure of pharyngeal function and airway protection during swallow (Logemann, 1995, 2007). As such, an instrumental evaluation of swallowing is generally recommended when dysphagia is suspected (Logemann, 1995, 2007). There are presently two gold standard instrumental evaluations of oropharyngeal swallow function: Videofluoroscopic swallowing study (VFSS), also known as the Modified Barium Swallow (MBS); and the Fiberoptic Endoscopic Evaluation of Swallowing (FEES) (Dodds, Logemann, & Stewart, 1990; Langmore, Schatz, & Olsen, 1988; Martin-Harris, Logemann, McMahon, Schleicher, & Sandidge, 2000). Patients with signs or symptoms of esophageal dysphagia may also be referred for high resolution manometry (HRM), pH monitoring, and additional radiographic and

endoscopic imaging of the upper gastrointestinal tract (Langmore, 1998; Logemann, 2007; Rommel & Hamdy, 2016).

VFSS is an imaging technique used for assessment of oropharyngeal swallow function, and is usually performed in the radiology department of a hospital (Dodds, Logemann, & Stewart, 1990; Logemann, 1995; Martin-Harris, Logemann, McMahon, Schleicher, & Sandidge, 2000). During VFSS, the patient is seated upright in a fluoroscope and administered food and liquid consistencies that have been mixed with barium sulfate, an inert contrast. A radiologist activates the fluoroscope to record x-ray videos as the patient swallows. This enables real-time assessment of airway protection and kinematics as a bolus passes through the upper aerodigestive tract and into the esophagus (Donner, 1985; Logemann, 1997).

During FEES, a patient is awake and positioned in their natural feeding position (Langmore, Schatz, & Olsen, 1988). A flexible endoscope is passed trans-nasally to enable direct visualization of the pharynx and laryngeal structures (Langmore, Schatz, & Olson, 1991). The patient is then given various food and liquid items to swallow. The endoscopic view is obscured by pharyngeal constriction during swallow, but swallow safety and efficiency can be inferred by presence of food or liquid residue in the pharynx and/or airway post-prandial (Langmore, Schatz, & Olsen, 1988; Logemann, 1995).

Rating airway protection during deglutition is a critical component of imaging studies (Rommel & Hamdy, 2016). To this end, the 8-Point Penetration-Aspiration Scale (PAS) has been widely used by dysphagia clinicians since its publication in 1996 (Rosenbek, Robbins, Roecker, Coyle, & Wood, 1996). Laryngeal penetration is defined as material entering the airway that does not pass below the vocal folds (Korpas & Jakus,

2000). Aspiration is defined as material entering the airway that passes below the vocal folds (Rosenbek, Robbins, Roecker, Coyle, & Wood, 1996). The PAS allows for quantification of airway invasion during swallow, and numerically increases with severity of dysfunction; a score of 1 is assigned when material does not enter the airway, and a score of 8 is assigned when there is aspiration with no attempt to eject material from the airway (Rosenbek, Robbins, Roecker, Coyle, & Wood, 1996).

While airway protection is of vital importance, it is not the sole indicator of oropharyngeal swallow function. The Modified Barium Swallow Impairment Profile (MBSImP) offers a standardized protocol for the VFSS, including a rating system that quantifies timing, kinematics, and efficiency of oropharyngeal swallow function (Martin-Harris, Brodsky, Michel, Castell, Schleicher, Sandidge, Maxwell, & Blair, 2008). The MBSImP has been considered best practice in VFSS among dysphagia clinicians since its publication in 2008 (Martin-Harris, Brodsky, Michel, Castell, Schleicher, Sandidge, Maxwell, & Blair, 2008; Martin-Harris, Canon, Bonilha, Murray, Davidson, & Lefton-Greif, 2020).

Most animal models of dysphagia utilize videofluoroscopy rather than videoendoscopy. The VFSS has been adapted for translational study of swallow function in the pig (German, Crompton, & Thexton, 2009; Holman, Campbell-Malone, Ding, Gierbolini-Norat, Griffioen, Inokuchi, Lukasik, & German, 2013), dog (Harris, Grobman, Allen, Schachtel, Rawson, Bennett, Ledyayev, Hopewell, Coates, Reinero, & Lever, 2017), rat (Russell, Ciucci, Hammer, & Connor, 2013) and mouse (Lever, Braun, Brooks, Harris, Littrell, Neff, Hinkel, Allen, & Ulsas, 2015; Lever, Simon, Cox, Capra, O'Brien, Hough, & Murashov, 2010). The 8-Point Penetration Aspiration Scale has also

been adapted for use in animal models (Holman, Campbell-Malone, Ding, Gierbolini-Norat, Griffioen, Inokuchi, Lukasik, & German, 2013; Rosenbek, Robbins, Roecker, Coyle, & Wood, 1996). Animal models of dysphagia offer a clinical phenotype, and enable evaluation of mechanism in a translatable, pre-clinical setting.

Dysphagia Management

The effectiveness with which dysphagia is managed has consequences for a patient's survival, quality of life, and ability to participate in cultural and religious aspects of food and drink (Coyle, Davis, Easterling, Graner, Langmore, Leder, Lefton-Greif, Leslie, Logemann, Mackay, Martin-Harris, Murray, Sonies, & Steele, 2009; Kim, Park, Park, & Kim, 2020; Lancaster, 2015; O'Keeffe, 2018). There are currently no pharmacological treatments for oropharyngeal dysphagia. Broadly speaking, oropharyngeal dysphagia management is limited to compensatory strategies and rehabilitative exercises (Logemann, 1995, 2008). Unfortunately, many compensatory and rehabilitative techniques lack the support of strong evidence.

Diet modification is a commonly used compensatory approach to dysphagia treatment (Castellanos, Butler, Gluch, & Burke, 2004). Diet modification refers to the prescription of altered food and liquid consistencies, including soft or pureed foods and thickened liquids (Gallegos, Brito-de la Fuente, Clave, Costa, & Assegehegn, 2017). The theoretical benefit of thickened liquids is that they enable patients that aspirate thin liquids (e.g., regular water) to continue oral alimentation without aspiration (Coyle, Davis, Easterling, Graner, Langmore, Leder, Lefton-Greif, Leslie, Logemann, Mackay, Martin-Harris, Murray, Sonies, & Steele, 2009). However, a large clinical trial that examined the safety and effectiveness of thickened liquids showed mixed results

(Logemann, 2008). A potential explanation is that although thickened liquids reduce aspiration detected by imaging, they unlikely prevent aspiration 100% of the time across feeding conditions (Steele, Ennis, & Dobler, 2021). If aspirated, thickened liquids are substantially more threatening to pulmonary health than thin liquids (Nativ-Zeltzer, Kuhn, Imai, Traslavina, Domer, Litts, Adams, & Belafsky, 2018). Additional concerns regarding the use of thickened liquids include increased risk of dehydration, increased patient costs, and negative impact on quality of life (Cichero, 2013; Logemann, 2008; O'Keeffe, 2018).

Postural techniques and swallowing strategies are commonly utilized compensatory treatments (Logemann, 2007). Postural techniques, such as a chin tuck or head turn during swallow, aim to maximize safety of bolus flow as it crosses the glottis (Logemann, 2008). Swallowing strategies are specific instructions to be followed during intake (Lazarus, 2017). For example, a patient may be instructed to take small sips, or avoid straws, based on the results of an instrumental assessment. Compensatory strategies can serve as a bridge to oral feeding following nothing by mouth, or *nil per os* (NPO), status, but do not restore function, and are often impractical in the long term; to prevent aspiration, a posture or strategy must be used 100% of the time, and some patients may be unwilling, or unable to do so (Lazarus, 2017).

Rehabilitative oropharyngeal exercises have been associated with improved swallow function in specific contexts, but more research is needed to standardize dosage guidelines (Agrawal, Kern, Edeani, Balasubramanian, Hyngstrom, Sanvanson, & Shaker, 2018; Krekeler, Rowe, & Connor, 2021; Park, Oh, Yoon, & Park, 2019). It is currently considered best practice to tailor exercise regimens to physiologic deficits identified on a

VFSS or FEES (Coyle, Davis, Easterling, Graner, Langmore, Leder, Lefton-Greif, Leslie, Logemann, Mackay, Martin-Harris, Murray, Sonies, & Steele, 2009; Logemann, 2008). However, this approach is subject to error, as highlighted by studies that showed inconsistent agreement in identification of swallowing impairment among dysphagia clinicians (Plowman & Humbert, 2018; Vose, Kesneck, Sunday, Plowman, & Humbert, 2018). Moreover, patients with a primary sensory impairment will not benefit from strengthening exercises, as weakness is not the etiology of their dysphagia (Labeit, Jung, Ahring, Oelenberg, Muhle, Roderigo, Wenninger, von Itter, Claus, Warnecke, Dziewas, & Suntrup-Krueger, 2023).

Neuromuscular electrical stimulation (NMES) was co-opted from the fields of physical and occupational therapy and applied to dysphagia rehabilitation (Langmore, McCulloch, Krisciunas, Lazarus, Van Daele, Pauloski, Rybin, & Doros, 2016). NMES has proven beneficial to limb rehabilitation, but its application to dysphagia treatment has shown mixed results (Langmore, McCulloch, Krisciunas, Lazarus, Van Daele, Pauloski, Rybin, & Doros, 2016). While some groups have shown that NMES aids swallow rehabilitation after stroke, multiple randomized clinical trials have demonstrated no significant effect (Bulow, Speyer, Baijens, Woisard, & Ekberg, 2008; Carnaby, LaGorio, Silliman, & Crary, 2020). When NMES was studied as a treatment for chronic dysphagia following acquired brain injury, the investigators concluded that submental and cervical NMES increased aspiration risk (Ludlow, Humbert, Saxon, Poletto, Sonies, & Crujido, 2007). A randomized clinical trial investigating NMES as a treatment for dysphagia after head and neck cancer showed that patients in the experimental group had worse Penetration Aspiration Scale scores (a standardized measure of airway protection during

swallow) than patients who received traditional swallowing therapy (Langmore, McCulloch, Krisciunas, Lazarus, Van Daele, Pauloski, Rybin, & Doros, 2016). Determining the risks and benefits of NMES is further complicated by lack of a standardized stimulation parameters; NMES equipment and training is available to clinicians through multiple companies, and there are brand-specific differences in stimulation protocols (Humbert, Michou, MacRae, & Crujido, 2012). More basic science studies are needed to evaluate the utility of electrical stimulation to swallow rehabilitation.

A prospective outcomes study showed that oral/dental status (presence of dental decay, frequency of toothbrushing, dependence for oral care) is a predictive factor of aspiration pneumonia (Langmore, Terpenning, Schork, Chen, Murray, Lopatin, & Loesche, 1998). As such, aggressive oral hygiene measures have been shown to reduce the risk of aspiration pneumonia among dysphagic patients (Wagner, Marchina, Deveau, Frayne, Sulmonte, & Kumar, 2016). The Frazier Water Protocol, developed at the Frazier Rehabilitation Institute in the 1980s, states that dysphagic patients are permitted regular water, with the caveat that water intake must occur after aggressive oral care, and at least thirty minutes after a meal (Bernard, Loeslie, & Rabatin, 2016; Gillman, Winkler, & Taylor, 2017). The Frazier Water Protocol is supported by a systematic review of health outcomes in humans, and studies showing that aspiration of plain water, in the absence of oral bacteria and food residue, is relatively benign (Bernard, Loeslie, & Rabatin, 2016; Gillman, Winkler, & Taylor, 2017; Steele, Ennis, & Dobler, 2021).

There is data to suggest that maximizing the sensory stimulus provided by a food or liquid bolus (temperature, taste, and/or carbonation), can improve swallow function

(Magara, Watanabe, Tsujimura, Hamdy, & Inoue, 2018; Selcuk, Uysal, Aydogdu, Akyuz, & Ertekin, 2007; Turkington, Ward, Farrell, Porter, & Wall, 2019; K. Yamamura, J. Kitagawa, M. Kurose, S. Sugino, H. Takatsuji, R. M. Mostafaezur, H. M. Zakir, & Y. Yamada, 2010). Administration of capsaicin prior to feeding can improve swallow function among dysphagic patients (Wang, Wu, Fang, Shen, Zhang, & Liu, 2019). Respiratory-swallow training has shown promise among patients with head and neck cancer (Martin-Harris, McFarland, Hill, Strange, Focht, Wan, Blair, & McGrattan, 2015). Expiratory muscle strength training (EMST) has led to improved swallowing outcomes in patient populations spanning neurodegenerative disease to head and neck cancer (Hutcheson, Barrow, Plowman, Lai, Fuller, Barringer, Eapen, Wang, Hubbard, Jimenez, Little, & Lewin, 2018; Park, Oh, Chang, & Kim, 2016; Pitts, Bolser, Rosenbek, Troche, Okun, & Sapienza, 2009; Plowman, Watts, Tabor, Robison, Gaziano, Domer, Richter, Vu, & Gooch, 2016).

The treatments for dysphagia, a prevalent but under-diagnosed condition linked to poor outcomes, are limited (Jukic Peladic, Orlandoni, Dell'Aquila, Carrieri, Eusebi, Landi, Volpato, Zuliani, Lattanzio, & Cherubini, 2019; Patel, Krishnaswami, Steger, Conover, Vaezi, Ciucci, & Francis, 2018; Plowman, Anderson, York, DiBiase, Vasilopoulos, Arnaoutakis, Beaver, Martin, & Jeng, 2023; Rommel & Hamdy, 2016; Zuercher, Moret, Dziewas, & Schefold, 2019). More work is needed to define the mechanisms of deglutition and its disorders. Specifically, more basic science studies are necessary to guide development of effective therapies for dysphagia.

Airway Protection

The pharyngeal swallow reflex protects the airway from food and liquid during feeding (Pitts, 2014). Airway protection during swallow is achieved through several actions (German, Crompton, & Thexton, 1998; Logemann, Kahrilas, Cheng, Pauloski, Gibbons, Rademaker, & Lin, 1992; Negus, 1942). As tongue base retraction propels a bolus from mouth to pharynx, the velum elevates. This elevation closes the velopharyngeal port, which seals the nasopharynx (preventing nasal regurgitation) and allows for generation of pressure in the pharynx, which aids downward bolus passage (Laitman & Reidenberg, 1993; Pitts, 2014). Contraction of the submental muscles brings the hyolaryngeal complex to an anterior and superior position under the tongue base (Kirchner, 1993; Pitts, 2014). During this movement of the larynx, contraction of intrinsic laryngeal adductor muscles prevents food or liquid from entering the subglottic airway (T. E. Dick, Y. Oku, J. R. Romaniuk, & N. S. Cherniack, 1993; Logemann, Kahrilas, Cheng, Pauloski, Gibbons, Rademaker, & Lin, 1992; Miyamaru, Kumai, Ito, & Yumoto, 2008; Pitts, 2014). Epiglottal deflection over the sealed larynx forms an additional barrier between the airway and ingested material (German, Crompton, & Thexton, 1998, 2009; Pitts, 2014). Superior to inferior contraction of the pharyngeal constrictors forms a peristaltic wave that propels the bolus through the pharyngeal tube (Dodds, Logemann, & Stewart, 1990; Doty & Bosma, 1956; Pitts, 2014; Spearman, Poliacek, Rose, Bolser, & Pitts, 2014). Following contraction of the inferior pharyngeal constrictor, the upper esophageal sphincter relaxes and the bolus passes into the esophagus (Pitts, 2014).

Cough is a protective behavior that removes mucus or foreign material from the lower airways (Fontana & Lavorini, 2006). Cough is a vagally mediated response to

aspiration as a result of activation of mucosal receptors and C-fibers in the larynx and trachea (Pitts, 2014). These afferents ascend to the nucleus tractus solitarius (NTS) and project to neurons in the pontomedullary respiratory network and the medial reticular formation (Bolser & DeGennaro, 1994; T. E. Dick, Y. Oku, J. R. Romaniuk, & N. S. Cherniack, 1993; Karlsson, Lanner, & Persson, 1990; Pitts, 2014; Pitts, Rose, Mortensen, Poliacek, Sapienza, Lindsey, Morris, Davenport, & Bolser, 2013). The cough reflex has been described as a three-phase motor pattern (Huff, Reed, Smith, Brown, Ovechkin, & Pitts, 2018). There is an inspiratory phase characterized by enhanced contraction of the diaphragm and laryngeal abductor (Fontana & Lavorini, 2006). During the compressive phase, subglottic pressure is generated by simultaneous contraction of the laryngeal adductor and abdominal expiratory muscles (Pitts, 2014; Pitts, Rose, Mortensen, Poliacek, Sapienza, Lindsey, Morris, Davenport, & Bolser, 2013). During the expulsive phase, the glottis opens and abdominal expiratory contraction continues (Fontana & Lavorini, 2006). Resultant forceful air expulsion scrubs the airway to remove foreign material (Smith Hammond, Goldstein, Zajac, Gray, Davenport, & Bolser, 2001). Studies in humans have shown that disordered cough is predictive of dysphagia following stroke and Parkinson's Disease (Pitts, Bolser, Rosenbek, Troche, & Sapienza, 2008; Smith Hammond, Goldstein, Horner, Ying, Gray, Gonzalez-Rothi, & Bolser, 2009).

The expiration reflex is elicited by stimulation of the laryngeal mucosa, and is not preceded by an inspiratory phase (Fontana & Lavorini, 2006; Korpas & Jakus, 2000). Also known as laryngeal cough, this response functions to expel foreign material that has penetrated the larynx but remained above the subglottis (Korpas, 1972). Omission of the inspiratory phase of cough prevents aspiration of the penetrating material into the lower

airway (Korpas, Misik, & Kalocsayova, 1975). Cough and laryngeal cough during feeding are overt, audible signs of aspiration, however aspiration may also be silent (e.g., no cough) (Logemann, 1995; Rommel & Hamdy, 2016).

Breathing

Breathing occurs automatically and is essential for life (Chang, Strohlic, Williams, Umans, & Liberles, 2015; Horton, Segers, Nuding, O'Connor, Alencar, Davenport, Bolser, Pitts, Lindsey, Morris, & Gestreau, 2018; Kirchner, 1993; Laitman & Reidenberg, 1993; Pilowsky, 2014; Richter, 1982; Richter, Manzke, Wilken, & Ponimaskin, 2003). In terms of airflow, breathing occurs in two phases: Inspiration and expiration (German, Crompton, & Thexton, 1998; Kirchner, 1993). The main function of inspiration is to conduct air from the atmosphere to the lower airways for gas exchange (West, 1972). Expiration then conducts air from the lower airways to the atmosphere for removal of carbon dioxide from the body (Palkovic, Marchenko, Zuperku, Stuth, & Stucke, 2020; West, 1972). This ventilation requires rhythmic, coordinated activation of several upper airway, thoracic, and abdominal muscles (Pilarski, Leiter, & Fregosi, 2019).

Analysis of breathing-related electromyogram (EMG) activity shows that breathing has a three-phase motor pattern: Inspiration, early (stage I) expiration, and late (stage II) expiration (T. E. Dick, Y. Oku, J. R. Romaniuk, & N. S. Cherniack, 1993; T. Pitts, M. J. Rose, I. Poliacek, J. Condrey, P. W. Davenport, & D. C. Bolser, 2015). Early expiration is also known as post-inspiration (Richter, 1982). Inspiration is defined as the period from the onset of breathing-related diaphragm activity to the peak of diaphragm EMG amplitude (A. Huff, M. D. Reed, K. E. Iceman, D. R. Howland, & T. Pitts, 2020;

Pitts, Iceman, Huff, Musselwhite, Frazure, Young, Greene, & Howland, 2022). In general, expiration is defined as the period from peak diaphragm amplitude to the onset of subsequent diaphragm activation (Gautier, Remmers, & Bartlett, 1973; Richter, 1982). Early expiration is the period from peak diaphragm amplitude to diaphragm quiescence, and late expiration is the period from the offset of diaphragm offset to the onset of subsequent diaphragm activity (T. E. Dick, Y. Oku, J. R. Romaniuk, & N. S. Cherniack, 1993; Richter, 1982).

The upper aerodigestive tract is active during the inspiratory and expiratory phases of the respiratory cycle (Kirchner, 1993; Pilarski, Leiter, & Fregosi, 2019; Richter, 1982). During inspiration, the glottis is abducted by the posterior cricoarytenoid muscle, and the UES is tonically contracted (Pitts, 2014; T. Pitts, M. J. Rose, I. Poliacek, J. Condrey, P. W. Davenport, & D. C. Bolser, 2015). In this configuration, inspired air meets less resistance from the larynx than the UES, and flows through the glottis to the lower airways for gas exchange (Negus, 1942; Pilarski, Leiter, & Fregosi, 2019). During expiration, the larynx is partially adducted by the thyroarytenoid muscle. Partial laryngeal adduction functions as an expiratory braking mechanism that helps match ventilation to metabolic demand (Doty & Bosma, 1956; Kirchner, 1993). The pharyngeal musculature can be active during either phase of the respiratory cycle, but is generally active during expiration (Doty & Bosma, 1956; Pitts, 2014).

Breathing is regulated by a distributed brainstem network (Krohn, Novello, van der Giessen, De Zeeuw, Pel, & Bosman, 2023). The dorsal respiratory group and ventral respiratory group have classically been defined as key regions of respiratory control, although there is evidence that neurons distributed throughout the brainstem and pons

contribute to respiratory pattern generation (T. E. Dick, Y. Oku, J. R. Romaniuk, & N. S. Cherniack, 1993; Krohn, Novello, van der Giessen, De Zeeuw, Pel, & Bosman, 2023; Richter, 1982). The dorsal respiratory group is made up of neurons in the nucleus tractus solitarius (NTS) and the nearby reticular formation, in the approximate region of the dorsal swallowing group described by Jean (Jean, 2001b; Pitts & Iceman, 2023). The dorsal respiratory group is the initial site of integration of afferent inputs from the glossopharyngeal and vagus nerves (T. E. Dick, Y. Oku, J. R. Romaniuk, & N. S. Cherniack, 1993; Pitts & Iceman, 2023). The ventral respiratory group is in the ventrolateral brainstem and contains key circuits of respiratory rhythm generation, including the preBötzinger complex and the Bötzing complex (Iovino, Mutolo, Cinelli, Contini, Pantaleo, & Bongianini, 2019; Krohn, Novello, van der Giessen, De Zeeuw, Pel, & Bosman, 2023; Pitts & Iceman, 2023; Smith, Ellenberger, Ballanyi, Richter, & Feldman, 1991).

Several sensory systems contribute to respiratory drive. Central chemoreceptors detect changes in arterial carbon dioxide levels by sensing pH in the surrounding cerebrospinal fluid (Buchanan & Richerson, 2009; Dean & Nattie, 2010; Guyenet & Bayliss, 2015; Marina, Turovsky, Christie, Hosford, Hadjihambi, Korsak, Ang, Mastitskaya, Sheikhabaei, Theparambil, & Gourine, 2018; Palkovic, Marchenko, Zuperku, Stuth, & Stucke, 2020; West, 1972). Peripheral chemoreceptors in the carotid and aortic bodies are mainly sensitive to changes in arterial partial pressure of oxygen (Krohn, Novello, van der Giessen, De Zeeuw, Pel, & Bosman, 2023; West, 1972). Afferent information from receptors in the larynx, trachea, lungs and chest wall are sensitive to temperature, pressure, stretch, and irritants ascends to the brainstem and

modulates breathing (Fontana & Lavorini, 2006; A. Huff, M. D. Reed, K. E. Iceman, D. R. Howland, & T. Pitts, 2020; Huff, Reed, Smith, Brown, Ovechkin, & Pitts, 2018; Krohn, Novello, van der Giessen, De Zeeuw, Pel, & Bosman, 2023; Sampson & Eyzaguirre, 1964). The brainstem network of pre-motor respiratory neurons generates the breathing motor pattern, and distributes the breathing motor command to distinct motor pools: Phrenic motor neurons in the cervical spinal cord, which drive inspiratory diaphragm activity; motor neurons in the thoracic spinal cord that drive expiratory abdominal activity; and cranial motor neurons in the medulla, which drive inspiratory and expiratory upper airway activity (Pilarski, Leiter, & Fregosi, 2019; Pitts, Iceman, Huff, Musselwhite, Frazure, Young, Greene, & Howland, 2022; Poliacek, Jakus, Knocikova, Barani, Halasova, & Visnovcova, 2008; Richter, 1982).

Opioids

Opioid receptors couple to G-proteins, and are inhibitory G-protein coupled receptors (GPCR) (Waldhoer, Bartlett, & Whistler, 2004). GPCRs consist of a seven-segment transmembrane protein with an extra-cellular receptor site and intracellular heterotrimeric protein complex that enables signal transduction following ligand binding (Connor & Christie, 1999). Four types of opioid receptors have been described (μ , κ , δ and nociceptin/orphanin), but most clinically relevant effects of opioids occur through activation of the μ -opioid receptor (MOR) (Waldhoer, Bartlett, & Whistler, 2004; Williams, Ingram, Henderson, Chavkin, von Zastrow, Schulz, Koch, Evans, & Christie, 2013). Opioid receptors are expressed throughout the central nervous system, including brainstem regions known to be important for breathing and swallowing (Blivis, Mentis, O'Donovan M, & Lev-Tov, 2007; Bolser & DeGennaro, 1994; George,

Zastawny, Briones-Urbina, Cheng, Nguyen, Heiber, Kouvelas, Chan, & O'Dowd, 1994; Irnaten, Aicher, Wang, Venkatesan, Evans, Baxi, & Mendelowitz, 2003; Lang & Marvig, 1989; Palkovic, Marchenko, Zuperku, Stuth, & Stucke, 2020; Zhuang, Gao, Gao, & Xu, 2017).

Opioids are commonly prescribed for acute or chronic pain management, post-operative pain control, or opioid maintenance therapy (Bateman, Saunders, & Levitt, 2023; Koehl, Zimmerman, & Bridgeman, 2019; Roughan & Flecknell, 2002).

Respiratory depression is a known complication of opioid use, and the main cause of death following overdose. Opioids also depress the gastrointestinal and immune systems (Bateman, Saunders, & Levitt, 2023; Foley, 1993; Roy, Ninkovic, Banerjee, Charboneau, Das, Dutta, Kirchner, Koodie, Ma, Meng, & Barke, 2011). Moreover, opioids depress cough and airway protective reflexes, and have been linked to esophageal dysfunction and aspiration (Patel & Vaezi, 2018; Patel, Goss, Hayat, Tombazzi, Naik, Slaughter, Aslam, Sarker, Higginbotham, & Vaezi, 2022; Savilampi, Ahlstrand, Magnuson, Geijer, & Wattwil, 2014; Steffens, Sung, Bastian, Edelman, Brackett, & Gunderson, 2020; Tagaito, Isono, & Nishino, 1998).

The opioid epidemic in the United States has persisted for over two decades, and opioid-related deaths have spiked dramatically since the COVID-19 pandemic (Bateman, Saunders, & Levitt, 2023; Cuadros, Branscum, Moreno, & MacKinnon, 2023; Flanagan, Wysong, Ramey, & Vallier, 2018; Upp & Waljee, 2020). Most opioid-related deaths occur due to opioid-induced respiratory depression (Bateman, Saunders, & Levitt, 2023; Ramirez, Burgraff, Wei, Baertsch, Varga, Baghdoyan, Lydic, Morris, Bolser, & Levitt, 2021). Aspiration pneumonia is also a cause of mortality following opioid use (Eizadi-

Mood, Yaraghi, Sharifian, Feizi, Hedaiaty, & Sabzghabae, 2015; Nicolakis, Gmeiner, Reiter, & Seltenhammer, 2020; Tabatabaei, Dorvashy, Soltani, Samsamshariat, Meamar, & Sabzghabae, 2021). Much research has been dedicated to opioid-induced respiratory depression, though its mechanisms remain unclear (Palkovic, Marchenko, Zuperku, Stuth, & Stucke, 2020). Due to a paucity of study, effects of opioids on pharyngeal swallow function are largely unknown. The studies in this dissertation provide a detailed investigation of pharyngeal swallow regulation, and systematically evaluate the effects of opioids on pharyngeal swallow using clinical-translational and basic science models.

CHAPTER 2

RAPID ACTIVATION OF ESOPHAGEAL MECHANORECEPTORS ALTERS THE PHARYNGAL PHASE OF SWALLOW: EVIDENCE FOR INSPIRATORY ACTIVITY DURING SWALLOW

¹Swallow is a complex behavior that consists of three coordinated phases: oral, pharyngeal, and esophageal. Esophageal distension (EDist) has been shown to elicit pharyngeal swallow, but the physiologic characteristics of EDist-induced pharyngeal swallow have not been specifically described. We examined the effect of rapid EDist on oropharyngeal swallow, with and without an oral water stimulus, in spontaneously breathing, sodium pentobarbital anesthetized cats ($n = 5$). Electromyograms (EMGs) of activity of 8 muscles were used to evaluate swallow: mylohyoid (MyHy), geniohyoid (GeHy), thyrohyoid (ThHy), thyropharyngeus (ThPh), thyroarytenoid (ThAr), cricopharyngeus (upper esophageal sphincter: UES), parasternal (PS), and costal diaphragm (Dia). Swallow was defined as quiescence of the UES with overlapping upper airway activity, and it was analyzed across three stimulus conditions: 1) oropharyngeal water infusion only, 2) rapid esophageal distension (EDist) only, and 3) combined

¹Frazure ML, Brown AD, Greene CL, Iceman KE, Pitts T. (2021). Rapid activation of esophageal mechanoreceptors alters the pharyngeal phase of swallow: Evidence for inspiratory activity during swallow. *PLOS ONE* 16(4): e0248994. doi:10.1371/journal.pone.0248994.

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stimuli. Results show a significant effect of stimulus condition on swallow EMG amplitude of the mylohyoid, geniohyoid, thyroarytenoid, diaphragm, and UES muscles. Collectively, we found that, compared to rapid cervical esophageal distension alone, the stimulus condition of rapid distension combined with water infusion is correlated with increased laryngeal adductor and diaphragm swallow-related EMG activity (schluckatmung), and post-swallow UES recruitment. We hypothesize that these effects of upper esophageal distension activate the brainstem swallow network, and function to protect the airway through initiation and/or modulation of a pharyngeal swallow response.

Introduction

Swallow is an important, complex behavior, controlled by a pattern generator in the medulla (Jean, 2001a, 1984; Kessler & Jean, 1985). A robust swallow pattern consists of three coordinated phases that propel the bolus in a rostral to caudal direction: oral, pharyngeal and esophageal (Atkinson, Kramer, Wyman, & Ingelfinger, 1957; T. Dick, Y. Oku, J. Romaniuk, & N. Cherniack, 1993; Ertekin & Palmer, 2000; Jean, 1984; Martin, Logemann, Shaker, & Dodds, 1994; Negus, 1948; Weerasuriya, Bieger, & Hockman, 1980). The pharyngeal phase of swallow is characterized by hyolaryngeal elevation, laryngeal adduction, and pharyngeal constriction, with concurrent relaxation of the upper esophageal sphincter (UES) and activation of inspiratory muscles (i.e. schluckatmung, or “swallow breath”); the pattern of muscle activation is rapid and stereotypic. (German, Crompton, & Thexton, 2009; Thexton, Crompton, & German, 2007b; Thexton, Crompton, Owerkowicz, & German, 2009). The sequential activation of the muscles involved in swallow is tightly coordinated to regulate pressures in the thoracic cavity and

upper airway (McConnel, Guffin, & Cerenko, 1991; McConnel, Guffin, Cerenko, & Ko, 1992; T. Pitts, M. Rose, I. Poliacek, J. Condrey, P. W. Davenport, & D. Bolser, 2015). These pressures must be highly regulated to control the passage of a bolus into the esophagus or air into the lungs via a dual valve system (Pitts, Rose, Mortensen, Poliacek, Sapienza, Lindsey, Morris, Davenport, & Bolser, 2013). In order for a bolus to enter the esophagus, the UES must relax, and the tongue and pharyngeal muscles activate to propel the bolus. This is aided by the diaphragm, such that negative intra-thoracic pressure paired with positive pressure in the oropharynx produces a pressure differential to optimize proper bolus movement into the esophagus. This must be accomplished while avoiding aspiration into the airway (T. Pitts, I. Poliacek, M. J. Rose, M. D. Reed, J. A. Condrey, H.-W. Tsai, G. Zhou, P. W. Davenport, & D. C. Bolser, 2018; Pitts, Rose, Mortensen, Poliacek, Sapienza, Lindsey, Morris, Davenport, & Bolser, 2013; Teresa Pitts, Melanie J Rose, Ivan Poliacek, Jillian Condrey, Paul W Davenport, & Donald C Bolser, 2015; Spearman, Poliacek, Rose, Bolser, & Pitts, 2014).

The oropharyngeal phase of swallow strongly influences the esophageal phase, either via direct excitation/disinhibition, by more diffuse neuromodulation, and/or afferent feedback (Cook, Dodds, Dantas, Massey, Kern, Lang, Brasseur, & Hogan, 1989; Goyal & Cobb, 1981; Goyal, Martin, Shapiro, & Spechler, 1993; Richter, 2001; Sanders, Kraus, Aviv, Racenstein, & Biller, 1987; Wang, Kadekda, Kahrilas, & Hirano, 2005). These afferents include oropharyngeal receptors, laryngeal/thoracic receptors, pulmonary stretch receptors, esophageal stretch receptors, and possibly thoracic-abdominal receptors (traveling through spinal dorsal root ganglia) (Ali, Laundl, Wallace, deCarle, & Cook, 1996; T. Dick, Y. Oku, J. Romaniuk, & N. Cherniack, 1993; Ezure, Oku, & Tanaka,

1993; Hiss, Treole, & Stuart, 2001; Huff, Reed, Smith, Brown, Ovechkin, & Pitts, 2018; Logemann, Pauloski, & Colangelo, 1998; Miller, Proud, & Behrle, 1952; Ootani, Umezaki, Shin, & Murata, 1995; Pommerenke, 1928; Rademaker, Pauloski, Colangelo, & Logemann, 1998; Schultz, Perlman, & VanDaele, 1994; Spearman, Poliacek, Rose, Bolser, & Pitts, 2014; Storey, 1968; Troche, Sapienza, & Rosenbek, 2008; Weerasuriya, Bieger, & Hockman, 1980; K. Yamamura, J. Kitagawa, M. Kurose, S. Sugino, H. Takatsuji, R.M. Mostafaezur, H.M. Zakir, & Y. Yamada, 2010). Motor contraction during swallow must adapt to the size of the bolus, based on afferent peripheral feedback. Distension of the pharynx by a bolus modulates both the oropharyngeal and esophageal phases of swallow (Lang, Medda, & Shaker, 2001). It is also well-reported that esophageal afferents modulate the esophageal phase of swallow, and in general, rapid esophageal distension (EDist) by solid bolus, air bolus, or balloon inflation makes the esophageal phase of swallow more powerful and prolonged (Enzmann, Harell, & Zboralske, 1977; Hwang, 1954; Lang, Medda, Babaei, & Shaker, 2014; Lang, Medda, Jadcherla, & Shaker, 2012; Lang, Medda, & Shaker, 2001). However, less is known about the effect of rapid esophageal distension on the pharyngeal phase of swallow, especially how it may alter diaphragm activity. Such effects would have the potential to induce or modulate subsequent/repetitive pharyngeal swallow in response to a bolus in the esophagus.

Several distinct reflexes that result from distension of the upper portion of the esophagus have been thoroughly described by Shaker's group (Lang, Haworth, Medda, Forster, & Shaker, 2016; Lang, Medda, Jadcherla, & Shaker, 2012; Lang, Medda, Jadcherla, & Shaker, 2016; Lang, Medda, Shaker, & Jadcherla, 2018; Wank & Neuhuber,

2001). The authors have divided these reflexes into two main sets: those that are activated by slow distension, and those that are activated by rapid distension. Slow esophageal distension activates the UES and esophageal peristalsis; these reflexes are mediated by muscular tension receptors. Rapid esophageal distension relaxes the UES, stimulates laryngeal adductor and elevator muscles, and stimulates some esophageal contractions; these reflexes are mediated by rapidly adapting mucosal touch receptors (Babaei, Dua, Naini, Lee, Katib, Yan, Hoffmann, & Shaker, 2012; Lang, Medda, Babaei, & Shaker, 2014; Lang, Medda, Jadcherla, & Shaker, 2012; Lang, Medda, Jadcherla, & Shaker, 2016; Lang, Medda, & Shaker, 2001; Szczesniak, Fuentealba, Burnett, & Cook, 2008) and have previously been categorized as belch and its component reflexes. These reported reflexes clearly indicate that esophageal sensory input can affect muscles involved in the pharyngeal phase of swallow, but these studies did not aim to specifically test the pharyngeal phase of swallow itself. Esophageal afferent information travels via the vagus nerve to the nucleus tractus solitarius (NTS) in the brainstem, where interneurons (some of which are premotor neurons) influence other esophageal or non-esophageal neurons involved in swallow. The esophageal motor nuclei are nearby in the nucleus ambiguus (NA) and the dorsal motor nucleus of the vagus.

Disorders of the pharyngoesophageal segment include esophageal web, cricopharyngeal bar, and generalized narrowing (Logan, Gawlik, Aden, Jarvis, & Dion, 2020). Different bolus size and viscosity change the distension required to move the bolus from the pharynx into the esophagus. While these disorders have been well described, their mechanistic effect on the activation of swallow, and the alteration of subsequent swallows in a series is not known. The current study tested the hypothesis that

activation of esophageal mechanoreceptors by rapid distension modulates the pharyngeal phase of swallow. This allows for direct comparison of the effects of esophageal distension, water infusion, and the combination of distension and water infusion on upper airway and diaphragm EMG activity during swallow.

Methods

Experiments were performed on 5 spontaneously breathing adult male cats (3.8 ± 0.2 kg, age 1-2 years). The protocol was approved by the University of Louisville Institutional Animal Care and Use Committees (IACUC), in compliance with the National Institutes of Health Guidelines. The animals were initially anesthetized with sodium pentobarbital (Lundbeck, Inc., Deerfield, IL) (35 mg/kg i.v.); supplementary doses were given as needed ($1-3$ mg/kg i.v.). The right femoral artery and vein were cannulated to monitor i.a. blood pressure and administer i.v. fluids, and a tracheostomy was performed. Physiologic levels of end-tidal CO_2 ($4-4.5\%$; Datax Engstrom; Datax Ohmeda, Inc; Madison, WI), body temperature (36.2 ± 0.7 °C; Homeothermic Blanket Control Unit; Harvard Apparatus; Holliston, MA), and arterial blood gas composition (i-STAT1; Abaxis; Union City, CA) were continually monitored and maintained (Pitts, Rose, Mortensen, Poliacek, Sapienza, Lindsey, Morris, Davenport, & Bolser, 2013). Arterial blood gas composition was measured once per hour. Mean \pm standard deviations for pH (7.4 ± 0.1), base excess (-4.3 ± 3.6 mmol/L), PCO_2 (30.9 ± 6.1 mmHg), PO_2 (105 ± 14.5 mmHg), HCO_3 (20.1 ± 3.4 mmol/L), and lactate (2.1 ± 4.3 mmol/L) were calculated by pooling data across experiments.

Electromyograms (EMGs) were recorded using bipolar insulated fine wire electrodes (A-M Systems stainless steel #791050) according to the technique of

Basmajian and Stecko (Basmajian & Stecko, 1962). Eight muscles were used to evaluate swallow: mylohyoid, geniohyoid, thyrohyoid, thyropharyngeus, thyroarytenoid, upper esophageal sphincter (UES), parasternal, and costal diaphragm. The digastric muscles were dissected away from the surface of the mylohyoid and electrodes were placed on the left mylohyoid. A small horizontal incision was made at the rostral end of the right mylohyoid followed by an incision following the midline for approximately 1cm to reveal the geniohyoid underneath. Electrodes were placed 1cm from the caudal insertion of the right geniohyoid muscle. The thyroarytenoid electrodes were inserted through the cricothyroid window into the anterior portion of the left vocal fold, which were visually inspected post-mortem. Rotation of the larynx and pharynx counterclockwise revealed the superior laryngeal nerve, which facilitated placement of the left thyropharyngeus muscle electrodes. The thyropharyngeus 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 thyroid cartilage within 5 mm of the rostral insertion of the muscle. To place the electrodes within the cricopharyngeus muscle, the larynx and pharynx were rotated counterclockwise to reveal the posterior aspect of the larynx. The tissue was palpated for the edge of the cricoid cartilage and electrodes were placed just cranial to the edge of this structure (for a bilateral recording). The left thyrohyoid electrodes were inserted approximately 1 cm rostral to the attachment to the thyroid cartilage. The sternal diaphragm was placed by elevation of the sternum and the electrodes placed along the dorsal surface.

Swallow was defined as quiescence of the UES with overlapping upper airway activity. Esophageal pressure was measured by placing a balloon catheter connected to a

pressure transducer. For distension and pressure recordings, a balloon attached to a thin polyethylene catheter (outer diameter 0.5-1.0 mm) attached to a syringe was placed into the upper esophagus through the mouth and attached to a pressure transducer (TA-100; CWE, Inc; Ardmore, PA). At least 1 hour was allowed between placement of the esophageal catheter and start of stimuli trials. Animals were euthanized with an overdose of sodium pentobarbital (3 mg/kg i.v.) until respiratory cessation, followed by 3cc i.v. of saturated potassium chloride until termination of cardiac activity.

Stimulus trials

Esophageal mechanoreceptor activation was produced by rapidly inflating the esophageal balloon with 3cc of air in less than 1 second, then maintaining this pressure for 5 seconds. Swallow was induced by infusing 3cc of water into the oropharynx via 1-inch-long thin polyethylene catheter (outer diameter 0.5-1.0 mm) placed at the back of the tongue (rostral to the faucial pillars). Each animal was subjected to three different stimulus conditions with at least 1 minute between each trial: 1) water only; 2) esophageal distension (EDist) only; and 3) combination: the esophagus was distended by balloon inflation for 5 seconds, and water was infused at the 2.5 second mark. Fig 2-1 displays representative swallows during each condition.

Data processing and statistical analysis

EMGs were recorded and analyzed using “Spike 2” Version 7 (Cambridge Electronic Design, United Kingdom). Moving averages of EMGs were integrated with a 20 ms time constant (Fig 2-1). Durations were measured as the time between the onset and the point where the signal returned to baseline (ms). EMG amplitude measures were normalized to the largest swallow and are presented as percent of maximum. Pressure

transducers were calibrated prior to each experiment, and here are presented as recorded. For all figures waveforms were exported to CorelDRAW 2020 (v22.1.1.523),

To assess swallow-breathing coordination, a Wilcoxon Signed Rank Test was used. An assigned coding system was used for the breathing phase in which the swallow occurred: inspiration (I; start to peak diaphragm activity) as “1”; early expiration (Yield (A. Huff, M. D. Reed, K. E. Iceman, D. R. Howland, & T. Pitts, 2020) or E1; peak to end diaphragm activity) as “2”; and mid/late-expiration (E2; end of diaphragm activity to start of next breath diaphragm activity) as “3”. For all tests a difference was considered significant if the *p*-value was less than or equal to 0.05.

A mean \pm standard deviation (SD) was calculated for each animal, and then averaged for each condition across animals (Table 2-1). Student t-tests or ANOVA were performed when appropriate. Pearson’s product moment correlations (*r*) were calculated comparing all amplitude and duration measures to determine relationships between the dependent variables (Table 2-2). Additionally, root mean square (RMS), a measurement of motor unit recruitment, was calculated using the following transfer equation: $V_{rms} = \sqrt{AVG}(V_{emg}^2)$, where V_{rms} is the voltage input of the EMG signal and AVG is the averaging time constant (75ms), as described by Sieck and Fournier (Sieck & Fournier, 1989) (Fig 2).

Results

Fig 2-1 illustrates anatomical placement of the recorded EMGs as well as example traces of swallows produced from each stimulus condition. The representative EMGs are aligned with the rostral-caudal direction of bolus flow. Respiratory cycles are displayed before and after each trial and the respiratory phase of each swallow is noted at the

bottom of the figure. Although portions of the thyropharyngeus and cricopharyngeus muscles both participate as part of the inferior pharyngeal constrictor and UES, we placed the electrodes for the thyropharyngeus to be representative of the inferior pharyngeal constrictor and the cricopharyngeus to be representative of the UES activity.

Each stimulus (water, EDist and combined stimulus) was effective in eliciting swallow. An average of 12.2 ± 3.4 stimuli were administered per animal. An average of 20.2 ± 7.3 total swallows were elicited per animal. Across all conditions 85% (52/61) of swallows occurred during expiration; 3% (2/61) occurred during inspiration; 3% (2/61) occurred during the transition from expiration-inspiration; and 8% (5/61) occurred during the transition from inspiration-expiration. There were no significant changes in swallow-breathing coordination across conditions.

Table 2-1 summarizes EMG amplitude (percent of maximum) and duration (ms) mean \pm SD for each muscle and condition, and results of the statistical comparisons. There were increases in EMG amplitude (% of maximum) during *water infusion compared to rapid EDist* in the mylohyoid (26%), geniohyoid (73%) and thyrohyoid (18%; approaching significant), and a significant decrease in UES amplitude (29%). There were increases in EMG amplitude (% of maximum) during *combined stimulus trials compared to rapid EDist* in the mylohyoid (23%; approaching significance), geniohyoid (88%), thyroarytenoid (40%) and the diaphragm (56%). Combined stimulus trials also significantly increased UES activity compared to water infusion by 45%.

There were increases in burst duration during *water infusion compared to rapid EDist* in the mylohyoid (16%) and geniohyoid (25%; approaching significance), and an increase in laryngeal elevation time by 17%. There was an increase in burst duration

during *combined stimulus trials compared to rapid EDist* in the geniohyoid (22%) and thyroarytenoid (57%), and an increase in total swallow time by 22% (approaching significance). Combined stimulus trials also increased thyropharyngeus duration by 21% and increased thyroarytenoid duration by 26% compared to water infusion.

Fig 2-2 illustrates RMS₇₅ analysis on UES activity with rapid esophageal distension and during a combined stimulation trial (Fig. 2-2a), and relative change in RMS₇₅ across conditions in the five animals (Fig. 2-2b). The recording in the figure displays an esophago-UES relaxation reflex, but this was not evoked by all stimuli or in all animals. It can also appear to resemble a very small swallow with activity of thyroarytenoid and thyropharyngeus muscles. There was a significant effect of condition on the RMS₇₅ of the UES activity [$F(2,12) = 17.248, p < 0.001$]; post-hoc testing revealed that the combined stimuli produced larger EMG recruitment than distension alone (Fig. 2-2b) and post-swallow activity in response to water ($p = 0.001; p < 0.001$ respectively).

Table 2-2 is a matrix showing all Pearson Product Moment Correlations for EMG amplitude and duration measures. Due to the relatively small amplitude and short duration of the swallows induced by esophageal distension, there were stronger correlations between EMG amplitude and duration than those reported in our previous publications (Alyssa Huff, Mitchell D Reed, Kimberly E Iceman, Dena R Howland, & Teresa Pitts, 2020; Huff, Reed, Smith, Brown, Ovechkin, & Pitts, 2018; T. Pitts, M. Rose, I. Poliacek, J. Condrey, P. W. Davenport, & D. Bolser, 2015; Spearman, Poliacek, Rose, Bolser, & Pitts, 2014) .

Discussion

Upper esophageal afferent feedback is an important factor in ongoing airway protection risk assessment. Our results confirm that rapid distension of the cervical esophagus (EDist) produces swallow, as shown by Lang, et al (Lang, Medda, Jadcherla, & Shaker, 2016), but also demonstrate that swallows induced by EDist have significantly reduced hyoid/laryngeal elevator EMG amplitude and duration when compared to swallows induced by oropharyngeal water stimulation and shorter laryngeal elevation time (Fig. 2-1; Table 2-1). Additionally, when the conditions of rapid EDist and water infusion were combined, the thyroarytenoid and diaphragm (schluckatmung) EMG activity increased and laryngeal closure time increased.

The muscular makeup of the esophagus varies by species. The esophagus in dogs, rodents, and sheep is composed entirely of striated muscle, but in cats and primates, the upper (proximal) portion of the esophagus is striated and controlled by cranial motor neurons, and the lower (distal) portion is smooth and controlled by the autonomic system (Goyal & Paterson, 1989; Jean, 2001a). In humans, the striated portion comprises the upper one-third of the esophagus, which transitions to incorporate more smooth muscle fibers, with the lower two-thirds consisting of entirely smooth muscle (Helleman, Vantrappen, Valembois, Janssens, & Vandenbroucke, 1968). In cats, the upper two-thirds is striated (Lang, Medda, & Shaker, 2001). The striated portion is innervated by motor neurons from the NA, while the smooth portion by is innervated by autonomic preganglionic neurons from the dorsal motor nucleus of the vagus that synapse with postganglionic motor neurons in the esophageal myenteric plexus (Collman, Tremblay, &

Diamant, 1993, 1992). Unlike the oropharyngeal phase of swallow, the esophageal phase is not an all-or-none activity, suggesting a difference in underlying central mechanisms.

Esophageal receptors have been extensively studied for secondary peristalsis (esophageal contraction that is experimentally induced in the absence of the oropharyngeal phase of swallow) (Enzmann, Harell, & Zboralske, 1977; Hwang, 1954; Lang, Medda, Jadcherla, & Shaker, 2012; Lang, Medda, Jadcherla, & Shaker, 2016; Lang, Medda, & Shaker, 2001). In the absence of swallow, activation of esophageal afferents alone stimulates esophageal secondary peristalsis; all esophageal peristalsis is secondary to esophageal stimulation and may therefore require at least a small esophageal bolus (Clerc, 1984; Clerc & Mei, 1983; Falempin, Mei, & Rousseau, 1978). When initiated from the upper (striated) portion of the esophagus, secondary peristalsis is controlled centrally, as evidenced by the fact that thoracic vagotomy (to sever afferents but preserve motor efferents to this portion) eliminates the reflex (Wank & Neuhuber, 2001). When initiated from the lower (smooth) portion of the esophagus, secondary peristalsis is controlled peripherally, as demonstrated by the fact that a peristaltic contraction can be evoked in an esophageal smooth muscle segment in the absence of any neural connection with the brainstem (Goyal & Paterson, 1989). For the primary peristalsis portion of swallow, the pattern in the smooth muscle esophagus is likely dependent on complex interactions between central and peripheral mechanisms (Gidda & Goyal, 1984; Lang, Medda, & Shaker, 2001; Vanek & Diamant, 1987). In species with a partial smooth muscle esophagus (including cats and humans), a swallowing wave in the esophagus can alter the subsequent esophageal wave (Vanek & Diamant, 1987), and afferent peripheral feedback during swallow allows esophageal smooth muscle peristaltic

contractions to adapt to the size of the bolus (Lang, Medda, Babaei, & Shaker, 2014). Indeed, swallow produces sequential action potentials in vagal preganglionic efferents (Gidda & Goyal, 1984) that presumably control the smooth muscle portion of the esophagus.

The sensory pathway of EDist-evoked pharyngeal activation is vagal, via the superior laryngeal nerve (SLN), and the recurrent laryngeal nerve caudal to the cricoid cartilage, but not the cervical vagus (Collman, Tremblay, & Diamant, 1992; Lang, Medda, Jadcherla, & Shaker, 2012). There are both rapidly and slowly adapting receptors in the esophageal mucosa (Lang, Medda, Jadcherla, & Shaker, 2016). Afferent innervation from these receptors is carried by myelinated A and unmyelinated C type fibers (Lennerz, Dentsch, Bernardini, Hummel, Neuhuber, & Reeh, 2007; Page & Blackshaw, 1998). These fibers are carried by the vagus nerve, project to the nodose ganglion (Collman, Tremblay, & Diamant, 1992; Lang, Medda, Jadcherla, & Shaker, 2012; Wank & Neuhuber, 2001), and end in the centralis subdivision of the nucleus tractus solitarius (NTS), which also contains esophageal interneurons, some of which are premotor neurons (Altschuler, Bao, Bieger, Hopkins, & Miselis, 1989; Wank & Neuhuber, 2001). Activation of esophageal afferents by balloon inflation in the upper esophagus stimulates discharge of esophageal interneurons in the NTS (Jean, 1972). Whether any of these esophageal neurons specifically project to oropharyngeal regions is unknown, however, they do converge in the NTS, where sensory information from other regions including the oral, pharyngeal, and laryngeal cavities is pooled and distributed to the swallow pattern generator.

Esophageal stimulation studies that used immunoreactivity of the immediate early gene c-Fos as a marker of neuronal activation showed activity in several brainstem regions, including those known to mediate swallow (Lang, Medda, & Shaker, 2011, 2010). Acid perfusion of the upper esophagus, which stimulated belch and/or other pharyngeal responses, activated most of the subnuclei of the NTS, particularly the intermediate, interstitial, and ventrolateral nuclei (Lang, Medda, & Shaker, 2010). Rapid balloon distension of the esophagus stimulated the same reflexes, and activated the same regions, in particular the caudal subnucleus of the NTS (Lang, Medda, & Shaker, 2011). In the cat, these subnuclei are the site of termination of afferents from the trachea (Kalia & Mesulam, 1980a, 1980b), and are also the primary pharyngeal premotor nuclei in rats (Bao, Wiedner, & Altschuler, 1995; Barrett, Bao, Miselis, & Altschuler, 1994). In contrast, acid perfusion of the lower esophagus, which stimulated secondary peristalsis, activated different subnuclei of the NTS, particularly the central subnucleus (Lang, Medda, & Shaker, 2010), as did slow balloon distension (Lang, Medda, & Shaker, 2011). The (pre)motor regions of the dorsal motor nucleus of the vagus and the nucleus ambiguus (NA) that were activated by the two categories of reflexes also differed. Rapid distension of the esophagus activated NA regions that contain motor neurons for muscles of the pharynx (Collman, Tremblay, & Diamant, 1993; Holstege, Graveland, Bijker-Biemon, & Schuddeboom, 1983; van Loveren, Saunders, Cassini, & Keller, 1985; Yoshida, Miyazaki, Hirano, Shin, Totoki, & Kanaseki, 1981), larynx (Kalia & Mesulam, 1980a, 1980b; Pasaro, Lobera, Gonzalez-Baron, & Delgado-Garcia, 1983; Yoshida, Miyazaki, Hirano, Shin, Totoki, & Kanaseki, 1981) and upper airway (Holstege, Graveland, Bijker-Biemon, & Schuddeboom, 1983).

Activation of esophageal receptors can stimulate a variety of behaviors including belch in order to prevent reflux of gastric contents, or to create a strong typical swallow and primary peristalsis pattern (Enzmann, Harell, & Zboralske, 1977; Hwang, 1954; Lang, Haworth, Medda, Forster, & Shaker, 2016; Lang, Medda, Jadcherla, & Shaker, 2012; Lang, Medda, Jadcherla, & Shaker, 2016; Lang, Medda, & Shaker, 2001; Lang, Medda, Shaker, & Jadcherla, 2018; Madsen, Wallin, Boesby, & Larsen, 1983). The main EDist-induced reflexes have been divided into two groups based on their responses to slow or rapid distension of the upper esophagus, although other stimuli may also activate them as well (Lang, Medda, & Shaker, 2001). One distinguishing factor between the groups of slow and rapid EDist-induced reflexes is the activity of the UES; UES relaxation and UES contraction/peristalsis are mediated differently. The cat esophagus contains mucosal rapidly adapting touch receptors (Harding & Titchen, 1975; Mei, 1970), and the belch response including UES relaxation is mediated by these receptors (Lang, Medda, & Shaker, 2001). Slowly adapting muscular tension receptors mediate UES contraction and peristalsis. Lidocaine applied to the esophageal mucosa inhibits or blocks UES relaxation, but not contraction (Lang, Medda, Jadcherla, & Shaker, 2016; Lang, Medda, & Shaker, 2001). Similarly, capsaicin (which selectively affects mucosal but not muscularis receptors) activates swallowing initially, then desensitizes the swallow response to rapid EDist, raising the threshold required for swallow initiation (Lang, Medda, & Shaker, 2001). When the mucosal layer was completely removed from the esophagus, rapid EDist-induced swallow was blocked, but UES contraction and secondary peristalsis were not (Szczesniak, Fuentealba, Burnett, & Cook, 2008). Systemic administration of the GABA_B receptor agonist baclofen produced the same

results, and also inhibited water-induced swallow and laryngeal adduction (Lang, Medda, & Shaker, 2001; Tsujimura, Sakai, Suzuki, Ujihara, Tsuji, Magara, Canning, & Inoue, 2017). Given these results, rapid EDist must primarily influence the oropharyngeal phase of swallow rather than the esophageal phase. Rapid EDist produces similar reflexes as the EDist-evoked oropharyngeal phase of swallow and accompanying UES relaxation reflex in the current study, therefore we would group these reflexes together.

The pharyngeal swallow pattern generator receives peripheral sensory input from vagal afferents including oropharyngeal receptors, laryngeal receptors, thoracic receptors, pulmonary stretch receptors, esophageal stretch receptors, and possibly thoracic-abdominal receptors [6-7, 17, 25-37]. The swallow sequence is thought to begin first with a synchronized inhibition across all muscles involved, under high peripheral feedback conditions (Doty & Bosma, 1956; Goyal & Cobb, 1981; Jean, 2001a; Sifrim, Janssens, & Vantrappen, 1994, 1992; Vanek & Diamant, 1987). This “deglutitive inhibition” is then removed in a rostrocaudal direction to allow a precise sequential wave of swallow muscle contractions. This activity travels quickly through the oropharynx to arrive at the UES. The esophagus, having also been inhibited at the start of the swallow sequence, remains inhibited during the oropharyngeal stage, but is excited once the oropharyngeal phase is completed. This inhibition of the esophagus involves the brainstem, at least at the onset of the synchronized inhibitory burst, but it may also be mediated by activation of oropharyngeal and/or laryngeal afferents (Lang, Medda, Babaei, & Shaker, 2014). Indeed, stimulation of the superior laryngeal nerve or inflation of a pharyngeal balloon also inhibit the esophageal stage (likely by a GABA-mediated mechanism) (Jean, 2001a, 1984, 1972; Wang & Bieger, 1991).

Studies of repeated rhythmic swallow show that swallows within a bout become stronger across repetitions, both in duration and amplitude. The last swallow in a bout will allow the completion of esophageal peristalsis (Jean, 2001a). While esophageal peristalsis is inhibited during the repetitive swallow bout due to deglutitive inhibition, rhythmic swallowing ultimately facilitates esophageal peristalsis after the last swallow occurs (Vanek & Diamant, 1987). Peripheral sensory activation decreases the velocity of esophageal peristalsis, making the duration of the whole esophageal phase of swallow longer, and the muscular contraction more powerful (Jean, 2001a, 1984, 1972). Whether that enhancement is caused by facilitatory or disinhibitory mechanisms is unknown.

Lang, Medda, Shaker, and colleagues (Lang, Medda, Jadcherla, & Shaker, 2016) found that EDist can induce pharyngeal swallow, and that in general, stronger and more proximal distensions are most likely to activate a pharyngeal swallow response (Lang, Medda, Jadcherla, & Shaker, 2016). This was also confirmed in a recent human study of intra-esophageal fluid injections, where swallows were most effectively induced by faster injections, larger fluid volumes, and when the injections were delivered to the upper portion of the esophagus (Taniguchi, Aoyagi, Matsuo, Imaeda, Hirimuta, & Saitoh, 2020). Interestingly, even with upper esophageal distension there appeared to be no increase in UES tone in these subjects. The present study further confirms that EDist can elicit pharyngeal swallow, and also compares swallow physiology across pharyngeal (water infusion), esophageal (balloon distension), and combined stimulus conditions. Like Shaker's group (Lang, Medda, Jadcherla, & Shaker, 2016), we determined activation of pharyngeal swallow through EMG recordings of pharyngeal and hyoid muscles. We also obtained EMG recordings of the diaphragm, which allowed for

description of inspiratory muscle activity during EDist-induced swallow (i.e. schluckatmung). Distinct types of motor units innervate muscle fibers which vary in metabolic and contractile properties. Type I (slow-twitch) fibers produce low voltage signatures and are fatigue resistant, and Type IIB (fast-twitch) fibers are involved in rapid and phasic activity, produce higher voltage signatures, and are prone to fatigue. As force increases, these are recruited in a specific order from smallest to largest (Henneman Size Principle (Henneman, 1957)). Studies from Sieck and colleagues (Mantilla, Seven, Zhan, & Sieck, 2010; Seven, Mantilla, & Sieck, 2014; Seven, Mantilla, Zhan, & Sieck, 2013; Sieck & Fournier, 1989) have used RMS to estimate central drive to the diaphragm, and demonstrate that the recruitment of motor units correlates well with the period of nonstationarity at the onset of the EMG signal. This is usually less than 75 ms, so we also employed the RMS₇₅ EMG analysis as a representation of central drive (Fig 2-2) (Seven, Mantilla, & Sieck, 2014; Seven, Mantilla, Zhan, & Sieck, 2013). The current data support the hypothesis that oropharyngeal stimulation combined with rapid distension increased drive to the upper esophageal sphincter (cricopharyngeus); we believe this reduces airway protection risk by limiting potential reflux.

Our results show that EDist alone elicits a pharyngeal swallow characterized by: decreased amplitude and duration of hyolaryngeal (mylohyoid and geniohyoid) and thyroarytenoid muscle contractions; decreased amplitude of diaphragm EMG; and decreased duration of laryngeal elevation. In contrast, when the swallow stimulus was stronger (water plus EDist; combined stimulation), the schluckatmung (diaphragm EMG) was characteristically ballistic (larger motor units recruited with the potential for larger force production) (Zehr & Sale, 1994), and the laryngeal adductors produced a longer and

stronger contraction. We hypothesize that this functions to protect the glottis from aspiration in the condition of negative intrathoracic pressure created by the increased inspiratory effort. We recently reported that electrical stimulation of the SLN inhibits swallow-related inspiratory activity (schluckatmung) (King, Shen, Musselwhite, Huff, Reed, Poliacek, Howland, Dixon, Morris, Bolser, Iceman, & Pitts, 2020), suggesting that SLN afferent feedback may modulate the swallow pattern to protect the airway from an incoming bolus. Combined with our current findings, this suggests that location-specific activation of SLN afferent modulations the swallow motor pattern to increase airway protection during aberrant feeding conditions.

Additionally, we found that hyolaryngeal elevator and pharyngeal muscles were strongly activated as a group. This was evidenced by amplitude correlations to each other, duration correlations to each other, and amplitude and duration correlations with themselves and each other. The amplitude of these muscles was also positively correlated to the amplitude of the laryngeal adductor muscle (thyroarytenoid), and with a more intense schluckatmung (higher amplitude but shorter duration). Also, laryngeal adductor (thyroarytenoid) amplitude was correlated with its own duration. Its duration was also positively correlated with the schluckatmung amplitude, but its amplitude was negatively correlated with schluckatmung duration. When the swallow stimulus was stronger, the schluckatmung (diaphragm EMG amplitude) was larger, and the laryngeal adductors produced a longer and stronger contraction, presumably in order to adequately protect the glottis from aspiration in the condition of negative intrathoracic pressure created by the increased inspiratory effort. Furthermore, the duration of the UES being open during swallow UES was positively correlated with its own post-swallow contraction amplitude

and with the schluckatmung amplitude and duration, but it was negatively correlated with all oropharyngeal EMG amplitudes and durations. Strong schluckatmung activation (amplitude and duration) was correlated with the UES being open longer during the swallow (duration), and with closing more forcefully after swallow (amplitude). These results are consistent with greater activation of oropharyngeal muscles, a more intense schluckatmung, and a longer total swallow duration during stronger swallow stimuli.

This strength of these correlations are in contrast with our previous publications (Alyssa Huff, Mitchell D Reed, Kimberly E Iceman, Dena R Howland, & Teresa Pitts, 2020; Huff, Reed, Smith, Brown, Ovechkin, & Pitts, 2018; T. Pitts, M. Rose, I. Poliacek, J. Condrey, P. W. Davenport, & D. Bolser, 2015; Spearman, Poliacek, Rose, Bolser, & Pitts, 2014). This is most likely due to the reduction in swallow amplitude and duration with the esophageal distension stimuli, which increased variability of the dataset revealing these relationships. It is not known if features are inherent to the regulation of the swallow pattern generator or present merely because amplitude and duration were both modified under these conditions. The addition of slow distension trials might also have aided interpretation of these results, and is a limitation of the current study.

Conclusion

We applied rapid balloon inflation in the cervical esophagus to examine the effects of proximal EDist on pharyngeal swallow physiology. Swallows elicited by EDist alone were characterized by decreased amplitude and duration of hyolaryngeal and thyroarytenoid muscle contractions, and decreased amplitude of diaphragm contraction; in general this swallow was smaller and shorter. This adapted swallow response could function as a clearing mechanism to help prevent aspiration of residual or refluxed

esophageal contents. Additionally, swallows elicited by the combined stimuli of both EDist and oral water infusion had stronger diaphragm and post-swallow UES activity, and increased laryngeal closure. Increased schluckatmung associated with these swallows could facilitate superior-inferior bolus propulsion, while increased laryngeal adduction protects against aspiration, and assessment of these features may aid in clinical decisions. These findings implicate brainstem integration of esophageal afferents in the initiation and modulation of pharyngeal swallow.

Table 2-1.

Means, standard deviation (SD), and *p*-values for swallow parameters during conditions of water infusion (W), esophageal distension (EDist), and combined stimuli (CS: W + EDist).

	Water (W)			Esophageal Distention (Edist)			Combined Stimuli (CS)			<i>p</i> -value*		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Edist vs W	Edist vs CS	W vs CS
Amplitude (% max)												
Hyoid/Laryngeal Elevators Mylohyoid	78	±	11	62	±	5	76	±	11	0.02	0.06	0.8
Geniohyoid	69	±	19	40	±	17	75	±	9	0.04	0.002	0.5
Thyrohyoid	77	±	8	65	±	16	76	±	5	0.07	0.2	0.8
Pharyngeal Thyropharyngeus	74	±	8	60	±	19	72	±	15	0.2	0.2	0.8
Laryngeal Adductor Thyroarytenoid	61	±	21	52	±	11	73	±	17	0.3	0.03	0.4
Schluckatmung Diaphragm	51	±	18	41	±	10	64	±	7	0.1	0.01	0.2
Cricopharyngeus (post-swallow UES)	55	±	20	78	±	12	80	±	7	0.02	0.4	0.02
	Water			Esophageal Distention			Combined Stimuli					
Duration (ms)	Mean	±	SD	Mean	±	SD	Mean	±	SD			
Mylohyoid	405	±	135	348	±	123	372	±	91	0.02	0.5	0.4
Geniohyoid	424	±	146	340	±	139	415	±	158	0.08	0.03	0.8
Thyrohyoid	433	±	256	315	±	200	360	±	69	0.5	0.6	0.6
Thyropharyngeus	331	±	87	297	±	46	402	±	118	0.4	0.1	0.02
Thyroarytenoid	326	±	35	263	±	53	412	±	67	0.2	0.02	0.05
Diaphragm	307	±	72	246	±	48	287	±	54	0.2	0.4	0.7
Cricopharyngeus (UES relaxation)	556	±	138	576	±	138	605	±	135	0.8	0.6	0.4
Total Swallow Time	522	±	192	482	±	151	590	±	125	0.5	0.09	0.3
Laryngeal Elevation Time	464	±	176	397	±	173	453	±	179	0.007	0.2	0.8

Amplitude is normalized to maximum of control and shown as a percentage. Reported *p*-values are from ANOVA and significant post-hoc tests. Significance is bolded at *p*-values ≤ 0.05 .

Table 2-2.

Pearson correlations comparing EMG amplitudes and durations during swallow with all data pooled across the three conditions.

		Amplitude							Duration								
		MyHy	GeHy	ThHy	ThPh	ThAr	Dia	UES	MyHy	GeHy	ThHy	ThPh	ThAr	Dia	UES		
Amplitude																	
Hyolaryngeal Elevators	MyHy		0.6	0.3	0.5	0.4	0.7	0.04	0.04	0.6	0.6	0.6	0.5	-0.6	-0.5		
	GeHy			0.7	0.7	0.7	0.6	0.05	-0.2	0.6	0.7	0.6	0.6	-0.6	-0.5		
	ThHy				0.4	0.4	0.4	-0.2	-0.1	0.4	0.4	0.3	0.3	-0.6	-0.4		
Pharyngeal	ThPh					0.6	0.2	0.01	0.1	0.4	0.6	0.5	0.3	-0.5	-0.4		
Laryngeal	ThAr						0.3	-0.04	-0.3	0.2	0.5	0.2	0.6	-0.6	-0.1		
	Dia							0.4	-0.4	0.3	0.4	0.3	0.6	-0.3	-0.2		
	UES									-0.6	-0.4	-0.2	-0.2	0.3	0.4	0.6	
Duration																	
Hyolaryngeal Elevators	MyHy										0.5	-0.01	0.1	-0.7	0.02	-0.6	
	GeHy			0.0–0.2	Negligible							0.7	0.7	0.2	-0.4	-0.9	
	ThHy			0.2–0.4	Weak									0.9	0.6	-0.6	-0.7
Pharyngeal	ThPh			0.4–0.6	Moderate										0.4	-0.3	-0.7
Laryngeal	ThAr			0.6–0.8	Strong											-0.5	-0.07
	Dia			0.8–1.0	Very Strong												0.6
	UES																

Amplitude is normalized to maximum of control and shown as a percentage. Reported *p*-values are from ANOVA and significant post-hoc tests. Significance is bolded at *p*-values ≤ 0.05 . (MyHy = mylohyoid; GeHy = geniohyoid; ThHy = thyrohyoid; ThPh = thyropharyngeus; ThAr = thyroarytenoid; Dia = diaphragm; and UES = upper esophageal sphincter).

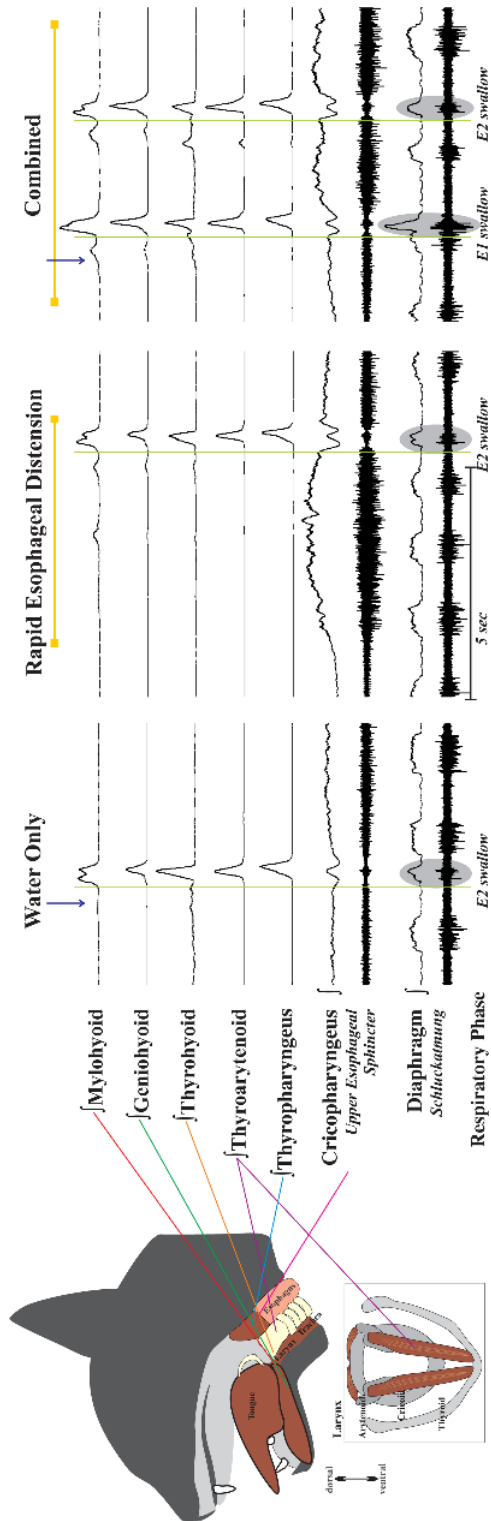


Figure 2-1. Representative examples of swallow across the three conditions.

The combined condition of esophageal distension plus water infusion resulted in a larger EMG amplitude of the thyroarytenoid and diaphragm muscles. Arrows indicate water infusion in the oropharynx, line indicated esophageal distension, and ovals indicate diaphragm activity during swallow (i.e. schluckatmung). Of note the first swallow in the combined condition has a swallow occurring in the transition from inspiration to expiration (E1 and/or post-I), all others are during late expiration (E2). Muscles are displayed as integrated traces, but the cricopharyngeus (UES) and diaphragm display raw EMG traces as well. *We hypothesize that the small activity during the UES relaxation is inferior pharyngeal constrictor activity, as the UES in the cat is relatively short.

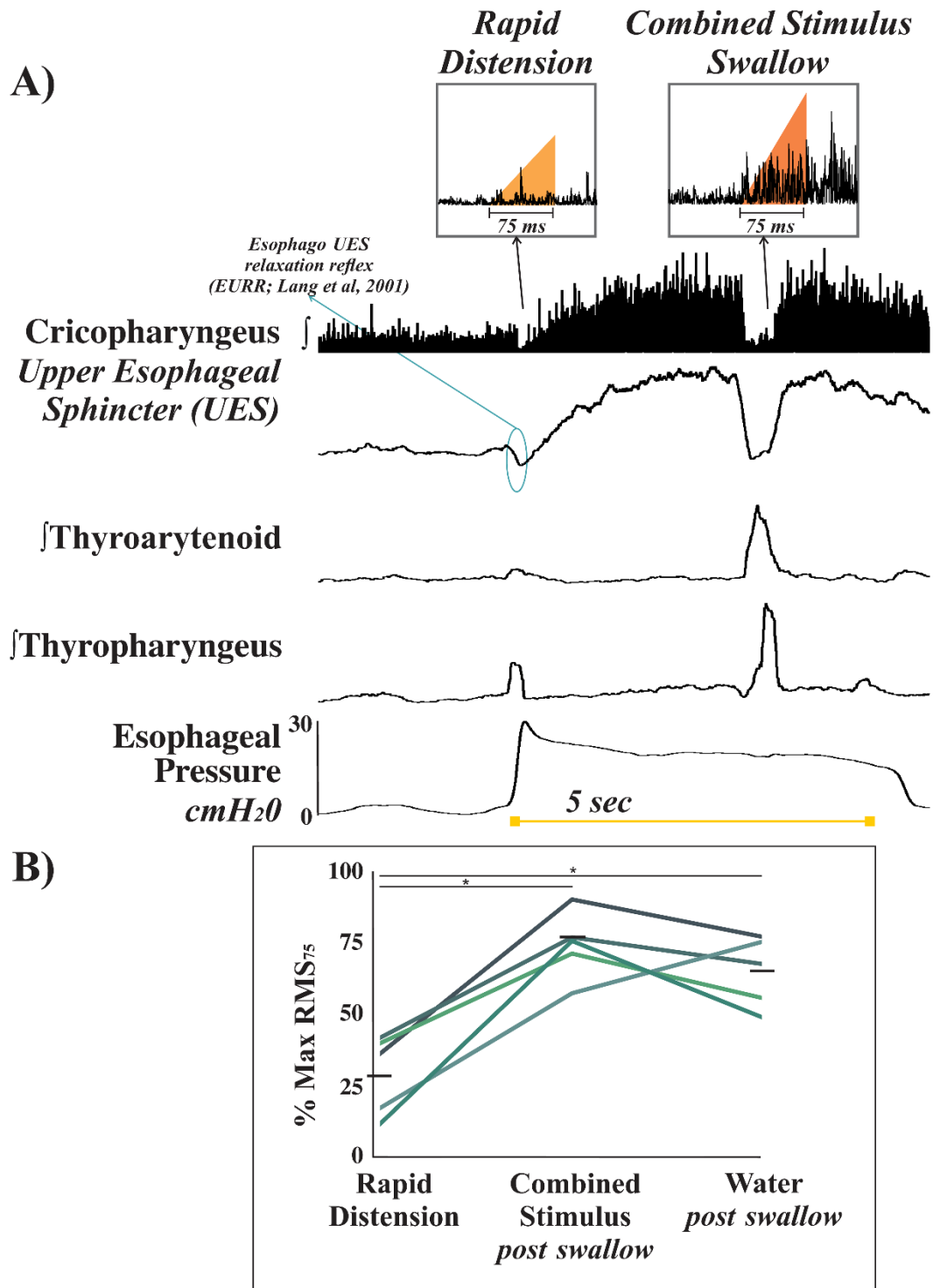


Figure 2-2. RMS75 analysis of upper esophageal sphincter (UES; cricopharyngeus) recruitment. A) Representative example of EMG activity and esophageal pressure during a combined stimulus trial. The root mean square calculation over 75ms (RMS₇₅)

represents motor unit recruitment of the UES after swallow. The triangles highlight integrated cricopharyngeus activity during rapid distension and post-swallow activity with a combined stimulus over 75ms. Oval highlights a esophago-UES relaxation reflex which is common with rapid esophageal distension. EMG's are displayed as integrated signals with the cricopharyngeus also displaying a rectified raw trace. B) Displays a line graph of individual animal's change in % of maximum RMS₇₅ across the three conditions, and the black horizontal lined display the group means. *There was a significant increase in UES recruitment during the combined and water conditions compared to rapid distension alone ($p < 0.05$).

CHAPTER 3
SEROTONIN THERAPIES FOR OPIOID-INDUCED DYSPHAGIA AND
RESPIRATORY DEPRESSION: SEX DIFFERENCES IN A RAT
ELECTROMYOGRAPHY MODEL

Opioids are well-known to cause respiratory depression, but the effects of opioids on swallow have not been characterized. We sought to test the effects of the opioid buprenorphine on pharyngeal swallow function and respiratory drive in male and female rats. We also evaluated utility of serotonin 5-HT_{1A} agonists (8-OH-DPAT and buspirone) to improve swallowing and breathing outcomes following buprenorphine administration. Experiments were performed on 44 freely breathing Sprague Dawley rats anesthetized with sodium pentobarbital. Bipolar fine wire electromyograms (EMGs) were inserted into the mylohyoid, thyroarytenoid, posterior cricoarytenoid, thyropharyngeus and diaphragm muscles to measure swallowing and breathing behaviors. We evaluated the hypotheses that swallow varies by stimulus, opioids depress swallow and breathing, and that 5-HT_{1A} agonists improve these depressions. Our results largely confirmed the hypotheses: 1) Swallow-related muscle activity was larger during swallows elicited by oral water infusion plus esophageal distension than by either stimulus alone. 2) Buprenorphine depressed swallow in both sexes, but most significantly in females. 3) Female animals were more susceptible to buprenorphine-induced respiratory arrest. 4) 8-OH-DPAT rescued breathing following buprenorphine-induced respiratory arrest, and pre-treatment with the partial 5-HT_{1A} agonist buspirone prevented buprenorphine-induced respiratory

arrest in female animals. 5) 8-OH-DPAT enhanced swallow-related mylohyoid drive, but did not restore excitability of the swallow pattern generator following total suppression by buprenorphine. Our results highlight the need for additional studies on sex-specific effects, and mechanisms of breathing and swallowing modulation by opioids and serotonin, in order to guide development of effective pharmacological therapies for humans.

Introduction

Precise coordination of the upper aerodigestive tract is essential for functional swallowing, breathing, and airway protection (German, Crompton, & Thexton, 1998; Kirchner, 1993; Shaker, 1995). Swallow is critical mechanism of energy intake for mammals (Negus, 1942). Breathing ventilates the lower airways to permit gas exchange, and is essential for survival (Chang, Strohlic, Williams, Umans, & Liberles, 2015). Several protective reflexes, including swallow, prevent ingested food and liquid from compromising the airway (Logemann, Kahrilas, Cheng, Pauloski, Gibbons, Rademaker, & Lin, 1992; Pitts, 2014; Shaker & Hogan, 2000). Functionality of the upper aerodigestive tract may be disrupted by numerous disease states and drugs, including opioids (Babaei, Szabo, Shad, & Massey, 2019; Lawal & Shaker, 2008; Logemann, 2007). The objective of our study was to systematically evaluate the effects of opioids on the upper aerodigestive tract and its dual role in breathing and swallowing.

Airway protection during swallow is achieved by synchronized laryngeal elevation and closure while ingested material is propelled through the pharynx (Logemann, Kahrilas, Cheng, Pauloski, Gibbons, Rademaker, & Lin, 1992). Activation of pharyngeal mechanoreceptors triggers transient relaxation of the upper esophageal

sphincter (UES), enabling ingested material to pass into the esophagus (Negus, 1942). Tonic at rest, the UES forms a functional barrier between the airway and any food contents in the esophagus (Negus, 1942; Shaker & Hogan, 2000). Failure to maintain separation of the airway from ingested material can result in aspiration of food or liquid into the lower airway (German, Crompton, & Thexton, 1998; Kirchner, 1993). Sequelae of aspiration can be fatal if airway obstruction (acute) or aspiration pneumonia (chronic) occur (Nativ-Zeltzer, Nachalon, Kaufman, Seeni, Bastea, Aulakh, Makkiyah, Wilson, Evangelista, Kuhn, Sahin, & Belafsky, 2022).

The upper aerodigestive tract is active during the inspiratory and expiratory phases of the respiratory cycle (Kirchner, 1993; Pilarski, Leiter, & Fregosi, 2019; Richter, 1982). During inspiration, the glottis is abducted by the posterior cricoarytenoid muscle, and the UES is tonically contracted. In this configuration, inspired air meets less resistance from the larynx than the UES, and flows through the glottis to the lower airways for gas exchange (Negus, 1942; Pilarski, Leiter, & Fregosi, 2019). During expiration, the larynx is partially adducted by the thyroarytenoid muscle. Partial laryngeal adduction functions as an expiratory braking mechanism that helps match ventilation to metabolic demand (Doty & Bosma, 1956; Kirchner, 1993). The pharyngeal musculature can be active during either phase of the respiratory cycle, but is generally active during expiration (Doty & Bosma, 1956).

Opioid receptors are inhibitory G-Protein Coupled Receptors (GPCRs), and are distributed throughout the pontomedullary respiratory network (Bolser & DeGennaro, 1994; Connor & Christie, 1999; Irnaten, Aicher, Wang, Venkatesan, Evans, Baxi, & Mendelowitz, 2003; Pasternak & Pan, 2013; Ramirez, Burgraff, Wei, Baertsch, Varga,

Baghdoyan, Lydic, Morris, Bolser, & Levitt, 2021; Waldhoer, Bartlett, & Whistler, 2004; Zhuang, Gao, Gao, & Xu, 2017). It is therefore unsurprising that respiratory depression is a serious complication of opioid use (Bateman, Saunders, & Levitt, 2023). The United States is facing a distressing opioid epidemic: Opioid-related deaths have increased steadily for two decades, and spiked each year since 2019 (Bateman, Saunders, & Levitt, 2023; Skolnick, 2022; Upp & Waljee, 2020). To complicate matters, adverse effects of opioid use are not limited to overdose events. Respiratory depression can also occur when therapeutic doses of opioids are administered in a controlled setting (Bateman, Saunders, & Levitt, 2023; Brown, 1985; Gerber & Apseloff, 1993; Oertel, Schneider, Rohrbacher, Schmidt, Tegeder, Geisslinger, & Lotsch, 2007).

In addition to well-known respiratory-depressant effects, opioids depress the immune system, gastrointestinal system, and airway defense mechanisms (e.g., cough) (Bateman, Saunders, & Levitt, 2023; Foley, 1993; Roy, Ninkovic, Banerjee, Charboneau, Das, Dutta, Kirchner, Koodie, Ma, Meng, & Barke, 2011). Clinical trials have reported esophageal dysfunction and aspiration in humans following opioid administration, and several reports have identified aspiration pneumonia as a serious complication of opioid use following both overdose and chronic use (Eizadi-Mood, Yaraghi, Sharifian, Feizi, Hedaiaty, & Sabzghabae, 2015; Nicolakis, Gmeiner, Reiter, & Seltenhammer, 2020; Patel & Vaezi, 2018; Savilampi, Ahlstrand, Magnuson, Geijer, & Wattwil, 2014; Steffens, Sung, Bastian, Edelman, Brackett, & Gunderson, 2020; Tabatabaei, Dorvashy, Soltani, Samsamshariat, Meamar, & Sabzghabae, 2021; Tagaito, Isono, & Nishino, 1998). While it is likely that aerodigestive dysregulation is a contributing factor to

aspiration pneumonia following opioid use, specific effects of opioids on pharyngeal swallow have been the subject of limited investigation.

Buprenorphine is a partial mu agonist used for post-operative pain management and opioid maintenance therapy (Elkader & Sproule, 2005; Jasinski, Pevnick, & Griffith, 1978; Shulman, Wai, & Nunes, 2019). Buprenorphine binds the mu opioid receptor with high affinity for long durations, but as a partial agonist, activates the receptor to a lesser extent than a full agonist (e.g., morphine, fentanyl)(Bateman, Saunders, & Levitt, 2023; Elkader & Sproule, 2005). Because of these pharmacological properties, buprenorphine is generally considered to be safer than full mu agonists (Fishman & Kim, 2018). Indeed, a ceiling effect of buprenorphine-induced respiratory depression has been demonstrated in humans, but to our knowledge, its effects on airway protection and deglutition are largely unknown (Dahan, van Lemmen, Jansen, Simons, & van der Schrier, 2022; Dahan, Yassen, Romberg, Sarton, Teppema, Olofsen, & Danhof, 2006). In the present study, we evaluated swallow and breathing function before and after buprenorphine administration to determine how a widely prescribed, clinically relevant opioid impacts aerodigestive function.

We initially tested three hypotheses: 1) Swallow motor pattern is modulated by aerodigestive afferent input to the medullary swallow pattern generator. 2) Administration of the opioid buprenorphine will result in a measurable decline of swallow function due to central depression of the swallow pattern generator. 3) Respiratory depression following systemic buprenorphine is not dose-dependent. During our initial dose response experiments, we found that unlike males, most females succumbed to respiratory arrest following high doses of buprenorphine. This striking sex

difference led us to consider potential counters to the potent respiratory depression we observed in female animals.

Basic science studies have implicated serotonin, a modulatory neurotransmitter, in the regulation of breathing and swallow (Bieger, 1991, 1981; Hashim & Bieger, 1987; Poliacek, Jakus, Knocikova, Barani, Halasova, & Visnovcova, 2008; Richter, Manzke, Wilken, & Ponimaskin, 2003). Like opioid receptors, serotonin receptors are G-Protein Coupled Receptors (GPCR) expressed throughout the brainstem swallow-breathing network (Pilowsky, 2014). Previous studies have demonstrated that the 5-HT_{1A} agonist 8-OH-DPAT produced excitatory respiratory effects in rats and rabbits (Iovino, Mutolo, Cinelli, Contini, Pantaleo, & Bongiani, 2019; Zhuang & Xu, 2022). Notably, Sahibzada and colleagues reversed morphine-induced apnea in male rats using a 5-HT_{1A} agonist (Sahibzada, Ferreira, Wasserman, Taveira-DaSilva, & Gillis, 2000). These promising reports, along with our primary results, led us to test three additional hypotheses: 4) Systemic administration of 8-OH-DPAT will restore breathing following buprenorphine-induced apnea through activation of 5-HT_{1A} receptors in female rats; 5) Pre-treatment with oral buspirone will preserve breathing following buprenorphine administration through action on 5-HT_{1A} receptors in female rats; 6) Systemic administration of a 5-HT_{1A} agonist will measurably improve swallow function following buprenorphine-induced depression of swallow

Methods

Study Design

All experiments were approved by the Institutional Animal Care and Use committee of the University of Louisville and conducted in accordance with the

American Physiological Society's Animal Care Guidelines (Drummond, 2009).

Experiments were performed using 44 adult Sprague Dawley ex-breeder rats [21 male (0.58 ± 0.16 kg) and 23 female (0.26 ± 0.19 kg)]. Our objectives were to evaluate 1) differential effects of pharyngeal and esophageal stimulation on oropharyngeal swallow initiation and motor pattern, 2) how protective responses to upper aerodigestive stimuli (e.g. swallow) are impacted by systemic opioid administration, and 3) the utility of 5-HT_{1A} agonists in the recovery/protection of swallow and respiratory drive following systemic opioid administration in rats.

The primary outcome measures are amplitudes of submental, laryngeal, pharyngeal, and inspiratory electromyograms (EMGs), and frequency of swallow occurrence. We also measured respiratory rate and heart rate. Analysis of variance (ANOVA) and post-hoc comparisons using the Tukey HSD test were performed when appropriate. Swallow-breathing coordination was evaluated by determining the phase of breathing at time of swallow initiation. Inspiration (I) was defined as the period from the onset of breathing-related diaphragm activity to the peak of the diaphragm burst. Expiration (E) was defined as the period from peak diaphragm activity to the onset of subsequent diaphragm activation, and further subdivided into early expiration (E1; the period from peak diaphragm amplitude to diaphragm quiescence) and late expiration (E2; the period from offset of diaphragm activity to the onset of subsequent diaphragm activity) (T. E. Dick, Y. Oku, J. R. Romaniuk, & N. S. Cherniack, 1993; Richter, 1982). Swallows that occurred during inspiration, early expiration and late expiration were coded as 1, 2 and 3 respectively, and a Wilcoxon signed-rank test was used to evaluate

differences in swallow-breathing coordination across conditions. For all measures, a difference was considered significant if the *p*-value was less than 0.05.

Rat Model

Animals were initially anesthetized with gaseous isoflurane (1.5% with 100% O₂) while a femoral intravenous (IV) cannula (0.6 mm inner diameter) was placed. Animals were then transitioned to sodium pentobarbital (initial dose 25 mg/kg IV), with supplementary doses (1-4 mg/kg IV) administered as needed. A dose of atropine sulfate (0.01 mg/kg IV) was given at the beginning of each experiment to reduce airway secretions from repeated tracheal stimulation. Following administration of atropine sulfate, a tracheostomy was performed. Body temperature was monitored and maintained at $36.5 \pm 0.5^\circ$ C with a heating pad (Homeothermic Monitor, Harvard Apparatus). Anesthetic level was evaluated by jaw tone, blink reflex, forelimb withdrawal reflex, and licking in response to oral water administration.

Electrophysiology Recording and Processing

All muscle activity was recorded via electromyography (EMG) using bipolar fine wire hook electrodes (A-M Systems stainless steel No. 791050) according to the technique of Basmajian and Stecko (Basmajian J, 1962). Four muscles were used to evaluate swallow: Mylohyoid, thyroarytenoid, thyropharyngeus, and costal diaphragm. These muscles span the actions of the pharyngeal phase of swallow: The mylohyoid elevates the hyolaryngeal complex, and is innervated by the trigeminal nerve (CN V); the thyroarytenoid adducts the larynx, and is innervated by the recurrent laryngeal branch of the vagus (CN X); the thyropharyngeus constricts the inferior pharynx, and is innervated by the glossopharyngeal and vagus nerves (CN IX, X); and diaphragm activation during

swallow (Schluckatmung) produces negative intrathoracic pressure (Miyamaru, Kumai, Ito, & Yumoto, 2008; Pitts & Iceman, 2023; Sakamoto, 2013; Yamaoka, Furusawa, Fujimoto, Iguchi, & Kumai, 1992). The anatomical location of each muscle, and representative traces of muscle activity during pharyngeal swallow, are shown in Figure 1. Breathing motor patterns were evaluated using the posterior cricoarytenoid (laryngeal abductor, CN X), thyroarytenoid (laryngeal adductor), thyropharyngeus, and costal diaphragm (Berkowitz, Sun, Chalmers, & Pilowsky, 1999; Doty & Bosma, 1956; Pilarski, Leiter, & Fregosi, 2019). Respiratory phase activity of inspiratory and expiratory upper airway muscles is shown in Figures 3-1 and 3-2.

Recording electrodes were placed surgically as follows: The digastric muscles were bluntly dissected away from the surface of the mylohyoid, and electrodes were placed in the medial portion of the mylohyoid. Thyroarytenoid muscle electrodes were placed through the cricothyroid window into the anterior third of the vocal folds, and examined post-mortem to ensure placement accuracy. The thyropharyngeus is a fan-shaped muscle that originates at the oblique line of the thyroid cartilage and courses posteriorly to the pharyngeal raphe where it meets the insertion of the contralateral thyropharyngeus. Electrodes were placed into the ventral portion of thyropharyngeus at the level of the rostral thyroid cartilage. To place electrodes in the posterior cricoarytenoid muscle, the esophagus was bluntly dissected from the trachea and the trachea was elevated, which enabled direct visualization of the dorsal larynx during electrode insertion. For costal diaphragm electrode placement, the xyphoid process was palpated and elevated. Needles (1" for males, 5/8" for females) were inserted directly caudal to the sternum, and electrodes were hooked under the xyphoid process, near the costal diaphragm muscle

attachment. Electrodes were placed in the left pectoralis muscle and right caudal gastrocnemius to record electrocardiogram (ECG) activity, which was used to measure heart rate and remove heart artifact from EMG traces. Correct placement of all electrodes was confirmed by visual inspection (after insertion, and post-mortem), and activation patterns during swallow and breathing, as previously published (Pitts, Iceman, Huff, Musselwhite, Frazure, Young, Greene, & Howland, 2022; T. Pitts, I. Poliacek, M. J. Rose, M. D. Reed, J. A. Condrey, H. W. Tsai, G. Zhou, P. W. Davenport, & D. C. Bolser, 2018; Pitts, Rose, Mortensen, Poliacek, Sapienza, Lindsey, Morris, Davenport, & Bolser, 2013; T. Pitts, M. J. Rose, I. Poliacek, J. Condrey, P. W. Davenport, & D. C. Bolser, 2015; Spearman, Poliacek, Rose, Bolser, & Pitts, 2014)

EMG signals were amplified (Grass P511 AC Amplifiers, Natus Neurology), band-pass filtered (200-5000 Hz), recorded at a 10 KHz sampling rate (1401 Power3 + ADC16 Expansion, Cambridge Electronic Design), and analyzed using Spike 2 (v8, Cambridge Electronic Design). EMGs were rectified, integrated (20-ms time constant) and exported to PowerPoint (v17, Microsoft) for figure creation. Peak EMG amplitude was measured for each muscle during each swallow or breath to determine swallow and respiratory drive. For comparison across animals, raw values were normalized as the percent change in amplitude relative to the mean peak EMG amplitude during control.

Experimental Protocols

Two experimental protocols were performed using two cohorts of male and female Sprague Dawley rats. A third protocol was performed using a cohort of female Sprague Dawley rats. A fourth protocol was performed using a cohort of male Sprague Dawley rats. 1) Cumulative buprenorphine dose response experiments were performed in

17 rats [10 male (0.5 ± 0.1 kg) and 7 female ($0.24 \text{ kg} \pm 0.1 \text{ kg}$)]. 2) The 5-HT_{1A} agonist 8-OH-DPAT was administered following buprenorphine administration in 15 rats [6 male ($0.7 \text{ kg} \pm 0.1 \text{ kg}$) and 9 female ($0.26 \text{ kg} \pm 0.2 \text{ kg}$)]. 3) The partial 5-HT_{1A} agonist buspirone was given orally one hour before buprenorphine administration in 7 female rats ($0.28 \text{ kg} \pm 0.1 \text{ kg}$). 4) In time control experiments for protocol A, saline vehicle infusions were administered IV in 5 male rats ($0.6 \text{ kg} \pm 0.1 \text{ kg}$). Following completion of the experimental protocol, euthanasia was induced by an overdose of sodium pentobarbital and 1 cc of saturated potassium chloride IV. In accordance with Institutional Animal Care and Use committee guidelines, a secondary method of euthanasia was induced via pneumothorax.

Experiment 1: Aerodigestive Stimuli Before and After Buprenorphine Administration

Pharyngeal mechanoreceptor activation was produced by infusing 1 cc of water into the oropharynx via a 0.5-inch-long thin polyethylene catheter (outer diameter 0.5-1.0 mm) placed at the base of tongue. Esophageal mechanoreceptor activation was produced by rapidly inflating an esophageal balloon with 0.5 cc of air in less than 1 second, then maintaining inflation for 5 seconds. The balloon was attached to thin polyethylene tubing (outer diameter 0.5-1.0 mm) attached to a syringe, and placed in the upper esophagus as follows: The esophagus was bluntly dissected from the trachea, and the balloon was inserted through a small incision in the caudal aspect of the thoracic esophagus and advanced proximally until just below the pharyngoesophageal segment, using the cricoid cartilage as a landmark. The esophageal catheter was secured to sternal tissue using 4-0 braided suture to ensure stable balloon placement throughout the experiment.

Each animal was subjected to three different stimulus conditions with at least 1 minute between each trial: 1) Water only, 2) Esophageal distension only, and 3) Esophageal distension plus water (combined stimulus), during which the esophagus was distended by balloon inflation for 5 seconds, and water was infused at the 2.5 second mark. Figure 1 displays representative swallows elicited by each stimulus.

One minute of eupnea was recorded prior to control stimulus trials. Animals then received a series of cumulative buprenorphine doses (0.01, 0.03, 0.1, and 0.3 mg/kg). Stimulus trials were repeated fifteen minutes after each dose of buprenorphine, in line with the work of Nielsen and Taylor, which demonstrates that fifteen to thirty minutes is the time to peak effect of IV buprenorphine in male Sprague Dawley rats (Nielsen & Taylor, 2005). We also recorded heart rate, respiratory rate and breathing motor patterns before and after each dose of buprenorphine.

Experiment 2: Systemic 8-OH-DPAT After Buprenorphine Administration

Female animals: Swallow was elicited by oral water infusion as described earlier. One minute of eupnea was recorded prior to control swallow trials. An initial dose of buprenorphine was administered (0.003 mg/kg IV), and following swallow trials, additional doses of buprenorphine were administered every fifteen minutes until opioid-induced apnea occurred (0.03 mg/kg IV among eight of nine animals in this cohort). Upon respiratory arrest, the 5-HT_{1A} agonist 8-OH-DPAT was administered (0.3 mg/kg IV). Following 8-OH-DPAT, presence or absence of respiratory effort was evaluated using EMG activity, and confirmed by direct visualization of the animal. Swallow trials were repeated eight minutes after 8-OH-DPAT in animals where breathing was restored. Finally, the competitive 5-HT_{1A} agonist was administered (1 mg/kg IV), and respiratory

effort was evaluated as above. We also recorded heart rate, respiratory rate and breathing motor pattern before and after administration of each drug. 8-OH-DPAT dosage was determined by pilot experiments not included in this manuscript.

Male animals: Swallow was elicited by oral water infusion. One minute of eupnea was recorded prior to control swallow trials. Two doses of buprenorphine were administered (cumulative 0.003 and 0.03 mg/kg IV), and swallow trials were repeated fifteen minutes after each dose. Animals were subsequently treated with 8-OH-DPAT (0.3 mg/kg) and WAY-100635 (1 mg/kg). Swallow trials were repeated eight minutes after administration of each drug. We also recorded heart rate, respiratory rate, and breathing motor pattern before and after administration of each drug.

Experiment 3: Buspirone Before Buprenorphine Administration

Following anesthesia with sodium pentobarbital and tracheostomy, thin polyethylene tubing (outer diameter 0.5-1.0 mm) was attached to a syringe, placed in the mouth, and advanced into the stomach. Buspirone (2.5 mg, crushed in distilled water) was then administered via orogastric gavage. One hour later, two doses of buprenorphine (cumulative 0.003 mg/kg and 0.03 mg/kg IV) were administered fifteen minutes apart. Fifteen minutes after the last dose of buprenorphine, WAY-100635 (1 mg/kg IV) was administered. We evaluated heart rate, respiratory rate, and breathing motor pattern before and after administration of each drug.

Experiment 4: Time Control

Each animal was subjected to 1) water only, 2) esophageal distension only, and 3) esophageal distension plus water (combined stimulus) stimulus trials as described in Experiment 1. Following control stimulus trials, four saline vehicle infusions were

administered (0.5 cc IV). Stimulus trials were repeated fifteen minutes after each sham infusion. We also recorded heart rate and respiratory rate before and after each saline infusion.

Results

First, we evaluated the effects of varied upper aerodigestive stimulation on oropharyngeal swallow initiation and motor pattern. Figure 3-1 demonstrates that oral water infusion, rapid esophageal distension, and esophageal distension plus water (combined stimulus) reliably elicit swallow, and that swallow-related mylohyoid (laryngeal elevator) and thyroarytenoid peak amplitudes are significantly larger during swallows elicited by esophageal distension plus water. There was no significant difference in swallow-related thyropharyngeus activity across stimulus conditions.

Second, we assessed the effects of systemic buprenorphine administration on oropharyngeal swallow in male and female rats. Figure 3-2 demonstrates that buprenorphine significantly reduces swallow-related laryngeal elevation in male rats (Fig. 3-2A), and significantly reduces swallow-related laryngeal elevation, laryngeal adduction and pharyngeal constriction in female rats. In contrast to female animals, thyroarytenoid (laryngeal adductor) and thyropharyngeus (pharyngeal constrictor) activity during swallow were not significantly impacted by buprenorphine in males. Frequency of swallow initiation was significantly reduced in both male and female animals following buprenorphine administration (Fig. 3-2B, 3-2D). To determine if these changes in function were due to effects of buprenorphine or effects of time, we performed a series of time control experiments ($N = 5$) in which animals received IV saline vehicle infusions instead of buprenorphine. There were no significant changes in swallow-related peak

amplitude (mylohyoid, thyroarytenoid, thyropharyngeus), or swallow frequency (Fig. 3-2B, 3-2D) across stimulus conditions (water, esophageal distension, esophageal distension plus water) and saline infusions among the time control group.

Third, we evaluated the effects of systemic buprenorphine administration on breathing and survival in male and female rats. Figure 3-3 shows that while buprenorphine had no significant effect on respiratory rate in male animals (Fig. 3-3B), respiratory rate was significantly reduced following buprenorphine administration in female animals (Fig. 3-3E). Female animals had a lower threshold for opioid-induced apnea (Fig. 3-3F), and there was a significant difference in the survival distributions of male and female animals along the buprenorphine dose response curve (Fig. 3-3G).

Fourth, we tested the utility of the 5-HT_{1A} agonist 8-OH-DPAT in the restoration of breathing following buprenorphine-induced apnea in female animals. Systemic administration of 8-OH-DPAT (0.3 mg/kg IV) restored breathing following respiratory arrest in 75% of female animals, and this effect was reversed by the competitive 5-HT_{1A} antagonist WAY-100635 (Fig. 4A). Unlike female animals, male animals maintained regular respiratory effort across all conditions (Fig. 3-5B, 3-5C).

Fifth, we wanted to determine if pre-treatment with oral buspirone (a partial 5-HT_{1A} agonist) would prevent buprenorphine-induced apnea in female animals. Animals that received 2.5 mg oral buspirone maintained stable respiratory effort following doses of buprenorphine that produced apnea in animals that did not receive buspirone, and demonstrated significantly higher median survival following opioid administration (Fig. 3-4D, 3-4E).

Finally, we used systemic 8-OH-DPAT to evaluate the utility of 5-HT_{1A} agonists in the restoration of swallow function following opioid-induced swallow depression. Swallow initiation was not recovered by IV 8-OH-DPAT following total suppression of swallow post-buprenorphine in female animals (Fig. 3-4C). However, among male animals that still initiated swallow, mylohyoid (laryngeal elevator) amplitude was significantly reduced after buprenorphine administration, with significant correction toward baseline following IV 8-OH-DPAT (Fig. 3-5A). There were no significant changes in swallow-related thyroarytenoid or thyropharyngeus amplitude across conditions in male animals.

Discussion

Swallow and breathing are vulnerable to depression by opioids, even following low doses of buprenorphine. Profound differences in sensitivity to buprenorphine between male and female rats indicates need for further evaluation of sex differences in humans. Our finding that 5-HT_{1A} agonists improve swallow and breathing measures following opioid administration expands on previous work in rats (Dutschmann, Waki, Manzke, Simms, Pickering, Richter, & Paton, 2009; Sahibzada, Ferreira, Wasserman, Taveira-DaSilva, & Gillis, 2000). We advocate that these results warrant further investigation to enable successful application to human medicine.

Buprenorphine Disproportionately Depresses Swallow and Breathing in Female Rats

A functional aerodigestive tract is dependent upon central integration of feedback from several afferent beds in the larynx, pharynx and esophagus (Lang, Medda, Jadcherla, & Shaker, 2016; Lang, Medda, & Shaker, 2019; Pitts, 2014; Shaker & Hogan, 2000). Our results support our first hypothesis, that swallow motor pattern is modulated

based on the location and intensity of peripheral aerodigestive stimulation. Following buprenorphine administration, pharyngeal swallow function was depressed, and laryngeal accommodation to a maximal stimulus was lost. Buprenorphine blunted aerodigestive responses such that often, no swallow or protective response was elicited by stimuli that effectively flooded the upper airway. When swallow did occur post-buprenorphine, there was significant reduction of EMG amplitudes, and altered swallow-breathing coordination. While swallow was depressed in both male and female animals, swallow was frankly depressed by buprenorphine in females. These findings support our second hypothesis, that the opioid buprenorphine would depress swallow function.

The swallowing musculature is driven by anatomically distinct motor nuclei: The mylohyoid is innervated by the trigeminal nerve (CN V); the thyroarytenoid is innervated by the recurrent laryngeal branch of the vagus nerve (CN X); and the thyropharyngeus is innervated by the glossopharyngeal and vagus nerves (CN IX, X) (Lang, Medda, Jadcherla, & Shaker, 2016; Miyamaru, Kumai, Ito, & Yumoto, 2008; Sakamoto, 2013). We hypothesize that buprenorphine centrally depresses swallow through its actions on opioid receptors throughout the brainstem swallow pattern generator. Buprenorphine is a partial mu-agonist, as well as a kappa-inverse agonist and delta-antagonist (Davis, Pasternak, & Behm, 2018). Mu-opioid receptors have been identified in several brainstem regions, including the nucleus tractus solitarius (NTS) (Bateman, Saunders, & Levitt, 2023; Irnaten, Aicher, Wang, Venkatesan, Evans, Baxi, & Mendelowitz, 2003; Ramirez, Burgraff, Wei, Baertsch, Varga, Baghdoyan, Lydic, Morris, Bolser, & Levitt, 2021; Zhuang, Gao, Gao, & Xu, 2017). Relatively few studies have investigated kappa- and delta-opioid receptors in the rat brainstem, and potential actions of these receptors on

regions important for swallow and breathing are unclear (George, Zastawny, Briones-Urbina, Cheng, Nguyen, Heiber, Kouvelas, Chan, & O'Dowd, 1994; Henderson, Keay, & Bandler, 2002). Our findings indicate that buprenorphine disrupts neural networks throughout the brainstem resulting in dysphagia, however more work is needed to determine the molecular basis and site of action of buprenorphine's effects on the swallow pattern generator.

The activity of each swallow-related muscle we measured represents a critical component of pharyngeal swallow: The mylohyoid (laryngeal elevator) helps lift the larynx above food or liquid as it passes through the pharynx; the thyroarytenoid (laryngeal adductor) functions to seal the larynx from ingested material; and the thyropharyngeus (pharyngeal constrictor) aids bolus clearance into the esophagus (Logemann, 2007; Logemann, Kahrilas, Cheng, Pauloski, Gibbons, Rademaker, & Lin, 1992; Pitts, Iceman, Huff, Musselwhite, Frazure, Young, Greene, & Howland, 2022). Clinically, even slight disturbances of aerodigestive regulation can result in aspiration related complications (e.g., aspiration pneumonia, death) (Langmore, 1998; Marik & Kaplan, 2003; Nativ-Zeltzer, Nachalon, Kaufman, Seeni, Bastea, Aulakh, Makkiyah, Wilson, Evangelista, Kuhn, Sahin, & Belafsky, 2022; Pitts, Rose, Mortensen, Poliacek, Sapienza, Lindsey, Morris, Davenport, & Bolser, 2013; Shaker, 1995; Wilson, 2012). Alterations in swallow-related EMG amplitude following buprenorphine administration were predictive of severe dysphagia, most remarkably in female animals.

Previous studies have reported that buprenorphine-induced respiratory depression has a ceiling effect (Dahan, Yassen, Romberg, Sarton, Teppema, Olofsen, & Danhof, 2006; Fishman & Kim, 2018). Our third hypothesis, that buprenorphine would not

produce dose-dependent respiratory depression, was refuted by experiments performed in female rats. These experiments demonstrated progressive respiratory depression and respiratory arrest as buprenorphine dosage increased. We obtained the opposite result in male rats, which showed no significant slow in respiratory rate following large doses of buprenorphine. Our finding that buprenorphine powerfully depresses swallow, and produces terminal respiratory arrest in female animals, suggest that even opioids considered to be safe can negatively impact survival.

To our knowledge, this study is the first to evaluate sex-specific effects of buprenorphine on breathing and swallowing. Our finding that breathing and swallowing are regulated differently in male and female animals is consistent with a limited, but growing number of studies dedicated to physiological sex differences. Studies by Huff and colleagues demonstrated evidence of sex-specific modulation of breathing and swallowing when a mechanical challenge or airway anesthesia was experimentally induced in rats (A. Huff, M. D. Reed, K. E. Iceman, D. R. Howland, & T. Pitts, 2020; Alyssa Huff, Mitchell D Reed, Kimberly E Iceman, Dena R Howland, & Teresa Pitts, 2020). A recent study comparing opioid-induced respiratory depression between male and female rats found that females demonstrated a greater degree of heroin-induced respiratory depression than males (Marchette, Carlson, Frye, Hastings, Vendruscolo, Mejias-Torres, Lewis, Hampson, Volkow, Vendruscolo, & Koob, 2023; Sarton, Teppema, & Dahan, 1999). The few studies performed in humans have also shown that females are more sensitive to morphine-induced respiratory depression than males. These reports, and the present results, highlight the importance of sex differences in the study of control of breathing and its depression by opioids.

5-HT_{1A}: A Promising Target for Opioid-Induced Depression of Breathing and Swallowing

Serotonin is a modulatory neurotransmitter synthesized in the brainstem raphe nuclei that has been shown to modulate breathing and swallow (Bieger, 1981; Hilaire, Voituron, Menuet, Ichiyama, Subramanian, & Dutschmann, 2010; Pilowsky, 2014; Richter, Manzke, Wilken, & Ponimaskin, 2003). The effects of serotonin are complex, and depend on the anatomical distribution of receptor subtypes on a given neuron or area (Pilowsky, 2014). The 5-HT_{1A} receptor is an inhibitory serotonin receptor subtype that is distributed throughout the brainstem and has been implicated in regions important for breathing and swallow (Bieger, 1991; Dutschmann, Waki, Manzke, Simms, Pickering, Richter, & Paton, 2009; Hashim & Bieger, 1987; Hilaire, Voituron, Menuet, Ichiyama, Subramanian, & Dutschmann, 2010; Iovino, Mutolo, Cinelli, Contini, Pantaleo, & Bongiani, 2019; Zhuang & Xu, 2022).

Experimentally, activation of medullary 5-HT_{1A} receptors has been shown to stimulate respiration (Iovino, Mutolo, Cinelli, Contini, Pantaleo, & Bongiani, 2019; Manzke, Dutschmann, Schlaf, Morschel, Koch, Ponimaskin, Bidon, Lalley, & Richter, 2009; Zhuang & Xu, 2022). Previous studies in rats have shown that 5-HT_{1A} agonists restore respiratory function following opioid-induced respiratory depression and arrest in males (Dutschmann, Waki, Manzke, Simms, Pickering, Richter, & Paton, 2009; Manzke, Dutschmann, Schlaf, Morschel, Koch, Ponimaskin, Bidon, Lalley, & Richter, 2009; Sahibzada, Ferreira, Wasserman, Taveira-DaSilva, & Gillis, 2000). Our experiments in female rats support our fourth and fifth hypotheses, that 8-OH-DPAT would restore breathing following opioid-induced apnea, and bupirone would preserve breathing

following buprenorphine administration. Of note, a clinical trial in humans was performed, and concluded that buspirone does not antagonize opioid-induced respiratory depression based on CO₂ rebreathing following morphine administration in healthy subjects (Oertel, Schneider, Rohrbacher, Schmidt, Tegeder, Geisslinger, & Lotsch, 2007). We do not claim that 5-HT_{1A} agonists restore respiratory function to normal range following opioid administration. However, our results do show that 8-OH-DPAT and buspirone extend survival by countering terminal respiratory arrest. We have replicated and expanded upon the results presented by Sahibzada and colleagues, and advocate for future research aiming to translate these findings to human medicine (Sahibzada, Ferreira, Wasserman, Taveira-DaSilva, & Gillis, 2000).

5HT_{1A} agonists have been shown to improve dysphagia symptoms and esophageal motility in humans (Di Stefano, Papathanasopoulos, Blondeau, Vos, Boecxstaens, Farre, Rommel, & Tack, 2012; Hanna, Feibusch, & Albright, 1997; Oertel, Schneider, Rohrbacher, Schmidt, Tegeder, Geisslinger, & Lotsch, 2007). Following total depression of breathing and swallowing in female rats, 8-OH-DPAT restored respiratory rhythmicity, but did not restore excitability of the swallow pattern generator. As we found in our initial dose-response experiments, male animals that were still stimuable for swallow following buprenorphine demonstrated significantly reduced mylohyoid amplitude. This decline in mylohyoid activation was reversed by subsequent 8-OH-DPAT administration. These findings support our hypothesis that 8-OH-DPAT would measurably improve buprenorphine-induced dysphagia, with the caveat that 8-OH-DPAT enhanced submental neural drive during swallow, but did not restore excitability of the swallow reflex following total suppression.

Despite reports from several groups that 5-HT_{1A} agonists stimulate breathing, the mechanisms through which inhibitory 5-HT_{1A} receptors modulate central pattern generators have not been definitively described (Dutschmann, Waki, Manzke, Simms, Pickering, Richter, & Paton, 2009; Iovino, Mutolo, Cinelli, Contini, Pantaleo, & Bongiani, 2019; Manzke, Dutschmann, Schlaf, Morschel, Koch, Ponimaskin, Bidon, Lalley, & Richter, 2009; Sahibzada, Ferreira, Wasserman, Taveira-DaSilva, & Gillis, 2000; Zhuang & Xu, 2022). It has been proposed that following opioid-induced depression of respiratory frequency, activation of 5-HT_{1A} receptors stimulates respiration by disinhibiting post-inspiratory neurons (Manzke, Dutschmann, Schlaf, Morschel, Koch, Ponimaskin, Bidon, Lalley, & Richter, 2009). It is possible that a different action is responsible for restoration of breathing following opioid-induced respiratory arrest, but more work is needed to elucidate the mechanism. We can attribute the effects we observed with buspirone and 8-OH-DPAT to action on 5-HT_{1A} receptors, as protective effects on breathing and swallowing were reversed by the competitive 5-HT_{1A} agonist WAY-100635.

There are currently no pharmacological treatments for pharyngeal dysphagia, a diagnosis that is challenging to treat and associated with poor outcomes (Marik & Kaplan, 2003; Martino, Foley, Bhogal, Diamant, Speechley, & Teasell, 2005; Patel, Krishnaswami, Steger, Conover, Vaezi, Ciucci, & Francis, 2018; Pezdirec, Strojjan, & Boltezar, 2019; Plowman, Anderson, York, DiBiase, Vasilopoulos, Arnaoutakis, Beaver, Martin, & Jeng, 2023; Thiyagalingam, Kulinski, Thorsteinsdottir, Shindelar, & Takahashi, 2021; Wilson, 2012; Zuercher, Moret, Dziewas, & Schefold, 2019). There is need for a drug that stabilizes breathing for patients who are prescribed opioids and have

risk factors for respiratory depression. Although naloxone effectively reverses opioid overdose, it is not a suitable concomitant therapy because it also reverses antinociception, and can precipitate withdrawal (Bateman, Saunders, & Levitt, 2023; Marchette, Carlson, Frye, Hastings, Vendruscolo, Mejias-Torres, Lewis, Hampson, Volkow, Vendruscolo, & Koob, 2023). Our pre-clinical data show that 5-HT_{1A} is a promising receptor for drug development aimed at treating dysphagia and opioid-induced respiratory arrest. Few 5-HT_{1A} agonists are currently available for use in humans, however buspirone is an FDA approved anxiolytic that may also be leveraged to treat disorders of breathing and swallowing (Wilson & Tripp, 2023). More research is needed on opioids, serotonin and the neural networks controlling breathing and swallowing in order to develop effective pharmacological therapies for humans.

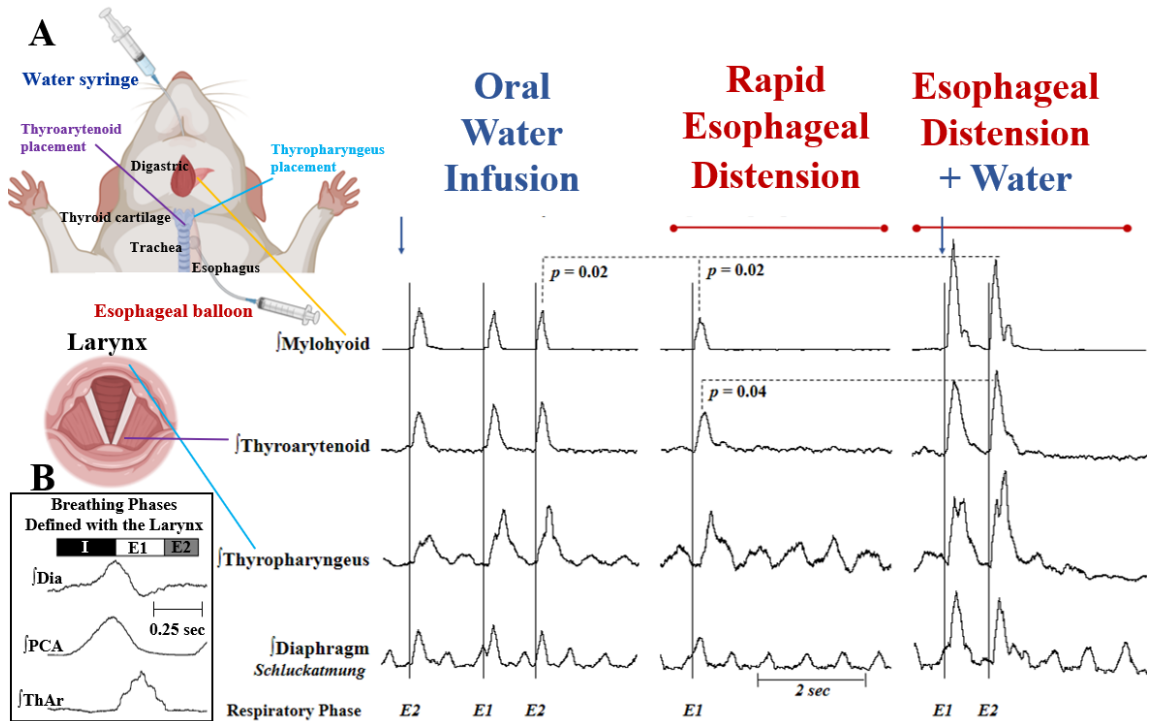


Figure 3-1. Simultaneous activation of pharyngeal and esophageal mechanoreceptors increases laryngeal drive during swallow. A) Electromyogram (EMG) activity was recorded from the mylohyoid, thyroarytenoid, thyropharyngeus and costal diaphragm in freely breathing (pentobarbital anesthetized) rats with intact vagi. Swallows were elicited with infusion of 1 cc of water into the oropharynx, rapid upper esophageal distension (0.5 cc balloon volume), and combined stimulus (esophageal distension plus water infusion). Traces are rectified and integrated (20-ms), and amplitudes are reported as percent of mean. Arrows indicate water infusion in the oropharynx, horizontal lines indicate esophageal distension, and vertical lines indicate swallow initiation. Representative traces of EMG activity during swallow show stimulus dependent modulation of the swallow motor pattern. Analysis of variance (ANOVA) showed differences in mylohyoid (laryngeal elevator) amplitude [$F(1.8, 40) = 7.93, p = 0.002$], mylohyoid burst duration [$F(2.0, 27) = 14.19, p < 0.0001$], and thyroarytenoid (laryngeal adductor) amplitude

[$F(1.4, 31) = 4.19, p = 0.037$] during swallow across the three stimulus conditions. Post-hoc comparisons using the Tukey HSD test indicated that the mean mylohyoid amplitude was larger during swallows elicited by esophageal distension plus water ($M = 118.1\%$, $SD = 17.1\%$) compared to swallows elicited by water alone ($M = 93.58\%$, $SD = 18.96\%$) or esophageal distension alone ($M = 91.60\%$, $SD = 24.71\%$). Mean mylohyoid burst duration was longer during swallows elicited by esophageal distension ($M = 304.3$ ms, $SD = 69.6$ ms, $p = 0.003$) and esophageal distension plus water ($M = 311.3$, $SD = 68.52$ ms, $p = 0.0009$) compared to swallows elicited by water alone ($M = 242.8$ ms, $SD = 58.02$ ms). Mean thyroarytenoid amplitude was larger during swallows elicited by esophageal distension plus water ($M = 103.8\%$, $SD = 12.85\%$) compared to swallows elicited by esophageal distension alone ($M = 91.46\%$, $SD = 10.43\%$). Diaphragm EMG activity reflects inspiratory activity during breathing and swallow. In 50% of animals, amplitude of diaphragm activity during swallows (i.e. Schluckatmung) elicited by esophageal distension and esophageal distension plus water increased qualitatively compared to swallows elicited by water alone, but the effect was not significant as a group. Respiratory phase was determined by diaphragm activity at the onset of swallow. Most swallows occurred during expiration, with no significant change in swallow-breathing coordination across stimulus conditions. B) Representative EMG example of breathing using laryngeal drive to define breathing phases. Traces are rectified and integrated (80-ms). The diaphragm (Dia) acts as an inspiratory pump. The inspiratory-phasic posterior cricoarytenoid (PCA) opens the glottis, which reduces resistance to inspired air. Activation of the thyroarytenoid (ThAr) during expiration partially closes the larynx, and functions as a braking mechanism for the expiratory phase. Inspiration (I) is

the period from the onset of diaphragm and PCA activation to the peak of the diaphragm burst. Early expiration (E1) is the period from the peak of the diaphragm burst to quiescence of the thyroarytenoid. Late expiration (E2) is the period from the end of the thyroarytenoid activation to the beginning of the next diaphragm burst. Breathing phases are defined by the larynx during eupnea. Because the thyroarytenoid is active during swallow, swallow-breathing coordination may be defined by diaphragm EMG activity.

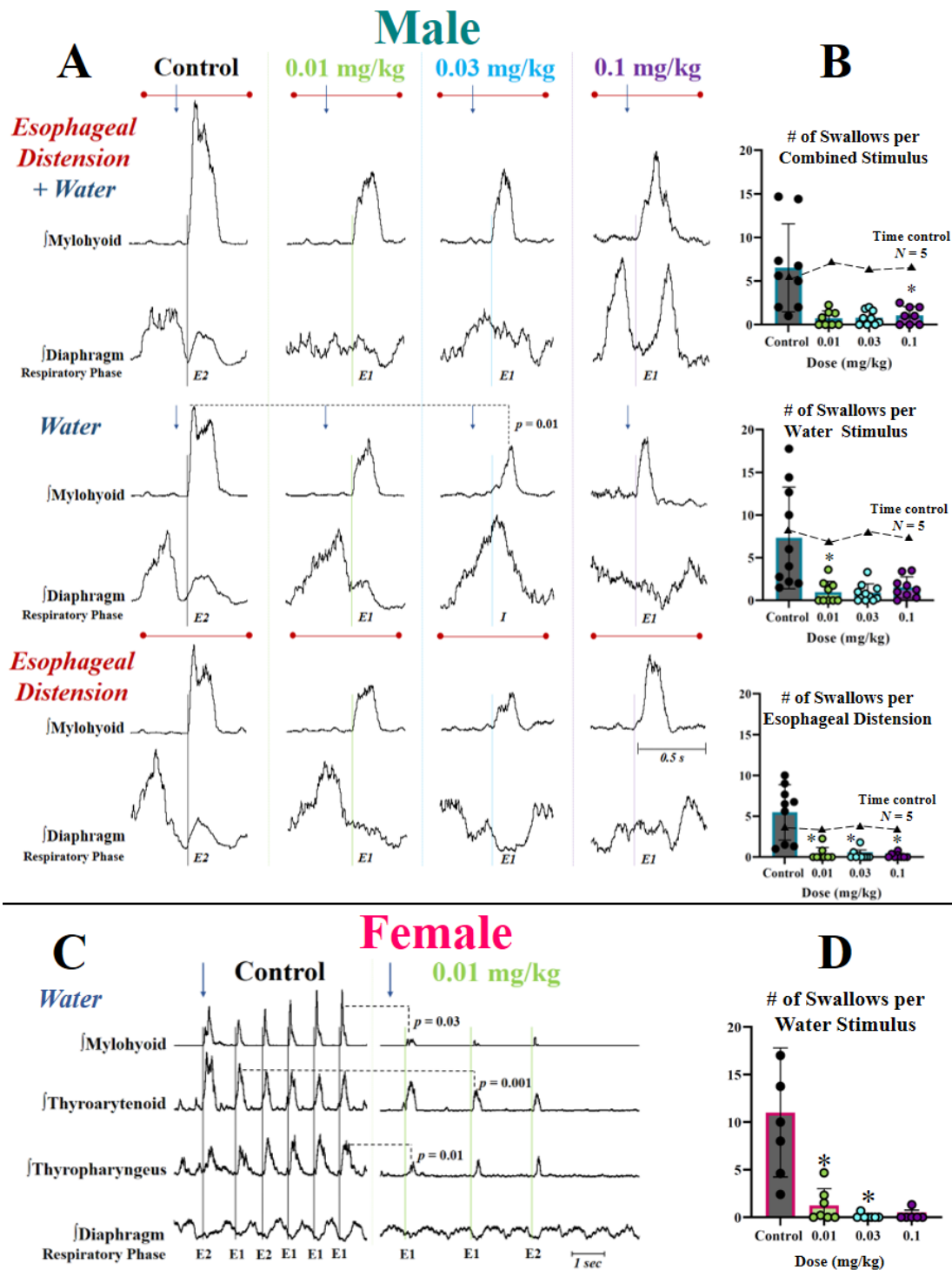


Figure 3-2. The opioid buprenorphine depresses swallow-related trigeminal drive in male rats, and swallow-related trigeminal, vagal and glossopharyngeal drive in female rats. To test the hypothesis that opioids depress swallow function, we performed cumulative dose

response experiments with buprenorphine (0.01, 0.03 and 0.1 mg/kg IV) in freely breathing (vagi intact) pentobarbital anesthetized male and female Sprague Dawley Rats.

A) Buprenorphine reduces laryngeal elevation during swallow, and alters swallow breathing coordination in male rats. Representative electromyogram (EMG) traces show mylohyoid (laryngeal elevator, innervation: CN V) and diaphragm activity during swallows elicited by esophageal distension plus water (top), water alone (middle) and esophageal distension alone (bottom) across buprenorphine doses in a male rat. Traces are rectified and integrated (20-ms time), and amplitudes are reported as percent of mean during control. Arrows indicate water infusion in the oropharynx, horizontal lines indicate esophageal distension, and vertical lines indicate swallow initiation. All three stimuli reliably elicit swallow, and analysis of variance (ANOVA) showed significant differences in mylohyoid amplitude [$F(2.2, 9.7) = 7.3, p = 0.01$] and burst duration [$F(1.3, 6) = 6.194, p = 0.04$] during swallow. Post-hoc comparisons using the Tukey HSD test indicated that peak mylohyoid amplitude was significantly reduced during swallows elicited by water after 0.03 mg/kg buprenorphine ($M = 43\%, SD = 15\%$) compared to control water swallows ($M = 93\%, SD = 19\%$). A trending reduction in peak amplitude during swallows elicited by esophageal distension plus water after 0.01 mg/kg ($M = 57\%, SD = 13\%, p = 0.09$) and 0.03 mg/kg buprenorphine ($M = 72\%, SD = 33\%, p = 0.06$) compared to control combined stimulus swallows ($M = 115\%, SD = 20\%$) did not reach significance. Mylohyoid burst duration was significantly reduced during swallows elicited by water after 0.01 mg/kg ($M = 196\text{ ms}, SD = 84\text{ ms}$) and swallows elicited by esophageal distension plus water after 0.03 mg/kg buprenorphine ($Mean = 170\text{ ms}, SD = 103\text{ ms}$), compared to control water ($M = 269\text{ ms}, SD = 56\text{ ms}$) and combined stimulus

($M = 337$ ms, $SD = 44$ ms) swallows, respectively. Swallow breathing coordination was determined by respiratory phase at time of swallow onset. Swallow initiation was categorized as occurring during inspiration (I), early expiration (E1), or late expiration (E2). During control, most swallows occurred during late expiration. A Wilcoxon signed-rank test detected a significant change in swallow breathing coordination following buprenorphine administration, with significantly more swallows occurring during early expiration following buprenorphine administration, across dose and stimulus conditions ($z = -3.71$, $p < 0.0001$). B) Buprenorphine reduces frequency of swallow occurrence in male rats. Plots show number of swallows elicited by water plus esophageal distension, water, and esophageal distension stimuli before and after buprenorphine administration in male rats. Each circle represents the average number of swallows per stimulus for an individual animal across the buprenorphine dose response curve. Triangles represent the pooled average number of swallows elicited by combined stimulus, water, and esophageal distension during separate time control experiments ($N = 5$) in which animals received a saline vehicle infusion (0.5 cc IV) instead of buprenorphine at each time point of the dose response. ANOVA showed a significant difference in frequency of swallow occurrence [$F(1.8, 13.6) = 11$, $p = 0.002$] among the buprenorphine experiments. Post-hoc comparisons using the Tukey HSD test showed that significantly fewer swallows occurred following: Oral water infusion and esophageal distension following 0.01 mg/kg buprenorphine; esophageal distension following 0.03 mg/kg buprenorphine; and esophageal distension plus water and esophageal distension alone after 0.1 mg/kg buprenorphine, compared to control, respectively. ANOVA showed no significant change in frequency of swallow occurrence among time control animals [$F(1.8, 7.3) = 3.3$], $p =$

0.1]. * indicates $p < 0.05$. C) Buprenorphine reduces laryngeal elevation, laryngeal closure, and pharyngeal constriction during swallow in female rats. Representative EMG traces of mylohyoid (laryngeal elevator, innervation: CN V), thyroarytenoid (laryngeal adductor, innervation: CN X), thyropharyngeus (pharyngeal constrictor, innervation: CN IX) and diaphragm depict swallows elicited by oral water infusion before and after buprenorphine administration in a female rat. Traces are rectified and integrated (20-ms), and amplitudes are reported as percent of mean during control. Arrows indicate water infusion in the oropharynx, and vertical lines indicate swallow initiation. ANOVA showed significant differences in peak mylohyoid [$F(1.7, 7.9) = 13.1, p = 0.004$], thyroarytenoid [$F(1.4, 6.5) = 28.2, p = 0.0009$], and thyropharyngeus [$F(1.9, 8.6) = 13.6, p = 0.002$], amplitudes during swallows elicited by oral water infusion. Post-hoc comparisons using the Tukey HSD test indicated that mylohyoid ($M = 33\%, SD = 14\%$), thyroarytenoid ($M = 68\%, SD = 3.5\%$) and thyropharyngeus ($M = 50\%, SD = 8\%$) amplitudes were significantly reduced during water swallows following 0.01 mg/kg buprenorphine compared to water swallows during control [mylohyoid: ($M = 95\%, SD = 20\%$), thyroarytenoid: ($M = 100\%, SD = 3\%$), thyropharyngeus: ($M = 101\%, SD = 19\%$)], respectively. Most swallows elicited by water occurred during expiration, with no significant change in swallow breathing coordination following 0.01 mg/kg buprenorphine. D) Buprenorphine reduces frequency of swallow initiation in female rats. Frequency plot shows number of swallows elicited by oral water infusion before and after buprenorphine administration in female rats. Each circle represents the average number of swallows per stimulus for an individual animal across the buprenorphine dose response curve. ANOVA showed a significant difference in frequency of swallow occurrence

[$F(1.8, 7.3) = 10.7, p = 0.008$]. Post-hoc comparisons using the Tukey HSD test showed that significantly fewer swallows occurred following 0.01 mg/kg and 0.03 mg/kg buprenorphine. A trending decrease in swallow frequency after 0.1 mg/kg was not statistically significant ($p = 0.09$). * indicates $p > 0.05$.

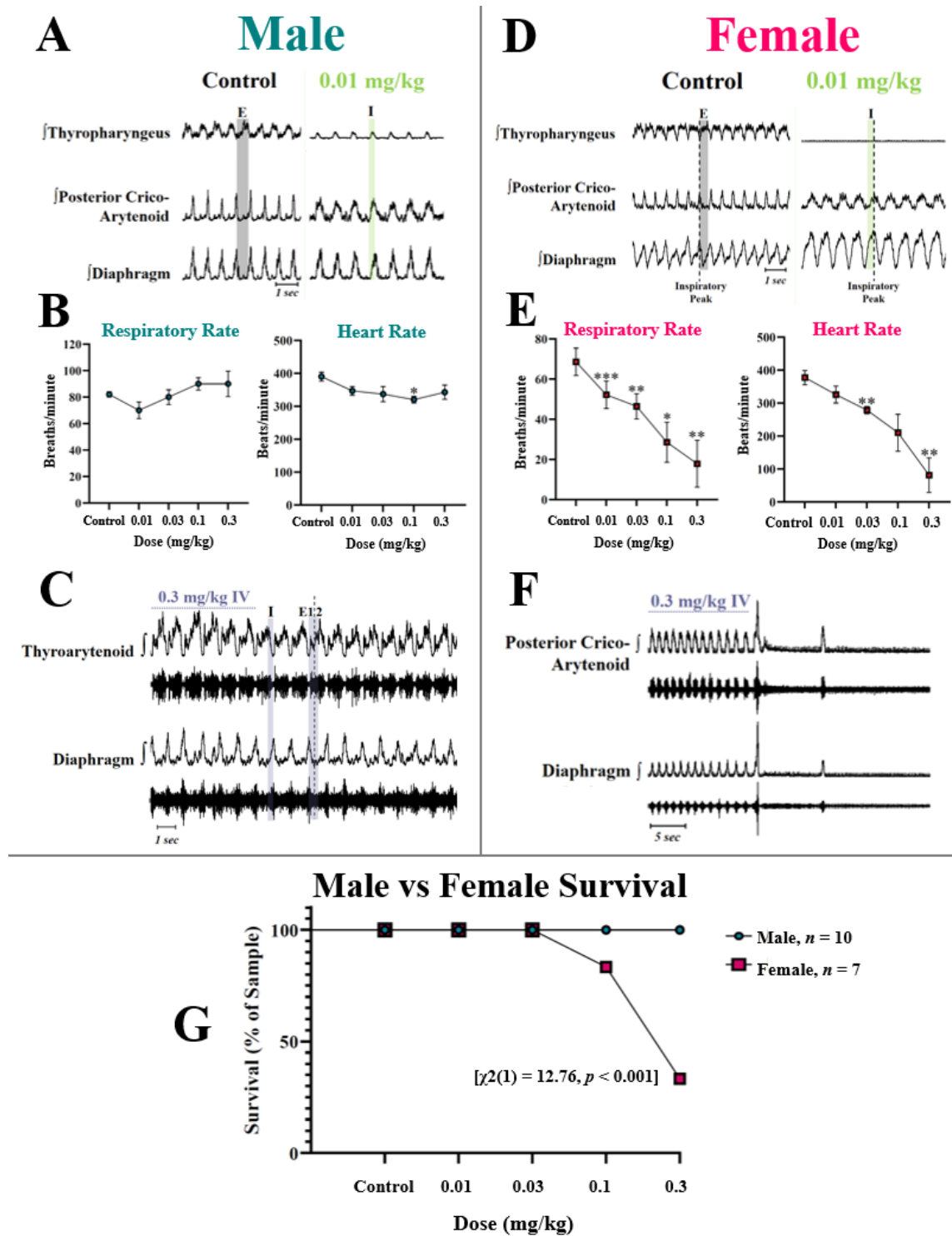


Figure 3-3. The opioid buprenorphine disproportionately depresses respiration in female rats. A) Representative electromyogram (EMG) traces show thyropharyngeus, posterior cricoarytenoid, and diaphragm activity during eupnea before and after

buprenorphine administration (0.01 mg/kg IV) in a male rat. Traces are rectified and integrated (20-ms). The thyropharyngeus (inferior pharyngeal constrictor) is active during both breathing and swallow. During control, the thyropharyngeus demonstrates phasic expiratory activity during breathing (gray bar). After buprenorphine, the activity of the thyropharyngeus shifts and is phasic during inspiration (green bar). A Wilcoxon signed-rank test detected a significant change in thyropharyngeus respiratory phase preference, with significantly more male animals demonstrating inspiratory activity following buprenorphine administration ($Z = -2.7, p = 0.004$). B) Plots show mean respiratory rate and heart rate among male rats across a cumulative buprenorphine dose response curve (0.01, 0.03, 0.1 and 0.3 mg/kg IV). Plotted values represent mean breaths per minute and beats per minute, respectively. Buprenorphine did not significantly impact respiratory rate in male animals. Analysis of variance (ANOVA) showed a significant difference in heart rate [$F(2.5, 19.2) = 3.8, p = 0.03$], and post-hoc comparisons using the Tukey HSD test indicated heart rate was significantly reduced following 0.1 mg/kg buprenorphine ($M = 320, SD = 30$) compared to control ($M = 390, SD = 42.4$). * indicates $p < 0.05$. C) Representative EMG traces showing thyroarytenoid and diaphragm activity during eupnea following high dose buprenorphine (0.3 mg/kg IV) in a male rat. Traces are rectified and integrated (20-ms). Horizontal dotted line indicates IV infusion. Vertical dotted line demarcates the beginning of diaphragm quiescence within a single breath cycle, which indicates the end of early expiration (E1) and the beginning of late expiration (E2). The thyroarytenoid (laryngeal adductor) is active during both breathing and swallow, and normally demonstrates phasic expiratory activity during eupnea. Traces depict stable respiratory activity in the upper airway and diaphragm after high dose

buprenorphine in a male animal. D) Representative electromyogram (EMG) traces show thyropharyngeus, posterior cricoarytenoid, and diaphragm activity during eupnea before and after buprenorphine administration (0.01 mg/kg IV) in a female rat. Traces are rectified and integrated (20 ms). Vertical dotted lines indicate peak diaphragm amplitude of a single breath, which marks the end of inspiration (I) and the beginning of early expiration (EI). During control, the thyropharyngeus is active during expiration (highlighted in gray). Breathing-related thyropharyngeus activity is lost following buprenorphine administration. Five female animals demonstrated reduced breathing-related thyropharyngeus activity after buprenorphine (0.01 mg/kg IV), but the effect was not significant as a group. E) Plots show mean respiratory rate and heart rate among female rats across a cumulative buprenorphine dose response curve (0.01, 0.03, 0.1, and 0.3 mg/kg IV). Plotted values represent mean breaths per minute and beats per minute, respectively. ANOVA showed a significant difference in respiratory rate among female animals [$F(1.5, 6.3) = 11, p = 0.01$], and post-hoc comparisons using the Tukey HSD test showed a significant decrease in respiratory rate following 0.01 mg/kg ($M = 52.1, SD = 18$), 0.03 mg/kg ($M = 46.4, SD = 16.5$), 0.1 mg/kg ($M = 28.6, SD = 26.3$), and 0.3 mg/kg buprenorphine ($M = 17.9, SD = 30.8$) compared to control ($M = 68.6, SD = 18$). ANOVA showed a significant difference in heart rate among female animals, and post-hoc comparisons using the Tukey HSD test showed a significant decrease in heart rate following 0.03 mg/kg ($M = 278.6, SD = 28.5$) and 0.3 mg/kg buprenorphine ($M = 81.4, SD = 139.3$) compared to control ($M = 377.1, SD = 57.1$). *** indicates $p < 0.001$, ** indicates $p < 0.01$, and * indicates $p < 0.05$. F) Representative EMG traces showing posterior cricoarytenoid and diaphragm activity during breathing, and apnea following

high dose buprenorphine (0.3 mg/kg IV) administration in a female rat. Traces are rectified and integrated (20 ms). The posterior cricoarytenoid (laryngeal abductor) demonstrates phasic activity during eupnea, and functions to open the glottis during inspiration. Traces depict loss of inspiratory effort in the upper airway and diaphragm following high dose buprenorphine. Breathing did not recover in this female animal. G) Plot of survival proportions among male and female rats following buprenorphine administration (cumulative .01, 0.03, 0.1 and 0.3 mg/kg IV). A log rank test detected a significant difference in the survival distribution between males and females. More than 70% of female animals succumbed to respiratory arrest following high dose buprenorphine (0.1 and 0.3 mg/kg), while all male animals maintained stable respiratory effort across doses.

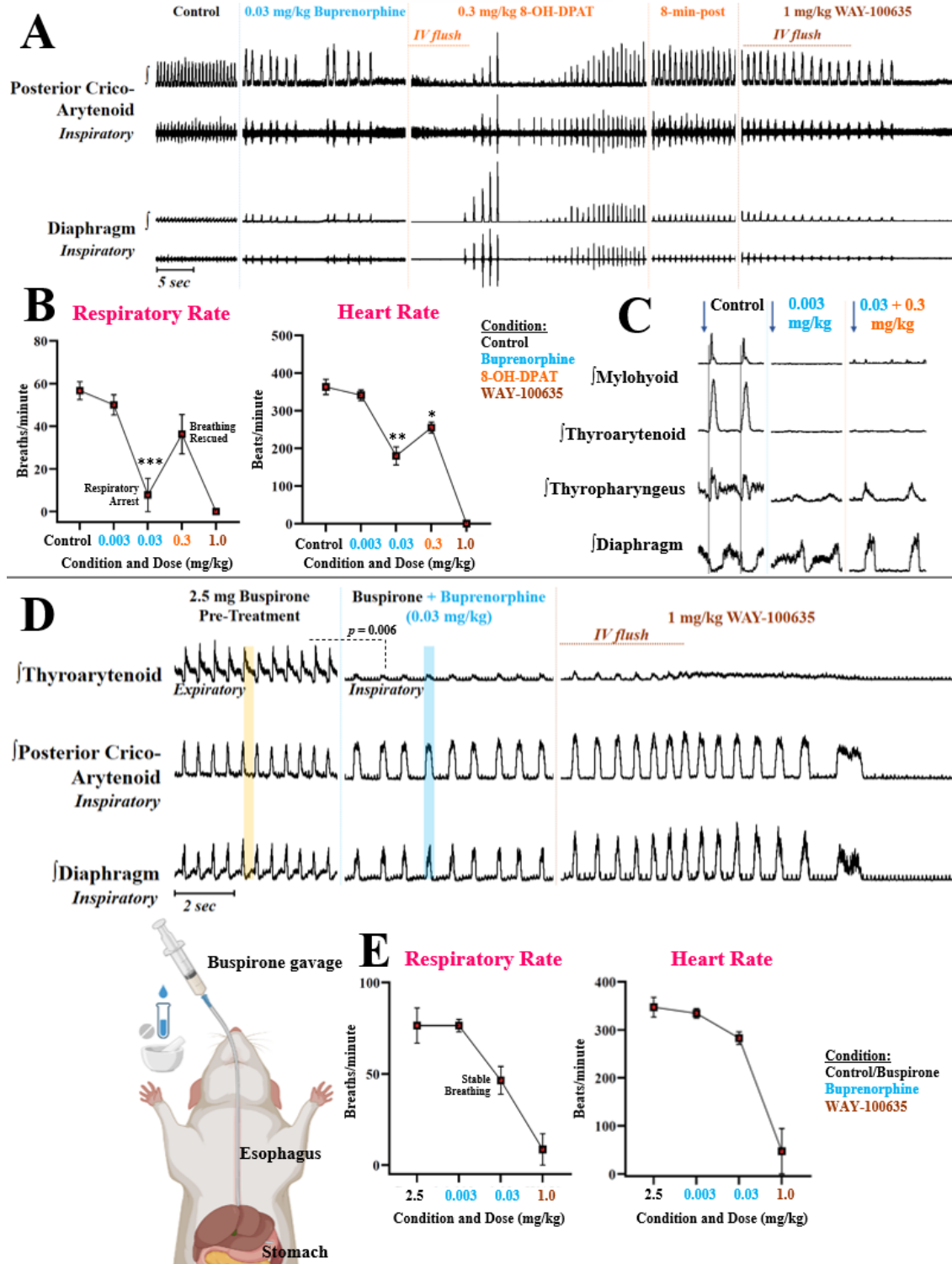


Figure 3-4. 5-HT_{1A} agonists restore and preserve breathing following buprenorphine

administration in female rats. A) To test the hypothesis that systemic administration of a 5-HT_{1A} agonist would restore breathing following opioid induced apnea, we performed experiments in $N = 9$ freely breathing pentobarbital anesthetized adult female Sprague Dawley rats. A) Representative traces from posterior cricoarytenoid (laryngeal abductor) and diaphragm electromyograms (EMGs) show activity during breathing in a female rat. Traces are rectified and integrated (20-ms). Following a period of eupnea (control), buprenorphine was titrated IV until apnea occurred (0.03 mg/kg in this animal). The 5-HT_{1A} agonist 8-OH-DPAT was then administered (0.3 mg/kg IV), and inspiratory effort was restored within 30 seconds of infusion. Breathing remained stable for over eight minutes following 8-OH-DPAT, and was abolished by administration of the competitive 5-HT_{1A} antagonist WAY-100635 (1 mg/kg IV). Eight of nine animals stopped breathing following buprenorphine administration (one animal resisted opioid induced apnea). 8-OH-DPAT restored breathing in six animals (no restoration in two animals), and this effect was reversed by WAY-100635. B) Plots show mean respiratory rate and heart rate among female rats after IV administration of buprenorphine, 8-OH-DPAT, and WAY-100635. Plotted values represent mean breaths per minute and beats per minute, respectively. Analysis of variance (ANOVA) detected significant differences in respiratory rate [$F(2, 13.8) = 18.7, p = 0.0001$] and heart rate [$F(2.6, 21.2) = 41.6, p < 0.0001$]. Post-hoc comparisons using the Tukey HSD test indicated that respiratory rate was significantly reduced following 0.03 mg/kg buprenorphine ($M = 7.8, SD = 30$) compared to control ($M = 56.7, SD = 12.5$). Respiratory rate increased in seven of nine animals following 0.3 mg/kg 8-OH-DPAT (compared to 0.03 mg/kg buprenorphine), but this effect was not statistically significant as a group ($p = 0.09$). Post-hoc comparisons

using the Tukey HSD test indicated that heart rate was significantly reduced following 0.03 mg/kg buprenorphine ($M = 180$, $SD = 71.9$) compared to control ($M = 363.3$, $SD = 56.1$), and significantly increased following 0.3 mg/kg 8-OH-DPAT ($M = 255$, $SD = 28.7$) compared to 0.03 mg/kg buprenorphine. *** indicates $p < 0.001$, ** indicates $p < 0.01$, $p < 0.05$. C) Representative traces from mylohyoid (laryngeal elevator), thyroarytenoid (laryngeal adductor), thyropharyngeus (pharyngeal constrictor) and diaphragm show response to oral water infusion before and after administration of buprenorphine (0.03 mg/kg IV) and 8-OH-DPAT (0.3 mg/kg IV) in a female rat. Traces are rectified and integrated (20-ms). Arrows indicate oral water infusion and vertical lines indicate swallow onset. Swallow initiation was suppressed following buprenorphine administration (0.03 mg/kg IV). 8-OH-DPAT administration did not restore responsiveness to oral water infusion following total swallow suppression by buprenorphine in female animals. D) To test the hypothesis that pre-treatment with the partial 5-HT_{1A} agonist buspirone would prevent opioid induced apnea in females, experiments were performed in $N = 7$ adult female Sprague Dawley rats. Representative traces from thyroarytenoid (laryngeal adductor), posterior cricoarytenoid (laryngeal abductor) and diaphragm show activity during eupnea in a female rat pre-treated with buspirone (2.5 mg orogastric gavage) and subsequently treated with buprenorphine (0.03 mg/kg IV) and WAY-100635 (1 mg/kg IV). Traces are rectified and integrated (20 ms), and amplitudes are reported as percent mean of control. Yellow rectangle highlights the expiratory phase of one respiratory cycle. Blue rectangle highlights the inspiratory phase of one respiratory cycle. Representative trace of thyroarytenoid activity depicts phasic expiratory activity during buspirone control, and reduced peak amplitude, phasic

inspiratory activity, and expiratory quiescence following buprenorphine administration. ANOVA detected significant differences in breathing-related thyroarytenoid amplitude across conditions [$F(1.3, 7.6) = 26.3, p = 0.0007$], and post-hoc comparisons using the Tukey HSD test indicated a significant decrease in breathing-related thyroarytenoid amplitude following 0.03 mg/kg buprenorphine (Mean = 66.4%, SD = 17.7%) compared to control ($M = 100\%, SD = 0\%$). A Wilcoxon signed-rank test detected a significant difference in laryngeal respiratory phase preference, with significantly more animals demonstrating phasic inspiratory thyroarytenoid activity following buprenorphine administration compared to control, when phasic thyroarytenoid activity is expiratory ($z = -1.86, p = 0.03$). EMG traces depict stable respiratory effort after a dose of buprenorphine that produced apnea in animals that did not receive buspirone (0.03 mg/kg IV, see panel A), and cessation of breathing following administration of the competitive 5-HT_{1A} antagonist WAY-100635. All seven animals pre-treated with buspirone maintained regular breathing across buprenorphine doses, and breathing was abolished in six animals following WAY-100635 (one animal resisted respiratory arrest). E) Plots show respiratory rate and heart rate among female rats pre-treated with buspirone following administration of buprenorphine (cumulative 0.003 and 0.03 mg/kg IV) and WAY-100635. Plotted values represent mean breaths per minute and beats per minute, respectively. There was no significant change in respiratory rate or heart rate following buprenorphine administration among female animals pre-treated with buspirone, compared to control. A log rank test detected a significant difference in survival between experimental groups, with higher median survival following buprenorphine

administration among female animals pre-treated with buspirone than animals that did not receive buspirone [$\chi^2(1) = 11.67, p < 0.001$].

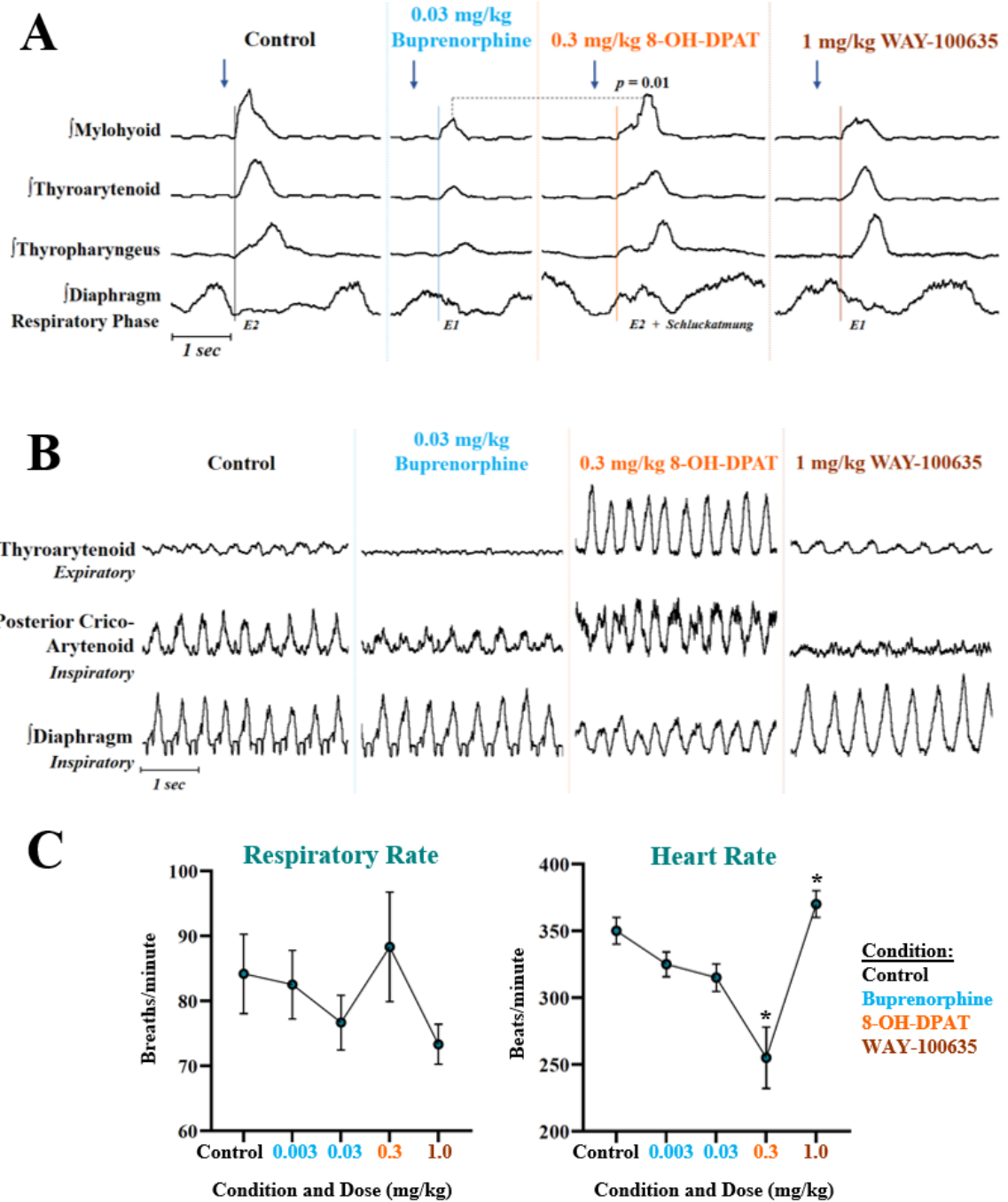


Figure 3-5. The 5-HT_{1A} agonist 8-OH-DPAT restores laryngeal elevation during swallow following buprenorphine administration in male rats. To test the hypothesis that systemic 8-OH-DPAT administration would result in correction toward baseline swallow measures following buprenorphine-induced alteration of swallow motor pattern, experiments were

performed in $N = 6$ spontaneously breathing pentobarbital anesthetized adult male Sprague Dawley rats. A) Representative electromyogram (EMG) traces of mylohyoid (laryngeal elevator), thyroarytenoid (laryngeal adductor), thyropharyngeus (pharyngeal constrictor) and diaphragm activity during swallow following buprenorphine (0.03 mg/kg IV), 8-OH-DPAT (0.3 mg/kg IV) and WAY-100635 (1 mg/kg IV) administration. Traces are rectified and integrated (20 ms), and amplitudes are reported as percent mean of control. Arrows indicate oral water infusion and vertical lines indicate swallow onset. Analysis of variance (ANOVA) showed a significant difference in peak mylohyoid amplitude during swallow across conditions [$F(1.7, 6.9) = 19.3$], $p = 0.002$], and post-hoc comparisons using the Tukey HSD test revealed a significant decrease in peak mylohyoid amplitude following 0.03 mg/kg buprenorphine ($M = 50.8\%$, $SD = 18\%$) compared to control ($M = 100\%$, $SD = 0\%$), and a significant increase in peak mylohyoid amplitude following 0.3 gm/kg 8-OH-DPAT ($M = 87.7\%$, $SD = 21.5\%$) compared to 0.03 mg/kg buprenorphine. Following administration of the competitive 5-HT_{1A} antagonist WAY-100635, swallow-related mylohyoid amplitude decreased in five of six animals ($M = 63.3\%$, $SD = 34.1\%$), and was no longer significantly different than mylohyoid amplitude following 0.03 mg/kg buprenorphine ($p = 0.8$). A Wilcoxon signed-rank test detected a significant change in swallow breathing coordination, with significantly more swallows occurring during early expiration (E1) following 0.03 mg/kg buprenorphine ($z = -3.3$, $p = 0.004$) compared to control, when most swallows occurred during late expiration (E2). This effect persisted following 0.3 mg/kg 8-OH-DPAT ($z = -2.7$, $p = 0.0004$) and WAY-100635 ($z = -3.1$, $p = 0.001$), with significantly more swallows initiated during early expiration (E1) at the group level. B) Representative electromyogram (EMG) traces of

breathing-related thyroarytenoid (laryngeal adductor), posterior cricoarytenoid (laryngeal abductor) and diaphragm activity during control and following IV administration of buprenorphine (0.03 mg/kg), 8-OH-DPAT (0.3 mg/kg), and WAY-100635 (1 mg/kg) in a male rat. Traces are rectified and integrated (50 ms). Traces depict reduction in breathing-related activation of laryngeal muscles (thyroarytenoid and posterior cricoarytenoid) following buprenorphine (0.03), and an increase in laryngeal drive during breathing following 8-OH-DPAT (0.3 mg/kg) that is reversed by the competitive 5-HT_{1A} agonist WAY-100635. This series of effects occurred in four of six animals, but was non-significant as a group. C) Plots show respiratory rate and heart rate among male rats following IV administration of buprenorphine (0.003 and 0.03 mg/kg), 8-OH-DPAT (0.3 mg/kg), and WAY-100635 (1 mg/kg). Plotted values represent mean breaths per minute and beats per minute, respectively. There were no significant differences in respiratory rate among male rats across doses. ANOVA indicated significant differences in heart rate across conditions, and post-hoc comparisons using the Tukey HSD test showed a significant decrease in heart rate following 8-OH-DPAT ($M = 255$, $SD = 62.2$) compared to control ($M = 350$, $SD = 26.8$), and a significant increase in heart rate following WAY-100635 ($M = 370$, $SD = 26.8$) compared to 8-OH-DPAT. Unlike female animals, all male animals maintained stable respiratory effort across conditions. * indicates $p < 0.05$.

CHAPTER 4
ASPIRATION FOLLOWING POST-OPERATIVE OPIOID ADMINISTRATION IN A
NOVEL MODEL OF DYSPHAGIA

Opioids are known to depress cough, but the effects of opioids on airway protection during swallow are not well understood. The purpose of our study was to test the effects of the opioid buprenorphine on pharyngeal swallow function following a routine surgery, and after clinical doses of buprenorphine alone (no surgery). We hypothesized that opioid administration would result in a measurable change in swallow function in both the post-operative and non-surgical groups. Experiments were performed on eight healthy adult cats that were trained to feed during videofluoroscopic swallow studies (VFSS). For the post-operative group, four females underwent routine spay surgery and received 0.015 mg/kg buprenorphine for 48-hours post-operatively. VFSS were performed after the last dose of buprenorphine and compared to control assessments. To evaluate the effects of buprenorphine alone, a non-surgical group received either 0.02 mg/kg or 0.04 mg/kg buprenorphine for 48-hours. VFSS were performed after buprenorphine administration and compared to control assessments. Airway protection during swallow was significantly affected in both groups, but most severe in the post-operative group where 75% (three of four) animals exhibited silent aspiration. We concluded that oropharyngeal swallow function is negatively impacted by the partial mu-opioid receptor agonist buprenorphine, most remarkably in the post-

operative setting. These findings have implications for the prevention and management of aspiration pneumonia in at-risk populations.

Introduction

Swallow is a complex behavior necessary for oral alimentation and airway protection (Jean, 2001b). Dysphagia, the medical term for disordered swallowing, occurs when the oral, pharyngeal and/or esophageal phases of swallowing are disrupted (Lawal & Shaker, 2008; Logemann, 2007). Dysphagia is associated with a variety of conditions including stroke, brain and spinal cord injury, neurodegenerative disease, head and neck cancer, critical illness, and polypharmacy (Martino, Foley, Bhogal, Diamant, Speechley, & Teasell, 2005; McRae, Morgan, Wallace, & Miles, 2022; Pezdirec, Strojjan, & Boltezar, 2019; Schwemmle, Jungheim, Miller, Kuhn, & Ptok, 2015; Takizawa, Gemmell, Kenworthy, & Speyer, 2016; Zuercher, Moret, Dziewas, & Schefold, 2019). Dysfunction of the swallowing mechanism can result in aspiration of food, liquid and secretions, chest infection, dehydration, malnutrition, and reduced quality of life (Kim, Park, Park, & Kim, 2020; Langmore, Terpenning, Schork, Chen, Murray, Lopatin, & Loesche, 1998; Thiyagalingam, Kulinski, Thorsteinsdottir, Shindelar, & Takahashi, 2021). People with dysphagia are more likely to require inpatient medical care, and experience significantly increased financial burden and mortality (Nativ-Zeltzer, Nachalon, Kaufman, Seeni, Bastea, Aulakh, Makkiyah, Wilson, Evangelista, Kuhn, Sahin, & Belafsky, 2022; Wilson, 2012).

Post-operative dysphagia is a known risk of head, neck, and gastrointestinal surgery. Specifically, post-operative dysphagia has been studied following anterior cervical discectomy and fusion (ACDF), head and neck cancer resection, tracheostomy,

esophagectomy, gastrectomy, and anti-reflux procedures (Goeze, Zaretsky, Lehner, Wermter, Mayer, Stuck, Birk, Neff, Fisher, Stover, Kramer, Ghanaati, Sader, & Hey, 2022; Greenberg, Stefanova, Reyes, Edelmuth, Harik, Thiesmeyer, Egan, Palacardo, Liu, Christos, Schnoll-Sussman, Katz, Finnerty, Fahey, & Zarnegar, 2022; Haller, Mehul Kharidia, Bertelsen, Wang, & O'Dell, 2022; Hayes, Gillman, Wright, Dorgan, Brennan, Walshe, Donohoe, Reynolds, & Regan, 2022; Nath, Yewale, Tran, Brebbia, Shope, & Koch, 2016; Skoretz, Anger, Wellman, Takai, & Empey, 2020). Additionally, post-operative dysphagia has been widely reported following cardiothoracic surgery, with mechanical damage to aerodigestive structures and the recurrent laryngeal nerve as proposed mechanisms (Barker, Martino, Reichardt, Hickey, & Ralph-Edwards, 2009; Ferraris, Ferraris, Moritz, & Welch, 2001; Plowman, Anderson, York, DiBiase, Vasilopoulos, Arnaoutakis, Beaver, Martin, & Jeng, 2023; Skoretz, Yau, Ivanov, Granton, & Martino, 2014). Dysphagia may result in pneumonia, which is associated with poor clinical outcomes post-operatively (Daly, Miles, Scott, & Gillham, 2016; Wang, Lu, Sun, Huang, Du, Jiao, Sun, & Xie, 2022).

Post-operative pneumonia is a common complication of non-cardiac abdominal surgery, with a prevalence of 1-18%, and estimated mortality of 21-24% (Arozullah, Khuri, Henderson, Daley, & Participants in the National Veterans Affairs Surgical Quality Improvement, 2001; Garibaldi, Britt, Coleman, Reading, & Pace, 1981; Kozlow, Berenholtz, Garrett, Dorman, & Pronovost, 2003). Advanced age, comorbid disease, and large volume intraoperative blood loss have been associated with increased risk of post-operative pneumonia (Arozullah, Khuri, Henderson, Daley, & Participants in the National Veterans Affairs Surgical Quality Improvement, 2001; Garibaldi, Britt, Coleman,

Reading, & Pace, 1981; Kozlow, Berenholtz, Garrett, Dorman, & Pronovost, 2003).

Dysphagia is a known risk factor for chest infection, but its role in pneumonia following non-cardiac, head, or neck surgery is unknown (Marik & Kaplan, 2003).

Systemic opioids are commonly used for post-operative pain management (Chou, Gordon, de Leon-Casasola, Rosenberg, Bickler, Brennan, Carter, Cassidy, Chittenden, Degenhardt, Griffith, Manworren, McCarberg, Montgomery, Murphy, Perkal, Suresh, Sluka, Strassels, Thirlby, Viscusi, Walco, Warner, Weisman, & Wu, 2016). Opioids depress the respiratory, gastrointestinal, and immune systems and are associated with several adverse effects, including opportunistic infection (Bateman, Saunders, & Levitt, 2023; Foley, 1993; Roy, Ninkovic, Banerjee, Charboneau, Das, Dutta, Kirchner, Koodie, Ma, Meng, & Barke, 2011). Furthermore, opioids suppress cough and upper airway reflexes, and have been linked to esophageal dysfunction and aspiration (Patel & Vaezi, 2018; Patel, Goss, Hayat, Tombazzi, Naik, Slaughter, Aslam, Sarker, Higginbotham, & Vaezi, 2022; Savilampi, Ahlstrand, Magnuson, Geijer, & Wattwil, 2014; Steffens, Sung, Bastian, Edelman, Brackett, & Gunderson, 2020; Tagaito, Isono, & Nishino, 1998). Surprisingly, the effect of opioids on oropharyngeal swallow, and potential impacts of opioid-induced aspiration, have been the subject of limited study.

Buprenorphine is a partial mu-opioid receptor agonist used to treat pain and opioid use disorder (Elkader & Sproule, 2005; Shulman, Wai, & Nunes, 2019).

Buprenorphine was approved for post-operative pain management in the United States in 1985, and is currently the most prescribed maintenance therapy for opioid addiction (Shulman, Wai, & Nunes, 2019). Additionally, buprenorphine is widely used for post-operative pain management in animals in veterinary and research settings (Roughan &

Flecknell, 2002). Pharmacological studies indicate that buprenorphine has a lower toxicity profile than other opioids, but there is a paucity of information on how buprenorphine, and opioids in general, affect oropharyngeal swallow function (Elkader & Sproule, 2005; Jasinski, Pevnick, & Griffith, 1978).

The purpose of our study was to systematically evaluate the effect of the opioid buprenorphine on airway protection during feeding in animals following a routine abdominal surgery, and in healthy animals that did not undergo surgery. We hypothesized that buprenorphine administration would cause a measurable change in oropharyngeal swallow function, resulting in airway invasion, in both the surgical and nonsurgical groups.

Methods

Experiments were performed on eight adult male and female short-hair domestic cats [2 male (5.3 ± 0.4 kg) and 6 female (3.4 ± 0.3 kg)]. Protocols were approved by the University of Louisville Institutional Animal Care and Use Committee (IACUC).

VFSS Collection Procedure

Cats were trained to feed during VFSS over a period of approximately four weeks. Animals were acclimated to the examiner, and offered preferred food rewards during brief training sessions (< 15 minutes) in their housing space three to five times per day. Animals were then acclimated to carrier-transport from animal care to our nearby fluoroscopy suite where they were temporarily housed during training and data collection (< 2 hours). Animals were offered test consistencies (water mixed with tuna fish and paté), and incrementally progressed from feeding in the kennel to feeding freely on a weighted table in the fluoroscopic field. Once animals fed continuously and without

direction in the fluoroscopic field, they were offered test consistencies mixed with barium sulfate. Animals were considered adequately trained for participation in an experimental protocol following 5 consecutive days of continuous feeding with barium-contrasted test consistencies during image recording.

Lateral plane VFSS were recorded at 30 frames per second using a Fluoroscanner[®] Insight mini-C-arm (Hologic; Bedford, MA, USA) during thin liquid and puree feeding. Our thin liquid consistency was made using water shaken with tuna fish and strained, and our puree consistency was made using Friskies Paté Turkey and Giblets Dinner (Nestlé Purina PetCare; St. Louis, MO, USA). Both thin liquid and puree consistencies were mixed with 40% by volume barium sulfate (Bracco Diagnostics; Milan, Italy).

Animals were food restricted for < 6 hours prior to image recording and had access to water ad libitum. Animals fed voluntarily and without restraint in a natural stance during image collection. A penny was included in-line with anatomy during all recordings to enable normalization to an object of known size (19.05 mm). Images were exported to a portable drive and uploaded to a data server following collection.

Spay Procedure

Four healthy adult female cats underwent routine ovariohysterectomy performed by a veterinarian specialized in Canine/Feline Practice as certified by the American Board of Veterinary Practitioners (ABVP). Animals received pre-anesthetic treatment with buprenorphine hydrochloride (0.015 mg/kg), acepromazine (0.015 mg/kg) and atropine sulfate (0.015 mg/kg), combined and administered intramuscularly (IM). Anesthesia was induced using isoflurane in a ventilated induction chamber administered via precision vaporizer (5% isoflurane, 5 l/min O₂). Once a light anesthetic plane was

achieved, animals were removed from the induction chamber, positioned sternally, received eye lubrication, and switched to a facemask for continued anesthesia delivery (5% isoflurane, 1 l/min O₂). When a deeper anesthetic plane was achieved, animals were intubated with a 3.5 mm cuffed endotracheal tube for maintenance of anesthesia (2-3% isoflurane, 1 l/min O₂) and a 22g intravenous (IV) catheter placed in the cephalic vein. Animals were then repositioned ventrodorsally, and the abdomen shaved and sterilely prepped and draped for surgery. A surgical monitor was used to continuously track pulse, electrocardiogram (ECG), oxygen saturation (SpO₂), and end-tidal carbon dioxide (ETCO₂), as well as automated, intermittent, non-invasive blood pressure (NIBP) measurements.

Surgical anesthetic depth was confirmed using reflex responses and eye position. A standard midline laparotomy approach (2-3 cm) was performed approximately one inch below the umbilicus and a spay hook used to assist in exteriorizing the left ovary and uterine horn. The suspensory ligament and associated vessels were double clamped with paired transfixion ligatures placed using 3-0 poliglecaprone 25 monofilament suture. The ligament was transected, and the stump inspected for bleeding prior to placement back into the peritoneal cavity. The procedure was then repeated on the right ovary and uterine horn. The broad ligaments were digitally broken down, and the uterine body was exteriorized and similarly double clamped at the cervix, double ligated, transected, and inspected for bleeding. The ovaries and attached uterus were removed and the uterine stump replaced into the peritoneal cavity. Routine closure in three layers (linea and subcutis – continuous; skin - subcuticular) was done and tissue glue (octyl/butyl cyanoacrylate) applied.

After completion of surgery, animals were administered cefazolin sodium (20 mg/kg, slow IV), taken off anesthesia and extubated following return of the pharyngeal swallow reflex. The IV catheter was removed, and patient monitoring continued until recovery was complete before returning to housing. Mean time under anesthesia was 1.3 ± 0.5 hours, mean length of intubation was 1.1 ± 0.5 hours, and mean length of surgery was 0.8 ± 0.4 hours. Buprenorphine (0.015 mg/kg, IM, q8-12h) was administered for post-operative pain control.

Post-Operative Experimental Protocol

Experiments were performed on four healthy adult female cats. Control VFSS were recorded during thin liquid and puree feeding prior to surgery and opioid administration. 48-hours after routine spay surgery, VFSS were repeated during thin liquid and puree feeding, one-hour after the last of five doses of buprenorphine (0.015 mg/kg). VFSS were then repeated one-week post-operatively (5-days after the last dose of buprenorphine).

Non-Surgical Experimental Protocol

Experiments were performed on four healthy adult male ($n = 2$) and female ($n = 2$) cats. Control VFSS were recorded during thin liquid and puree feeding prior to opioid administration. Animals were randomly assigned to a lower (0.02 mg/kg) or higher (0.04 mg/kg) end dose of buprenorphine within the feline clinical range (0.01 - 0.04 mg/kg intramuscular). Animals received our surgical buprenorphine protocol (five doses every eight to 12-hours for 48-hours) without surgery to evaluate the effects of buprenorphine alone. VFSS were recorded after 24- and 48-hours on buprenorphine. Assessments were timed one-hour after buprenorphine injection to allow for time to peak effect. Follow up

VFSS were performed 24-hours, 72-hours, and 5-days after the last dose of buprenorphine. This protocol was repeated one month later with swapped dose assignments such that all four animals were evaluated at lower (0.02 mg/kg) and higher (0.04 mg/kg) doses of buprenorphine.

Quantitative Measures

Images were viewed and analyzed using RadiANT DICOM Viewer (Medixant; Poznan, Poland). Oral phase duration was defined as the time from the beginning of lapping behavior to pharyngeal swallow initiation and measured in milliseconds (ms). Pharyngeal phase duration was defined as the time from hyolaryngeal excursion onset through upper-esophageal sphincter (UES) closure and return of pharyngeal air space, and measured in ms. Prior to pharyngeal swallow initiation, bolus width was measured from the tongue base to the epiglottic rim using the digital imaging and communications in medicine (DICOM) viewer's length measurement tool, and reported as pharyngeal distension in mm. Prior to primary peristalsis initiation, the width of the food-filled, proximal third, striated portion of the esophagus was measured using the length measurement tool and reported as esophageal distension in mm. Bolus area was measured prior to pharyngeal swallow initiation using a closed polygon measurement tool and reported in mm². Oral to pharyngeal phase ratios were calculated by counting the number of tongue laps prior to pharyngeal swallow initiation. Pharyngeal to esophageal phase ratios were calculated by counting the number of pharyngeal swallows prior to primary peristalsis initiation. Feeding bout length was calculated by counting the number of swallows in a feeding bout.

Quantitative measures were calculated during thin liquid and puree feeding across time points. Results are expressed as means \pm standard deviation (SD). Analyses were made within groups (post-operative and non-surgical) using analysis of variance (ANOVA) with SPSS software (IBM; Chicago IL, USA). LSD post-hoc tests were performed when appropriate. For all statistical analyses, a difference was considered significant if the *p*-value was less than or equal to 0.05.

Safety and Efficiency Ratings

We adapted a categorical Airway Invasion Scale (AIS, Table 4-1) from Rosenbek and colleagues' 8-Point penetration aspiration scale (Rosenbek, Robbins, Roecker, Coyle, & Wood, 1996) and German and colleagues' infant mammalian penetration-aspiration scale (Holman, Campbell-Malone, Ding, Gierbolini-Norat, Griffioen, Inokuchi, Lukasik, & German, 2013) to rate airway protection during swallow in our translational cat model (Table 4-1). Novel ratings that reflect volume of aspiration were added to our AIS.

A Timing and Efficiency Scale (Table 4-1) was adapted from Martin-Harris and colleagues' Modified Barium Swallow Impairment Profile (MBSImP), a standardized videofluoroscopy protocol (Martin-Harris, Brodsky, Michel, Castell, Schleicher, Sandidge, Maxwell, & Blair, 2008). Two of the 17 physiologic components of swallow described in the MBSImP (Table 4-1) may be applied to lateral plane videofluoroscopy in the cat: Initiation of pharyngeal swallow and pharyngeal residue (Martin-Harris, Brodsky, Michel, Castell, Schleicher, Sandidge, Maxwell, & Blair, 2008). A novel rating that reflects bolus spillage to the upper airway prior to swallow initiation was added to our Timing and Efficiency Scale (Table 4-1). A Wilcoxon signed-rank test was used to identify statistically significant differences in ratings of airway protection, initiation of

pharyngeal swallow and pharyngeal residue with SPSS software (IBM; Chicago IL, USA).

Reliability and Blinding

AIS scores were made by two speech-language pathologists with certificate of clinical competence (CCC) from the American Speech Language and Hearing Association (ASHA) and at least five years of experience (MF, TP). Images were de-identified prior to rating. Following a one-hour training session with practice-rating of representative VFSS samples in the cat, raters scored images recorded during thin liquid and puree feeding from a de-identified data set containing footage from 10 animals under the following conditions: Control, post-operative buprenorphine, buprenorphine without surgery, and post cervical spinal cord injury (cSCI). An inter-rater reliability analysis was performed using Cohen's kappa coefficient to determine consistency among raters (Tang, Hu, Zhang, Wu, & He, 2015). 30% of videos were repeated at random to allow for assessment of intra-rater reliability. A two-way random intraclass correlation coefficient was used to determine intra-rater reliability (Shrout & Fleiss, 1979). Reliability statistics were performed using SPSS software (IBM; Chicago IL, USA). Both intra- and inter-rater reliability were considered adequate if equal to or greater than 80%. Ratings of timing and efficiency were made by an ASHA certified speech pathologist (MF) using the same de-identified data set following demonstration of inter- and intra-rater reliability greater than 80%.

Results

Post-Operative Buprenorphine (Fig 4-1)

First, we assessed the effect of post-operative opioid administration on oropharyngeal and esophageal swallow function. Figure 4-1 demonstrates a decline in airway protection during pharyngeal swallow after 48-hours of post-operative buprenorphine (0.015 mg/kg). Most animals demonstrated aspiration (liquid entering the airway and passing below the vocal folds) without protective response (no cough, throat clear, or cessation of feeding) during post-operative thin liquid feeding. Most animals (75%) demonstrated delayed pharyngeal swallow initiation during both thin liquid and puree feeding following post-operative buprenorphine. A Wilcoxon signed-rank test detected a significant difference in bolus depth at time of initiation of pharyngeal swallow (IPS); elevated IPS ratings indicate that ingested material spilled deeper into the pharynx or larynx before swallow onset post-operatively than during control ($z = -2.3, p = 0.02$). Pharyngeal swallow duration increased by $118 \pm 12\%$ during post-operative puree feeding (Fig 4-1), extending the transit time of food through the pharynx, a shared space for breathing and swallow. Maximum esophageal distension increased by $117 \pm 5\%$ during post-operative puree feeding (Fig 4-1), with more food accumulating in the esophagus before clearance by primary peristalsis. All animals demonstrated return to functional baseline one-week post-operatively (5-days after the last dose of buprenorphine).

Non-Surgical: 0.02 mg/kg Buprenorphine

Next, we assessed the effect of non-surgical opioid administration on oropharyngeal and esophageal swallow function using a lower-end clinical dose of

buprenorphine (0.02 mg/kg). After 24-hours on buprenorphine, elevated AIS ratings did not reach statistical significance ($z = -1.9, p = 0.059$). There was laryngeal penetration during thin liquid feeding in three of four animals. A Wilcoxon signed-rank test detected a difference in bolus depth at the time of initiation of pharyngeal swallow (IPS); elevated IPS ratings indicate that ingested material spilled deeper into the larynx or pharynx before a swallow was initiated after 24-hours on buprenorphine than during pre-buprenorphine control ($z = -2.7, p = 0.008$). Analysis of variance (ANOVA) showed a significant difference in pharyngeal swallow duration [$F(2, 8) = 4.62, p = 0.04$] and pharyngeal distension [$F(2, 9) = 4.1, p = 0.05$]. LSD post-hoc testing revealed that pharyngeal swallow duration was significantly longer during thin liquid feeding after 24-hours on buprenorphine ($M = 325.6, SD = 37.6$) than during control ($M = 271.7, SD = 11.4$). LSD post-hoc testing revealed that pharyngeal distension before swallow was significantly increased after 24-hours on buprenorphine ($M = 12.7, SD = 0.9$) compared to control ($M = 10.7, SD = 1$).

One male animal demonstrated total feeding refusal after 48-hours on 0.02 mg/kg buprenorphine. The following results pertain to the three animals that fed voluntarily after 48-hours of opioid administration. Fig 4-2 demonstrates elevated AIS ratings during thin liquid feeding, with laryngeal penetration in all animals. A Wilcoxon signed-rank test detected a difference in bolus depth at the time of initiation of pharyngeal swallow (IPS); IPS ratings were significantly higher after 48-hours on buprenorphine than during control ($z = -2.2, p = 0.03$). Pharyngeal swallow duration significantly increased during thin liquid feeding after 48-hours on buprenorphine compared to control (Fig 4-2). LSD post-hoc testing revealed a trending increase ($p = 0.07$) in maximum pharyngeal

distension during puree feeding after 48-hours on buprenorphine ($M = 12.2$, $SD = 0.9$) compared to control ($M = 10.7$, $SD = 1$).

All animals resumed voluntary feeding within 24-hours of the last dose of buprenorphine. All animals demonstrated return to functional baseline within 5-days of the last dose of buprenorphine. Measures of duration are reported in ms. Measures of distension are reported in mm.

Non-Surgical: 0.04 mg/kg Buprenorphine

We also assessed the effect of a higher-end clinical dose of buprenorphine (0.04 mg/kg) on oropharyngeal and esophageal swallow function in a non-surgical setting. One male animal (same from 0.02 mg/kg experiment) demonstrated total feeding refusal after 24-hours on 0.04 mg/kg buprenorphine. The following results pertain to the three animals that fed voluntarily following 24-hours of opioid administration. A Wilcoxon signed-rank test detected a difference in bolus depth at the time of initiation of pharyngeal swallow (IPS); elevated IPS ratings indicate ingested material spilled deeper into the pharynx or larynx before triggering swallow after 24-hours on buprenorphine than during control ($z = -2.2$, $p = 0.03$). ANOVA showed that there was a significant difference in pharyngeal swallow duration [$F(2, 7) = 27.6$, $p < 0.001$]. LSD post-hoc testing indicated that pharyngeal swallow duration was significantly increased during thin liquid feeding after 24-hours on buprenorphine ($M = 328.9$, $SD = 7.7$) than during control ($M = 271.7$, $SD = 11.4$).

The same male animal demonstrated total feeding refusal after 48-hours on 0.04 mg/kg buprenorphine. The following results pertain to the three animals that fed voluntarily following 48-hours of opioid administration. AIS ratings were elevated in two

of three animals, but the effect was not statistically significant as a group (Fig 4-2). A Wilcoxon signed-rank test detected a difference in bolus depth at the time of initiation of pharyngeal swallow (IPS); IPS ratings were significantly higher following 24-hours on buprenorphine than during control ($z = -2.2, p = 0.03$). Pharyngeal swallow duration was longer during thin liquid feeding after 48-hours on buprenorphine than during control, extending the time of liquid transit through the pharynx (Fig 4-2). All animals resumed voluntary feeding within 24-hours of the last dose of buprenorphine. All animals demonstrated return to functional baseline within 5-days of the last dose of buprenorphine. Measures of duration are reported in ms.

Reliability

The inter-rater reliability for the raters was found to be Kappa = 0.97 ($p < 0.001$), 95% CI (0.94, 0.98). The average two-way random intraclass coefficient (ICC) for MF was ICC = 0.99 [$F(32, 32) = 625.9, p < 0.001$], 95% CI (0.99, 0.99). The average two-way random intraclass coefficient for TP was ICC = 0.97 [$F(32, 32) = 67.2, p < 0.001$], 95% CI (0.94, 0.99).

Discussion

The goal of this study was to evaluate the effects of opioids on swallow function a) post-operatively and b) in healthy normal animals. Swallow function was evaluated using VFSS in a) adult cats treated with buprenorphine following routine spay surgery and b) adult cats given buprenorphine without surgery. The present results demonstrate that a) clinical doses of buprenorphine produce dysphagia in healthy animals and b) post-operative operative opioid administration produces severe dysphagia without clinical signs/symptoms (cough, cessation of feeding) in some animals.

Potential Mechanisms of Opioid Induced Dysphagia

Opioids have been used medicinally for thousands of years (Waldhoer, Bartlett, & Whistler, 2004). In antiquity, opium was commonly used as an antidiarrheal treatment for dysentery due to opioid induced inhibition of gastrointestinal peristalsis and transit (Pasternak & Pan, 2013). Today, opioids are most known for their analgesic effects and abuse potential, however depression of respiratory and gastrointestinal systems are widely recognized side effects of clinical concern (Gharavi, Hedrich, Wang, & Hassan, 2015).

Opioid receptors couple to G-proteins, and are classified as G-protein coupled receptors (GPCR) (Waldhoer, Bartlett, & Whistler, 2004). GPCRs consist of a seven-segment transmembrane protein with an extra-cellular receptor site and intracellular heterotrimeric protein complex that enables signal transduction following ligand binding (Connor & Christie, 1999). Four types of opioid receptors have been described (mu, kappa, delta and nociceptin/orphanin), but most clinically relevant effects of opioids occur through activation of the mu-opioid receptor (MOR) (Waldhoer, Bartlett, & Whistler, 2004; Williams, Ingram, Henderson, Chavkin, von Zastrow, Schulz, Koch, Evans, & Christie, 2013).

Buprenorphine binds the mu-opioid receptor with high affinity for long durations (Elkader & Sproule, 2005). Buprenorphine is considered a safe alternative for post-operative pain management and opioid maintenance therapy because it is a partial agonist with an apparent ceiling effect at high doses (Shulman, Wai, & Nunes, 2019). However, rising hospital visits and poison control cases following buprenorphine abuse, and potential for lethal drug-drug interactions with central nervous system depressants (e.g.,

benzodiazepines), suggest that adverse effects are possible following buprenorphine use (Gharavi, Hedrich, Wang, & Hassan, 2015). Full mu-agonists (e.g., morphine) are known to depress respiration, gastrointestinal motility, cough, and related airway protective behaviors, but there is limited information on how partial mu-agonists (e.g., buprenorphine) affect these physiological functions (Bateman, Saunders, & Levitt, 2023; Foley, 1993; Pasternak & Pan, 2013; Tagaito, Isono, & Nishino, 1998). We are the first to study specific effects of buprenorphine, and opioids in general, on airway protection during swallow in a large animal model.

Opioid receptors are expressed throughout the central nervous system (Pasternak & Pan, 2013). Opioid administration has been shown to disrupt medullary respiratory, cough, and emetic pattern generators, and spinal locomotor pattern generators (Blivis, Mentis, O'Donovan M, & Lev-Tov, 2007; Bolser & DeGennaro, 1994; Lang & Marvig, 1989; Ramirez, Burgraff, Wei, Baertsch, Varga, Baghdoyan, Lydic, Morris, Bolser, & Levitt, 2021). The swallow pattern generator is distributed throughout the medulla, consists of the nucleus tractus solitarius (NTS), nucleus ambiguus (NA), reticular formation (RF) and various cranial nerve motor nuclei, and receives dense inputs from neighboring regions (Jean, 2001b; Pitts & Iceman, 2023). As the NTS and NA are enriched with mu-opioid receptors, and opioid administration has been shown to affect other central pattern generators, it is unsurprising that opioids have a deleterious impact on swallow function (Irnatén, Aicher, Wang, Venkatesan, Evans, Baxi, & Mendelowitz, 2003; Zhuang, Gao, Gao, & Xu, 2017).

Post-Operative Aspiration

Instrumentation is required to definitively evaluate airway protection during swallow (Logemann, 2007; Rommel & Hamdy, 2016). During videofluoroscopic swallowing studies (VFSS), the lateral fluoroscopic view includes the oral cavity, pharynx, larynx, trachea and proximal esophagus during intake of food and liquid items contrasted with barium sulfate, enabling real-time assessment of airway protection, kinematics and bolus transit during swallow (Donner, 1985; Logemann, 1997).

Because VFSS allows for visualization of all three phases of swallow, and does not require insertion of a scope, it is an ideal instrumentation method for animal studies (German, Crompton, Gould, & Thexton, 2017; Lever, Brooks, Thombs, Littrell, Harris, Allen, Kadosh, & Robbins, 2015). Translational models of VFSS have been described in the mouse, rat, dog and neonate pig (Cullins & Connor, 2019; Harris, Grobman, Allen, Schachtel, Rawson, Bennett, Ledyayev, Hopewell, Coates, Reiner, & Lever, 2017; Lever, Brooks, Thombs, Littrell, Harris, Allen, Kadosh, & Robbins, 2015; Stevens, Mayerl, Bond, German, & Barkmeier-Kraemer, 2021). While electrophysiology of swallow and airway protective behaviors in the anesthetized cat are foundational to models of neural control in humans, swallow function in awake, voluntarily feeding cats has been the subject of limited study (Kobara-Mates, Logemann, Larson, & Kahrilas, 1995; Pitts & Iceman, 2023; Sampson & Eyzaguirre, 1964). Adult cats have similar mechanisms of airway protection as humans, and can feed freely in the field of a fluoroscope (Kobara-Mates, Logemann, Larson, & Kahrilas, 1995; Pitts, Rose, Mortensen, Poliacek, Sapienza, Lindsey, Morris, Davenport, & Bolser, 2013). As such, a cat model of VFSS stands to offer highly translatable information regarding airway

protection during normal and disordered swallow. Furthermore, an animal model enables study of central mechanisms using injury or pharmacology not possible in humans (German, Crompton, Gould, & Thexton, 2017).

We adapted our airway invasion scale (AIS) from existing clinical and animal-model scales of airway protection (Holman, Campbell-Malone, Ding, Gierbolini-Norat, Griffioen, Inokuchi, Lukasik, & German, 2013; Rosenbek, Robbins, Roecker, Coyle, & Wood, 1996). After viewing several VFSS images of normal and disordered swallows in the cat, we found that some instances of aspiration involved large amounts of liquid in the airway, while others involved small, or trace amounts of liquid in the airway. To distinguish between these scenarios, we developed ratings that account for volume of aspiration. Our assessment of inter-rater reliability showed almost perfect agreement between raters, and our assessment of intra-rater reliability found a high degree of reliability between ratings. The AIS will allow researchers to use the adult cat model to evaluate swallow function and therapeutic outcomes in a variety of translational disease models.

Our results indicate increased frequency of airway invasion during thin liquid and puree feeding following opioid administration in both post-operative and non-surgical groups. All airway invasion detected following opioid administration occurred without protective response. This is understandable, as mu-opioid agonists are known to suppress cough, and cough is a major protective response to aspiration (Kamei, 1996; Kamei, Mori, Ogawa, & Kasuya, 1989; Karlsson, Lanner, & Persson, 1990; Pitts, Rose, Mortensen, Poliacek, Sapienza, Lindsey, Morris, Davenport, & Bolser, 2013). Importantly, pharyngeal swallow function declined following opioid administration,

resulting in increased airway invasion and depressed protective reflexes. We speculate that opioid induced oropharyngeal dysphagia is a) under-detected due to suppression of overt indicators of aspiration (e.g., cough) and b) a contributing factor to post-operative aspiration pneumonia.

Although airway protection during swallow declined in both the surgical and non-surgical groups, the most severe aspiration occurred during thin liquid feeding following post-operative buprenorphine administration. Following trauma or elective surgery, the stress/inflammatory response is an adaptive process in which cardiometabolic, neuroendocrine, and immune responses are mounted to protect immediate survival (Finnerty, Mabvuure, Ali, Kozar, & Herndon, 2013; Priebe, 2016). Metabolic inflammatory markers peak two days post-injury and return to baseline one-week post-injury (Kohl & Deutschman, 2006). Interestingly, animals in our post-operative group demonstrated severe aspiration two days after spay surgery, with return to baseline swallow function one-week after surgery. We speculate that post-operative inflammation may have contributed to the severe dysphagia detected following post-operative buprenorphine administration.

Swallowing Disorder Following Buprenorphine Administration

We detected changes in onset of swallow initiation, swallow duration, and distension reflexes following buprenorphine administration in both post-operative and non-surgical groups. Most animals demonstrated delayed pharyngeal swallow initiation following opioid administration. Bolus location at time of pharyngeal swallow initiation spans from the oral tongue to the pyriform sinus during normal feeding, and is variable by consistency and at the population level. Because the glottis is open at rest, there is

increased likelihood of airway invasion when a bolus spills to the pharyngeal cavity prior to swallow initiation. Risk of airway invasion with posterior bolus spillage is further increased in the setting of sensory or motor impairment. Our results show that increased depth of food/liquid spillage, and delayed onset of pharyngeal swallow resulted in increased airway invasion before swallow following opioid administration.

Pharyngeal swallow duration significantly increased following opioid administration. The laryngeal vestibule must remain tightly closed for the duration of pharyngeal swallow for transport of food/liquid from mouth to esophagus to occur without aspiration into the airway. Increased duration of pharyngeal swallow indicates decreased oropharyngeal efficiency, and extends the period in which food/liquid may invade the airway during the swallow.

Pharyngeal distension prior to swallow initiation significantly increased in the non-surgical group, and esophageal distension prior to primary peristalsis significantly increased in the post-operative group. These data suggest that the sensitivity of pharyngeal and esophageal distension reflexes is decreased following opioid administration. This conclusion is consistent with reports that esophageal circuitry is enriched with mu-opioid receptors, and human studies demonstrating disordered peristalsis and achalasia following opioid administration (Babaei, Szabo, Shad, & Massey, 2019; Patel, Callaway, & Vaezi, 2019; Patel & Vaezi, 2018; Patel, Goss, Hayat, Tombazzi, Naik, Slaughter, Aslam, Sarker, Higginbotham, & Vaezi, 2022; Sanchez, Olivier, Gediklioglu, Almeida, Gaeta, Nigro, de la Rosa, Nguyen, Lalehzari, Regala, Njei, Deng, Ciarleglio, & Masoud, 2022; Snyder & Vela, 2020). According to the dual-valve hypothesis, airway protection is maintained by precise gating of the glottis and

upper esophageal sphincter (UES) during ventilation and ingestion (Pitts, 2014). During breathing, the glottis is open to permit airflow into the lower airways, and the UES is closed to prevent ingestion of air. During swallow, the glottis is closed to prevent aspiration of food/liquid, and the UES is open to permit bolus passage into the esophagus. Following completion of pharyngeal swallow, the UES returns to its tonic state to prevent reflux of swallowed material into the pharynx or larynx. We speculate that increased filling of the pharynx and esophagus increases the likelihood of airway invasion and laryngopharyngeal reflux following opioid administration.

Feeding bout length was unaffected following post-operative buprenorphine, but was significantly reduced following buprenorphine without surgery. Animals in the non-surgical group required increased direction and fed for shorter periods of time compared to control, and one animal demonstrated total feeding refusal during opioid administration. Reduced intake and food refusal are clinical indicators of dysphagia. We propose two explanations for this phenomenon: A) Reduced feeding length was a compensatory mechanism in the non-surgical group, who demonstrated less severe aspiration than the post-operative group and b) poor feeding was a function of higher-end clinical doses of buprenorphine administered to the non-surgical group.

Conclusion

Oropharyngeal swallow function is negatively impacted by the partial mu-opioid receptor agonist buprenorphine, a drug that is considered safe for pain management and treatment of opioid addiction disorder (Fishman & Kim, 2018; Koehl, Zimmerman, & Bridgeman, 2019). We propose that risk for dysphagia related chest infection following opioid administration is greatest in the post-operative setting. We hypothesize that

decline in swallow function following opioid administration occurs due to central disturbance of the swallow motor pattern, and depression of aerodigestive sensation and protective reflexes. Our findings have implications for prevention of aspiration pneumonia in post-surgical cases. In the event of post-operative opioid administration, instrumental evaluation of swallowing is indicated for patients at increased risk for dysphagia related chest infection, including persons who are immunocompromised or aged.

Table 4-1.

Rating scales adapted for interpretation of videofluoroscopic swallowing study outcomes in adult cats.

A	Score	Airway Invasion Scale (AIS)
	0	Material does not enter the airway
	1	Material enters the larynx, remains above the vocal folds, and is ejected from the airway
	2	Material enters the larynx, remains above the vocal folds, and is not ejected from the airway
	3	Material enters the larynx, contacts the vocal folds, and is ejected from the airway
	4	Material enters the larynx, contacts the vocal folds, and is not ejected from the airway
	5	Material enters the larynx, passes below the vocal folds, and is ejected back to the larynx or pharynx
	6	Material enters the larynx, a small amount passes below the vocal folds, and is not ejected despite effort
	7	Material enters the larynx, a large amount passes below the vocal folds, and is not ejected despite effort
	8	Material enters the larynx, a small amount passes below the vocal folds, and no effort is made to eject
	9	Material enters the larynx, a large amount passes below the vocal folds, and no effort is made to eject
B	Score	7-Point Infant Mammalian Penetration Aspiration Scale from Holman et al., 2013
	1	Material does not enter the airway
	2	Material is in the supraglottic space, remains above the vocal folds, and passively leaves the airway before the epiglottis returns to rest position
	3	Material is in the supraglottic space, a small amount remains above the vocal folds after epiglottis in rest position
	4	Material is in the supraglottic space, a larger amount remains above the vocal folds after epiglottis in rest position
	5	New material is in the supraglottic space, then passes below the vocal folds, and is actively ejected, above the vocal folds
	6	New material is in the supraglottic space, then passes below the vocal folds and is not ejected from the trachea despite effort
	7	New material is in the supraglottic space, then passes below the vocal folds, and no effort is made to eject (silent aspiration)
C	Score	Timing and Efficiency Scale
		<i>Initiation of the pharyngeal swallow</i>
	0	Bolus head at ramus of mandible
	1	Bolus head in valleculae
	2	Bolus contacts laryngeal surface of epiglottis
	3	Bolus head in pyriform sinus
	4	Bolus spillage to larynx or subglottis
	5	Absent swallow initiation
		<i>Pharyngeal residue</i>
	0	No residue
	1	Trace residue coating the pharynx
	2	Collection of residue in the pharynx
	3	Majority of bolus remains in the pharynx
	4	Minimal to no pharyngeal clearance
D	Score	MBSImP Component Ratings from Martin-Harris et al., 2008
		<i>Initiation of the pharyngeal swallow</i>
	0	Bolus head at posterior angle of ramus (first hyoid excursion)
	1	Bolus head in valleculae
	2	Bolus head at posterior laryngeal surface of epiglottis
	3	Bolus head in pyriforms
	4	No visible initiation at any location
		<i>Pharyngeal residue</i>
	0	Complete pharyngeal clearance
	1	Trace residue within or on pharyngeal structures
	2	Collection of residue within or on pharyngeal structures
	3	Majority of contrast within or on pharyngeal structures
	4	Minimal to no pharyngeal clearance

A) Airway Invasion Scale (AIS) adapted from the B) 7-Point Infant Mammalian Penetration Aspiration Scale (Holman, Campbell-Malone, Ding, Gierbolini-Norat, Griffioen, Inokuchi, Lukasik, & German, 2013). C) Timing and Efficiency Scale adapted from the D) Modified Barium Swallow Impairment Profile (MBSImP) (Martin-Harris, Brodsky, Michel, Castell, Schleicher, Sandidge, Maxwell, & Blair, 2008)

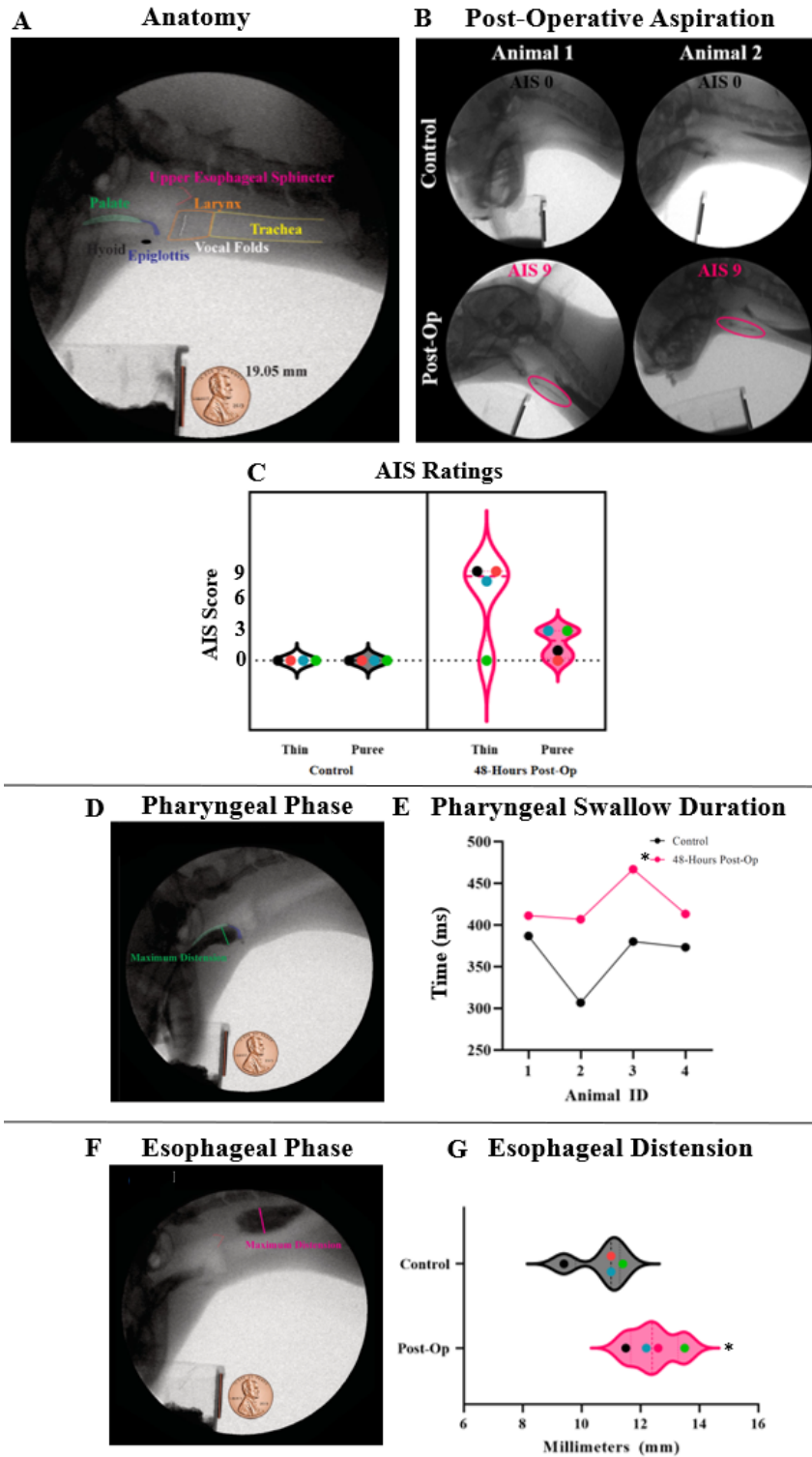
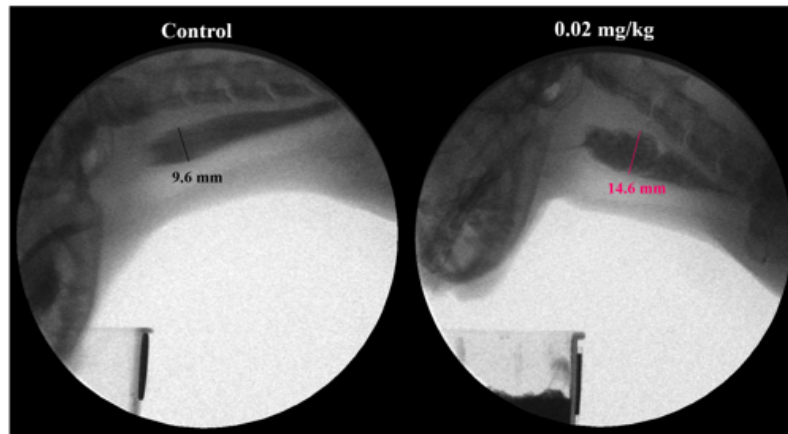


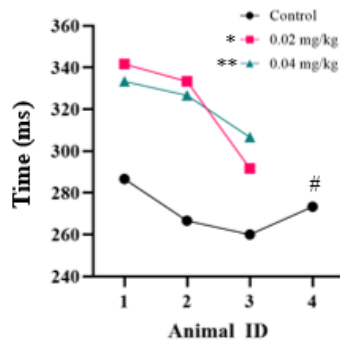
Figure 4-1. Aspiration following post-operative buprenorphine (0.015 mg/kg).

Airway protection during swallow is compromised following post-operative opioid administration. Swallow was evaluated using lateral plane videofluoroscopy which allows for visualization of the A) oropharynx, pharynx, esophagus, larynx and trachea and D) oropharyngeal and F) esophageal phases of swallow. A penny was included in-line with anatomy during all recordings for calibration of measurements. Swallowing studies were obtained in voluntarily feeding cats treated with intramuscular buprenorphine (0.015 mg/kg) 48-hours after routine ovariohysterectomy. Most animals demonstrated decline in airway protection, with B) large volume aspiration without protective response during thin liquid feeding in two of four animals and C) elevated AIS ratings during thin liquid and puree feeding in three of four animals post-operatively. A Wilcoxon signed-rank test detected a statistically significant change in AIS ratings, with more scores indicative of airway invasion 48-hours post-operatively ($z = -2.21, p = 0.03$). Analysis of variance (ANOVA) showed differences in pharyngeal swallow duration [$F(2, 9) = 8.1, p = 0.01$] and maximum esophageal distension before primary peristalsis [$F(2, 9) = 7.01, p = 0.015$] during puree feeding. E) LSD post-hoc test results revealed that pharyngeal swallow duration was significantly longer during post-operative puree feeding ($M = 424.4, SD = 28.3$) compared to control ($M = 361.7, SD = 37.1$). F) Maximum esophageal distension before peristalsis was significantly increased during post-operative puree feeding ($M = 12.4, SD = 0.86$) compared to control ($M = 10.7, SD = 0.89$). Swallow metrics returned to functional baseline 1-week post-operatively (5-days after the last dose of buprenorphine). Swallow durations are reported in ms. Distension measures are reported in mm. $*p < 0.05$.

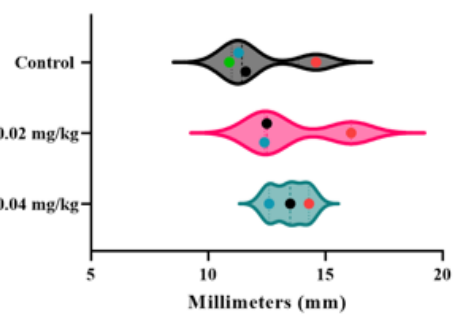
A Esophageal Distension Before Peristalsis



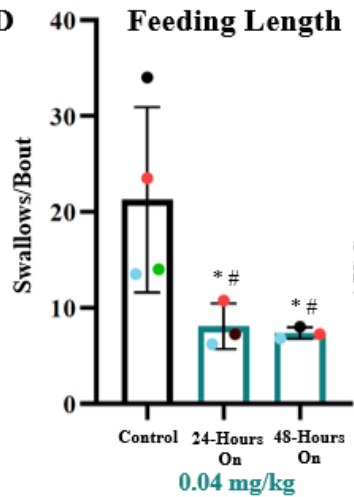
B Pharyngeal Swallow Duration



C Esophageal Distension



D Feeding Length



E Airway Invasion Scale Ratings

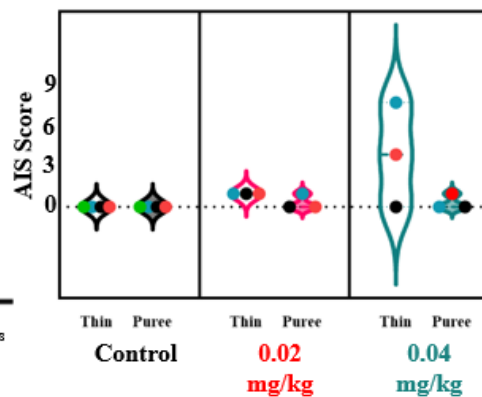


Figure 4-2. Pharyngeal and esophageal inefficiency following non-surgical buprenorphine administration (0.02 and 0.04 mg/kg). Swallow efficiency is reduced after opioid administration without surgery. A) Representative videofluoroscopic image depicts increased maximum esophageal distension after 48-hours on a lower-end clinical dose of buprenorphine (0.02 mg/kg), suggesting decreased sensitivity of esophageal distension reflexes. B) Pharyngeal swallow duration increased during thin liquid feeding after clinical doses of buprenorphine. Analysis of variance (ANOVA) showed differences in pharyngeal swallow duration in the 0.02 mg/kg [$F(2, 8) = 4.62, p = 0.04$] and 0.04 mg/kg [$F(2, 7) = 27.6, p < 0.001$] data sets. LSD post-hoc test results showed that pharyngeal swallow duration was significantly longer after 48-hours on 0.02 mg/kg ($M = 322.2, SD = 26.8$) and 0.04 mg/kg buprenorphine ($M = 322.2, SD = 13.9$) compared to control ($M = 271.7, SD = 11.4$). C) Maximum esophageal distension before peristalsis increased on average following 48-hours on 0.02 mg/kg ($M = 13.7, SD = 2.1$) and 0.04 mg/kg buprenorphine ($M = 13.5, SD = 0.8$) compared to control ($M = 12.1, SD = 1.7$), but did not reach statistical significance. D) Feeding bout length significantly decreased during thin liquid feeding after buprenorphine administration. ANOVA showed differences in feeding length in the 0.04 mg/kg data set [$F(2, 7) = 5.3, p = 0.04$]. LSD post-hoc test results showed that there were significantly fewer swallows per bout after 24-hours ($M = 8.1, SD = 2.4$) and 48-hours ($M = 7.4, SD = 0.6$) on 0.04 mg/kg buprenorphine compared to control ($M = 21.3, SD = 9.7$). One male animal demonstrated total feeding refusal at both time points after 0.04 mg/kg buprenorphine. E) A Wilcoxon signed-rank test indicated a statistically significant change in AIS ratings, with more scores indicative of airway invasion after 48-hours on 0.02 mg/kg buprenorphine ($z = -2,$

$p = 0.05$). The mild but significant elevation in AIS ratings indicates that thin liquid laryngeal penetration occurred in all three animals that fed at this time point. AIS ratings were elevated in two of three animals after 48-hours on 0.04 mg/kg buprenorphine, but the effect was non-significant as a group. There was non-transient penetration to the vocal folds in one animal and small volume aspiration in one animal. Swallow duration are reported in ms. Distension measures are reported in mm. $*p < 0.05$, $**p < 0.01$, # indicates feeding refusal.

CHAPTER 5

DISCUSSION

This series of studies concludes with four main points. The swallow motor pattern is modulated by afferent input from receptors spanning the upper aerodigestive tract. Opioids depress pharyngeal swallow function, and the effects are sex-specific. Sensitivity to opioid-induced respiratory depression is sex-specific. 5-HT_{1A} agonists counter opioid-induced depression of breathing and swallowing.

Modulation of the Swallow Motor Pattern

Stimulation of pharyngeal mechanoreceptors has long been known to elicit the pharyngeal swallow reflex (Doty, 1951; Negus, 1942). Chapter Two demonstrates that rapid activation of esophageal mechanoreceptors also reliably elicits pharyngeal swallow in cats (Frazure, Brown, Greene, Iceman, & Pitts, 2021). The results in Chapter Two showed that there were significant differences in the motor pattern of swallows elicited by stimulation of the pharynx, swallows elicited by stimulation of the esophagus, and swallows elicited by simultaneous stimulation of the pharynx and esophagus. In general, a maximal stimulus resulted in more forceful laryngeal closure during swallow; simultaneous stimulation of both pharyngeal and esophageal receptors resulted in significantly increased electromyogram (EMG) amplitude and duration of thyroarytenoid activation during swallow.

Chapter Three replicated this result using a rat model. Consistent with Chapter Two, Chapter Three shows that rapid activation of esophageal mechanoreceptors reliably

elicits the pharyngeal swallow reflex in rats. Again, we found that a maximal stimulus resulted in a more forceful swallow; simultaneous activation of pharyngeal and esophageal mechanoreceptors resulted in significantly increased EMG amplitudes of laryngeal elevator and laryngeal adductor muscles during swallow.

The important point is that a functional aerodigestive tract is dependent upon central integration of information from several afferent beds in the larynx, pharynx and esophagus, which enables dynamic modulation of breathing and swallowing based on peripheral conditions. Even slight disturbances of aerodigestive regulation can compromise airway protection, resulting in aspiration and its sequelae. Of experimental and clinical relevance, understanding the relationship between the location and intensity of an aerodigestive stimulus and the response it elicits enables nuanced evaluation of the severity and mechanisms underlying dysphagia.

Opioids Depress Pharyngeal Swallow Function

Opioids are known to depress lower gastrointestinal motility (Bateman, Saunders, & Levitt, 2023; Foley, 1993). Manometry studies, and symptomology, in humans have shown that chronic opioid use can result in significant esophageal dysfunction (Babaei, Szabo, Shad, & Massey, 2019; Patel, Callaway, & Vaezi, 2019; Patel & Vaezi, 2018; Patel, Goss, Hayat, Tombazzi, Naik, Slaughter, Aslam, Sarker, Higginbotham, & Vaezi, 2022; Penagini, Picone, & Bianchi, 1996). Chapters Three and Four illustrate that opioids also depress pharyngeal swallow function.

To our knowledge, Chapter Three is the first electromyographic study of pharyngeal swallow function before and after opioid administration. The results show that opioids depress excitability of the swallow reflex, with a significant reduction in

swallow number following even low doses of buprenorphine. When swallows were elicited after opioid administration, there were significant changes in swallow motor pattern, and swallow no longer adapted in response to a maximal stimulus.

Chapter Three is the first study to evaluate sex-specific effects of opioids on pharyngeal swallow function. Female animals were more sensitive to depression of swallow than males following buprenorphine administration. Following buprenorphine administration, evoked swallows in females displayed reduced EMG amplitudes in laryngeal elevator, laryngeal adductor, and pharyngeal constrictor muscles. In contrast, only laryngeal elevator muscle activity was reduced following buprenorphine in males. Mechanistically, these results indicate sex-specific effects of opioids on the swallow pattern generator, as neural drive was reduced to three separately innervated muscles in females, compared to one muscle in males (Miyamaru, Kumai, Ito, & Yumoto, 2008; Sakamoto, 2013; Yamaoka, Furusawa, Fujimoto, Iguchi, & Kumai, 1992). Clinically, these findings suggest that women may be more vulnerable to complications of opioid-induced dysphagia (e.g., aspiration pneumonia) than men.

Chapter Four is the first study to utilize the videofluoroscopic swallow study in unrestrained, freely feeding cats. This is also the first study to demonstrate aspiration following opioid administration in a clinical-translational model. The results show that clinical doses of buprenorphine deleteriously impacted airway protection and swallow timing in otherwise healthy animals. Post-operative buprenorphine administration resulted in more severe dysphagia; a clinical dose of buprenorphine following a minor abdominal surgery led to aspiration in 75% (three of four) animals, and large volume aspiration in 50% (two of four) animals. There was no protective response (e.g., cough,

expiration reflex, cessation) to aspiration. This suggests that patients treated with opioids following a surgical procedure may be vulnerable to silent aspiration which could cause pneumonia, a common post-operative complication associated with poor outcomes.

Sex-Specific Sensitivity to Opioid-Induced Respiratory Depression

The few studies that have compared sensitivity to opioid-induced respiratory depression between sexes have found that females are more susceptible to respiratory depression than males. These studies were performed in humans and rats, and used full mu-opioid receptor agonists to induce respiratory depression (Dahan, Sarton, Teppema, & Olievier, 1998; Marchette, Carlson, Frye, Hastings, Vendruscolo, Mejias-Torres, Lewis, Hampson, Volkow, Vendruscolo, & Koob, 2023; Sarton, Teppema, & Dahan, 1999). Chapter Two is the first study to demonstrate sex-specific, dose-dependent respiratory depression following administration of buprenorphine, a partial mu-opioid receptor agonist previously thought to have a ceiling effect on respiratory depression. These results show that even the safest opioids can produce significant respiratory depression, especially when abused (i.e., large doses) or combined with other drugs. Sex differences should be evaluated when determining a drug's side effect profile.

5-HT_{1A} Agonists Counter Opioid-Induced Depression of Breathing and Swallowing

In modern medicine, opioids are commonly used for analgesia and anesthesia (Bateman, Saunders, & Levitt, 2023). Opioids have well known, serious side effects including respiratory depression, addiction potential, and overdose lethality (Bateman, Saunders, & Levitt, 2023; Flanagan, Wysong, Ramey, & Vallier, 2018; Foley, 1993; Ramirez, Burgraff, Wei, Baertsch, Varga, Baghdoyan, Lydic, Morris, Bolser, & Levitt,

2021; Upp & Waljee, 2020). Over 20 million Americans are affected by substance use disorder, a disease characterized by inability to control substance use, including prescription medications and illegal drugs (Cuadros, Branscum, Moreno, & MacKinnon, 2023). Opioids have driven an increase in drug-related deaths in the last two decades, a public health crisis known as the United States opioid epidemic (Bateman, Saunders, & Levitt, 2023; Flanagan, Wysong, Ramey, & Vallier, 2018). Respiratory depression is the cause of most deaths related to opioid use, and can occur when opioids are used illicitly, or as prescribed (Bateman, Saunders, & Levitt, 2023; Brown, 1985; Gerber & Apseloff, 1993; Ramirez, Burgraff, Wei, Baertsch, Varga, Baghdoyan, Lydic, Morris, Bolser, & Levitt, 2021). Bateman and colleagues advocate that research focused on countering opioid-induced respiratory depression is needed to curb deaths related to opioid use (Bateman, Saunders, & Levitt, 2023).

Chapter Three demonstrates that 5-HT_{1A} receptors, a serotonin receptor subtype, are a promising target for countering opioid-induced respiratory arrest. Previous reports have shown that 5-HT_{1A} agonists restore respiratory rhythmicity following opioid administration in male rats (Dutschmann, Waki, Manzke, Simms, Pickering, Richter, & Paton, 2009; Hilaire, Voituron, Menuet, Ichiyama, Subramanian, & Dutschmann, 2010; Manzke, Dutschmann, Schlaf, Morschel, Koch, Ponimaskin, Bidon, Lalley, & Richter, 2009; Sahibzada, Ferreira, Wasserman, Taveira-DaSilva, & Gillis, 2000). Chapter Three shows that IV administration of the 5-HT_{1A} agonist 8-OH-DPAT rescued breathing following opioid-induced respiratory arrest in female rats. These studies are the first to evaluate 5-HT_{1A} agonists as a counter to opioid-induced respiratory arrest in female animals.

Chapter Three also demonstrates that pre-treatment with the partial 5-HT_{1A} agonist buspirone prevented cessation of breathing after doses of buprenorphine that produced apnea in untreated animals. The experiments in Chapter Two expand on previous investigations of buspirone as a counter to opioid-induced respiratory depression with two key innovations: Route of administration, and primary outcome (Dutschmann, Waki, Manzke, Simms, Pickering, Richter, & Paton, 2009; Oertel, Schneider, Rohrbacher, Schmidt, Tegeder, Geisslinger, & Lotsch, 2007; Sahibzada, Ferreira, Wasserman, Taveira-DaSilva, & Gillis, 2000).

Clinically, buspirone is an oral medication that reaches peak plasma concentrations one hour after ingestion. Previously published studies in animals report enhancement of breathing after *intravenous* buspirone (Sahibzada, Ferreira, Wasserman, Taveira-DaSilva, & Gillis, 2000). Chapter Three evaluated the effectiveness of *oral* buspirone as a counter to opioid-induced respiratory depression. The positive results show that despite relatively low bioavailability after first-pass metabolism, and partial agonist activity at 5-HT_{1A} receptors, buspirone effectively stabilizes breathing in an animal model of opioid-induced respiratory arrest (Wilson & Tripp, 2023).

A human study evaluated buspirone as a treatment for morphine-induced respiratory depression (Oertel, Schneider, Rohrbacher, Schmidt, Tegeder, Geisslinger, & Lotsch, 2007). Following treatment with either buspirone and morphine, or placebo and morphine, minute expiratory volume, end-tidal carbon dioxide levels, and ventilatory rate were measured as subjects breathed into a plastic bag. The researchers found that buspirone did not significantly impact the hypercapnic response following morphine administration, and concluded that buspirone does not prevent morphine-induced

respiratory depression in humans (Oertel, Schneider, Rohrbacher, Schmidt, Tegeder, Geisslinger, & Lotsch, 2007). In contrast, the experiments in Chapter Three used survival as the primary outcome measure of respiratory function following opioid administration. Chapter Three demonstrates that buspirone pre-treatment significantly increased median survival following buprenorphine administration in female animals. Together, these results suggest that while buspirone does not prevent measurable changes in respiratory function following opioid administration, it does prevent respiratory arrest. More work is needed to in order to successfully translate these findings to humans.

Oropharyngeal dysphagia is a debilitating disease for which there are no pharmacological treatments (Logemann, 2007). Chapters Three and Four demonstrate that opioid administration may result in severe oropharyngeal dysphagia, an effect that has received limited attention in clinical settings. Chapter Three shows that the 5-HT_{1A} agonist 8-OH-DPAT improved some aspects of swallow function following buprenorphine-induced dysphagia; 8-OH-DPAT enhanced submental EMG amplitude when swallows occurred, but did not restore excitability of the swallow reflex following total suppression. This result suggests that 5-HT_{1A} agonists may be beneficial for the treatment of opioid-induced dysphagia, and warrants further investigation.

Clinical Significance

The studies in this dissertation provide data that are highly translatable to the evaluation and treatment of swallowing disorders. Chapters Two and Three demonstrate that afferent information from the esophagus modulates oropharyngeal swallow. This finding has implications for dysphagia management practice patterns, as oropharyngeal

dysphagia and esophageal dysphagia are currently treated as separate conditions (Lawal & Shaker, 2008; Rommel & Hamdy, 2016).

Chapters Three and Four have implications for improved screening and prevention of opioid-induced dysphagia and aspiration pneumonia, especially in post-surgical cases. Chapter Three offers a pharmacological approach to dysphagia management, which has traditionally relied on behavioral and rehabilitative therapies (Logemann, 2007). Chapter Four describes a novel clinical-translational, large-species model of dysphagia that will enable more basic study of dysphagia following a variety of pathologies, including spinal cord injury.

As described above, Chapter Three demonstrates that serotonin 5-HT_{1A} receptors are a promising target for researchers pursuing a counter to opioid-induced respiratory arrest. Sex-specific effects observed in rats have potential implications for the treatment of dysphagia and respiratory diseases, and warrant further investigation in humans (Marchette, Carlson, Frye, Hastings, Vendruscolo, Mejias-Torres, Lewis, Hampson, Volkow, Vendruscolo, & Koob, 2023).

Future Directions

Improved understanding of the neuronal mechanisms driving deglutition and respiration will enable development of novel treatments for their disorders. Patch clamp electrophysiology, immunocytochemistry, and optogenetics are routinely used to study control of breathing, and should be leveraged to study pharyngeal swallow (Huff, Karlen-Amarante, Oliveira, & Ramirez, 2023; Huff, Karlen-Amarante, Pitts, & Ramirez, 2022; Wollman, Hill, Hasse, Young, Hernandez-De La Pena, Levine, & Fregosi, 2022; Wollman, Flanigan, & Fregosi, 2022). Phenotyping the brainstem swallow network

would produce breakthroughs in knowledge of deglutition and dysphagia. Sex differences should be considered during future investigations of breathing and swallowing in both basic science and human studies.

Well-designed clinical trials are needed to translate advances in basic science to medicine. The partial 5-HT_{1A} agonist buspirone should be evaluated as a treatment for oropharyngeal dysphagia in a human clinical trial. Buspirone should also be re-evaluated as a counter to opioid-induced respiratory depression in humans, especially among people at increased risk for opioid-induced respiratory arrest (e.g., post-operative patients, older adults, people with substance use disorder). Future research should focus on pharmacological approaches to dysphagia therapy.

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APPENDIX A: ABBREVIATIONS

CN V	Cranial Nerve V: Trigeminal
CN VII	Cranial Nerve VII: Facial
CN IX	Cranial Nerve IX: Glossopharyngeal
CN X	Cranial Nerve X: Vagus
CN XII	Cranial Nerve XII: Hypoglossal
UES	Upper Esophageal Sphincter
sEMG	Surface Electromyography
LES	Lower Esophageal Sphincter
NTS	Nucleus Tractus Solitarius
NA	Nucleus Ambiguus
COPD	Chronic Obstructive Pulmonary Disease
VFSS	Videofluoroscopic Swallowing Study
MBS	Modified Barium Swallow
FEES	Fiberoptic Endoscopic Evaluation of Swallowing
HRM	High Resolution Manometry
PAS	Penetration-Aspiration Scale
MBSImP	Modified Barium Swallow Impairment Profile
NPO	Nothing By Mouth
NMES	Neuromuscular Electrical Stimulation
EMST	Expiratory Muscle Strength Training
EMG	Electromyogram
GPCR	G-Protein Coupled Receptor
MOR	Mu-Opioid Receptor

EDist	Esophageal Distension
MyHy	Mylohyoid
GeHy	Geniohyoid
ThHy	Thyrohyoid
ThPh	Thyropharyngeus
Thar	Thyroarytenoid
PS	Parasternal
Dia	Diaphragm
IACUC	Institutional Animal Care and Use Committee
SD	Standard Deviation
RMS	Root Mean Square
I	Inspiration
E1	Early Expiration
E2	Mid/Late Expiration
CS	Combined Stimuli: Water + Esophageal Distension
ANOVA	Analysis of Variance
IV	Intravenous
ECG	Electrocardiogram
PCA	Posterior Cricoarytenoid
ACDF	Anterior Cervical Discectomy and Fusion
SpO ₂	Oxygen Saturation
ETCO ₂	End Tidal Carbon Dioxide
NIBP	Non-invasive Blood Pressure
AIS	Airway Invasion Scale
IPS	Initiation of Pharyngeal Swallow
PR	Pharyngeal Residue
CCC	Certificate of Clinical Competence
ASHA	American Speech Language and Hearing Association

cSCI

Cervical Spinal Cord Injury

RF

Reticular Formation

CURRICULUM VITAE

NAME: Michael Lee Frazure

ADDRESS: Department of Physiology – Kentucky Spinal Cord Injury
Research Center
511 South Floyd Street,
University of Louisville
Louisville, KY 40202

DOB: Louisville, KY – May 3, 1991

EDUCATION &
TRAINING: B.A, English
University of Louisville
2009-2013

M.S, Communicative Sciences and Disorders
University of Louisville
2015-18

M.S., Physiology and Biophysics
University of Louisville
2020-2022

Ph.D., Physiology and Biophysics
University of Louisville
2020-2023

AWARDS Certificate of Recognition for Outstanding Presentation,
American Physiological Society Respiration Section Trainee
Subcommittee, Long Beach, CA
2023

Abstract of Distinction, American Physiological Society
Summit Meeting, Long Beach, CA
2023

1st Place Poster Presentation, Kentucky Speech, Language and Hearing Association Convention, Lexington, KY
2023

2nd Place Poster Presentation, Kentucky Speech, Language and Hearing Association Convention, Louisville, KY
2022

Outstanding Thesis Award, Division of Communicative Disorders, University of Louisville, Louisville, KY
2018

Top Praxis Score for the Speech Language Pathology Class of 2018, University of Louisville, Louisville, KY
2018

2nd Place Poster Presentation, Kentucky Speech, Language and Hearing Association Convention, Lexington, KY
2018

Leon V. Driskell Award for Excellence in Writing, Department of English, University of Louisville, Louisville, KY
2013

Hallmark Scholarship, University of Louisville, Louisville, KY
2009-2013

**PROFESSIONAL
EXPERIENCE:**

Speech-Language Pathologist
University of Louisville Hospital
2018-2023

Speech-Language Pathologist
Norton Women's and Children's Hospital
2018-2020

Speech-Language Pathologist
Baptist Floyd Hospital
2018-2019

LICENSURE:

License to practice Speech Language Pathology, KY Board of Speech Language Pathology and Audiology, Frankfort, KY (248098)

National Certificate of Clinical Competence (CCC), American Speech and Hearing Association, Rockland, MD (14316642)

PROFESSIONAL SOCIETIES:

American Physiological Society (00318360)

PUBLICATIONS:

Pitts, T., Iceman, K.E., Huff, A., Musselwhite, M.N., **Frazure, M.**, Young, K.C., Greene, C.L., & Howland, D.R. (2022). Laryngeal and swallow dysregulation following acute cervical spinal cord injury. *J Neurophysiol*, 128(2).

Frazure, M., Brown, A., Greene, C., Iceman, K., & Pitts, T. (2021). Rapid activation of esophageal mechanoreceptors alters the pharyngeal phase of swallow: evidence for inspiratory activity during swallow. *PLoS One*, 16(4).

Frazure, M., Mattingly, R., Pitts, T., & Smith, A. (2018). Traumatic brain injury: investigating misconceptions among graduate students in physical therapy, occupational therapy and speech-language pathology. *Journal of Physiotherapy and Physical Rehabilitation*, 3(3).

PRESENTATIONS: *Oral Presentations*

International

Frazure, M. (2023). Development of serotonin therapies for opioid induced depression of breathing and swallowing. Short talk at the Respiratory Section Trainee Breakfast at the *American Physiological Society Summit*, April 21, 2023, Long Beach, CA.

Frazure, M. (2022). Potential mechanisms of dysphagia following clinical doses of buprenorphine. Short talk at the *Symposium for Craniofacial Neuroscience*, November 11, Virtual: San Diego, CA.

Frazure, M. (2022). Aspiration following opioid administration in a novel model of dysphagia. Short talk at the Respiratory Section Trainee Breakfast at the *Experimental Biology*, April 3, 2022, Philadelphia, PA.

Frazure, M. (2021). Impaired swallow function and airway protection after a clinical dose of buprenorphine. Short talk at the *Symposium for Craniofacial Neuroscience*, November 12, Virtual: Chicago, IL.

Regional

Frazure, M. (2023). Altered swallow and respiratory drive following opioid administration. One-hour presentation at *Kentucky Spinal Cord Injury Research Center* seminar. March 10, Louisville, KY.

Frazure, M. (2023). Potential mechanisms of dysphagia following opioid induced dysphagia. One-hour presentation at *Speech Language Pathology Research Seminar Series*. February 3, Louisville, KY.

Frazure, M. (2022). Investigating effects of opioids on aerodigestive mechanoreceptors. Short talk at *Ohio River Control of Breathing* meeting. September 23, Lexington, KY.

Frazure, M. (2021). Expiratory bulbospinal neuron plasticity after chronic spinal cord injury. Journal club presentation at *Ohio River Control of Breathing* meeting. September 20, Virtual: Louisville, KY.

Abstract/Poster Presentations

International

Frazure, M., Morimoto, I., Greene, C., Iceman, K., & Pitts, T. (2023). The 5-HT_{1A} agonist 8-OH-DPAT reverses the suppression of breathing but not swallowing caused by opioid administration in female rats. Poster presentation at the *American Physiological Society Summit* annual meeting, April 21, Long Beach, CA.

Morimoto, I., **Frazure, M.,** Iceman, K., Koike, T., & Pitts, T. (2023). Sex differences in swallowing response to Gaussian stochastic surface electrical stimulation. Poster presentation at the *American Physiological Society Summit* annual meeting, April 21, Long Beach, CA.

Frazure, M., Greene, C., Iceman, K., & Pitts, T. (2022). Opioids impact feeding behavior and response to aerodigestive afferent stimulation. Oxford Conference, October 17-21, Odawara, Japan.

Morimoto, I., **Frazure, M.,** Iceman, K., Koike, T., & Pitts, T. (2022). Modulation of swallowing patterns using Gaussian stochastic mechanosensory stimulation. Oxford Conference, October 17-21, Odawara, Japan.

Iceman, K., **Frazure, M.**, Strecker, C., Greene, C., Howland, D., & Pitts, T. (2022). Acute and chronic laryngeal deficits following cervical spinal cord injury. Oxford Conference, October 17-21, Odawara Japan.

Frazure, M., Young, K., Musselwhite, M., Greene, C., Iceman, K., & Pitts, T. (2022). Global dysphagia scale detects swallowing disorder following a clinical dose of buprenorphine. Poster presentation at *Experimental Biology*, April 2-5, Philadelphia, PA.

Musselwhite, M.N, Young, K., **Frazure, M.**, Greene, C., Iceman, K., & Pitts, T. (2022). The 5-HT_{1A} agonist 8-OH-DPAT enhances submental laryngeal elevator EMG amplitude during swallow. Poster presentation at *Experimental Biology*, April 2-5, Philadelphia, PA.

Frazure, M., Young, K., Musselwhite, M., Greene, C., Iceman, K., & Pitts, T. (2021). Swallowing disorder following a clinical dose of buprenorphine in adult cats. Poster presentation at *Society for Neuroscience*, November 9, Virtual: Chicago, IL.

Musselwhite, M.N., **Frazure, M.**, Young, K., Greene, C., Iceman, K., Howland, D.R., & Pitts, T. (2021). Swallow synchrony is restored with a 5-HT_{1A} agonist after cervical spinal cord injury. Poster presentation at *Society for Neuroscience*, November 9, Virtual: Chicago, IL.

Regional

Frazure, M., Morimoto, I., Iceman, K., Greene, G., Pitts, T. (2023). Mechanisms of depressed breathing and swallowing following buprenorphine administration: Identifying a preventative treatment for opioid induced dysphagia and respiratory arrest. Poster presentation at *Kentucky Speech Language and Hearing Association* annual meeting, February 16, Lexington, KY.

Brennan, M., **Frazure, M.**, Iceman, K., Greene, G., Howland, D., Pitts, T. (2023). A novel model of dysphagia following spinal cord injury. Poster presentation at *Kentucky Speech Language and Hearing Association* annual meeting, February 16, Lexington, KY.

Morimoto, I., **Frazure, M.**, Iceman, K., Pitts, T. (2023). Measuring swallowing responses using Gaussian probabilistic surface electrical stimulation. Poster presentation at *Kentucky*

Speech Language and Hearing Association annual meeting, February 16, Lexington, KY.

Fielder, N., **Frazure, M.**, & Pitts, T. (2022). Baclofen effects on swallow. Poster presentation at *Research!Louisville*, September 19, Louisville, KY.

Frazure, M., Young, K., Greene, C., Iceman, K., Pitts, T. (2022). Model development of opioid induced dysphagia. Poster presentation at the *Kentucky Spinal Cord and Head Injury Research Trust Symposium*, May 18-19, Louisville, KY.

Frazure, M., Young, K., Musselwhite, M., Greene, C., Iceman, K., & Pitts, T. (2022). Aspiration following opioid administration in a novel model of dysphagia. Poster presentation at the *Kentucky Speech-Language-Hearing Association* annual meeting, February 17, Louisville, KY.

Frazure, M., Pitts, T., & Smith, A. (2018). Traumatic brain injury: investigating misconceptions among graduate students in physical therapy, occupational therapy, and speech-language pathology. Poster presentation at the *Graduate Student Regional Research Conference*, March 2, Louisville, KY.

Frazure, M., Pitts, T., & Smith, A. (2018). Traumatic brain injury: investigating misconceptions among graduate students in physical therapy, occupational therapy, and speech-language pathology. Poster presentation at the *Kentucky Speech-Language-Hearing Association* annual meeting, February 22, Lexington, KY.

TEACHING:

Course Instructor

CMDS 600: Research Methods in Speech-Language Pathology, University of Louisville, Louisville, KY.
Fall 2022

Guest Lectures

“Evidence Based Practice for the Speech Language Pathologist,”
CMDS 668: Professional Issues taught by Dr. Rhonda Mattingly,
University of Louisville, Louisville, KY
January 2022

“The Importance of Research in Communicative Sciences and Disorders,” CMDS 600: Research Methods in Speech Language Pathology taught by Dr. Teresa Pitts, University of Louisville, Louisville, KY

December 2021

“A Practical Guide to the Clinical Fellowship: The Road to Licensure and Certification,” CMDS 668: Professional Issues taught by Dr. Rhonda Mattingly, University of Louisville, Louisville, KY
April 2019

“Voice Assessment for the Speech Language Pathologist: Endoscopic, Auditory and Perceptual Measures,” CMDS 663: Voice Disorders taught by Dr. Suzanne King, University of Louisville, Louisville, KY
February 2019

Research Mentoring

In Morimoto, MS
2022-2023

Michael Brennan, MS
2022-2023

Nathan Fielder, BS
2022-2023