CURRICULUM VITAE: SHAINA DEVI HOLMAN, B.S.

Graduate Research Assistant, Department of Neural and Pain Sciences
University of Maryland School of Dentistry

Curriculum Vitae

May 1, 2013

<u>Contact Information</u>
650 West Baltimore Street, Baltimore MD 21201
443-799-7714
swahl001@umaryland.edu

<u>Education</u>
Duke University, Trinity School of Arts and Sciences, 2004-2008
B.S. with distinction, Biological Anthropology and Anatomy, Minors in Biology and Chemistry
Honors Thesis Advisor: Steven Churchill, Ph.D., Dept. of Biological Anthropology and Anatomy, Duke University

University of Maryland Graduate School, 2008-2013
Ph.D., Biomedical Sciences (diploma on hold until 2015)
Dissertation Advisor: Rebecca German, Ph.D., Dept. of Physical Medicine and Rehabilitation, Johns Hopkins School of Medicine
Dissertation Defended on January 11, 2013

University of Maryland School of Dentistry, 2008-present
D.D.S. Candidate, Class of 2015

<u>Employment History</u>
Research Assistant, Summer 2003, Duke University
PI: William Parker, PhD, Department of Surgery
Project: Determining the features of *E. coli* that are necessary for biofilm formation

Undergraduate Research Assistant, Summer 2004, Summer 2006, Fall 2006, Duke University
PI: Bruce Bondurant, PhD, Department of Radiation Oncology
Project: Testing radiation sensitive liposomes as cancer drug transporters

Undergraduate Research Assistant, January 2007-May 2008, Duke University
PI: Steven Churchill, PhD, Department of Biological Anthropology and Anatomy
Project/Honors Thesis title: To determine the link between diet and tooth sharpness in carnivores

Undergraduate Research Assistant, Summer 2007, University of North Carolina, Chapel Hill School of Dentistry
PI: Shad Smith, PhD, Department of Neuroscience
Project: Doing a behavioral analysis in mice after administering anti-anxiety drugs

Graduate Research Assistant (lab rotation), Summer 2008, University of Maryland School of Dentistry
PI: Mark Scheper, DDS, PhD, Department of Oncology and Diagnostic Sciences
Project: Determining if there is a synergistic effect between Farnesol and Chemotherapeutic Agents

Graduate Research Assistant (lab rotation) Summer 2009, Johns Hopkins University
PI: Rebecca German, PhD, Department of Physical Medicine and Rehabilitation
Project: Evaluating regional differences in mechanical strain along the geniohyoid muscle in infant pigs

Professional Society Memberships
American Student Dental Association, 2008-present
American Association of Dental Research, 2009-present
Dysphagia Research Society, 2012-present

Honors and Awards
Howard Hughes Pre-college Scholarship in the Biological Sciences, Duke University, 2003
Principal's Leadership Award, East Chapel Hill High School, 2004
J.J. Vance Memorial Scholarship, East Chapel Hill High School, 2004
Academic All-American- Lacrosse, East Chapel Hill High School, 2004
Undergraduate Research Support Grant, Duke University, Spring 2007
Undergraduate Research Support Grant, Duke University, Fall 2007
Undergraduate Research Support Grant, Duke University, Spring 2008
Trinity Undergraduate Research Fellowship, Duke University, Spring 2008
Citizenship Award, Duke University, Order of Omega, Greek Honor Society, 2008
Hinman Student Research Symposium, Travel Award, University of Maryland School of Dentistry, 2009
Hinman Student Research Symposium, Travel Award, University of Maryland School of Dentistry, 2010
AADR Student Research Fellowship, 2010
AADR Student Research Fellowship, 2011
AADR Student Research Fellowship, 2012
Dysphagia Research Society 2013 Springer Publishing Junior Investigator Scholarship

Local and National Leadership
Founder, President (2006-07), Vice President (2005-06), Duke pre-Dental Society
President, School of Dentistry Class of 2012, 2008-2009, University of Maryland School of Dentistry
President, Student Research Group, Maryland chapter- 2010- 2012

Secretary-elect, National Student Research Group- 2011-2012
Web Content Coordinator, Student Research Group, Maryland chapter- 2012-2013
Secretary, National Student Research Group- 2012-2013
Web Content Coordinator/Newsletter Editor, Student Research Group- 2013-2014

Teaching Service
2011-2012 Mentor for Danielle Waranch, dental student
      Supervised and mentored day-to-day activities
      Taught her how to analyze electromyography data and perform statistical analysis
      Poster presented won 3rd place at the Summer Research Program Poster
      Competition 2012
      Abstract accepted for poster presentation at 2013 IADR/AADR Annual Meeting
      Co-author on 2 manuscripts

2011-2013 Mentor for Estela Gierbolini-Norat, post-baccalaureate student
      Supervised and mentored day-to-day activities
      Taught her how to solder electrodes, handle animals, analyze data
      Abstract accepted for poster presentation at 2013 DRS Annual Meeting
      Co-author on 4 manuscripts

Grant Support
2/1/2009-1/31/2013 PI: Sharon Gordon, Ph.D.
   "Training in Oral and Craniofacial Biology"
   NIH/NIDCR T32 DE07309

2/1/2013-6/1/2015 PI: Shaina Holman
   "Oral Sensation During Pharyngeal Swallowing in Infant Mammals"
   NIH/NIDCR F30 DE021944
   Total Direct Costs: $122,236
   Annual Direct Costs: $48,032

Peer-reviewed journal articles
1. Bollinger RR, Everet ML, **Wahl SD**, Lee YH, Omdorff PE. 2006. Secretory IgA and mucin-mediated biofilm formation by environmental strains of Escherichia coli: role of type 1 pili. Mol Immunol 43(4):378-387.

2. Hartstone-Rose A, **Wahl SD**. 2008. Using radii-of-curvature for the reconstruction of extinct South African carnivoran masticatory behavior. Comptes Rendus Palevol 7(8):629-643.

3. German RZ, Campbell-Malone R, Crompton AW, Ding P, **Holman SD**, Konow N, Thexton AJ. 2011. The concept of hyoid posture. Dysphagia 26(2):97-98.

4. **Holman SD**, Konow N, Lukasik SL, German RZ. 2012. Regional Variation in Geniohyoid Muscle Strain During Suckling in the Infant Pig. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 317(6):359-370.

5. **Holman SD**, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Griffioen AM, Inokuchi H, Lukasik SL, German RZ. 2012. Development, reliability and validation of an infant mammalian penetration-aspiration scale. Dysphagia. Epub Nov 7 2012.

6. **Holman SD,** Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, German RZ. 2013. Duration of action of bupivacaine hydrochloride used for peripheral sensory nerve block to the greater palatine and nasopalatine nerves in infant pigs. Journal of Veterinary Dentistry (*in press*)

7. Ding P, Campbell-Malone R, **Holman SD**, Lukasik SL, Fukuhara T, Gierbolini-Norat EM, Thexton A, German RZ. 2013. Unilateral superior laryngeal nerve lesion in an animal model of dysphagia and its effect on suckling and swallowing. Dysphagia. (*in press*)

8. Ding P, Campbell-Malone R, **Holman SD**, Lukasik SL, Gierbolini-Norat EM, Thexton A, German RZ. 2013. The effect of unilateral SLN lesion on swallowing threshold volume. The Laryngoscope. *(in press)*

9. **Holman SD**, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, Waranch, DR, German RZ. 2013. Swallowing kinematics after palatal local anesthesia in infant pigs. The Laryngoscope. *(in press)*

10. **Holman SD,** Waranch, DR, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, German RZ: Sucking and swallowing rates after palatal anesthesia: an electromyographic study in infant pigs. Journal of Neurophysiology. *(in press)*

Peer-reviewed journal articles in review
1. **Holman SD**, Wietecha MS, Gullard A, Peterson JM: Research experiences impact U.S. dental students' attitudes toward research and its integration in the curriculum. (Journal of Dental Education- in review)

Undergraduate Honors Thesis
Wahl, SD. 2008. The link between diet and tooth sharpness in carnivores. Duke University

Abstracts and Proceedings (*=presenter)
1. **Wahl SD***, Bollinger R, Everet M, Lee Y, Omdorff P, Parker W (2003). Secretory IgA and mucin-mediated biofilm formation by environmental strains of esherichia coli: Role of type 1 pili. Howard Hughes Research Fellowship poster session, Durham, NC. (poster)

2. **Wahl SD***, Hartstone-Rose A (2008). The link between dental morphology and diet in extant carnivores
Visible Thinking poster session, Durham, NC.(poster)

3. **Wahl SD***, Jabra-Rizk M, Scheper M (2008). Synergistic interactions between farnesol and chemotherapeutic agents. Student Research Forum and Poster Day Competition Summer, Baltimore, MD. (poster)

4. **Wahl SD***, Jabra-Rizk M, Scheper M (2009). Syngergistic interactions between farnesol and chemotherapeutic agents. Hinman Student Research Symposium Memphis, TN. (poster)

5. **Wahl SD***, Konow N, German RZ (2009). Regional differences in length change in geniohyoid during infant mammalian suckling. Student Research Forum and Poster Day Competition Summer, Baltimore, MD. (poster)

6. **Wahl SD***, Konow N, German RZ (2010). Regional differences in length change in geniohyoid during infant mammalian suckling. AADR Annual Meeting/NIDCR trainee poster session, Washington, DC. (poster)

7. **Wahl SD***, Konow N, German RZ (2010). Regional differences in length change in geniohyoid during infant mammalian suckling. Hinman Student Research Symposium, Memphis, TN. (oral)

8. Ding P*, Campbell-Malone R, **Holman SD**, Fukuhara T, Feng W, German RZ (2011). Aspiration Mechanism for Unilateral Superior Laryngeal Nerve Lesion in Animal Model. Dysphagia Research Society Annual Meeting, San Antonio, TX. (oral)

9. **Holman SD***, Campbell-Malone R, Ding P, Lukasik S, German RZ (2011). The impact of perioral local anesthesia on suckling and swallowing in infant pigs. IADR/AADR/CADR General Session. San Diego, CA. (poster + oral)

10. **Holman SD***, Campbell-Malone R, Ding P, Lukasik S, German RZ (2011). The role of perioral sensation during suckling and swallowing in infant mammals. Graduate Research Conference, Baltimore, MD. (poster)

11. **Holman SD***, Campbell-Malone R, Ding P, Lukasik S, Waranch DR, German RZ (2012). The impact of palatal sensation during swallowing in infant mammals. AADR Annual Meeting. Tampa, Fl. (poster)

12. **Holman SD***, Campbell-Malone R, Ding P, Lukasik S, Waranch DR, German RZ (2012). The impact of palatal sensation during swallowing in infant mammals. Graduate Research Conference, Baltimore, MD. (poster)

13. Waranch DR*, **Holman SD**, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, German RZ (2012). The Impact of Palatal Anesthesia on Muscle Activity Timing during Swallowing. Student Research Forum and Poster Day Competition. Baltimore, MD. (poster)

14. **Holman SD**, Wietecha MS*, Gullard A, Petersen J (2013). Dental students' attitudes toward research and its integration in the curriculum. UIC College of Dentistry Clinic and Research
Day. Chicago, IL. (poster)- *1st place Winner of Pre-doctoral Clinical and Behavioral Science Research, Awarded by Dentsply*

15. **Holman SD**, German RZ* (2013). Sensorimotor interactions between the oral cavity and pharyngeal function during mammalian feeding. The Society for Integrative & Comparative Biology Annual Meeting. San Francisco, CA. (oral)

16. **Holman SD***, Waranch DR, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, German RZ (2013). Infant Pig Suckling Following Palatal Local Anesthesia. IADR/AADR/CADR General Session. Seattle, WA. (oral)

17. Waranch DR*, **Holman SD**, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, German RZ (2013). The Impact of Palatal Anesthesia on Muscle Activity Timing during Swallowing. IADR/AADR/CADR General Session. Seattle, WA. (poster)

18. Ding P*, Campbell-Malone R, **Holman SD**, Lukasik SL, Gierbolini-Norat EM, Thexton AJ, German RZ (2013).The Effect of Unilateral Superior Laryngeal Nerve Lesion on Sucking and Swallowing in an Animal Model. Dysphagia Research Society Annual Meeting. Seattle, WA. (oral)

19. **Holman SD***, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, German RZ (2013). Altered airway protection and swallowing kinematics following palatal anesthesia in infant pigs. Dysphagia Research Society Annual Meeting. Seattle, WA. (oral)

20. Griffioen AM*, Campbell-Malone R, Ding P, **Holman SD**, Lukasik SL, German RZ (2013). Validation of a novel method for semi-automated digitizing of videofluoroscopic images for kinematic analysis. Dysphagia Research Society Annual Meeting. Seattle, WA. (poster)

21. Gierbolini-Norat EM*, **Holman SD**, Campbell-Malone R, Ding P, Lukasik SL, German RZ (2013). Variation in the timing and frequency of sucking and swallowing over an entire feeding session in the infant pig *Sus scrofa*. Dysphagia Research Society Annual Meeting. Seattle, WA. (poster)

22. **Holman SD***, Waranch DR, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, German RZ (2013). Infant Pig Suckling Following Palatal Local Anesthesia. Graduate Research Conference. Baltimore, MD (poster)

23. **Holman SD**, Wietecha MS*, Gullard A, Petersen J (2013). Dental students' attitudes toward research and its integration in the curriculum. Annual Session of the American Dental Association. New Orleans, LA. (poster- to be presented)

<u>Invited Seminars and Speeches</u>
Holman, SD, *The Career of Dentistry*. Piccowaxen Middle School Career Day. Piccowaxen Middle School. October 2011

Holman, SD, *Dentistry*. Women and Math 2012. University of Southern Maryland. October 2011

Holman, SD, *Dental Research*. Dentistry Today 2012. University of Maryland School of Dentistry. June 2012

Holman, SD, *Dentistry*, Career Fridays. City Springs Charter School. November 2012.

Holman, SD. *Duke University*. 8th Grade College Day 2012. Lansdowne Middle School. November 2012

Holman, SD, *Dentistry*, Career Fridays. City Springs Charter School. April 2013.

Holman, SD, *Dentistry*. Women and Math 2012. University of Southern Maryland. April 2013

Firstly, I would like to thank my research mentor, Dr. Rebecca Z. German for her mentorship and guidance throughout my training. I have learned so much about science and also about life while being a member of your lab. Thank you for pushing me hard and supporting me along the way.

Next I would like to thank Dr. Sharon Gordon and Dr. Norman Capra for their encouragement to pursue this degree. I will always be grateful to you, Dr. Capra, for introducing me to Dr. German. And I thank you, Dr. Gordon, for introducing me to the AADR which I intend to be active with for the rest of my career. Thank you both for supporting the dual degree program and giving me this opportunity.

I would like to thank the members of my dissertation committee: Dr. Ianessa Humbert, Dr. Radi Masri, Dr. Jin Ro and Dr. Maureen Stone. Thank you for challenging me and mentoring me through the development of this work. You all helped develop my scientific thinking and gave me new perspectives on my project that were extremely valuable.

The friendships and support system in the German lab also played a crucial role in the success of this project. I am very thankful for all your input on my project at every step along the way. I would like to thank Dr. Ceiling Ding, Estela Gierbolini-Norat, Takako Fukuhara, Annie Griffioen, Haruhi Inokuchi and Stacey Lukasik for their hard work, support and encouragement throughout my past few years. I would especially like to thank Dr. Regina Campbell-Malone for her day-to-day support and for our conversations that helped develop my thinking as a scientist.

Lastly, I would like to thank my family. Mom, I never even knew women were underrepresented in the sciences until I was 16 because I had you to look up to and encourage me. Your stories of graduate school helped motivate me when I reached challenges along the way. Dad, I've learned everything about management and leadership from you and those lessons proved to be very helpful when leading experiments and managing relationships. Thank you for encouraging me to earn this degree and supporting me through the ups and downs. Sarah, thank you for always listening to me when I need someone to talk to, and for encouraging me as I have pursued this degree. You motivate me more than you know. Kerri, thanks for your clinical perspective on my project and for being my cheerleader throughout the last several years.

And to my husband, Trevor- this year makes 10 years that you have been in my life. It seems like just yesterday you were there watching my induction into the National Honor Society in the 11th grade and now I have earned my Ph.D.! Thank you for always giving me a reason to smile at the end of the day and for being a vital part of my support system. You always help me to keep my perspective on life and to motivate me to work harder each day.

ABSTRACT

Title of Dissertation: The Role of Palatal Sensation during Sucking and Pharyngeal Swallowing in the Infant Pig

Shaina Holman, Doctor of Philosophy Candidate, 2013

Dissertation Directed by: Rebecca German, Ph.D., Professor, Department of Physical Medicine and Rehabilitation, Johns Hopkins University School of Medicine

Swallowing dysfunction in infants can be caused by differences in craniofacial anatomy, neurological disorders or prematurity. These conditions result in difficulty initiating pharyngeal swallow cycles and/or a lack of airway protection during the swallow that can result in aspiration. In order to provide swallowing rehabilitation for these infants, we need to know more about the sensory and motor interactions that occur during the normal infant swallow. The overall aim of this dissertation was to understand how reducing palatal sensation would affect the oral and pharyngeal phase of the swallow. We hypothesized that after a palatal injection of local anesthesia both phases would show significant changes in movements of the tongue, hyoid and epiglottis that may result in less airway protection during the pharyngeal swallow. We also hypothesized we would observe changes in muscle activity to explain the mechanism of the altered kinematics. We used an infant pig model of mammalian feeding to test these hypotheses. Electromyographic (EMG) electrodes were implanted into several hyoid and pharyngeal muscles. We fed the pigs while simultaneously recording EMG and lateral videofluoroscopy captured at 60 frames per second. We evaluated these recordings during feeding sessions with no treatments and compared them to feeding sessions following a palatal anesthesia (0.5% bupivacaine hydrochloride) or saline injection. In order to evaluate airway protection before and after the treatments, we developed the infant mammalian penetration-aspiration scale (IMPAS). A novel method was developed

to test local anesthesia duration in infants that proved bupivacaine hydrochloride lasted at least one hour after injection before the return of oral reflexes. Using these methods, we demonstrated that reducing palatal sensation has profound effects on frequency, kinematics and motor function during the oral and pharyngeal swallow. Preliminary data suggests that the infant pharyngeal swallow may also be capable of motor learning. These studies demonstrate an important role for trigeminal sensation in the normal initiation and coordination of the sucking and swallowing CPGs in the brainstem. Future studies are needed to determine if manipulating oral sensory receptors can lead to novel dysphagia rehabilitation strategies in human patients.

The Role of Palatal Sensation during Sucking and Pharyngeal Swallowing in the Infant
Pig

by
Shaina Devi Holman

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland in partial fulfillment
Of the requirements for the degree of
Doctor of Philosophy
2013

Table of Contents

CHAPTER 1: INTRODUCTION

Swallowing dysfunction in infants is often caused by differences in craniofacial anatomy, neurological disorders or prematurity (Miller, 2011a). Infants that suffer from swallowing dysfunction often can suffer from inadequate nutrition or aspiration which can become life threatening (Tutor and Gosa, 2012). In order to provide swallowing rehabilitation for these infants, we need to know more about the sensory and motor interactions that occur during the normal infant swallow.

Mammalian feeding is a complex sensorimotor behavior with both rhythmic and reflexive components (German et al., 2009; Thexton et al., 2012). Infant mammals are a good model for studying feeding that allows us to understand how the sensory and motor aspects of feeding interact because it is a relatively more simple behavior than adult feeding. The rhythmic component consists of the pure suck cycles that both extract milk from the nipple and transport it through the oropharynx prior to a swallow. The reflexive component is the pharyngeal swallow where the bolus is transported into the esophagus. The terminal suck cycle before a pharyngeal swallow is the suck-swallow cycle. Throughout a feeding session, sucking is an ongoing rhythmic behavior and reflexive swallows are interspersed throughout. Although we know some of the sensory mechanisms that elicit the swallow, including bolus volume, temperature, taste, and carbonation (Butler et al., 2011; Michou et al., 2012; Yamamura et al., 2010), it is unknown how specific sensory information gathered during the oral transport process impacts the onset of the reflexive pharyngeal swallow.

Oropharyngeal swallowing is a highly coordinated, centrally patterned, motor function that requires sensory input from four cranial nerves in the oral cavity and

pharynx (Miller, 2008; Steele and Miller, 2010). Central patter generators, or CPGs, are thought to control both swallowing and sucking. During sucking, sensory information about the bolus is sent to the sucking CPG in the brainstem that regulates the motor output necessary to transport the bolus to the oropharynx (Barlow, 2009; Steele and Miller, 2010). There are two groups of interneurons identified in the brainstem that make up the swallowing CPG. A group of interneurons in and around the nucleus tractus solitarius (NTS) contains neurons that trigger the CPG. Another group of interneurons in and around the nucleus ambiguus (NA) has the neurons that regulate the rhythmic output of the motor neurons.

Sensory information from the oropharynx synapses in the swallowing pattern generator in the brainstem to initiate the motor function necessary for a pharyngeal swallow (Barlow, 2009; Steele and Miller, 2010). There are three sensory nerves that will synapse in the NTS (Steele and Miller, 2010): the facial (VII), glossopharyngeal (IX) and vagus (X) nerves (Fig. 1). There are four motor nerves that will leave the NA and innervate the muscles involved in swallowing: the facial (VII), glossopharyngeal (IX), vagus (X), and hypoglossal nerves (XII; Fig. 1). Descending input from the cortex can synapse in the swallowing CPG and initiate swallows and it can alter the timing of movements during the pharyngeal swallow in adults (Fig. 1). Despite this finding, it has been demonstrated in decerebrate animals that swallowing can be generated in the absence of cortical input (Jean, 2001).

The fifth cranial nerve, the trigeminal nerve (V2 & V3 in particular), supplies sensation to the oral cavity and also sends projections to the NTS (Fig. 1). This means it could influence the swallowing pattern generator and thus affect the pharyngeal phase of

the swallow (Sessle and Storey, 1972). Although oral sensation from the trigeminal nerve

projects to the region of the brainstem where the swallowing pattern generator is located,

its role in the triggering of the pharyngeal swallow is not clear. Sensory feedback in the

pharyngeal and esophageal phases of the swallow is known to impact swallow reflex

timing, but the sensory information from the oral phase is often overlooked in the context

of swallow reflex initiation and dynamics (Lang, 2009). Using an infant pig model,

swallowing frequency was found to be directly related to oral and oropharyngeal

stimulation from increased frequency of feeding rather than bolus volume (German et al.,

2004a). It is not clear how sensory information from the oral cavity regulates the

activation and coordination of the many muscles that are needed for suckling and

swallowing.
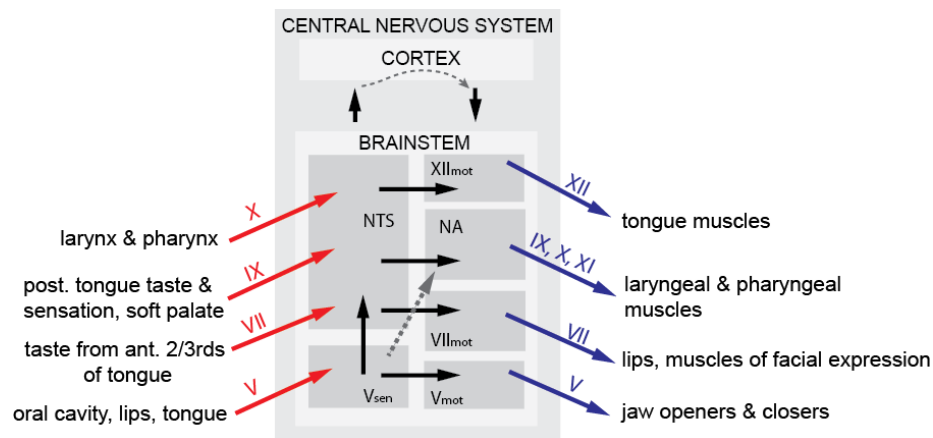
Figure 1 Sensory and Motor Pathways involved in Swallowing. The diagram shows sensory nerves synapsing in the brainstem in their respective nuclei. Sensory information also activates the cortex and there is descending cortical input to the NA. The NA sends motor neurons to the muscles involved in swallowing. We know that there are projections from Vsen to the NTS and cortex, but we do not know if Vsen is linked to the NA either directly or indirectly. (NTS= nucleus tractus solitarius; Vsen= trigeminal sensory nucleus; XIImot= hypoglossal motor nucleus; NA= nucleus ambiguus; VII= facial motor nucleus; Vmot= trigeminal motor nucleus).

Although much is known about the development of swallowing and the neurophysiology of swallowing, very little is known about the role of oral sensation in the initiation of the pharyngeal swallow. Clinical studies in adults have shown that altering intraoral sensation changes the recruitment of craniofacial muscles (Miller et al., 1985) and highly variable changes in swallowing initiation, oral containment time and intraoral pressure (Pelletier and Dhanaraj, 2006; Tei et al., 2004; Yagi et al., 2008). Clinical studies often anesthetize the oral cavity along with the oropharynx which makes it impossible to determine if the changes are due to a deficit in trigeminal, glossopharyngeal or vagus sensation (Mansson and Sandberg, 1974). Very little is understood about which sensory nerves are involved, and to what extent, in order to initiate a swallow. Oral sensation from the trigeminal nerve is thought to be important for swallow initiation (Hollshwandner et al., 1975) and programming the motor pattern of the swallow in adults (Humbert et al., 2012a; Lowell et al., 2008). Additionally, the clinical studies are all in adults with few studies looking at the role of oral sensation during swallowing in infants.

Intraoral and perioral stimulation administered manually or by a machine has been shown to help premature babies develop their suckling ability (Barlow et al., 1992; Barlow et al., 2001; Finan and Barlow, 1996). The same finding was found in decerebrate neonatal rats that exhibited rhythmic jaw movement following stimulation to the anterior oral cavity (Thexton and Griffiths, 1979 ; Thexton et al., 1980). It is not known if or to what extent oral stimulation can influence the pharyngeal swallow. If oral stimulation did help to improve swallowing kinematics or initiation, then this would be an ideal

dysphagia rehabilitation strategy to develop since the oral region is an easily accessible area to stimulate (Ogura et al., 2007).

There are three regions in the oral cavity that are dense with mechanoreceptors and have been consistently shown to be important during feeding: the hard palate, the tongue and the perioral region (Capra, 1995; Halata et al., 1999). These regions hold the nipple steady while milk is being extracted. The hard palate is especially important because when the tongue compressed the nipple against it, enough intraoral pressure is generated to initiate a swallow. Reduced intraoral pressure has been linked to increased risk of aspiration in adults (Hirota et al., 2010; Pelletier and Dhanaraj, 2006). In a study of 10 adult subjects, covering the hard palate or anesthetizing it caused decreased lingual pressure during swallowing and altered bolus propulsion time in half of the subjects (Kodaira et al., 2006). It is not known how palatal sensation could affect these same parameters in infants and it is not known how palatal sensation affects other pharyngeal swallowing kinematics. This study investigates how reduced sensation from the hard palate will alter pharyngeal swallowing initiation and airway protection.

Infant pigs have been used successfully as a model for understanding human feeding because musculature, nerves and feeding mechanism are evolutionarily conserved in all mammals (Crompton et al., 2008; German and Crompton, 2000; German et al., 2009; Thexton et al., 2009). Pigs are also favored over other non-primate mammals due to their "head-up" posture while swallowing which resembles humans. Recent studies have utilized a pig model to advance the understanding of human oral anatomy and function (Ross et al., 2007). Other studies have shown that there is little qualitative variation within this age range (German et al., 2004a). We chose to use an infant pig

model to understand infant sucking and swallowing physiology since it is an established model for studying feeding.

The following studies aimed to assess the role of palatal sensation during swallowing in infants. We gave a sensory nerve block to the greater palatine and nasopalatine nerves, which provide sensation to the hard palate, in an infant pig model. We then assessed swallowing function using videofluoroscopy and electromyography. We evaluated swallowing and sucking frequencies, kinematics and airway protection. In order to evaluate these parameters, we developed a penetration-aspiration scale for infant mammals and also demonstrated the duration of palatal anesthesia in infant mammals. Understanding of the effects of sensory inputs from the oral cavity during swallowing and suckling will significantly enhance our understanding of sensorimotor links between the oral and pharyngeal phase of food processing and swallowing. As a result of this study, innovative strategies using oral and oropharyngeal stimulation could be developed to rehabilitate infants suffering from dysphagia. These regions are easily accessible as opposed to the pharynx or larynx. It will also help health care professionals, including dentists, take necessary precautions when working with dysphagic patients.

CHAPTER 2: DEVELOPMENT, RELIABILITY AND VALIDATION OF AN INFANT MAMMALIAN PENETRATION-ASPIRATION SCALE[1]

ABSTRACT

A penetration-aspiration scale exists for assessing airway protection in adult videofluoroscopy and fiberoptic endoscopic swallowing studies, however no such scale exists for animal models. The aim of this study was threefold to 1) develop a Penetration-Aspiration Scale (PAS) for infant mammals, 2) test the scale's intra- and inter-rater reliability, and 3) to validate the use of the scale for distinguishing between abnormal and normal animals. After discussion and reviewing many videos, the result was a 7-Point Infant Mammal PAS. Reliability was tested by having 5 judges score 90 swallows recorded with videofluoroscopy across two time points. In these videos, the frame rate was either 30 or 60 frames per second and the animals were either normal, had a unilateral superior laryngeal nerve (SLN) lesion, or had hard palate local anesthesia. The scale was validated by having one judge score videos of both normal and SLN lesioned pigs and testing the difference using a t-test. Raters had a high intra-rater (average kappa of 0.82, intraclass correlation coefficient (ICC) of 0.92) and high inter-rater reliability (average kappa of 0.68, ICC= 0.66). There was a significant difference in reliability for videos captured at 30 and 60 frames per second for scores of 3 and 7 ($p<0.001$). The scale was also validated for distinguishing between normal and abnormal pigs ($p<0.001$). Given the increasing number of animal studies using videofluoroscopy to study dysphagia, this scale provides a valid and reliable measure of airway protection during

swallowing in infant pigs that will give these animal models increased translational significance.

INTRODUCTION

Penetration and aspiration can occur in the absence of adequate airway protection during swallowing. Penetration occurs when material passes into the airway, but remains at or above the vocal folds in the supraglottic space. Aspiration occurs when material passes below the vocal folds and into the trachea. In clinical studies of swallowing dysfunction, the incidence of both penetration and aspiration are associated with increased risk of developing pneumonia, which can be life threatening (Pikus et al., 2003). The 8-Point Penetration-Aspiration Scale (PAS) (Rosenbek et al., 1996), used in clinical research studies, is an established and validated instrument for measuring the airway protection during swallowing (Baijens et al.; Bulter et al., 2011; Hey et al., 2011; Robbins et al., 1999; Rofes et al., 2011). No such scale, however, currently exists for animal models of swallowing dysfunction.

Animal models are an important tool for understanding both normal and abnormal swallowing. Such models are valuable for collecting data on swallowing dysfunction that is not possible in humans, for both logistic and ethical reasons (Ciucci et al., 2011; German et al., 2009; Lever et al., 2012; Watrous and Suter, 1983). For example, we can study swallow function in animals using videofluoroscopy without the time limitation that is necessary in clinical studies due to limits of radiation exposure. This model also permits multiple and unlimited videofluoroscopy recordings over several days or even months. In animal models, researchers can generate pathological conditions through

nerve lesions, anesthesia or other means and then test the effect of that specific condition

on function in the same individual (Bennett et al., 2012; Klein et al., 1994). The other

advantage of animal models is the ability to control extraneous and confounding factors

that frequently co-occur in human clinical conditions (Aluffi et al., 2001; Kark et al.,

1995).

A valuable animal model of studying infant swallowing is the infant pig (*Sus scrofa*). The main reason for studying infant pigs in swallowing dysfunction studies is

because the anatomy of the infant pig parallels other infant mammals, which enables us

to draw clinically relevant conclusions to the treatment of human infants with swallowing

dysfunction (Hiiemae, 2000). For example, a model of superior laryngeal nerve (SLN)

lesion in the infant pig is currently being used to understand how loss of sensory

information carried by this nerve affects the motor control of swallowing (Ding et al.,

2011; Ding et al., 2012). In this model, we have documented both penetration and

aspiration in the infant pig (Ding et al., 2011).

The aim of this paper was to 1) develop a novel PAS for the infant pig model of

swallowing dysfunction based on the standard clinical research scale, 2) assess the intra-

and inter-rater reliability, and 3) test the validity of this scale for differentiating abnormal

versus normal animals.

METHODS

Summary of methods used for unilateral SLN lesion and palatal local anesthesia

The videofluoroscopic recordings utilized to develop this scale and assess reliability and validity were from ongoing studies in the lab. These methods are summarized below.

The pigs that had a unilateral SLN lesion had undergone two surgeries. The pigs were 10-16 days old and 5-6 kg in weight. During the first surgery they were given general anesthesia (5% Isoflurane) and intubated and then underwent surgery that lasted 3-5 hours. During this surgical procedure, electromyographic (EMG) electrodes were implanted into hyolaryngeal muscles and a radio-opaque marker was sutured to the hyoid bone and thyroid cartilage. These hyolaryngeal muscles include mylohyoid, genioglossus, geniohyoid, digastrics, thyrohyoid, sternothyroid, sternohyoid and cricothyroid. After this surgical procedure was completed, radio-opaque markers were placed into the tongue, gingiva, hard palate and soft palate and a radio-opaque marker was placed with an intraoral approach on the epiglottis. The animal recovered from surgery after 1-5 hours and was then fed swine milk formula (Land O'Lakes Solustart Pig Milk Replacer) containing barium using a bottle with a 'pig nipple' (Nasco, Fort Atkinson, WI) while investigators recorded both lateral videofluoroscopy at 30 or 60 frames per second and EMG signals from the electrodes placed into the hyoid musculature. This first recording was for control data. After 1-2 days the pigs underwent another surgery where the SLN was cut on the right side before it branches into the internal and external branches that supply the larynx and cricothyroid muscles. After the animal recovered, the animal was again fed using procedures identical to those for control data collection. After all the

necessary recordings were completed, the animal was euthanized and the location of the EMG electrodes and markers were confirmed by dissection.

The pigs with palatal local anesthesia underwent one surgery to place EMG electrodes into their hyoid musculature and radio-opaque markers placed into the same structures as with the SLN lesioned pigs. Starting a day after surgery, these pigs were fed while recording lateral videofluoroscopy and from the EMG electrodes at 4 time points, 2 hours apart across a day for control data. Early on the next day, the pig was given a 0.5ml injection of 0.5% bupivacaine, a long-lasting local anesthetic, at 3 locations: the nasopalatine foramen, and left and right greater palatine foramina. The technique used was a standard dental local anesthesia nerve block. Bupivacaine starts being effective 15 minutes post-injection and lasts for 3-5 hours in small dogs, which is considered to be comparable in infant pigs due to their similar size (Reuss-Lamky, 2007). Starting one hour after the nerve blocks the animal was fed using the same procedures as were used for the SLN lesion animals. They were fed 4 total times, every 2 hours. Animals were counterbalanced so that half of the pigs were recorded on day 1 post-surgery with no local anesthesia and on day 2 with local anesthesia, while the other half of pigs were recorded on day 1 post-surgery with local anesthesia and on day 2 without local anesthesia. After all the recordings were completed, the animal was euthanized and the location of the electrodes and markers were confirmed by dissection. All of these procedures were approved by the Johns Hopkins Medical Institute IACUC.

Development of the Scale

In order to adapt the clinical PAS to the infant mammal, we examined a number of infant pig videofluoroscopic images of normal swallows, as well as swallows with

clear penetration and aspiration. The infant pigs studied were all from the previously

described studies.

Our infant mammal PAS was based on the current clinical 8-Point PAS (1; Table

1). The result was a 7-Point Infant Mammal PAS (IMPAS) (Table 2). As with the PAS,

this scale was multidimensional. It took into account the depth of the bolus into the

airway, whether it was above or below the vocal folds, as well as the animal's response to

the bolus, whether it was expelled passively, actively or not at all. Similar to the PAS

used in clinical research, our scale was ordinal, meaning that lower scores represent less

severe conditions (more airway protection during swallowing), and higher scores reflect

more severe conditions (less airway protection during swallowing). Below is a

description of each score.

Table 1 8-Point Penetration-Aspiration Scale from Rosenbek et al 1996.

Score	Description
1	Material does not enter the airway
2	Material enters the airway, remains above the vocal folds, and is ejected from the airway
3	Material enters the airway, remains above the vocal folds, and is not ejected from the airway
4	Material enters the airway, contacts the vocal folds, and is ejected from the airway
5	Material enters the airway, contacts the vocal folds, and is not ejected from the airway
6	Material enters the airway, passes below the vocal folds and is ejected into the larynx or out of the airway
7	Material enters the airway, passes below the vocal folds, and is not ejected from the trachea despite effort
8	Material enters the airway, passes below the vocal folds, and no effort is made to eject

No Penetration: A score of 1 on the IMPAS is the equivalent to the score of 1 on the

clinical PAS (Table 1). During these swallows no material enters the airway. The

material, or in this case milk, flows over the epiglottis as it moves caudally to protect the airway (Fig. 2a&b).

Penetration: A score of 2 on the IMPAS is similar to the score 2 on the clinical 8-Point PAS. The score of 2 on the clinical scale is when material enters the airway, remains above the vocal folds and then is ejected. On the clinical scale this material is ejected either passively or by a cough. In the infant pig we observed a similar case where material would enter the airway, remain above the vocal folds and passively leave the airway, often before the epiglottis returned to its upright position (Fig. 2c).

Scores 3 and 4 on the IMPAS are similar to the score of 3 on the clinical PAS. In the clinical scale, material enters the airway and remains above the vocal folds. This was also seen in infant pigs; however, we observed two distinct conditions where milk remained above the vocal folds. Occasionally, a very small amount of milk was seen on the caudal side of the epiglottis by its base forming a small triangle (Fig. 2d). In other cases, a substantial amount of material remained in the airway, above the vocal folds, however it was traveling towards the vocal folds (Fig. 2e). Thus we divided this category into two scores as having more material closer to the vocal folds was perceived as a more severe condition that would increase the risk of aspiration as it does in human infants (Friedman and Frazier, 2000). The score of 3 is a small amount of material above the vocal folds on the inverse side of the epiglottis forming a small triangle by the base of the epiglottis and a score of 4 is a large amount of material.

It is important to note that for scores 2-4 "material" may be old or new material (Table 2). In clinical videofluoroscopic swallowing studies (VFSS), swallows are often isolated; however in the infant pig videofluoroscopy recordings are made across a feeding

session where there are multiple swallows per second with no break. As a result there is

often milk residue in the airway from previous swallows. Since any milk in the airway

above the vocal folds was due to a failed swallow, it was determined that it should be

scored regardless of whether it was from a previous swallow or the current swallow.

Aspiration: A score of a 5 and 6 on the ISPAS is similar to a score of 6 and 7 on the

clinical 8-Point PAS. A score of 6 on the clinical PAS describes material passing below

the vocal folds, and then being ejected into the larynx or out of the airway. A score of 7

was where material passed below the vocal folds and was not ejected despite effort.

Although we did not observe a score of 6 or 7 in our videofluoroscopic recordings, past

observations of coughing in pigs with SLN lesions meant these two scores are possible.

Further, aspiration following sensory or motor nerve lesions could trigger a coughing

reflex that may or may not be successful. For this reason a score of 5 on the IMPAS is

when material passes below the vocal folds and is ejected into the larynx or out of the

airway. A score of 6 is when material passes below the vocal folds and is not ejected

despite effort.

Table 2 The 7-Point Infant Mammal Penetration-Aspiration Scale

Score	Description
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)

Silent aspiration is described as a score of 7 on the IMPAS (Fig. 2f). This score is the equivalent of a score 8 on the clinical 8-Point PAS. This was often seen after nerve lesions in the infant pig model.

With scores of 5, 6 and 7, *new* milk must be visualized moving from the supraglottic space to below the vocal folds. There were some instances where milk, from a previously failed swallow, was visualized in the trachea moving above, and then back below the vocal folds. This was not a score of a 5, 6 or 7 because for those scores new material must be in the supraglottic space then move below the vocal folds. If material is in the supraglottic space and joins milk residue from below the vocal folds, forming a solid stream of milk, then it is aspiration and is scored as a 5, 6 or 7 depending on whether or not there is effort to eject that material.
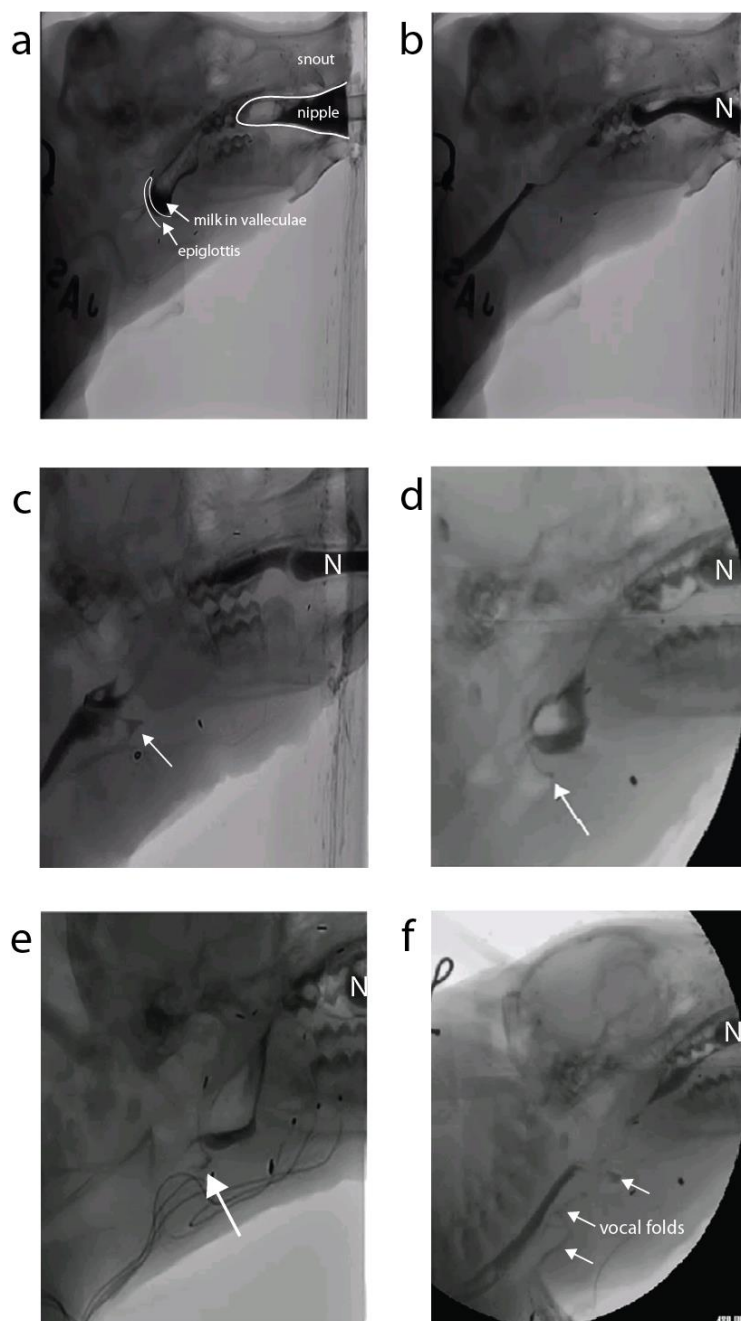
Figure 2 Videofluoroscopic images from each score on the 7-Point Infant Mammal PAS a) This videofluoroscopic image shows the epiglottis is in the upright position with milk in the valleculae right before a swallow is initiated. The anatomy is clearly outlined and labeled for orientation. b) In the image, milk is being emptied out of the valleculae, the epiglottis is fully flipped to protect the airway, and milk continues into the esophagus. This is a score of 1. c) The epiglottis has flipped fully to protect the airway, however, milk penetrates the airway as identified by the arrow. When the epiglottis returns to its rest position, there is no milk left in the supraglottic space. This is a score of 2. d) The epiglottis is in its upright position after a swallow. The arrow points to a small amount of milk visible on the caudal side of the epiglottis forming a triangle at the base. When this occurs it is scored as a 3. e) The epiglottis has returned to its rest position after a swallow.

The arrow points at a large amount of milk residue left on the caudal side of the epiglottis, but above the vocal folds. This is scored as a 4. f) In the image, the epiglottis is flipped to protect the airway during the swallow. Milk is clearly visible crossing the vocal folds and entering the larynx and is identified by the arrows. The nipple is in the far right of all the images and is labeled with the letter "N".

No equivalent score of 4 and 5 on the clinical PAS was included in the infant pig scale because milk was always clearly either above or below the vocal folds and never just contacted them.

Guidelines for users of the scale

In order to ensure consistency and higher reliability, specific directions were developed for judges scoring videos. The swallow being scored begins at the start of the epiglottal flipping (posterior/inferior movement) and ends immediately before the next epiglottal posterior/inferior movement. The rationale for this definition was that in some cases the events leading to a score of 3, 4 or 7 did not develop until after the epiglottis had returned to its upright position, but before the next swallow occurred. Judging was also based only on a score where material was clearly visible in the airway. Sometimes due to the poor quality of the video, judges would describe seeing very small residues of material in the airway. This resulted in variable scoring of the videos. Judges were not permitted to alter the contrast or brightness of the image since that could alter the amount of material visualized or create artifacts. When scoring videos, judges were given as much time to review the swallow as needed and they could view it multiple times and in slow motion.

Determining the reliability of the scale

In order to test the reliability of the IMPAS, five judges scored 90 videofluoroscopy recordings. Images of swallows were randomly selected from 10 infant pigs and 30 total feeding sessions from the previously described ongoing studies. From each feeding session, three swallows were randomly clipped out of the sequence and separated into their own video file.

The judges had various levels of experience and education with respect to analysis of VFSS data, but all had experience with either animal or clinical swallowing research. The judges were given the 90 videofluoroscopic recordings of individual swallows in a randomized order to score according to the scale. They were instructed to score all of the videos within 48 hours and then, as was done with the development of the clinical PAS, to score them all again after a two week hiatus, also within a 48 hour period (Rosenbek et al., 1996). The judges were given the same set of videos for their second scoring, but in a different, randomized order. All videos were viewed using MaxTRAQ Version 2.2.4.1 (Innovision Systems, Inc.). All judges also were given the same five videos that were examples of scores 1, 2, 3, 4 and 7 to reference during their scoring of the videos.

In order to assess inter- and intra-rater reliability, Cohen's kappa and two-way mixed intraclass correlation coefficient (ICC) with absolute agreement were calculated using SYSTAT 13 (SYSTAT, 2009) and IBM SPSS Statistics 20, respectively. Cohen's kappa was also calculated by scale score in order to determine if there were differences in intra- and inter- rater reliability based on each score. Rosenbek et al 1996 used kappa to calculate reliability, however, we also calculated ICC since the scale is ordinal and that calculation takes into account the degree of difference. Percentage of agreement and the

stratification of the scores were also calculated. We also tested for statistically significant differences in the Cohen's kappa score given for videos captured at 30 vs. 60 frames per second using an Analysis of Variance test (ANOVA) and post-hoc Tukey's test. The statistical analysis was carried out by an independent investigator who had not judged the videos.

Assessing validity of the scale

A separate study was conducted to assess if the scale could distinguish normal and abnormal pigs. A blinded judge, who was not one of the five judges used to assess reliability, scored 39 swallows from six intact infant pigs and 39 swallows from three abnormal infant pigs. The abnormal pigs had a unilateral SLN lesion. Each swallow was from a different randomly selected feeding session. The scores were graphed to see if there was a distinct difference in those between normal and abnormal infant pigs. A two sample t-test was calculated to determine if the normal and abnormal pigs were statistically significantly different with an alpha set at 0.05.

RESULTS

Reliability of the scale

In an initial attempt to score the 90 videos using the scale, the intra- and inter-rater reliability was low and was deemed unacceptable. The intra-rater reliability measured using Cohen's kappa averaged 0.65 and the inter-rater reliability ranged from 0.36-0.67 with an average of 0.58. Following that preliminary analysis, difficulties and problems with the scale were discussed. Ten videos that were a source of significant

variation were reviewed in our group in order to better define the seven categories. The subsequent clarification and revision to the scale resulted in the version presented here.

When the scoring was performed again, the results showed much higher inter and intra-rater reliability scores. Intra-rater reliability calculations showed an average Cohen's kappa of 0.82 and an average ICC of 0.92 with 86% agreement (Table 3). The intra-rater reliability by score was calculated using Cohen's kappa (Table 3) and showed higher reliability for scores 1, 2 and 7 (0.90, 0.82, 0.83 respectively) as compared to scores 3 and 4 (0.74, 0.75 respectively). Inter-rater reliability was, as expected, was lower than the intra-rater reliability. For the first scoring, the average Cohen's kappa value was 0.70 and the ICC was 0.89. For the second scoring the average Cohen's kappa value was 0.66 and the ICC was 0.87 (Tables 4 and 5). The Cohen's kappa values ranged from 0.65-0.76 for the first ranking and ranged from 0.58-0.84 for the second ranking. No scores of 5 or 6 were observed by the judges.

Table 3 Intra-rater reliability by score. Percentage of agreement is shown for each judge between the first time they did the scoring and when they did scoring two weeks later. Cohen's kappa is also calculated overall and by score for each judge.

Judge	Agreement n	Agreement %	ICC	Cohen's kappa	Cohen's kappa by scale score 1	2	3	4	5	6	7
1	75	83.33	0.87	0.78	0.90	0.82	0.68	0.65	-	-	0.71
2	79	87.78	0.97	0.84	0.80	0.80	0.84	0.85	-	-	0.96
3	82	91.11	0.96	0.89	0.92	0.87	0.86	0.87	-	-	0.88
4	79	87.78	0.95	0.84	0.91	0.85	0.73	0.82	-	-	0.86
5	72	80.00	0.87	0.74	0.94	0.77	0.57	0.58	-	-	0.73
AVERAGE	77.4	86.00	0.92	0.82	0.90	0.82	0.74	0.75	-	-	0.83

Table 4 Inter-rater reliability for each pair of judges at first scoring. This round of scoring was completed within one week of a training session for the judges where examples were reviewed. The average Cohen's kappa was 0.70 and the average percentage of agreement was 76.7%. Most scores that did not agree differed by only one score.

Judge pair	1-2	1-3	1-4	1-5	2-3	2-4	2-5	3-4	3-5	4-5	average
Two scores agree											
n	66	69	68	71	62	63	61	78	65	65	66.8
%	73.3	76.7	75.6	78.9	68.9	70.0	67.8	86.7	72.2	72.2	74.2
Number of scores that differ by											
1	20	19	22	14	22	24	24	11	20	21	19.7
2	4	2	0	3	5	3	3	1	4	3	2.8
3	0	0	0	2	1	0	2	0	1	1	0.7
>3	0	0	0	0	0	0	0	0	0	0	0.0
Cohen's Kappa	0.65	0.70	0.68	0.72	0.60	0.61	0.58	0.83	0.64	0.64	0.66

Table 5 Inter-rater reliability for each pair of judges at second scoring. This scoring was completed two weeks after the first scoring. The average Cohen's kappa is lower, but still acceptable at 0.66 with a percentage of agreement of 74.2%. As before, most scores that were not in agreement differed by one score.

Judge pair	1-2	1-3	1-4	1-5	2-3	2-4	2-5	3-4	3-5	4-5	Average
Two scores agree											
n	66	69	70	71	67	67	67	70	73	70	69.0
%	73.3	76.7	77.8	78.9	74.4	74.4	74.4	77.8	81.1	77.8	76.7
Number of scores that differ by											
1	20	17	18	19	18	22	21	20	16	19	19.0
2	3	4	1	0	5	1	1	0	1	0	1.6
3	1	0	1	0	0	0	1	0	0	1	0.4
>3	0	0	0	0	0	0	0	0	0	0	0.0
Cohen's Kappa	0.65	0.70	0.71	0.73	0.67	0.67	0.67	0.71	0.76	0.71	0.70

The Cohen's kappa for each score showed higher reliability for scores 1, 2, 4 and 7 (0.86, 0.68, 0.67 0.80 respectively) than for 3 (0.50) in the first scoring (Table 6). In the second scoring, reliability was lower for both scores 3 and 4 (0.46, 0.59 respectively) than for scores of 1, 2 and 7 (Table 7). An examination of the distribution and differences

of scores between the first and second scoring showed that if there was not agreement,

they usually only differed by one score (Table 8).

Table 6 Interjudge Cohen's kappa by scale score for first scoring. The interjudge kappa by score shows high reliability (0.60+) for scores, except for a score of 3 which is more variable and has a lower average Cohen's kappa value. There were no scores of 5 or 6 observed.

Judge	Scale score						
Pair	1	2	3	4	5	6	7
1-2	0.94	0.77	0.57	0.58	-	-	0.73
1-3	0.90	0.64	0.46	0.63	-	-	0.81
1-4	0.83	0.67	0.46	0.75	-	-	0.81
1-5	0.86	0.70	0.58	0.70	-	-	0.75
2-3	0.84	0.64	0.49	0.60	-	-	0.73
2-4	0.88	0.62	0.31	0.71	-	-	0.73
2-5	0.85	0.59	0.44	0.66	-	-	0.79
3-4	0.84	0.72	0.49	0.65	-	-	0.86
3-5	0.81	0.69	0.68	0.75	-	-	0.94
4-5	0.85	0.72	0.52	0.65	-	-	0.81
average	0.86	0.68	0.50	0.67	-	-	0.80

Table 7 Interjudge Cohen's kappa by scale score for second scoring. The interjudge Cohen's kappa by score shows high reliability (0.60+) for scores, except for a score of 3 and 4 which are more variable and have lower average Cohen's kappa value. This indicates that the training may not be retained over two weeks and is important for scoring 3's and 4's. There were no scores of 5 or 6 observed.

Judge	Scale score						
Pair	1	2	3	4	5	6	7
1-2	0.77	0.61	0.45	0.52	-	-	0.81
1-3	0.92	0.65	0.33	0.58	-	-	0.89
1-4	0.82	0.62	0.40	0.68	-	-	0.88
1-5	0.77	0.64	0.75	0.71	-	-	0.81
2-3	0.79	0.49	0.30	0.53	-	-	0.81
2-4	0.78	0.56	0.38	0.56	-	-	0.69
2-5	0.72	0.48	0.49	0.55	-	-	0.63
3-4	0.89	0.77	0.75	0.84	-	-	0.88
3-5	0.84	0.63	0.37	0.45	-	-	0.68
4-5	0.89	0.65	0.35	0.47	-	-	0.78
Average	0.82	0.61	0.46	0.59	-	-	0.78

Table 8 Distribution of first and second grading scores for all raters collectively. The table shows the frequency of scores at the first scoring and two weeks later at the second scoring. No scores of 5 or 6 were observed.

first grading score

		1	2	3	4	5	6	7	Total	% of total	% agree
second grading score	1	**75**	4						79	29.3	94.9
	2	9	**68**	1				1	79	29.3	86.1
	3	1	3	**29**	4				37	13.7	78.4
	4		1	3	**36**			5	45	16.7	80.0
	5					**0**			0	0	0
	6						**0**		0	0	0
	7			1	1			**28**	30	11.1	93.3
	Total	85	76	34	41	0	0	34	270		
	% of total	31.5	28.1	12.6	15.2	0	0	12.6			
	% agree	88.2	89.5	85.3	87.8	0	0	82.35			

There was no significant difference in Cohen's kappa for videos captured at 30 vs. 60 frames per second (p=0.16). When comparisons were made by score, there were no statistically significant differences in frame rate for scores 1, 2 and 4, however, there was a statistically significant difference for scores 3 and 7 (p<0.001, Fig. 3). For a score of 3, the videos captured at 60 frames per second had a significantly lower reliability than those captured at 30 frames per second (0.30 and 0.56 respectively). For a score of 7 the reliability of videos captured at 60 frames per second was significantly higher than at 30 frames per second (1.00 and 0.693 respectively).
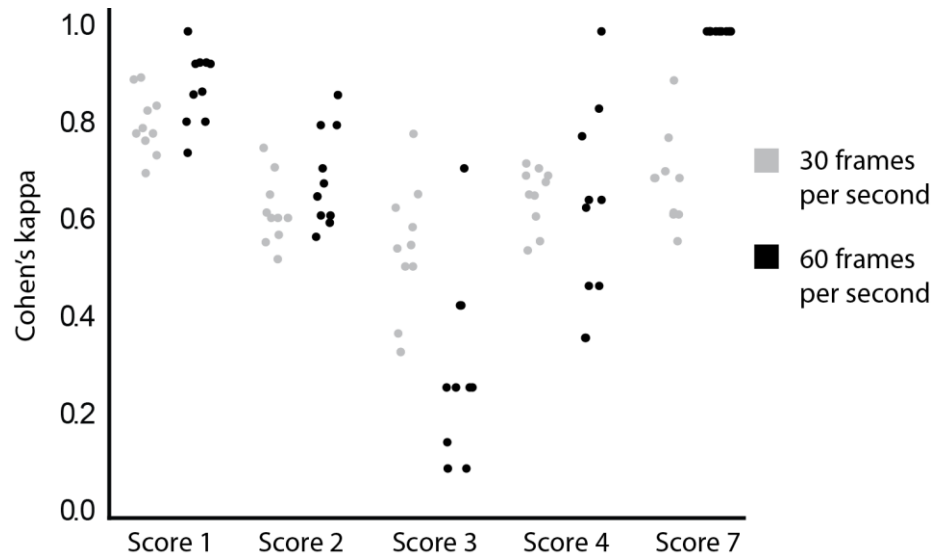
Figure 3 Distribution of intraclass correlation coefficients (ICCs) for videos captured at 30 and 60 frames per second by 7-point infant mammalian penetration-aspiration scale score The figure shows the distribution of ICCs, by score and by video capture rate. There is no significant difference in ICC between videos captured at 30 and 60 frames per second for scores 1, 2 and 4. There is a significant difference for scores 3 and 7 (p<0.001).

Validity of the Scale

When the scale was used to score 39 swallows from intact animals and 39 swallows from abnormal animals, there was a clear difference in the distribution of scores (Fig.4). In intact pigs, 61.6% scored a 1, 33.3% scored a 2, 5.1% scored a 3 and none scored 4's or 7's. Again, there were no scores of 5 or 6. This was a stark contrast to the abnormal pigs where 46.2% scored a 1, 2.6% scored a 2, 7.7% scored a 3, 2.6% scored a 4 and 41.0% scored a 7. In addition, a two sample t-test determined that the normal and abnormal pigs were significantly different (t= -4.89, p<0.001). The mean for normal pigs was 1.42 with a standard deviation of 0.60 and the mean for abnormal pigs was 3.72 with a standard deviation of 2.86.
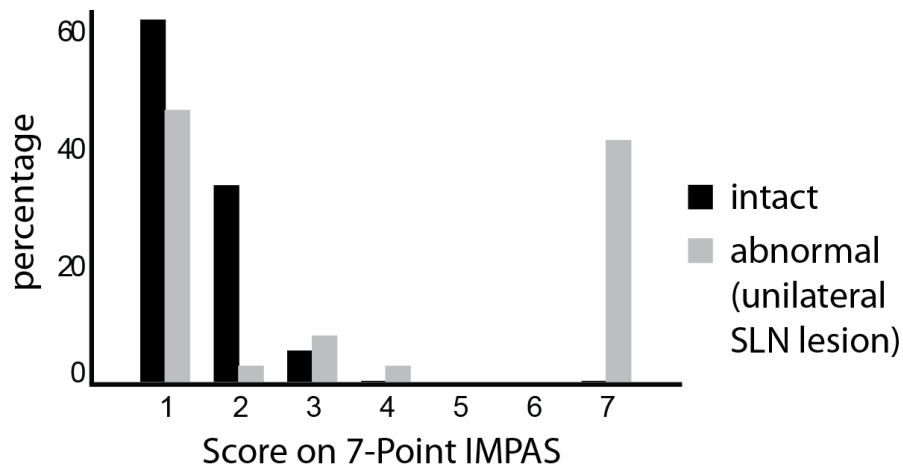
Figure 4 Distribution of scores given to control animals and animals with a unilateral superior laryngeal nerve lesion The graph shows a clear distinction between scores given to normal and abnormal animals.

DISCUSSION

The IMPAS will allow researchers to use the infant pig model and other infant mammalian models for assessing the pathophysiology of swallowing dysfunction and outcomes of rehabilitation. The Cohen's kappa result was interpreted as follows: 0-0.20= slight, 0.20-0.40= fair, 0.40-0.60= moderate, 0.60-0.80= substantial, 0.80-1.00= almost perfect strength of agreement (Landis and Koch, 1977). The inter-rater reliability assessment demonstrated substantial strength of agreement and the intra-rater reliability demonstrated "almost perfect strength" of agreement(Landis and Koch, 1977). The ICC for intra and inter-rater reliability were also very high (0.92 and 0.88 respectively). The inter-rater reliability by score demonstrated that scores 3 and 4 were harder to score reliably and that reliability decreased after the two week break. This underscores the importance of training of judges, especially when multiple judges are used. Whenever the scale is used, inter- and intra-rater reliability should be calculated and assessed to determine functional significance of results.

A significant difference was found in reliability of the IMPAS for videos captured at 30 vs. 60 frames per second only for scores 3 and 7. For scores of 7, although the difference was statistically significant, it was most likely not functionally significant since the reliability was still high (0.60+). For a score of 3, there was lower reliability for videos at 60 frames per second which was not expected since these videos can capture behaviors with a higher time resolution. A score of 3 may appear differently depending on the capture rate or resolution of the video. While clinical videofluoroscopic swallowing studies utilize 30 frames per second recording, animal studies are able to take advantage of the higher 60 frames per second setting that is an option on most videofluoroscopic units. This suggests that the score of 3 needs to be defined clearly to raters by having a detailed description of the size of the bolus and having examples.

The data also showed that this scale can be used to distinguish normal swallowing from abnormal swallowing. The distribution of scores for normal swallows indicates scores of both 1 and 2 are seen and do not indicate a pathological condition. A score of 2 occurred during approximately 1/3 of all swallows. Very rarely a score of 3 occurred. Although a score of 2 is penetration, it is often seen in normal pigs and may be a result of their developing coordination between sucking, swallowing and breathing although it is not observed in normal infant feeding (Newman et al., 1991). Both infant humans and pigs feed with an upright posture, so posture, or gravity, should not affect the rate of penetration. The rate of scores of 2 in the normal, healthy infant pigs is a notable difference between infant human and pig swallows. The rate of scores of 2 in infant pigs is actually more comparable to adult humans who have scores of 2 normally in about 20% of swallows and does not indicate abnormal swallowing (Robbins et al., 1999). In

the experience of the authors, a 4 and 7 also may occur in a normal, healthy animal, but these are extremely rare conditions. The distribution of scores for infant pigs with a unilateral SLN lesion shows more frequent scores of 4 and many scores of 7 indicating silent aspiration. This difference was statistically significant ($p<0.001$) when tested using a two-way t-test. As the infant pig model is used to model different causes of swallowing dysfunction, this scale can be used to describe the extent of airway protection.

We found that it is important for all judges to first review the data and discuss it in a group before doing any scoring in order to maximize the reliability of the results. After the judges scored the videos and the inter- and intra-rater reliability scores were low, we had further training based on that data in order to achieve high inter- and intra-rater reliability. After concluding the assessment of inter- and intra-rater reliability, a schematic was developed to help train judges that use this scale in the future (Fig. 5). Because the IMPAS is a multidimensional scale, it may help judges to score the videos.
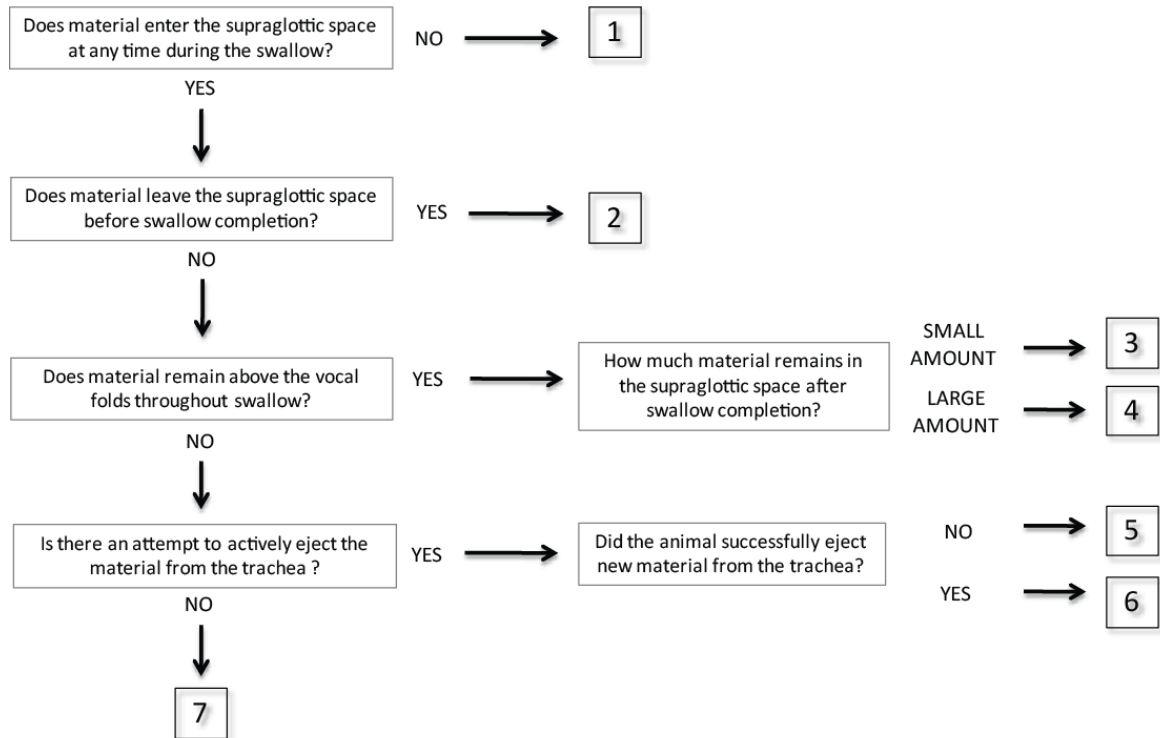
Figure 5 Schematic representation of the 7-Point Infant Mammal Penetration-Aspiration Scale This schematic can be used by the judges as a systematic way to approach scoring the videos.

It is important to note that this scale does not quantify all possible events during swallowing, rather it groups them into categories based on extent of airway protection. Instead of grouping swallows as a) normal, b) having penetration or c) having aspiration, the seven categories described allow for a more precise description of the swallow observed. While this study focused on using videofluoroscopy to assess swallow function, it should be emphasized that videofluoroscopy should be used along with other methods. Videofluoroscopy is essential for visualizing penetration and aspiration, however, ultrasound and electromyography are other important tools that can allow researchers to understand the mechanisms of swallowing dysfunction in animal models (Konow et al., 2010; Kuo et al., 2012; Macrae et al., 2012). Endoscopy, a valuable

research tool in humans, is not possible in many animal models because of the extensive nasal conchae.

There are many opportunities for swallowing research to advance by using the 8-Point PAS for the infant pig model. This scale could be adapted for other infant mammal models such as cats, rabbits and monkeys that are already being used to study feeding and swallowing (Iriarte-Diaz et al., 2011; Medda et al., 1997; Tsujimura et al., 2011). Mammals, both infants and adults, share a common pharyngeal anatomy with an intranasal larynx and a soft palate and epiglottis that contact (Hiiemae, 2000). Despite differences in chewing and oral transport, it is expected that the same scores described in the IMPAS would also be seen in other mammals since they share a common pharyngeal anatomy. Using this scale, new studies can be designed to further understand swallowing neurophysiology by using a pathological animal model. Along with other advanced technology we can further understand what causes penetration and aspiration.

CHAPTER 3: DURATION OF ACTION FOR BUPIVACAINE USED FOR PERIPHERAL SENSORY NERVE BLOCK TO THE GREATER PALATINE AND NASOPALATINE NERVES IN INFANT PIGS[2]

ABSTRACT

Bupivacaine hydrochloride is frequently used in veterinary dental procedures to reduce the amount of general anesthesia needed and to reduce post-procedural pain. We aimed to develop a novel method to test local anesthetic duration in mammals. Six infant pigs (2-3 weeks old) were placed under deep/surgical anesthesia with 3% isoflurane and oxygen while we injected 0.5 mL of 0.5% bupivacaine hydrochloride to each of the three nerves. They were then maintained under light anesthesia with 0.5-1.0% Isoflurane. Starting 15 minutes post-injection, seven sites in the oral cavity were stimulated using a pointed dental waxing instrument, including three sites on the hard palate. The response, or lack of response, to the stimulus was recorded in video and in written record. The bupivacaine lasted between 1-3 hours before the animals responded to the sensory stimulation with a reflexive movement. This study provides evidence that bupivacaine used to anesthetize the hard palate has a relatively short and variable duration of action far below what is expected based on its pharmacokinetic properties.

INTRODUCTION

Bupivacaine hydrochloride (Marcaine[TM], Hospira, Inc., Lake Forest, IL) is a long-lasting amide-type local anesthetic commonly used for peripheral sensory nerve blocks to provide anesthesia and analgesia during dental procedures in animals (Greene, 2002). Although the procedures are performed under general anesthesia, the use of a local

[2] Holman SD**,** Campbell-Malone R, Ding P, Gierbollini-Norat EM, Lukasik SL, German RZ: Duration of action of bupivacaine hydrochloride used for peripheral sensory nerve block to the greater palatine and nasopalatine nerves in infant pigs. (Journal of Veterinary Dentistry- in press)

anesthetic can help reduce the amount of general anesthesia needed and reduce post-procedural pain (Jonnavithula et al., 2010; Obaya et al., 2010). Local anesthetics are also used during head and neck surgeries in humans to reduce post-operative pain (Bateman et al., 2006; Jonnavithula et al., 2007; Jonnavithula et al., 2010; Nicodemus et al., 1991; Obaya et al., 2010; Prabhu et al., 1999). Although bupivacaine hydrochloride is typically used, it is not known how long it is effective since it is difficult to evaluate when sensation returns in an animal.

The pharmacological properties of bupivacaine hydrochloride describe its half-life of 2.7 hours in adults and 8.1 hours in neonates (Marcaine[TM] [Package Insert] Lake Forest, IL: Hospira, Inc., 2011). In infants, protein binding and clearance of bupivacaine is already reduced due to its immature liver development (Mazoit, 2006). In dentistry, bupivacaine administered as a nerve block in adult humans has a duration of action of 6-8 hours, however 5-7 as a local infiltration (Gordon et al., 2010). We hypothesize that in mammalian infants it would last longer based on its pharmacokinetic properties.

We aimed to develop a novel method of testing duration of local anesthesia by testing oral reflexes following palatal local anesthesia injection. Using an infant pig (*Sus scrofa*) model, we aim to determine the duration of action of a peripheral sensory nerve block using 0.5 mL of 0.5% bupivacaine to block the greater palatine nerves and nasopalatine nerve to achieve anesthesia of the hard palate while simultaneously administering isoflurane, a general anesthetic.

METHODS

This study included six infant pigs, *Sus scrofa*, 2-3 weeks old weighing 3.0 to 5.5 kg. At this age the animals are comparable to human infants 6 months to 1 year-of-age as judged by tooth eruption, weaning status, and skeletal development (Book and Bustad, 1074; Weaver et al., 1969). This experimental design was adapted from Krug and Losey, 2011 (Krug and Losey, 2011) who tested the area of skin desensitization following a mental nerve block in dogs. All procedures were approved by JHU IACUC (SW10M212).

We induced general anesthesia and maintained stage III, plane III, or a deep/surgical anesthesia, with 3% isoflurane and oxygen administered through a mask. The pigs were intubated and continued to receive a lower dose of isoflurane (0.5-1.2%) until in stage III, plane I, or a lighter stage of anesthesia characterized by occasional movements of the legs, jaw and tongue. Occasionally a blink reflex or swallow reflex was also observed.

The protocol started with sensory stimulation testing in the control state, prior to placement of the nerve blocks. We performed sensory stimulation testing by using a pointed dental waxing instrument (3 P.K. Thomas Waxing Instrument, Hu-Friedy, Chicago, IL) to stimulate seven different regions in the oral cavity, including three on the hard palate: (1) gingiva labial to the maxillary central incisors (CI) at the muco-gingival junction (MGJ), (2) gingiva buccal to the maxillary molar at the MGJ, (3) gingiva labial to the mandibular CIs (4) gingiva buccal to the mandibular molar at the MGJ (5) gingiva palatal to the maxillary CIs in the region of the incisive papilla on the hard palate, (6) gingiva palatal to the maxillary right molar on the hard palate and (7) gingiva palatal to

the maxillary left molar on the palate (Fig. 6). Each location was tapped up to five times until a clear response was observed. The stimulation testing was also recorded on video so that the responses could be reviewed and verified.

During control sensory stimulation testing, a reflexive movement in response to stimulation was observed at the majority of these locations. A positive response was defined as a twitch in a nearby muscle, movement of the head and legs, or a reflexive movement of the jaw (Thexton, 1973; 1974). If no stimulation was observed, the concentration of inspired isoflurane was lowered until reflexive movements were seen in the majority of the locations. If the pigs moved significantly, the concentration of inspired isoflurane was increased. Vital signs including respiratory rate, temperature, heart rate, oxygen saturation and electrocardiogram (ECG) were recorded regularly throughout the procedure. We adjusted the concentration of isoflurane to maintain constant heart rate and respiratory rate throughout the study.
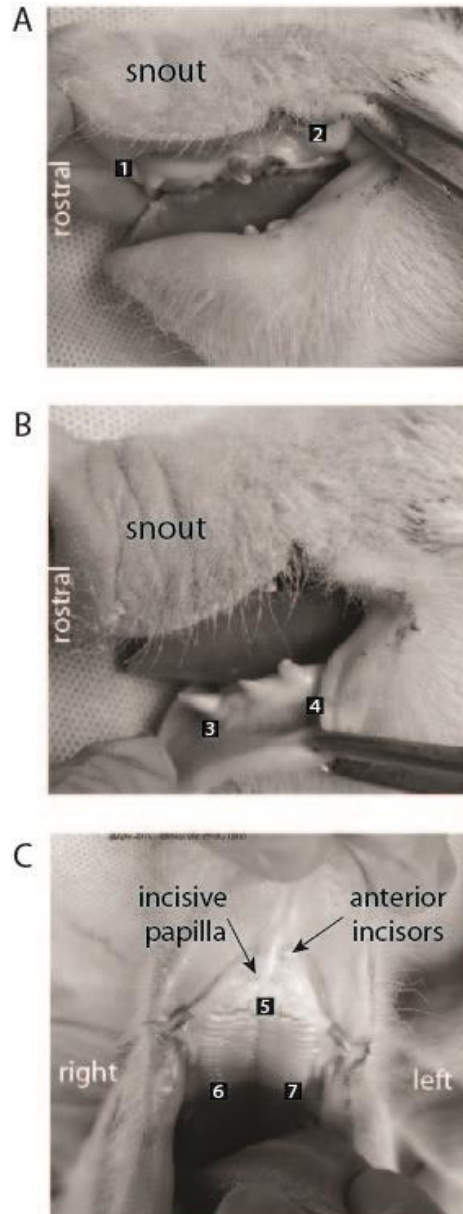
Figure 6 The seven locations stimulated for the sensory stimulation testing. Using a pointed dental waxing instrument (3 P.K. Thomas Waxing Instrument, Hu-Friedy, Chicago, IL), seven different regions in the oral cavity were stimulated: (1) gingiva labial to the maxillary central incisors (CIs) at the muco-gingival junction (MGJ), (2) gingiva buccal to the maxillary molar at the MGJ, (3) gingiva labial to the mandibular CIs (4) gingiva buccal to the mandibular molar at the MGJ (5) gingiva palatal to the maxillary CIs in the region of the incisive papilla on the hard palate, (6) gingiva palatal to the maxillary right molar on the hard palate and (7) gingiva palatal to the maxillary left molar on the palate.

After the control sensory stimulation testing, the inspiratory concentration of

isoflurane was increased to 2-3.5% to return the animal to the stage III, plane III, or a

deep/surgical anesthesia, and the sensory nerve blocks were administered. The nasopalatine foramen, where the nasopalatine nerve exits the maxilla, is located just deep to the incisive papilla on the hard palate, palatal to the central incisors. The greater palatine foramina, where the greater palatine nerves exit the maxilla, are located approximately 1 cm palatal to the first molars. At the nasopalatine foramen and each greater palatine foramen, 0.5 mL of 0.5% bupivacaine hydrochloride (5mg/ml, Hospira, Inc. Lake Forest, IL) was injected slowly using a 25g needle. For consistency, the same investigator (SDH) administered all regional blocks. The maximum dose recommended for infiltration was 2-4 mg/kg (personal communication. Eric Hutchinson, DVM). The total dose of bupivacaine was the same for each animal, 7.5 mg. After the injections were completed, the animal was returned to stage III, plane I, or a lighter stage of anesthesia, for the sensory stimulation testing.

Starting 15 minutes post-injection, the investigator (SDH) started the sensory stimulation testing and the location of the reflexive responses were noted. This was done every 10-30 minutes until a response was seen from any of the three regions of the palate. The amount of time between sensory testing was maximized to prevent over-stimulation of the site. Once a response was observed, testing was then performed every 10-20 minutes until a reflexive movement was seen in response to the palatal stimuli in at least two of the three locations for three consecutive sensory stimulation tests. At this point, the sensory nerve block was determined to be ineffective and the animal was allowed to recover. Three pigs underwent the same procedure twice, at least three days apart.

Two animals in this study were also used for a feeding study. The local anesthetic testing described was performed twice each for pigs A and B- once before and once after

the feeding study, six days apart. As part of the feeding study, we surgically implanted electromyographic (EMG) electrodes in several hyolaryngeal muscles, and radio-opaque markers on the hyoid bone, hard palate and thyroid cartilage as well as in the tongue, and maxillary gingiva. A Weck Hemoclip Ligating Clip (Pilling Weck, Research Triangle Park, NC) was placed onto the epiglottis by an intra-oral approach. After this surgery the animals were given ampicillin (0.16 mL of 250 mg/mL) and buprenorphine (0.17 mL of 0.3 mg/mL) twice daily and metacam (0.1 mL of 5 mg/mL) once daily.

For Pig B's first sensory stimulation testing, the pig could not be intubated after repeated attempts by both a veterinary technician and veterinarian, so intravenous injections of propofol were given for sedation as recommended by the veterinarian. The pig was given 1 milliliter (10 mg), half the dose usually recommended to maintain light anesthesia, every 5-10 minutes. When the pig started moving more frequently and to a larger extent, the next dosage was administered. Sensory stimulation testing was always performed 5 minutes after the previous injection of propofol.

Pigs C, D, E and F were also used for a feeding study, and the duration of action of the nerve blocks was tested before that study commenced. Prior to testing the duration of local anesthetic action, a short (10 minute maximum) surgery was performed to suture radio-opaque markers to the hyoid bone and thyroid cartilage. A Weck Hemoclip Ligating Clip was placed onto the arytenoid cartilages by an intra-oral approach. In Pigs C and F, additional radio-opaque markers were inserted intraorally into the maxillary gingiva, hard palate, soft palate, posterior pharyngeal wall and tongue. After these markers were placed the animals were given ampicillin (0.16 mL of 250 mg/mL) and

buprenorphine (0.17 mL of 0.3 mg/mL) twice daily and metacam (0.1 mL of 5 mg/mL) once daily. Pig C underwent sensory stimulation testing twice, four days apart.

RESULTS

Bupivacaine's duration of action in the infant pigs ranged from 65-190 minutes, or approximately 1-3 hours (Table 1, Fig. 7). This is based on the time until the first response from any region of the hard palate. The average time of the last sensory stimulation test where there was no response in the hard palate was 94 minutes after bupivacaine was administered (standard deviation: 39 mins, range: 45-150 mins). The first response to the sensory stimulation test was seen on average 119 mins after bupivacaine injection (standard deviation: 39 mins, range: 65-190 mins). A consistent response in at least two of the three regions of the hard palate tested was seen on average 167 mins after administering bupivacaine (standard deviation: 64 mins, range: 115-310 mins). The bupivacaine consistently lasted at least 1 hour in all pigs. Individual variation was clear, as well as variation between trials in the two animals with replicates. In a few instances response to sensory stimulation testing could be elicited from only one location, and then during the next test there would be no response at the same location. There was always a response from the site buccal to the maxillary molar at the MGJ.

Table 9 Duration of action for bupivacaine blocks of the nasopalatine and greater palatine nerves by animal. The first column is the last time where no response was seen in any of three palatal regions tested following sensory stimulation test. The second column is the time of the first sensory stimulation test where at least one region responded to the test. The third column is the time when the response to the sensory stimulation test showed a response in two or more of the three palatal regions and was repeatable for three tests in a row. The last four rows summarize the central tendency of the results by giving the average, standard deviation and range.

	last test with no response (minutes)	first test with response (minutes)	consistent response (minutes)
Pig A- 1	100	130	310
Pig A- 2	75	125	145
Pig B- 1	135	150	150
Pig B- 2	170	190	190
Pig C- 1	95	115	115
Pig C- 2	60	80	120
Pig D	45	65	120
Pig E	65	85	220
Pig F	105	135	135
AVERAGE	**94**	**119**	**167**
STD DEV	**39**	**39**	**64**
RANGE	**45-170**	**65-190**	**115-310**
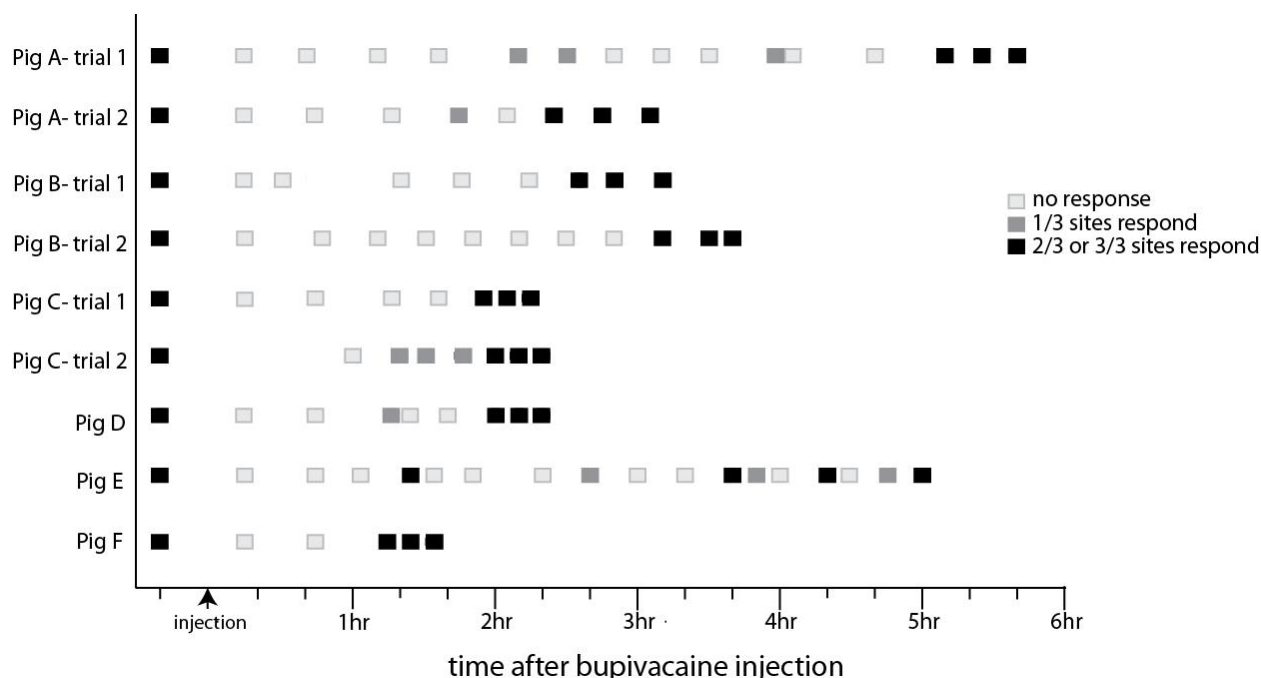
Figure 7 Duration of action for bupivacaine blocks of the nasopalatine and greater palatine nerves. Light grey squares represent sensory stimulation tests where no response was noted on the hard palate in the three locations tested. Dark grey squares represent sensory stimulation tests where one of three locations on the hard palate responded to the test. Black squares represent tests where two or three locations on the hard palate responded to the sensory stimulation.

DISCUSSION

Bupivacaine hydrochloride is a long-lasting local anesthetic drug commonly used in veterinary dental procedures to reduce post-procedure pain (Jonnavithula et al., 2010; Obaya et al., 2010). The duration of action of bupivacaine was determined to be 1-3 hours in the infant pig before oral reflexes returned, which is substantially less than predicted based on its pharmacokinetic properties.

It is most likely that our method of sensory stimulation testing elicited oral reflexes that had low thresholds for excitation. When bupivacaine is administered, it first blocks the C fibers, then Aδ fibers and last Aβ fibers. As it begins to be metabolized, sensation returns in the reverse order. Bupivacaine's duration of action will be different

depending on the measured outcome. It is unclear exactly which types of sensory fibers were stimulated during sensory testing when a reflexive response was observed. One explanation for the relatively short duration of action is the oral reflexes observed were elicited by stimulating mechanoreceptors and not nociceptors. The duration of action may have been longer if were measuring an outcome based on nociceptor stimulation. Alternatively, the short duration of action observed in our study relative to past studies could reflect variation in the metabolism rate of local anesthetics between infant pigs and infant humans, although this is unlikely based on their shared anatomy and development.

Variability in our findings may be explained due to a fluctuating level of inhalational anesthesia or physiological variation across individuals in how they metabolize bupivacaine. For Pig B, the second sensory testing was done with propofol instead of isoflurane which could have influenced the duration of action of bupivacaine. It is not known how general anesthetics can influence local anesthetic metabolism. There were instances during the sensory stimulation testing where a response was positive and then could not be elicited on subsequent testing. In one case, Pig E had a strong response (2 out of 3 sites) to the sensory stimulation test which could not be reproduced on two following tests. In this case the site may have been over-stimulated and the animal stopped responding.

Local anesthetics are a valuable tool for animal researchers. In particular, local anesthetics have been used to understand the functional significance of sensory input from the oral cavity and pharynx during feeding (Huang et al., 1993). In order to use local anesthetics as a research tool, we must understand its pharmacological properties, including its duration of action. Using the methods established in this study, any local

anesthetic can be tested in any mammalian animal model. In the future this will allow local anesthetics to be used in a wide variety of animal studies.

It is not known how the duration of action of bupivacaine hydrochloride in infant pigs will compare to adult pigs or other mammals. This study demonstrates a novel methodology for testing local anesthetic duration in mammals and could be performed in different species of different ages and weights to determine the range of variation. It is also noteworthy that the sensory testing procedure does not result in any visible damage to tissues, such as bleeding or swelling. Using local anesthetics as a research tool is especially useful since they are completely reversible, and testing them using the methods described also result in no visible injury to oral structures.

Our study had an objective, reflexive endpoint to determine the duration of the sensory nerve blocks. The duration of action of bupivacaine in infants reported in this study, as measured by their oral reflexes, should be taken into consideration for veterinary procedures that aim to maximally reduce postoperative pain. Further research is needed to understand how combining general anesthesia or monitored anesthetic care with propofol can increase the duration of a bupivacaine sensory nerve block in the oral cavity. The infant pig model can be used for future studies to answer these clinical questions.

CHAPTER 4: SUCKLING AND SWALLOWING RATES AFTER PALATAL LOCAL ANESTHESIA: AN ELECTROMYOGRAPHIC STUDY IN INFANT PIGS[3]

ABSTRACT

Infant mammalian feeding consists of rhythmic suck cycles and reflexive pharyngeal swallows. Although we know how oropharyngeal sensation influences the initiation and frequency of suck and swallow cycles, the role of palatal sensation is unknown. We implanted EMG electrodes into the mylohyoid muscle (MH), a muscle active during suckling, and the thyrohyoid muscle (TH), a muscle active during swallowing, in eight infant pigs. Pigs were then bottle-fed while simultaneously recording lateral videofluoroscopy and from the electrodes. Two treatments were administered prior to feeding and compared to control feedings: 1) palatal anesthesia (0.5% bupivacaine hydrochloride) and 2) palatal saline. Using the timing of MH and TH activity, we tested for differences between treatment and control feedings for swallowing frequency and suck cycle duration. Following palatal anesthesia, 4 pigs could not suck and exhibited excessive jaw movement. We categorized the 4 pigs that could suck after PLA as Group A and those who could not as Group B. Group A had no significant change in suck cycle duration and a higher swallowing frequency after palatal saline(p=0.021). Group B had significantly longer suck cycles after palatal anesthesia (p<0.001) and a slower swallowing frequency (p<0.001). Swallowing frequency may be a way to predict group membership since it was different in control feedings between groups (p<0.001). The qualitative and bimodal group response to palatal anesthesia may reflect a developmental

---

difference. This study demonstrates that palatal sensation is involved in the initiation and frequency of suck and swallow cycles in infant feeding.

INTRODUCTION

Infant mammalian feeding is a complex sensorimotor behavior with both rhythmic and reflexive components (German et al., 2009; Thexton et al., 2012). Because it is relatively simpler than adult feeding, infant mammalian feeding is a good model for understanding how the sensory and motor aspects of feeding interact (German and Crompton, 2000; German et al., 2004b). The rhythmic component consists of the pure suck cycles that both extract milk from the nipple and transport it through the oropharynx prior to a swallow. The reflexive component is the pharyngeal swallow where the bolus is transported into the esophagus. This reflexive component is inserted into a suck cycle and collectively that cycle is termed a suck-swallow cycle (Thexton et al., 2012). Throughout a feeding session, sucking is an ongoing rhythmic behavior and suck-swallow cycles occur every 1-3 suck cycles early during a feeding session and are less frequent towards the end. Although we know some of the sensory mechanisms that elicit the swallow in adults, including bolus volume, temperature, taste, and carbonation (Butler et al., 2011; Michou et al., 2012; Yamamura et al., 2010), it is unknown how specific sensory information gathered during the oral transport process impacts the onset of the reflexive pharyngeal swallow in infants.

During sucking, sensory information about the bolus is sent to the sucking pattern generator in the brainstem that regulates the motor output necessary to transport the bolus to the oropharynx (Barlow, 2009; Steele and Miller, 2010). Sensory information from the oropharynx synapses in the swallowing pattern generator in the brainstem to initiate the motor function necessary for a pharyngeal swallow (Barlow, 2009; Steele and Miller,

2010). Although oral sensation from the trigeminal nerve projects to the region of the brainstem where the swallowing pattern generator is located, its role in the triggering of the pharyngeal swallow is not clear. During sucking, three regions of the oral cavity, supplied by branches of the trigeminal nerve, are either in contact with the nipple, or deform as a result of the negative pressure: the hard palate, the tongue and the lips. To what extent sensation carried by the trigeminal nerve, from these regions in the oral cavity, is involved in sucking, swallowing and their coordination is unknown.

Taste and mechanical sensation from the oral cavity and oropharynx impact the threshold for stimulating the initiation of an oral transport cycle in adults (Steele and Miller, 2010) and of a suck cycle in infants (German et al., 1997). Based on past studies in infant pigs, the initiation of a suck cycle is dependent on milk being present in the nipple and on the frequency of milk delivery when using an automated feeding system (German et al., 1997). Clinically, oral stimulation helps infants struggling to feed to develop stronger sucking (Finan and Barlow, 1998). Regulation of swallowing frequency is better known. Swallowing frequency relies on sensation from the superior laryngeal nerve (CNX), as well as the glossopharyngeal and facial nerves that is relayed to the brainstem (Steele and Miller, 2010). There is also descending cortical input to the brainstem that regulates swallowing frequency. In infant pigs, the frequency of delivery of a bolus, from an automated feeding system, can change the frequency of swallowing, indicating that swallowing may also be dependent on trigeminal sensation (German et al., 2004a).

The central pattern generator (CPG) for sucking and the CPG for swallowing appear to communicate in infant pigs (Thexton et al., 2012). After a pharyngeal swallow,

there is a set amount of time before the next suck cycle begins that is dependent on the duration of the oral transport cycle (Thexton et al., 2012). In that study, a suck cycle that contained a swallow (suck-swallow cycle) was divided into two predictable phases: phase 1 was from the start of the suck-swallow cycle to the start of the pharyngeal swallow, and phase 2 was from the start of the pharyngeal swallow to the end of the suck-swallow cycle. Suck-swallow cycle length was correlated with phase 2 length, but not phase 1; however, it is not known what role oral sensation plays in maintaining this temporal relationship. Understanding these relationships in the periphery will provide data for the functional significance of central connections.

We evaluated the impact of palatal sensation on sucking and swallowing frequencies in an infant pig model (German et al., 2004b) by anesthetizing the palate and evaluating how that altered or reduced sensation affected a) swallowing frequency, b) suck cycle duration, and c) suck cycles per swallow. Additionally, we included a saline injection treatment, intended as a sham treatment. The study utilized a repeated measures model. We hypothesized that with palatal anesthesia sucking and swallowing frequencies would be reduced because of the reduced sensation. We also evaluated if the suck-swallow cycle length was correlated with the time from the start of the suck-swallow to the pharyngeal swallow (phase 1) or with the time from the pharyngeal swallow to the end of the suck-swallow cycle (phase 2) when the hard palate was anesthetized.

MATERIALS AND METHODS

Animal Model

This study included eight infant pigs obtained from Tom Morris Farms (Reisterstown, MD) that were 2-3 weeks old and weighed 3.0-5.5 kg. This was based on power

calculations from previous studies (Ding et al., 2013a; Ding et al., 2013b; Thexton et al., 2009). At this age the animals were comparable to 6 months-1 year-old human infants as judged by tooth eruption, weaning status, and skeletal development (Book and Bustad, 1974; Weaver et al., 1969). Although pigs are precocious relative to humans, the developmental patterns of feeding infants are remarkably similar across most species of mammals (German and Crompton, 2000). Infant pigs have been used in previous studies of normal feeding and swallowing neurophysiology, and thus a large body of comparable data exists for comparison with the results of this study (Campbell-Malone et al., 2011; Ding et al., 2013a; German et al., 2004b; Thexton et al., 2007; Thexton et al., 2009). The pigs were trained to feed from a bottle for 4-6 days prior to the start of the study. All procedures were approved by the JHU IACUC (SW10M212).

Electromyographic Electrode Implantation

After trained to feed from a bottle, the pigs underwent surgery to implant fine wire bipolar electromyographic (EMG) electrodes into several oropharyngeal and hyolaryngeal muscles (Fig. 1). The EMG electrodes recorded from motor units in the vicinity of that implanted electrode (Loeb and Gans, 1986). Animals were intubated and anesthetized with 2-3% Isoflurane until they were in Stage III, Plane III anesthesia. During surgery, electrodes were implanted into the thyrohyoid muscle (TH).. Additionally, patch EMG electrodes were placed on the ventral surface of the anterior mylohyoid (MH) muscle. Lastly, three 2-mm piezoelectric crystals (Sonometrics, London, ON) were inserted into the midline of the genioglossus muscle (Holman et al., 2012b; Konow et al., 2010)(Fig. 1). All muscles were accessed through a midline incision

and the electrodes exited through a window on the left lateral neck. In previous studies

where the electrodes exited the midline incision, the wires often broke due to the

movement in that region during head flexion. One radio-opaque marker was sutured to

the hyoid bone and another one was sutured to the thyroid cartilage. The same surgeons

performed all surgeries. After surgery, the suture lines were wrapped in VetWrap^{TM} (3M,

St. Paul, Minnesota). A small metal sphere (12.69 mm in diameter) was placed on the

lateral neck under the VetWrap^{TM} which would later be used to correct for absolute size

of the images during videofluoroscopy data analysis. The electrodes were plugged into a

connector that exited the bandage on the back of the animal. The procedures followed for

EMG surgery and analysis are those used in several previous studies (German et al.,

2009; Konow et al., 2010; Thexton et al., 2012). Analyses of kinematics as well as animal

discomfort show that these procedures and instrumentation have minimal, if any, impact

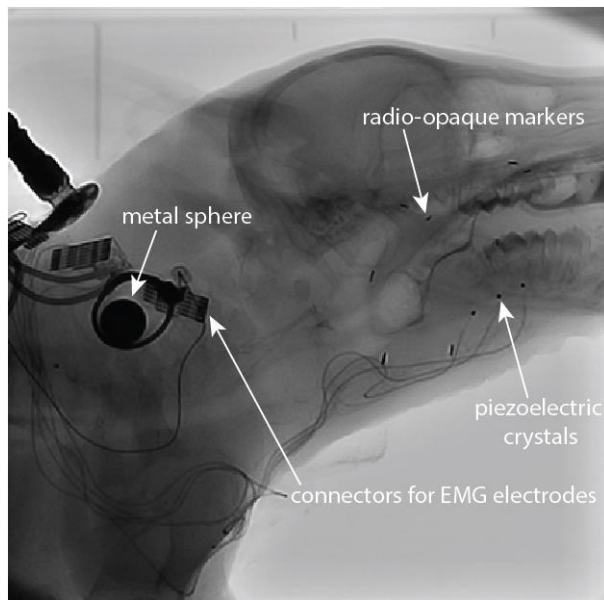on feeding ability in mammals (Dutra et al., 2010; Thexton et al., 2007).

Figure 7 Videofluoroscopy Image. One frame from the 60 frames per second
videofluoroscopy recording showing the infant pig with EMG electrodes and radio-
opaque markers.

After the electrodes were implanted, the animal had several intraoral radio-opaque markers (<0.5 cm long) placed into the tongue and other soft tissue structures (Fig. 7). The markers were inserted in the midline of the anterior hard palate, the soft and hard palate junction, the tongue approximately 10mm deep to the foramen cecum, and the right and left mucogingival junction by the maxillary molar. Previous studies have demonstrated that these markers are not harmful to the animals and that they do not affect their oral motor function (German and Franks, 1991; Thexton, 1981). After the markers were placed, the animals were taken off the general anesthesia and quickly recovered in approximately 20 minutes. The entire surgical procedure took 2-4 hours. Once the animals were standing and vocalizing, following surgery, they were offered milk every hour until they were able to feed normally. They were subsequently fed every four hours. The following medications were administered post-operatively and were continued for the duration of the experiment: ampicillin (0.16 mL of 250 mg/mL) and buprenorphine (0.17 mL of 0.3 mg/mL) twice daily and meloxicam (0.1 mL of 5 mg/mL) once daily. The implantation of radio-opaque markers has been previously shown to not impact feeding kinematics (Thexton et al., 1998b; Thexton et al., 2007).

Treatments

The feeding study began the day after electrodes were implanted and lasted for four days. Each day, the animal was placed under stage III, plane III, or deep/surgical anesthesia for 20 minutes. A laryngoscope was used to visualize the epiglottis and a Weck Hemoclip Ligating Clip (Pilling Weck, Research Triangle Park, NC) was placed in the midline on

the tip of the epiglottis. The epiglottis marker typically falls off after two days; in these cases it was replaced. After this, either no treatment was given (control) or one of two treatments was administered. The two treatments were: (1) saline and (2) anesthesia nerve blocks bilaterally to the greater palatine nerves and to the nasopalatine nerve, for three total injections per animal. Both the nasopalatine and greater palatine nerves are branches of the maxillary branch of the trigeminal nerve (cranial nerve V). The nasopalatine nerve provides sensation to the anterior 1/3 of the hard palate and the two greater palatine nerves provide sensation to the posterior 2/3 of the hard palate. The local anesthetic used was 0.5% bupivacaine hydrochloride (Marcaine[TM], 5mg/ml, Hospira, Inc. Lake Forest, IL). Injections were administered using standard veterinary dental techniques (Reuss-Lamky, 2007). 0.5ml was injected to each site as a nerve block (Jonnavithula et al., 2010; Reuss-Lamky, 2007). Nerve blocks to the hard palate have a high rate of success since there are no alternative spaces for the anesthetic to travel without affecting the sensory nerves and their branches. There are also no muscles in the area, ensuring that only sensory nerves are blocked. The same experimenter administered all injections. The duration of anesthesia with these nerve blocks is from 1-3 hours in anesthetized infant pigs before oral reflexes are observed (Holman et al., 2013). The order of the treatments and control was randomized for each pig using a random number generator. On either the second or third day of the study there was no treatment given. This would be on the day after an injection (anesthesia or saline) is given in order to rule out a lingering effect of treatment. The last day of the study was always a treatment day (anesthesia or saline). After the position of the epiglottis marker was verified, and the

treatment given, if necessary, the pig was removed from the general anesthesia and typically recovered in 5-10 minutes.

Feeding Sessions

Each pig was freely fed milk containing barium from a pig nipple in a plastic feeding box 30-60 minutes post-treatment. During the feeding session we recorded lateral videofluoroscopy at 60 frames per second (Allura FD20, Philips Healthcare, Best, The Netherlands) equipped with a high-resolution digital flat-panel detector (154x154 micron pixel-pitch, 30x40 cm) and electromyography (10 kHz) recorded with a synchronization signal on a Powerlab 30/16 (AD instruments, Colorado Springs, CO). The milk was made using eight ounces of prepared pig milk replacer (Land O Lakes Solustart pig milk replacer, St. Paul, MN) with 1/3 cup of barium powder. The bottle was warmed in a hot water bath for approximately 2 minutes to ensure that each bottle was the same temperature. Temperature and the amount of barium in the bottles was standardized since both taste, texture and temperature have been shown to alter swallowing kinematics (Cichero et al., 2010; Ebihara et al., 2011; Lee et al., 2012). The pig was fed until 20 swallows were recorded or until five 15-second videos were recorded. At the conclusion of the study, the animals were euthanized and the location of the electrodes was verified during a post-mortem dissection conducted by one of the experimenters not involved in electrode placement.

Data Analysis

We analyzed EMG recordings from the mylohyoid and thyrohyoid muscles in order to address our aims. Based on the results of the post-mortem dissection and the quality of

the EMG recordings, two EMG signals (from one of MH and one TH electrode per pig)

were selected for data analysis. The timing of MH and TH EMG activity in each feeding

session was used to identify suck and swallow cycles (Fig. 8). A pharyngeal swallow was

identified by the mid-point of activity in the TH and a suck cycle was identified by the

mid-point activity in the MH (Thexton et al., 2012). The pigs were latching onto and

suckling from the bottle throughout the entire feeding sequence. We were simultaneously

recording lateral videofluoroscopy at 60 frames per second where we could clearly see

the sucking and swallowing cycles. There were two types of suck cycles (Fig. 2): a) the

pure suck cycle and b) the suck-swallow cycle (Thexton et al., 2012). The MH activity

immediately before the TH activity was identified as the start of the suck-swallow cycle.

The next MH activity was the end of the suck-swallow cycle. The MH activity observed

before the suck-swallow defined the start of the pure suck cycle. Midpoint activity was

assigned to the middle of the signal burst of MH and TH, even if the burst was skewed in

any way (Fig. 8). This method represents a departure of the methods used in Thexton et

al. since the feedings in this study were recorded over several days and there quality of

the signal changed over this time period (Thexton et al., 2012). All labeling was done in

LabChart® 7 (AD instruments, Colorado Springs, CO).

Swallow frequency was measured as the inverse of the time between pharyngeal

swallows, or TH activity (Thexton et al., 2012). The number of sucking, or oral transport

cycles per pharyngeal swallow was calculated by counting the number of suck cycles

(MH activity) between the start of a pharyngeal swallow and the start of the next

pharyngeal swallow. The MH activity that was the start of a suck-swallow cycles was not

included since they always occurred with a pharyngeal swallow. We began counting after

the first complete swallow.
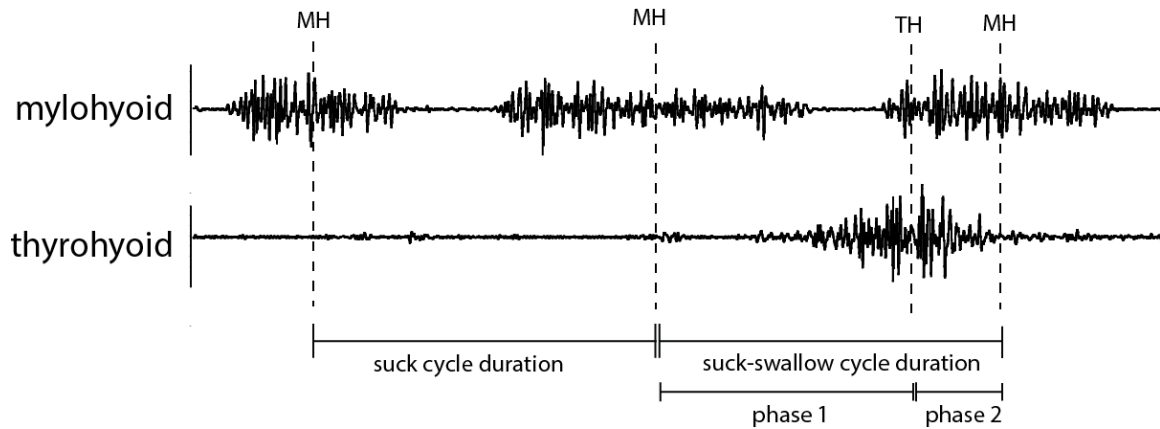
Figure 8 Electromyographic recording of mylohyoid and thyrohyoid with cycles labeled. Electromyographic recordings are shown with labeled midpoints of mylohyoid (MH) and thyrohyoid (TH). Also labeled are the suck cycle duration and suck-swallow cycle durations as well as phase 1 and 2 lengths.

Differences between treatments for each of these dependent variables, were

evaluated using a General Linear Model analysis of variance (ANOVA) with the

independent, repeated measures variable being anesthetic treatment (control, anesthesia,

saline), and individual as a random variable. The unit of analysis is a cycle. The inclusion

of individual as a random factor accounted for the differences within individuals. This

model is less susceptible to extreme outliers than one using averages over individuals as

the unit of analysis (German et al., 2008). A post-hoc Tukey's Honestly Significant

Difference (HSD) test evaluated for specific differences. All statistical analysis was

performed using SYSTAT 13 (Systat Software, Inc., Chicago, IL)

We also tested whether the suck-swallow cycle and phase relationships described

by Thexton et al. (Thexton et al., 2012) in control animals was modified after palatal

saline and palatal anesthesia. They defined a cycle as the suck-swallow cycle described

previously (Fig. 2). They divided the cycle into two phases. Phase 1 was from the start of the suck-swallow cycle to the swallow as defined by the midpoint of TH activity (Fig. 2). Phase 2 was the time from the swallow as defined by TH to the end of the suck-swallow cycle (Fig. 2). Following Thexton et al., we used a linear regression test for relationships between phase duration and cycle length. This was done for each treatment. Thexton et al. found that cycle length was correlated with phase 2 duration. Linear regression determined if the slope of that line was statistically significantly different from zero, or was a flat line (Thexton et al., 2012). If it was not a flat line, then phase length is independent of cycle length, and if it was significantly different from zero, there was a significant relationship between those variables.

Testing Blockade of the Greater Palatine Nerve

In a study of three euthanized infant pigs we tested if the block of the greater palatine nerve also anesthetized the lesser palatine nerve. The goal of a nerve block, often used in dentistry, is to deposit the local anesthetic into the foramen that the sensory nerve exits. The local anesthetic will block the propagation of an action potential to the central nervous system. By depositing the anesthetic at the foramen, before it branches, the operator has effectively blocked all sensory information from that nerves branches to the central nervous system. The lesser palatine nerve is very small relatively to the greater palatine nerve and provides sensation to the anterior soft palate. Immediately following euthanasia (within 20 minutes),we injected a mixture of 0.1 ml india ink with 0.9 ml 0.5% bupivacaine hydrochloride as a nerve block bilaterally to the greater palatine nerves. After injection, the mucosa was lifted from the hard palate using a scalpel to

expose the greater and lesser palatine nerves bilaterally. The ink sticks to the nerve and its distribution was used to assess the distribution of the anesthetic *in vivo*. By confirming that the local anesthetic, with the ink, was able to enter the foramen that the nerve exits, we can confirm that no sensation could be transmitted from that sensory nerve, or its branches on the palate, to the brainstem.

RESULTS

Qualitative Difference between Individuals

With palatal anesthesia, four of the eight pigs could not suck normally. These pigs were still able to collect a bolus in the valleculae, after multiple oral transport cycles, and swallow; however, their method of transport was qualitatively distinct, characterized byvery little surface deformation of the tongue and masticatory-like jaw movements. Sucking is a very well described behavior that is rhythmic with vertical tongue movement that either strips milk out of the nipple or creates pressure changes within the oral cavity. There is very little, if any, rhythmic jaw movement (da Costa et al., 2010; German et al., 1992; German et al., 2004b).

This difference in gross feeding behavior was immediately obvious during the feeding session, and was an all or none occurrence. Normally, infants have little or no jaw movement, latch onto the nipple, and show significant surface deformation. There were three characteristics that defined this behavioral alternative, all of which always appeared in an individual: excessive jaw movement, failure to latch onto the nipple and little surface deformation of the tongue. Since such a distinct, qualitative difference in a normally consistent behavior was observed, the eight pigs were divided into two groups.

Pigs in Group A could suck with normal characteristics after palatal anesthesia. Pigs in Group B showed all three of the alternative features described above during feeding after palatal anesthesia. No individual had only a subset of these characteristics and there were no intermediate forms of these characteristics. This particular behavior did not ever occur with the palatal saline treatment. For the statistical analysis of the dependent variables measured in this study, in addition to having treatment (control, anesthesia, saline) as a fixed factor, group (A or B) was also evaluated for effects. The interaction between treatment and group was also tested. The individual (pig) was tested in the model nested within group to account for the random variation between animals.

Swallow Frequency

There was a difference overall between treatments (control, anesthesia and saline) among the 8 pigs (p<0.001, Table 10A), however, there was no statistical difference in swallowing frequency between Group A and Group B (Table 10B). The specific tests revealed significant differences among all three treatments with p<0.001 for each comparison. The frequency was lowest after palatal anesthesia, followed by control and then palatal saline (Table 10A).

There were also significant interactions between treatment and group. Among Group A there was a higher swallowing frequency in the pigs with palatal saline compared to control (p=0.021, Table 10C). There was no significant difference with palatal anesthesia compared to control or palatal saline. In the Group B, swallow frequency was lower with palatal anesthesia compared to control (p<0.001) and compared to palatal saline (p=0.001, Table 10D). There was no difference between

control and palatal saline. The control feedings were significantly different in Group A and Group B (p<0.001). There was a significant difference between the groups after palatal anesthesia (p<0.001) and palatal saline (p<0.001).

Table 10 Swallowing Frequency (swallows per second). A) Mean, range and standard deviation for swallowing frequency by treatment (control, palatal anesthesia, palatal saline), B) Mean, range and standard deviation for swallowing frequency by group, C) Mean, range and standard deviation for swallowing frequency in Group A by treatment, D) Sample size, mean, range and standard deviation for swallowing frequency in Group B by treatment. Groups with different letters next to the mean and standard deviation are statistically significantly different from each other (p<0.05). Significant differences across tables are not shown.

A

Swallow frequency (swallows/sec) by treatment			
treatment	n	mean±SD	range
Control	150	1.17 ± 0.94^a	0.33-6.54
Palatal Anesthesia	149	2.71 ± 3.40^b	0.26-26.52
Palatal Saline	151	0.91 ± 0.59^c	0.23-3.96

B

Swallow frequency (swallows/sec) by group			
treatment	n	mean±SD	range
Group A	226	1.23±0.80	0.41-6.54
Group B	224	1.96±2.98	0.23-26.52

C

Group A Swallow frequency (swallows/sec)			
treatment	n	mean±SD	range
Control	75	0.69 ± 0.93^d	0.15-2.25
Palatal Anesthesia	76	0.82±1.45	0.32-2.46
Palatal Saline	75	0.98 ± 2.38^e	0.48-2.35

D

Group B Swallow frequency (swallows/sec)			
treatment	n	mean±SD	range
Control	75	1.13 ± 1.52^f	0.27-3.08
Palatal Anesthesia	73	0.26 ± 0.23^g	0.04-3.79
Palatal Saline	76	1.26 ± 1.41^f	0.25-4.29

Suck Cycle and Suck-swallow Cycle Durations

For suck cycle duration, there was a significant difference overall between treatments (p<0.001, Table 11A). There was no significant difference between groups (Table 11B). After palatal anesthesia, suck cycle duration was longer compared to control (p<0.001). After palatal saline, suck cycle duration was longer compared to control (p=0.050). Suck cycles after palatal anesthesia were longer than those after palatal saline with p<0.001.

There was also a significant interaction between group and treatment (p<0.001, (Table 11C&D). There were no significant differences between treatments in Group A (Table 11C). There was significantly longer suck cycles in Group B after palatal anesthesia compared to control (p<0.001) and to palatal saline (p<0.001, Table 11D). There was no significant difference between control and palatal saline. There was also no difference between control suck cycle durations in the 2 groups. There was a significant difference between the reaction to palatal anesthesia in Group A and Group B with longer cycles in Group B (p<0.001). Likewise, there was a significantly longer cycle duration in response to palatal saline in Group A compared to Group B (p=0.001).

For suck-swallow cycle duration, overall there was a significant difference between treatments (p<0.001, Table 11E) and there was no significant difference between groups (p=0.281, Table 11E&F). There was no significant difference between palatal saline and control (p=0.104), however cycles were longer with palatal anesthesia relative to both control (p<0.001) and palatal saline (p<0.001).

There was a significant interaction between treatment and group (p<0.001, Table 11G&H). There were no significant differences in suck-swallow cycle duration between treatments in Group A. In Group B the suck-swallow cycles were longer with palatal

anesthesia relative to control (p<0.001). There was also a significant difference between

palatal saline and palatal anesthesia cycle durations (p<0.001). There was no difference

between control and palatal saline cycle durations. There was no significant difference in

cycle durations during control feedings between Group A and Group B. There was,

however, a significant difference between cycle durations after palatal anesthesia

between Group A and Group B (p<0.001) and palatal saline (p=0.012).

Table 11 Suck cycle durations (seconds). A) Mean, range and standard deviation for
suck cycle duration by treatment (control, palatal anesthesia, palatal saline), B) Mean,
range and standard deviation for suck cycle duration by group, C) Suck cycle duration
mean, range and standard deviation for Group A by treatment , D) Suck cycle duration
sample size, mean, range and standard deviation for Group B by treatment, E) Mean,
range and standard deviation for suck-swallow cycle duration by treatment, F) Mean,
range and standard deviation for suck-swallow cycle duration by group, G) Suck-swallow
cycle duration for Group A by treatment, H) Suck-swallow cycle duration for Group B by
treatment. Groups with different letters next to the mean and standard deviation are
statistically significantly different from each other (p<0.05). Significant differences
across tables are not shown.

A

Suck cycle duration (seconds) by treatment			
treatment	n	mean±SD	range
Control	160	0.24±0.05[A]	0.15-0.40
Palatal Anesthesia	156	0.29±0.11[B]	0.19-0.86
Palatal Saline	160	0.25±0.05[C]	0.18-0.47

B

Suck cycle duration (seconds) by group			
treatment	n	mean±SD	range
Group A	240	0.26±0.05	0.16-0.47
Group B	236	0.26±0.09	0.15-0.86

C

Group A suck cycle duration (seconds)			
treatment	n	mean±SD	range
Control	80	0.24±0.04	0.16-0.38
Palatal Anesthesia	80	0.26±0.04	0.19-0.42
Palatal Saline	80	0.26±0.06	0.19-0.47

D	Group B suck cycle duration (seconds)			
	treatment	**n**	**mean±SD**	**range**
	Control	80	0.23±0.05^D	0.15-0.40
	Palatal Anesthesia	76	0.36±0.14^E	0.19-0.86
	Palatal Saline	80	0.23±0.03^D	0.18-0.33

E	Suck-swallow cycle duration (seconds) by treatment			
	treatment	**n**	**mean±SD**	**range**
	Control	160	0.24±0.04^A	0.15-0.36
	Palatal Anesthesia	159	0.29±0.10^{A,B}	0.18-0.98
	Palatal Saline	160	0.25±0.05^B	0.17-0.52

F	Suck-swallow cycle duration (seconds) by group			
	treatment	**n**	**mean±SD**	**range**
	Group A	240	0.25±0.05	0.17-0.52
	Group B	239	0.26±0.09	0.15-0.98

G	Group A suck-swallow cycle duration (seconds)			
	treatment	**n**	**mean±SD**	**range**
	Control	80	0.24±0.04	0.17-0.36
	Palatal Anesthesia	80	0.25±0.03^E	0.18-0.34
	Palatal Saline	80	0.26±0.06^F	0.17-0.52

H	Group B suck-swallow cycle duration (seconds)			
	treatment	**n**	**mean±SD**	**range**
	Control	80	0.23±0.05^C	0.15-0.36
	Palatal Anesthesia	79	0.32±0.14^{C,D,E}	0.19-0.98
	Palatal Saline	80	0.23±0.03^{D,F}	0.19-0.33

Oral Transport Cycles per Swallow

Overall there was a significant difference in the number of oral transport cycles between all of the factors tested. There was also a difference overall between treatments ($p<0.001$, Table 12A). After palatal anesthesia there were more cycles per swallow

relative to control (p<0.001) and palatal saline (p<0.001). There were also more

following control relative to palatal saline (p=0.022). Overall there was a significant

difference between groups (p<0.001) with Group B having more oral transport cycles per

swallow (Table 12B).

Table 12 Oral transport cycles per swallow. A) Mean, range and standard deviation for oral transport cycles per swallow by treatment, B) Mean, range and standard deviation for oral transport cycles per swallow by group A) Sample size, mean, range, standard deviation for calculations of oral transport cycles per swallow in Group A by treatment (control, palatal anesthesia, palatal saline). B) Sample size, mean, range, standard deviation for calculations of oral transport cycles per swallow in Group B by treatment. Groups with different letters next to the mean and standard deviation are statistically significantly different from each other (p<0.05). Significant differences across tables are not shown.

A

Oral transport cycles per swallow by treatment			
treatment	n	mean±SD	range
Control	160	4.21±3.75^A	1-21
Palatal Anesthesia	160	8.56±10.10^B	0-63
Palatal Saline	160	2.79±2.44^C	0-16

B

Oral transport cycles per swallow by group			
treatment	n	mean±SD	range
Group A	240	4.03±2.98^D	1-21
Group B	240	6.35±9.04^E	0-63

C

Group A Oral transport cycles per swallow			
treatment	n	mean±SD	range
Control	80	5.1±3.9	1-21
Palatal Anesthesia	80	3.8±2.5	1-12
Palatal Saline	80	3.2±1.8	1-7

D

Group B Oral transport cycles per swallow			
treatment	n	mean±SD	range
Control	80	3.3±3.3^F	1-18
Palatal Anesthesia	80	13.4±12.4^G	0-63
Palatal Saline	80	2.4±2.9^F	0-16

There was also a significant difference among the interactions between group and treatment (p<0.001, Table 12C&D.) In Group A there was no difference in number of oral transport cycles per pharyngeal swallow among the treatments. In the Group B, after palatal anesthesia there were more oral transport cycles per swallow compared to both control (p<0.001) and palatal saline (p<0.001). This difference was extreme with a wide range of values for the pigs with palatal anesthesia, often with over 20 transport cycles. There was no difference between palatal saline and control. There was no difference in sucks per swallow between Group A and Group B's response to control or palatal saline. There was a significant difference between Groups A and B after palatal anesthesia with Group B pigs having more oral transport cycles per swallow.

Cycle and Phase Relationships

For phase I, there was no significant relationship between phase length and cycle duration (Fig. 3). In Group A the time between the suck-swallow cycles (cycle duration) was not correlated phase 1 length for control (r^2=0.025, p=0.159, Fig. 9A), palatal anesthesia (r^2=0.037, p=0.086, Fig. 9C) or palatal saline (r^2=0.012, p=0.330, Fig. 9E). For Group B, no correlation was seen for control (r^2=0.007, p=0.457, Fig. 9A), but with palatal anesthesia (r^2=0.054, p=0.039, Fig. 9C) and palatal saline (r^2=0.059, p=0.029, Fig. 9E) there was a minimal correlation that was significantly different from zero. The Group B with palatal anesthesia had a wider range of durations for cycle length and a couple of outliers showing extremely large phase 1 lengths.

For phase 2, there were significant relationships for all treatments in both groups between phase length and cycle duration. For Group A there was a statistically

significantly relationship between cycle duration and phase 2 length that was different from zero for control (r^2=0.307, p<0.001, Fig. 9B), palatal anesthesia (r^2=0.412, p<0.001, Fig. 9D) and palatal saline (r^2=0.799, p<0.001, Fig. 9F). For Group B this relationship was also observed between cycle duration and phase 2 for control (r^2=0.668, p<0.001, Fig. 9B), palatal anesthesia (r^2=0.839, p<0.001, Fig. 9D), and palatal saline (r^2=0.259, p<0.001, Fig.9F). The Group B with palatal anesthesia had a wider range of values for cycle duration and phase 2 length that maintained the same linear relationship seen with the control and palatal saline treatments. It also had a greater average slope.

Figure 9 Correlation between cycle and phase length. A) The correlation between the time from suck-swallow cycle to post-swallow suck cycle (cycle) and the suck-swallow cycle to pharyngeal swallow (phase 1) for the control feedings. B) The correlation between cycle length and the time from pharyngeal swallow to post-swallow suck cycle (phase 2) for the control feedings. C) The correlation between phase 1 and cycle duration for palatal local anesthesia. D) The correlation between phase 2 and cycle duration for palatal local anesthesia. E) The correlation between phase 1 and cycle duration for palatal saline. F) The correlation between phase 2 and cycle duration for palatal saline.
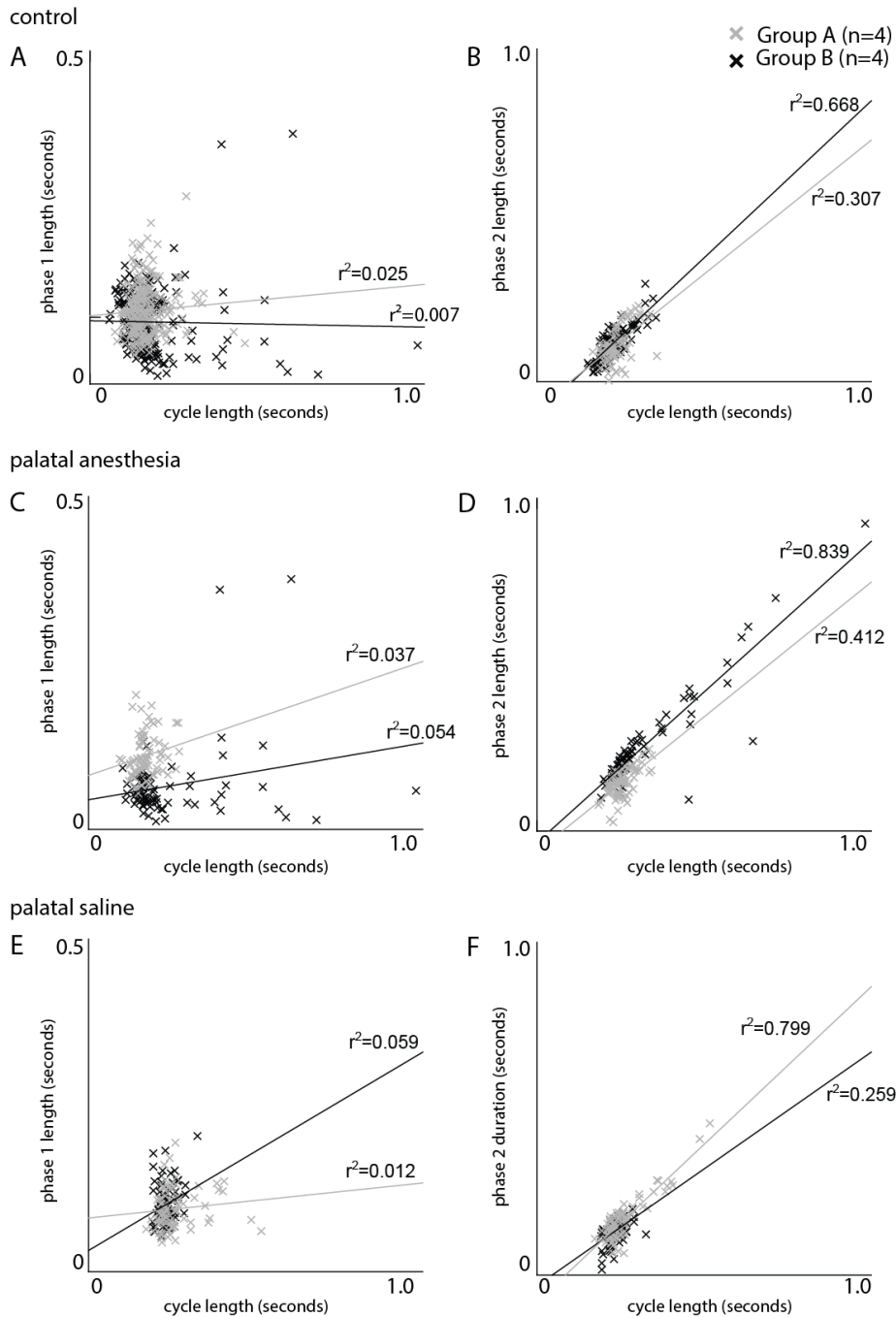
Blockade of the Lesser Palatine Nerve

In two of six cases (3 infant pigs with 2 greater palatine nerve blocks, bilaterally) the india ink reached the lesser palatine nerve and foramen following the greater palatine nerve block.

DISCUSSION

Two distinct reactions to palatal local anesthesia

All individuals suckled normally during control and saline treatments; but with a reduction of palatal sensation only half of the infants had normal function. We hypothesize that the extreme jaw movement seen in dysfunctional suckling was a less efficient compensation to reestablish some form of intraoral transport. The obliteration of the typical vertical, wave-like tongue movement is consistent with the documented link between palatal sensation and tongue rhythmicity (Thexton, 1973). Because jaw movement, vertical tongue movement and latching were either all affected or all normal, suggests a central link amongst them.

This movement is not part of normal infant or adult behavior. Adult pig drinking involves suction, with the tongue remaining in their mouth, and significant surface deformation of the tongue (Herring and Scapino, 1973; Thexton et al., 1998b). Mastication includes jaw movement in all planes (Herring and Scapino, 1973). Although the amount of jaw opening after palatal anesthesia was similar to mastication, they were limited to the sagittal plane (Herring and Scapino, 1973).

The difference between the two behavioral groups is similar to a difference observed in two sets of same-aged infant opossums. In these animals, two distinct oral transport mechanisms were described (German and Crompton, 1996). In infants that were

younger (45 days old), they had a sucking mechanism similar to infant pigs. The infants

that were older (65 days) and separated from their mother transitioned to a feeding

mechanism where they protruded their tongue to feed from the nipple, in a manner

similar to lapping. Even though lapping is only seen in adult opossums, they made that

transition when separated from their mothers. Adult pigs, drink using a sucking

mechanism, not a lapping one, where the tongue remains in the oral cavity and the head is

down and snout immersed in milk (Herring and Scapino, 1973). The non-sucking

behavior we observed after palatal anesthesia is consistent with previous findings of two

different feeding mechanisms existing simultaneously, prior to the adult mechanism, that

is not exclusively dependent on maturation, but also environmental or in this case a

sensory change.

The reason for the existence of two distinct groups of animals with different

feeding mechanisms after palatal anesthesia is unclear. One explanation is that half of the

infants had the anterior soft palate anesthetized due to the lesser palatine nerve being

anesthetized We reject this explanation because this sensory nerve is relatively very

small and only provides sensation to the anterior region of the soft palate. Another

explanation is that there is a developmental difference and that the feeding mechanism

exhibited in Group B after anesthesia could be an alternative feeding strategy seen in

more developed animals. The variation in feeding kinematics in normal pigs, even after

decerebration (German et al., 2009; Thexton et al., 2007), is large and well documented.

Another potential explanation is that the rate of adaptation was different between animals

and that in the time between the injection and feeding session, some animals were able to

adapt and start sucking quicker than others. There was no correlation between the order of treatments and whether the pigs had a Group A or B response to anesthesia.

One explanation for the different responses to anesthesia could be their rate of development. Developmental differences in the role of oropharyngeal sensation during respiration have been noted in previous studies, and the same may be seen for the swallowing reflex as well (Litmanovitz et al., 1994; Penatti et al., 2006). Additionally, early studies of swallowing in decerebrate cats showed substantial variation in recordings from motor nerves following different oropharyngeal sensory stimulations that resulted in swallows in infants (Sumi, 1967). That study showed that the brain stem developed postnatally for the first three months of life and that there was a large range of variation between animals. This could be due to developmental changes in myelination as is seen with other oropharyngeal sensory nerves (Miller and Dunmire, 1976). In the present study, the variable response to anesthesia could reflect a varying stage of development of the reflex as was seen in the previous studies.

An early study of rabbit chewing and swallowing found that in response to lingual nerve stimulation, there were two distinct responses from hypoglossal nerves- either excitatory or inhibitory (Sumi, 1970). The complexity of the hypoglossal nerve and its functional linkage to sensation from the trigeminal nerve branches in the tongue could explain why the tongue reacted so differently to anesthesia in the infant pigs. In half of the pigs (Group B) the tongue was moving significantly more and was unable to latch to the nipple. Like sensation from the tongue, sensation of the palate is also linked to motor output from the tongue. More studies are needed to further understand the changing

myelination of the trigeminal nerve and relative role of the sensory and motor nerves during swallowing throughout early postnatal development.

Sucking and Swallowing after Palatal Local Anesthesia

All infants were able to adapt or compensate to reduced palatal sensation and elicit swallows. The reduction in swallowing frequency after palatal anesthesia in Group B was most likely due to the inefficient oral transport cycles. Alternatively, palatal stimulation may influence the threshold to initiate the swallow. With anesthesia it was more difficult to reach that threshold. We found that the only parameter that was different in Group A and Group B during control feedings was swallowing frequency. Group B pigs had a slower swallowing frequency during control feedings. This could be a factor that could predict whether a pig will have a Group A or Group B response to anesthesia. Further testing of this hypothesis is needed.

The vomeronasal organ, which detects pheromones, was also most likely affected by the nasopalatine nerve block. In a study of opossums, they found that blocking the nasopalatine canal resulted in a change of food preference, but did not report any change in mastication or feeding ability (Poran, 1998). It is unknown if the same would be seen in infant pigs, however, we did not notice any less willingness to feed after palatal anesthesia.

Sucking and Swallowing following Palatal Saline

Palatal saline could potentially provide analgesia due to pressure on the nerve, or pain due to the expansion of the soft tissues covering the palate. Although originally intended as a sham treatment, the possibility exists that it was another palatal sensory disruption.

Little information exists to clarify these results. A study evaluating palatal sensory nerve blocks following palatoplasty in children found that a palatal saline injection produced variable results that were different from untreated patients, demonstrating that the saline treatment disrupted sensation (Jonnavithula et al., 2010). It is important to note that there was no way to know if the effects of both palatal saline and anesthesia were due to volume of fluid or the sensory effects that both most likely had.

The palatal saline treatment results were variable both between Groups A and B, and over the different outcome measures. Swallowing frequency increased in only in Group A with palatal saline, which indicates that there could have been a painful reaction that caused heightened sensory input that triggered swallow cycles more frequently. There was a statistically significant difference between Group A and Group B swallow frequency after palatal saline and after control feedings which points to a physiologically significant difference in their reactions.

The results indicate that palatal saline caused a sensory disruption that affected the initiation of suck cycles and did so in all pigs regardless of Group causing them to less frequent. Further studies are needed in order to determine if the palatal saline treatment caused pain or anesthesia and to determine how long that effect would have remained.

Cycle and Phase Relationships after Palatal Sensory Disruption

Thexton et al. postulated that a suck-swallow cycle could be divided into two phases in a manner similar to other rhythmic activities in mammals, one of which was stable in length, the other of which is function of the length of the entire cycle (Frigon and Gossard, 2009; Thexton et al., 2012). The relationship between suck-swallow cycle and

both phase lengths in the control and treatments was that described by Thexton et al. and Ding et al. (Ding et al., 2013a; Thexton et al., 2012). Our results support and refine these hypotheses. We found a stable phase 1 and a linear relationship between phase 2 and cycle length. The only exception was an extremely small slope that is marginally from zero.

This pattern of phase relationships exists even without sensory feedback in other models (Frigon and Gossard, 2009; Gossard et al., 2011). While we found this pattern in the palatal saline and control, we did find one interesting difference in the response to palatal anesthesia. Group B with palatal anesthesia had a higher slope than Group A with palatal anesthesia indicating longer phase 2 lengths. Despite significant differences in their oral transport cycle lengths, the same linear, temporal relationship existed between cycle and phase lengths. Thus, while palatal anesthesia lengthened cycles and phase 2 for some animals, it did not change the basic pattern of phase relationships. This is in contrary to Ding et al.'s finding that the relationship between cycle and phase 1 length changed after a unilateral superior laryngeal nerve lesion in infant pigs (Ding et al., 2013a). It is evident that the pattern generator for the pharyngeal swallow must communicate with the pattern generator for the oral transport or suck cycles so it delays the onset of that next suck cycle in a predictable way. This relationship is mediated by the SLN, and possibly other sensory nerves, but not the greater palatine or nasopalatine nerves. While one neurophysiology study indicated this network may exist and may indeed influence the coordination of sucking and swallowing, the interactions between the sucking pattern generator and the swallowing pattern generators in the brainstem need to be further investigated (Sessle and Storey, 1972).

Implications for Feeding Neurophysiology and the Role of the Central Nervous System

This study provides evidence that trigeminal sensation directly affects the frequency of swallowing, oral transport and the coordination of relevant pattern generators in the brainstem. Oropharyngeal sensory nerves synapse in and around the nucleus tractus solitarius (NTS) in the brainstem which is part of the central pattern generator for swallowing. The motor program for the swallow originates in and around the nucleus AMBIGUUS (NA) in the brainstem, which is also part of the central pattern generator for swallowing, and is influenced by both the NTS and descending cortical input. Trigeminal afferents synapse in the trigeminal sensory nucleus in the brainstem, however, it is known that it also sends information to the NTS which means it could also influence swallowing centers within the NTS or NA (Capra, 1995; Sweazey and Bradley, 1989). Alternatively, trigeminal sensation could influence the NA by altering descending cortical input. There is evidence from fMRI that stimulating oral sensory receptors activates primary and secondary somatosensory cortex and thalamus (Lowell et al., 2008). It is clear that these interactions are not simple, and that feeding has complex regulation. Further investigation is needed to understand the exact connectivity between these nuclei in the brainstem and the cortex.

The relative importance of the hard palate sensation for initiating oral transport, or suck, cycles is a key finding since most feeding therapies for infants having trouble sucking are aimed at the perioral region. This study also showed that not only was initiation of the suck cycle affected in all animals, but in half of the animals, palatal

anesthesia led to the inability to suck due to pathologically altered jaw and tongue movements.

The coordination between the swallowing and sucking pattern generators is fundamental to protecting the airway from aspiration (Barlow, 2009; Lau and Hurst, 1999). Our findings suggest that the swallowing and sucking pattern generators communicate to coordinate the start of the suck cycle that follows a pharyngeal swallow and that this communication occurs even after sensory disruption to the hard palate.

Palatal sensation has a clear impact on normal oral and pharyngeal function in infant pigs . What requires further study are the causes of the individual differences that produced Group A and Group B. A developmental difference could be the reason why two distinct feeding mechanisms are seen after palatal anesthesia. Further evaluation of this data will help us understand how the motor pattern of the swallow, kinematics and airway protection is affected by palatal anesthesia. Additional experiments as a function of developmental time could test this hypothesis.

CHAPTER 5: SWALLOWING KINEMATICS AND AIRWAY PROTECTION AFTER PALATAL LOCAL ANESTHESIA IN INFANT PIGS[4]

ABSTRACT

Objective: Abnormal kinematics during swallowing can result in aspiration which may become life threatening. We tested the role of palatal sensation in the motor control of pharyngeal swallow in infants. Study Design: In eight infant pigs, we reduced palatal sensation using local anesthesia (PLA) and measured the impact on swallowing kinematics and airway protection. Methods: The pigs drank milk containing barium while we simultaneously recorded videofluoroscopy and electromyography from fine wire bipolar electrodes in several hyolaryngeal muscles. We compared these results to control feedings and feedings following palatal saline injections (PSA). Results: After PLA, four pigs had extreme jaw movements and abnormal tongue movement uncharacteristic of sucking. For this reason, we evaluated differences between these "Group B" pigs and the others that could suck normally after PLA, "Group A". In the four Group A pigs, after PLA there was less hyoid elevation ($p<0.001$) but normal jaw and tongue movements. In Group B, in addition to greater jaw movement ($p<0.001$) there was more anterior and superior tongue movement ($p<0.001$) and a larger range of hyoid movement ($p<0.001$). Conclusion: The airway was protected in all of the pigs, indicating that these changes allowed successful adaptation to the reduction in palatal sensation. However, the oral and pharyngeal phases of the swallow were functionally linked and trigeminal sensation influenced the motor control of the pharyngeal swallow.

---

[4] Holman SD, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, Waranch, DR, German RZ. Swallowing kinematics after palatal local anesthesia in infant pigs. The Laryngoscope- *in press*.

INTRODUCTION

Prematurity, neurological deficits or differences in craniofacial anatomy can cause swallowing dysfunction(Arvedson et al., 2010; Miller, 2011b), with a sequelae of aspiration(Steele et al., 2011; Tutor and Gosa, 2012). While much is known about swallowing biomechanics (Crompton et al., 1997; Crompton et al., 2008), less is known about the integrated sensorimotor neurophysiology in either normal or abnormal swallowing.

Infant swallowing physiology is characterized by rhythmic suck cycles and suck-swallows, where the bolus passes past the laryngeal opening and into the esophagus. With each suck-swallow cycle there is significant rhythmic tongue movement, a lesser amount of jaw opening, and hyolaryngeal elevation. Oral sensory input from the trigeminal nerve is necessary for motor function during pharyngeal swallowing in adults (Humbert et al., 2012a), but its role in in infants is unknown.

Using an infant pig model, we tested the role of palatal sensation on the motor function and kinematics of the suck-swallow. We injected palatal local anesthesia (PLA) and saline (PSA) and then compared swallowing kinematics and motor function after these treatments to normal (control) feeding sessions in the same animal. We hypothesized that they would adapt to PLA with unaltered airway protection. We expected altered tongue and jaw movement since palatal stimulation elicits reflexive jaw and tongue movements in decerebrate animals (Thexton, 1973). The PSA treatment was designed as a sham, and we did not expect significant differences from the control feedings.

MATERIALS AND METHODS

Surgical Procedure

Eight pigs, S*us scrofa*, a standard model, (German et al., 2004b; Thexton et al., 2007; Thexton et al., 2009) were obtained from Tom Morris Farms (Reisterstown, MD) at 2-3 weeks old (3-5kg). All procedures were approved by JHMI IACUC (SW10M212).

After successfully learning to bottle feed using a pig nipple (Nasco, Fort Atkinson, WI), we implanted fine wire bipolar electromyographic (EMG) electrodes in the genioglossus (GG), thyrohyoid (TH), and cricothyroid (CT) muscles. An EMG patch electrode was sutured to the anterior surface of the mylohyoid (MH) muscle. Intraoral radio-opaque markers were inserted in the midline of the anterior hard palate, hard/soft palate junction, tongue (~10mm deep to the foramen cecum), and right and left mucogingival junction superior to the maxillary first molar (Fig. 10). Husbandry and the specifics of the methods of EMG electrode and marker implantation followed Thexton et al. (Thexton et al., 2012). This methodology has been used in other pig studies and does not alter feeding(Dutra et al., 2010; German et al., 2004b).
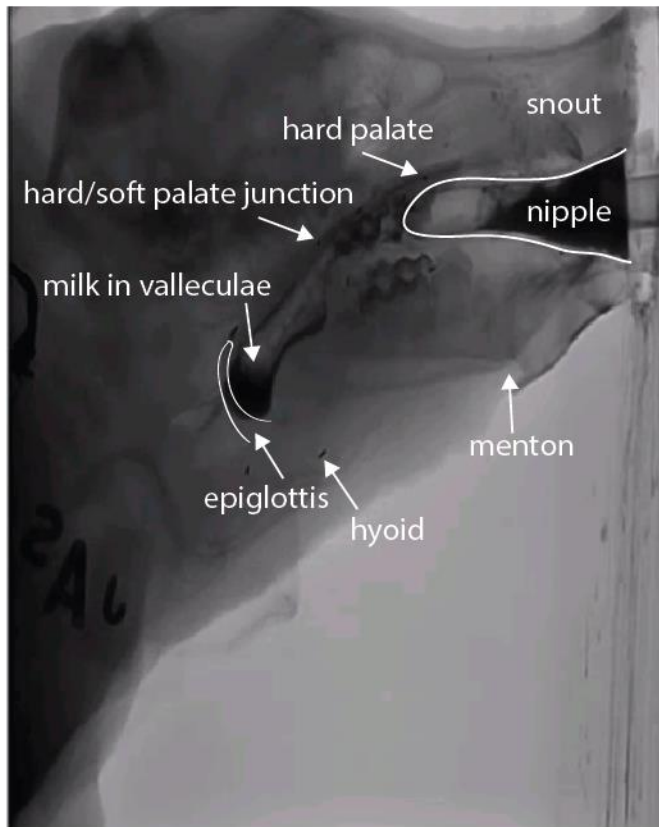
Figure 10 Videofluoroscopy image with radio-opaque and anatomical markers identified. The radio-opaque markers on the hard palate, hard/soft palate junction and hyoid are clearly identified. Additionally, the radiological point of the menton is identified. The epiglottis is outlined and the nipple and snout are labeled for orientation. There are additional radio-opaque markers in the image that were not evaluated in the present study.

Treatments

The day after surgery, experiments began and the animal was placed under 3% Isoflurane to achieve deep/surgical anesthesia for 20 minutes. A Weck Hemoclip Ligating Clip (Pilling Weck, Research Triangle Park, NC) was placed on the epiglottis (Crompton et al., 2008).

There were two treatments: (1) saline (PSA) and (2) local anesthesia (PLA) given on separate days. The local anesthesia injections were 0.5 ml of 0.5% bupivacaine hydrochloride (Marcaine™, 5mg/ml, Hospira, Inc. Lake Forest, IL) and the saline

injection was 0.5 ml of saline. A standard clinical dental injection technique and amount was used (Jonnavithula et al., 2010; Reuss-Lamky, 2007). These injections were to three locations: the right and left greater palatine nerves and the nasopalatine nerve. In the process of anesthetizing the greater palatine nerve, it is likely that a small portion of the lesser palatine nerve, supplying sensation to the soft palate was also blocked. In a post-mortem study of infant pigs, this occurred in 1/3 of cases.

The day after an injection was given was a recovery day during which no experiments were conducted. The order of the treatments was randomized. After the epiglottis marker was either placed or verified, and the treatment was given, recovery from general anesthesia took 5-10 minutes. Bupivacaine hydrochloride has a minimal duration of action of 1 hour until oral reflexes are observed in infant pigs(Holman et al., 2013).

Recordings of Feeding Sessions

The pigs were bottle fed while standing unrestrained in a plastic feeding box 30 minutes to 1 hour post-injection while lateral videofluoroscopy was recorded at 60 frames per second. The recording device (Allura FD20, Philips Healthcare, Best, The Netherlands) was equipped with a high-resolution digital flat-panel detector (154x154 micron pixel-pitch, 30x40 cm). The pigs were fed until 20 swallows had been recorded or at least five 15 second recordings. Bottles contained eight ounces of milk formula (Land O Lakes Solustart pig milk replacer, St. Paul, MN) with 1/3 cup powdered barium. Temperature and the amount of barium in the bottles was standardized (Cichero et al., 2010; Ebihara et al., 2011).

Videofluoroscopy Data Analysis

One judge evaluated the videofluoroscopy recordings using MaxTRAQ Version 2.2.4.1 (Innovision Systems, Inc., Columbiaville, MI). Each swallow was scored according to the Infant Mammalian Penetration-Aspiration Scale (IMPAS, Table 13) (Holman et al., 2012a). The scores were tested for differences between treatments using a Kruskal-Wallis Test and a Dwass-Steel-Critchlow-Fligner Test using SYSTAT 13 (SYSTAT, 2009). Holman et al. 2012 reports intra-rater reliability for this scale with an average of 86% agreement with an intraclass correlation coefficient of 0.92(Holman et al., 2012a).

Table 13 7-Point Infant Mammalian Penetration Aspiration Scale after Holman et al. 2013.

	Score	Description
normal	1	Material does not enter the airway
penetration	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
aspiration	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)

We calculated the total distance the hyoid marker and mandible (menton, the most anterior and inferior point on the mandible) moved relative to the palate markers. These are standard measurements in both animal and human videofluoroscopy studies(Crompton et al., 1975; Palmer et al., 1997; Thexton et al., 1998a). The maximum anterior/posterior and dorsal/ventral movements of tongue and hyoid were calculated relative to an x-axis between the hard/soft palate junction marker (origin) and the hard palate marker. The distances were corrected using the maximum diameter of a radio-opaque sphere in the video. Intra-rater reliability of auto-digitizing points from infant pig lateral videofluoroscopy has shown the average variability to be 0.67 millimeters with a standard deviation of 0.83 millimeters (Unpublished data, AM Griffioen).

Electromyographic Data Analysis

Based on the results from the post-mortem dissection and the quality of the EMG recordings, one channel each for MH, GG, TH and CT was chosen for data analysis per pig. The time of the midpoint of each burst of muscle activity in the EMG recordings was identified for a maximum of 20 suck-swallow cycles per feeding session in LabChart® Pro (AD instruments, Colorado Springs, CO, Fig. 2)(Thexton et al., 2012). The CT was labeled at the start of its activity due to its gradually increasing signal (Fig. 11). We evaluated timing differences during the suck cycle (MH to GG), between the suck and swallow (MH to TH), and during the pharyngeal swallow (TH to CT). When interpreting the significant differences in this data, we kept in mind that the total duration of epiglottal movement during the pharyngeal swallow is on average 208.72 ms (n=457 swallows, Unpublished data, SD Holman).
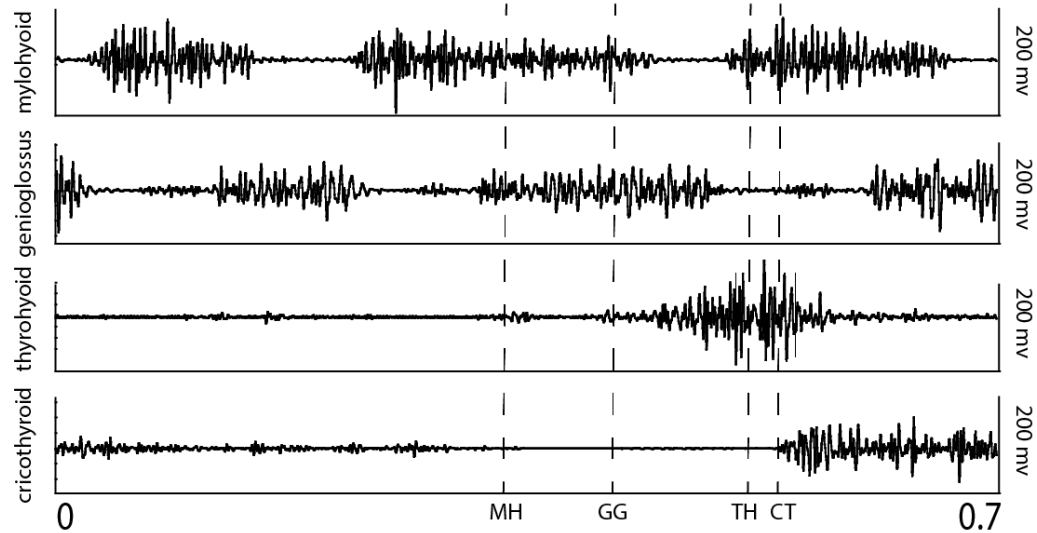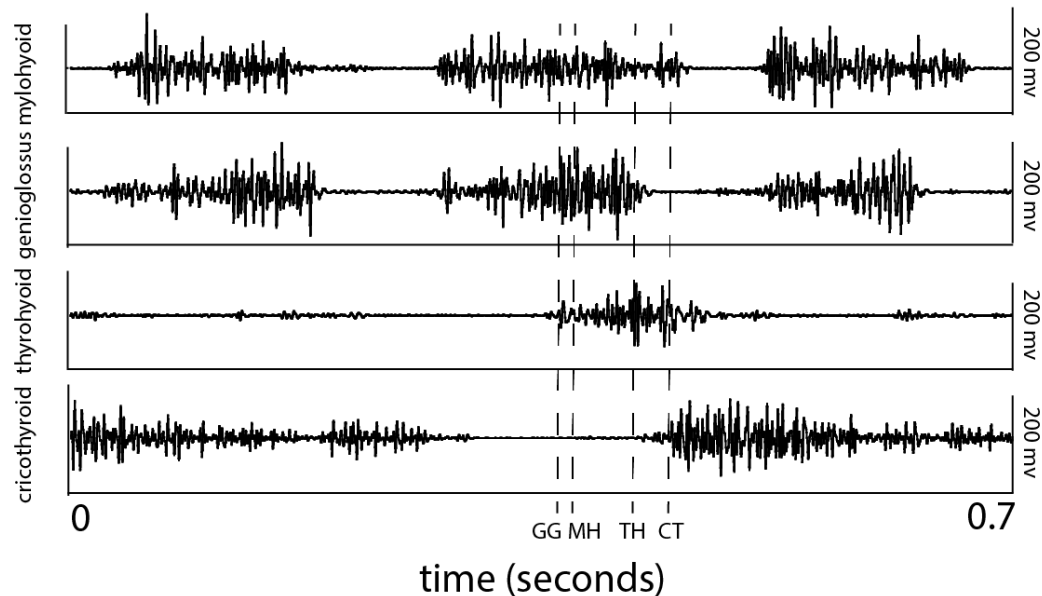
Figure 11 EMG signals in control and local anesthesia for Pig H (Group B). A) EMG signals during a control feeding. B) EMG signals in the same pig that was not sucking following a palatal anesthesia (PLA) treatment. With PLA the mylohyoid (MH) and genioglossus (GG) are active at roughly the same time with the mid-point of GG activity actually occurring before MH. In the control feeding the MH is always active well before GG. There is also a lot less time between oral (MH) and pharyngeal (TH) muscle activity. The dashed lines indicate how the muscle was labeled. The MH, GG and TH were labeled at the midpoint of their activity while the cricothyroid (CT) was labeled at the start due to the triangular nature of its EMG signal. The time point at each label was used to calculate timing differences.

Statistical Testing

Differences between treatment and control feedings, for each dependent variable (except the IMPAS results), were evaluated using a General Linear Model ANOVA and post-hoc Tukey's HSD test with an alpha of 0.05. The sample size was approximately 480 swallows with 20 swallows per treatment per animal.

RESULTS

Two groups: Group A and Group B

The pigs were divided into two exclusive groups for statistical analyses: Group A and Group B. Group B pigs (n=4) had noticeably larger movements of the mandible, did not latch onto the nipple and had less surface deformation of the tongue during oral transport cycles after PLA (Fig. 12)[5]. Group A pigs (n=4) had no noticeable change in jaw movement, latching ability or tongue movement with PLA. This clear qualitative distinction had no intermediate feeding mechanism. Group was added as a fixed factor in the ANOVA and also tested for an interaction with treatment. Individual (pig) was nested in group to account for it as a random factor.

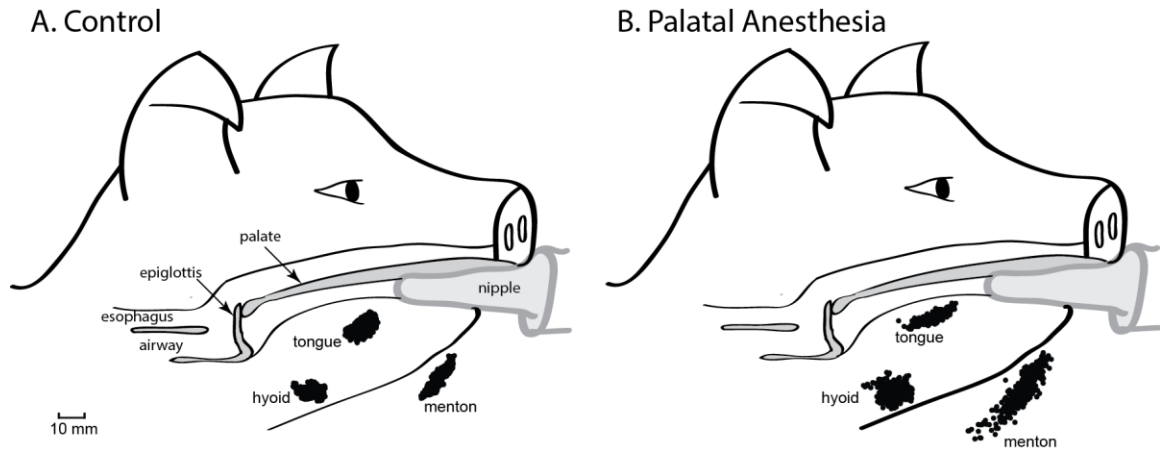[5] SD Holman et al- In review at Journal Neurophysiology

Figure 12 Tongue, hyoid and menton movements in a Group B pig during a control feeding and a feeding following PLA. Each data point represents the 2-D location of a specific anatomic marker. Each cluster of data points represents a complete record of movement for a given marker over the course of a suck-swallow cycle. The black points represent hyoid movement, the dark grey points represent tongue movement and the light grey points represent menton movement. A) The range of hyoid, tongue and mandible movements in a control feeding from one of the Group B pigs. B) The range of hyoid, tongue and mandible movements in a feeding after palatal anesthesia (PLA) in a Group B pig. The menton and hyoid markers had a larger range of motion in the Group B pig after PLA. The tongue marker had more movement in the Group B pigs after PLA.

Airway Protection

Group A, after PLA and PSA, had significantly more airway protection ($p<0.001$

for PLA and PSA) relative to control feedings (Fig. 13). There was only marginally

increased airway protection in Group B with PLA or PSA ($p=0.068$). There were only a

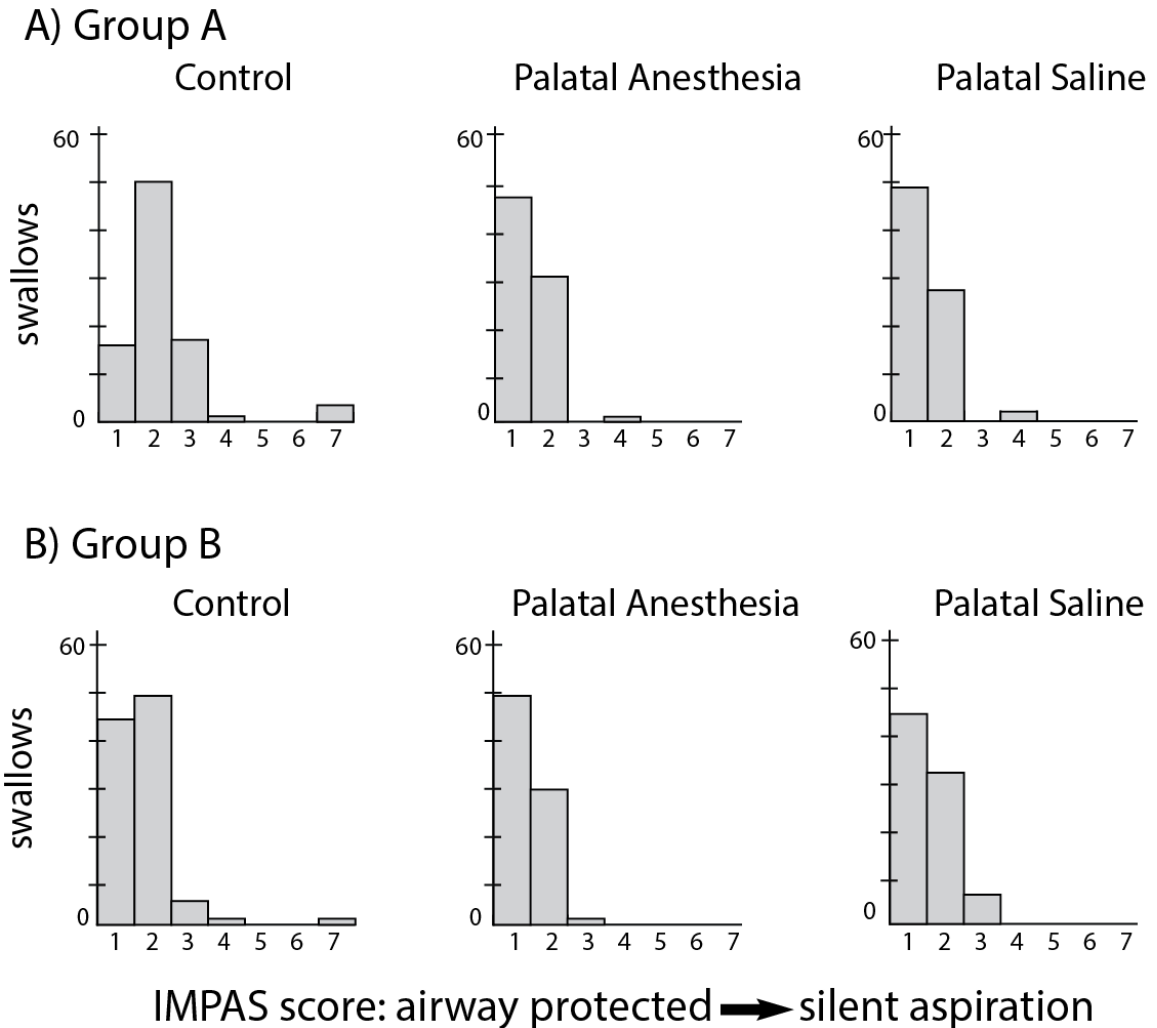few incidents of aspiration across all animals and coughing was never observed.

Figure 13 IMPAS scores for Group B and Group A. A) For Group A there was more airway protection after palatal anesthesia (p<0.001) and saline (p<0.001) compared to control feedings. B) For Group B there was no significant differences among the three treatments (p=0.068).

Oral Transport

There was a significant difference in extent of jaw opening between treatments (p<0.001) and in the interaction between treatment and group (p<0.001, Fig. 14), but not between the groups. There was significantly more jaw movement after PLA relative to control (p<0.001) and PSA (p<0.001) and no difference between control and PSA.
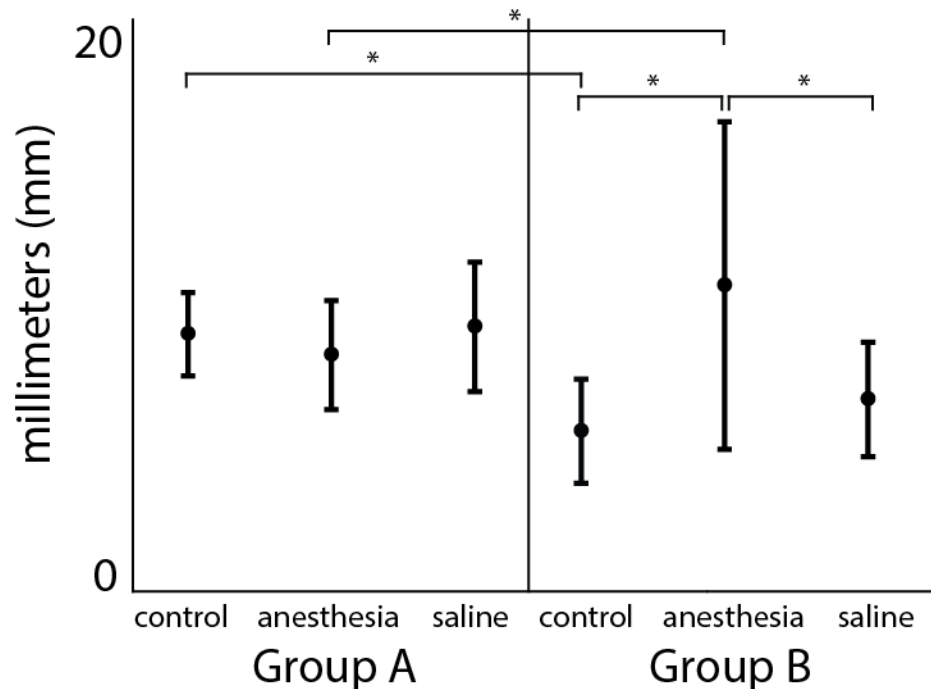
Figure 14 Jaw opening during the suck-swallow. On the left is the range of jaw opening movement in Group A between treatments (n=4). On the right is the range of jaw opening movement in Group B between treatments (n=4). There was significant more jaw opening in Group B pigs after palatal anesthesia (PLA, p<0.001).

Group A had no significant difference in jaw opening between treatments. Group B had significantly more jaw opening with PLA relative to control (p<0.001) and PSA (p<0.001, Table 14, Fig. 12, Fig. 14). No difference in jaw opening existed between control and PSA. There was significantly more jaw opening in the control feedings in Group A compared to Group B (p<0.001, Table 14, Fig. 14). Group B had significantly more jaw opening after PLA than Group A (p<0.001, Table 14, Fig. 14). One Group A pig (Pig C) was excluded from the dataset because the menton was not in view during the videofluoroscopy recording.

Table 14 Sample sizes, means, standard deviations and ranges for the dependent variables evaluated. The grey represents Group A and the white represents Group B. While we aimed to have a n of 80 for each group, sample sizes were lower if that marker was not in the frame during a swallow or if the EMG signal was not clear enough to label. The menton was not in view for one pig for all treatments. The GG signal was either not clear or not available for 3 pigs.

		n	Mean ± SD	range
Time between oral muscle activity (MH to GG) (ms)	**CON**	60	67.9±27.2	17-153
	PLA	60	66.4±19.5	1-106
	PSA	60	64.3±20.0	17-124
Time between oral muscle activity (MH to GG) (ms)	**CON**	40	63.8±12.9	32-93
	PLA	40	-6.2±30.9	-47-65
	PSA	40	54.5±7.3	43-74
onset of pharyngeal muscle activity (MH to TH) (ms)	**CON**	80	125.9±44.2	56-282
	PLA	80	110.3±32.3	54-202
	PSA	80	94.4±29.8	44-194
onset of pharyngeal muscle activity (MH to TH) (ms)	**CON**	80	113.1±30.7	30-166
	PLA	80	64.1±55.7	6-376
	PSA	80	101.2±30.1	53-204
time between pharyngeal muscle activity (TH to CT) (ms)	**CON**	80	41.4±15.9	8-108
	PLA	80	43.4±18.6	10-108
	PSA	80	43.2±17.2	9-66
time between pharyngeal muscle activity (TH to CT) (ms)	**CON**	80	26.5±12.4	6-59
	PLA	80	30.9±13.3	0-55
	PSA	80	28.6±7.5	10-46
Range of hyoid motion (mm)	**CON**	77	7.4±1.7	4.8-11.0
	PLA	80	6.6±1.6	2.8-9.5
	PSA	80	7.3±2.5	2.9-13.6
Range of hyoid motion (mm)	**CON**	80	6.5±1.6	3.6-9.6
	PLA	60	8.5±1.5	5.5-12.3
	PSA	80	7.1±1.8	2.7-10.0
Jaw opening distance (mm)	**CON**	57	9.0±1.5	6.2-12.1
	PLA	60	8.3±1.9	4.2-11.8
	PSA	40	9.5±2.2	5.0-11.7
Jaw opening distance (mm)	**CON**	80	5.6±1.8	2.6-11.0
	PLA	58	14.5±7.9	3.1-31.7
	PSA	79	6.8±1.8	3.5-12.0
Tongue x-axis distance (A/P) (mm)	**CON**	79	7.1±2.0	1.0-10.5
	PLA	61	8.1±1.8	1.8-11.4
	PSA	34	7.7±1.4	2.6-10.0
Tongue x-axis distance (A/P) (mm)	**CON**	67	9.0±4.0	0.1-19.9
	PLA	59	12.3±6.3	1.4-27.3
	PSA	74	8.9±3.6	3.2-17.7
Tongue y-axis distance (D/V) (mm)	**CON**	79	7.1±2.3	0.3-12.7
	PLA	61	7.9±2.0	1.9-12.1
	PSA	34	7.8±1.7	1.7-10.6
Tongue y-axis distance (D/V) (mm)	**CON**	67	8.7±4.7	2.4-28.3
	PLA	59	15.5±6.9	0.9-31.9
	PSA	74	6.8±2.3	1.7-15.7
Hyoid x-axis (A/P) (mm)	**CON**	79	5.7±2.3	0.4-10.3

	PLA	61	7.0±1.8	2.8-12.4
	PSA	34	5.8±1.8	2.0-8.3
Hyoid x-axis (A/P) (mm)	**CON**	67	6.6±3.2	0.9-16.2
	PLA	59	13.7±8.9	1.6-48.5
	PSA	74	7.5±2.4	2.6-14.9
Hyoid y-axis (D/V) (mm)	**CON**	79	5.6±2.2	0.6-12.9
	PLA	61	5.4±2.9	1.5-14.6
	PSA	34	4.4±2.2	1.2-8.9
Hyoid y-axis (D/V) (mm)	**CON**	67	5.8±3.9	0.3-26.0
	PLA	59	13.9±8.8	2.0-34.5
	PSA	74	6.0±3.0	0.7-20.9

Significant differences existed in anterior tongue movement based on treatment ($p<0.001$) and group ($p<0.001$). Pigs had significantly more anterior tongue movement after PLA relative to control ($p<0.001$) and PSA ($p=0.004$). Group B also had more movement than Group A ($p<0.001$, Table 14).

Superior tongue movement differed between group, treatment and in interaction ($p<0.001$ for all). There was more movement after PLA relative to control ($p<0.001$) and PSA ($p<0.001$), but no difference between control and PSA. There was also significantly move movement in Group B than Group A ($p<0.001$).

In Group A there were no significant differences between treatments. In Group B had significantly move movement after PLA relative to both control ($p<0.001$) and PSA ($p<0.001$) and no difference between control and PSA (Table 14). There was also no difference between groups during control feedings or PSA feedings. There was significantly more tongue movement after PLA in Group B than Group A ($p<0.001$, Table 2).

The GG EMG activity was acceptable only for five of the eight animals in the study. In two cases the electrodes were not in the correct muscle, and in the other case the signal was not clear due to erratic tongue movements after PLA. For the remainder, the time between MH and GG activity was significantly different between treatment, group

and their interaction (p<0.001 for all). There was less time between MH and GG after

PLA compared to both control (p<0.001) and PSA (p<0.001), but no difference between

control and PSA. There was also less time between MH and GG for Group B relative to

Group A (p<0.001).

In Group A there was no difference between treatments. In Group B there was

less time between MH and GG after PLA relative to control (p<0.001) and PSA

(p<0.001, Fig. 11, Fig. 15, Table 14) with no difference between control and PSA. There

was no difference between groups during control or PSA feedings. There was less time

between MH and GG during PLA feedings in Group B relative to Group A (p<0.001).
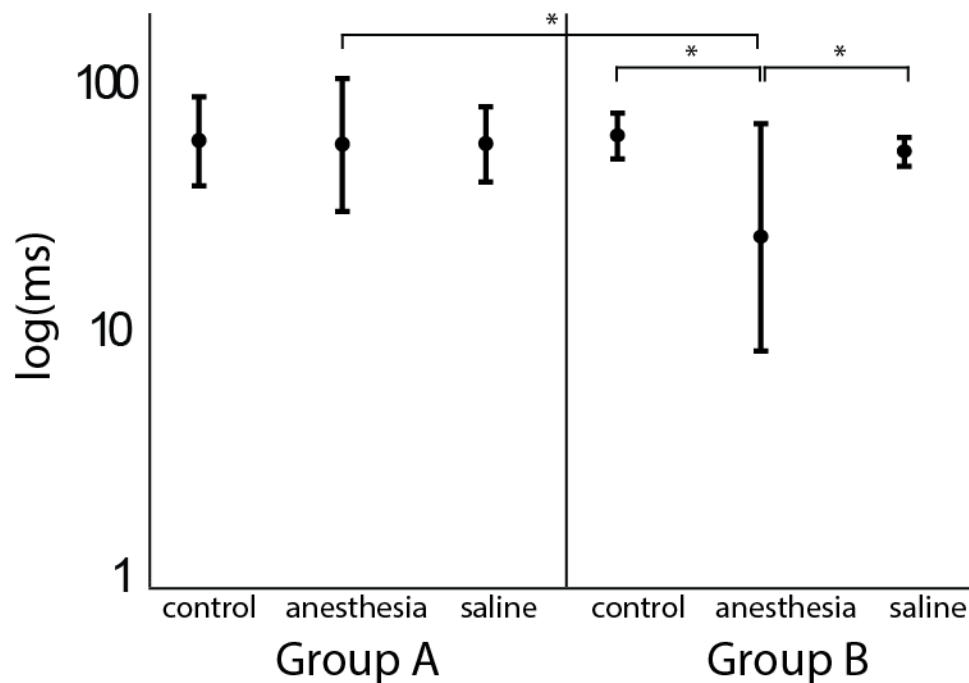

Figure 15 Time between mylohyoid and genioglossus muscle activity during oral phase of the swallow. On the left is the time difference between mylohyoid (MH) and genioglossus (GG) muscle activity in Group A between treatments (n=4). On the right is the time difference in Group B between treatments (n=4). There was significantly less time between MH and GG activity in Group B pigs after palatal anesthesia (PLA) compared to control and saline (PSA, p<0.001). There was also less time between MH and GG activity in Group B pigs after PLA compared to Group A pigs after PLA (p<0.001).

Time between start of suck-swallow and pharyngeal swallow

For the lag between MH and TH activity, significant differences between treatments (p<0.001), groups (p<0.001) and in the interaction between treatment and group (p<0.001) existed. The differences between treatments all had p<0.001 with control feedings having more time, followed by PSA and then PLA with the least amount of time. Group A had a longer lag than Group B (p<0.001).

Within Group A there was no significant difference between control and PLA. Significantly less time existed between MH and TH muscle activity after PSA compared to control (p<0.001) and PLA (p=0.024, Table 14, Fig. 16). In Group B There was less time after PLA relative to control (p<0.001) and PSA (p<0.001, Table 2, Fig. 16). There was no difference between control and PSA feedings. There was no difference between groups during control feedings or PSA feedings. There was less time after PLA in Group B compared to Group A (p<0.001, Table 14, Fig. 16).
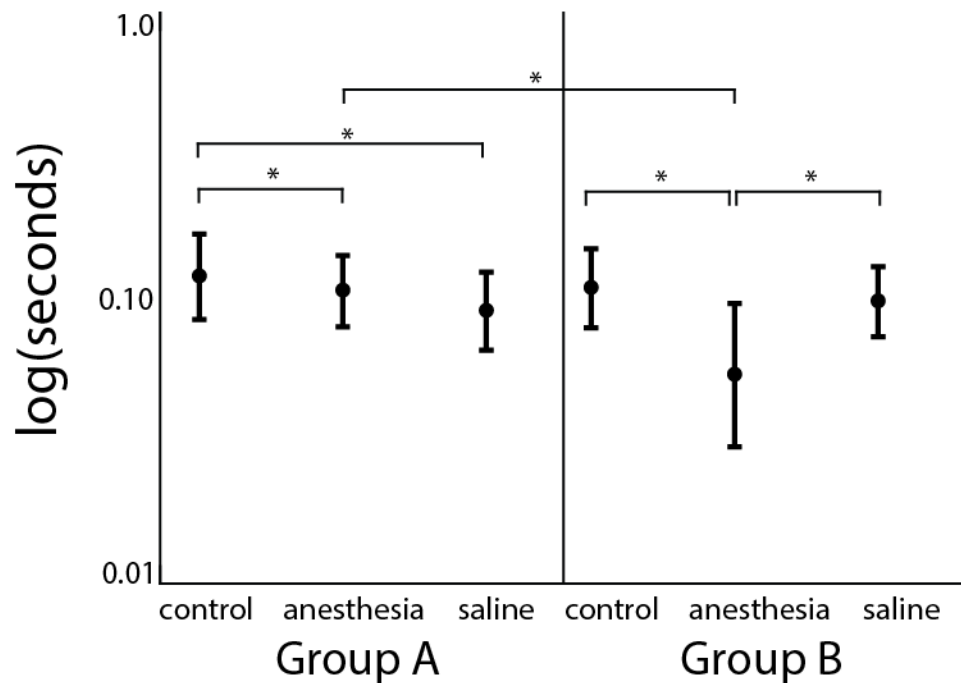
Figure 16 Time between mylohyoid and thyrohyoid muscle activity during the swallow. On the left is the time difference between mylohyoid (MH) and thyrohyoid (TH) muscle activity in Group A between treatments (n=4). On the right is time difference in Group B between treatments (n=4). There was less time between MH and TH in Group A after palatal anesthesia (PLA, p=0.024) and saline (PSA, p<0.001) compared to control. There was also less time between MH and TH activity in Group B after PLA compared to both control (p<0.001) and PSA (p<0.001). There was less time between MH and TH activity in Group B after PLA than Group A after PLA.

Pharyngeal Swallow

There were significant differences in range of hyoid movement between treatments (p=0.010) and in the interaction between treatment and group (p<0.001). There was significantly more hyoid movement after PLA compared to control (p=0.007) but no other differences between treatments.

In Group A there was less hyoid movement after PLA compared to control (p<0.001) and PSA (p=0.015, Table 14, Fig. 17), however, no difference between control and PSA. In Group B there was more movement after PLA relative to control (p<0.001) and PSA (p<0.001, Table 14, Fig. 17). There was also more hyoid movement after PSA

relative to control (p=0.006, Table 2, Fig. 17). Additionally, there was more hyoid

movement during control feedings in Group A relative to Group B (p<0.001, Table 14,

Fig. 17). There was also more hyoid movement after PLA in Group B relative to Group A

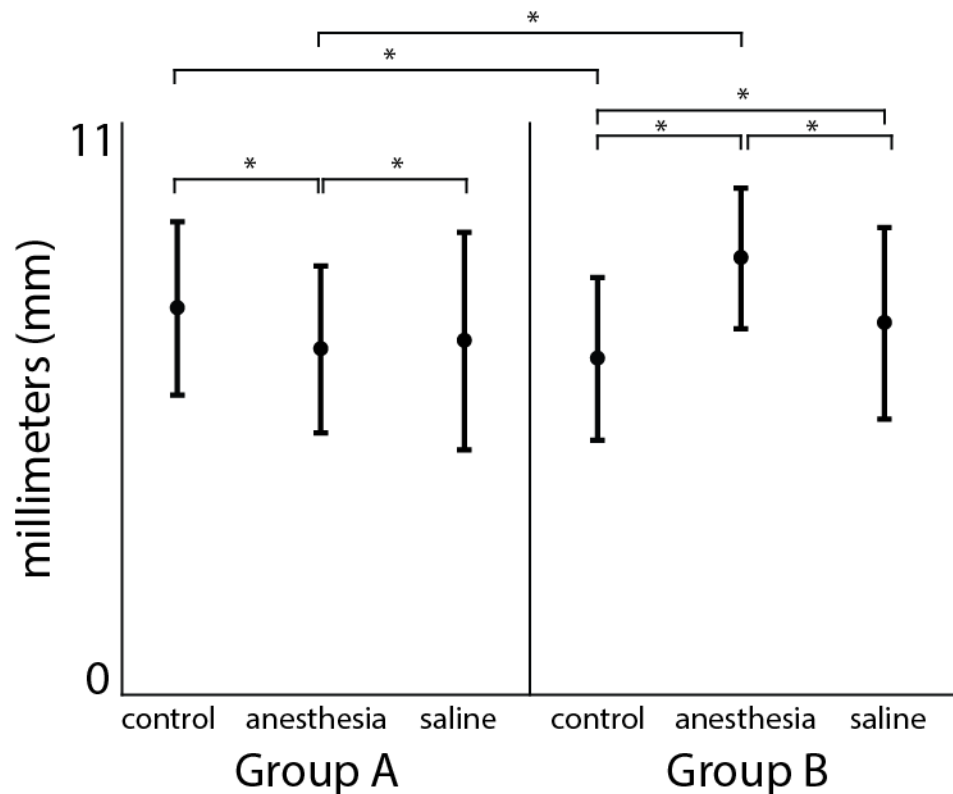(p<0.001, Table 14, Fig. 17). There was no difference between groups after PSA.

Figure 17 Range of hyoid movement during the pharyngeal swallow. On the left is
the range of hyoid movement in Group A between treatments (n=4). On the right is the
range of hyoid movement in Group B between treatments (n=4). In Group A there was
significantly less movement after palatal anesthesia (PLA) compared to control (p<0.001)
and saline (PSA, p=0.015). In Group B there was more movement after PLA compared to
control (p<0.001) and PSA (p<0.001). There was also more hyoid movement after PSA
relative to control (p=0.006). There was significantly less movement during control
feedings in Group B compared to Group A (p<0.001). There was significantly move
movement during PLA feedings in Group B compared to Group A (p<0.001).

There were significant differences in anterior hyoid movement based on

treatment, group and their interaction, all with p<0.001. There was more movement after

PLA relative to both control (p<0.001) and PSA (p<0.001) with no difference between

control and PSA. There was also more movement in Group B relative to Group A (p<0.001).

In Group A no differences existed between treatments. In Group B there was more movement after PLA relative to control (p<0.001) and PSA (p<0.001) but no difference between control and PSA (Table 14). There was no difference in anterior hyoid movement during control or PSA feedings in Group A compared to Group B. There was more movement after PLA in Group B compared to Group A (p<0.001).

There were significant differences in superior hyoid movement based on treatment, group and their interaction, all with p<0.001. There was significantly more elevation after PLA compared to both control (p<0.001) and PSA (p<0.001) with no difference between control and PSA. There was also more movement in Group B than Group A (p<0.001).

In Group A there were no differences between treatments. In Group B there was more movement after PLA relative to control (p<0.001) and PSA (p<0.001) with no difference between control and PSA (Table 14). There was no difference between control feedings or PSA feedings in Group A and B. There was more movement in the PLA feedings in Group B than Group A (p<0.001).

There was a significant difference in the time between TH and CT overall with regard to group (p<0.001) and treatment (p<0.001). There was significantly more time between TH and CT after PLA relative to control (p=0.024) and after PSA relative to control (p=0.021). There was no significant difference between PSA and PLA. There was also more time between TH and CT in Group B relative to Group A overall (p<0.001, Table 2).

DISCUSSION

Swallowing kinematics and airway protection following palatal anesthesia

PLA affected kinematics and airway protection differently in Group A and Group B. In Group B, where the pigs did not latch onto the nipple after PLA, the increased hyoid elevation could be due to the increased range of tongue motion and jaw opening. The tongue is an elevator of the hyoid bone and its increased range of motion could have caused the hyoid bone to elevate further(Matsuo and Palmer, 2010). The muscles responsible for jaw opening in Group B are also hyoid elevators (Matsuo and Palmer, 2010; Pearson Jr et al., 2012; Pearson et al., 2011b). This increased hyoid elevation and less time between the oral and pharyngeal muscle activity could have provided a slight increase in airway protection(Medda et al., 2003).

After PLA in Group A, who after PLA were still able to latch, despite the reduced hyolaryngeal elevation, the airway was more protected. This suggests that the response after PLA was to provide better airway protection than during control feedings. There are many other ways that mammals protect their airway during swallowing and not all of these airway protection mechanisms were measured in this study.

Swallowing kinematics and airway protection following palatal saline

Although initially intended as a sham treatment, it is not clear if the PSA was truly a sham. PSA treatment could have another effect on sensory nerves: 1) anesthesia, from pressure on these sensory nerves, or 2) pain, consistent with clinical observation. It is unknown how long either effect could last following PSA, confounding the interpretation of these results.

With PSA, Group A had significantly more airway protection which may stem from reduced time between oral and pharyngeal muscle activity. With PSA, Group B had no change in airway protection, perhaps due to increased hyoid elevation. This was a physiologically different response between the groups, not just to anesthesia, but also to saline. Yet the airway remained protected despite significant kinematic differences, demonstrating that the infant was able to adapt to both treatments. There are multiple mechanisms of airway protection, and unfortunately, not all could be measured in this study.

While the significant temporal difference in TH and CT activity with the PSA treatment were statistically significant, it was only a 5 ms difference. This small magnitude is likely not biologically significant, and may not reflect adaptation to the altered palatal sensation.

Overall Impact of Sensory Modification

Airway protection, although increased in both groups and treatments, was not abnormal prior to treatment. It is unknown how PLA or PSA could affect airway protection in an animal model with significant penetration or aspiration. Applying these treatments to a pathologic model could answer this question(Ding et al., 2013a).

Trigeminal sensation influenced the motor output in the brainstem. Alternatives exist for the mechanism responsible for this result. The trigeminal sensory nerves that project to the nucleus tractus solitaries (Capra, 1995) could influence the nucleus ambiguus output and therefore the pharyngeal swallow. A second hypothesis is that descending input from the cortex to the swallowing CPG influences the motor output for

the pharyngeal swallow (Lowell et al., 2008). Further study is necessary to understand fully the mechanisms underlying these differences.

Limitations of the Study

We only evaluated EMG data from a few muscles of many that are active during swallowing. We also evaluated the kinematics of a few key structures, but not all that are in motion during a swallow. There are other airway protection mechanisms during swallowing that we did not measure, such as vocal fold closure and epiglottis movement.

CONCLUSION

This study is the first to document the role of sensory information as a functional link between the oral and pharyngeal phases in the infant suck-swallow. Further studies are needed to determine if there are other regions in the oral cavity where sensation is also linked to pharyngeal function. These results strongly support the use of oral sensory stimulation devices, but more research is needed to quantify the impact that sensory stimulation may have on disordered swallowing.

CHAPTER 6: KINEMATICS OF THE PHARYNGEAL SWALLOW FOLLOWING PALATAL ANESTHESIA IN INFANT PIGS: EVIDENCE OF MOTOR LEARNING

ABSTRACT

Adaptive motor learning during swallowing occurs in adults, but its existence or prevalence in infants is unknown. Understanding the underlying motor control of infant swallowing will facilitate the effectiveness of rehabilitation strategies. A reduction in palatal sensation due to local anesthesia (PLA) impacts swallowing and produces an increase in both hyoid elevation and jaw opening during swallowing. To examine the potential for motor learning during infant feeding, the time course of the response of swallowing to palatal anesthesia was measured in four pigs while feeding and recording lateral videofluoroscopy. On one day they were fed and recorded to assess normal kinematics and on a second day they were given PLA and fed every 20 minutes until sucking returned and then an hour later. We quantitatively evaluated hyoid and mandibular movements as well as airway protection over time after PLA for evidence of motor learning. All pigs had, on average, more hyoid elevation and jaw opening 30 minutes after PLA. None of the pigs showed signs of adaptive learning over the course of the feeding session 30 minutes after PLA. In three of four pigs, when sucking returned 50-90 minutes after PLA injection, the hyoid remained elevated early in the feeding and decreased over time. Despite this change, jaw opening immediately returned to baseline levels. This is the first evidence that infant mammalian swallowing may also be capable of motor learning. More studies are needed to see if suprahyoid EMG amplitude is correlated to increased hyoid elevation which may explain these findings.

INTRODUCTION

Swallowing is a complex behavior involving many muscles and relying on sensory input from several branches of cranial nerves (Humbert and German, 2012; Steele and Miller, 2010). The relationship between sensory input and motor output during swallowing is not fully understood (Humbert and German, 2012; Steele and Miller, 2010). Swallowing dysfunction can result in nutritional deficiency, delay growth and even lead to aspiration pneumonia (Langmore et al., 1998; Tutor and Gosa, 2012). For this reason, understanding the motor control of swallowing and how it ensures adequate airway protection during swallowing is vital. In the future, this knowledge can be used to develop rehabilitation strategies for those with swallowing dysfunction.

Recently, principles of motor learning have been applied to swallowing research which will lead to a further understanding of the motor control of the pharyngeal swallow (Humbert and German, 2012). In order to demonstrate motor learning, there must be evidence of a feed-forward mechanism of adaptation. In a feed-forward loop, the system predicts the next movement before it acquires sensory feedback (Humbert and German, 2012). Feed-forward loops have recently been demonstrated in hyolaryngeal elevation during swallowing in adults (Humbert et al., 2012b), but no research has investigated this phenomenon in infants. If infants also have feed forward loops, then it could be the basis of new swallowing rehabilitation techniques.

Demonstrating feed-forward mechanisms of adaptation during the pharyngeal swallow would indicate that the system is capable of motor learning (Humbert and German, 2012). In Humbert et al.'s studies of motor learning in pharyngeal swallowing, one outcome measure that demonstrated motor learning was hyoid elevation. Adaptive

motor learning was observed as healthy adult participants incrementally increased hyo-laryngeal elevation to counter the perturbation which was an electrical stimulation applied to the anterior neck. After several swallows, the hyo-laryngeal elevation returned to baseline levels. Adaptive motor learning was again demonstrated during a "catch trial" when the perturbation was abruptly removed and there was more hyo-laryngeal elevation than baseline levels during that swallow. The perturbation was applied again for several trials and then removed abruptly, this time for several trials. When the perturbation was initially removed, there was higher hyo-laryngeal elevation than during baseline, as was seen during the catch trial, but over time levels returned to baseline. This showed error-based learning.

Palatal sensation plays a significant role in the motor control of the normal infant pharyngeal swallow (Chapter 4). A recent study found that when the palate is anesthetized in infants, half of infants respond by no longer being able to suck and having extreme jaw movements, more hyoid elevation and little surface tongue deformation during the pharyngeal swallow (Chapters 3 and 4). These adaptations are necessary in order to ensure adequate airway protection and execute a pharyngeal swallow (Chapters 3 and 4). In these infants, it is unknown if or how hyoid elevation, jaw opening and airway protection adapts over time to the reduced sensory input from the hard palate. It is also unknown how their feeding changes when the sensation begins to significantly return as the anesthetic wears off one hour post-injection (Chapter 2). The extent of hyolaryngeal elevation is linked to aspiration risk, therefore, we are particularly interested in understanding what sensory information is required for the motor output to the muscles that will elevate the hyoid and larynx (Steele et al., 2010; Tutor and Gosa, 2012).

In this study we investigated adaptive motor learning in an infant pig model. The perturbation was a local anesthetic injection to the palate which would reduce palatal sensation. As an outcome measures we evaluated hyoid elevation, mandibular movement and airway protection. Since the mechanism of the increased hyoid elevation during swallowing after PLA is still unclear, we had two alternative hypotheses that would both suggest that motor learning was occurring. The first hypothesis was that, like Humber et al. after the perturbation we would observe (1) an initial spike in hyoid elevation that would return to baseline over time after PLA. When the anesthetic had worn off significantly after one hour, we hypothesized that sucking would return and there would be (2) less hyoid elevation that would return to baseline after several swallows.

Our second alternative hypothesis was that hyoid elevation was an indirect measure of the amplitude of suprahyoid muscle motor output. Further studies need to demonstrate a direct correlation between hyoid elevation and amplitude of EMG activity from the suprahyoid muscles during infant feeding. If this was the case, then we hypothesized that (1) hyoid elevation would increase from baseline over time and then reach a constant level, and that (2) when the perturbation was removed hyoid elevation would at first remain elevated and over the course of multiple swallowing cycles decrease to baseline levels. This would be the same trend that would be expected if suprahyoid surface EMG was recorded during the experiments described in Humbert et al (Humbert et al., 2012b).

Instead of abruptly removing the perturbation, we waited until one hour after the injection, when we predicted that the local anesthetic has worn off enough so that sucking would return (Chapter 2). Since we used local anesthesia, it was impossible to have a

catch trial where the perturbation was abruptly removed for only one swallow. Increased jaw opening was hypothesized to be correlated to increased hyoid elevation. We further hypothesized that higher hyoid elevation would be correlated to larger jaw movements and lower rates of penetration of the airway during the swallow.

MATERIALS AND METHODS

Experimental Procedure

Five infant pigs were used in this study as a model of infant swallowing, although one was later excluded. They weighed 3-5 kg (8-12 lbs) and were 2-3 weeks old. For a week prior to the study the pigs were trained to feed pig milk formula (Land O Lakes Solustart pig milk replacer, St. Paul, MN) from a bottle with a pig nipple (Nasco, Fort Atkinson, WI). Four days prior to the study radio-opaque markers were placed in the midline of the tongue deep to the foramen cecum, in the right and left maxillary gingiva, the midline of the anterior hard palate, the junction of the hard and soft palate, the soft palate, the posterior pharyngeal wall and the arytenoids cartilages. These pigs also underwent a brief surgical procedure to attach a radio-opaque marker to the superficial fascia covering the hyoid bone and another to the thyroid cartilage. Following the surgical procedure the animal was given ampicillin (0.16 mL of 250 mg/mL) and buprenorphine (0.17 mL of 0.3 mg/mL) twice daily and metacam (0.1 mL of 5 mg/mL) once daily. These markers have been previously shown to not affect swallowing kinematics (German and Franks, 1991; Thexton, 1981).

On day 1 of the study the infant pigs were given Isoflurane until deep/surgical general anesthesia was achieved. A radio-opaque Weck Hemoclip Ligating Clip (Pilling

Weck, Research Triangle Park, NC) was attached to the epiglottis using an intraoral approach (Crompton et al., 2008) and then local anesthesia injections were given to the hard palate. 0.5 ml of 0.5% bupivacaine hydrochloride (Marcaine^{TM}, 5mg/ml, Hospira, Inc. Lake Forest, IL) was administered as a nerve block to the right and left greater palatine nerves and the nasopalatine nerve to fully block sensation to the hard palate. After the treatment was administered the animal was taken off isoflurane and recovered within ten minutes.

Starting 30 minutes after the local anesthesia injection, the pigs were fed freely while recording lateral videofluoroscopy at 60 frames per second. The videofluoroscopy unit (Allura FD20, Philips Healthcare, Best, The Netherlands) was equipped with a high-resolution digital flat-panel detector (154x154 micron pixel-pitch, 30x40 cm). The milk formula they were fed was mixed with barium in the ratio of 8 ounces of milk to 1/3 cup barium powder. The milk was also heated in boiling water for at least one minute prior to the study. Controlling for the amount of barium and temperature is important since those factors have been shown to alter swallowing kinematics (Cichero et al., 2010; Ebihara et al., 2011). The pigs that continued in the study were non-sucking infants, or unable to suck after local anesthesia (Chapter 3 & 4). One pig in the study was excluded since it was able to adapt immediately to the anesthetized palate and suck which resulted in a total sample size of four. After non-sucking behavior was observed, the pigs were fed every 20 minutes until they started sucking, as evidenced by rhythmic vertical tongue movement and reduced mandibular movement. The pigs were fed once more after suckling was observed, one hour later. For each recording a minimum of 20 swallows or five 15 second recordings were done. On day 2 of the study there was no treatment

administered and the animals were fed twice, 2 hours apart, while recording lateral

videofluoroscopy.

Data Collection

The 14-bit video images were exported in DICOM format and then converted to

AVI. The video recordings were analyzed using MaxTRAQ Version 2.2.4.1 (Innovision

Systems, Inc.). Each swallow for a maximum of 20 swallows per feeding session were

scored on the Infant Mammalian Penetration-Aspiration Scale (IMPAS) to evaluate

protection of the airway (Holman et al., 2012a). These scores were plotted over time to

observe changes due to adaptation.

The markers placed on the hyoid, hard/soft palate junction and the hard palate

were digitized to find their Cartesian coordinates in each frame of the videos. The

menton, or most anterior and inferior point on the mandible was digitized by hand frame

by frame to find its x and y coordinate. The movements of the hyoid and menton were

graphed over time to observe adaptation in the extent and rhythmicity of their

movements.

Data Analysis

The distance of hyoid elevation for each swallow was calculated by determining

frame where the hyoid started to elevate and the frame where the hyoid was maximally

elevated and then calculating the distance between them. We determined when the hyoid

started to elevate and was maximally elevating by plotting the distance between the hyoid

and hard/soft palate junction marker over time. The total distance moved by the hyoid per

swallow was corrected for magnification by using a radio-opaque 12.69mm metal sphere placed on the animal's neck under the bandage. The hyoid elevation per swallow was graphed over time to observe adaptation over the course of a feeding.

Similarly, we graphed the distance between the menton and hard palate marker over time to determine when jaw opening started and ended during the swallow. The start of hyoid elevation would almost always occur during jaw opening. From these measurements we calculated the total distance the jaw opened during each pharyngeal swallow and graphed that over time to observe adaptation over the course of a feeding.

RESULTS

Overall Pattern of Behavior

As expected, 30 minutes following PLA four of the five pigs were not able to suck, had increased hyoid elevation and increased jaw opening. The one pig that was sucking was excluded from this study because the goal was to determine adaptation of the non-sucking response. The time point where the pigs were able to suck again occurred at 50 minutes post-injection in Pigs A, C and D and 90 minutes post-injection in Pig B. In Pigs A, C and D we noticed a trend where there was initially increased hyoid elevation at the beginning of control feeding sessions.

Kinematic changes in jaw and hyoid movement

In all pigs there was a constant level of jaw opening during both control feedings. In Pigs A, C and D, hyoid elevation was initially higher for the first few pharyngeal swallows. In Pig B, hyoid and jaw opening movements were constant.

During the first feedings in all the pigs after PLA there was more hyoid elevation and jaw opening relative to control feedings. There was no noticeable change in the extent of hyoid elevation and opening over time (Figs. 17-20). This high level of hyoid elevation and jaw opening lasted for 3 total feeding sessions in Pig C (until 90 minutes post-injection), but only for 1 feeding session in Pigs A, B and D (until 50 minutes post-injection). In Pigs A, C and D, when sucking returned after PLA, hyoid elevation was initially higher than control feedings, but not higher than the previous feeding. The return to baseline hyoid movement occurred over 20 total swallow cycles in that feeding session. The feeding sessions one hour later appeared very similar to control feedings. In Pig C where there were three feeding sessions recorded with no sucking after PLA, the second and third feedings still had more hyoid elevation than during control feedings.

Jaw opening did not exhibit the same trends. During control feedings, when the pigs were sucking there was very little jaw opening with no changes over the duration of the feeding. During the PLA treatment, when the pigs were not sucking there was significant jaw opening, with no changes over the duration of the feeding. With resumption of sucking, in all pigs, jaw movement immediately returned to baseline.

Airway Protection

A score of 1 on the IMPAS is when milk passes to the esophagus with no milk penetrating the airway (Holman et al., 2012a). A score of 2 on the IMPAS is when milk penetrates the airway during the pharyngeal swallow, but stays above the vocal folds and is ejected out of the supraglottic space before the next swallow (Holman et al., 2012a). The IMPAS score in control animals was a variable ratio of scores of 1, 2 and 3 with no score over 3. With PLA, the scores were immediately either the same (Pig D), lower

(Pigs B and C) or higher (Pig A) than the control feedings while hyoid and jaw movement was increased. In pigs A-C, when they began sucking after PLA, the IMPAS scores were higher (higher ratio of scores of 2) compared to when they were not sucking after PLA. Pig D had almost exclusively scores of 1 during all feedings.
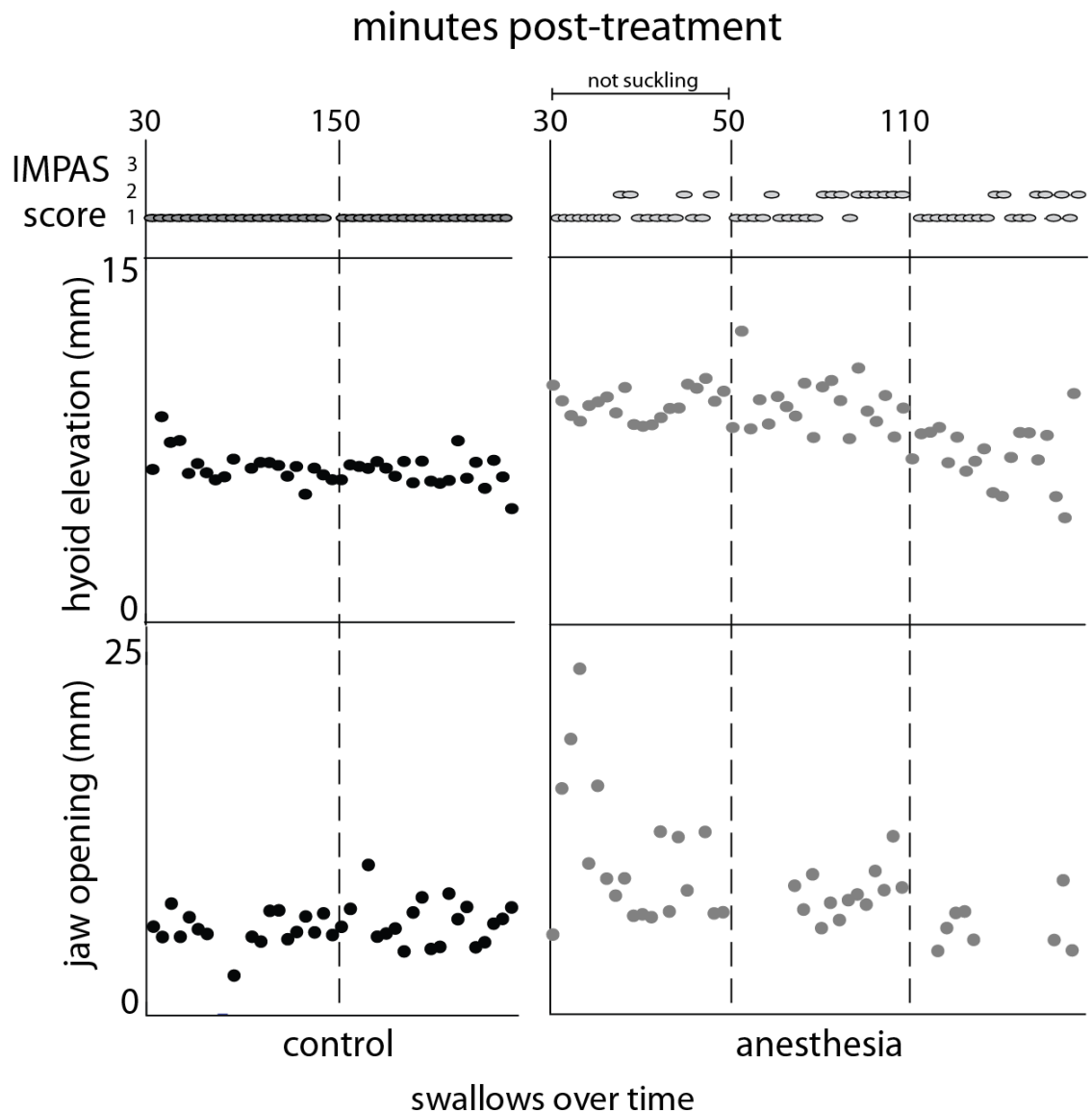

Figure 17 Pig A hyoid elevation, mandibular excursion and IMPAS data over time. On the left, in black, is data from the two control feedings. On the right, in grey, is the data from the feedings after palatal local anesthesia. Each point represents a swallow and they are plotted over time.

Figure 18 Pig B hyoid elevation, mandibular excursion and IMPAS data over time.
On the left, in black, is data from the two control feedings. On the right, in grey, is the data from the feedings after palatal local anesthesia. Each point represents a swallow and they are plotted over time.

Figure 19 Pig C hyoid elevation, mandibular excursion and IMPAS data over time.
On the left, in black, is data from the two control feedings. On the right, in grey, is the
data from the feedings after palatal local anesthesia. Each point represents a swallow and
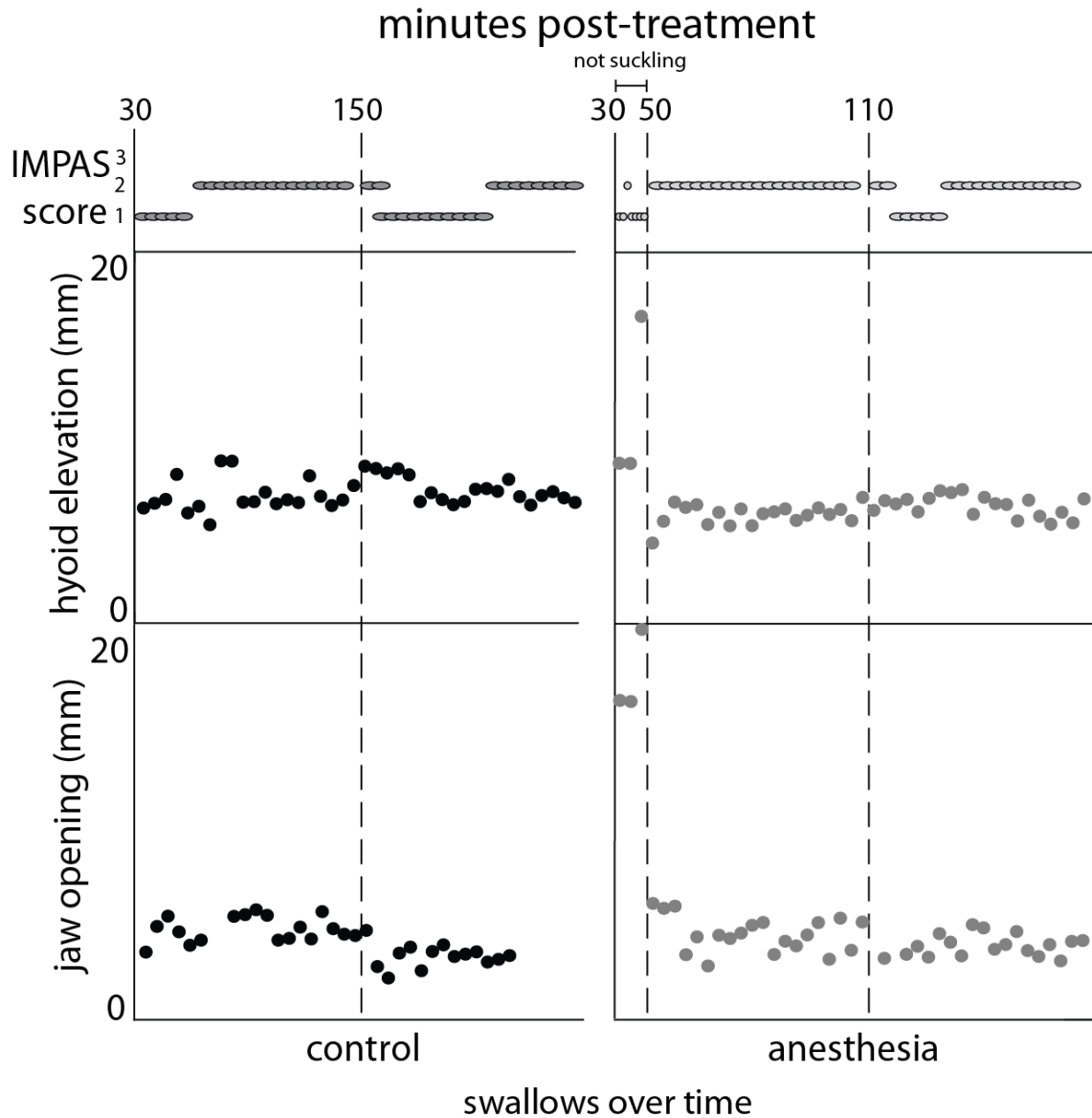they are plotted over time.

Figure 20 Pig D hyoid elevation, mandibular excursion and IMPAS data over time. On the left, in black, is data from the two control feedings. On the right, in grey, is the data from the feedings after palatal local anesthesia. Each point represents a swallow and they are plotted over time.
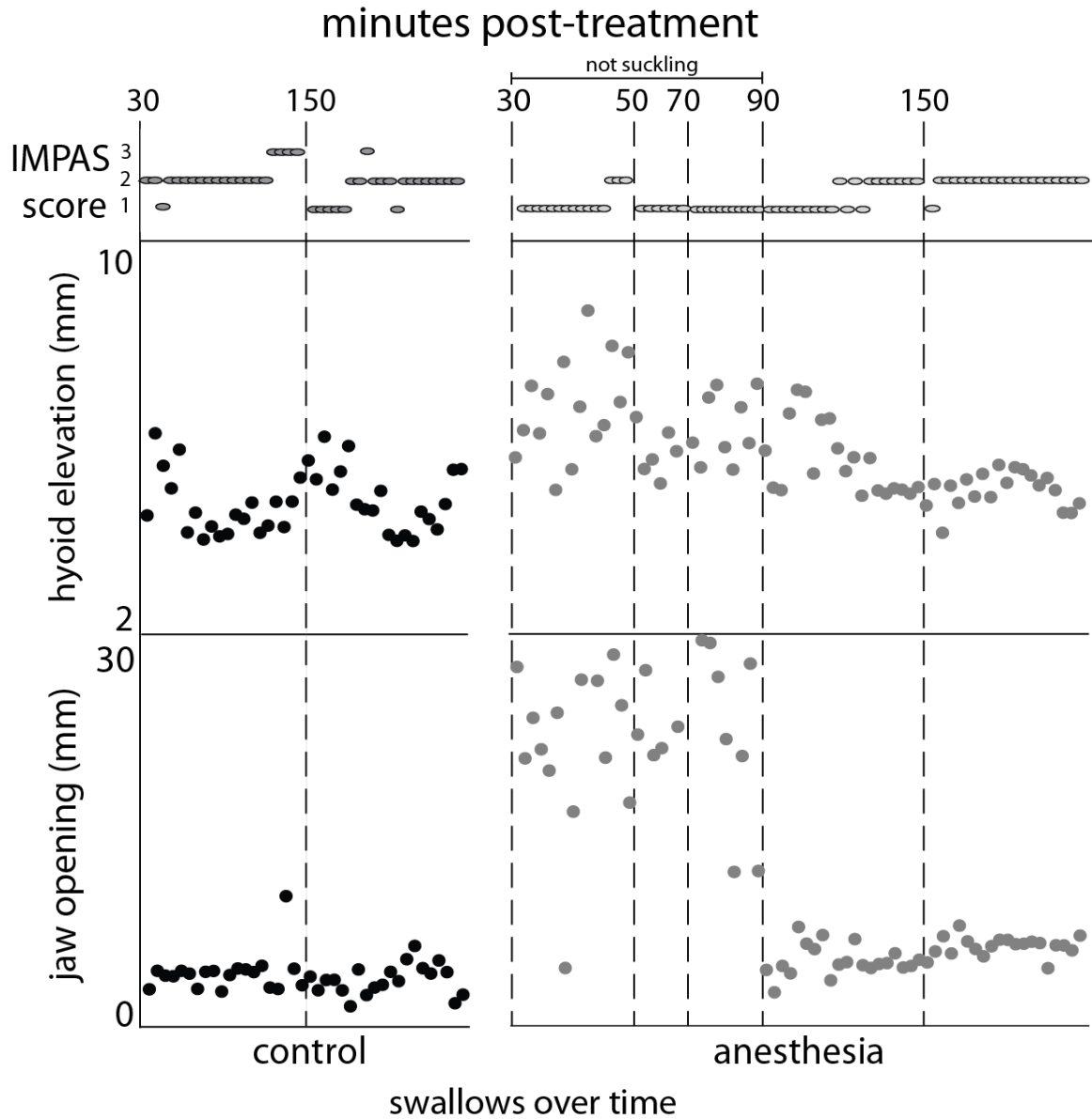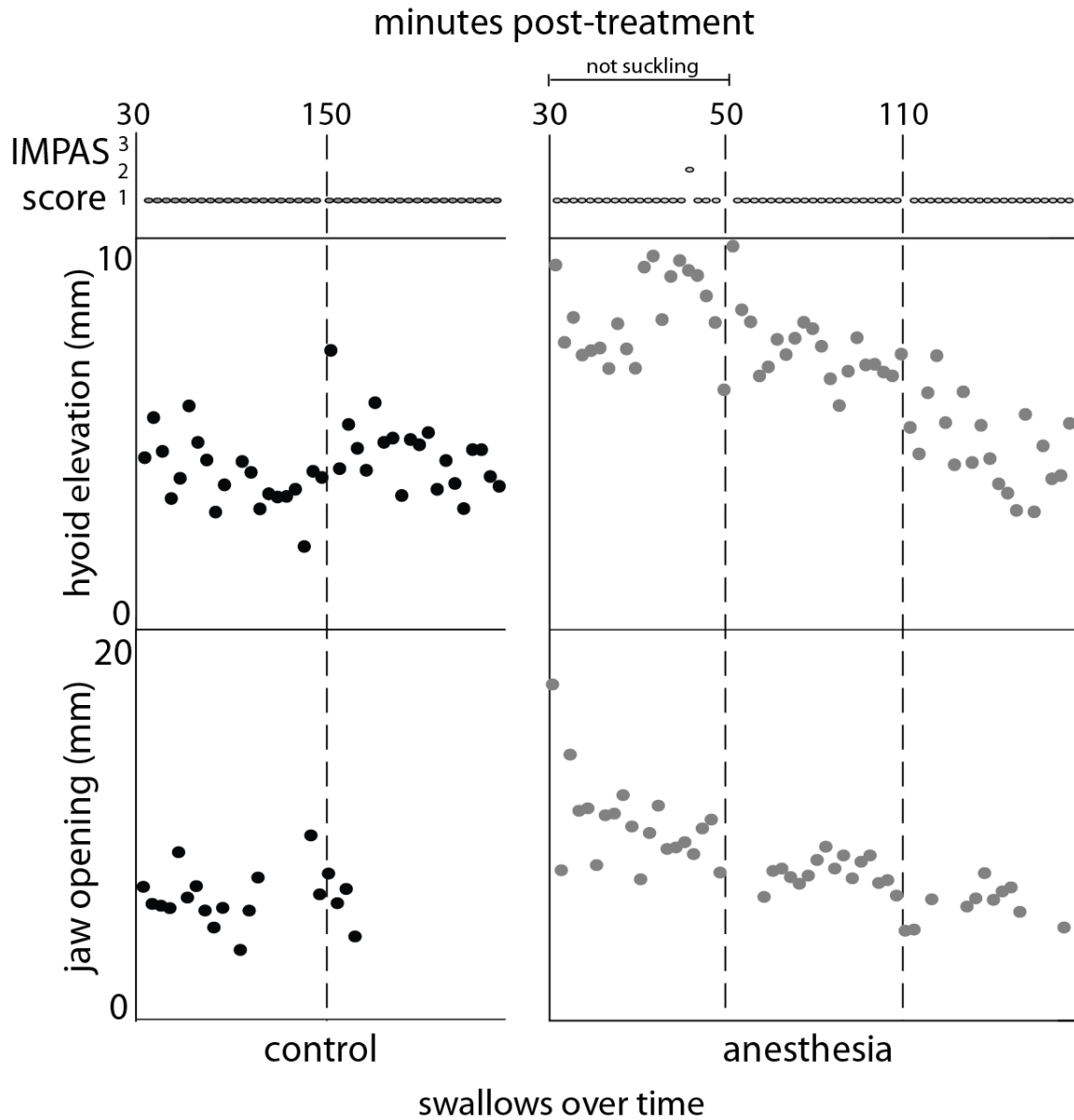
DISCUSSION

Overall patterns of behavior

None of the four animals included in this study were able to suck 30 minutes post-local anesthesia injection. The ability to suck returned after 50-90 minutes, which is consistent with data that suggests oral reflexes return following palatal bupivacaine hydrochloride nerve blocks 1 hour post-injection[6] (chapter 2). We hypothesize that the reason sucking returned was because the local anesthetic had worn off enough so it could be initiated again. If this hypothesis were correct, the return of sucking, likely due the reduced or absent impact of the anesthesia, suggests that there is a threshold for oral sensation necessary to elicit a suck cycle. Once the sensation to the palate returns to a point where the threshold can be reached, then suck cycles are initiated. Alternatively, the system could have adapted and been able to initiate suck cycles even though palatal sensation was still reduced and had not yet reached this threshold.

During control feedings in three animals, we noted that the hyoid was more elevated earlier in the feeding, but this trend was not seen when we evaluated control data from eight other infant pigs. It is, however, consistent with data about feeding rates in general, where the beginning can be faster & more vigorous at the beginning of a feeding session (German et al., 1997). Hyoid movement was not correlated with jaw opening during control feedings. This implies that hyoid movement was most likely due to elevated tongue, (geniohyoid, hyoglossus, styloglossus or genioglossus muscle), activity. Further data analysis of control feedings from eight pigs revealed that hyoid elevation is also not correlated with the frequency of swallowing, although swallowing frequency is

---

[6] Holman et al- in review at Journal of Veterinary Dentistry

also higher earlier in a feeding session[7]. Another explanation could be fatigue in the muscles that elevate the hyoid over time reduces the hyoid's elevation. Additional studies evaluating electromyography data could test these various hypotheses.

Evidence of adaptive motor learning

In order to asses if there was motor learning, we looked for evidence of feed-forward loops during the pharyngeal swallow (Humbert and German, 2012). Our results for hyoid movement partially confirmed the second alternative hypothesis. When the perturbation was given, which in this case was palatal anesthesia, the hyoid elevated higher which we hypothesize was due to an increase in the force generated in the suprahyoid muscles. We predicted that over time the hyoid would gradually elevate more and more, away from baseline levels. We did not see this trend. Over the course of the feeding session with no sucking, the hyoid remained elevated, as predicted, with no signs of returning to baseline. We hypothesize that this is due to the force in the suprahyoid muscles remaining elevated.

When the pigs started to suck again, as predicted, hyoid elevation remained elevated relative to baseline or control levels and then decreased over the course of the feeding session towards control levels. If there was no feed-forward loop the hyoid would have immediately returned to baseline. The decrease over time within a feeding session could be further evidence of a feed-forward mechanism of motor learning in the suprahyoid muscles, or it could be a normal pattern due to muscle fatigue or satiation as discussed previously. In all three pigs with higher levels of hyoid elevation relative to

---

[7] Unpublished data, SD Holman

control after the return of sucking (Pigs A, C and D), the last feeding had normal levels of hyoid elevation.

The jaw opening movement did return abruptly to baseline in all cases. This finding rejected our hypothesis that jaw and hyoid movements were directly correlated. EMG from other hyoid muscles may help address which muscles are primarily controlling hyoid movements after PLA.

Even though we did not see a gradual increase in hyoid elevation over time following the anesthesia, it is possible that since the perturbation was not as abrupt in onset as with previous motor learning studies, that we missed this adaptive learning process. Since we did see that hyoid elevation remained increased even after the anesthetic wore off enough that sucking and mandibular movement returned to baseline levels, we believe that there is evidence of adaptive motor learning in response to the sensory perturbation.

An alternative hypothesis for why the hyoid remained elevated after the animal began sucking is that enough sensation had returned to initiate suck cycles, but the sensation that was still absent was enough to still disrupt hyoid elevation during the pharyngeal swallow. Even though we saw that there was a return to normal mandibular movement, those suck cycles may still be affected in other ways, such as being longer than usual or having changes in surface tongue deformation.

According to this latter hypothesis there is also a threshold of palatal sensation needed for normal hyolaryngeal elevation. Even though suck cycles could be initiated, there was insufficient palatal sensation for normal hyoid elevation. In the one pig that did

immediately return to normal levels, that pig may have reached the threshold for normal

hyoid elevation more quickly due to a faster metabolism of the anesthetic.

Airway protection

In three of the animals the airway protection was higher (lower IMPAS score)

with PLA and high jaw movement. The IMPAS score dropped, indicating less airway

protection when the animals returned to normal sucking. Thus, the increased hyoid

elevation when the pigs were not sucking after PLA could be correlated to heightened

airway protection. This follows findings from other studies in adults (Steele et al., 2010)

and the previous study from infant pigs which found this trend although it was not

statistically significant (Chapter 4). It should be noted that IMPAS scores of 1 and 2 are

typically seen during normal feeding, and that even though a score of 2 is a more severe

condition, it is still not pathological (Holman et al., 2012a). Future studies of dysphagic

animals would be needed to see if there is a clinically significant increase in airway

protection following PLA.

Future directions

A number of changes in study design could clarify the significance of the data

described here. A smaller time interval between feeding sessions would increase accuracy

as to the timing of the change to sucking, as the anesthetic significantly wears off after 1

hour. Future studies should also give the anesthesia treatment on the first day of

experiments and then use the same time intervals for the control feeding sessions on the

following day. In addition, the same volume of milk could be given at each feeding to

account for satiation. Ideally there would be many more feeding sessions before the anesthetic wears off, but there is also the risk that the pig will be satiated and refuse to be fed. A better understanding of the role of satiation and muscle fatigue over time during a control feeding session would clarify these results.

Another method to test if this is a feed-forward mechanism would be to administer anesthesia more abruptly using a topical anesthetic, however it would be impossible to only anesthetize the palate or any other oral structure without also anesthetizing the tongue and oropharynx. It would also not be possible to abruptly remove it. Sensory stimulation could be a useful tool for investigating how oral sensory receptors influence motor learning in the pharyngeal swallow. It is not known if oral sensory stimulation (not oropharyngeal) can influence the pharyngeal swallow, but if it does, it could be abruptly given and removed to test for motor learning by demonstrating feed-forward loops.

If infant swallowing is capable of adaptive motor learning, further research will be needed to understand what sensory input is needed in order to have error based learning. It is possible that by anesthetizing the hard palate, there is not enough sensation to have error based learning. There are many other branches of sensory nerves that are known to influence the motor control of the pharyngeal swallow (vagus, glossopharyngeal and facial nerves). By using anesthetics or by ligating nerves we can further understand which sensation is needed to have adaptive motor learning.

Conclusions

In conclusion, this preliminary study of adaptive motor learning during infant pig pharyngeal swallowing has demonstrated that motor learning may be possible. Further studies should evaluate how infant pig feeding adapts over time during control feedings so that studies of motor learning can provide clearer conclusions. Additional studies using different types of sensory and motor perturbations could help further our understanding of the sensorimotor control of infant swallowing. More research is needed in this field since demonstrating motor learning in infant swallowing could help make dysphagia rehabilitation strategies significantly more effective.

CHAPTER 7: DISCUSSION

We conclude that palatal sensation in infants plays an important role in the initiation and motor control of the suck-swallow and pharyngeal swallow cycles. This demonstrates an important role for trigeminal sensation in the sucking and swallowing CPGs in the brainstem. This is a significant advancement for dysphagia research since the oral cavity is easily accessible in infants and would be the ideal place to target in dysphagia rehabilitation.

We were able to develop a novel method of quantifying the incidence of penetration and aspiration in the infant pig feeding model which will allow us to further investigate questions about sensory and motor relationships during swallowing in infants. The scale was a 7-point scale based on the 8-point clinical scale. We called the scale the Infant Mammalian Penetration-Aspiration Scale (IMPAS). We are currently in the process of evaluating the incidence of penetration and aspiration in an infant pig that has had single and double superior laryngeal nerve (SLN) lesions. Being able to quantify the incidence of penetration and aspiration will help make the research have translational significance. Another study is evaluating the rehabilitative potential of thickened fluids on those pigs that have had the SLN lesion. Using the IMPAS the researchers can evaluate the change in incidence before and after rehabilitation. The development of a penetration-aspiration scale for the infant pig model that is based on the clinical scale will help to expand the use of animal models in dysphagia research. This will also help us to quantify the value of rehabilitative interventions.

We also developed a novel method for measuring the duration of action of local anesthesia in an animal model. It is difficult to assess the duration of anesthesia in infants and animals since we cannot ask them when a local anesthetic wears off like you can do

in adult humans. Prior to this study, the duration of local anesthetics in infants had only been estimated based on pharmacokinetic properties and indirect measures of pain after a surgical procedure (Jonnavithula et al., 2007; Jonnavithula et al., 2010). In veterinary dentistry bupivacaine hydrochloride is often used to reduce post-operative pain and the amount of general anesthesia needed for surgical procedures. It is helpful to know that soon after surgery that oral reflexes are not affected and that they should be able to eat without difficulty. Being able to estimate the duration of action of local anesthetics in infant animals will allow them to be used as a research tool for future studies of oral motor functions. Local anesthetics are reversible and can be used on both sensory and motor nerves. Local anesthetics have already been used in pigs to study mastication (Huang et al., 1993). The future use of local anesthetics in dysphagia research can help us address many basic neurophysiological questions about the relative importance of the different sensory and motor nerves involved in swallowing.

We found that reduced palatal sensation influences rates of sucking and swallowing. We were surprised to find that after reducing palatal sensation, half of our sample size could no longer initiate sucking cycles at all and developed an alternative feeding strategy entirely. This alternative feeding strategy showed significantly more mandibular and hyoid movement during oral transport and the pharyngeal swallow. There was also abnormal tongue activity that was reflected in its EMG activity and kinematics. These oral transport cycles were highly inefficient and caused swallowing frequency and swallowing frequency to be significantly reduced. In this study we demonstrated a direct connection between palatal sensation and the suck CPG since the frequency and ability to produce normal cycles was significantly affected. We also demonstrated a link between

altered palatal sensation and swallowing initiation since after palatal saline swallowing frequency was higher in half of the pigs. This data supports the fact that trigeminal sensory nerves influence the pharyngeal swallowing threshold that is largely controlled by sensory input from the facial, glossopharyngeal and vagus nerves.

We also demonstrated that reduced palatal sensation influences pharyngeal swallowing kinematics and airway protection. When pigs sucked after palatal anesthesia, they had reduced hyoid elevation and when they were not sucking they had heightened hyoid elevation. After anesthesia all the pigs had more airway protection, having less incidence of penetration. This was only statistically significant in the pigs that were sucking. There were many other ways that the swallows compensated for the altered oral sensation by anesthesia and saline that were different in the pigs that were sucking after anesthesia and not sucking after anesthesia. It is unclear how the swallow would have changed if the control animals had swallowing dysfunction. Future studies should investigate if the alternative oral transport strategy in the pigs that could not suck after anesthesia could help protect the airway in dysphagic infants that are aspirating.

We also investigated how some of the extreme changes in oral feeding behavior and pharyngeal motor function changed over time after palatal anesthesia. During these studies we saw that after sucking returned, the hyoid elevation during the pharyngeal swallow was still affected. More studies need to be done to determine if this was evidence of motor learning or if palatal sensation was still reduced enough to affect the swallow even after the suck cycles were no longer as severely affected. No other study of infant swallowing as looked at swallows over time, and the use of an animal model

allows us to collect a lot more swallow cycles and more frequently over the course of the day or multiple days than would be possible in human infants due to radiation exposure.

More studies are needed to understand the relative input from the perioral region and the anterior tongue which are also innervated by the trigeminal nerve and contact the nipple during swallowing. We also do not know if stimulating sensation from these regions can influence the pharyngeal swallow, so more studies are need to understand the nature of these connections. Stimulation studies would help make these findings translational. Neurophysiology studies are also needed to understand the exactly connections between the branches of the trigeminal sensory nerves that synapse in the brainstem and how they are able to influence the NA. If we can understand these connections better then we can understand how to rehabilitate patients with neurological or anatomical differences.

Overall, two novel methods were developed to advance animal models of dysphagia, and we demonstrated that reducing palatal sensation has profound effects on the frequency and motor function of the oral and pharyngeal swallow. We also collected preliminary data that suggests that the infant pharyngeal swallow may be capable of motor learning. Future studies are needed to determine if manipulating oral sensory receptors can lead to novel dysphagia rehabilitation strategies in human patients.

APPENDIX I: REGIONAL VARIATION IN LENGTH CHANGE OF THE INFANT PIG GENIOHYOID MUSCLE DURING SUCKLING[8]

ABSTRACT

The geniohyoid muscle (GH) is a critical suprahyoid muscle in most mammal oropharyngeal motor activities. We aimed to use sonomicrometry to evaluate regional strain (i.e. changes in length) along the muscle during one activity, suckling in infant pigs, and compare the results to existing information on strain heterogeneity in hyoid musculature. We tested the hypothesis that during rhythmic activity, the GH would behave as a single unit. Therefore, we used pairs of sonomicrometry transducers to divide the muscle into three regions from anterior to posterior. The results showed differences in strain among the regions within a feeding cycle; however, no region consistently shortened or lengthened over the course of a cycle. Moreover, regional strain patterns were not correlated with timing of the suck cycles, neither 1) relative to a swallow cycle nor 2) to the time in feeding sequence (early or late). Previous studies showed more consistent patterns of regional variation in the sternohyoid (SH) and GH during swallowing and head shaking (Konow et al., 2010; Wentzel et al., 2011). In combination, these findings suggest that the regional changes in strain during patterned suckling behavior function to stabilize the hyoid bone, whereas the predictable regional strain differences in reflexive behaviors may be necessary for faster and higher amplitude movements of the hyoid bone.

---

[8] Holman SD, Konow N, Lukasik SL, German RZ (2012). Regional Variation in Geniohyoid Muscle Strain During Suckling in the Infant Pig. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* 317(6):359-70.This manuscript is included with permission from the publisher John Wiley and Sons.

INTRODUCTION

Movement of the hyoid bone is critical in the execution of many vital behaviors including mastication, swallowing, suckling, phonation and respiration (Thexton et al., 2007; van Lunteren et al., 1987a; van Lunteren and Dick, 2000; Yokoba et al., 2003). Given that the hyoid bone in mammals either has few or no articulations with other bones, the muscles responsible for hyoid movement must also stabilize this bone in a multiple vectored sling (Campbell-Malone et al., 2011; Crompton et al., 1975; German et al., 2011; Hiiemae et al., 2002; Pearson et al., 2011a). These muscles are commonly referred to as "strap muscles" due to their simple architecture as long, relatively thin muscles with parallel fibers. Although the strap muscles traditionally have been described as anatomically simple, recent studies have identified regional variation in the mechanical action of two strap muscles, the sternohyoid muscle (SH) and geniohyoid muscle (GH), during reflexive head shaking (Wentzel et al., 2011) and in the sternohyoid during swallowing (Konow et al., 2010). In order to fully understand muscle strain, we recorded length change and muscle activity, since muscle activity alone does not necessarily correlate to length change (Ahn et al., 2003; Konow et al., 2010). Active muscles have varying patterns of mechanical action since they can be lengthening (eccentric contraction), remaining the same length (isometric contraction) or shortening (concentric contraction).

Pharyngeal swallowing and reflexive head shaking are both episodic, non-voluntary actions, which have been characterized as reflexes (Miller, 2002; Prochazka et al., 2000). The fact that regional mechanical variation is seen in these muscles during

reflexive behaviors raises the question if regional variation also occurs in these hyoid muscles during steady rhythmic behaviors such as respiration, mastication and suckling. To address this question, we measured the regional mechanical action of the GH during rhythmic suckling in the infant pig. The GH originates on the genial tubercles of the mandible and attaches to the hyoid bone. While there are other hyoid muscles that can move the hyoid bone, anatomical evidence suggests that the GH likely is the muscle that has the greatest potential for moving the hyoid bone anteriorly (Pearson et al., 2011a).

The first hypothesis we tested was that there are predictable differences in mechanical action between the anterior, mid-belly and posterior regions of GH during suckling. It is known that the presence of rhythmic oral movements influences the timing of muscle activity during a pharyngeal swallow (German et al., 2009). Preliminary data suggests that pharyngeal swallows can also influence the timing of muscle activity during ongoing oral rhythmic behaviors (Thexton, pers comm.). Given the latter finding, we also tested the hypothesis that suck cycles occurring before and after the pharyngeal swallow would be characterized by different patterns of mechanical action. Lastly, we tested the hypothesis that during rapid early feeding, all suck cycles would be influenced by frequent pharyngeal swallows and GH would have a similar mechanical action as compared to suck cycles later in a feeding.

MATERIALS AND METHODS

All experiments were performed using infant pigs ranging from 10-16 days old and 5-6 kg in weight. The animals were obtained from Tom Morris Farms (Reisterstown,

MD). All procedures were approved by the ACUC #SW07M14. The experimental
methods are detailed in Konow et al. (2010) and briefly described here.

All animals underwent surgery to place four 2-mm piezoelectric crystals
(Sonometrics, London, ON) along the anterior-posterior axis of the GH. While the animal
was anesthetized with 2-3% Isoflurane gas, four crystals were equidistantly implanted
along the length of the GH (approx 17 mm apart). In between each crystal, a bipolar
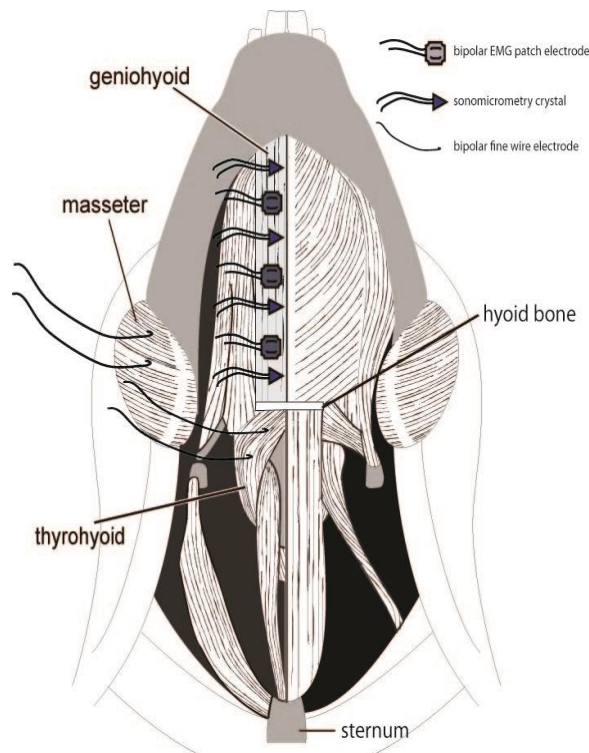patch electrode was sutured to the muscle surface (Fig. 21).

**Figure 21 Placement of the sonomicrometry crystals, bipolar EMG patch electrodes
and bipolar fine wire electrodes in the GH.** The right side of the diagram shows the
muscles that are visualized after removing the skin. The left side of the diagram shows
the muscles visualized after removal of the mylohyoid muscle, sternohyoid muscle and
omohyoid muscles.

Surrounding muscles were exposed with blunt dissection and fine wire bipolar electrodes were placed in midbelly of the masseter and thyrohyoid muscles. The masseter is active during suckling and the thyrohyoid is active during swallowing. Identification of their normal pattern of activity made identification of suck and swallow cycles possible (Thexton et al., 2007). The electrode and crystal wires were routed through a ventral midline incision. The wires were wrapped in Vet wrap (3M) with the mini connector left exposed on the dorsal neck surface, where it was easily connected to the equipment for recording during feeding.

Animals rested after surgery until fully awake, standing and alert, and were then fed every 2-4 hours throughout that day and the next day. During these feedings, the pigs stood in a clear Plexiglas box while electromyography (EMG) and sonomicrometry measurements were recorded synchronously. A 'pig nipple' was used with the bottle (Nasco, Fort Atkinson, WI). At the conclusion of study, each animal was euthanized following JHU IACUC standards, and a post-mortem dissection was performed to evaluate placement of the electrodes and crystals.

Data generated from the crystals and electrodes were digitized via a Powerlab 16/30 and inspected in the proprietary LabChart v.6.1.3 software (AD instruments, Colorado Springs, CO). Swallows were identified based on activity from the thyrohyoid muscle and sucks were identified based on activity from the masseter muscle (Fig. 22; (German et al., 2009)). The data was recorded at 10 kHz. For each feeding, suck cycles were extracted over 200ms with the 100ms mark at peak masseter activity. This enabled suck cycle comparisons over time.

The sample for this study included four female infant pigs (mean body mass 5.53 ± 0.49) with four feeding sessions (experiments) for each pig. Both animal and feeding session were considered random factors in our statistical analysis. There were two fixed factors, time in feeding sequence (early/late) and position of suck cycle relative to the swallow (before/after). The first factor, time, had two levels, early or late, relative to the entire feeding session. The frequency of both suck and swallow cycles was much higher early in the session than later (Fig. 22). The second fixed factor was position of a suck cycle either immediately before or immediately following a swallow. There is some evidence (Thexton, pers comm.) that suck cycles occur as a function of their position relative to a swallow. Therefore, we selected suck cycles either right before or directly following a pharyngeal swallow but we did not include intermediate suck cycles. The two crossed factors produced four groups: Early/Before, Early/After, Late/Before and Late/After. We extracted 20 suck cycles for each of these four groups from each of the 16 experiments for a total of 1280 suck cycles. Thus our unit of analysis was a suck.
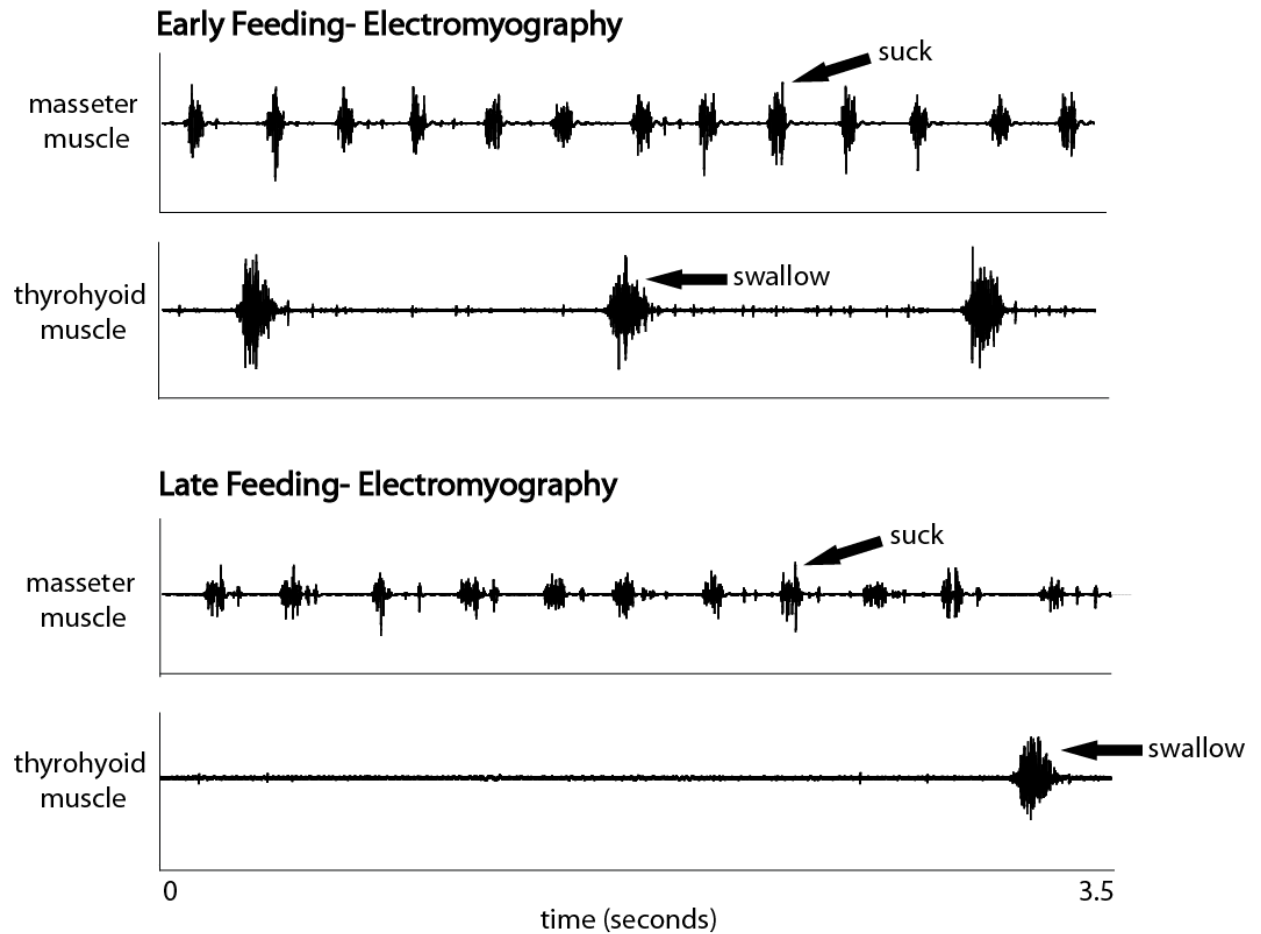
Figure 22 Electromyography from the masseter and thyrohyoid muscles used as markers of suckling and swallowing. Masseter activity was a marker for suck cycles and thyrohyoid activity was a marker for pharyngeal swallows. A 200ms time frame was extracted, centered around the peak activity of the masseter, and will be considered one suck cycle. Suckling that occurs early in a feeding session is more rapid with more frequent swallowing as compared to later in the same feeding session. If there is an influence from the pharyngeal swallow on the suck cycle, then this influence would be seen during early feeding suck cycles that occur before and after pharyngeal swallows.

The shortening and lengthening strain in the three regions of the GH were compared using a cross correlation function (CCF). The CCF calculates the peak-to-peak time lag between two waveforms, and the strength of the correlation at that lag (Konow et al., 2010). The output from a CCF is a lag-score, i.e. the timing between two waveforms, and the pair-wise correlation between the values of the two waveforms. The correlation is

a standard measure of similarity, and the lag-score measures how many units the target wave must shift, either earlier (+) or later (-) to produce the maximum absolute value of the correlation possible with the reference wave. Although the overall data were sampled at 10 kHz, using LabChart's sampling and data extraction routines, we collected a 200 ms window that consisted of 20 points of 10 ms each.

In order to test our hypothesis that are predictable regional differences in the mechanical action along the GH, three sets of comparisons were made between the three regions of the GH for each suck cycle: anterior to posterior, anterior to middle and middle to posterior GH. Therefore, three lag scores with associated pair-wise correlations were calculated for each suck cycle. We tested for differences in lag-score among the tree muscle regions, using a nested analysis of variance (ANOVA) (German et al., 2008). Following Konow et al. (2010), we separated negative and positive correlations. This is because a negative and positive correlation indicates biologically different relationships. A positive correlation indicates that the two regions were lengthening and/or shortening in synchrony (with the given lag). A negative correlation indicates regional strain heterogeneity, i.e. that length changes were out of phase in the two regions, and that one region was lengthening whilst the other was shortening. Comparisons were also made between suck cycles early and late in a feeding session and between suck cycles occurring before and after the pharyngeal swallow. We tested for differences in these groups using a multi-factorial ANOVA. To determine specific differences among groups, and the values of those differences we used post-hoc tests of Least Squares Mean Differences.

RESULTS

In each suck cycle, at least one of the three muscle regions shortened or lengthened. However, the pattern of length change within each region was highly variable over multiple cycles (Fig. 23A-L). For some sucks, two regions changed length in-phase while the other changed length out of phase (Fig. 23J). In another suck cycle during the same feeding sequence, i.e., occurring within a few seconds, all regions were in synchrony (Fig. 23F). In some suck cycles, only one region changed length (Fig. 23D), whereas in other cycles, all three sections changed length (Fig. 23L).
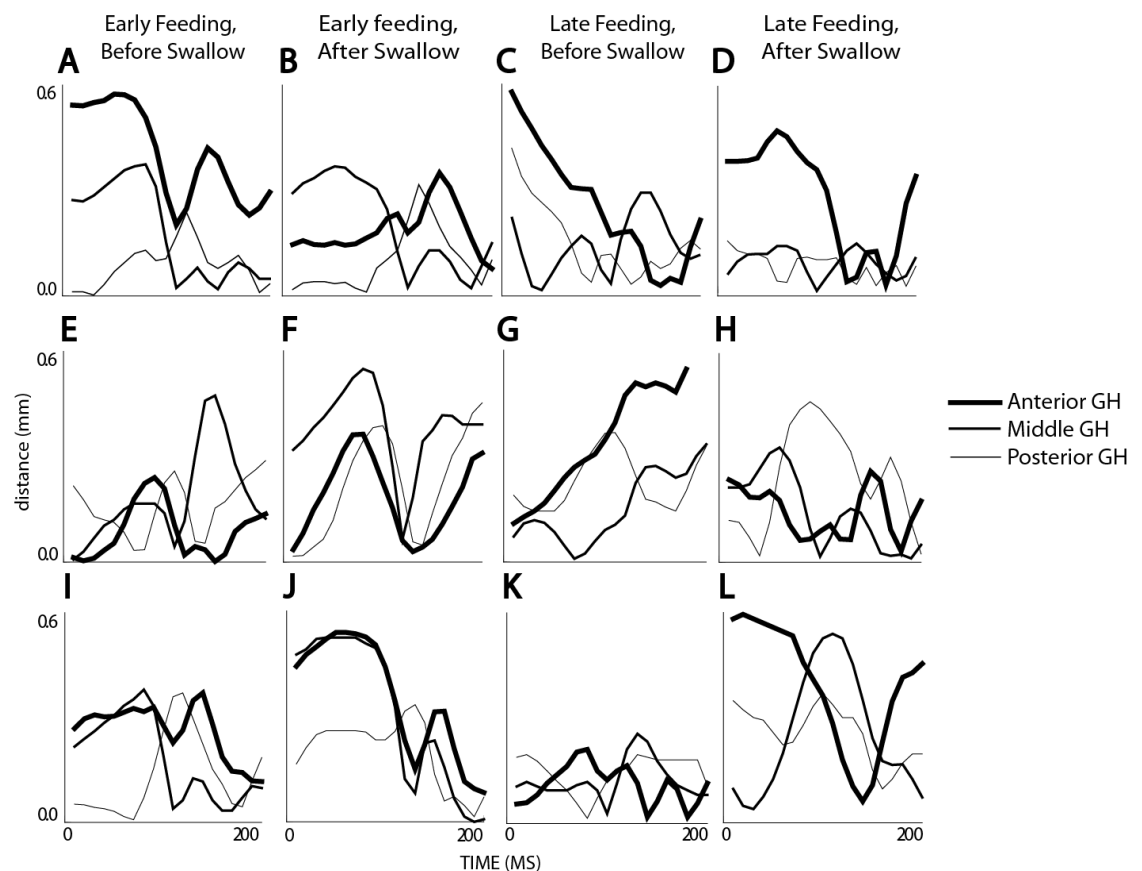

Figure 23 Each column shows three suck cycles from the same animal and experiment across the four treatments of the two factors. The high levels of variability in mechanical action in three regions of the GH were not consistent from cycle to cycle.

These differences occurred from cycle to cycle. Figure 24 illustrates the typical degree of variation across five selected cycles that occurred in the same experiment, from the same treatment group (Early/Before). While the anterior region shortened from mid-cycle in sucks #1, 4 and 5, there were different patterns of mechanical action in sucks # 2 and 3.
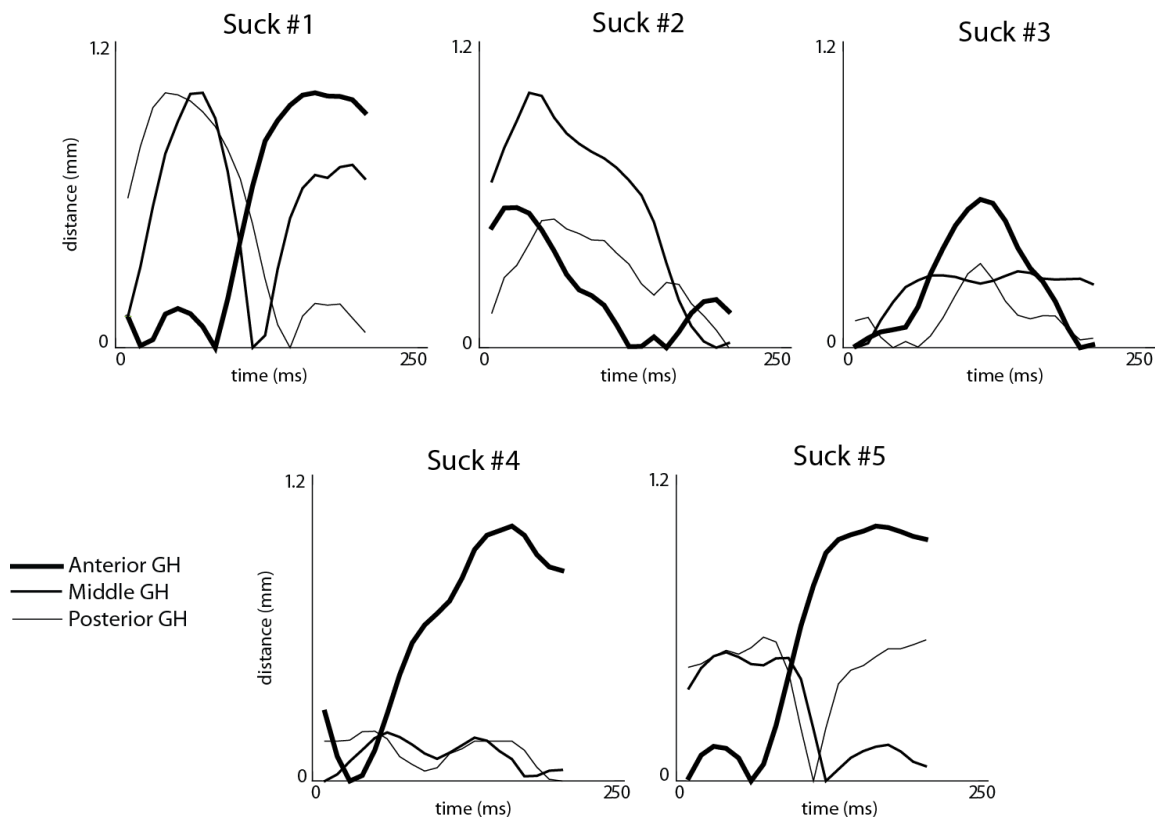
Figure 24 Five suck cycles from the same experiment, Early/Before. Substantial variation existed in the shortening patterns among the three GH regions, as well as within each region over successive sucks.

There was no discernible or consistent pattern of the timing lags in the CCF analyses. While the correlations tended to be strong, i.e., above 0.5 or below -0.5, all values of lag were possible in each of the four groups (Fig. 25). Furthermore, there were

an equal number of positive and negative correlations, with equal numbers of length

changes that were in and out of phase equally.
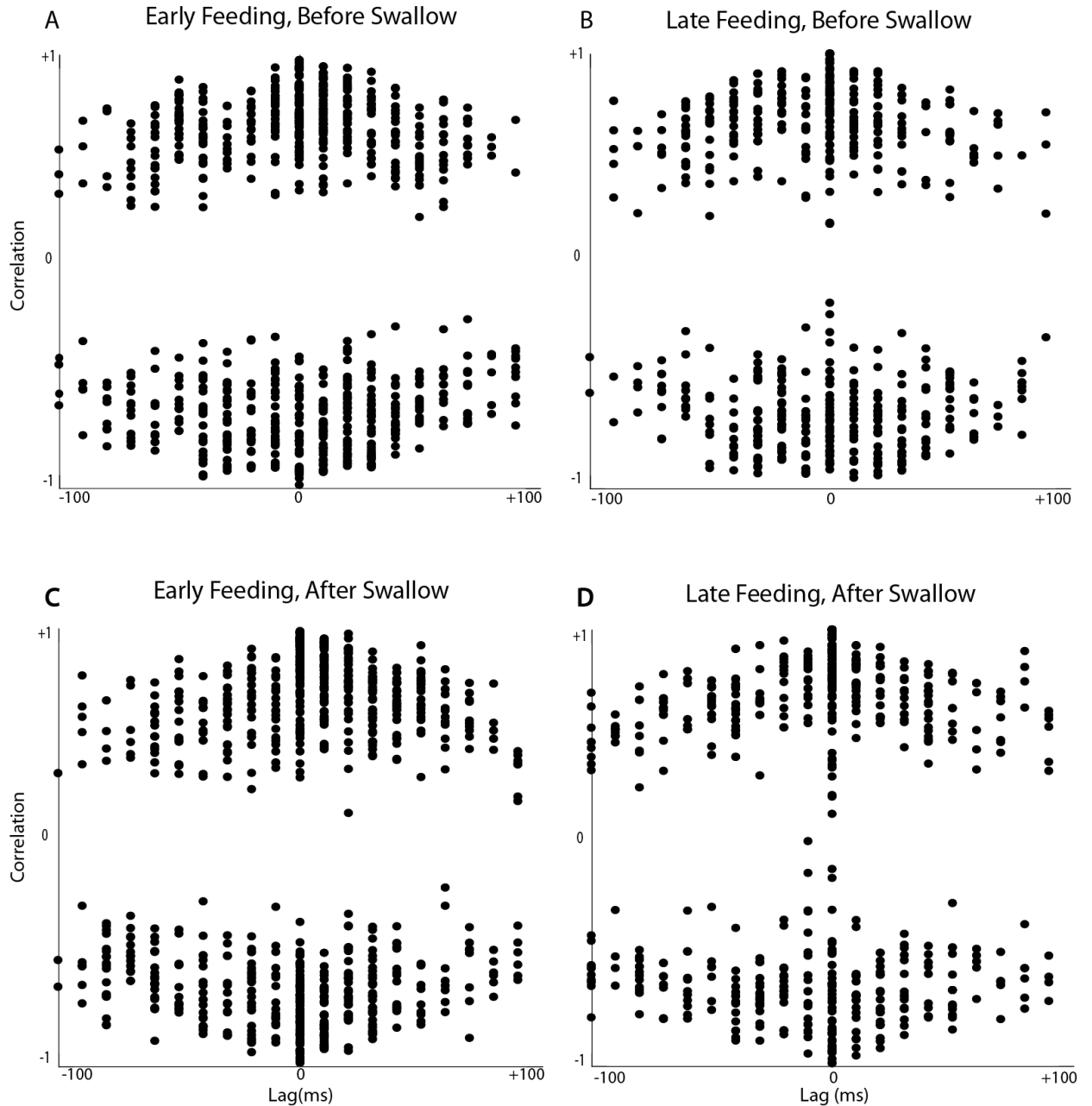

Figure 25 The lag and correlation values from cross correlation analyses (CCFs) within suck cycles. The results are separated for suck cycles occurring early and late in a feeding as well as before and after pharyngeal swallow cycles. There is an equal distribution of positive and negative correlations and a wide, consistent range of lag values regardless of their classification.

Subsequent ANOVA testing indicated significant differences in the lag values among the three regions of the muscle for both positive and negative correlations (Tables 15 and 16). When the correlations were positive, there was also a significant difference between early and late suck cycles. When the correlation was negative there were statistically significant differences between the suck cycles occurring before and after the pharyngeal swallow. Further testing of group means showed that the significant differences were either less than or at approximately 10 ms, the level of time resolution in these data.

Table 15 ANOVA results for positive CCF correlation lag values

	F ratio	p-value	LSMD
After/Before	1.257	0.285	
Early/Late	33.366	*0.000	0.856
Region (Anterior/Middle/Posterior)	26.120	*0.000	
Anterior/Middle to Anterior/Posterior			1.369
Middle/Posterior to Anterior/Posterior			0.652
Middle/Posterior to Anterior/Middle			0.717
Early/Late, Region- interaction	4.747	*0.009	
After/Before, Region- interaction	2.391	0.092	
After/Before, Early/Late, Region- interaction	0.367	0.693	

*p-value < 0.5; LSMD= Least Squares Mean Difference

Table 16 ANOVA results for negative CCF correlation lag values

	F ratio	p-value	LSMD
After/Before	9.236	*0.002	0.826
Early/Late	0.014	0.970	
Region (Anterior/Middle/Posterior)	5.295	*0.005	
Anterior/Middle to Anterior/Posterior			0.295
Middle/Posterior to Anterior/Posterior			1.156
Middle/Posterior to Anterior/Middle			1.451
Early/Late, Region- interaction	0.649	0.523	
After/Before, Region- interaction	7.910	*0.000	
After/Before, Early/Late, Region- interaction	1.433	0.239	

*p-value < 0.5; LSMD= Least Squares Mean Difference

DISCUSSION

Unpredictable heterogeneity within the geniohyoid during suckling

Our results show that while there is heterogeneity in the mechanical action of GH during suck cycles, there is no consistent pattern or temporal relationship among the three regions studied. In all three regions there was an equal probability of shortening or lengthening at any time in the suck cycle. The variation observed was not explained by satiation (i.e. sucks sampled from early vs. late in the feeding session) or by the influence of a pharyngeal swallow (before or after a swallow). Even though there were some statistically significant differences found among the timing of mechanical action changes in the three muscle regions, these differences were less than 10 ms which was the level of temporal resolution of these data. .

Geniohyoid anatomy and muscle fiber distribution

The existence of heterogeneity in GH mechanical action is consistent with descriptions of the anatomy of this muscle in several species. The GH has regional anatomic separations in dogs, mice, rats, opossums and tree shrews (Lakars and Herring, 1987; Mu and Sanders, 1998). In these taxa, there is a thin, complete, transverse myoseptum within the muscle. In dogs, the two regions of the GH are innervated by separate primary nerve branches and each region has different distributions of slow and fast twitch muscle fibers (Mu and Sanders, 1998). The functional significance of this myoseptum remains unknown, but we hypothesize that it could be the basis of independent contraction in the different muscle regions. It is unknown whether pigs have such a band, several bands or none. The results of this study indicate that there are

regional differences along the muscle which could be related to a myoseptum, different primary nerve supply or distribution of muscle fibers.

Functional significance of heterogeneity in muscle strain of geniohyoid

The regional variation in hyoid strap-musculature is consistent during functions other than suckling. Length changes in SH during swallowing in infant pigs were consistent and predictable (Konow et al., 2010). Consistent, yet different, patterns in length change exist in both the SH and GH during reflexive head shake movements in infant pigs (Wentzel et al., 2011). Studies of the GH during respiration have focused on overall length change and muscle activity, and not potential regional variation (van Lunteren et al., 1987a; Wiegand and Latz, 1991; Yokoba et al., 2003).

The functional importance of heterogeneity in muscle strain is unknown although it has been hypothesized that it could potentially increase force or the ability to fine-control torque around a joint (Higham and Biewener, 2011; Lakars and Herring, 1987). The differences and similarities among behaviors involving contraction of the hyoid strap muscles (i.e. swallowing, suckling and head movements) suggest a degree of control that permits these relatively simple muscles to function in a number of different ways. In order to further understand the implications of our present findings, experiments must be conducted in animals during other rhythmic and reflexive activities. Regional variation may be more predictable for some behaviors than others.

The position of the hyoid bone is primarily maintained by a sling of hyoid muscles with antagonistic actions that help move and stabilize the bone during these centrally patterned actions (Crompton et al., 1975; German et al., 2011; Konow et al., 2010; Pearson et al., 2011a). Regional specialization of the GH potentially makes this

muscle more capable of stabilizing the hyoid during rhythmic patterned activities (Higham and Biewener, 2011). During suckling, the hyoid bone moves rhythmically and the geniohyoid muscle is principally responsible for the superior and anterior hyoid bone movement. As is true with every central pattern generated behavior, there is constant sensory feedback from the environment that will alter muscle activity to keep the overall function intact (Dellow and Lund, 1971). In the case of suckling, sensory information from the bolus is sent to the suckling pattern generator in the brainstem via afferents from the trigeminal nerve and the muscle activity of the oral and hyoid muscles is adjusted (Barlow, 2009). Regional variation in the length-trajectory of the strap muscles may serve to ensure that the movement of the hyoid during suckling is not significantly altered when processing different types and sizes of boluses.

Future Directions

It will be necessary to compare the regional variation in GH and other hyoid muscles during rhythmic oral activities (respiration, suckling, mastication) and comparing them to short latency reflexes (swallowing, head shaking, coughing) to fully understand the implications of our findings. Further evaluation of muscle fiber unit composition and the potential existence of transverse myosepta could also contribute to this complex function of the geniohyoid. The findings presented here should also be examined quantitatively along with electromyography data from the different regions in order to evaluate the relationship between regional changes in mechanical action and patterns in muscle activity. This study supports recent findings that the hyoid musculature

may have a complex function that is dependent on the nature of the task and hyoid

movement necessary.

Bibliography

Ahn A, Monti R, Biewener A (2003). In vivo and in vitro heterogeneity of segment length changes in the semimembranous muscle fo the toad. *J Physiol* 549(Pt 3)*:*877-888.

Aluffi P, Policarpo M, Cherovac C, Olina M, Dosdegani R, Pia F (2001). Post-thyroidectomy superior laryngeal nerve injury. *European Archives of Oto-Rhino-Laryngology* 258(9)*:*451-454.

Arvedson J, Clark H, Lazarus C, Schooling T, Frymark T (2010). Evidence-Based Systematic Review: Effects of Oral Motor Interventions on Feeding and Swallowing in Preterm Infants. *Am J Speech Lang Pathol* 19(4)*:*321-340.

Baijens L, Speyer R, Passos V, Pilz W, Roodenburg N, Clave P Swallowing in Parkinson Patients versus Healthy Controls: Reliability of Measurements in Videofluoroscopy. *Gastroenterol Res Pract* 2011 October 3. [Epub ahead of print](

Barlow S, Finan D, Rowland S (1992). Mechanically evoked perioral reflexes in infants. *Brain Res* 599(1)*:*158-160.

Barlow SM, Dusick A, Finan DS, Coltart S, Biswas A (2001). Mechanically evoked perioral reflexes in premature and term human infants. *Brain Research* 899(251-254.

Barlow SM (2009). Central pattern generation involved in oral and respiratory control for feeding in the term infant. *Curr Opin Otolaryngol Head Neck Surg* 17(3)*:*187-193.

Bateman MC, Conejero AJ, Mooney EK, Rothkopf DM (2006). Short-stay cleft palate surgery with intraoperative dexamethasone and marcaine. *Annals of Plastic Surgery* 57(3)*:*245-247.

Bennett SW, Lanovaz JL, Muir GD (2012). The biomechanics of locomotor compensation after peripheral nerve lesion in the rat. *Behavioural Brain Research* 229(2)*:*391-400.

Book SA, Bustad LK (1074). The fetal and neonatal pig in biomedical research. *J Anim Sci* 38(5)*:*997-1002.

Book SA, Bustad LK (1974). The fetal and neonatal pig in biomedical research. *J Anim Sci* 38(5)*:*997-1002.

Bulter S, Stuart A, Leng X, Wilhelm E, Rees C, Williamson J *et al.* (2011). The Relationship of Aspiration Status with Tongue and Handgrip Strenght in Healthy Older Adults. *J Gerontol A Biol Sci Med Sci* 66A(4)*:*452-458.

Butler SG, Stuart A, Case LD, Rees C, Vitolins M, Kritchevsky SB (2011). Effects of Liquid Type, Delivery Method, and Bolus Volume on Penetration-Aspiration Scores in

Healthy Older Adults During Flexible Endoscopic Evaluation of Swallowing. *Annals of Otology, Rhinology & Laryngology* 120(5):288-295.

Campbell-Malone R, Crompton AW, Thexton AJ, German RZ (2011). Ontogenetic Changes in Mammalian Feeding: Insights from Electromyographic Data. *Integrative and Comparative Biology* 51(2):282-288.

Capra N (1995). Mechanisms of oral sensation. *Dysphagia* 10(235-247.

Cichero J, Nicholson T, Dodrill P (2010). Liquid Barium is not Representative of Infant Formula: Characterisation of Rheological and Material Properties. *Dysphagia* Epub ahead of print(

Ciucci M, Russell J, Schaser A, Doll E, Vinney L, Conner N (2011). Tongue force and timing deficits in a rat model of Parkinson disease. *Behavioural Brain Research* Epub ahead of print(

Crompton A, Cook P, Hiiemae K, Thexton A (1975). Movement of the hyoid apparatus during chewing. *Nature* 258(5530):69-70.

Crompton AW, German RZ, Thexton AJ (1997). Mechanisms of swallowing and airway protection in infant mammals (*Sus domesticus* and *Macaca fascicularis*). *Journal of Zoology* 241(89-102.

Crompton AW, Thexton AJ, German RZ (2008). Development of the movement of the epiglottis in infant and juvenile pigs. *Zoology* 111(339-349.

da Costa SP, van der Schans CP, Boelema SR, van der Meij E, Boerman MA, Bos AF (2010). Sucking patterns in fullterm infants between birth and 10 weeks of age. *Infant Behavior and Development* 33(1):61-67.

Dellow P, Lund J (1971). Evidence for central timing of rhythmical mastication. *J Physiol* 215(1):1-13.

Ding P, Campbell-Malone R, Holman S, Fukuhara T, Feng W, German R Aspiration Mechanism for Unilateral Superior Laryngeal Nerve Lesion in Animal Model. Dysphagia Research Society Annual Meeting2011, San Antonio, TX.

Ding P, Chen Y, Lukasik S, German R Mechanism of Epiglottal Movement during the Infant Pharyngeal Swallow. Dysphagia Research Society Annual Meeting2012, Toronto, ON Canada.

Ding P, Campbell-Malone R, Holman S, Lukasik S, Fukuhara T, Gierbolini-Norat E *et al.* (2013a). Unilateral superior laryngeal nerve lesion in an animal model of dyphagia and its effect on suckling and swallowing. *Dysphagia* (in press, accepted 1/16/13)(

Ding P, Campbell-Malone R, Holman SD, Lukasik SL, Gierbolini-Norat EM, Thexton AJ *et al.* (2013b). The effect of unilateral SLN lesion on swallowing threshold volume. . *The Laryngoscope (accepted 1/22/13, in press).*

Dutra EH, Caria PH, Rafferty KL, Herring SW (2010). The buccinator during mastication: A function and anatomical evaluation in minipigs. *Archives of Oral Biology* 55(627-638.

Ebihara S, Kohzuki M, Sumi Y, Ebihara T (2011). Sensory stimulation to improve swallowing reflex and prevent aspiration pneumonia in elderly dysphagic people. *J Pharmacol Sci* 115(2)*:*99-104.

Finan DS, Barlow SM (1996). The actifier: A device for neurophysiological studies of orofacial control in human infants. *Journal of Speech & Hearing Research* 39(4)*:*833.

Finan DS, Barlow SM (1998). Intrinsic dynamics and mechanosensory modulation of non-nutritive sucking in human infants. *Early Human Development* 52(181-197.

Friedman B, Frazier JB (2000). Deep laryngeal penetration as a predictor of aspiration. *Dysphagia* 15(3)*:*153-158.

Frigon A, Gossard J-P (2009). Asymmetric control of cycle period by the spinal locomotor rhythm generator in the adult cat. *J Physiol* 587(19)*:*4617-4627.

German RZ, Franks HA (1991). Timing in the movement of jaws, tongue and hyoid during feeding in the hyrax, *Procavia syriacus*. *J Exp Zool* 257(34-42.

German RZ, Crompton AW, Levitch LC, Thexton AJ (1992). The mechanism of suckling in two species of infant mammal: miniature pigs and long-tailed macaques. *J Exp Zool* 261(322-330.

German RZ, Crompton AW (1996). Ontogeny of suckling mechanisms in opossums (Didelphis virginiana). *Brain, behavior and evolution* 48(3)*:*157-164.

German RZ, Crompton AW, Hertweck DW, Thexton AJ (1997). Determinants of rhythm and rate in suckling. *Journal of Experimental Zoology* 278(1-8.

German RZ, Crompton AW (2000). The Ontogeny of Feeding in Mammals. In: Feeding: Form, Function and Evolution in Tetrapod Vertebrates. K Schwenk editor. San Diego: Academic Press, pp. 449-457.

German RZ, Crompton AW, Owerkowicz T, Thexton AJ (2004a). Volume and rate of milk delivery as determinants of swallowing in an infant model (Sus scrofia). *Dysphagia* 19(147-154.

German RZ, Crompton AW, Thexton AJ (2004b). The role of animal models in understanding feeding behavior in infants. *The International journal of orofacial myology : official publication of* 30(20-30.

German RZ, Crompton AW, Thexton AJ (2008). Variation in EMG activity: a hierarchical approach. *Integr Comp Bio* 48(272-282.

German RZ, Crompton AW, Thexton AJ (2009). Integration of the reflex pharyngeal swallow into rhythmic oral activity in a neurologically intact pig model. *J Neurophysiol* 102(2)*:*1017-1025.

German RZ, Campbell-Malone R, Crompton AW, Ding P, Holman SD, Konow N *et al.* (2011). The concept of hyoid posture. *Dysphagia* 26(2)*:*97-98.

Gordon S, Mischenko A, Dionne R (2010). Long-Acting Local Anesthetics and Perioperative Pain Management. *Dent Clin N Am* 54(611-620.

Gossard J-P, Sirois J, Noue P, Cote M-P, Menard A, Leblond H *et al.* (2011). Chapter 2: The spinal generation of phases and cycle duration. *Progress in Brain Research* 188(15-29.

Greene SA (2002). Veterinary Anesthesia and Pain Management Secrets Philadelphia, PA: Hanley & Belfus, Inc.

Halata Z, Cooper BY, Baumann KI, Schwegmann C, Friedman RM (1999). Sensory nerve endings in the hard palate and papilla incisiva of the goat. *Experimental Brain Research* 129(2)*:*218-228.

Herring SW, Scapino RP (1973). Physiology of feeding in miniature pigs. *Journal of Morphology* 141(4)*:*427-460.

Hey C, Pluschinski P, Stanschus S, Euler HA, Sader RA, Langmore S *et al.* (2011). A Documentation System to Save Time and Ensure Proper Application of the Fiberoptic Endoscopic Evaluation of Swallowing (FEES). *Folia Phoniatrica et Logopaedica* 63(4)*:*201-208.

Higham T, Biewener A (2011). Functional and architectural complexity within and between muscles: regional variation and intermuscular force transmission. *Phil Trans R Soc B* 366(1477-1487.

Hiiemae K, Palmer J, Medicis S, Hegener J, BS J, Lieberman D (2002). Hyoid and tongue surface movements in speaking and eating. *Arch Oral Biol* 47(1)*:*11-27.

Hiiemae KM (2000). Feeding in Mammals. In: Feeding: Form, Function and Evolution in Tetrapod Vertebrates. K Schwenk editor. San Diego: Academic Press, pp. 411-448.

Hirota N, Konaka K, Ono T, Tamine K, Kondo J, Hori K *et al.* (2010). Reduced tongue pressure against the hard palate on the paralyzed side during swallowing predicts Dysphagia in patients with acute stroke. *Stroke* 41(12)*:*2982-2984.

Hollshwandner C, Brenman H, Friedman M (1975). Role of afferent sensors in the initiation of swallowing in man. *J Dent Rest* 54(83-88.

Holman SD, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Griffeon AM, Inokuchi H *et al.* (2012a). Development, reliability and validation of an infant mammalian penetration-aspiration scale. *Dysphagia* E pub November 7, 2012(

Holman SD, Konow N, Lukasik SL, German RZ (2012b). Regional Variation in Geniohyoid Muscle Strain During Suckling in the Infant Pig. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* 317(6)*:*359-370.

Holman SD, Gierbolini-Norat EM, Lukasik SL, Campbell-Malone R, Ding P, German RZ (2013). Duration of action of bupivacaine hydrochloride used for palatal sensory nerve block in infant pigs. *Journal of Veterinary Dentistry* (in press, accepted 1/2/13) (

Huang X, Zhang G, Herring SW (1993). Effects of Oral Sensory Afferents on Mastication in the Miniature Pig. *J Dent Res* 72(980-986.

Humbert I, Lokhande A, Christopherson H, German R, Stone A (2012a). Adaptation of swallowing hyo-laryngeal kinematics is distinct in oral versus pharyngeal sensory processing. *J Appl Physiol* 2012 Mar 8 [Epub ahead of print](

Humbert IA, Christopherson H, Lockhande A, German RZ, Gonzalez-Fernandez M, Celnik P (2012b). Human hyolaryngeal movements show adaptive motor learning during swallowing. *Dysphagia* Epub ahead of print(

Humbert IA, German RZ (2012). New directions for understanding neural control in swallowing: the potential and promise of motor learning. *Dysphagia* Epub ahead of print(

Iriarte-Diaz J, Reed DA, Ross CF (2011). Sources of Variance in Temporal and Spatial Aspects of Jaw Kinematics in Two Species of Primates Feeding on Foods of Different Properties. *Integrative and Comparative Biology* 51(2)*:*307-319.

Jean A (2001). Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiological reviews* 81(2)*:*929-969.

Jonnavithula N, Durga P, Kulkarni DK, Ramachandran G (2007). Bilateral intra-oral, infra-orbital nerve block for postoperative analgesia following cleft lip repair in paediatric patients: comparison of bupivacaine vs bupivacaine–pethidine combination. *Anaesthesia* 62(6)*:*581-585.

Jonnavithula N, Durga P, Madduri V, Ramachandran G, Nuvvula R, Srikanth R *et al.* (2010). Efficacy of palatal block for analgesia following palatoplasty in children with cleft palate. *Pediatric Anesthesia* 20(8):727-733.

Kark AE, Kissin MW, Wenig BI (1995). Superior laryngeal nerve injury to the editor. *Head & Neck* 17(6):542-543.

Klein BG, Duffin JR, Kraje B (1994). Neonatal infraorbital nerve damage and the development of eating behavior in the rat. *Behavioural Brain Research* 60(25-33.

Kodaira Y, Ishizaki K, Sakurai K (2006). Effect of palate covering on bolus-propulsion time and its contributory factors. *Journal of Oral Rehabilitation* 33(1):8-16.

Konow N, Thexton AJ, Crompton AW, German RZ (2010). Regional differences in length-change and electromyographic heterogeneity in the sternohyoid muscle during infant mammalian swallowing. *Journal of Applied Physiology* 109(2):439-448.

Krug W, Losey J (2011). Area of desensitization following mental nerve block in dogs. *J Vet Dent* 28(8):146-150.

Kuo P, Holloway RH, Nguyen NQ (2012). Current and future techniques in the evaluation of dysphagia. *Journal of Gastroenterology and Hepatology* 27(5):873-881.

Lakars T, Herring S (1987). Polymorphous geniohyoid muscles of mice, rats and hamsters. *Arch Oral Biol* 32(6):421-427.

Landis JR, Koch GG (1977). The Measurement of Observer Agreement for Categorical Data. *Biometrics* 33(1):159-174.

Lang I (2009). Brain stem control of the phases of swallowing. *Dysphagia* 24(3):333-348.

Langmore SE, Terpenning MS, Schork A, Chen Y, Murray JT, Lopatin D *et al.* (1998). Predictors of aspiration pnuemonia: How important is dysphagia? *Dysphagia* 13(69-81.

Lau C, Hurst N (1999). Oral feeding in infants. *Current Problems in Pediatrics* 29(4):105-124.

Lee SI, Yoo JY, Kim M, Ryu JS (2012). Changes of Timing Variables in Swallowing of Boluses With Different Viscosities in Patients With Dysphagia. *Archives of Physical Medicine and Rehabilitation* 0).

Lever T, Simon E, Cox K, Capra N, O'Brien K, Hough M *et al.* (2012). A Mouse Model of Pharyngeal Dysphagia in Amyotrophic Lateral Sclerosis. *Dysphagia* 25(2):112-126.

Litmanovitz I, Dreshaj I, Miller MJ, Haxhiu MA, Martin RJ (1994). Central chemosensitivity affects respiratory muscle responses to laryngeal stimulation in the piglet. *J Appl Physiol* 76(403-408.

Loeb G, Gans C (1986). Electromyography for experimentalists Chicago: University of Chicago Press.

Lowell S, Poletto C, Knorr-Chung B, Reynolds R, Simonyan K, Ludlow C (2008). Sensory stimulation activates both motor and sensory components of the swallowing system. *Neuroimage* 42(285-295.

Macrae PR, Doeltgen SH, Jones RD, Huckabee M-L (2012). Intra- and inter-rater reliability for analysis of hyoid displacement measured with sonography. *Journal of Clinical Ultrasound* 40(2):74-78.

Mansson I, Sandberg N (1974). Manometry of the pharynx and the esophagus in relation to laryngectomy. *JFORL J Fr Otorhinolaryngol Audiophonol Chir Maxillofac* 23(8):737 - 743.

Matsuo K, Palmer J (2010). Kinematic linkage of the tongue, jaw and hyoid during eating and speech. *Arch oral biol* 55(325-331.

Mazoit J-X (2006). Pharmacokinetic/Pharmacodynamic Modeling of Anesthetics in Children: Therapeutic Implications. *Pediatric Drugs* 8(3):139.

Medda B, Lang I, Dodds W, Christl M, Kern M, Hogan W *et al.* (1997). Correlation of electrical and contractile activities of the cricopharyngeus muscle in the cat. *Am J Physiol* 273(2):G470-G479.

Medda BK, Kern M, Ren J, Xie P, Ulualp SO, Lang IM *et al.* (2003). Relative contribution of various airway protective mechanisms to prevention of aspiration during swallowing. *Am J Physiol Gastrointest Liver Physiol* 284(6):G933-939. Epub 2003.

Michou E, Mastan A, Ahmed S, Mistry S, Hamdy S (2012). Examining the role of carbonation and temperature on water swallowing performance: a swallowing reaction-time study. *Chem Senses* 37(799-807).

Miller AJ, Dunmire CR (1976). Characterization of the postnatal development of superior laryngela nerve fibers in the postnatal kitten. *Journal of Neurobiology* 7(6):483-494.

Miller AJ, Vargervik K, Phillips D (1985). Neuromuscular adaptation of craniofacial muscles to altered oral sensation. *American Journal of Orthodontics* 87(4):303-310.

Miller AJ (2002). Oral and pharyngeal reflexes in the mammalian nervous system: Their diverse range in complexity and the pivotal role of the tongue. *Crit Rev Oral Biol Med* 13(409-425.

Miller AJ (2008). The neurobiology of swallowing and dysphagia. *Dev Disabil Res Rev* 14(2)*:*77-86.

Miller CK (2011a). Feeding Issues and Interventions in Infants and Children with Clefts and Craniofacial Syndromes. *Semin Speech Lang* 32(02)*:*115-126.

Miller CK (2011b). Feeding Issues and Interventions in Infants and Children with Clefts and Craniofacial Syndromes. *Semin Speech Lang* 32(02)*:*115,126.

Mu L, Sanders I (1998). Neuromuscular specializations of the pharyngeal dilator muscles: I. *Anat Rec* 250(2)*:*146-153.

Newman L, Cleveland R, Blickman J, Hillman R, Jaramillo D (1991). Videofluoroscopic analysis of the infant swallow. *Invest Radiol* 26(870-873.

Nicodemus HF, Ferrer MJ, Cristobal VC, deCastro L (1991). Bilateral infraorbital block with 0.5% bupivacaine as post-operative analgesia following cheiloplasty in children. *Scand J Plast Reconstr Surg Hand Surg* 25(3)*:*253-257.

Obaya GM, Refaie A, Aboushanab O, Ibraheem N, Abdelazees M (2010). Addition of dexmedetomidine to bupivacaine for greater palatine nerve blocks prolongs postoperative analgesia after cleft palate repair. *European Journal of Anaesthesiology* 27(3)*:*280-284.

Ogura K, Kimoto S, Yamaguchi H, Kobayashi K (2007). Perception thresholds for electrical stimulation of the palatal mucosa. *Int J Prosthodont* 20(4)*:*423-431.

Palmer JB, Hiiemae KM, Liu J (1997). Tongue-jaw linkages in human feeding: a preliminary videofluorographic study. *Arch Oral Biol* 42(6)*:*429-441.

Pearson Jr WG, Hindson DF, Langmore SE, Zumwalt AC (2012). Evaluating Swallowing Muscles Essential for Hyolaryngeal Elevation by Using Muscle Functional Magnetic Resonance Imaging. *International Journal of Radiation Oncology*Biology*Physics* Epub before print).

Pearson W, Langmore S, Zumwalt A (2011a). Evaluating the Structural Properties of Suprahyoid Muscles and their Potential for Moving the Hyoid. *Dysphagia:*1-7.

Pearson W, Langmore S, Zumwalt A (2011b). Evaluating the Structural Properties of Suprahyoid Muscles and their Potential for Moving the Hyoid. *Dysphagia* 26(4)*:*345-351.

Pelletier C, Dhanaraj G (2006). The effect of taste and palatability on lingual swallowing pressure. *Dysphagia:*121-128.

Penatti EM, Berniker AV, Kereshi B, Cafaro C, Kelly ML, Niblock MM *et al.* (2006). Ventilatory response to hypercapnia and hypoxia after extensive lesion of medullary serotonergic neurons in newborn conscious piglets. *J Appl Physiol* 101(1177-1188.

Pikus L, Levine M, Yang Y-X, Rubesin S, Katzka D, Laufer I *et al.* (2003). Videofluoroscopic studies of swallowing dysfunction and the relative risk of pneumonia. *AJR Am J Roentgenol* 180(1613-1616.

Poran NS (1998). Vomeronasal organ and its associated structures in the opossum Monodelphis domestica. *Microscopy Research and Technique* 43(6):500-510.

Prabhu PPK, Wig J, Grewal S (1999). Bilateral infraorbital nerve block is superior to peri-incisional infiltration for analgesia after repair of cleft lip. *Scand J Plast Reconstr Hand Surg* 33(83-87.

Prochazka A, Clarac F, Loeb GE, Rothwell JC, Wolpaw JR (2000). What do reflex and voluntary mean? Modern views on an ancient debate. *Exp Brain Res* 130(417-432.

Reuss-Lamky H (2007). Administering dental nerve blocks. *J Am Anim Hosp Assoc* 43(298-305.

Robbins J, Coyle J, Rosenbek J, Roecker E, Wood J (1999). Differentiation of normal and abnormal airway protection during swallowing using the penetration-aspiration scale. *Dysphagia* 14(228-232):228-232.

Rofes L, Arreola V, Almirall J, Cabre M, Campins L, Garcia-Peris P *et al.* (2011). Diagnosis and management of oropharyngeal dysphagia and its nutritional and respiratory complications in the elderly. *Gastroenterol Res Pract* 2010 Aug 3 [Epub ahead of pring](

Rosenbek JC, Robbins JA, Roecker EB, Coyle JL, Wood JL (1996). A penetration-aspiration scale. *Dysphagia* 11(2):93-98.

Ross CF, Dharia R, Herring SW, Hylander WL, Liu Z-J, Rafferty KL *et al.* (2007). Modulation of mandibular loading and bite force in mammals during mastication. *J Exp Biol* 210(1046-1063.

Sessle B, Storey A (1972). Periodontal and facial influences on the laryngeal input to the brain stem of the cat. *Arch oral biol* 17(1583-1595.

Steele C, Bailey G, Chau T, Molfenter S, Oshalla M, Waito A *et al.* (2010). The relationship between hyoid and laryngeal displacement and swallowing impairment. *Clin Otolaryngol* 36(30-36.

Steele CM, Miller AJ (2010). Sensory Input Pathways and Mechanisms in Swallowing: A Review. *Dysphagia* 25(4):323-333.

Steele CM, Bailey GL, Chau T, Molfenter SM, Oshalla M, Waito AA *et al.* (2011). The relationship between hyoid and laryngeal displacement and swallowing impairment. *Clinical Otolaryngology* 36(1)*:*30-36.

Sumi T (1967). The nature and postnatal development of reflex deglutition in the kitten. *The Japanese Journal of Physiology* 17(200-210.

Sumi T (1970). Changes of hypoglossal nerve activity during inhibition of chewing and swallowing by lingual nerve stimulation. *Pflugers Arch* 317(303-309.

Sweazey RD, Bradley RM (1989). Responses of neurons in the lamb nucleus tractus solitarius to stimulation of the caudal oral cavity and epiglottis with different stimulus modalities. *Brain Res* 480(133-150.

Tei K, Yamazaki Y, Kobayashi M, Izumiyama Y, Ono M, Totsuka Y (2004). Effects of bilateral lingual and inferior alveolar nerve anesthesia effects on masticatory function and early swallowing. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 97(5)*:*553-558.

Thexton A (1973). Oral reflexes elicited by mechanical stimulation of palatal mucosa in the ca. *Archives of Oral Biology* 18(971-980.

Thexton A (1974). Jaw opening and jaw closing reflexes in the cat. *Brain research* 66(425-433.

Thexton A, Griffiths C (1979). Reflex oral activity in decerebrate rats of different age. *Brain Res* 175(1-9.

Thexton A, Crompton A, German R (2012). EMG activity in hyoid muscles during pig suckling. *J Appl Physiol* 112(1512-1519.

Thexton AJ, Griffiths C, McGarrick J (1980). Evidence for peripheral activation of the trigeminal rhythm generator in the decerebrate rat, obtained by fourier analysis of conditioning testing curves. *Archives of Oral Biology* 25(7)*:*491-494.

Thexton AJ (1981). Tongue and hyoid movement in the cat Tokyo; Chicago: Quintessence Publishing Co.

Thexton AJ, Crompton AW, German RZ (1998a). Transition from suckling to drinking at weaning: a kinematic and electromyographic study in miniature pigs. *Journal of Experimental Zoology* 280(5)*:*327-343.

Thexton AJ, Crompton AW, German RZ (1998b). Transition from suckling to drinking at weaning: A kinematic and electromyographic study in miniature pigs. *Journal of Experimental Zoology* 280(5)*:*327-343.

Thexton AJ, Crompton AW, German RZ (2007). Electromyographic activity during the reflex pharyngeal swallow in the pig: Doty and Bosma (1956) revisited. *Journal of Applied Physiology* 102(2)*:*587-600.

Thexton AJ, Crompton AW, Owerkowicz T, German RZ (2009). Impact of rhythmic oral activity on the timing of muscle activation in the swallow of the decerebrate pig. *J Neurophysiol* 101(3)*:*1386-1393.

Tsujimura T, Yamada A, Nakamura Y, Fukuhara T, Yamamura K, Inoue M (2011). The Digastric Muscle is Less Involved in Pharyngeal Swallowing in Rabbits. *Dysphagia:*1-6.

Tutor JD, Gosa MM (2012). Dysphagia and aspiration in children. *Pediatr Pulmonol* 47(4)*:*321-337.

van Lunteren E, Haxhiu M, Cherniack N (1987a). Mechanical function of hyoid muscles during spontaneous breathing in cats. *J Appl Physiol* 62(582-590.

van Lunteren E, Dick TE (2000). Heterogeneity within geniohyoid motor unit subpopulations in firing patterns during breathing. *Respiratoin and Physiology* 124(1)*:*23-33.

Watrous BJ, Suter PF (1983). Oropharyngeal dysphagias in the dog: A cinefluorographic analysis of experimentally induced and spontaneously occurring swallowing disorders. *Veterinary Radiology* 24(1)*:*11-24.

Weaver ME, Jump EB, McKean CF (1969). The eruption pattern of permanent teeth in miniature swine. *Archives of Oral Biology* 14(3)*:*323-IN312.

Wentzel S, Konow N, German RZ (2011). Regional differences in hyoid muscle activity and length dynamics during mammalian head shaking. *J of Exp Zool* 315(3)*:*111-120.

Wiegand DA, Latz B (1991). Effect of geniohyoid and sternohyoid muscle contraction on upper airway resistance in the cat. *J Apply Physiol* 71(4)*:*1346-1354.

Yagi S, Fukuyama E, Soma K (2008). Involvement of Sensory Input from Anterior Teeth in Deglutitive Tongue Function. *Dysphagia* 23(3)*:*221-229.

Yamamura K, Kitagawa J, Kurose M, Sugino S, Takatsuji H, Mostafeezur RM *et al.* (2010). Neural Mechanisms of Swallowing and Effects of Taste and Other Stimuli on Swallow Initiation. *Biol Pharm Bull* 33(11)*:*1786-1790.

Yokoba M, Hawes H, Easton P (2003). Geniohyoid muscle function in awake canines. *J Appl Physiol* 95(2)*:*801-807.