Published in final edited form as:

Dysphagia. 2017 February ; 32(1): 73–77. doi:10.1007/s00455-016-9778-7.

Animal Models for Dysphagia Studies: What have we learnt so far

Rebecca Z. German^{1,*}, A.W. Crompton², Francois D. H. Gould¹, and Allan J. Thexton³

¹Department of Anatomy and Neuroscience, Northeast Ohio Medical University, Rootstown, OH 444272

²Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138

³Division of Physiology, King's College, Guy's Campus, London SE1 9RT, UK

Abstract

Research using animal models has contributed significantly to realizing the goal of understanding dysfunction and improving the care of patients who suffer from dysphagia. But why should other researchers and the clinicians who see patients day in and day out care about this work? Results from studies of animal models have the potential to change and grow how we think about dysphagia research and practice in general, well beyond applying specific results to human studies. Animal research provides two key contributions to our understanding of dysphagia. The first is a more complete characterization of the physiology of both normal and pathological swallow than is possible in human subjects. The second is suggesting of specific, physiological, targets for development and testing of treatment interventions to improve dysphagia outcomes.

Keywords

Animal Models; Performance; Pathophysiology; deglutition; deglutition disorders

A model for dysphagia research

The main goal of dysphagia research is to provide better care, if not cure our patients suffering from one of the many forms of swallowing impairment which in turn interfere both with the quality of life and survival itself. Research using animal models has contributed significantly to realizing this goal, and has the potential to contribute to future advances. The clinicians and researchers who work with animals understand this. But why should other researchers and the clinicians who see patients day in and day out care about this work? Surely we can all read about the results *after* they have translated to human subjects? Results from studies of animal models have the potential to change and grow how we think about dysphagia research and practice in general, well beyond applying specific results to human studies.

*Corresponding author: rzgerman@jhu.edu, 410-502-4461.

Conflicts of Interest: The authors have no conflicts of interest to disclose.

At a recent meeting (*Swallowing Think Tank, Dec. 2–3, 2016, Swallowing Systems Core at University of Florida, Humbert & Plowman, organizers, pers. com.*), a number of researchers asked and discussed some fundamental questions, including: *What is a swallow?* and *What is normal?* Through discussions of the existence of distinct dysphagia phenotypes, and of how to define, study, and measure them across the bench-to-bedside continuum, aspects of a conceptual framework emerged. In particular (fig 1):

The interaction of the first two boxes, the risk factors with the underlying physiology or pathophysiology, are the root causes producing the failure. These interactions can be important to the diagnosis and treatment plan, represented here by the clinical decision box. One significant contribution of animal research to the outcome box is to dissect out and provide detail on those first boxes, in particular the underlying physiology that is the causal mechanism of both accurate and acceptable performance, as well as performance failure (fig 2). Swallowing is a motor function that requires the combined accurate movement or kinematics of a number of separate anatomical structures. Kinematics, in turn, are generated by muscle activity, which itself is generated by signals from the Central Nervous System (CNS) sent through peripheral nerves to the relevant muscles.

However, this is not simply a unidirectional pathway. Performance generates sensory signals that return to the CNS and can modify the next set of outgoing signals. This loop, represented in fig 3, is the causal mechanism of each of the events that constitutes a swallow. Performance does not necessarily equate with a complete swallow, but with a subset of the activities within a swallow.

Animal models are uniquely able to facilitate understanding and testing of the causal mechanisms that produce good or bad performance in a normal, intact, and healthy individual. For failures in good performance, animal models can determine where in the fig. 3 loop that failure occurred. At each stage of the loop in fig. 3, animal models can provide more detailed data, in greater sample sizes with fewer ethical concerns than can a human sample. Although treatment is possible without understanding the complete mechanism of failure, understanding the full pathophysiology will accelerate the development of effective treatments by facilitating hypothesis driven, physiology based treatment testing in a scientifically rigorous manner.

Animal Research and the generation of performance

Numerous studies exist for each step of the loop in fig 3. Excellent kinematic studies of human function exist, but a number of limitations on those studies do not exist in animal work. The amount of permissible radiation exposure is strictly limited in humans but not in animals. Frail and ill patients often cannot sustain a Videofluoroscopic Swallowing Study (VFSS), whereas it is possible in most animal models of disease and frailty. Higher imaging speeds, 100–500 frames per second, are possible in animals, as compared to the 30 or 60 fps in human studies. Animal studies can have implanted radio-opaque markers that permit detailed kinematic analyses of tongue movements not possible humans [5, 14, 31].

Muscle function is responsible for the kinematics or movements that are the basis of swallowing function. Yet, accurate recording and measurement of the electrical activity of muscles in humans is difficult. While surface electrodes are not invasive, they provide only a broad measurement of the underlying muscle signals. They cannot differentiate between the activities in large categories of muscles, such as the individual members of the infra- or supra-hyoid groups that are critical for swallowing. Measurements of tongue muscle activity are nearly impossible without indwelling fine-wire bipolar electrodes. Such electrodes have been used to examine oral and pharyngeal function, although rarely and then in only a few anatomically accessible muscles [4, 6, 20, 21]. In animals, surgical placement of electrodes in even deeply sited muscles is possible. The quality and quantity of data from such studies facilitates determination of the relationship between the moving structure and the muscle generating that movement [24, 26, 27, 41, 48–50].

Information on neural control, both in terms of direct measurement of neural function and indirectly through lesion studies, has become more possible in human subjects through recent innovative techniques such as TMS [33–35, 39]. However, understanding specific aspects of neural control is still the realm of implanted electrodes, neural stimulation, and lesions, none of which are possible in human subjects. Stimulation studies can provide information on how specific nerves or CNS regions control or inhibit swallowing [13, 43, 46, 47]. Lesion studies can both mimic human injury, and specify the extent of functional involvement of different regions of the brain [17, 45]. Finally, direct recordings from the CNS through chronically implanted electrodes can provide basic information on the spatial/temporal activity of those regions and so clarify the background to a number of human neural insults [1–3, 25].

***Ceteris Paribus* and understanding pathophysiology**

The hallmark of reproducible, evidence based research is the concept of *ceteris paribus*, literally “other things equal” [30]. In studies of normal individuals, we can control many of the factors in the first two boxes in fig 3, including age, gender, and other demographic variables. In studies of clinically compromised individuals, demographic factors become more difficult to control, and further complications, such as co-morbidities and treatment compliance are introduced. We can limit studies to a statistical sample of a particular disease entity, such as Parkinson’s disease, or a condition, such as preterm birth, or an injury, such as traumatic brain injury. However, these diagnoses are themselves complex categories, with a variable extent of pathology and etiology. For some of these, it is not clear where in the loop (Fig 3) the problem arises. Identifying the causal pathway deficit that produces failures in swallowing and deglutition failures in the complex, hierarchical, interacting physiological dysfunctions that result from disease etiologies is often not straightforward. Human clinical subjects represent the same wonderful variation, the same marvelously complicated packages that characterize all human endeavor. Although it is possible to collect data for each step in fig 3 from human subjects, because of ethical limitations, the data are not as complete, nor the datasets as large, as they can be from animal subjects.

Animals offer the opportunity for clean and uncomplicated studies of the impact of insult and the effect of intervention at different stages in presumptive etiological pathways. *Ceteris*

paribus is inherent in the design of the study. The SOD1-G93A transgenic rodents are validated models of oral-stage dysphagia for bulbar deficits of Amyotrophic Lateral Sclerosis [28, 32]. There are multiple models of Parkinson's disease, including (6-OHDA)-induced DA depletion [8, 37], surgical lesions [42] and PINK KO genetic models [22, 23]. Surgical or pharmacological models of stroke in rodents have been common, but are now being developed and validated for examining dysphagia [19, 44]. Injuries that are associated with dysphagia, such as damage to branches of the vagus nerve, recurrent or superior laryngeal nerve [11, 12, 16, 38], or other sensory deficits [18], can be relatively easily duplicated in animal models.

In general, a number of concerns in human studies are much reduced. If the impact of an insult is subtle, and effect size is small, or swamped by inter-individual variation, having a large sample may be important to see the effect. Large sample sizes, either in number of individuals, or number of swallows, are relatively easy to obtain in animal models. Another issue is that swallowing problems are often considered doubly hidden. Firstly, unlike other sensorimotor tasks such as locomotion or reaching, some form of imaging is necessary to view the swallow. The gold standard for such imaging is the VFSS, which has radiation risks [36]. Secondly, silent aspiration, where the patient is unaware of the problem is a significant issue [10, 40, 52]. Uncovering these hidden problems is significantly easier with an animal model and a large sample size.

For age-based studies, either of the very old [29, 42] or the very young [7, 9, 15], animal models answer both ethical and logistic challenges. The old and the young tend to be frailer than young or mid-aged adults. Furthermore, the separation of the effects of age, either young or old, from other clinical issues is difficult in human beings [51]. From the purely practical point of view, aging or normal development in humans represents changes that can occur over years. Some animal models represent a compressed timeline that makes longitudinal work possible. For developmental studies, the changes in airway protection necessitated by weaning are difficult to study on a human ontogenetic time scale.

Animal Models and Human Dysphagia

Animal models can provide unique data and insight into both normal and abnormal swallowing that is not possible in human subjects. This ability is due in part to ethical limitations in human research. Alternatively, because of the ability to control the sample, design and methodology in animal studies, different aspects may be clarified. Removing comorbidities and human compliance concerns enhances the reliability of the results. Yet, in the end, much of the value of animal research lies in what it tells us about human dysphagia. The results in the studies cited here, and the ongoing research programs that generated these results, are worth understanding. They can generate the specific hypotheses that can be tested in human subjects. They can validate non-invasive methodologies, or map results from invasive methodologies onto non-invasive methodologies that can be used with frail patients.

The justification for animal research depends on workers in human clinical research and the clinicians who apply that research understanding and appreciating how the translation from bench to bedside is made. In this regard, animal research provides two key contributions to

our understanding of dysphagia. The first is a more complete characterization of the physiology of both normal and pathological swallow than is possible in human subjects. The second is suggesting of specific, physiological, targets for development and testing of treatment interventions to improve dysphagia outcomes.

Acknowledgments

The work of the authors has been supported by multiple grants from the NIH over the last 40 years, including: AR18140, DC3604, DC6953, DC9980, DE5526, DE 5738, DE7325 and HD8856. We dedicate this paper to our late colleague and mentor, Dr. Karen Hiimeae.

Literature Cited

1. Arce-McShane FI, Hatsopoulos NG, Lee JC, Ross CF, Sessle BJ. Modulation dynamics in the orofacial sensorimotor cortex during motor skill acquisition. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2014; 34(17):5985–97. [PubMed: 24760857]
2. Arce-McShane FI, Ross CF, Takahashi K, Sessle BJ, Hatsopoulos NG. Primary motor and sensory cortical areas communicate via spatiotemporally coordinated networks at multiple frequencies. *Proc Natl Acad Sci U S A*. 2016; 113(18):5083–8. [PubMed: 27091982]
3. Arce FI, Lee JC, Ross CF, Sessle BJ, Hatsopoulos NG. Directional information from neuronal ensembles in the primate orofacial sensorimotor cortex. *J Neurophysiol*. 2013; 110(6):1357–69. [PubMed: 23785133]
4. Barkmeier JM, Bielamowicz S, Takeda N, Ludlow CL. Laryngeal activity during upright vs. supine swallowing. *J Appl Physiol* (1985). 2002; 93(2):740–5. [PubMed: 12133886]
5. Best MD, Nakamura Y, Kijak NA, Allen MJ, Lever TE, Hatsopoulos NG, Ross CF, Takahashi K. Semiautomatic marker tracking of tongue positions captured by videofluoroscopy during primate feeding. *Conf Proc IEEE Eng Med Biol Soc*. 2015; 2015:5347–50. [PubMed: 26737499]
6. Blitzer A, et al. Recommendations of the Neurolaryngology Study Group on laryngeal electromyography. *Otolaryngology--head and neck surgery: official journal of American Academy of Otolaryngology-Head and Neck Surgery*. 2009; 140(6):782–793. [PubMed: 19467391]
7. Campbell-Malone R, Crompton AW, Thexton AJ, German RZ. Ontogenetic changes in Mammalian feeding: insights from electromyographic data. *Integrative and comparative biology*. 2011; 51(2): 282–8. [PubMed: 21653542]
8. Ciucci MR, Russell JA, Schaser AJ, Doll EJ, Vinney LM, Connor NP. Tongue force and timing deficits in a rat model of Parkinson disease. *Behav Brain Res*. 2011; 222(2):315–20. [PubMed: 21459116]
9. Crompton AW, Thexton AJ, German RZ. Development of the movement of the epiglottis in infant and juvenile pigs. *Zoology*. 2008; 111:339–349. [PubMed: 18387794]
10. Daniels SK. Letter by Daniels Regarding Article “Silent Aspiration Risk is Volume-Dependent”. *Dysphagia*. 2012
11. Ding P, Campbell-Malone R, Holman SD, Lukasik SL, Thexton AJ, German RZ. The effect of unilateral superior laryngeal nerve lesion on swallowing threshold volume. *The Laryngoscope*. 2013; 123(8):1942–7. [PubMed: 23670486]
12. Ding P, Fung GS, Lin M, Holman SD, German RZ. The effect of bilateral superior laryngeal nerve lesion on swallowing: a novel method to quantitate aspirated volume and pharyngeal threshold in videofluoroscopy. *Dysphagia*. 2015; 30(1):47–56. [PubMed: 25270532]
13. Fukuhara T, Tsujimura T, Kajii Y, Yamamura K, Inoue M. Effects of electrical stimulation of the superior laryngeal nerve on the jaw-opening reflex. *Brain Res*. 2011; 1391:44–53. [PubMed: 21466791]
14. German RZ, Crompton AW, Levitch LC, Thexton AJ. The mechanism of suckling in two species of infant mammal: miniature pigs and long-tailed macaques. *J Exp Zool*. 1992; 261:322–330. [PubMed: 1629663]

15. German RZ, Crompton AW, Thexton AJ. The coordination and interaction between respiration and deglutition in young pigs. *Journal of Comparative Physiology (A)*. 1998; 182:539–547.
16. Gould FD, Lammers AR, Ohlemacher J, Ballester A, Fraley L, Gross A, German RZ. The Physiologic Impact of Unilateral Recurrent Laryngeal Nerve (RLN) Lesion on Infant Oropharyngeal and Esophageal Performance. *Dysphagia*. 2015 Aug 19.2015 epub ahead of print.
17. Hiraba H, Sato T. Cortical control for mastication in cats: changes in masticatory movements following lesions in the masticatory cortex. *Somatosens Mot Res*. 2005; 22(3):171–81. [PubMed: 16338825]
18. Holman SD, Campbell-Malone R, Ding P, Gierbolini-Norat EM, Lukasik SL, Waranch DR, German RZ. Swallowing kinematics and airway protection after palatal local anesthesia in infant pigs. *The Laryngoscope*. 2013;doi 10.
19. Ikeda J, Kojima N, Saeki K, Ishihara M, Takayama M. Perindopril increases the swallowing reflex by inhibiting substance P degradation and tyrosine hydroxylase activation in a rat model of dysphagia. *Eur J Pharmacol*. 2015; 746:126–31. [PubMed: 25445054]
20. Inokuchi H, Gonzalez-Fernandez M, Matsuo K, Brodsky MB, Yoda M, Taniguchi H, Okazaki H, Hiraoka T, Palmer JB. Electromyography of swallowing with fine wire intramuscular electrodes in healthy human: activation sequence of selected hyoid muscles. *Dysphagia*. 2014; 29(6):713–21. [PubMed: 25142242]
21. Inokuchi H, Gonzalez-Fernandez M, Matsuo K, Brodsky MB, Yoda M, Taniguchi H, Okazaki H, Hiraoka T, Palmer JB. Electromyography of Swallowing with Fine Wire Intramuscular Electrodes in Healthy Human: Amplitude Difference of Selected Hyoid Muscles. *Dysphagia*. 2016; 31(1):33–40. [PubMed: 26487062]
22. Kelm-Nelson CA, Yang KM, Ciucci MR. Exercise Effects on Early Vocal Ultrasonic Communication Dysfunction in a PINK1 Knockout Model of Parkinson's Disease. *J Parkinsons Dis*. 2015; 5(4):749–63. [PubMed: 26577653]
23. Kelm-Nelson CA, Stevenson SA, Ciucci MR. Data in support of qPCR primer design and verification in a Pink1 $-/-$ rat model of Parkinson disease. *Data Brief*. 2016; 8:360–3. [PubMed: 27331115]
24. Lang IM, Dana N, Medda BK, Shaker R. Mechanisms of airway protection during retching, vomiting, and swallowing. *American journal of physiology Gastrointestinal and liver physiology*. 2002; 283(3):G529–36. [PubMed: 12181164]
25. Lang IM, Medda BK, Shaker R. Differential activation of medullary vagal nuclei caused by stimulation of different esophageal mechanoreceptors. *Brain research*. 2011; 1368:119–33. [PubMed: 20971087]
26. Lang IM, Medda BK, Jadcherla S, Shaker R. The role of the superior laryngeal nerve in esophageal reflexes. *American journal of physiology Gastrointestinal and liver physiology*. 2012; 302(12):G1445–57. [PubMed: 22403790]
27. Lang IM. The Physiology of Eructation. *Dysphagia*. 2016; 31(2):121–33. [PubMed: 26694063]
28. Lever TE, Simon E, Cox KT, Capra NF, O'Brien KF, Hough MS, Murashov AK. A mouse model of pharyngeal dysphagia in amyotrophic lateral sclerosis. *Dysphagia*. 2010; 25(2):112–26. [PubMed: 19495873]
29. Lever TE, Brooks RT, Thombs LA, Littrell LL, Harris RA, Allen MJ, Kadosh MD, Robbins KL. Videofluoroscopic Validation of a Translational Murine Model of Presbyphagia. *Dysphagia*. 2015; 30(3):328–42. [PubMed: 25783697]
30. Lilienfeld AM. The Fielding H. Garrison Lecture: Ceteris paribus: the evolution of the clinical trial. *Bull Hist Med*. 1982; 56(1):1–18. [PubMed: 7046852]
31. Liu Z, Yamamura B, Shcherbatyy V, Green J. Regional volumetric change of the tongue during mastication in pigs. *J Oral Rehabilitation*. 2008; 35(8):604–12.
32. Ma D, Shuler JM, Kumar A, Stanford QR, Tungtur S, Nishimune H, Stanford JA. Effects of Tongue Force Training on Bulbar Motor Function in the Female SOD1-G93A Rat Model of Amyotrophic Lateral Sclerosis. *Neurorehabil Neural Repair*. 2016
33. Macrae PR, Jones RD, Huckabee ML. The effect of swallowing treatments on corticobulbar excitability: a review of transcranial magnetic stimulation induced motor evoked potentials. *J Neurosci Methods*. 2014; 233:89–98. [PubMed: 24932964]

34. Magara J, Michou E, Raginis-Zborowska A, Inoue M, Hamdy S. Exploring the effects of synchronous pharyngeal electrical stimulation with swallowing carbonated water on cortical excitability in the human pharyngeal motor system. *Neurogastroenterol Motil.* 2016; 28(9):1391–400. [PubMed: 27061591]
35. Michou E, Mistry S, Jefferson S, Tyrrell P, Hamdy S. Characterizing the mechanisms of central and peripheral forms of neurostimulation in chronic dysphagic stroke patients. *Brain stimulation.* 2014; 7(1):66–73. [PubMed: 24314909]
36. Morishima Y, Chida K, Watanabe H. Estimation of the Dose of Radiation Received by Patient and Physician During a Videofluoroscopic Swallowing Study. *Dysphagia.* 2016; 31(4):574–8. [PubMed: 27318941]
37. Nuckolls AL, Worley C, Leto C, Zhang H, Morris JK, Stanford JA. Tongue force and tongue motility are differently affected by unilateral vs bilateral nigrostriatal dopamine depletion in rats. *Behav Brain Res.* 2012; 234(2):343–8. [PubMed: 22796604]
38. Park AM, Bhatt NK, Paniello RC. Paclitaxel inhibits post-traumatic recurrent laryngeal nerve regeneration into the posterior cricoarytenoid muscle in a canine model. *Laryngoscope.* 2016
39. Pisegna JM, Kaneoka A, Pearson WG Jr, Kumar S, Langmore SE. Effects of non-invasive brain stimulation on post-stroke dysphagia: A systematic review and meta-analysis of randomized controlled trials. *Clin Neurophysiol.* 2016; 127(1):956–68. [PubMed: 26070517]
40. Ramsey D, Smithard D, Kalra L. Silent aspiration: what do we know? *Dysphagia.* 2005; 20(3): 218–25. [PubMed: 16362510]
41. Ravosa MJ, Vinyard CJ, Gagnon M, Islam SA. Evolution of anthropoid jaw loading and kinematic patterns. *Am J Phys Anthropol.* 2000; 112(4):493–516. [PubMed: 10918126]
42. Russell JA, Ciucci MR, Hammer MJ, Connor NP. Videofluorographic assessment of deglutitive behaviors in a rat model of aging and Parkinson disease. *Dysphagia.* 2013; 28(1):95–104. [PubMed: 22763806]
43. Satoh Y, Tsuji K, Tsujimura T, Ishizuka K, Inoue M. Suppression of the swallowing reflex by stimulation of the red nucleus. *Brain Res Bull.* 2015; 116:25–33. [PubMed: 26012722]
44. Sugiyama N, Nishiyama E, Nishikawa Y, Sasamura T, Nakade S, Okawa K, Nagasawa T, Yuki A. A novel animal model of dysphagia following stroke. *Dysphagia.* 2014; 29(1):61–7. [PubMed: 23907747]
45. Thexton AJ, Crompton AW, German RZ. Electromyographic activity during the reflex pharyngeal swallow in the pig: Doty and Bosma (1956) revisited. *Journal of Applied Physiology.* 2007; 102(2):587–600. [PubMed: 17082375]
46. Tsuji K, Tsujimura T, Magara J, Sakai S, Nakamura Y, Inoue M. Changes in the frequency of swallowing during electrical stimulation of superior laryngeal nerve in rats. *Brain Res Bull.* 2015; 111:53–61. [PubMed: 25542096]
47. Tsujimura T, Tsuji K, Ariyasinghe S, Fukuhara T, Yamada A, Hayashi H, Nakamura Y, Iwata K, Inoue M. Differential involvement of two cortical masticatory areas in modulation of the swallowing reflex in rats. *Neurosci Lett.* 2012; 528(2):159–64. [PubMed: 22982202]
48. Vinyard CJ, Williams SH, Wall CE, Johnson KR, Hylander WL. Jaw-muscle electromyography during chewing in Belanger's treeshrews (*Tupaia belangeri*). *Am J Phys Anthropol.* 2005; 127(1): 26–45. [PubMed: 15486965]
49. Vinyard CJ, Wall CE, Williams SH, Johnson KR, Hylander WL. Masseter electromyography during chewing in ring-tailed lemurs (*Lemur catta*). *Am J Phys Anthropol.* 2006; 130(1):85–95. [PubMed: 16345068]
50. Wall CE, Vinyard CJ, Johnson KR, Williams SH, Hylander WL. Phase II jaw movements and masseter muscle activity during chewing in *Papio anubis*. *Am J Phys Anthropol.* 2006; 129(2): 215–24. [PubMed: 16278877]
51. Walston J, Hadley EC, Ferrucci L, Guralnik JM, Newman AB, Studenski SA, Ershler WB, Harris T, Fried LP. Research Agenda for Frailty in Older Adults: Toward a Better Understanding of Physiology and Etiology: Summary from the American Geriatrics Society/National Institute on Aging Research Conference on Frailty in Older Adults. *Journal of the American Geriatrics Society.* 2006; 54(6):991–1001. [PubMed: 16776798]

52. Wirth R, et al. Oropharyngeal dysphagia in older persons - from pathophysiology to adequate intervention: a review and summary of an international expert meeting. Clin Interv Aging. 2016; 11:189–208. [PubMed: 26966356]

Figure 1.
Dysphagia flow chart.

Figure 2.
Components of underlying physiology that generate performance failure.

Figure 3.
Relationship among underlying components for both normal and pathologic function.