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Age-related Alterations in Swallowing Biomechanics

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Abstract

Background: Aging rodent models allow for the discovery of underlying mechanisms of cranial muscle dysfunction. Methods are needed to allow quantification of complex, multivariate biomechanical movements during swallowing. Videofluoroscopic swallow studies (VSS) are the standard of care in assessment of swallowing disorders in patients and validated quantitative, kinematic, and morphometric analysis methods have been developed. Our purpose was to adapt validated morphometric techniques to the rodent to computationally analyze swallowing dysfunction in the aging rodent.

Methods: VSS, quantitative analyses (bolus area, bolus velocity, mastication rate) and a rodent specific multivariate, morphometric computational analysis of swallowing biomechanics were performed on 20 swallows from 5 young adult and 5 old Fischer 344/Brown Norway rats. Eight anatomical landmarks were used to track the relative change in position of skeletal levers (cranial base, vertebral column, mandible) and soft tissue landmarks (upper esophageal sphincter, base of tongue).

Results: Bolus area significantly increased and mastication rate significantly decreased with age. Aging accounted for 77.1% of the variance in swallow biomechanics, and 18.7% of the variance was associated with swallow phase (oral vs pharyngeal). *Post hoc* analyses identified age-related alterations in tongue base retraction, mastication, and head posture during the swallow.

Conclusion: Geometric morphometric analysis of rodent swallows suggests that swallow biomechanics are altered with age. When used in combination with biological assays of age-related adaptations in neuromuscular systems, this multivariate analysis may increase our understanding of underlying musculoskeletal dysfunction that contributes to swallowing disorders with aging.

Graphical Abstract

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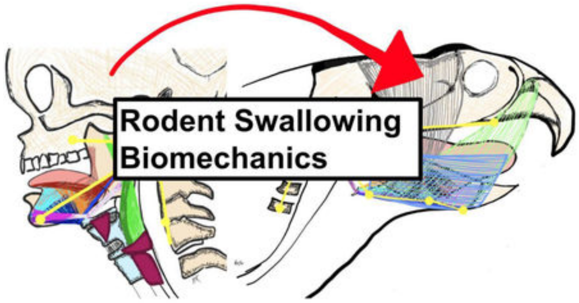

Keywords

Aging; swallowing; mastication; dysphagia; biomechanics

1. Introduction

In the next 35 years, it is projected that over 89 million people will be 65 years and older in the United States.(Ortman and others 2014; Prevention 2013) Swallowing disorders (dysphagia) in elderly people impede the ability to eat a meal, a function fundamental to quality of life, and may contribute to aspiration pneumonia and death.(Baum and Bodner 1983; Clark and others 2003; Clark and Solomon 2012) Swallowing function is typically examined using an instrumental evaluation, such as the modified barium swallow study that uses videoflururoscopy and allows for visualization of the bolus as it moves through the oral cavity and pharynx. Methodologies have been developed to assess and quantify swallowing impairment (Modified Barium Swallowing Tool [MBSImp])(Martin-Harris and others 2008) and biomechanics (Computational Analysis of Swallowing Mechanics [CASM]) in humans,(Ellis and others 2018; Garand and others 2018; May and others 2017; Pearson and others 2016a; Pearson and others 2016b; Pearson and Zumwalt 2013; Schwertner and others 2016; Thi Tu Anh and others 2018) but underlying physiological and biological mechanisms of age-related swallow dysfunction have not yet been identified.

Because use of human subjects is often precluded in examination of age-related muscular degeneration in the cranial motor system, aging rodent models have been used to study underlying biochemical, molecular, and cellular mechanisms contributing to age-related dysphagia. Degeneration of muscles involved in mastication and swallowing have been reported in aged rodents, and include alterations in the myosin heavy chain composition, atrophy and death of myofibers and myonuclei, fragmentation of the neuromuscular junction, and impaired regenerative potential.(Connor and others 2012; Cullins and Connor 2017; Elkerdany and Fahim 1993; Johnson and Connor 2011; Kletzien and others 2018a; Kletzien and others 2018b; Kletzien and others 2013; Krekeler and others 2018; Ono and others 2010; Randolph and others 2014; Randolph and Pavlath 2015; Russell and others 2013; Sambasivan and others 2009) These age-related changes of the musculoskeletal system may be associated with alterations in muscle contractile properties and contribute to weakness and fatigue in the aged tongue, masseter, and pharynx. However, previous studies have lacked the ability to link age-related changes in muscle to masticatory and swallowing biomechanics, and thus causal mechanisms remain undiscovered.

To assess swallowing function in aging rodents, prior studies have used validated quantitative measures of rat deglutition (bolus area, bolus velocity, and mastication rate) to quantify mastication and swallowing because they can be easily extracted from videofluoroscopic swallow studies (VSS).(Lever and others 2015a; Lever and others 2015b; Russell and others 2013) Although these more traditional VSS analyses are useful in assessment of swallowing function and quantification of swallowing kinematics and bolus dynamics, they do not provide biomechanical information that may be valuable in the determination of underlying musculoskeletal dysfunction that contributes to dysphagia. Because age-related degeneration of muscles in the head and neck may alter swallowing biomechanics, the development of methodology to study oropharyngeal swallow mechanics in the rodent model is crucial. In this manner, it can be determined how anatomic and physiologic changes with aging affect movement biomechanics and overall swallow function. Our purpose was to develop a computational tool to assess swallowing biomechanics in a rodent model and determine whether aging induces changes in the swallowing mechanics of rats.

2. Methods

2.1. Animals

The animal care and use protocol for this study was approved by the University of Wisconsin School of Medicine and Public Health Animal Care and Use Committee, and performed in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and the Public Health Service policy on care and use of laboratory animals. Five young adult (6 mo. old) and 5 old (29 mo. old) Fischer 344/BN rats were obtained from the National Institute on Aging Rodent Colony (Charles River Laboratories) 2 weeks prior to the VSS. Rats were housed in pairs, in standard polycarbonate cages in a light controlled environment with a 12:12 hour light-dark reversed light cycle.

2.2. Videofluoroscopic Swallow Study (VSS)

VSS was performed to assess swallowing function in all rats. An L-shaped platform was secured to the inside of a single rat’s clear polycarbonate home cage. A mixture of 5 g of peanut butter and 5 mL of barium sulfate (EZ-M Varibar Nectar) was placed on the platform at the level of the mouth. The rat moved freely within the cage and ingested the peanut butter/barium mixture *ad libitum* for a maximum of 5 minutes to obtain 2, high-quality videofluoroscopic swallows per rat. Images were obtained on a C-ARM fluoroscope model OEC 9800 (GE Medical Systems-OEC) at a rate of 30 frames per second. A high-quality videofluoroscopic swallow was defined by: 1) the rat was in the sagittal plane for the entirety of the swallow, 2) the entire act of deglutition (procurement, ingestion [oral phase, pharyngeal phase, esophageal phase] could be observed, and 3) all anatomical coordinates could be visualized, and reliably mapped throughout the entire swallow (Fig. 1; Fig. 3).

2.3. Quantitative Spatial and Temporal Analysis

Quantitative analysis (Russell and others 2013) of bolus area (bolus size measured following swallowing initiation; mm^2; spatial), bolus velocity (speed of the head of bolus from initiation of the swallow to the 4^{th} cervical vertebrae; mm/s; temporal), and mastication rate (following bolus procurement, the rate of 5 total opening and closing cycles of the mandible; cycles/s: temporal) were determined in 20 swallows (10 young adult; 10 old). Quantitative measures were averaged from each of the 2 swallows per rat. Three raters (HK, AJH, SMW) examined 10% of all swallows to achieve an inter-rater reliability score r>0.75.

2.4. Computational Analysis of Swallowing Mechanics-Rodent (CASM-R)

A well-established and validated methodology to characterize covariant swallowing mechanics in humans served as the foundation for the development of CASM-R.(Natarajan and others 2017; Pearson and Zumwalt 2013; Schwertner and others 2016; Thi Tu Anh and others 2018) Because anatomical and VSS protocol differences between humans and rodents exist, it was necessary to develop a rodent-specific multivariate morphometric methodology for the analysis of swallowing biomechanics (Fig. 2A-B).(Cenci and others 2002; Russell and others 2013; Treuting and others 2018) Eight anatomical landmarks were identified that characterized the morphology of rodent swallowing mechanics (Fig. 2C). Coordinates 1-3 and 5-7 tracked the relative change in position of 3 skeletal levers (cranial base, vertebral column, mandible), and coordinates 4 and 8 tracked soft tissue landmarks (upper esophageal sphincter [UES] and base of tongue, respectively). Together, the 8 coordinates mapped muscle groups involved in the displacement of the pharynx, tongue, and jaw (Fig. 2D).

Coded videos were converted from .ima to .avi files and trimmed. Twenty swallows (10 young adult; 10 old) were individually uploaded into MATLAB and the 8 coordinates were mapped in every frame of the swallow using a semi-automated tracking tool (Fig 3). Magnification and contrast-correction of videos were utilized to ensure precise, and identical placement of each anatomical coordinate marker at each frame throughout the entirety of the swallow. Frames depicting the oral (mastication and tongue pump) and pharyngeal (bolus movement through the pharynx until UES entrance) phases of the swallow were recorded. Upon completion of coordinate mapping, a .txt file was extracted from MATLAB with the x- and y-position of each coordinate for every frame of the swallow, and a .mp4 video was created that displayed each coordinate-mapped landmark of the individual swallow. Individual .txt files of coordinates were concatenated to generate a single file of all coordinates (n=976), and classifier variable (age and swallowing phase) were assigned to each of the 8 coordinates for every frame of the swallow. Intra-rater (HK) reliability, r>0.95, of coordinate mapping of 15% of all swallows was achieved. Inter-rater (HK, MJC) reliability, r>0.91, was achieved for coordinate mapping for 15% of the swallows throughout the entire process of deglutition.

2.5. Statistical Analysis

Two-tailed t-tests were used to compare quantitative spatial and temporal measures between each age group, and the critical value of obtaining statistical significance was set at α=0.05.

CASM-R data were uploaded into MorphoJ, an integrated geometric morphometric software program, to evaluate the overall shape change associated with multivariate components underlying swallowing biomechanics in an aging rodent model.(Klingenberg 2011) A Procrustes fit was first generated following a matrix transformation to align the coordinates along the cervical vertebrae (coordinates 2 &3; Fig 4) to assess the distribution of shape change during the swallow, adjusting for image rotation and size differences that may exist among rats. A canonical variate analysis was then used to determine biomechanical differences associated with age and swallowing phase. *Post hoc* discriminant function analysis was performed to visualize biomechanical differences (eigenvectors scaled according to the Mahalanobis distance at each coordinate) associated with the classifier variables for CV1 and CV2. The critical value of obtaining statistical significance was set at $\alpha<0.006$ by performing a Bonferroni correction to account for multiple comparisons of 10 coordinates.

3. Theory

Swallowing mechanics have traditionally been studied using conventional quantitative and kinematic measures of specific anatomical structures involved in swallowing. Because the swallow is a complex mechanism involving numerous skeletal and cartilaginous structures and multiple muscle groups, it should be analyzed using a multivariate approach that allows for the analysis of the overall oropharyngeal conduit shape change across the duration of the swallow.(Pearson and Zumwalt 2013) Development of a rodent-based platform that is analogous to that used to analyze human swallows, allows for the assessment of biomechanics across numerous pathological phenotypes, including genetic and transgenic rodent models, and will improve our understanding of the underlying biological mechanisms that manifest as biomechanical alterations and ultimately a disordered swallow.

4. Results

4.1. Quantitative Swallowing Analysis

With aging, bolus area significantly increased ($t_{18}=-2.126$, p=0.048; Fig. 5A) and mastication rate was significantly reduced ($t_{18}=6.072$, $p<0.001$; Fig. 5B) in old rats compared with young adult rats. No difference in bolus velocity was observed ($t_{18}=-0.524$, p=0.607; Fig. 5C).

4.2. Swallowing Biomechanics

Canonical variate analysis by age and swallowing phase resulted in 77.14% of the variance associated with age (CV1; D=1.907, $p<0.0001$; Fig. 6A) and 18.74% of the variance associated with swallowing phase (CV2; D=1.1835, $p<0.0001$; Fig 6B). Post hoc discriminant function analysis suggested that with aging, tongue base retraction was reduced, masticatory movements were more variable, and increased flexion and caudal movement of the head, was observed in old rats compared to young rats during the swallow (Fig. 6C).

5. Discussion

In this initial study to assess the utility of Computational Analysis of Swallowing Mechanics-Rodent (CASM-R) to examine differences in biomechanics during the oral and pharyngeal phases of the swallow in an aging rodent model, geometric morphometric analysis of 20 swallows identified biomechanical alterations in old rats. Future research will combine biological assays of neuromuscular systems involved in swallowing with multivariate morphometric analyses of anatomical components of the rodent swallow to increase our understanding of underlying dysfunctions that contribute to swallowing disorders. Resulting data may aid in the development of targeted therapies that combat observed biomechanical swallowing alterations.

Age-related alterations in bolus area and mastication rate have been observed in a rat model using conventional and validated kinematic measurements of swallowing function. However, application of CASM-R allows us to go beyond those measures to characterize the biomechanics and resulting covariant shape changes of the swallowing mechanism that occur with age. When examined directly, our morphometric findings suggest swallowing biomechanics change with increasing age and may contribute to altered kinematics and bolus flow dynamics. Eigenvectors of landmarks representing the base of tongue and cervical vertebrae indicate reduced base of tongue retraction and a flexed, caudal movement of the head and neck during the aged swallow. A reduction in base of tongue retraction may contribute to an accumulation of material in the oropharynx and thus larger bolus sizes for subsequent swallows. Posterior movement of the head and neck in combination with increased flexion may be a compensatory mechanism to initiate the oropharyngeal swallow and propulsion of large boluses through the pharynx. Additionally, eigenvectors overlaid on the mandible masticatory mechanics are also altered with age, and may be related to slower mastication rates in old rats.

The quantitative and biomechanical results of this study further our understanding of age-related swallowing disorders when viewed in conjunction with previously published findings. In the rat model, we have reported age-related reductions in tongue force, longer tongue muscle contraction times, increased tongue muscle fatigue, alterations in tongue and masticatory muscle biochemical properties, and increases in apoptosis of myonuclei.(Becker and others 2015; Connor and others 2012; Connor and others 2009; Cullins and Connor 2017; Kletzien and others 2018a; Kletzien and others 2015; Kletzien and others 2018b; Kletzien and others 2013; Krekeler and others 2018; Nagai and others 2008; Russell and Connor 2014; Schaser and others 2015) These biological alterations in tongue muscles are likely contributors to the biomechanical alterations noted in the aging swallow and require further study in a common cohort of animals. In the future, to decipher whether biomechanical changes are muscular, skeletal, or musculoskeletal of origin, skeletal analyses of cranial, mandibular, and cervical bones should also be included. It may also be advantageous to use implantable markers to detect movements of posterior, medial, and anterior tongue and the hyoid bone to further understand swallowing biomechanics with age and disease.

6. Conclusion

Geometric morphometric analysis of rodent swallows found that swallow biomechanics are altered with age. When used in future mixed methods studies that combine these analyses with cellular, molecular, and physiological assays of age-related adaptations in neuromuscular systems, we may increase our understanding of underlying dysfunctions that contribute to swallowing disorders with aging and neuromuscular diseases.

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Abbreviations:

VSS	videfluoroscopic swallow studies
CASM-R	Computational Analysis of Swallowing Mechanics-Rodent

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Highlights

- Bolus area and mastication rate (kinematic data) change with age
- Swallow biomechanics are altered with age
- Age-related changes in biomechanics contributes to swallowing dysfunction in rodents

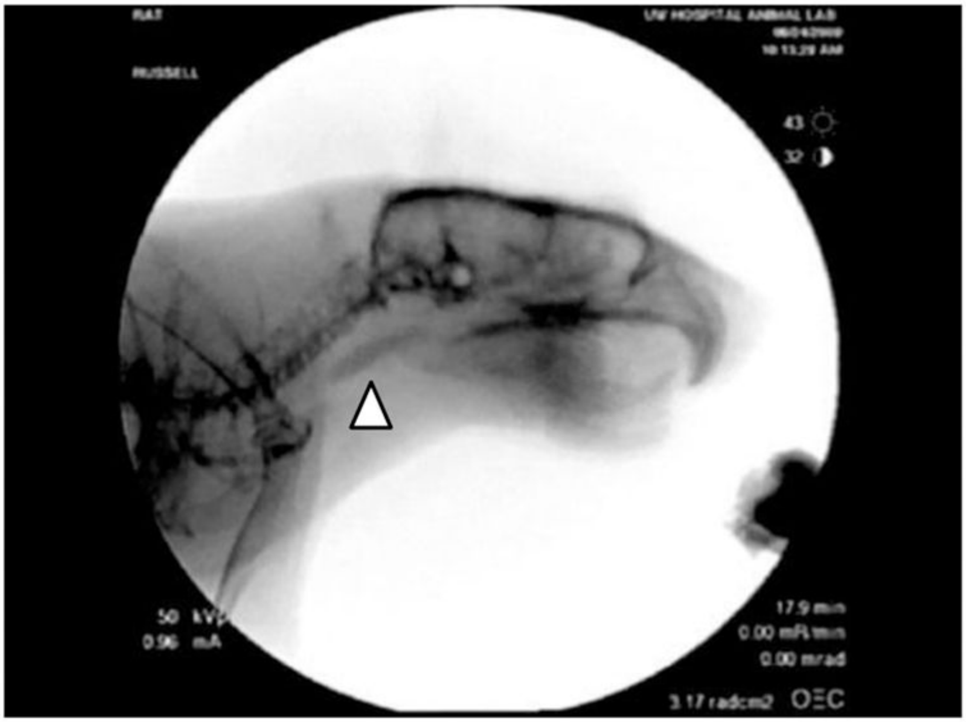


Fig. 1.
Representative still frame from a rat undergoing VSS. (arrowhead = bolus)

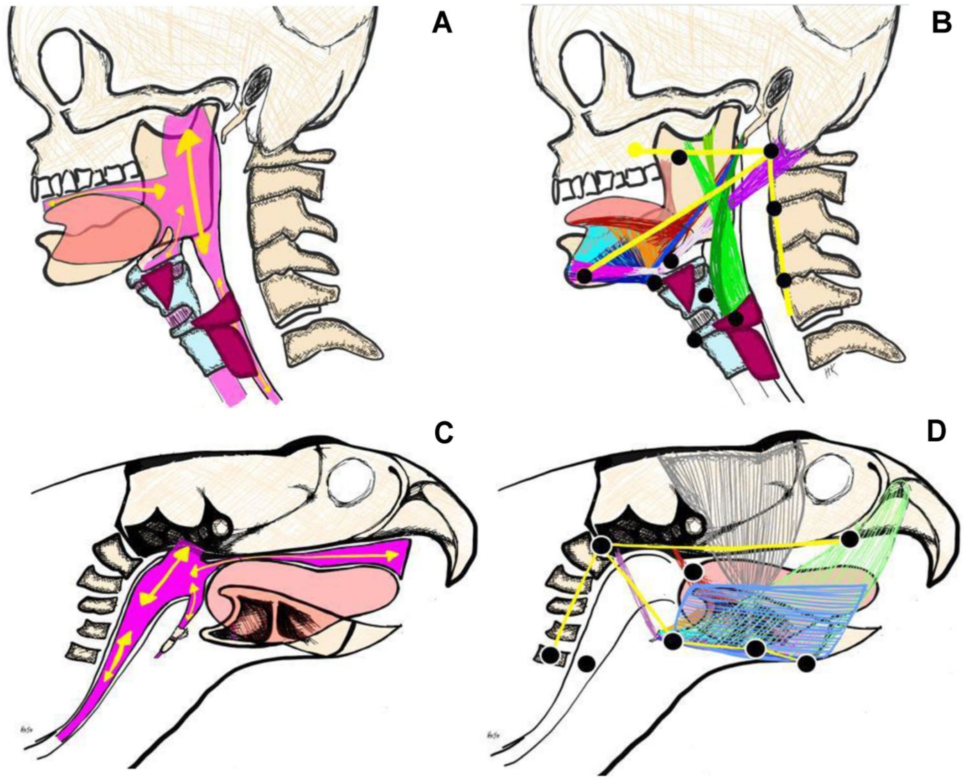


Fig. 2.

Differences in geometry between human (A,B) and rodent (C,D) swallowing anatomy, in the oropharyngeal conduit through which the bolus is transported (A,C) and in the orientation of the muscles involved in propulsion and transport of the bolus. Anatomical landmarks to characterize swallowing mechanics in human (CASM; B) and rodent (CASM-R; D) map muscle groups involved in tongue, jaw, and pharyngeal displacement.

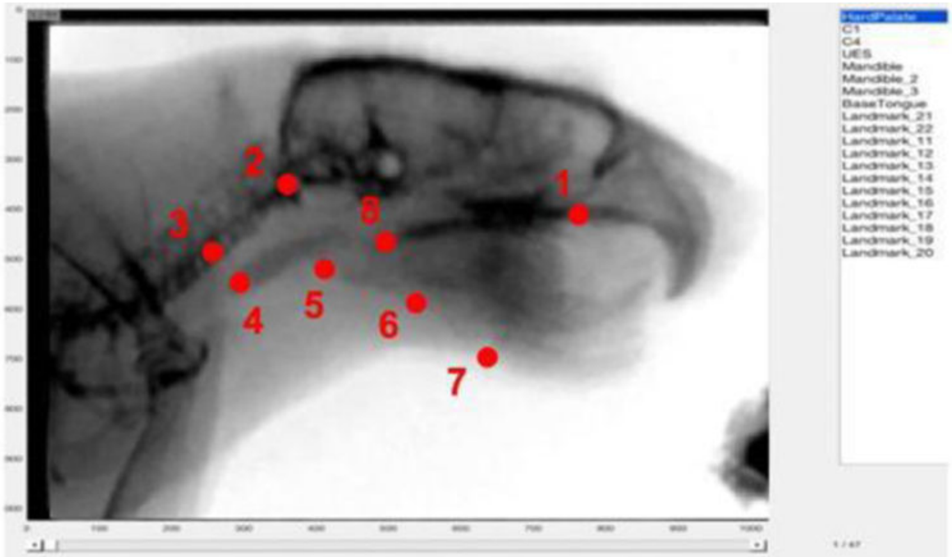


Fig. 3.

Rodent specific MATLAB semi-automatic tracker tool. Overlaid are markers identifying the 8 anatomical coordinates used to map the biomechanics of the rodent swallow.

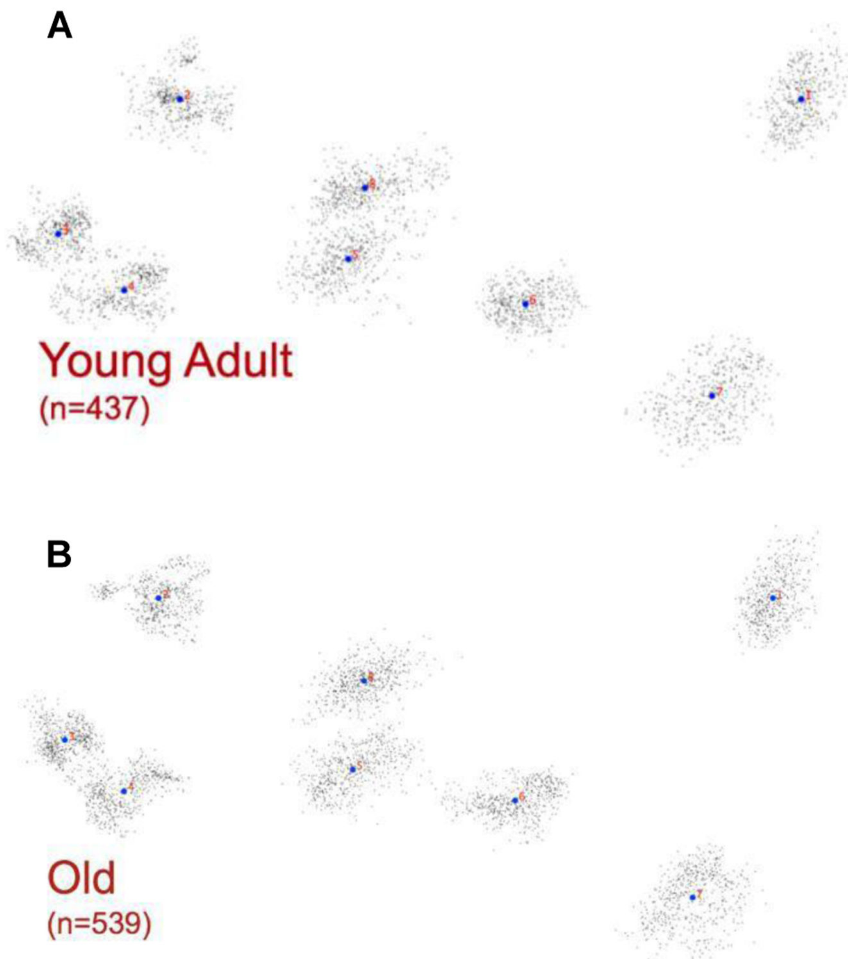


Fig. 4.

Distribution of shape change between young (A) and old (B) rats during the swallow was first assessed with a Procustes fit. Black dots represent individual landmark positions for every frame of every swallow, while the blue dots represent the mean position of each landmark.

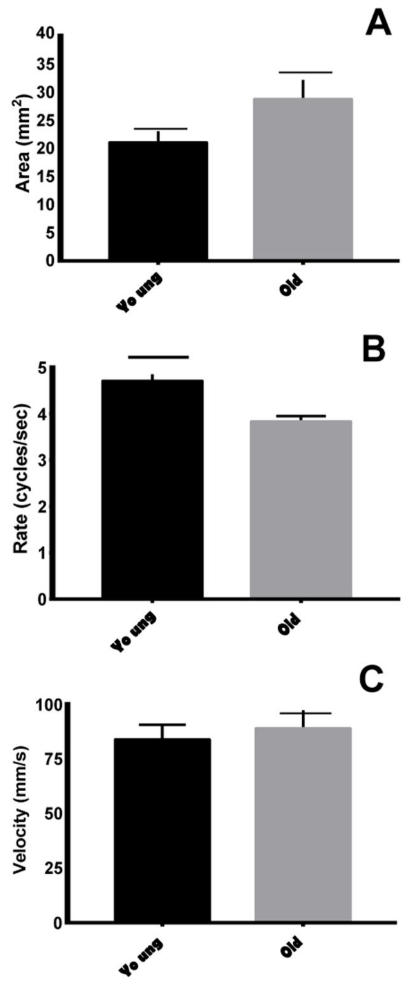
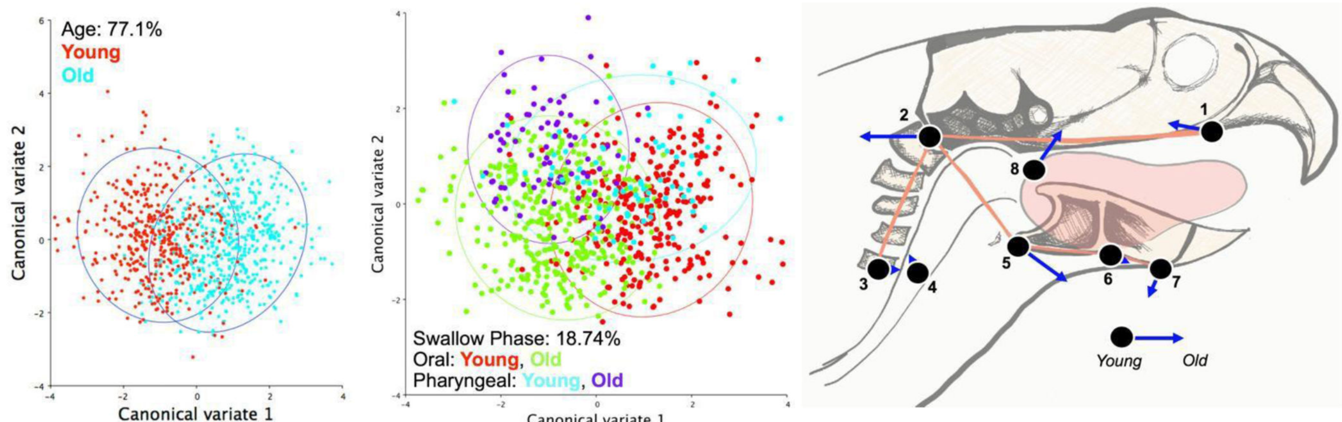


Fig. 5.

Swallowing kinematics. With age, bolus area increased (A) and mastication rate declined (B). No differences were observed in bolus velocity (C).



suggest

Fig. 6.

Swallowing Biomechanics. Aging accounted for 77.1% of the variance in shape change during the oropharyngeal swallow (A), while swallowing phase (oral vs pharyngeal by age) accounted for 18.74% of the variance (B). Overlaid are eigenvectors showing the magnitude and direction of shape change of young versus old swallowing biomechanics following Discriminant Function Analysis, and suggests reduced tongue base retraction (8), more variable masticatory movements (5,7), and compensatory head movements (2) with increasing age (C).