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SHORT COMMUNICATION

Anatomical and physiological variation of the hyoid musculature during swallowing in infant pigs

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ABSTRACT

The function of a muscle is impacted by its line of action, activity timing and contractile characteristics when active, all of which have the potential to vary within a behavior. One function of the hyoid musculature is to move the hyoid bone during swallowing, yet we have little insight into how their lines of action and contractile characteristics might change during a swallow. We used an infant pig model to quantify the contractile characteristics of four hyoid muscles during a swallow using synchronized electromyography, fluoromicrometry and high-speed biplanar videofluoroscopy. We also estimated muscle line of action during a swallow using contrast-enhanced CT-scanned muscles animated to move with the hyoid bone and found that as the hyoid elevated, the line of action of the muscles attached to it became greater in depression. We also found that muscles acted eccentrically and concentrically, which was correlated with hyoid movement. This work contributes to our understanding of how the musculature powering feeding functions during swallowing.

KEY WORDS: Fluoromicrometry, DiceCT, Feeding, Muscle, Biomechanics

INTRODUCTION

The ability of muscles to generate movement depends on a variety of factors, including their leverage, timing of activity and contractile characteristics (Biewener, 1989; Biewener and Gillis, 1999; Hutchinson et al., 2005; Maynard Smith and Savage, 1956). These factors have the potential to vary with development, behavior and/or environment, which makes understanding muscle function challenging. This is especially critical in muscles that show variation in activity within a given behavior, muscles that power multiple behaviors and/or muscles with functions that may change through ontogeny (Cheng et al., 2008; Earhart and Stein, 2000; Gillis and Biewener, 2000; Gillis and Blob, 2001; Mayerl et al., 2021). For example, muscle firing patterns differ between infant mammals feeding on milk versus those drinking from a bowl or eating solid food (Thexton et al., 1998). Furthermore, the leverage of a given muscle has the potential to change within a given activity as its orientation relative to the joint changes (Hutchinson et al., 2005; Kargo and Rome, 2002). Importantly, knowledge of a muscle's leverage and activity, without insight into the contractile characteristics of a muscle during an activity, provides an incomplete picture of the function of a given muscle.

The possibility for variation in muscle function within a single event is especially high for movements with many degrees of freedom, such as movements of the hyoid bone (German et al., 2011). The hyoid in mammals is suspended by a suite of muscles, with limited to no bony constraints to its movement (Thexton et al., 2007). However, controlled movement of the hyoid is essential for a variety of behaviors, including breathing, vocalization and swallowing (German et al., 2011). Although we have a detailed understanding of the timing of muscle activity during swallowing, we have little to no insight into the contractile characteristics of the hyoid musculature such as whether and when these muscles are firing concentrically, eccentrically or isometrically when active. The results that do exist, for geniohyoid and sternohyoid, plus more extensive data on the jaw adductors, indicate that contractile characteristics and muscle activity are regionally variable within a muscle, and the muscles themselves have been demonstrated to be highly heterogeneous (Herring et al., 1991; Holman et al., 2012; Konow et al., 2011; Lakars and Herring, 1987).

Here, we used an infant pig model to evaluate the contractile characteristics of four muscles attached to the hyoid during swallowing. Our muscles of interest include those hypothesized to primarily move the hyoid anteriorly (geniohyoid) and posteriorly (thyrohyoid and omohyoid), as well as elevate the hyoid (stylohyoid). This allowed us to evaluate the possibility that muscles with different orientations might have different levels and patterns of variation in contractile characteristics. We measured muscle activity using electromyography (EMG) and contractile characteristics using fluoromicrometry (Camp et al., 2016), synchronized with hyoid kinematics obtained from biplanar videofluoroscopy. Our null expectation was that each muscle acted primarily concentrically to move the hyoid along its line of action. By digitally reconstructing the orientation of these muscles at rest (when the hyoid is depressed) and at maximal hyoid elevation, we determined changes in the lines of action of these muscles throughout a swallow. In doing so, this work provides insight into the complexity of hyoid function and contributes to our understanding of how the musculature powering feeding acts during infancy.

MATERIALS AND METHODS

Animal housing and care

Infant pig care followed standard procedures (Mayerl et al., 2019, 2020a), whereby four pigs (*Sus scrofa* Linnaeus 1758) were obtained at 1 day of age (Yorkshire/Landrace, Shoup Investments, Orville, OH, USA) and trained to drink infant pig formula (Solustart Pig Milk Replacement, Land O'Lakes, Arden Mills, MN, USA) via a bottle and modified nipple (NASCO Farm & Ranch, Fort Atkinson, WI, USA). Animals used in this study were part of a

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larger study involving muscle function in infant feeding (Mayerl et al., 2021). All animal care and procedures were approved by the NEOMED Institutional Animal Care and Use Committee (protocol no. 19-03-222).

Surgical procedures

Details on surgical procedures can be found in Mayerl et al. (2021). Throughout the duration of the experiment, infant pigs underwent three separate procedures. (1) At 5 days of age, tantalum markers were implanted under isoflurane anesthesia into the subdermal space dorsal to the snout, in the hard palate, tongue midline (anterior, middle, and posterior locations), soft palate and palatopharyngeal arches.

Each animal also underwent two surgeries for the implantation of electrodes and fluoromicrometry beads. (2) One surgery occurred between 6 and 8 days of age, and (3) the second occurred between 20 and 22 days of age. Although we collected data following both sterile procedures, the data presented here were collected after the second sterile surgery, with the first being mentioned for completeness. In both sterile surgeries, electrodes and 0.8 mm tantalum fluoromicrometry beads were placed in muscles of interest. Bipolar electrodes were constructed by twisting two individually insulated wires together with slightly different lengths (1 mm). For younger pigs, we sutured a radiopaque (3 mm) bead at the base of the hyoid bone and adjacent to the thyroid eminence, and then placed electrodes on the left side of the animal in four muscles attached to the hyoid bone that span the anteroposterior axis of muscles involved in swallowing: geniohyoid, stylohyoid, thyrohyoid and omohyoid. Electrodes were inserted to the right side of the animal during the surgery occurring when pigs were older. We sutured electrodes to connective tissue in the body as a means of strain relief. All electrodes were connected to a microconnector that exited the body at the posterior margin of the incision. Microconnectors were attached to a 25-pin D connector outside of the body and cables were secured with Vetwrap to minimize the risk of pigs pulling out cables or injuring themselves.

Data collection

Shortly after animals recovered from surgery (approximately 12–24 h), we recorded digitally synchronized videofluoroscopy with EMG. Video data were collected using a high-speed camera (XC1M, XCitex, Cambridge, MA, USA) at 100 Hz during fluoroscopic exposure (GE 9400C-Arm, 70 kV, 5 mA) using standard XROMM data collection procedures (Brainerd et al., 2010). Pigs were placed in a radiolucent box and fed infant pig formula mixed with barium from a bottle and nipple *ad libitum*. We recorded at least 20 swallows per pig per age, starting after the first 10 s of feeding, which occurs at a faster rate than normal (Gierbolini-Norat et al., 2014). All EMG signals were amplified and recorded at 10 kHz (MA-300, Motion Laboratory Systems, Baton Rouge, LA, USA) on a 16-channel Powerlab (16/35, ADInstruments, Colorado Springs, CO, USA). Animals were euthanized at the conclusion of experiments and electrode placements were verified postmortem via manual dissection.

Anatomical data were collected using DiceCT (Gignac et al., 2016) on a pig that was previously euthanized at 21 days of age. For the pig to fit in the scanner and to reduce noise as much as possible, we removed the right half of the animal and the skull cap using a bandsaw. To reduce diffusion time, the pig was initially perfused using a 5% I2Ki solution through the common carotid artery and then placed in a 10% formalin bath for 14 days for tissue fixation. After tissues were fixed, the pig was placed in a 2.5% I2Ki solution, which was changed every 14 days, and CT scanned (35 µm, 70 kVp, 22 µA) until the scan showed complete and even staining throughout the tissue.

Data processing

The beginning of the swallow was identified as the frame where the bolus accumulated in the valleculae prior to passing the epiglottis, following published procedures (Mayerl et al., 2020b,c,d). The end of the swallow was identified as the frame in which the epiglottis returned to a resting position. To analyze muscle activity patterns, we first eliminated any channels with high levels of baseline noise. Within a sequence, if movement artifact was present (identified through synchronized EMG with X-ray video), that section of the recording was not used for analyses. For channels and sequences that were remaining, we applied a band-pass filter to reduce background noise and documented muscle onset and offset manually in LabChart (ADInstruments), by measuring the point in time where the signal was at least 30% above baseline levels. Muscles involved in swallowing typically only show one burst of activity within a swallow (Doty and Bosma, 1956; Thexton et al., 2007; Delozier et al., 2018), so the first occurrence where this threshold was reached was determined as onset, and when values reached below the threshold, this was determined as offset.

Kinematic data from the entire feeding sequence of identified swallows (the start of the first swallow through the end of the last swallow) were processed in lateral and dorsoventral views using XMALab (Knörlein et al., 2016) via a combination of manual tracking and automatic tracking from DeepLabCut (Laurence-Chasen et al., 2020; Mathis et al., 2018), which was then evaluated and corrected as necessary. Markers inserted into the nose and hard palate had intermarker distance standard deviations of less than 0.03 mm and were assigned as a rigid body. Following published procedures (Mayerl et al., 2020b), Kinematic data were filtered using a 10 Hz low-pass filter to reduce noise, and *XYZ* points of individual markers and the skull rigid body were exported, as well as undistorted video from each camera. Exported data was loaded into Autodesk Maya (Autodesk, San Rafael, CA, USA). We created an anatomical coordinate system (ACS) parented to the skull rigid body placed at the midline of the animal at the anterior tip of the hard palate. In this ACS, translations in the *X* plane indicated anteroposterior movements, *Y* translations indicated mediolateral movement and *Z* translations indicated dorsoventral movement. We measured hyoid kinematics relative to this ACS (oRel, XROMM MAYA tools) and exported them for analysis relative to muscle length changes. Exported hyoid kinematics were processed through a custom MATLAB script that synchronized 3D hyoid movements with EMG data. Hyoid movements were set at zero at the beginning of the swallow, and deviations prior and following this setpoint reflected changes from the standardized position. As the hyoid bone primarily moves dorsoventrally and anteroposteriorly, we extracted translations of the hyoid in these dimensions for analysis.

Fluoromicrometry data exported from XMALab were processed using a custom MATLAB script that calculated the distance between two markers and synchronized it with the timing of muscle activity for each swallow. For each set of swallows, we calculated and analyzed the raw intermarker distance during muscle activity, as well as intermarker distance interpolated throughout activity to percentage of activity to account for differences in length of muscle activation. We analyzed intermarker distance for both the entire swallow and the period of time when each muscle was active. For each muscle, muscle length was set at zero at the frame the muscle began firing, with subsequent lengths reflecting deviations from the initial length.

DiceCT data were segmented using Avizo 9.4 (FEI Visualizations Science Group, Hillsboro, OR, USA). Muscle fibers and endomysium were identified as high-density material and were surrounded by lower density material (perimysium), which was below the minimum gray-scale value threshold to be included in segmented volumes. We segmented the entire muscle for each muscle we recorded EMG data on. Segmented muscles were exported as individual .obj files and were imported into Autodesk Maya, along with models of the skull, hyoid, and left and right mandibles of a scanned infant pig with an intact cranium. Each set of muscles were grouped together and then rotated and translated to align them with the skull. To do so, we positioned the hyoid between all segmented muscles, with geniohyoid running parallel to the long axis of the body. To measure muscle lines of action, we created a set of axes with the anteroposterior axis aligned to be parallel to the long axis of the body, the dorsoventral axis set as being perpendicular to the hard palate and the mediolateral axis being orthogonal to those two. As muscle lines of action have been reported in detail elsewhere (Mayerl et al., 2021), here we focused primarily on dorsoventral angles relative to the hyoid at rest and following elevation. We then translated the axis to the location of the insertion of each muscle at the hyoid and measured the two-dimensional angle for each axis direction relative to the orientation of the muscle line of action. To measure the line of action of the muscles relative to the hyoid following hyoid elevation, we animated muscles using inverse kinetic chains in Maya so that their insertion on the hyoid moved without their origins on their respective structures moving. We then elevated and moved the hyoid anteriorly to match the mean hyoid elevation and anterior movement exhibited by pigs and measured the resulting lines of action (Fig. 1). These data allowed us to approximate muscle orientation as a function of time in the swallow.

Statistical analyses

The data used in this study included multiple modalities. Duration of muscle activity (determined from EMG), muscle lengthening and shortening (determined from microfluorometry), and hyoid kinematic movement (determined from high-speed videofluoroscopy) were digitally synchronized at a time resolution of 100 Hz. These data were combined with the anatomical data (derived from DICE-CT) to determine changes in muscle line of action relative to muscle length and activity.

We did not compute statistics for muscle orientations between maximum hyoid elevation and at rest (maximal depression), as we had only one measurement per condition. We also present hyoid kinematics qualitatively, as we did not test for differences in hyoid kinematics between conditions.

Owing to interindividual variation in fluoromicrometry bead and electrode placement, we analyzed the relationships between hyoid movements and muscle contractile placements on an individual basis. For each individual, we used cross-correlation analyses to test the relationship between hyoid movements and muscle shortening patterns, modified for discontinuous time series following Olsen et al. (2019). Additionally, we separated hyoid elevation/depression and anterior/posterior movement into separate axes and calculated circular distribution statistics (R package CircStats, https://cran.r-project.org/package=CircStats) to acquire statistical interpretations of the strength of the relationship between hyoid movement and muscle contractile patterns.

RESULTS

The hyoid elevates and moves anteriorly at the initiation of the swallow

At the beginning of the swallow the hyoid elevated and moved anteriorly before switching directions to depress and move posteriorly toward the end of the swallow (Fig. 2). Across all individuals, the hyoid elevated from its resting position on average by 5.07±1.78 mm, and moved anteriorly from its resting position an average of 2.44±0.60 mm. The hyoid began elevating prior to the onset of the swallow as identified by X-ray video and did not finish its return to maximal depression by the time the epiglottis of the pig returned to its resting position, and the swallow was finished (Fig. 2).

Muscle orientation changes during a swallow to act more in depression

After hyoid elevation, all muscles exhibited changes in the dorsoventral orientation (Fig. 1, Table S1). Geniohyoid, which had a small (−12 deg) depression line of action at rest, substantively increased its line of action for depression at maximal hyoid

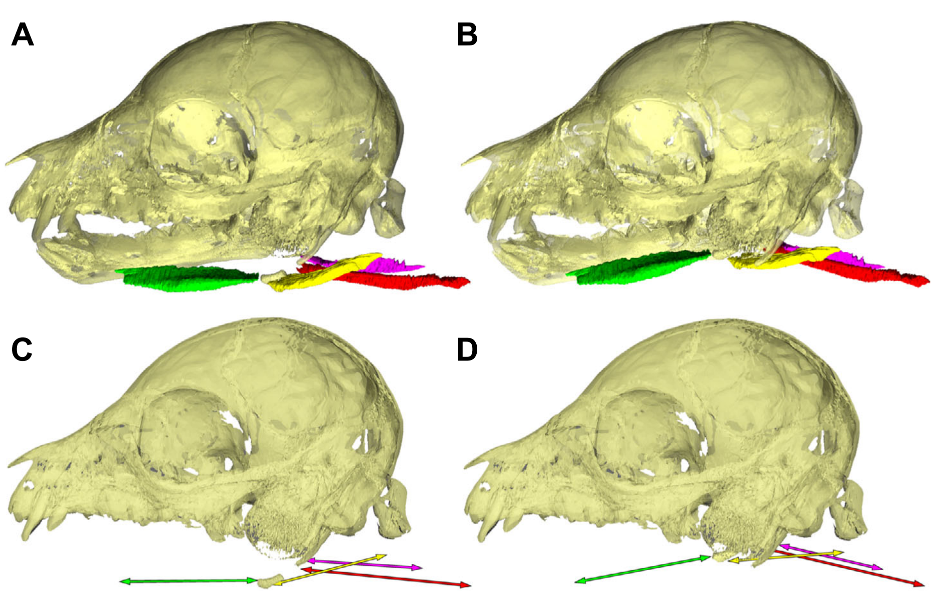

Fig. 1. Muscles and their vectors at rest, and when the hyoid bone is elevated to its peak position. Lateral view of muscles of interest (A, C) at rest and (B,D) following mean hyoid elevation/retraction during a swallow. Top: skull with muscles; bottom: skull with muscle vectors. Green, geniohyoid; yellow, stylohyoid; red, omohyoid; pink, thyrohyoid.

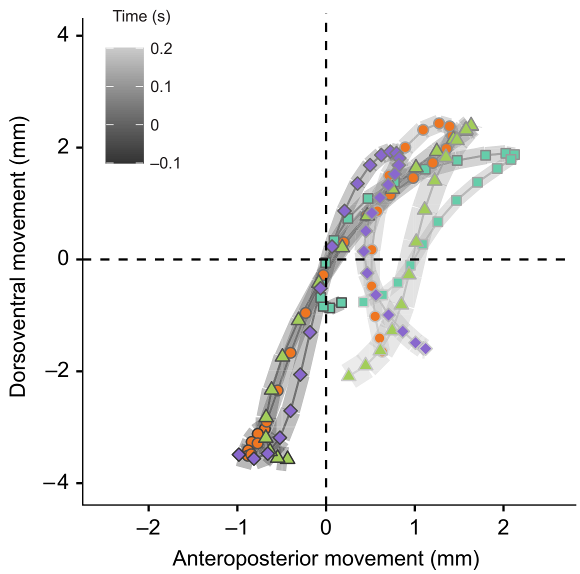

Fig. 2. Mean hyoid movements in dorsoventral and anteroposterior directions (lines)±standard deviation (shaded areas) for four pigs (different symbols). The hyoid moves primarily dorsoventrally and begins elevating prior to the onset of the swallow as identified by X-ray video (0,0 on the plot). It makes a quick turn following maximal elevation and has not reached its resting point by the time the epiglottis has finished its movement during the swallow. Darker colors indicate the beginning of the swallow, lighter colors indicate the end. Blue points, pig 1; orange points, pig 2; green points, pig 3; purple points, pig 4.

elevation (−18 deg). Stylohyoid was oriented 25 deg in elevation, which decreased to 19 deg at maximal hyoid elevation. Thyrohyoid and omohyoid, which were slight elevators at rest (3.5 and 2.8 deg, respectively), became slight depressors at maximal hyoid elevation (−5.2 and – 3.1 deg, respectively).

Muscle length changes occur in synchrony with hyoid movements

Across all muscles of interest, and among all individuals, we found a high degree of correlation between changes in muscle length and changes in hyoid movements, although geniohyoid exhibited markedly lower correlations in dorsoventral movements of the hyoid (Table S2).

Geniohyoid

The correlation between geniohyoid shortening patterns and hyoid movement varied across individuals, probably owing to the high levels of regional heterogeneity in muscle firing timing (Holman et al., 2012; Mayerl et al., 2021), although circular distribution statistics found a significant relationship between hyoid movement and geniohyoid shortening patterns (Tables S3, S4). Geniohyoid shortened during hyoid elevation and anterior movement and lengthened as the hyoid depressed and moved posteriorly.

Stylohyoid

Stylohyoid shortened for the first 50% of its contraction, which occurred at the beginning of hyoid elevation, and was eccentric for the second half during hyoid retraction (Fig. 3). Cross-correlation analyses indicated high correlations between stylohyoid shortening patterns and dorsoventral (−0.93, −0.78) and anteroposterior hyoid movements (−0.92, −0.79; Table S2). Similarly, circular distribution statistics found a significant relationship between stylohyoid shortening and all hyoid movements (Fig. S1, Tables S3, S4).

Thyrohyoid

Thyrohyoid was concentric initially and was eccentric for the latter half of its activity (Fig. 3). Cross-correlation between hyoid anteroposterior movement and muscle shortening was high in one individual, and medium in another (−0.90, −0.48), with a medium strength cross-correlation for hyoid dorsoventral movements in both individuals (−0.66, −0.49; Table S2). However, circular distribution statistics found that thyrohyoid shortening patterns during activity were significantly associated with anteroposterior and dorsoventral movements of the hyoid (Fig. S1, Tables S3, S4).

Omohyoid

Omohyoid was eccentric for the majority of its activity, with concentric contractions occurring in the last ∼15% of activity during hyoid depression at the conclusion of the swallow (Fig. 3). This shortening pattern was similar between individuals, and cross-correlation analysis found substantive correlations between omohyoid shortening patterns and hyoid movements (Table S2). Circular distribution statistics similarly found a significant relationship between omohyoid shortening patterns and hyoid movements (Fig. S1, Tables S3, S4).

DISCUSSION

Our results highlight that the orientation, and thus the function, of muscles changes throughout a swallow. Similarly, the contractile patterns of the muscles involved in the swallow vary throughout a swallow and are tightly coupled with movements of the hyoid. Although this result is in and of itself not surprising, what this work demonstrates is that almost half of any single muscle's activity during swallowing may function to stabilize the hyoid, rather than to move the hyoid itself. This indicates that the position of the hyoid at any given point during a swallow is tightly regulated by an array of muscles acting both concentrically and eccentrically and that the position of the hyoid is actively regulated. Furthermore, we have focused on four of the muscles controlling hyoid movements, yet many other muscles likely play a role in the position of the hyoid. Additionally, most research to date, including ours, has examined hyoid motions only in translation, even though muscles insert onto the hyoid at different points, and have the potential to rotate, as well as translate the bone. These possibilities further support the hypothesis that the position and orientation of the hyoid is a tightly regulated 'posture' actively maintained by multiple agonist–antagonist relationships (German et al., 2011).

Muscle function powering hyoid movement is complex

Movements of the hyoid are complex and controlled by several antagonists acting in concert to elicit a swallow. For example, EMG activity of two muscles acting in nearly opposite directions, omohyoid and geniohyoid, overlap extensively. Here, we show that as geniohyoid contracts concentrically at the initiation of the swallow to pull the hyoid anteriorly, omohyoid is acting eccentrically to stabilize hyoid movement. In contrast, at the conclusion of the swallow both muscles are still active, but their contractile patterns have switched, where omohyoid is acting concentrically to pull the hyoid posteriorly and geniohyoid is acting eccentrically to stabilize the hyoid. The contractile characteristics of stylohyoid suggest that during concentric activity at the beginning of the swallow, stylohyoid is likely primarily responsible for the elevation of the hyoid. In contrast, during its eccentric activity it is acting to stabilize the hyoid during hyoid depression. Similarly, thyrohyoid function changes throughout the duration of its activity. We propose that thyrohyoid acts to coordinate movements of the

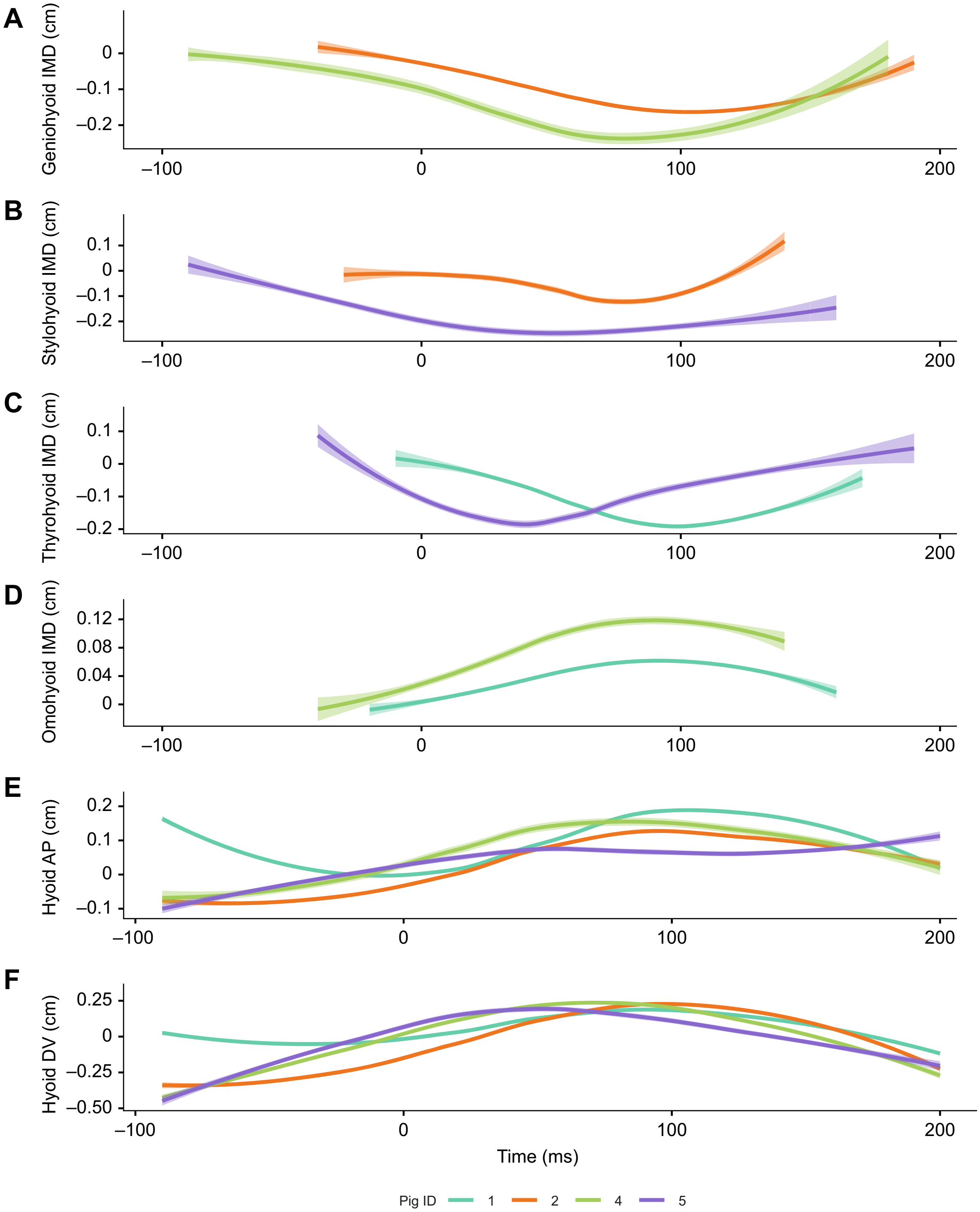

Fig. 3. Muscle length changes during activity through a swallow are correlated with hyoid translations. Muscle length changes during EMG activity for the (A) geniohyoid, (B) stylohyoid, (C) thyrohyoid and (D) omohyoid, as well as hyoid movements in (E) anteroposterior and (F) dorsoventral movements, with time (s) on the *x*-axis. Increasing values with time in E indicate hyoid anterior movement, and increasing values with time in F indicate hyoid elevation. AP, anteroposterior; DV, dorsoventral; IMD, intermarker distance.

thyroid cartilage with the hyoid during its initial concentric activity, and subsequently functions to pull the hyoid posteriorly during its eccentric activity, at which time the sternothyroid likely acts in concert to pull the thyroid cartilage posteriorly. Future work coordinating thyrohyoid and sternothyroid contractile patterns would test this hypothesis.

Although the infrahyoid muscles are often assumed to function primarily as hyoid depressors (Zhu et al., 2017), our results indicate that the function of the infrahyoid muscles are far more complex, and that they only act as depressors during the concentric portion of their activities. By explicitly examining the muscle lines of action relative to the hyoid, our results demonstrate that at rest, infrahyoid muscles function as poor depressors, and only after the hyoid has been elevated can they function to depress the hyoid. Understanding how these muscles function to move the hyoid across species with differing morphologies is a potentially fruitful avenue of future research.

Hyoid muscle function is variable within a muscle and between behaviors

The importance of understanding the interactions between muscle vectors (e.g. moment arms), muscle activity and muscle contractile characteristics have long been appreciated in locomotor research (Biewener, 1989; Mayerl et al., 2017; Maynard Smith and Savage, 1956; Westneat, 1994), and in research investigating mastication (Herring et al., 1991; Hylander et al., 2005; Taylor and Vinyard, 2009; Vinyard et al., 2008). However, very little attention has been paid to the function of the muscles associated with the hyoid. In infants, geniohyoid and sternohyoid length changes and muscle firing patterns have been evaluated and were demonstrated to be highly variable within the location of a muscle (Holman et al., 2012; Konow et al., 2010). Similarly, the posterior mylohyoid and digastric have been shown to have regional variation in muscle firing patterns, and function differently during chewing and swallowing in adult primates (Orsbon et al., 2018). Our work supplements the conclusions drawn by previous work by illustrating that the length changes of the hyoid musculature during activity, in conjunction with movements of the hyoid, can also vary throughout a behavior. We also focused on how the hyoid musculature functions during a swallow, but many of the muscles involved in swallowing are also active during isolated sucks (Mayerl et al., 2021; Thexton et al., 1998), and we have a limited understanding of how these muscles function during sucking. Finally, although we present muscle length changes and activity during swallowing, movement of the hyoid is essential for a broad range of behaviors including swallowing, vocalization and breathing (German et al., 2011), and there is thus also potential for muscles powering hyoid movement to vary in function depending on behavior.

Ontogenetic and evolutionary implications

Infant feeding is a complex behavior in which suction is generated to acquire milk from a teat, the tongue moves in a wave-like motion to transport milk through the oral cavity, and then the hyoid, tongue and pharyngeal musculature initiate and power a swallow (German et al., 1992; Mayerl et al., 2020c, 2021; Thexton et al., 2007). However, this process differs fundamentally from feeding in adult mammals (German and Crompton, 1996; Hiiemae and Crompton, 1985), and the hyoid musculature, tendons and craniofacial bones change in morphology and function as mammals grow (Herring, 1985; Hurov et al., 1988). Testing the interactions between ontogeny and muscle function remains a promising prospect for future research.

These results have implications for future research on the evolution of feeding and swallowing across tetrapods. Nearly all research on the function of the hyoid musculature has been limited to mammals (Holman et al., 2012; Konow et al., 2010; Orsbon et al., 2018), yet many of the muscles involved in mammalian feeding and swallowing are homologous to those found across vertebrates. Explicitly analyzing how muscles that have evolved within mammals, such as the cricothyroid, differ in function and variation from those found across vertebrates (i.e. mylohyoid and geniohyoid) could provide novel insights into the evolution of feeding behaviors. Furthermore, analyzing how the lines of action of the hyoid musculature vary across vertebrates in conjunction with their muscle firing and shortening patterns would elucidate potential interactions between evolutionary history, physiology and neuromotor function.

Conclusions

Our work illustrates the complexity of hyoid movements during swallowing. These movements are controlled by multiple muscles oriented in different directions acting in concert to regulate the position of the hyoid at any point in time. The tight regulation of hyoid movements has functional implications for all behaviors involving the hyoid, including respiration, speech, feeding and swallowing. These data also suggest that interventions used in human swallowing research such as surface electrical stimulation of the hyolaryngeal muscles likely result in muscle functions that do not reflect their contractile characteristics during normal swallowing (Humbert et al., 2006). Future research should examine how the muscles involved in hyoid movement function during these behaviors from an evolutionary framework.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.J.M., R.Z.G.; Methodology: C.J.M., C.E.E., F.D.H.G.; Software: C.J.M., T.L.H.; Validation: C.J.M., C.J.V., R.Z.G.; Formal analysis: C.J.M.; Investigation: C.J.M., K.E.S., A.M.C., L.E.B., C.E.E., T.L.H.; Resources: C.J.M., T.L.H., R.Z.G.; Data curation: C.J.M., K.E.S., A.M.C., L.E.B., T.L.H., C.J.V.; Writing - original draft: C.J.M.; Writing - review & editing: C.J.M., K.E.S., L.E.B., C.E.E., F.D.H.G., C.J.V., R.Z.G.; Visualization: C.J.M.; Supervision: C.J.M.; Project administration: C.J.M., C.J.V., R.Z.G.; Funding acquisition: C.M.

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Data availability

Data used in statistical analyses are available from figshare: doi:10.6084/m9.figshare.16860589

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