Regional differences in length change and electromyographic heterogeneity in sternohyoid muscle during infant mammalian swallowing

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1Department of Physical Medicine and Rehabilitation, Johns Hopkins University, Baltimore, Maryland; 2Division of Physiology, King’s College, Guy’s Campus, London, United Kingdom; and 3Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts

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Konow N, Thexton A, Crompton AW, German RZ. Regional differences in length change and electromyographic heterogeneity in sternohyoid muscle during infant mammalian swallowing. J Appl Physiol 109: 439–448, 2010. First published June 10, 2010; doi:10.1152/japplphysiol.00353.2010.—A complex sling of muscles moves and stabilizes the hyoid bone during many mammalian behaviors. One muscle in this sling, the sternohyoid, is recruited during food acquisition, processing, and swallowing, and also during nonfeeding behaviors. We used synchronous sonomicrometry and electromyography to investigate regional (intramuscular) changes in length and electromyographic (EMG) activity of the sternohyoid during swallowing in the infant pig. The simple straplike architecture of the sternohyoid led us to hypothesize that limited regional variation in length and muscle activity would be present. We found statistically significant regional differences in EMG activity, and, with respect to length dynamics, the sternohyoid did not behave homogeneously during swallowing. The midbelly region typically shortened while the anterior and posterior regions lengthened, although in a minority of swallows (12.5%) the midbelly lengthened simultaneously with the end-regions. Despite its nonpennate architecture and evolutionarily conservative innervation, the mammalian sternohyoid appears to contain previously unrecognized populations of regionally specialized motor units. It also displays differential contraction patterns, very similar to the sternohyoid of nonmammalian vertebrates.

motor unit; muscle mechanics; electromyography; sonomicrometry; feeding; hyolingual

THE STERNOHYOID MUSCLE (SH) is one of the largest muscles involved in producing the complex hyoid movements seen in mammals. These movements form the basis of many oropharyngeal behaviors, including respiration and airway protection (36, 38, 42, 58), phonation (25–27, 46), and feeding (4, 5, 33). The hyoid of many terrestrial mammals lacks a robust articulation with other bones, and the muscles attaching to it are responsible not only for its active movement but also for resisting secondarily induced movement and for hyoid stabilization (7). During respiration, electromyographic (EMG) activity often occurs in the SH without shortening of the muscle (59). Moreover, during ventilation, there is significant variability both in SH activity (57) and in the muscle length change dynamics (60). These results raise the question of whether there are similarly variable patterns during mammalian feeding behaviors, such as sucking, mastication, and swallowing, where the SH is known to be active (22, 50, 53). We wanted to determine whether this variability was inherent to all behaviors involving SH activity or only characterized nonfeeding behavior. Variability during respiration but not during swallowing or other feeding behaviors would suggest that there are some important distinctions in the neural control of these two behavioral categories. If this variability characterizes both behavioral categories, then it will be important to determine which muscles exhibit variation, particularly among the spindle-poor muscles of the tongue, pharynx, and larynx. Using the infant pig model of swallowing, we examined the EMG activity and muscle dynamics of the anterior (hyoid), midbelly, and posterior (sternal) SH regions during swallowing. We tested the hypothesis that, during the swallow, EMG electrodes in different SH regions would detect broadly equivalent patterns of muscle activity (hypothesis 1). Because the SH is characterized as a simple, parallel-fibered muscle, with the relative simple function of depressing the hyoid bone (7, 11, 12, 30, 53), it seems unlikely that there would be variation within the muscle. We also tested the hypothesis that there would be regional equivalence in the timing of shortening or lengthening of the SH (hypothesis 2). As is true for hypothesis 1, the most likely scenario is that the muscle shortens consistently across its length. Given the existing data on variable relationships between the electrical and mechanical activity of the SH during ventilation (59), we also hypothesized that, during swallowing, there would be a similar lack of correlation between regional EMG and mechanical activity (hypothesis 3). Finally, within each feeding session, we hypothesized that there would be differences in both regional EMG activity and length dynamics of the SH in swallows occurring during the early period of voracious sucking and during the later period of more relaxed sucking (hypothesis 4). Our expectation was that such differences in behavior over a feeding session could be detected at a gross level, as a change in cycle length (19).

MATERIALS AND METHODS

All experimental work was done with Johns Hopkins University Animal Care and Use Committee approval (no. SW07M14). Infant pigs (n = 5, 10–16 days old, 5–6 kg body wt; see Table 1) were obtained from Tom Morris Farms (Reisterstown, MD) and housed in the animal care facility at Johns Hopkins University, School of Medicine.

To place electrodes, each animal was first induced with 5% isoflurane administered via face mask and then intubated and maintained at a deep plane of general anesthesia. All procedures were conducted under aseptic conditions. The SH, genioglossus (GG), and thyrohyoid (TH) muscles were identified (47) and exposed by blunt dissection. We implanted four 2-mm piezoelectric crystals (Sonometrics, London, ON, Canada) at equidistant intervals (~17 mm apart) into the left SH (Fig. 1). This instrumentation divided the muscle into anterior (hyoid insertion end), mid (belly), and posterior (sternal origin end) regions. Midway between each pair of crystals, we sutured bipolar...
EMG patch electrodes (34) onto the surface of the muscle. These electrodes were offset laterally, 3–4 mm from the anterior-posterior line of the crystals, to avoid recording from any muscle fibers that might have been damaged during crystal placement. We also implanted two sets of fine-wire bipolar electrodes (3) into the GG midway between its mandibular origin and hyoid insertion and two sets into the TH close to its thyroid origin.

The electrode and crystal wires were led out through a submandibular incision to the dorsum of the neck. Before surgery, the crystal wires were mounted into a skin button connector (Sonometrics), and the electrode wires were soldered to microconnectors (Glen-Air, Glendale, CA). To prevent undue tension on the electrodes and crystals, the external wires were looped over the skin of the animals between layers of Vetwrap bandage (3M); the connectors were then sutured to the bandage on the dorsum of the animal. When anesthesia was discontinued, recovery was allowed for 3–5 h, to eliminate the inhalational anesthetic agent, before food was offered and data were recorded. At the end of the experimental period of 36–48 h, the animal was placed in a deep plane of anesthesia and was euthanized by intracardiac injection of barbiturate. Postmortem dissections were performed to verify the position and the physical condition of the crystals and electrodes.

Experimental feeding methodology and data recording. Animals were hand-fed an infant pig formula from a standard baby bottle fitted with a pig nipple (Nasco, Fort Atkinson, WI) from the time of their arrival at the animal facility and at 3–4 h intervals during the day. During collection of data, animals were fed in a standard medium-sized pet carrier but were otherwise unrestrained.

The EMG signals were amplified 1,000× with an MA-300 EMG System (Motion Lab Systems) with a 25-Hz high-pass filter and no other filtering engaged. Data were recorded via a Powerlab 16/30 onto a PC running LabChart v. 6.1.3 (AD Instruments, Colorado Springs, CO). The analog inputs to PowerLab (i.e., the voltage-differential outputs from the EMG amplifiers and from the sonomicrometer) were digitized at 10 kHz. The output voltages of the sonomicrometer reflected three crystal-pair distances sampled at 508 Hz, from identical output ranges, and with a transmit pulse of 220 ms and inhibit-delay set at 2.2–2.6 mm, depending on signal condition. In this way, data on the changes in regional muscle length dynamics and the regional EMG were acquired in total synchrony. Sections of data that contained uninterrupted feeding cycles, without noise or artifacts, were cropped in LabChart and saved for further processing.

Data extraction. The zero time point (t₀) of each swallow was defined as the point at which activity in a TH electrode reached 20% of its maximum. We chose the TH EMG as t₀ reference because it has the least variation in timing of all the hyoid muscles, across several levels of analysis, i.e., between electrodes, among successive swallows, and among individuals (22, 52, 55). Moreover, its activity follows epiglottal flexion with a constant 100-ms latency (50, 52), and it has therefore been used as a reliable marker of swallowing in other studies (37, 52, 55).

Data from all channels were extracted within a time period of 400 ms, starting 200 ms before TH 20% activity. We refer to the TH EMG as our reference wave. The main criterion for accepting data from a feeding session was that the sonomicrometry signals were free from secondary harmonics (a common effect of intrascalar crystal movement), but also that the EMG signals were noise- and artifact free. At least three of such data traces, usually from the first two experimental days, were selected for each individual animal. The early and late stages of each feeding session were then cropped into separate files to ensure that data for individual swallows were extracted separately for the early and late portions of the feeding sessions. This was done to parse out any behavioral differences arising over time, such differences being a likely result of the animal becoming satiated (17). Swallows from each trace were labeled consecutively and exported as raw unfiltered data in separate ASCII files.

Data processing. A script in Matlab R2008a (v.7.6.0.324, The Mathworks, Natick, MA) was coded to perform several processing stages on each of the EMG waves: 1) baseline correction to compensate for any amplifier drift away from zero; 2) signal rectification; and 3) reset-integration at 0.01-s intervals. The amplitude level that best discriminated between EMG signal and noise was then determined.

### Table 1. Summary of animals used and data collected

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight, kg</th>
<th>Sequences (day)</th>
<th>n (total 479)</th>
<th>EMG Triplets</th>
<th>Distance Triplets</th>
<th>SD1-SH1</th>
<th>SD2-SH2</th>
<th>SD3-SH3</th>
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<td>6.0</td>
<td>7 (1–3)</td>
<td>121</td>
<td>49</td>
<td>54</td>
<td>7</td>
<td>57</td>
<td></td>
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<tr>
<td>2</td>
<td>5.7</td>
<td>3 (1–3)</td>
<td>83</td>
<td>3</td>
<td>3</td>
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<td>3 (2)</td>
<td>91</td>
<td>39</td>
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<tr>
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<td>3 (2)</td>
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<td>81</td>
<td>73</td>
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<td></td>
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<tr>
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<td>5.0</td>
<td>6 (1, 2)</td>
<td>102</td>
<td>55</td>
<td>35</td>
<td></td>
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</tr>
</tbody>
</table>

We collected measurements from a total of 479 infant pig swallows and extracted data on sternohyoid electromyographic (EMG) activity (EMG triplets) and length dynamics (distance triplets) from 3 muscle regions. We also compared sternohyoid EMG (SH) and length dynamics (SD) for the anterior (SD1-SH1), midbelly (SD2-SH2), and posterior (SD3-SH3) muscle regions.
with a randomization technique (49). Data for which the signal could not be discerned from the noise were at this stage automatically excluded from analyses. Another Matlab script was then used to define the timing of 20% peak TH EMG and calculate and plot median and quartile waves of regional SH EMG and muscle dynamics. This process was repeated separately for swallows originating from the early and from the late stages of each feeding sequence.

The processed data were then subjected to pairwise cross-correlation analyses (35, 52, 67). The cross-correlation function (CCF) yielded two sets of values: the lag, which is a measure of the relative timing between the two waves, and the $r$ value, which is the linear correlation between the two waves at that lag. In these experiments, the single lag value obtained from the analysis refers to the specific time-displacement of one wave relative to another that produced the highest absolute $r$ value from the CCF.

For each variable, being either a length or an EMG wave, a CCF was calculated relative to peak TH EMG (Fig. 2) for each swallow, being the unit of analysis (see below). The Matlab script returned lag scores and $r$ values for the closest and strongest correlation between the waves in either direction away from zero lag. For these analyses, 20% of the maximum activity in the rectified TH EMG was used as the $t_0$ reference. The $r$ value indicates the strength of the correlation obtained at any given lag between the waves. Both lags and $r$ values have a sign (positive or negative). The lag sign indicates the order in which the waves peak; a negative lag indicates that the second wave occurred before the first. A negative $r$ value indicates that optimum correlation between the two waves was an inverse. Given the convex shape of a rectified EMG wave, a CCF among single EMG bursts only produces positive $r$ values. In contrast, distance measurements often resulted in more complex waves, and the $r$ value sign was used to determine whether the muscle region shortened or lengthened.

We avoided direct comparisons among the target EMG and muscle length waves for two reasons. Using TH EMG as a measure of timing, and as the reference wave, provided a more stable and repeatable time synchronization. In the SH EMG waves, a subsidiary peak would often give a spurious high correlation, which did not occur when using TH as the reference wave. More importantly, a lack of independence would complicate subsequent statistical pairwise comparisons among the three regional EMG and muscle dynamics waves.

**Hypothesis testing and statistical analyses.** There were several null hypotheses. Hypothesis 1 was that all SH regions have identical timing of EMG activity relative to the TH reference wave. Hypothesis 2 was that all SH regions have identical patterns of length change relative to the reference wave. Hypothesis 3 was that with a constant lag, there would be a lack of correlation between EMG activity and length dynamics within each sternohyoid region. Hypothesis 4 was that these lag and correlation relationships between EMG and length dynamics would be altered from early voracious to later relaxed feeding due to satiation.

Data were extracted from a minimum of three feeding sessions from each of the five animals (Table 1). From each session, we processed at least 30 swallows from each of the early and the late stages of feeding. The resulting data, containing lag and correlation values from the pairwise CCFs, came from a total of 489 swallows. First, the data were organized into five data sets: 1) triplet EMG variables, one for each region ($n = 91$); 2) triplet length change variables, one for each SH region ($n = 225$); and three data sets containing paired distance and EMG variables for the anterior ($n = 118$), midbelly ($n = 208$), and posterior ($n = 157$) SH regions. The reduced sample sizes were due to the amount of data that could be recovered for each set after cross-correlation analyses. For example, if data were missing for the anterior regional EMG electrode (analysis 3), it would still be possible to analyze the distance-to-EMG relationship for the other regions (analyses 4 and 5) but not for triplet EMG (analysis 1). If, in this example, the distance measurements for the anterior region existed, analysis 2 would still be possible. The different samples for each of the data sets also yielded slightly different distributions of EMG timing in the analyses (viz. Fig. 3C).

All subsequent analyses were carried out as repeated-measure models using the mixed model module of SYSTAT 12 (Chicago, IL). These data sets were used in six different analyses aimed at testing the following temporal relationships, in order to address our four hypotheses: 1) in EMG activity among the three SH regions (hypothesis 1); 2) in muscle length among the three SH regions (hypothesis 2); 3) between EMG activity and length in the anterior SH (hypothesis 3); 4) between EMG activity and length in the midbelly SH (hypothesis 3); 5) between EMG activity and length in the posterior SH (hypothesis 3); 6) between regional EMG and length of early and late swallowing (hypothesis 4).

The first two analyses tested among the three SH regions for similarity in EMG differences and for similarity in length dynamics. This took region (anterior/midbelly/posterior) as the repeated measure, stage (early/late) as the fixed factor, and animal as well as experiment nested within animal as random factors. The response variable was the lag of either length dynamics or EMG activity to the TH reference wave. The unit of analysis was the individual swallow (15). If a significant main effect of region was detected, the hypothesis testing module in SYSTAT was used to determine the pattern of differences. Statistically, this is more powerful than carrying out all possible pairwise combinations of post hoc testing (10).
The tests for regional muscle dynamics were more complex because the length-change waves were not simple convex shapes, like a rectified EMG burst. Here, the sign of the \( r \) value was a critical factor in determining the regional timing relationships. Thus an additional fixed factor, namely, \( r \) value sign, was included in these models. A significant effect of \( r \) value sign, or interaction between \( r \) value sign and region, required additional hypothesis testing among multiple groups. The specific hypotheses tested were dictated by the original null hypotheses that all regions shortened identically as a function of time, as well as whether a main effect or an interaction was significant. The specific SYSTAT commands for these comparisons are available from the authors.

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The final three analyses (analyses 3–5) tested whether the regional length dynamics were attributable to the intraregional EMG. We compared regional muscle activity and length dynamics, using a full repeated-measures mixed model. In these cases, the response variable was the lag of the regional muscle activity relating to the preceding sucking activity, the burst around \( t_0 \) appears to be the swallow-related EMG of the SH.

RESULTS

Swallowing behavior during bottle-feeding. Feeding sessions lasted 1–4 min, with all animals feeding readily at 3-h intervals. During feeding, an animal would break off sucking several times, only to resume feeding immediately. Early feeding was fast paced, often with a swallowing frequency approaching 4 Hz (Fig. 4A). There were rarely more than two suckles between early swallows, and swallows often occurred in couplets, as indicated by the TH activity in Fig. 4A. During the more quiescent feeding later in a session (Fig. 4B), swallows occurred at 0.5–2 Hz, with up to 10 sequential suckles between each swallow and no sign of swallowing couplets.

Sternohyoïd swallowing EMG activity. The pattern of regional activity in the SH during swallowing was highly variable (analysis 1). In many swallows, there was no discernable EMG activity in one, two, or all three SH regions. In other swallows, the timing of activity was variable. Only in 91 swallows (18.6%) did all three regions exhibit activity that was measurably different from the baseline. The difficulty of analysis was also compounded by the presence of SH EMG activity with two distinct timings, as measured by the lag from \( t_0 \). In each muscle region there was one grouping of EMG bursts that occurred approximately at the same time as the peak TH EMG and another that occurred \( \sim 200 \) ms after peak TH activity (Fig. 5). The first group did not overlap at all with the second set of bursts (Fig. 3). We hypothesized that this bimodal pattern represented a true biological pattern, and, consequently, we analyzed these two bursts separately.

Fig. 3. Histogram of timing difference between SH and TH EMG activity during swallowing. The processed EMGs from both muscles were cross-correlated, and the histograms show the lags (in ms) of SH regional EMG, plotted relative to zero time (\( t_0 \)) (i.e., peak TH activity). In all 3 SH regions, the 2 most frequent peak correlation timings are \( \sim 200 \) ms after TH EMG activity and coincident with TH EMG activity, respectively. Whereas the early SH EMG burst may represent muscle activity relating to the preceding sucking activity, the burst around \( t_0 \) appears to be the swallow-related EMG of the early SH.

Fig. 4. Normalized sonomicrometric (Sono) and electromyographic data from early and late feeding activity. Swallows are clearly marked by activity in the TH. Early feeding sequence (A) shows primary swallows (dark gray shading) with differential regional length patterns and secondary swallows with more uniform regional length patterns (light gray shading), with suck occurring during the swallows. Late sequence (B) shows more similar regional muscle dynamics and isolated sucks between swallows (viz. GG activity without TH in nonshaded areas). SHA, sternohyoïd anterior region; SHB, belly region; SHP, posterior region. Time between x-axis tick marks is 1 s.
1.56,
P
F
were present (muscle activity between the early and late stages of feeding individuals. However, regional differences in the timing of differences among individuals or among experiments nested in experiments. The overall result was that differences between the negative correlations in the anterior and posterior regions (F1,1660 = 0.92, P = 0.762) or the positive correlations in these two regions (F1,1660 = 1.345, P = 0.247). However, the midbelly regional lag was different from the end-regions for both the negative (F2,660 = 20.70, P < 0.0001) and positive (F2,660 = 66.17, P < 0.0001) correlations. There were random effects of individual variation and among experiments nested within individuals. The overall result was that behavioral differences were present (F1,270 = 7.53, P < .01).

**Regional length change in sternohyoid during swallowing.** For 225 swallows (46% of all swallows), all three regional lengths were recorded and used in analysis 2. This analysis of muscle length was complicated by the existence, at specific lags, of strong negative correlations between regional length and TH activity. Meanwhile, at the same lag, intraregional muscle lengths could have entirely opposite dynamics, resulting in positive as well as negative r values. In the example shown in Fig. 6, the change in length of the belly region was negatively correlated with TH activity, whereas the anterior and posterior regions were positively correlated. Such differences in muscle behavior were found for all regions. Lengthening was most commonly found in the anterior and posterior regions, the median pattern of length change for these regions being followed closely by the quartile profiles (Fig. 7, A and D), i.e., with limited variation. Meanwhile, in the midbelly region, there were two distinct patterns of length dynamics (Fig. 7, B and C). Most frequently (87.5%), the midbelly region was at its longest early and shortened over the course of the swallow (Fig. 7B). Less frequently (12.5%), the midbelly length with TH EMG yielded positive coefficients for anterior and posterior regions but a negative coefficient for the midbelly. In this case, t0 was set when TH reached 20% peak activity.

When the lag values were separated on the basis of negative and positive correlation with the reference wave (r value sign), a clear pattern of difference emerged (Fig. 8). For the anterior and posterior muscle regions, the positive correlations clustered around t0 (zero lag), indicating that the shortening was directly correlated with the reference wave. Moreover, inverse correlations (negative r values) only occurred at high negative or high positive lags, so that peak lengthening in these two regions occurred at significantly different timing from t0. Conversely, in the midbelly, when the lag from the reference wave was small, the r values were strongly negative (Fig. 6 and Fig. 8B). Positive r values only occurred when the peak regional muscle length occurred with a timing that was significantly different from t0. Inclusion of a fixed factor for the r value sign in the mixed model showed that positive r values (i.e., SH regional lengthening during TH EMG activity) represented a different pattern of length dynamics from those with a negative r value (i.e., shortening during TH EMG activity).

Statistical testing for the lag timing of regional length dynamics revealed a marginal significance in the main effect of region (F2,660 = 4.54, P < 0.02). The main model tested other factors and found a significant differences in lag between the positive and negative r values (F1,660 = 35.24, P < 0.0001) but no difference due to stage (early/late, F1,660 = 1.81, P = 0.179). There was a significant interaction between r value sign and region (F2,660 = 33.71, P < 0.0001). Testing of several specific hypotheses clarified the relationships among the different treatments. There were no statistically significant differences between the negative correlations in the anterior and posterior regions (F1,660 = 0.92, P = 0.762) or the positive correlations in these two regions (F1,660 = 1.345, P = 0.247). However, the midbelly regional lag was different from the end-regions for both the negative (F2,660 = 20.70, P < 0.0001) and positive (F2,660 = 66.17, P < 0.0001) correlations. There were random effects of individual variation and among experiments nested within individuals. The overall result was that...
the timing of length change in the midbelly was different from
the timing of length change in the ends, irrespective of the
value sign for the correlation.

Correlation between regional SH muscle length and EMG
activity. Within each SH region, the relationship between EMG
activity and muscle length differed across all regions (Fig. 9).
Given that the regional length dynamics had both positive and
negative correlations with the reference wave (TH EMG), the
length dynamics were again separated into two groups based
on whether length changes were in or out of phase with the
reference wave. In these analyses (analyses 3–5), the lags of
both regional EMG and length relative to the reference wave
were used to test the hypothesis that the timing of regional
activity was constant, irrespective of whether the length dy-
namics were negatively or positively correlated with activity.

In the anterior region (analysis 3), the difference between
lags of the positively correlated lengths and lags of SH EMG,
both relative to the reference wave (Fig. 9A, top), were not
statistically different from zero ($F_{1,225} = 0.032, P = 0.86$).
Thus the longest regional muscle lengths occurred simulta-
neously with peak regional EMG. The lags of the negatively
correlated lengths (Fig. 9A, bottom) differed significantly
from the timing of the peak EMG ($F_{1,225} = 30.96, P < 0.0001$)
but were also multimodal in timing.

In the belly region (analysis 4), when the length changes
were out of phase with the reference wave (i.e., shortening
occurred; Fig. 9B, bottom), the lag difference between regional
length change and EMG was bimodal but did not differ from
zero ($F_{1,402} = 0.024, P = 0.88$). Regional lengthening in phase
with the reference wave (Fig. 9B, top) did differ significantly in
timing relative to the regional EMG activity ($F_{1,402} = 76.56,$
$P < 0.0001$) but was strongly bimodal. Thus shortening of this
SH muscle region (identified by out-of-phase lags) occurred
with muscle activity in this region. The peak muscle length,
thus, positive $r$ value, occurred at a different timing than the
muscle activity in this region.

In the posterior region (analysis 5), the length changes
that were out of phase with the reference wave (i.e., shortening
occurred; Fig. 9C, bottom) ($F_{1,303} = 86.11, P < 0.0001$) as well as the length changes that were in phase (e.g.,
Fig. 9C, top) ($F_{1,303} = 22.65, P < 0.0001$), differed signifi-
cantly from the timing of EMG activity in the posterior SH
region. For the out-of-phase (negatively correlated) length
dynamics, shortening occurred after peak regional EMG
activity. The difference in timing between the in-phase (positively
correlated) length dynamics and EMG activity was different
from zero, and the muscle region attained its peak length
before the onset of EMG activity and shortened thereafter.
midbelly region (analysis 4) there was no stage difference ($F_{1,225} = 1.69, P = 0.19$), but in the posterior region (analysis 5) a stage effect was present ($F_{1,225} = 40.58, P < 0.0001$). The posterior regional relationship was the reverse of that in the anterior region, with early swallows having EMG and distance changes closer in time but further apart in later swallows.

**DISCUSSION**

**Sternohyoid length dynamics.** Our results show that the functional patterns of the pig SH during swallowing are very complex. The muscle exhibited highly variable regional EMG activation and timing, with two distinct burst periods within the 400-ms sampling window. Peak timing of the earliest burst differed between the posterior region and the rest of the muscle. This falsified hypothesis 1. SH length-change patterns were also variable. In the majority of swallows analyzed, the origin and insertion regions lengthened while the belly was shortened, while in fewer swallows, all regions lengthened, consistently with a different timing of peak length change in the belly region. This falsified hypothesis 2. Lengthening of the anterior region and shortening of the belly region were likely explained by intraregional EMG, and the tightest correlations between peak EMG and length change were found in the posterior region during early vigorous swallowing and in the anterior region during late quiescent swallows. Given the overall regional differences in length dynamics and EMG activity, we rejected the hypothesis that all regions of the SH behaved identically during swallowing. Clear differences were seen between early and late swallowing activity, as cycle lengths increased and swallow couplets disappeared with the onset of satiation. Overall, these complex muscle dynamics are not consistent with a traditional view of the SH as a simple, fusiform-fibered strap muscle with uniform and conserved innervation pattern (40, 41).

The SH was active on two different occasions during the 400-ms time window bracketing the swallow. The EMG burst occurring simultaneously with the TH EMG peak appeared to be part of the swallowing process, and there were regional differences in this earliest burst. The late burst is more likely part of the following sucking activity than a component of the swallow sensu stricto (50).

Hyoid muscle activity during swallowing occurs sequentially, with activity in the anterior/superior group of muscles preceding activity in the posterior/inferior muscles (50). In the present data, there was also an intramuscle staggering of the time at which EMG activity was recorded, with activity in the posterior region of the SH being detected later than activity in the anterior and midbelly.

**Sternohyoid EMG heterogeneity.** Aside from the timing variability, there was also variation in the actual presence of EMG signals in all three SH regions. Such variability characterizes SH activity during swallowing, both in the pig (8, 15, 18, 22, 50–52) and across mammalian species and behaviors (14, 16, 20, 39, 58, 61, 66). This raised a concern of ours that any regional pattern of SH activity could be obscured by variable firing and that we would not have sufficient statistical power. Still, the statistically significant patterns detected suggest some degree of consistency, activity occurring with the same timing across many swallows. We hypothesize that only a small proportion of the total motor unit pool is recruited for...
any given swallow, and that the variability in the recorded signal is an inherent function of using selectively recording patch electrodes where only a small sample can be detected from the total of a motor unit pool, which furthermore is only sparsely activated.

**Fiber type composition and minority type function.** The maximum rate of muscle shortening of a 17-mm segment, shown in the median data of Fig. 7B, is 0.35 mm in 40 ms. This converts to ~0.5 muscle lengths per second. Given our evidence of such shortening being due to single brief bursts of EMG activity (Fig. 5) and since there is no obvious heavy load upon the muscle, this rate of shortening suggests that the SH muscle fibers involved might be of the slow-contracting variety (type I); the value of 0.5 lengths/s is an intermediate of available data for type I fibers in rabbit (0.8 lengths/s) and in horse or human (0.3 lengths/s), whereas fast (type II) fibers in those animals contract at ~2–3 lengths/s (45).

The suggestion that type I muscle activity might form the basis of the SH response in swallowing contrasts with the general fiber type composition of this muscle. In many species, the SH has a predominantly fast muscle fiber type composition (9, 62, 64), with ~15% slow fibers. This ratio suggests that SH response in swallowing may be expressed using some of the type I minority of the muscle fibers and, therefore, that the known difficulties in recording swallow-related EMG activity might be due primarily to sparse motor units populations exhibiting swallow-related activity within the entire motor unit pool of the SH.

**Regional EMG differences or traveling action potentials.** The data in Fig. 10 indicate that in the anterior region of the SH the quantified EMG reaches 50% amplitude at about \( t_0 = 50 \) ms, in the midbelly the EMG reaches 50% at about \( t_0 + 30 \) ms, and in the posterior region EMG reaches 50% at about \( t_0 + 70 \) ms. Thus depolarization lags 120 ms between the anterior-most and posterior-most EMG electrodes, which were ~34 mm apart in the pig SH. If the action potentials were conducted between these two muscle parts, they would travel at <0.4 m/s, a velocity comparable to action potentials in the slow limb muscle fibers of the adolescent pig (0.17 m/s; see Ref. 54).

The timing of SH EMG activity in a given swallow may consequently vary by electrode location. This is a plausible explanation of the difference between the pattern of EMG activity in the SH belly (temporally close to TH EMG; Fig. 5) and the significantly later EMG activity previously reported for the SH (15, 31, 50), all studies in which positioning of EMG electrodes were less specific. However, because of evidence of a longitudinally distributed pattern of motor terminals (41), we cannot exclude the possibility that the differentially timed EMG activity reflects potentials arising in the regions containing the electrodes at different times, rather than being conducted to them from distant end plates. However, the current evidence suggesting the involvement of conducted potentials in very slowly conducting and slowly contracting muscle fibers is in part circumstantial and clearly requires further investigation.

**Sternohyoid EMG and length dynamics.** Across the three muscle regions, there were no entirely consistent relationships between regional length dynamics and EMG activity, although the relationships were more consistent at the end-regions of the muscle. Moreover, no region displayed direct causal relationships between activity and length dynamics for both lengthening and shortening muscle dynamics. However, both lengthening of the anterior region and shortening of the midbelly occurred simultaneously with peak EMG, suggesting an eccentric contraction for the former and an isotonic contraction for the latter. The posterior region showed the most straightforward functional relationship between muscle dynamics and activity. Shortening in this region frequently occurred simultaneously with or following EMG with an ~60-ms lag. If the intraregional activity is to be the immediate cause of the corresponding muscle dynamics, then the lag from EMG to onset of length change should, at minimum, exceed 15 ms in order to incorporate electromechanical delay (32, 43, 68).

Moreover, if the recorded activity is only a sample of all activity present, the sample might not have an obvious causal relationship to isotonic shortening or to isometric tension of whole sections of muscle. There are other plausible scenarios explaining the relationships between regional muscle dynamics and activity. In Fig. 9, the bottom right-hand quadrants contain cases in which regional SH stretching could be caused by deactivation of the previous intraregional EMG burst (i.e., the preceding suck; see Fig. 4B), combined with contraction of a hyoid protractor (i.e., passive regional lengthening of the SH). Conversely, the top left-hand quadrants in Fig. 9 are where regional SH shortening may be due to offset of EMG in hyoid protractor (i.e., elastic recoil of the SH region).

Given the reflex nature of the mammalian swallow (52), we hypothesized that swallows from early and late in a feeding sequence would not differ, except in their frequency of occurrence (19). However, in the SH there was evidence of regional differences in timing relationships that occurred early in the feeding sequence versus those that occurred later. The time during the feeding sequence was not significant in relation to the midbelly region, where the relationship between length dynamics and EMG activity was strongest. However, this relationship did change from the beginning to the end of a feeding sequence in the anterior and posterior regions. The swallow “couplets” detected early during feeding represent a high level of interindividual variation that was independent of standardized food treatments.

**Mammalian hyoid functional complexity.** The hyoid musculature includes eight muscles with divergent force vectors, which not only move the hyoid bone (4, 11, 12, 24) but also stabilize it during various oropharyngeal behaviors (7, 51). During an infant pig swallow, the hyoid bone moves in a figure-eight pattern, first anterodorsally, then posteroventrally,
followed by anterodorsally again (51). However, contrasting with textbook notions of SH contraction causing a posterior/ventral hyoid motion late in the human swallow (14), we only found an overall shortening of the SH during a minority of swallows.

On the contrary, during the majority of swallows, the belly of the SH lengthened overall while active. Figure 10C represents a scenario involving initially low-level SH recruitment coincident with muscle lengthening. This pattern could be caused either with SH activity or entirely passively, but in either case due to antagonist muscle activity. If the latter, cessation of muscle activity in the antagonist geniohyoid could result in elastic recoil of the SH. Low-level muscle activity could indicate that the active lengthening permits the muscle to reach the plateau of its length-tension curve, whereby force production is optimized, which is the case in the SH muscle of some lepidosaurs (21).

Sternohyoid muscle dynamics and functional evolution. Regional differences in muscle dynamics, although common in penimate and segmented vertebrate locomotor musculature (1, 2, 23, 44), remain unprecedented in simple strap musculature. Contrary to our initial hypotheses (hypotheses 1 and 2), our findings in the SH suggest that heterogeneous muscle dynamics occurs more frequently than expected or, alternatively, that this strap muscle contains unrecognized compartmentalization or innervation complexity. Although these characteristics are true for the geniohyoid muscle (50, 57, 63), which antagonizes the SH, available data on the SH contradict all these speculations.

The anatomic position of the SH, set between antagonist suprathyroid and synergist infrathyroid musculature, suggests a biomechanical explanation for the labile and complex muscle dynamics (30, 56, 59). The labile muscle activity pattern may result from two different functional roles. The SH not only actively lengthens or shortens but also counteracts antagonist stress or undergoes elastic recoil, supported by our finding of abrupt EMG offset (29). Each of these scenarios could explain weak correlations between regional length dynamics and muscle activity in our study.

From an evolutionary perspective, it is not surprising that the mammalian SH displays differential contractile properties and heterogeneous activity. These patterns are the case in different behaviors among unrelated taxa, including mammalian respiration (56, 59, 60), fish feeding (6, 28, 65), and amphibian lingual retraction (13).

While ancestral hyoid muscles, including the SH and geniohyoid, have labile EMGs, several novel mammalian muscles (48), including the TH, have highly stereotyped EMGs during swallowing (50). These novel muscles assume the role in mammals that the ceratohyals assume in pretetrapods, to stabilize the hyoid. Thus a stereotypical EMG may reflect a functional constraint, whereas a labile EMG in old hyoid muscles, including the SH, may represent a historical legacy.

We present important insight into how heterogeneous muscle activity governs the differential regional muscle dynamics that in turn generate the complex hyoid motion patterns that drive mammalian feeding behaviors. Our results highlight that experiments on antagonist muscles coupled with data on muscle architecture and fiber type and distribution could improve existing quantifications of mammalian hyoid apparatus functions.

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