RESEARCH ARTICLE

Regional Variation in Geniohyoid Muscle Strain During Suckling in the Infant Pig

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ABSTRACT

The geniohyoid muscle (GH) is a critical suprahyoid muscle in most mammalian oropharyngeal motor activities. We used sonomicrometry to evaluate regional strain (i.e., changes in length) in the muscle origin, belly, and insertion during suckling in infant pigs, and compared the results to existing information on strain heterogeneity in the hyoid musculature. We tested the hypothesis that during rhythmic activity, the GH shows regional variation in muscle strain. We used sonomicrometry transducer pairs to divide the muscle into three regions from anterior to posterior. The results showed differences in strain among the regions within a feeding cycle; however, no region consistently shortened or lengthened over the course of a cycle. Moreover, regional strain patterns were not correlated with timing of the suck cycles, neither (1) relative to a swallow cycle (before or after) nor (2) to the time in feeding sequence (early or late). We also found a tight relationship between muscle activity and muscle strain, however, the relative timing of muscle activity and muscle strain was different in some muscle regions and between individuals. A dissection of the C1 innervations of the geniohyoid showed that there are between one and three branches entering the muscle, possibly explaining the variation seen in regional activity and strain. In combination, our findings suggest that regional heterogeneity in muscle strain during patterned suckling behavior functions to stabilize the hyoid bone, whereas the predictable regional strain differences in reflexive behaviors may be necessary for faster and higher amplitude movements of the hyoid bone. *J. Exp. Zool. 00:1–11, 2012.* © 2012 Wiley Periodicals, Inc.

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Movement of the hyoid bone is a critical component in many vital behaviors including mastication, swallowing, suckling, phonation, and respiration (Crompton et al., '75; Pearson et al., 2011; German et al., 2011). Given that the hyoid bone in mammals either has few or no articulations with other bones, the muscles responsible for hyoid movement must also stabilize this bone in a multiple vectored sling (Crompton et al., '75; Hiiemae et al., 2002; Campbell-Malone et al., 2011; German et al., 2011; Pearson et al., 2011). These muscles are commonly referred to as "strap muscles" due to their simple architecture as long, relatively thin muscles with parallel fibers. Although the strap muscles traditionally have been described as anatomically simple, recent studies have identified regional variation in the muscle strain of two strap muscles, the sternohyoid muscle (SH) and geniohyoid muscle (GH), during reflexive head shaking (Wentzel et al., 2011) and in the sternohyoid during swallowing

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(Konow et al., 2010). To better understand muscle strain, we recorded length change and muscle activity, since muscle activity alone does not necessarily correlate to length change (Ahn et al., 2003; Konow et al., 2010). Active muscles have varying patterns of muscle strain since they can be lengthening (eccentric contraction), remaining the same length (isometric contraction), or shortening (concentric contraction).

Pharyngeal swallowing and reflexive head shaking are both short latency, episodic actions that occur in response to a sensory stimulus, which have been characterized as reflexes (Prochazka et al., 2000; Miller, 2002). The fact that regional muscle strain variation is seen in these muscles during reflexive behaviors raises the question if regional variation also occurs in these hyoid muscles during central pattern generated rhythmic behaviors such as respiration, mastication, and suckling. To address this question, we measured the regional muscle strain and muscle activity of the GH during rhythmic suckling in the infant pig. The GH originates on the genial tubercles of the mandible and attaches to the hyoid bone. While there are other hyoid muscles that can move the hyoid bone, anatomical evidence suggests that the GH likely is the muscle with the greatest potential for moving the hyoid bone anteriorly (Pearson et al., 2011).

The first hypothesis we tested was that there are predictable differences in muscle strain between the anterior, midbelly, and posterior regions of GH during suckling. We tested if the muscle strain was correlated with whether the suck cycle occurred before or after a swallow, or if it correlated with being early or late in a feeding session. Preliminary data suggest that pharyngeal swallows influence the timing of muscle activity during ongoing oral rhythmic behaviors (Thexton et al., 2012) and early in a feeding session, pharyngeal swallowing is so rapid that all suck cycles would be influenced by frequent pharyngeal swallows causing the GH to have a similar muscle strain. The second hypothesis tested was that muscle activity corresponds to muscle strain in the three regions studied. We further tested how the three regions within an individual and among individuals compare to one another. Lastly, the GH was dissected to see if differences in C1 nerve branching patterns would indicate different distributions of motor units along the length of the muscle in different individuals.

MATERIALS AND METHODS

Experimental Design

All experiments were performed using infant pigs ranging from 10 to 16 days old and 5–6 kg in weight. The animals were obtained from Tom Morris Farms (Reisterstown, MD). All procedures were approved by the ACUC no. SW07M14. The experimental methods are detailed in Konow et al. (2010) and therefore briefly described here.

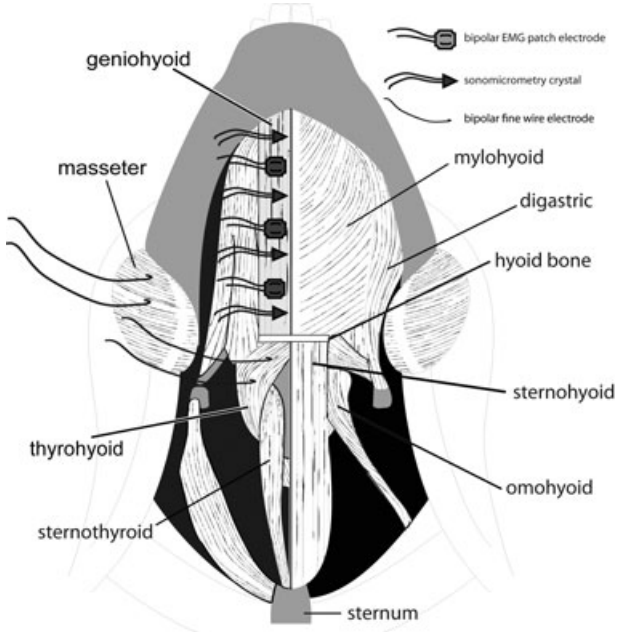

Figure 1. Placement of the sonomicrometry crystals, bipolar EMG patch electrodes, and bipolar fine-wire electrodes in the GH. The right side of the diagram shows the muscles that are visualized after removing the skin. The left side of the diagram shows the muscles visualized after removal of the mylohyoid muscle, sternohyoid muscle, and omohyoid muscles.

All animals underwent surgery to place four 2-mm piezoelectric crystals (Sonometrics, London, ON) along the anterior–posterior axis of the GH. Each of these crystals acted as transponders. While the animal was anesthetized with 2–3% Isoflurane gas, four crystals were equidistantly implanted along the length of the GH (approximately 17 mm apart). In between each crystal, and 3-4 mm from the anterior–posterior midline of the muscle, a fine-wire bipolar patch electrode was sutured to the muscle surface (Fig. 1).

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

Animals rested after surgery until fully awake, standing, and alert, and were then fed every 2–4 hr throughout that day and

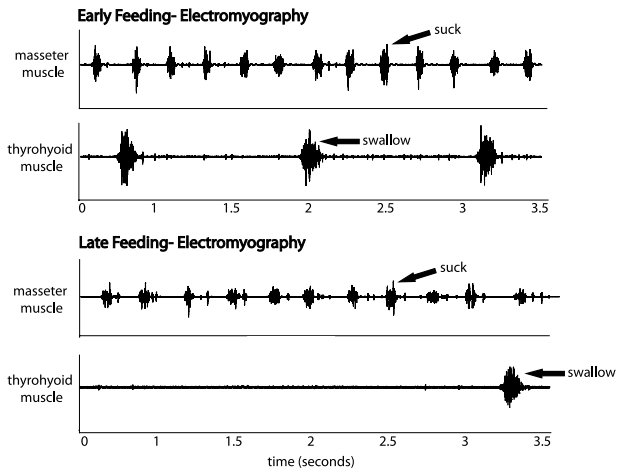

Figure 2. Electromyography from the masseter and thyrohyoid muscles used as markers of suckling and swallowing. Masseter activity was a marker for suck cycles and thyrohyoid activity was a marker for pharyngeal swallows. A 200-msec time frame was extracted, centered around the peak activity of the masseter, and will be considered one suck cycle. Suckling that occurs early in a feeding session is more rapid with more frequent swallowing as compared to later in the same feeding session. If there is an influence from the pharyngeal swallow on the suck cycle, then this influence would be seen during early feeding suck cycles that occur before and after pharyngeal swallows.

the next day. During these feedings, the pigs stood in a clear Plexiglas box while electromyography (EMG) and sonomicrometry measurements were recorded synchronously. A "pig nipple" was used with the bottle (Nasco, Fort Atkinson, WI). Data generated from the sonomicrometry crystals and EMG electrodes were digitized via a Powerlab 16/30 and inspected in the proprietary LabChart v.6.1.3 software (AD instruments, Colorado Springs, CO). The data were recorded at 10 kHz. At the conclusion of study, each animal was euthanized following JHU IACUC standards, and a postmortem dissection was performed to evaluate placement of the electrodes and crystals.

Data Analysis

The first hypothesis we tested was that regional length changes along geniohyoid are not the same during suckling. Additional testing aimed to determine if regional length changes were predictable based on the timing of the suck cycle and its relationship to the swallow cycle. First, suck cycles were extracted from the raw sonomicrometry data. Swallows were identified based on activity in the thyrohyoid muscle (German et al., 2009) and sucks were identified based on activity in the masseter muscle (Fig. 2). For each feeding, suck cycles were extracted over 200 msec with the 100-msec mark centered at peak masseter activity. This extraction procedure enabled suck cycle comparisons over time.

The sample for this study included three female infant pigs (mean body mass 5.53 ± 0.49) with four feeding sessions (experiments) for each pig. Both animal and feeding session were considered random factors in our statistical analysis. There were two fixed factors, time in feeding sequence (early/late) and position of suck cycle relative to the swallow (before/after). The first factor, time, had two levels, early or late, relative to the entire feeding session. The frequency of both suck and swallow cycles was much higher early in the session than later (Fig. 2). The second fixed factor was position of a suck cycle either immediately before or immediately following a swallow. There is some evidence (Thexton et al. 2012) that suck cycles occur as a function of their position relative to a swallow. Therefore, we selected suck cycles either right before or directly following a pharyngeal swallow but we did not include intermediate suck cycles. The two crossed factors produced four groups: Early/Before, Early/After, Late/Before, and Late/After. We extracted 20 suck cycles for each of these four groups from each of the 16 experiments for a total of 1,280 suck cycles. Thus, our unit of analysis was a suck.

The muscle strains in the three regions of the GH were compared using a cross-correlation function (CCF). The CCF calculates the peak-to-peak time lag between two waveforms, and the strength of the correlation at that lag (Konow et al., 2010). The output from a CCF is a lag-score, i.e., the timing between two waveforms, and the pair-wise correlation between the values of the two waveforms. The correlation is a standard measure of similarity, and the lag-score measures how many units the target wave must shift, either earlier (+) or later (–) to produce the maximum absolute value of the correlation possible with the reference wave. Although raw data were sampled at 10 kHz, using LabChart's sampling and data extraction routines, we collected a 200-msec window that consisted of 20 points of 10 msec each.

In order to test our hypothesis, that there are predictable regional differences in the muscle strain along the GH, three sets of comparisons were made between the three regions of the GH for each suck cycle: anterior to posterior, anterior to middle, and middle to posterior GH. Therefore, three lag-scores with associated pair-wise correlations were calculated for each suck cycle. Following Konow et al. (2010), we separated negative and positive correlations since each indicate biologically different relationships. A positive correlation indicates that the two regions were lengthening and/or shortening in synchrony (with the given lag). A negative correlation indicates regional strain heterogeneity, i.e., that length changes were out of phase in the two regions, and that one region was lengthening while the other was shortening. Comparisons were also made between suck cycles early and late in a feeding session and between

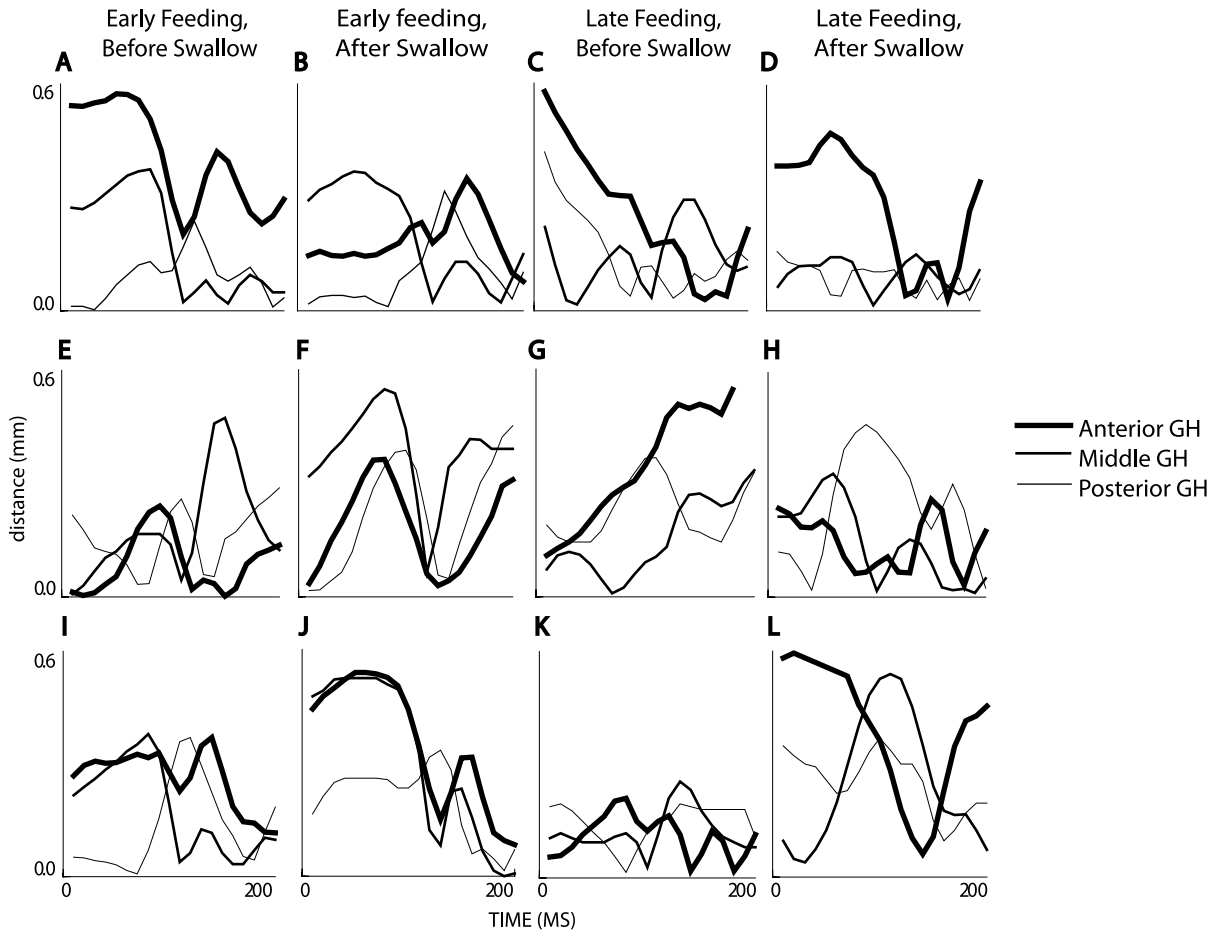

Figure 3. Each column shows three suck cycles from the same animal and experiment across the four treatments of the two factors. The high levels of variability in muscle strain in three regions of the GH were not consistent from cycle to cycle.

suck cycles occurring before and after the pharyngeal swallow. We tested for differences in lag-score among the three muscle regions, using a nested analysis of variance (ANOVA) (German et al., 2008). To determine specific differences among groups, and the values of those differences we used a post-hoc Tukey's test.

The second hypothesis we tested was whether regional length change correlates with muscle activity. Additional testing aimed at determining if these changes were predictable based on the region and individual. First, sequences of three to five suck cycles occurring between swallow cycles were isolated from the raw EMG and sonomicrometry data. The EMG data was rectified and integrated in LabChart using a reset interval of 10 msec. Data from three pigs were used and the cycles were randomly selected for a total of 18 suckling sequences. CCFs were calculated to test the timing difference between EMG activity and muscle shortening. The CCF results were limited to positive correlations and positive and negative lag values. A multifactorial ANOVA was calculated based on the lag values. The fixed factor was the interaction between animal and the region of muscle being evaluated. This resulted in nine total groups. The specific differences among these nine groups were evaluated using post-hoc tests of Least Squares Mean Differences.

Lastly, in order to further understand the anatomy of the geniohyoid muscle, the muscle was dissected in four infant pig cadavers. These four pigs were euthanized following other studies in our lab and were not the same animals used for the experiments previously described. The hypoglossal nerve was isolated and tracked to find the C1 motor branch that supplies the muscle. The anatomy was described and the exact location where the nerves branched and entered the muscle was noted. Photographs were also taken.

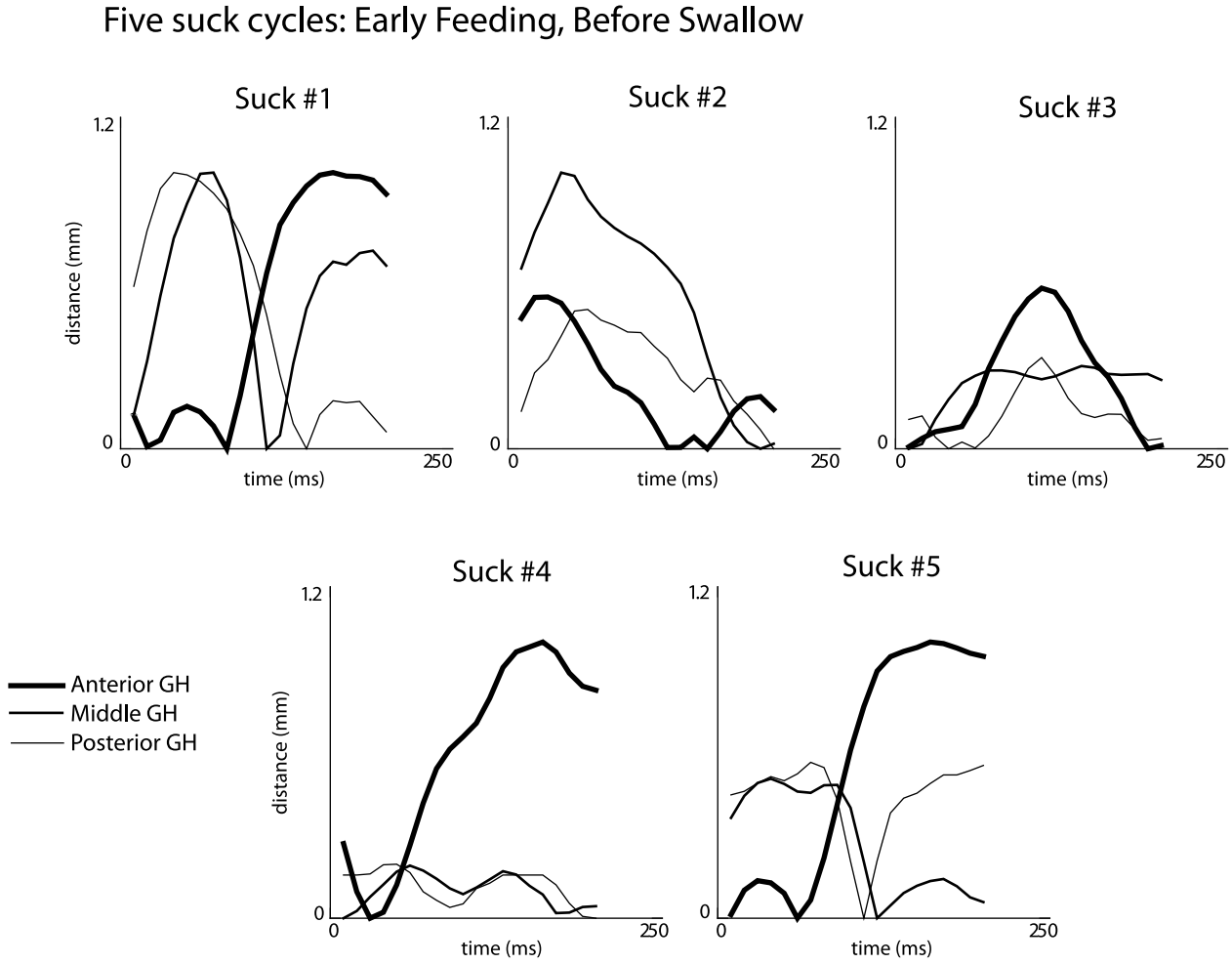

Figure 4. Five suck cycles from the same experiment, Early/Before. Substantial variation existed in the shortening patterns among the three GH regions, as well as within each region over successive sucks.

RESULTS

The first hypothesis we tested was that there are predictable regional changes in muscle strain along the length of the GH during suckling. In each suck cycle, at least one of the three muscle regions shortened or lengthened. However, the pattern of length change within each region was highly variable over multiple cycles (Fig. 3A–L). For some sucks, two regions changed length in-phase while the other changed length out of phase (Fig. 3J). In another suck cycle during the same feeding sequence, i.e., occurring within a few seconds, all regions were in synchrony (Fig. 3F). In some suck cycles, only one region changed length (Fig. 3D), whereas in other cycles, all three sections changed length (Fig. 3L).

These differences occurred from cycle to cycle. Figure 4 illustrates the typical degree of variation across five cycles that occurred in the same experiment, from the same treatment group (Early/Before). While the anterior region lengthened from mid-cycle in sucks no. 1, 4, and 5, there were different strain patterns in sucks #2 and 3.

There was no discernible or consistent pattern of the timing lags in the CCF analyses. While the correlations tended to be strong, i.e., above 0.5 or below –0.5, all values of lag were possible in each of the four groups (Fig. 5). Furthermore, there were an equal number of positive and negative correlations, with equal numbers of length changes that were in and out of phase.

Subsequent ANOVA testing indicated significant differences in the lag values among the three regions of the muscle for both positive and negative correlations (Tables 1 and 2, Fig. 6). For positive correlations, the timing lags were statistically

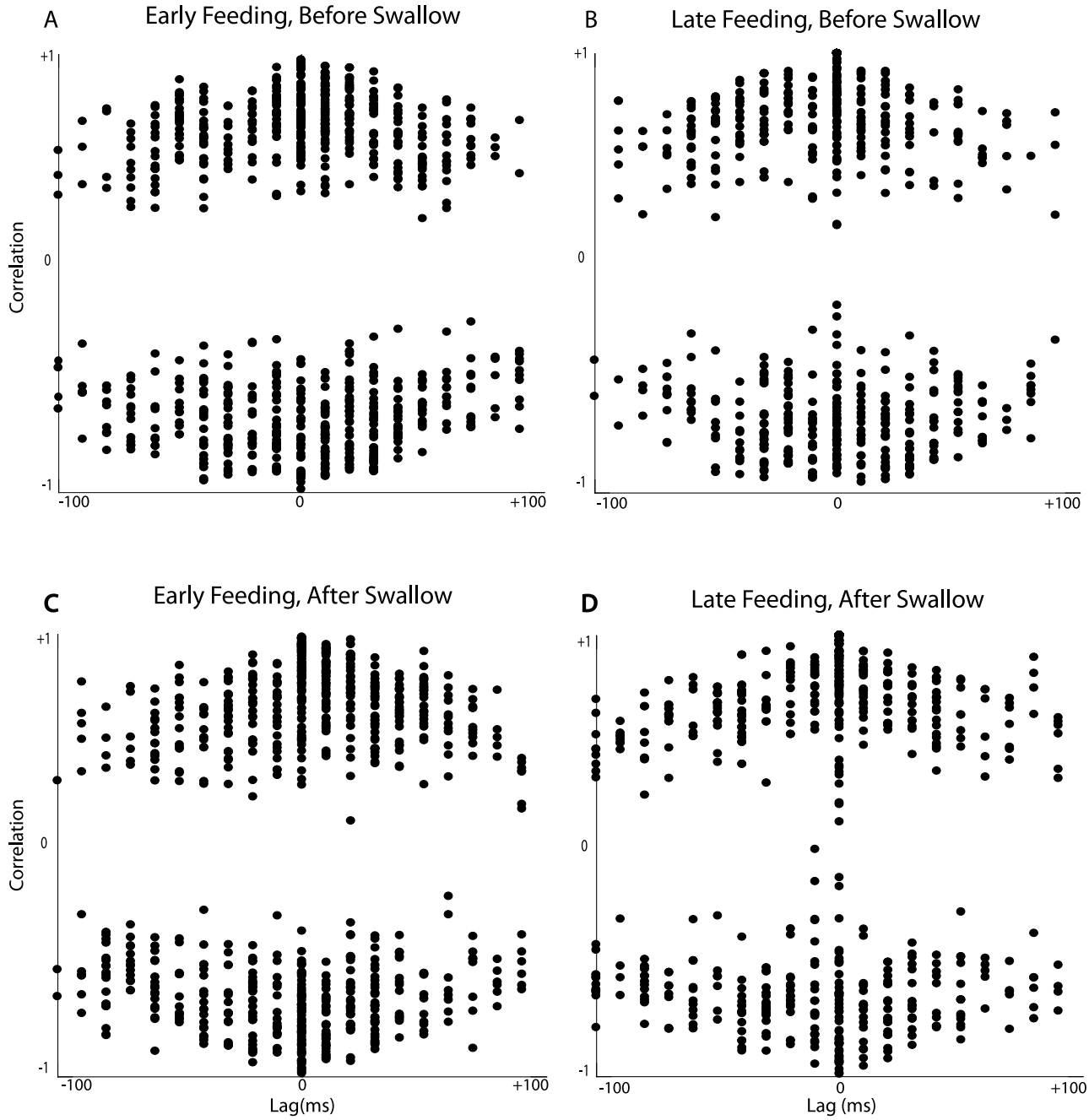

Figure 5. The lag and correlation values from cross-correlation analyses (CCFs) within suck cycles. The results are separated for suck cycles occurring early and late in a feeding as well as before and after pharyngeal swallow cycles. There is an equal distribution of positive and negative correlations and a wide, consistent range of lag values regardless of their classification.

significantly different in the three muscle regions being compared. The timing lag for the comparison between the muscle strain in the anterior and posterior region was the only value that was different from zero ($p = 0.011$) with a lag averaging −8.06 msec. Given that a suck cycle is approximately 200 msec, these differences are not likely to be functionally significant. For negative correlations, the comparison between regional strain timing in the middle to posterior region was the only

Table 1. Analysis of variance (ANOVA) and Tukey's Honestly Significant Difference Test results for positive CCF correlation lag values—regional length changes.

	F ratio	*T*	*P* value
After/Before	2.140		0.144
Early/Late	33.205		*<0.001
Early/Late		5.762	*<0.001
Region (Anterior/Middle/Posterior)	25.997		*<0.001
Anterior/Middle to Anterior/Posterior		7.558	*<0.001
Middle/Posterior to Anterior/Posterior		−4.251	*<0.001
Middle/Posterior to Anterior/Middle		4.009	*<0.001
Early/Late, Region-interaction	4.717		*0.009
Early Anterior/Middle to Late Anterior/Middle		4.515	*<0.001
Early Anterior/Posterior to Late Anterior/Posterior		3.884	*0.001
Early Middle/Posterior to Late Middle/Posterior		1.083	0.888
After/Before, Region-interaction	2.337		0.097
After/Before, Early/Late, Region-interaction	0.349		0.706

p-value < 0.05.

Table 2. Analysis of variance (ANOVA) and Tukey's Honestly-Significant-Difference Test results for negative CCF correlation lag values—regional length changes.

	F ratio	*t*	*P* value
After/Before	9.236		*0.002
After/Before		−3.039	*0.002
Early/Late	0.014		0.907
Region (Anterior/Middle/Posterior)	5.295		*0.005
Anterior/Middle to Anterior/Posterior		−1.163	0.476
Middle/Posterior to Anterior/Posterior		−2.554	*0.029
Middle/Posterior to Anterior/Middle		−3.233	*0.004
Early/Late, Region-interaction	0.649		0.523
After/Before, Region-interaction	7.910		*<0.001
After Anterior/Middle to Before Anterior/Middle		0.786	0.970
After Anterior/Posterior to Before Anterior/Posterior		−4.502	*<0.001
After Middle/Posterior to Before Middle/Posterior		−1.878	0.416
After/Before, Early/Late, Region-interaction	1.433		0.239

p-value < 0.05.

region that was statistically significant from the other two regional comparisons with a lag of 0.764 msec, however none of the three comparisons made resulted in lag values that were significantly different from zero. This supports the hypothesis that these are not functionally significant differences in timing.

When the correlations were positive, there was also a significant difference in lag between early and late suck cycles (Table 1, Fig. 7). The average lag for early suck cycles was 2.9 msec and the average lag for late suck cycles was −5.6 msec. Neither of these values were statistically significant from zero, which indicates that while they were statistically significantly different from each other, they are most likely not functionally significantly different from each other. Lastly, there was a statistically significant difference for the interaction of timing and muscle region (Table 1, Fig. 8). When further tested, the data showed that when comparing the anterior and middle regions, the lag was significantly larger earlier in the feeding session than later ($p < 0.000$). The same trend was seen when comparing the anterior and posterior muscle regions ($p < 0.000$). When these values were tested, none of them were statistically significant from zero, which again leads us to believe this is not functionally significant.

When the correlation was negative, there were statistically significant differences between the suck cycles occurring before and after the pharyngeal swallow (Table 2, Fig. 7). The average lag for suck cycles after a swallow was −5.6 msec and the average lag for a suck cycle before a swallow was 3.7 msec, which once again are relatively very low values. As seen previously, these values were not statistically significant from zero, which supports the hypothesis that these are not functionally significant values. Lastly, there was a statistically significant interaction between the timing of the suck cycle relative to the swallow and the muscle region (Table 2, Fig. 8). The lag between the anterior and posterior regions was lower and negative for the suck cycles occurring after the swallow and slightly positive, although not statistically significantly different from zero, when the suck cycle occurred before the swallow.

The second hypothesis we tested was that the timing of regional EMG activity and strain was consistent and different in the three regions of the geniohyoid muscle. The

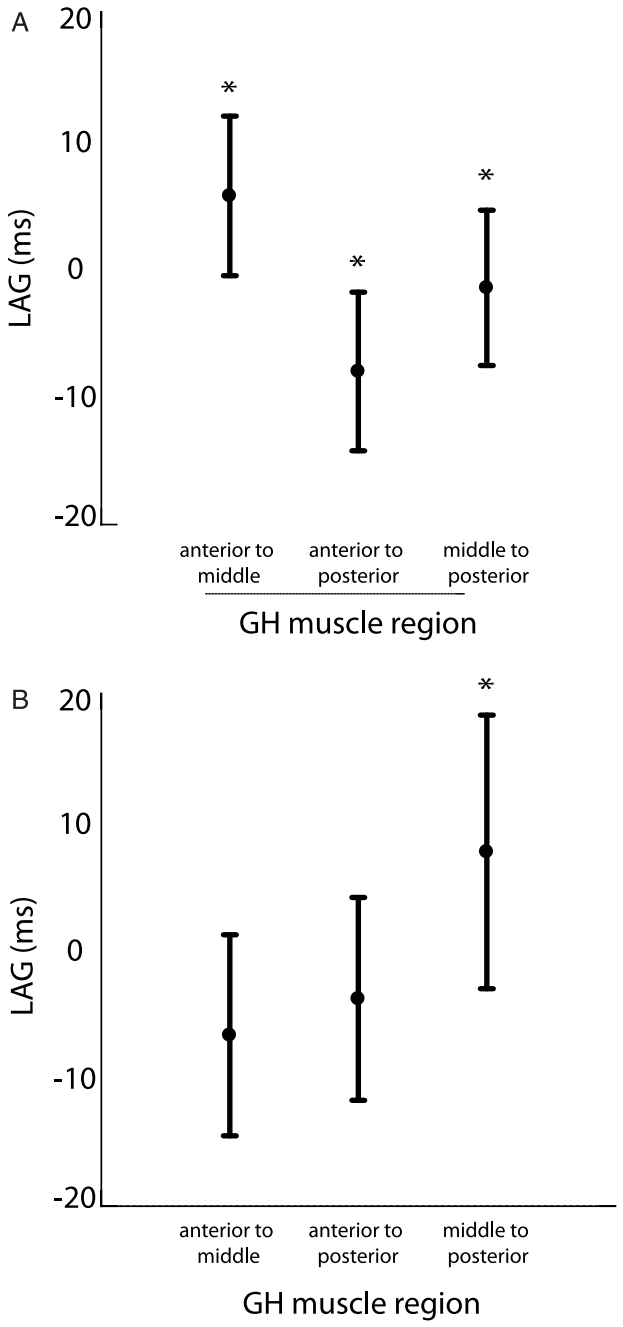

Figure 6. Statistically significant differences in regional strain. (A) For positive correlations, significant differences exist in timing of muscle strain change in all three regions. (B) For negative correlations, the timing of muscle strain change between the middle and posterior muscle regions was significantly different then the other regions.

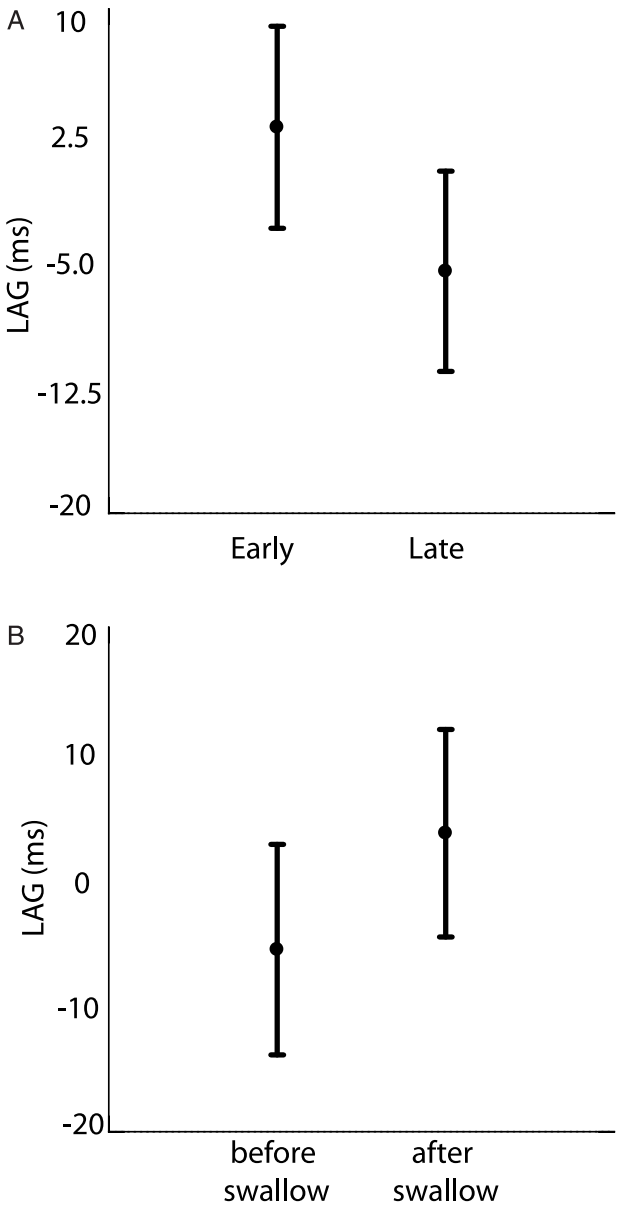

Figure 7. Statistically significant differences in suck cycles. (A) For positive correlations, there was a significant difference in lag between suck cycles that occurred early in a feeding as opposed to later in the feeding ($p < 0.00$). (B) For negative correlations, there was a significant difference in lag between suck cycles that were before a swallow as opposed to those after a swallow ($p = 0.002$).

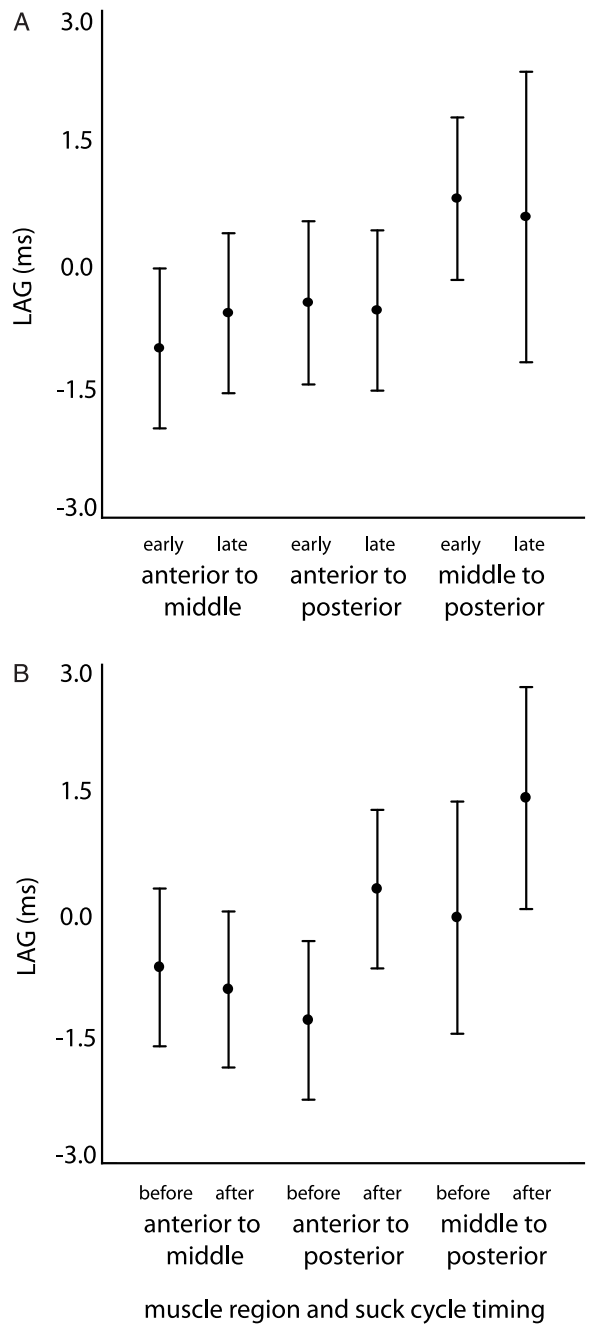

Figure 8. Statistically significant interactions between regional strain and timing of the suck cycles. (A) For positive correlations, there was a significant difference in lag between suck cycles occurring early in a feeding as opposed to late in a feeding when comparing the anterior to middle muscle regions and the anterior and posterior muscle regions. (B) For negative correlations, there was a significant difference in lag between suck cycles occurring before a swallow as opposed to after a swallow when comparing the anterior and posterior muscle regions.

results indicated that EMG activity caused a change in muscle strain with a tight correlation of timing in the three regions. The time lag between EMG activity and regional length change while predictable in each region was also different in each region of the muscle and different in the three animals (Fig. 9, Table 3). In Pig A, there was no difference between the timing of EMG and muscle lengthening in the three regions. In Pig B, the EMG and length change timing in the anterior region was marginally not statistically significantly different from that in the middle region ($p = 0.069$), but not different from the posterior region. That anterior region had a positive lag value while the other two regions had lag values that were not different from zero. In Pig C, the anterior region was statistically significantly different than the other two regions ($p < 0.05$). That anterior region had a negative lag value while the other two regions had positive lag values.

The dissections of the GH in four infant pig cadavers, for a total of eight geniohyoid muscles with C1 branches, had significant variation (Fig. 10). Each C1 had between one and three branches before it entered the muscle belly. Of the eight GHs, there were three that had one C1 branch, two that had two C1 branches, and three that had three C1 branches.

DISCUSSION

Unpredictable Heterogeneity Within the Geniohyoid During Suckling

Our results show that while there is heterogeneity in the muscle strain of GH during suck cycles, there is no consistent pattern or temporal relationship among the three regions studied. In all three regions, there was an equal probability of shortening or lengthening at any time in the suck cycle. The variation observed was not explained by satiation (i.e., sucks sampled from early vs. late in the feeding session) or by the influence of a pharyngeal swallow (before or after a swallow). Even though there were some statistically significant differences found among the timing of muscle strain changes in the three muscle regions, these differences were less than 10 msec, which was the level of temporal resolution of these data. It is plausible, however, that these very low differences in timing of length change could reflect the physiology of these muscles and their rate of contraction. This idea should be further explored in order to understand the functional significance of these data.

Geniohyoid Anatomy and Muscle Fiber Distribution

The fact that the C1 motor nerves branch before supplying the geniohyoid muscle supports our finding of the existence of active strain heterogeneity in GH. This anatomy was also consistent with descriptions of the anatomy of this muscle in several other species. The GH has regional anatomic separations in dogs, mice, rats, opossums, and tree shrews (Lakars and Herring, '87; Mu and Sanders, '98). In these taxa, there is a thin, complete, transverse myoseptum within the muscle. In dogs, the two regions of the

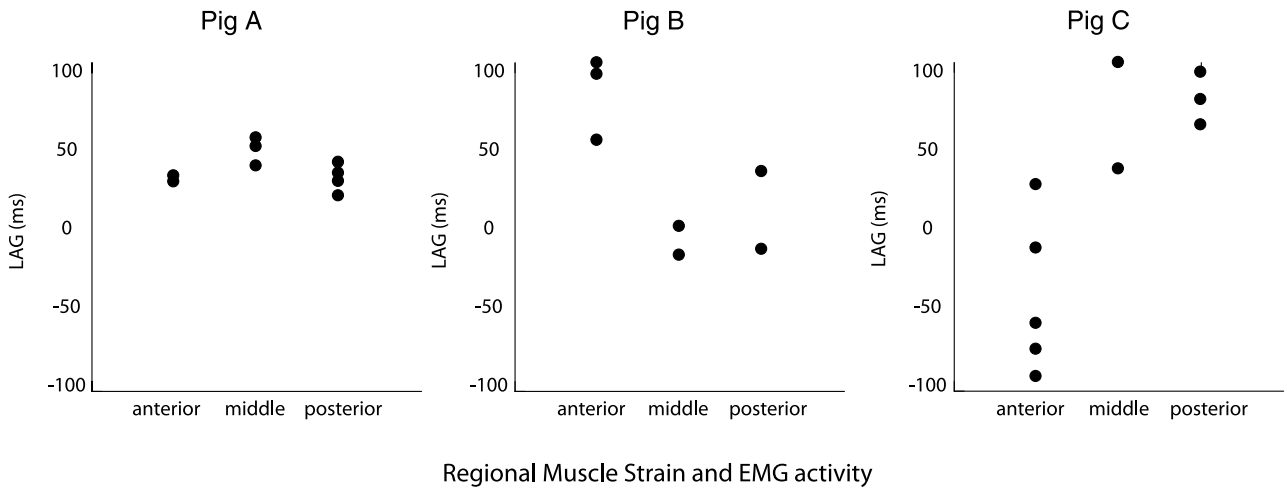

Figure 9. The lag values from the cross-correlation analyses (CCFs) when comparing regional muscle strain and EMG activity. The graphs show a tight relationship between the timing of muscle activity and muscle lengthening in the three regions of the muscle. The timing differences are different in the three regions based on the animal.

GH are innervated by separate primary nerve branches and each region has different distributions of slow and fast twitch muscle fibers (Mu and Sanders, '98). The functional significance of this myoseptum remains unknown, but we hypothesize that it could be the basis of independent contraction in the different muscle regions. However, no such myoseptum was observed in any of the animals studied. The results of this study indicate that there are regional differences along the muscle, which are not related to a myoseptum, but a different primary nerve supply or distribution of muscle fibers.

Regional Relationships Between Muscle Strain and EMG Activity

The regional changes in muscle strain along the length of GH were compared to the timing of EMG activity. There was a tight correlation between length change and EMG activity in all three regions of the muscle in the three animals tested. There were also differences found among the three regions in the three animals, but these were not consistent between animals. The fact that there was a tight relationship between length change and EMG activity demonstrates that the sonomicrometry crystals were detecting significant length change caused by muscle activity. The differences among animals may be due to a differential distribution of motor units along the length of the muscle. The different branching patterns found in the C1 nerve support this hypothesis.

Functional Significance of Heterogeneity in Geniohyoid Muscle Strain

The regional variation in hyoid strap-musculature is consistent during functions other than suckling. Length changes in SH during swallowing in infant pigs were consistent and predictable (Konow et al., 2010). Consistent, yet different, patterns in length change exist in both the SH and GH during reflexive head shake movements in infant pigs (Wentzel et al., 2011). Studies of the GH during respiration have focused on overall length change and muscle activity, and not potential regional variation (van Lunteren et al., '87; Wiegand and Latz, '91; Yokoba et al., 2003).

The functional implications of heterogeneity in muscle strain remain unknown although it has been hypothesized that it could potentially increase force or the ability to fine-control torque around a joint (Lakars and Herring, '87; Higham and Biewener, 2011). The differences and similarities among behaviors involving contraction of the hyoid strap muscles (i.e., swallowing, suckling, and head movements) suggest a degree of control that permits these architecturally simple muscles to function in a number of different ways. In order to further understand the implications of our present findings, experiments must be conducted in animals during other rhythmic and reflexive activities. Regional variation may be more predictable for some behaviors than others.

The position of the hyoid bone is primarily maintained by a sling of hyoid muscles with antagonistic actions that help move and stabilize the bone during these centrally patterned actions (Crompton et al., '75; Konow et al., 2010; German et al., 2011; Pearson et al., 2011). Regional specialization of the GH potentially makes this muscle more capable of stabilizing the hyoid during rhythmic patterned activities (Higham and Biewener, 2011). During suckling, the hyoid bone moves rhythmically and the geniohyoid muscle is principally responsible for the superior and anterior hyoid bone movement. As is true with every central

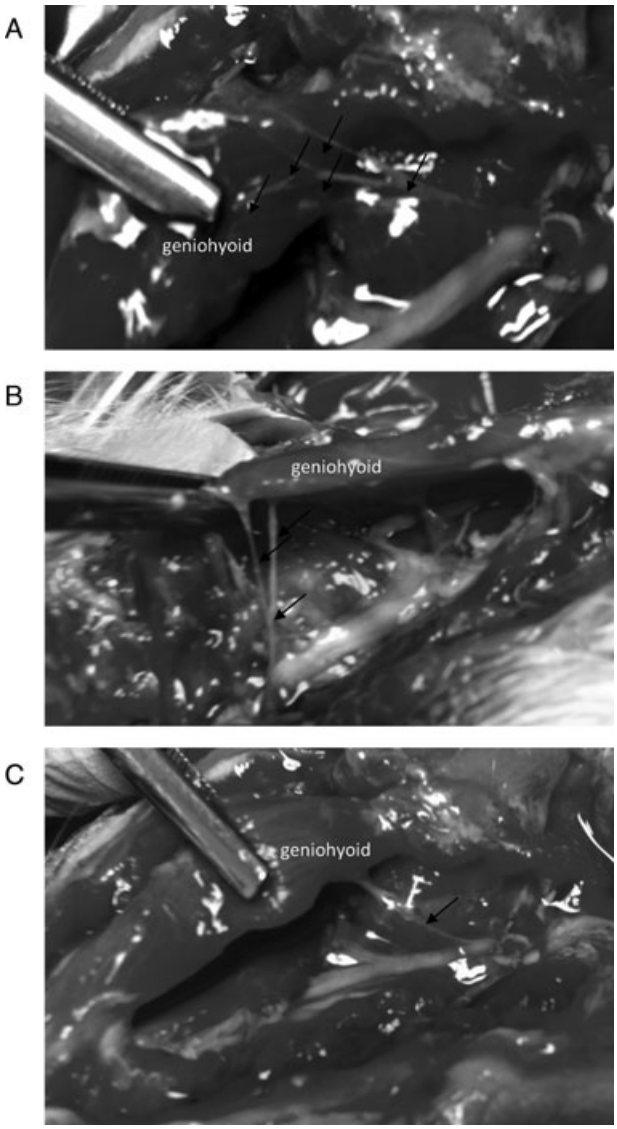

Figure 10. Nerve dissection photos (A) C1 with three branches, (B) C1 with two branches, and (C) C1 with one branch.

pattern generated behavior, there is constant sensory feedback that will alter muscle activity to keep the overall function intact (Dellow and Lund, '71). In the case of suckling, sensory information from the bolus is sent to the suckling pattern generator in the brainstem via afferents from the trigeminal nerve and the muscle activity of the oral and hyoid muscles is adjusted (Barlow, 2009). Regional variation in the length-trajectory of the strap muscles may serve to ensure that the movement of the hyoid during suckling is not significantly altered when processing different types and sizes of boluses.

Table 3. Analysis of variance (ANOVA) results for positive CCF correlation lag values and post-hoc Tukey's test results—regional length change to EMG activity.

p	*F* ratio	*t*	*p* value
Length change × animal	8.195		<0.001
Region 1 to 2: length change to EMG—Animal A		−0.647	0.999
Region 2 to 3: length change to EMG—Animal A		0.758	0.997
Region 1 to 3: length change to EMG—Animal A		−0.014	1.000
Region 1 to 2: length change to EMG—Animal B		3.360	0.069
Region 2 to 3: length change to EMG- Animal B		−0.628	0.999
Region 1 to 3: length change to EMG—Animal B		2.672	0.228
Region 1 to 2: length change to EMG—Animal C		−4.464	0.008
Region 2 to 3: length change to EMG—Animal C		−0.379	1.000
Region 1 to 3: length change to EMG—Animal C		−5.589	0.001

[a] *p* value < 0.05.

Future Directions

It will be necessary to compare the regional variation in GH and other hyoid muscles during rhythmic oral activities (respiration, suckling, mastication) and comparing them to short latency reflexes (swallowing, head shaking, coughing) to fully understand the implications of our findings. Further evaluation of muscle fiber unit composition could also contribute to this complex function of the GH. This study supports recent findings that the hyoid musculature may have a complex function that is dependent on the nature of the task and hyoid movement necessary.

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