Electromyographic activity during the reflex pharyngeal swallow in the pig: Doty and Bosma (1956) revisited

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Electromyographic activity during the reflex pharyngeal swallow in the pig: Doty and Bosma (1956) revisited. J Appl Physiol 102: 587–600, 2007. First published November 2, 2006; doi:10.1152/japplphysiol.00456.2006.—The currently accepted description of the pattern of electromyographic (EMG) activity in the pharyngeal swallow is that reported by Doty and Bosma in 1956; however, those authors describe high levels of intramuscle and of interindividual EMG variation. We reinvestigated this pattern, testing two hypotheses concerning EMG variation: 1) that it could be reduced with modern methodology and 2) that it could be explained by selective detection of different types of motor units. In eight decerebrate infant pigs, we elicited radiographically verified pharyngeal swallows and recorded EMG activity from a total of 16 muscles. Synchronization signals from the video-radiographic system allowed the EMG activity associated with each swallow to be aligned directly with epiglottal movement. The movements were highly stereotyped, but the recorded EMG signals were variable at both the intramuscle and interanimal level. During swallowing, some muscles subserved multiple functions and contained different task units; there were also intramuscle differences in EMG latencies. In this situation, statistical methods were essential to characterize the overall patterns of EMG activity. The statistically derived multimuscle pattern approximated to the classical description by Doty and Bosma (Doty RW, Bosma JF. J Neurophysiol 19: 44–60, 1956) with a leading complex of muscle activities. However, the mylohyoid was not active earlier than other muscles, and the geniohyoid muscle was not part of the leading complex. Some muscles, classically considered inactive, were active during the pharyngeal swallow.

The work of Doty and Bosma (10) led to a consensus that the mylohyoid muscle was the lead muscle in the sequential pattern of muscle activation. However, they (10) very carefully describe their classic figure as only a “schematic summary” in which the “contours of the rise and fall of activity are not considered accurate.” They also repeatedly emphasize the variability of the signals they recorded, stating that “there was wide variation in the duration of activity in various muscles” and that “not two EMGs of deglutition from the same electrodes gave identical or even similar unit patterns.” Such comments suggested that reinvestigation was justified. Our initial hypothesis was that the reported variation was largely due to the technology available at that time. The study by Doty and Bosma (10) predated both the description of the size principle in the recruitment order of motor units and the finding of exceptions to that order (7). It also predated the discovery of different task units within individual muscles (13, 58) and the discovery that different initiating stimuli could alter the subsequent pattern of activity in swallowing (41). These studies raise the possibility that the variability reported by Doty and Bosma (10) also included what were then unrecognized factors.

We tested two hypotheses: 1) that the variation in EMG activity reported by Doty and Bosma (10) could be reduced if the pharyngeal swallows were elicited using a single natural stimulus and more recent EMG recording techniques; and 2) that the reported variation in EMG activity could be explained by the selective recording characteristics of the electrodes detecting different types of motor units. Although not used in the Doty and Bosma (10) study, we used the decerebrate infant pig model for two reasons. First, considerable information is available on the feeding mechanisms of the intact piglet (16–
18, 51). Second, our ultimate aim was to use these data in comparison with conscious suckling to demonstrate the effect of cerebrocortical influences on the pharyngeal swallow.

MATERIALS AND METHODS

Animals and Surgical Procedures

All experiments that generated data for this paper were approved by the Harvard University Institutional Animal Care and Use Committee (IACUC; 23–05). Videoradiographic and EMG data were collected in a series of experiments using eight decerebrated infant mini-pigs (2 groups, Hartford strain and Hartford/DuRoc strain, each of 4 animals). We also used some qualitative radiographic data from nondecerebrate siblings of these animals, collected for another study, as an intact/decerbrate comparison. The animals were between 20 and 30 days of age (preweaning). Three different surgical procedures were carried out on each animal under halothane anesthesia (1–5% in oxygen administered by face mask) before recording swallowing.

1) Under general anesthesia, a microsurgical hematological clip was placed on the tip of the epiglottis (Fig. 1). This technique has been used in other studies (29, 30, 32) as well as by the authors without any noticeable adverse effects. The process of attachment took <60 s and was usually carried out immediately before the second procedure. Two animals were allowed to recover before the second procedure; they were reanesthetized 24–36 h later for the second surgical procedure, and during the intervening time, normal feeding was recorded videoradiographically.

2) The second procedure was decerebration. Under deep general anesthesia and aseptic conditions, bilateral craniotomies were performed. Under direct vision, the cerebral hemispheres and all other structures above the level of the inferior colliculi were removed by suction. We introduced Gelfoam (Pharmacia and Upjohn, Kalamazoo, MI) into the cranial space to control bleeding before closing the midline incision. We then gave each animal 10–20 ml isotonic fluids subcutaneously. The decerebrates were allowed to recover for 12–24 h before carrying out the third procedure, with subcutaneous fluids administered every 6–8 h.

3) Under general anesthesia and aseptic conditions, the supra- and infrathyroid muscles were exposed and identified following a standard atlas of pig anatomy (44). We implanted duplicate bipolar wire electrodes (1) into the muscles with the separation between the recording surfaces oriented parallel to the long axis of the muscle fibers and with the emerging wires sutured to nearby epimysium. Electrodes were inserted into eight of the following muscles at a time: m. geniohyoideus (at its mandibular origin), m. omohyoideus (superior belly), m. stylohyoideus, m. styloglossus, m. cricothyroideus, m. digastricus, m. hyoglossus (at its origin from the hyoid), m. constrictor pharyngis medialis, m. thyropharyngeus, m. cricopharyngeus, m. sternothyroideus, m. sternohyoideus, m. thyrohyoideus, or m. pterygoideus medialis muscles as previously described (53). Electrode insertion into the palatopharyngeus was accomplished by intraoral visualization of the surface anatomy of the muscle followed by intraoral manual palpation of the point of the needle carrying the bipolar electrodes during insertion from the surgically exposed access to the styloid muscles. In the case of the sheetlike m. mylohyoideus, the duplicate electrodes placed were bipolar patch electrodes (35) sewn to the surface of the muscle midway between the midline raphe and the mandible (this is ~10 mm medial to the medial edge of the pig digastric and ~9 mm lateral to the lateral edge of the deeper placed geniohyoid, so that confounding sources of activity were at a considerable distance from the electrode compared with the immediate electrode contact with mylohyoid fibers). In all except one animal, both mylohyoid electrodes were placed on the rostral two-thirds of the muscle; in one animal, one of the electrodes was placed over the most caudal muscle fibers. Subsequently, in this paper, the common names are used for the muscles; the term “inferior constrictor” is used to indicate m. thyropharyngeus and excludes m. cricopharyngeus. In all cases, the duplicate electrodes inserted into each muscle were laterally displaced from each other with respect to the orientation of the muscle fibers. We easily identified the relevant muscles using a single menton-manubrium incision. All electrode leads exited the surgical wound via the midline incision. After the final surgical procedure, the animals were given analgesics and were left for a minimum of 12 h to eliminate the inhalational anesthetic agent before swallowing was recorded.

The experimental period lasted 1–2 days after which the animals were euthanized following IACUC standards. A postmortem was then performed to confirm the position and state of the electrodes (independently of the worker originally placing the electrodes).

Experimental Feeding Methodology and Data Recording

From their time of arrival at the Harvard University vivarium, animals were fed infant pig formula from a standard baby bottle, fitted
with a special pig nipple (Nasco, Fort Atkinson, WI). Two additional animals, used for a different study but each with an epiglottal marker, were videofluorographed during conscious feeding. These animals drank milk containing radio-opaque barium, which provided a visual record of the vallecular space. Videoradiographs of these two additional animals were used to assess the level of vallecular filling necessary to elicit a swallow, before and after decerebration. Following decerebration, the preparations were positioned in the X-ray beam using a custom-designed body sling. This held the animal in a near normal prone position with the head supported approximately in the same position as the intact animal suckling on an artificial teat.

Most of the decerebrates lacked anterior oral function and the ability to suckle. They were fed using a small catheter (inserted into the posterior oral cavity under radiographic control), and we could deliver milk containing barium from a syringe in 1-ml aliquots directly into the valleculae. At each decerebrate feeding (4–6 times per day, not all of which involved recording), each animal received up to 80 ml of milk substitute. This produced a visually similar distension of the belly at each feed, similar to satiation in the intact animal. There was no visible reflux. The fluid balance of the decerebrates was supplemented with subcutaneous fluids (10–20 ml isotonic saline) administered every 3–4 h during the 24–48-h experimental period. Together the two sources maintained frequent voiding of urine during the experimental period.

The movements of swallowing were recorded using digital video radiography (Siemens Tridoros 150G3 cineradiographic apparatus with a Sony DCR-VX1000 digital video camera) in the lateral (sagittal) plane. EMG signals from the selected muscles were amplified (×400 to ×10,000) using an MA-300 EMG System (Motion Lab Systems) with a band pass of 20 Hz to 2 kHz and a 60-Hz notch filter. The EMG signals were then recorded on a TEAC RD-14ST digital data recorder together with the synchronization signals from the X-ray apparatus; an effective digitization rate of 6 kHz was used. Sections of EMG data that corresponded to cineradiographic recordings of sequences of swallows were then transferred to a computer. During feeding sessions the signals were displayed using a Quikview program designed for the TEAC data recorder and were, in addition, played out on a thermal Graphitec Arraycorder (WR3600).

Description of Recording Protocol

The unit of analysis for this study was a single pharyngeal or “reflex” swallow. In this study, we used the start of the rapid, caudally directed movement of the epiglottal tip (as recorded by lateral view digital video radiography) as the marker for the swallow. A period of multichannel EMG activity was subsequently aligned to this event. This first epiglottal movement was followed by a faster return movement. For convenience, in this paper the first caudally directed movement of the epiglottis is referred to as the epiglottal “flip.” While visually distinctive and easily identifiable, this movement could only be detected as an event occurring within a single NTSC videoframe. A single measurement of the onset of the movement could, however, be subject to a potential error of 67 ms (based on NTSC rate of 29.97 frames/s).

Individual swallows were elicited serially over a period of 1–3 min with a minimum of a few seconds delay between each swallow. Fluoroscopy allowed us to confirm that after each swallow the mouth, pharynx, and upper esophagus were clear of milk before delivering further milk. A set of serially elicited individual swallows was termed a sequence and consisted of 12–35 swallows. The sequences varied in length of time for technical reasons (e.g., stopping recording to reset catheter position or to refill delivery syringe, etc.). We recorded one or more sequences in each animal two or three times a day. Swallows with additional complex oral movements (described in RESULTS) or with inadequate EMG signals (see Data Analysis) were excluded from analysis. Because not all data from each animal were suitable for analysis, sequences were selected to produce a data set with approximately equal numbers of swallows per animal. The criteria for data selection were first, adequate videoradiographic images of successive individual swallows and second, artefact- and noise-free matching EMG records. After that, the data were accepted in the order in which they were recorded.

Although two electrodes were inserted into each of the selected muscles in each animal, the number of electrode recordings at any one time was limited to 15 because one of the 16 channels on the digital tape recorder was reserved for the digital camera synchronization signal. Consequently, duplicate recordings were possible in only seven muscles at a time. We use “electrode swallow” as a measure of sample size. This term indicates the number of EMG recordings of swallows obtained for each muscle, i.e., if two electrodes were present in the muscle and two swallows were elicited, this was counted as four electrode swallows. Details of the number of electrode swallows for the different muscles are given in Table 1.

Data Analysis

Selectivity of electrodes, the ability to detect an EMG signal only from the target muscle, has not been a problem in our previous work (49, 53). However, the simultaneous activation of a number of closely apposed muscles in the swallow, reported by Doty and Bosma (10), suggested that the selectivity of the electrodes should be confirmed. Therefore, we used cross-correlation analysis on the raw EMG data recorded from pairs of duplicate electrodes that were in close proximity to each other in small muscles (in omohyoid and in cricopharyngeus) to determine if there were any significant signals in common. If signals were to spread from nearby muscles or from distant motor units in the same muscle, there would be a significant cross-correlation between recordings from the two electrodes. We found no significant signal detected simultaneously by both electrodes. The peak correlation due to activation of motor units detectable by both electrodes was always offset by 1–2 ms, consistent with an earlier or later detection of traveling potentials by one of the two electrodes. These results confirmed a high level of selectivity of the recording electrodes.

We processed the band-pass filtered and amplified EMG activities in several steps, which were all combined into a single program by D. Hertweck. This program also presented a display of the resultant quantified EMG signals and allowed sections of the data to be indexed with reference to the time of the onset of the epiglottal flip (obtained from the radiographic data, as described below). The initial step was rectification and constant time (10 ms)-reset integration (53), after which two additional processes were applied to the data. The first defined a noise threshold statistically and rejected the background activity (50), and the next extracted the average EMG responses from the data as detailed below (53). We worked with 1-s blocks of selected EMG data in the form of a data matrix consisting of n swallows and 100 time units (each representing a 10-ms reset integration period) for each electrode.

The timing of the start of epiglottal flip during each swallow was identified from the frame count on the videotape and the corresponding time established in the EMG data stream, using the synchronization signal. The justification for the use of this event as a timing signal is given in detail in the APPENDIX. We first collected the EMG signals for a period starting 300 ms before each epiglottal flip and ending 700 ms after the flip (Fig. 2A). We divided the 1-s block into 100 10-ms reset integration periods and used these 100 periods as the basic time unit of this study. We next normalized the processed EMG activity recorded by each electrode in each swallow to a maximum amplitude of 100 units (Fig. 2B). This equalized signal amplitude across electrodes and swallows, despite inherent amplitude variation due to differences in the distance of the electrodes from the nearest active
muscle fibers (6, 14), and it also allowed amplitude-independent comparisons to be made across animals. We proposed to test the hypothesis that the variation described by Doty and Bosma (10) was the result of early methodology. To quantify the variation in our data, we first processed the data as indicated in Fig. 2. We summarized the data from each electrode by the median (50th percentile) level of activity in each of the 100 time bins of the 1-s analysis period. We plotted these medians against time to produce a “typical” EMG profile or burst for each muscle (Fig. 2C). Variation around this typical burst was measured by finding the 25th and 75th percentiles (upper and lower quartiles) for each time bin using the same data; the difference between the two is the standard interquartile range (IQR) measure of variability (Fig. 2C). The IQR was calculated for each of the 100 time bins. Thus, for each electrode, there were 100 measures of median activity and 100 measures of variability (IQR). To summarize the overall variation of the signals recorded by each electrode, we first summed the 100 measures of median activity and 100 measures of variability (Fig. 2C). The ratio was expressed as a percentage to provide an index of signal variability as a single number, analogous to the coefficient of variation. We set 200% as an upper limit of variability. If within a sequence the variability of the signal recorded by an electrode was greater than 200%, then we excluded that data from further analysis as being unstable or excessively noisy; the measure was also used for illustrative purposes.

We also calculated the extent of overlap between the signals detected by two electrodes in the same muscle as the area of overlap of the two median profiles expressed as a percentage of the overall area enclosed by the two median profiles.

A summary profile of EMG activity was generated for each muscle, using the overall matrix of all the acceptable EMG records obtained from that muscle in all animals. The final profile or kernel of activity for a muscle was then represented by the successive medians of all the individual amplitude values in each integration period. We used these profiles of median activity, over the 100 time bins, to test for timing differences between the activities in different muscles. Several logistic and methodological concerns complicated the among-muscle analysis of data. Standard parametric statistics were inappropriate because of

### Table 1. Data for muscles investigated

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Delay Relative to Leading Complex, ms</th>
<th>No. of Electrode Swallows</th>
<th>No. of Animals</th>
<th>Index of Overall Variability, %</th>
<th>Range of Overlap of Signals from Duplicate Electrodes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyoglossus</td>
<td>0</td>
<td>251</td>
<td>5</td>
<td>272.0</td>
<td>4.8–66.0</td>
</tr>
<tr>
<td>Stylohyoid</td>
<td>0</td>
<td>352</td>
<td>4</td>
<td>211.8</td>
<td>18.5–86.5</td>
</tr>
<tr>
<td>Mylohyoid</td>
<td>0</td>
<td>314</td>
<td>5</td>
<td>189.3</td>
<td>18.4–77.3</td>
</tr>
<tr>
<td>Medial constrictor</td>
<td>0</td>
<td>318</td>
<td>5</td>
<td>175.6</td>
<td>7.6–70.9</td>
</tr>
<tr>
<td>Styloglossus</td>
<td>0</td>
<td>60</td>
<td>1</td>
<td>263.1</td>
<td>25.5–45.4</td>
</tr>
<tr>
<td>Medial pterygoid</td>
<td>20</td>
<td>146</td>
<td>1</td>
<td>198.7</td>
<td>24.1–68.7</td>
</tr>
<tr>
<td>Palatopharyngeus</td>
<td>30</td>
<td>263</td>
<td>4</td>
<td>264.4</td>
<td>22.2–44.9</td>
</tr>
<tr>
<td>Omohyoid</td>
<td>30</td>
<td>464</td>
<td>7</td>
<td>189.3</td>
<td>18.7–92.7</td>
</tr>
<tr>
<td>Anterior digastic</td>
<td>40</td>
<td>146</td>
<td>3</td>
<td>382.9</td>
<td>8.8–60.3</td>
</tr>
<tr>
<td>Thyrohyoid</td>
<td>50</td>
<td>222</td>
<td>3</td>
<td>150.2</td>
<td>5.7–74.8</td>
</tr>
<tr>
<td>Inferior constrictor</td>
<td>70</td>
<td>234</td>
<td>3</td>
<td>143.2</td>
<td>23.8–80.0</td>
</tr>
<tr>
<td>Geniohyoid</td>
<td>120</td>
<td>278</td>
<td>3</td>
<td>347.5</td>
<td>17.4–56.2</td>
</tr>
<tr>
<td>Cricothyroid</td>
<td>180</td>
<td>146</td>
<td>1</td>
<td>200.8</td>
<td>22.5–44.5</td>
</tr>
<tr>
<td>Sternohyoid</td>
<td>250</td>
<td>153</td>
<td>2</td>
<td>375.0</td>
<td>19.5–68.6</td>
</tr>
<tr>
<td>Sternohyoid</td>
<td>270</td>
<td>74</td>
<td>2</td>
<td>286.2</td>
<td></td>
</tr>
<tr>
<td>Cricopharyngeus</td>
<td>320</td>
<td>224</td>
<td>3</td>
<td>154.8</td>
<td>18.8–70.1</td>
</tr>
</tbody>
</table>

The delays relative to the leading complex were calculated by cross correlation. See MATERIALS AND METHODS for calculation of index of variability and range of signal overlap. In the case of muscles where the reliable data could only be derived from a single animal, the median profiles of mylohyoid and omohyoid activity of those animals were compared with the group profiles; in all cases >95% of the profile was contained within the group profile, and linear correlations were also better than r = 0.95.

### Fig. 2. Method used to summarize the pattern of electromyographic (EMG) activity in a series of swallows: synthetic data. The time at which epiglottal movements started in successive swallows was established radiographically first. The associated EMG activity (100 reset integration periods of 10 ms each), extending from 300 ms before to 700 ms after the start of each epiglottal movement, was selected (A). The EMG data for each swallow were then aligned to the time of onset of epiglottal movement and scaled in amplitude to a maximum of 100 units (B). Because the EMG data from successive swallows had a highly skewed amplitude distribution, medians and quartiles of the scaled data were used to describe the amplitude profile of the grouped values (C). If the EMG signal had virtually the same amplitude-time profile on each occasion the reflex was elicited, the quartiles should be close to the median and the median peak close to 100 on the vertical scale. In the synthetic example shown, the median has an amplitude of ~70 scale units. Note that the quartiles indicate the limits containing 50% of all recorded values, i.e., half the values were outside these limits.
the nonnormality of the data because of the repeated-measures nature of the variables (through time in a sequence) and because duplicate recordings were not always available from all muscles in an animal. The lack of independence also made many standard nonparametric tests problematic. To determine the relative time delay between the different muscles, the median profiles of activity among the 16 muscles were consequently compared using cross-correlation analysis (SYSTAT, 2004).

RESULTS

The decerebrate infant pig did not normally exhibit the classical limb and jaw rigidity of the precollicular decerebrate adult cat. Spontaneous locomotion was variably present, and the behavior was similar to that of the decerebrate rat (36, 48, 64).

Videoradiographic Appearance

The delivery of three to six successive 1-ml aliquots of milk with barium by catheter to the dorsal surface of the posterior tongue elicited a pharyngeal swallow in all the decerebrate pigs. Occasionally only one aliquot would elicit a swallow. Therefore, a swallow followed delivery with a variable latency. In nearly all cases the radiographic shadows of the filled valleculae, immediately before reflex emptying, were substantially larger than those seen in intact animals (51). In the two animals from which we recorded pre- and postdecereration, the radiographic shadows of the valleculae immediately before swallowing were considerably larger after decerebration.

In the decerebrate, we found two types of swallowing activity.

1) Complex swallows. In ~10% of the swallows in two animals, rhythmic tongue responses were elicited in association with the emptying of the vallecular space. These rhythmic responses were similar to those seen in normal suckling, although they were slower and less precise and were reduced in amplitude. In two other animals, emptying of the vallecular space was periodically followed by a terminal muscle jerk similar to a hiccup. Data associated with complex swallows of either of the above types were excluded from the study because videoradiographically they were not pure reflex pharyngeal swallows.

2) Simple swallows. In the majority of swallows, the epiglottis was initially in an intranarial position with the marked tip above the soft palate (Fig. 1). As the swallow started, the epiglottal tip flexed rapidly in a caudal direction from this position, taking it below the level of the nearly horizontally running palatopharyngeus. We refer to this as the epiglottal flip. In three individuals, the epiglottis occasionally remained flexed and so within the pharyngeal cavity. In these cases, there was no epiglottal flip during the swallow. All swallows lacking an epiglottal flip were excluded from analysis because we relied on the epiglottal flip for analysis purposes.

In all the pharyngeal swallows included in the EMG analysis, there was some early penetration of the milk into the piriform fossae. The bulk of the bolus passed below the level of the radio-opaque marker on the tip of the epiglottis as it traversed the pharynx. This did not differ from the equivalent action in intact infant pigs suckling on a bottle nor did we find obvious differences in this action in the videoradiographs of swallows in the two animals for which we collected pre- and postdecereration data.

![Fig. 3. EMG recordings from 2 electrode sites in the superior belly of the omohyoid muscle during 4 successive swallows. All records are aligned to the time of the epiglottal flip which started at 300 ms. The top 2 records (A,B) are the raw EMG signals that correspond to the 1st of the 4 quantified records (C,D). This muscle is about 10 mm long and 5 mm in diameter in the infant pig so that the 2 bipolar electrodes were within a few of millimeters of each other.](http://japl.physiology.org/)

Intramuscle Signal Variation

The individual EMG signals recorded from bipolar electrodes during successive swallows normally consisted of bursts with durations of the order of 300 ms. In the case of the cricoid-related muscles, the burst lasted longer (400–500 ms). The signals varied in successive swallows for two reasons. First, different components of the burst varied irregularly in amplitude, and second, there were variably present ancillary bursts of activity. These variations were evident in the processed EMG activity when recorded by a single bipolar electrode in successive swallows (Fig. 3B). The signals also differed between intramuscular sites when they were recorded simultaneously by duplicate bipolar electrodes in the same muscle (Fig. 3, A and B).

The indexes of signal variability for an individual electrode ranged from a low of 19% to over 200% (all records exceeding the 200% limit were excluded; see MATERIALS AND METHODS) although in most cases the indexes were in the range 40%–140%. Examples of the statistical summary graphs (median and quartile profiles) of successive EMG records with indexes in this range are shown in Fig. 4. Figure 4 also illustrates a large difference in the latency of EMG activity at two different anteroposterior sites in the same mylohyoid muscle. The activity at the anterior site commenced ~50 ms before the start of the epiglottal flip (dotted line at 300 ms), while the activity in the posterior fibers commenced nearly 100 ms after that event. The area of overlap of the two signals was 18.4%. The overlap of the duplicate EMG recordings from a single mylohyoid muscle in other animals (with more closely approximated dual electrodes) varied from 48% to 77%. Although Fig. 4 illustrates the activity recorded with maximum separation of
duplicate patch electrodes in the mylohyoid muscle, analogous but smaller time differences in signals from more closely positioned duplicate wire electrodes were obtained in all other muscles; this is illustrated in part by the range of signal overlap found (Table 1). While some duplicate recordings showed very large degrees of overlap (up to 92.7%), others overlapped by <10%. In the case of sternothyroid recordings, swallow-to-swell differences in the signals from a single electrode produced very high indexes of variability (>200%). We consequently excluded large numbers of recordings (because their indexes of variability exceeded the acceptance limits), and this led to a lack of reliable duplicate recordings for this muscle.

The number of electrode swallows recorded in the named muscles varied from 60 to 464 with a median of 223 responses. There were several reasons for the variation in sample size. The two most significant were the exclusion criteria and the fact that we could only record from 15 electrodes simultaneously. Occasionally intact electrodes at the correct site could not be verified postmortem. The pattern of EMG activity in each of the 16 muscles was represented by the median and the quartile measures derived from all electrode swallows for that muscle, i.e., for a named muscle it included all the data from different electrodes in that muscle in different animals (Table 1). In the case of muscles for which acceptable data could only be obtained from one animal, the data from two other muscles were tested against the group data to confirm that the animal was not behaving very differently from the group (see legend to Table 1).

Examples of the general responses (Fig. 5) showed that the median profiles of activity from different muscles had peak values that varied substantially from a high of 67 scale units to a low of 23 scale units. The level of signal variation, indicated by the distance between the upper and lower quartiles, was also large and varied with the muscle. The calculated indexes of variability for the pooled data, i.e., data for that muscle pooled from all electrodes, from all sequences and from all animals, ranged from 143% to 375% depending on the muscle (Table 1).

Finally, the overall activity for each of the 16 muscles investigated was summarized graphically by plotting the grand medians of the EMG activity for each muscle on the same time scale (Fig. 6).

**Intermuscle Motor Pattern**

The median profiles for some muscles overlapped in time (Fig. 6), while others were located at clearly different times within the 1-s period examined in relation to the swallow. In addition, the peak value of these profiles varied considerably. The first group of muscles activated included hyoglossus, stylohyoid, mylohyoid, middle pharyngeal constrictor, and styloglossus. On the basis of cross-correlation analysis, there were no significant differences in the timings of these five muscles. All started activity before the beginning of epiglottal movement. With zero relative time lag, the average correlation coefficient (Pearson) among pairs of muscles in this group was 0.976. The next three muscles (medial pterygoid, palatopharyngeus, and omohyoid) had a slightly later timing. The pairwise cross-correlation of the activity profiles of these three muscles with the first five (hyoglossus, stylohyoid, mylohyoid, middle pharyngeal constrictor, and styloglossus) showed a time lag of <30 ms. With this time lag, the average correlation coefficient of the activity profiles of these muscles with the five leading muscles was 0.981 for medial pterygoid, 0.964 for palatopharyngeus, and 0.987 for omohyoid.

The activity profiles of the remaining muscles had an obvious lag from the first group of five. Three muscles had a small but significant timing lag: anterior digastric lagged by 40 ms, thyrohyoid by 50 ms, and the inferior pharyngeal constrictor by 70 ms. The average correlation coefficients with the five leading muscles were 0.929, 0.978, and 0.981, respectively. The next two muscles, geniohyoid and cricothyroid, lagged by 120 and 180 ms with average cross-correlations of 0.979 and 0.880. Finally, there was a group of muscles with significantly longer lags relative to the first group. These muscles started to show activity well after the start of the epiglottal movement at 300 ms (Figs. 5 and 6). Sternothyroid lagged the first five muscles by 250 ms with a correlation of 0.788. Sternohyoid lagged by 270 ms with a correlation coefficient of 0.782. The last muscle was cricopharyngeus, which lagged the first group by 320 ms with a correlation coefficient of 0.910.
DISCUSSION

Nature of the Elicited Swallow

When intact conscious animals suckled, the swallows started significantly before the radiographically defined vallecular space was completely filled with fluid. In contrast, after decerebration, substantial or complete filling of the vallecular space with liquid (3–6 ml) was normally required to elicit a pharyngeal swallow. This indicated that the threshold for the response had been increased and suggested that a source of central facilitation had been lost. A compensatory greater peripheral sensory input would then be required in these animals in order to reach the threshold for eliciting vallecular emptying. This is consistent with decerebration transecting the facilitatory pathways that descend from cerebral structures to the brain stem swallow-generating circuits (3, 37, 45, 63). However, the serotoninergic activity in decerebrates (27), which facilitates motor output, may also suppress sensory processing (23). This aspect consequently cannot be ignored as a potential factor raising the threshold for vallecular emptying.

Because analysis was restricted solely to those swallows that conformed to simple emptying of the valleculae, it was unsurprising that the movements appeared to be constant in form and amplitude. However, the recorded EMG signals showed considerable variation, as also described by Doty and Bosma (10). For a given muscle, in the present study, the variability in the recorded EMG signals existed at several levels. There was variation between the signals recorded in successive swallows by a single electrode (within-electrode variation), variation between the signals recorded simultaneously from duplicate electrodes in the same muscle (within muscle), and variation in the sampled activity of that muscle between animals (within animals).

Nature of the EMG Signals

An initial consideration in the analysis of the variation in the recorded EMG signals is that the occurrence of larger potentials in the records did not necessarily reflect the activation of larger motor units. This is because the size principle in motor unit recruitment is obscured when recording with small-surface-area intramuscular electrodes (14). Consequently, over the time course of a burst of EMG activity, the successive changes in signal amplitude were largely a function of the distance between the muscle fibers (active at that time) and the tips of the recording electrodes (6, 14). Records showing visually distinct differences in the profiles of EMG activity in successive swallows (Fig. 3D) reflected the detection of different motor units active at different times. This indicated that the order of recruitment of many of the motor units was not fixed, as has been found in other situations (7, 43), but that it varied or rotated from swallow to swallow. The recorded variation does not imply that the entire motor pool behaved in exactly the same way.
the same way so that the whole muscle changed activity from swallow to swallow. It only indicated that each record, representing a small sample of the total activity occurring in that region of the muscle, could reflect slight changes in the selection of muscle fibers that were activated in that region. Consequently, the larger the sample number (or number of electrodes or sequences or animals), the better would be the approximation to the average activity of that muscle.

The different patterns of the within-muscle variation in EMG signals reflected different organizational or morphological aspects of the muscles being studied. In many cases, the two sets of signals detected by duplicate electrodes (e.g., in the superior belly of the omohyoid; Fig. 3) had different patterns of activity, even though the electrodes were within a few millimeters of each other. This suggested a high level of selectivity of the electrodes. However, the overall period or duration of EMG activity detected by many of the duplicate electrodes was similar in timing, relative to epiglottal movement. We concluded that the differences between the signals detected by the two electrodes were mainly due to the detection, by each electrode, of different motor units activated within the swallow-related burst.

In some muscles, signals from duplicate electrodes showed pronounced site-specific differences in the time of onset and the time of peak activity (e.g., mylohyoid, Fig. 4), so that the two signals showed limited overlap in the time of activity. The temporal overlap of signals recorded by duplicate electrodes varied considerably with the muscle (Table 1). No set of data reached 100% overlap, which indicated that no two electrodes in the same muscle recorded exactly the same signals. Some of these differences could be simply due to each of the duplicate electrodes detecting signals from motor units with differing recruitment thresholds. Other site-specific differences showed very large latency differences, and these required a different explanation. The recording sites did not have to be widely separated (as in Fig. 4) because the latency of the median responses obtained from duplicate electrodes in other muscles could periodically differ by as much as 80 ms. This included the omohyoid, a muscle in which the electrodes were within a few millimeters of each other because of its small size.

Duplicate records from different widely separated antero-posterior sites on the large sheetlike mylohyoid muscle (Fig. 4) showed large latency differences (≥100 ms) when the EMG activity was recorded using relatively large area and therefore less selective or multiunit patch electrodes (35). The large latency differences were consequently more likely to be due to differences in the tasks undertaken by the motor units in the two different regions of the muscle than due to threshold differences of individual units. In the mylohyoid, one of the most important muscles in the Doty and Bosma (10) scheme, two strands of evidence supported this view. First, the distribution of muscle fiber types differs between the rostral two-thirds and the caudal one-third of the mylohyoid (8). Second, in conscious feeding, EMG recordings from the posterior region of the mylohyoid not only show activity occurring at the same time as the anterior fibers but also exhibit an extra later burst of activity considered to have a different function (38).

The considerable difference in timing of mylohyoid activity (Fig. 4) could have reflected the existence of differences between task units in the anterior and in the posterior regions of the pig mylohyoid. The human mylohyoid shows segmental innervation and probable functional differences between the anterior and posterior muscle fibers (42), indicating a potential parallel with the current study. However, in humans, the muscle fiber types are distributed relatively homogeneously, which makes further comparison uncertain.

The variability in the recorded EMG signals in this study was influenced by both methodological and biological factors. One factor was the potential variation in identification of the timing of the epiglottal flip, due to the video time resolution of 29.97 frames/s, i.e., positions were recorded at 33.367-ms intervals. Because of the large numbers of observations, this potential variation had a relatively small impact on the median response relative to variation in the EMG signals, as outlined in the appendix. Other sources of variation in recorded EMG signals arose because recordings came from varying small samples of motor units, from different threshold motor units, from different task units, and possibly from some units showing postdecerebration changes.

While task unit differences are relatively common in the muscle system under study (34, 55, 56, 62), it was surprising that different task units should have been activated in what is conventionally viewed simply as reflex emptying of the valleculae. Although the reflex pharyngeal swallow was largely isolated by the process of decerebration, we occasionally elicited additional rhythmic tongue and/or jaw activity. This is

![Diagram](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAAIoAAADhCAMAAAAAW1pSAAAABlBMVEX///...)

**Fig. 6.** The pattern of EMG activity in 16 oropharyngeal muscles over the 1,000-ms period associated with the reflex emptying of the prefilled vallecular space. The profiles represent median values derived from the data from all included sequences in all animals. All data were synchronized to the time at which the epiglottis started to move caudally (vertical line at 300 ms). The reduction in the size of the peak levels from 100 scale units is a function of the temporal variation of the signal (see DISCUSSION). The relative time delays of the activities in the different muscles were obtained by cross correlation (see Table 1).
consistent with the neural substrates for those activities existing at the brain stem level (5, 9, 48). Although we defined pharyngeal swallows on the basis of radiographic evidence, this did not guarantee the total absence of all trace of EMG activity representative of the more complex, complete swallow. Our criteria of no rhythmic tongue or jaw movement only guaranteed that such activity was sufficiently reduced to the point where it did not produce obvious movements. The sporadic occurrence of EMG activity in what appeared to be different task units may therefore have represented the reduced expression of the intraoral transport component of the complete swallow.

This may explain some of the differences between the present study’s findings and those of Doty and Bosma (10). We could, on radiographic grounds, exclude everything except isolated reflex pharyngeal swallows, but Doty and Bosma (10) lacked such methodology and could not. It seems likely that a conscious encephalon preparation (cervical cord transected at C1–C2), as used by Doty and Bosma (10), would have generated some form of rhythmic oral activity following electrical stimulation of the superior laryngeal nerve or following placement of fluid in the mouth. Such activity would not necessarily have been discernable by simple external observation so that EMG activity, reflecting weak rhythmic muscle activity, would not have been separable from the EMG activity of the reflex pharyngeal swallow. This is particularly relevant to differences in the timing of geniohyoid activity, which Doty and Bosma (10) place in the leading complex but the present study places 120 ms later.

Variable recruitment thresholds and task differences in some of the motor units presented a problem in generating and testing an across-animal summary of EMG signals. We used rank order statistics (median, quartiles) because they are uninfluenced by sporadic extreme values and are applicable to data that are not normally distributed. Significantly, even after combining multiple sets of data from different electrodes in successive swallows in different sequences in different animals, the profiles of activity for several muscles, including thyrohyoid and inferior pharyngeal constrictor, showed only limited variability (Table 1; Fig. 5). This contrasts with the data from muscles such as anterior digastric, sternothyroid, sternohyoid, and geniohyoid, which showed considerable variation. The differences in variability in the pattern of activation of different muscles could be explained by three factors. One factor is the presence of variable threshold motor units. The second may stem from the additional tasks of these muscles in relation to respiration and hyoid posture, i.e., primarily the maintenance of airway patency during inspiration (12, 43, 59–61). These multiple functions of oropharyngeal muscles significantly complicate the problem of establishing their pattern of activity purely in relation to the pharyngeal swallow. A third possible factor (dealt with in more detail in the Appendix in relation to determination of swallow time markers) is the uneven expression of plateau potentials in the motoneuron pool (19, 27); these events can advance and extend the period of EMG activity recorded in response to a recurring natural stimulus (2).

Although in an individual swallow, a possible error in establishing the exact time of the onset of epiglottal movement is obviously a factor in the variability of the combined signals, that potential error is common to all the EMG signals recorded at that time, and it cannot, therefore, differentially affect the variability of EMG signals in the specific muscles noted in the previous paragraph.

Relationship of the Present Findings to the Traditional Pattern of EMG activity

The calculated pattern of activity (Fig. 6) has an obvious similarity to the pattern of activity described by Doty and Bosma (10). Our results support their concept of a “leading complex of muscles,” active early in the swallow. However, important differences exist in the muscles of the leading complex in the two studies. Doty and Bosma (10) include the superior constrictor, palatopharyngeus, palatoglossus, posterior intrinsic tongue muscles, styloglossus, stylohyoid, geniohyoid, and mylohyoid in their leading complex. Significantly they indicate that the mylohyoid sometimes leads the complex by 30–40 ms.

While the leading complex in our study (Fig. 6; Table 1) included some of Doty and Bosma’s leading complex (stylohyoid, mylohyoid, and styloglossus), it differed in four significant respects. First, we found that the hyoglossus and the middle pharyngeal constrictor were part of the leading complex. Second, the principal activity of the palatopharyngeus was found to occur 30 ms later than the other leading muscles. Third, geniohyoid was clearly not a member of the leading complex in the pharyngeal swallow. Finally, in view of the prominence that is traditionally given to the mylohyoid as the lead muscle in the pattern of EMG activity in the pharyngeal swallow, there was no evidence that the mylohyoid muscle activity consistently occurred in advance of the other muscles of the leading complex (Figs. 5 and 6; Table 1).

The pattern of EMG activity in the mylohyoid was similar to that of other muscles of the leading complex, but it was not the leading muscle. The upper quartile profile of mylohyoid EMG activity (Fig. 5) indicated that some motor units were activated 100 ms earlier than defined by the median profile of this muscle. The same pattern was also evident in hyoglossus, anterior digastric, and geniohyoid muscles (Fig. 5) amongst others. The periodic occurrence of early activity was not specific to mylohyoid but a general pattern of variation in several muscles. In the case of the geniohyoid (Fig. 5), the profile of the upper quartile of activity showed that some motor units were active long before the start of the epiglottal flip and long before (as well as long after) any of the leading complex of muscles. This extended period of activity of a minority of motor units may be due to the fact that the functions of the geniohyoid are not confined to deglutition but include airway dilation (60) and jaw depression. Similarly, the extended activity of a subset of motor units in sternohyoid and sternothyroid muscles may be related to their additional functions in relation to respiration and hyoid posture (12, 55, 59–62).

The difficulty in determining any first or leading muscle is due to the variability we found. By definition, 50% of the data lay outside the limits of the upper and lower quartiles. Given this variability, if the EMG records from a number of the leading complex muscles were selected by inspection, the onset of activity in those muscles could be obtained in almost any order. This indicates that the only way to establish a reliable order of activation is to obtain large numbers of
Conversely, the pattern of EMG activity of the complete input, independent of any descending encephalic influences. The patterns of activity recorded in these muscles had two obvious and consistent characteristics. The peak activity of the median profile was low, and these muscles had high indexes of variability (Fig. 5; Table 1). These characteristics could have been produced by the overall data sets for a muscle containing signals from motor units with different latencies. Records showing different latencies or times of peak activity (Figs. 3 and 4) suggest dissimilar motor unit groups in the same muscle. This means that the peak EMG signals in different swallows from different electrodes in different animals will not coincide, and as a result, the median of the data set will have a reduced peak value. The use of rank order statistics to summarize the EMG responses in the pharyngeal swallow normally limits excessive influence from outlier values that reflect a minority of motor units behaving very differently from the norm. If, however, there is a substantial subsample of the motor unit pool consisting of units that behave differently, the median values of peak activity are substantially depressed, and the variability is high, e.g., digastric, sternohyoid, sternothyroid (Table 1; Fig. 5). We hypothesize that only a limited number of motor units of digastric, sternohyoid, and sternothyroid fire in phase with the reflex pharyngeal swallow, while other motor units are subject to different sources of excitation.

The excitatory activity of the sternohyoid was preceded by a period of reduced activity, which may reflect central disfacilitation or inhibition (Fig. 5). These findings do not conflict with the description of inhibition of sternohyoid motor units during swallowing (31). The reduction in sternohyoid activity (Fig. 5), also just visible in Fig. 6 at the time of peak thyrohyoid activity, corresponded to the pattern found by Lang et al. (31). Furthermore, the subsequent increase in sternohyoid activity shown by Lang et al. corresponds in time to the period of increased activity shown in Figs. 5 and 6.

Our results do not support the first hypothesis we proposed, that the variation in EMG activity reported by Doty and Bosma (10) could be substantially eliminated by using more modern experimental methods. The second hypothesis, that the variation in EMG activity could be largely explained by the recording characteristics of intramuscular electrodes, selectively detecting different types of motor units, was well supported. In behaviors such as swallowing, where the muscles studied subserved multiple functions and contained different task units, we found that statistical methods were essential to characterize the swallow-related patterns of EMG activity. Despite the signal variability encountered (Figs. 3–5, Table 1), nonparametric methods enabled the central tendency of the data (Fig. 6) to be established, providing the number of observations was large.

In this study, the overall pattern of EMG activity in the pharyngeal swallow (Fig. 6) represented the response of the brain stem pattern generator to a purely peripheral sensory input, independent of any descending encephalic influences. Conversely, the pattern of EMG activity of the complete conscious swallow depends on activity of encephalic structures in addition to that of the brain stem. This underscores one of the problems of precise comparison of our results to those of Doty and Bosma (10). They used both anesthetized intact and unanesthetized isolated encephalon preparations. In the case of the anesthetized animals, it is likely that the higher centers and some of the brain stem circuits would have been pharmacologically depressed by the different anesthetics used (ether, urethane); in this situation, Doty and Bosma (10) find changes in the phase relationships between some of the muscles. In contrast, their isolated encephalon preparation would have been capable of both conscious sensation and action above the level of cervical cord section. The comparison is then further complicated by the periodic reference to responses of the “unanesthetized medulla” (10), which may imply the use of a further reduced neural preparation analogous to that used in the present study. However, Doty and Bosma (10) make no great distinctions between swallowing elicited in the different preparations. This, taken together with the general similarities of the results obtained by Doty and Bosma and in the present study with decerebrates, suggests that the expression of experimentally elicited swallowing activity is largely independent of the experimental preparation.

Another source of difference in results is due to the model species used in both studies. Although Doty and Bosma (10) report differences in swallowing activity (in anterior digastric, posterior digastric, palatoglossus, palatopharyngeus, plus laryngeal and cricothyroid muscles) among the three species investigated (cat, dog, monkey), they also summarize the situation as there being “no great species variation save in the larynx.” In view of the similarities between the findings of the present study and those of Doty and Bosma (10) discussed above, it would appear that the generalities of the temporal pattern can now be considered to be common to four species.

The differences in the eliciting stimuli in Doty and Bosma’s (10) study and ours also do not seem to impact on results. Doty and Bosma use different eliciting stimuli (pharyngeal stroking, electrical stimulation of the superior laryngeal nerve, and sometimes “rapidly injected water”) but found “no difference in temporal pattern, duration, or amplitude” between swallow EMGs elicited in this way. This view is, however, contested by a subsequent study (41). However, to the extent that Doty and Bosma (10) occasionally injected fluid to elicit the swallow, there is some comparability with our methodology.

The major difference between the two studies was our use of videoradiography to identify purely reflex pharyngeal swallows with no associated rhythmic oral movement. Some modification of the temporal pattern is highly probable if significant rhythmic oral activity is present, whether induced reflexly by peripheral sensory input to the decerebrate brain stem or by descending activity in the intact animal. The present study is the first step in trying to establish if and to what extent such a modification occurs. Comparison of the current data, which quantifies the decerebrate response to fluid in the valleculae, with the EMG response of intact sucking animals will permit the identification of those components of the swallow that are dependent on descending encephalic influences. Such data may ultimately clarify some aspects of the dysphagia associated with cerebral pathology.
APPENDIX

Methods for Establishing the Timing of the Reflex Pharyngeal Swallow

Introduction. A time marker common to all swallows was essential for the analysis of the EMG activity in the 16 different muscles studied. Unfortunately, in this study the reflex pharyngeal swallow had a variable latency so that the delivery of fluid to the valleculae could not be used as the timing event. We therefore examined two other potential signals as alternative time markers for the swallow: 1) the objectively defined onset of the EMG signal in one of the muscles; and 2) the onset of epiglottal flexion, determined from the videoradiographs.

The EMG responses were frequently variable in form, whereas the epiglottal flexion appeared to be a simple regularly reproducible event. The following is an analysis of the characteristics of these alternative time markers.

Timing derived from an EMG signal. The EMG activity was first processed as described in MATERIALS AND METHODS. Then the EMG signals in successive swallows were tested to establish their level of reproducibility. Because of the current acceptance of the mylohyoid as a lead muscle in swallowing, the following description concentrates on that muscle as a source of a timing signal. The profiles of mylohyoid EMG activity in the three swallowing shown in Fig. 7 were recorded by the same electrode within a 30-s period. In the absence of any other marker, the signals were aligned to the time of their onset, defined as the time at which the activity exceeded 1% of maximum signal amplitude. The times at which each signal then reached 10%, 20%, and 100% amplitude levels varied, in some cases differing by >200 ms between responses (Fig. 7).

Such signal variation has the potential to affect the time location of markers for swallowing. We tested the impact of this potential time variation in the reference EMG on the analysis of other muscles using data from duplicate bipolar electrodes in the mylohyoid. The time in each swallow at which the mylohyoid EMG activity reached 10% of peak amplitude was used as the reference time for that swallow (similar results were obtained using other levels). The procedures outlined in MATERIALS AND METHODS were then used to process the activity recorded from the thyrohyoid muscle for a sample of 20 reflex swallows, using the time markers obtained separately from the two mylohyoid electrodes. This process generated (for each of the 2 reference electrodes) a set of median and quartile profiles of the thyrohyoid EMG activity. The results of this analysis (Fig. 8, A and B) indicate that the variability of the thyrohyoid data was a function of which reference electrode was used to generate the time marker. The time of onset, determined from the upper and lower quartiles in Fig. 8B, indicates that in half of the records the onset time varied by ~60 ms and so, by definition, in the remaining half of the records the onsets varied by >60 ms. No arbitrary allowance could be made for the different latencies of response at the two mylohyoid sites.

Timing from epiglottal movement. An alternative time marker for the start of the reflex swallow was the onset of epiglottal movement from the videofluorographic data. While visually distinctive and easily identifiable, this movement could only be detected as an event occurring within a single NTSC videoframe. The video time resolution was determined by the recording rate of 29.97 frames/s, or 33.367-ms intervals. Epiglottal flexion had a sudden onset and was complete in two videoframes so that a single measurement of the onset of the movement could be subject to a potential error of ±1 frame, i.e., a total likely error for a single measurement of ~67 ms.

However, when the same thyrohyoid EMG data (that were processed using mylohyoid EMG markers and shown in Fig. 8, A and B) were reprocessed using the onset of epiglottal movement as the timing marker, variability in the summary values was reduced. The index of variability was 66% with the epiglottal marker and 155% and 114% for the two different mylohyoid EMG signals.

The same analysis was also employed in four animals using two mylohyoid EMG signals in each animal to generate the timing signals for processing swallow-related omohyoid EMG activity. In this case the magnitude of the interquartile range was used as the variable of interest, and this was examined in relation to the three possible treatments. While there was no statistical difference between the results of processing data using two randomly allocated mylohyoid electrodes to provide the timing signals (Fig. 9), there was a significantly lower level of variability when the onset of epiglottal movement was used as the time marker (P < 0.001).

Sources of variability. These results show that when the objectively defined onset of the accurately recorded EMG activity was used to define the timing of the swallow, the variability in the processed data was greater than when the less accurately determined epiglottal movement was used. This was a wholly counterintuitive result, the explanation of which rests primarily on two factors, the variability in the profile of the EMG response and the effect of large numbers in reducing the mean error of epiglottal timing.

First, the profile of the recorded EMG activity varied between swallows (Fig. 7) so that, even if the conventional first sign of activity was used as the timing event, the major part of each burst of EMG activity followed with a highly variable delay. This suggested that deriving a time marker from a simple threshold level would not be error free. The unusual pattern of the variably prolonged initial activity followed with a highly variable delay. This suggested that deriving a time marker from a simple threshold level would not be error free. The unusual pattern of the variably prolonged initial activity followed with a highly variable delay. This suggested that deriving a time marker from a simple threshold level would not be error free. The unusual pattern of the variably prolonged initial activity followed with a highly variable delay. This suggested that deriving a time marker from a simple threshold level would not be error free.
of epiglottal flexion (C). In the absence of any external index, the individual elevated activity. Therefore, it occurs with a shorter latency or against a background of motor units. Some EMG activity associated with the swallow may thereupon the potential for uneven facilitation of different ing a given muscle exhibit plateau potentials to the same extent (33).

19. 22, 40). Because trigeminal and hypoglossal sensory input, and are known to occur in a number of cranial motor fibers (11, 19, 21, 27), are enhanced by excitation from peripheral sensory input, and are known to occur in a number of cranial motoneurons, including trigeminal and hypoglossal (22, 40). Because intraoral mechanical stimuli in decerebrates can have excitatory effects (46), it is likely that deliveries of fluid would have periodically increases the reliability of the statistically determined timing of the median pattern. The level of variation in the signal was consequently reduced to below the level that was obtainable using the EMG signal for timing purposes (Fig. 9). On the other hand, if there was an undetected systematic error in timing, this would apply equally to all muscles studied and so would have no effect on their relative timings, although it would affect the accuracy of the time relationship between the presumed start of epiglottal movement and the start of the entire EMG pattern.

A variable pattern of this type would be consistent with many of the records obtained in this study. As indicated previously, it was difficult to derive a reliable time marker from the EMG signals. This timing inaccuracy was the likely cause of the variability shown in the processed signals of Fig. 8, A and B. This view is supported by the finding that the variability could be reduced when an alternative time marker (Fig. 8C) was used.

A further problem with using EMG derived timing is that, as described in the body of the paper, EMG activity recorded by duplicate electrodes in the same muscle did not necessarily have the same latency (e.g., Fig. 4), so that there was often a systematic error between recordings from two sites in a muscle. This intramuscle difference in latency was quite independent of temporal variability in the EMG signals obtained from a single electrode. For example, in two electrodes recording from palatopharyngeus, in a sample of 20 swallows timed from epiglottal movement, the IQR was low in each electrode recording (22.9% and 20.7%), but there was a 100-ms difference in latency between signals recorded at the two sites. Such latency differences were independent of the use of epiglottal movement as a timing marker because, if the onsets of signals at one electrode are taken as the time markers, the signals at the other electrode still differed on average by 100 ms, and there was no a priori way of knowing which signal should be used to generate the time marker.

In contrast to the frequently complex EMG signals with differing latencies, epiglottal movement was simple but was assessed subjectively and at low resolution. However, high-resolution measurements can be obtained using a low-resolution system (25), providing that the number of observations is large.

With the use of the onset of epiglottal movement as the time marker, the time location of the EMG activities for a single swallow would be subject to any individual error made in the identification of the timing of the reference event. If, however, the sample were sufficiently large, the error around the timing of the event would tend to have a mean of zero (25). Consequently, the combination of data from a number of recording sequences from a number of animals increases the reliability of the statistically determined timing of the median pattern. The level of variation in the signal was consequently reduced to below the level that was obtainable using the EMG signal for timing purposes (Fig. 9). On the other hand, if there was an undetected systematic error in timing, this would apply equally to all muscles studied and so would have no effect on their relative timings, although it would affect the accuracy of the time relationship between the presumed start of epiglottal movement and the start of the entire EMG pattern.

### Fig. 8. Thyrohyoid EMG data processed using the time at which the EMG activity recorded by 2 electrodes in the mylohyoid muscle each attained 10% of their peak amplitudes (A and B) and again processed using the time of onset of epiglottal flexion (C). In the absence of any external index, the individual mylohyoid timings were all aligned to 50 ms so that the median thyrohyoid values in A and B commence at ~100 ms. The data in C were processed as in the main text, with the epiglottal flexion located at 300 ms.

### Fig. 9. Differences in least-squares mean variation of recorded mylohyoid activity. Three different references are used to calculate variation, two different electrodes in the same mylohyoid muscle, and the epiglottal flip. Variation is measured by ln(intraquartile range) over 100 time units for 4 animals. The least-square means are calculated from an ANOVA testing for differences among references but also including individual and time bin as random factors. The error bars indicate one SE on either side of the mean variation. Epi, epiglottal; Mylo, mylohyoid.
Conclusion: In the final analysis, the epiglottal movement represented the mechanical effect of pooled muscle activity. Despite the possibility of indirect muscle action and intervening mechanical elements, the movement represented the overall effect of a very large number of different motor units. Conversely, the recorded EMG activity was a direct but very small sample of motor units drawn from the total population of motor units of a single muscle, where some units were likely to have expressed plateau potential-related activity and where the latencies could differ with recording site within a given muscle. The ability to use large numbers of low precision measures to derive a higher precision measure (25) consequently applies to epiglottal movement where there is a single source of measurement error. However, it does not apply as well to measurements where there is both uncertainty in definition of onset, variability in timing of sequential events, and variability in those measures by intramuscular site.

In the circumstances of this study, epiglottal movement was the preferred time marker, but this may not be the case in other experimental situations.

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