Role of peripheral reflexes in the initiation of the esophageal phase of swallowing

Ivan M. Lang, Bidyut K. Medda, Arash Babaei, and Reza Shaker

Dysphagia Research Institute, Division of Gastroenterology and Hepatology and Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin

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Lang IM, Medda BK, Babaei A, Shaker R. Role of peripheral reflexes in the initiation of the esophageal phase of swallowing, Am J Physiol Gastrointest Liver Physiol 306: G728–G737, 2014. First published February 20, 2014; doi:10.1152/ajpgi.00411.2013.—The aim of this study was to determine the role of peripheral reflexes in initiation of the esophageal phase of swallowing. In 10 decerebrate cats, we recorded electromyographic responses from the pharynx, larynx, and esophagus and manometric data from the esophagus. Water (1–5 ml) was injected into the nasopharynx to stimulate swallowing, and the timing of the pharyngeal and esophageal phases of swallowing was quantified. The effects of transection or stimulation of nerves innervating the esophagus on swallowing and esophageal motility were tested. We found that the percent occurrence of the esophageal phase was significantly related to the bolus size. While the time delays between the pharyngeal and esophageal phases of swallowing were not related to the bolus size, they were significantly more variable than the time delays between activation of muscles within the pharyngeal phase. Transection of the sensory innervation of the proximal cervical esophagus blocked or significantly inhibited activation of the esophageal phase in the proximal cervical esophagus. Peripheral electrical stimulation of the pharyngoesophageal nerve activated the proximal cervical esophagus, peripheral electrical stimulation of the vagus nerve activated the distal cervical esophagus, and peripheral electrical stimulation of the superior laryngeal nerve (SLN) had no effect on the esophagus. Centripetal electrical stimulation of the SLN activated the cervical component of the esophageal phase of swallowing before initiation of the pharyngeal phase. Therefore, we concluded that initiation of the esophageal phase of swallowing depends on feedback from peripheral reflexes acting through the SLN, rather than a central program.

METHODS AND MATERIALS

Experimental Protocols

The following experimental protocols were designed to address the hypotheses presented above.

Hypothesis 1: initiation of the esophageal phase of swallowing depends on peripheral reflexes, not a central program. We investigated the temporal relationship between the pharyngeal and esophageal phases of swallowing in protocol 1. If the esophageal phase of swallowing is controlled by peripheral reflexes, and not a central program, then the percent occurrence of the esophageal phase should be related to peripheral feedback, i.e., bolus size. On the other hand, if initiation of the esophageal phase is centrally controlled by the occurrence of the pharyngeal phase, then occurrence of the esophageal phase should be as variable as occurrence of the pharyngeal phase.

Protocol 1: determination of the relationship between bolus size and temporal measures of the esophageal phase of swallowing. Water was injected into the nasopharynx to stimulate pharyngeal swallows, and the volume of fluid injected was related to the percent occurrence of the esophageal phase at different locations in the esophagus.

Hypothesis 2: peripheral reflexes are essential for initiation of the esophageal phase of swallowing. We tested whether the loss of sensory innervation of the proximal cervical esophagus affected initiation of the esophageal phase of swallowing in protocol 2. If the link between the pharyngeal and esophageal phases of swallowing is purely central, then elimination of esophageal afferents should not prevent initiation of the esophageal phase of swallowing. In addition,
to confirm that we only eliminated sensory innervation, we defined the motor innervation to the feline esophagus in protocol 3.

Protocol 2: role of sensory innervation of the proximal cervical esophagus in initiation of the esophageal phase of swallowing. We determined the effects of cutting the sensory innervation of the proximal cervical esophagus on the esophageal phase of swallowing. Swallowing was stimulated by injection of water into the nasopharynx, and we transected the most proximal extent of the recurrent laryngeal nerve (RLNp).

Protocol 3: determination of the motor innervation of the feline esophagus. We determined the effects of stimulating the three nerves, i.e., SLN, vagus nerve (X), and pharyngoesophageal nerve (PEN), that innervate the esophagus on esophageal motility. The SLN, X, or PEN was transected and stimulated peripherally to determine which was the motor nerve to the cervical esophagus in the cat.

Hypothesis 3: afferent stimulation of the SLN can activate esophageal peristalsis independent of swallowing. The only type of study that has been used to conclude that the esophageal phase of swallowing is initiated independent of peripheral feedback assumed that SLN stimulation activated the central program of the esophageal phase. However, if SLN stimulation can activate the esophageal phase independent of the pharyngeal phase, as tested in protocol 4, then the only data supporting central control of initiation of the esophageal phase of swallowing is in doubt.

Protocol 4: effects of centripetal electrical stimulation of SLN afferents on esophageal motility. The RLNp or SLN was transected and stimulated centripetally to determine whether afferents mediated by these nerves could activate esophageal peristalsis independent of the pharyngeal phase of swallowing.

Techniques

Animal preparation. The experiments were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin. Ten decerebrate cats that had been fasted overnight were anesthetized using isoflurane (3%), the ventral neck region was exposed, the trachea was intubated, and the carotid arteries were ligated. The skull was exposed, and a trephine was used to make a hole over a parietal lobe. The hole was enlarged using rongeurs, the lateral sinuses were filled with bone wax, the exposed brain was ligated, and the brain was severed midcorticallly using a metal spatula. The forebrain was then suctioned out of the skull, and the blood vessels of the circle of Willis were coagulated by suction through cotton balls soaked in warm saline. The boney sinuses were filled with bone wax, the exposed brain was covered with paraffin oil-soaked cotton balls, and the skin over the skull was sown closed. The anesthesia was removed, and the animals were placed supine on a heating pad (Harvard Homeothermic monitor) to maintain body temperature between 38 and 40°C.

To monitor the pharyngeal and esophageal phases of swallowing, we recorded from muscles representative of the different phases. For the pharyngeal phase, we recorded from muscles representative of the different sets of muscles activated, i.e., pharyngeal, laryngeal, and hyoid. Thus we recorded in n (number) animals the electromyographic (EMG) activity from the cricopharyngeus (CP, n = 10), cricothyoides (CT, n = 10), geniohyoides (GH, n = 10), and thyrohyoides (TH, n = 10). For the esophageal phase, using a solid-state manometric catheter (Gaeltec Medical Instruments). The individual recording sites were 3 cm apart, with the most proximal site 1 cm from the CP. The criteria for identification of esophageal peristalsis was a manometric response that propagated at least two recording sites, and the criterion for identification of response to nerve stimulation was a doubling of the voltage; for the CP, the threshold was a 25% change in response. In all cases, the response must have been temporally correlated with the stimulus. The electrical activity was filtered (band pass = 0.1–3.0 kHz) and amplified 1,000–10,000 times before it was fed into the computer.

ESOPHAGEAL AND BLOOD PRESSURE MANOMETER. The intraluminal pressures of the esophagus were recorded using a solid-state manometric catheter (Gaeltec Medical Instruments). The individual recording sites were 3 cm apart, with the most proximal site 1 cm from the CP. The criterion for identification of esophageal peristalsis was a manometric response that propagated at least two recording sites, and the criterion for identification of response to nerve stimulation was a doubling of the voltage.

This catheter was used to inject water into the nasopharynx, thereby providing a controlled stimulus of the pharyngeal swallow. The femoral artery and vein were cannulated to monitor blood pressure and maintain hydration with infusion of saline.

The effects of cutting the RLNp in nine animals were tested. The RLNp (Fig. 1), the most proximal branch of the recurrent laryngeal nerve (RLN) just caudal to the cricoid cartilage, joins the SLN within the larynx and transmits afferent information from the proximal cervical esophagus to the brain (21, 25, 30). This nerve is readily visible and easily transected as it travels from the proximal cervical esophagus to enter the larynx at the lateral side of the caudal border of the cricoid cartilage.

In seven animals, the effects of electrical stimulation of nerves (Fig. 1) innervating the esophagus, i.e., the X, SLN, PEN, and RLNp, on esophageal pressure were tested. The X was identified and isolated in the cervical region at the level of the CP. The SLN was identified and isolated as it traverses from the lower border of the nodose ganglion to the dorsal border of the TH. The PEN was identified and isolated as it exits the cranial border of the nodose ganglion and moves caudal along the midline of the thyropharyngeus. The RLNp was identified as described above.

Recording techniques. ELECTROMYOGRAPHY. Bipolar Teflon-coated stainless steel wires (AS 632, Cooner Wire, Chatsworth, CA), bared for 2–3 mm, were placed in each muscle and fed into differential amplifiers (model 1800, A-M Systems). The threshold for identification of an EMG response of all muscles except the CP was a doubling of the voltage; for the CP, the threshold was a 25% change in response. In all cases, the response must have been temporally correlated with the stimulus. The electrical activity was filtered (band pass = 0.1–3.0 kHz) and amplified 1,000–10,000 times before it was fed into the computer.

Fig. 1. Innervation of the esophagus by the vagus nerve (X), recurrent laryngeal nerve (RLN), superior laryngeal nerve (SLN), and pharyngoesophageal nerve (PEN). The RLNp, the most proximal 1–2 cm of the RLN, joins the SLN under the cricoid cartilage. NG, nodose ganglion.
response temporally associated with the stimulus, and not respiration. Inspiration caused a negative pressure artifact, and expiration caused minimal changes in recorded esophageal pressure. Blood pressure was recorded using a Statham pressure transducer. The output signals from these devices were attached to low-level direct-coupled preamplifiers (model P122, Grass) set at 3-Hz high-frequency-cutoff filtration. The manometric signals were stored on a computer.

**Stimulation techniques.** STIMULATION OF SWALLOWING. Water was injected into the nasopharynx at volumes of 1–5 ml at 1 ml/s. We waited ≥2 min between swallows. Each animal received at least two stimulations of swallowing at each volume before and after the RLNP was cut.

**Electrical stimulation of nerves.** Each nerve was placed on a bipolar electrode and stimulated at 1–5 V for 0.2 ms at 10–30 Hz for 3–10 s using a Grass S88 stimulator.

**Data reduction and analysis.** The mean and SE of the percent occurrences and time delays between the beginning of CP inhibition and the beginning of the responses of the other muscles to stimulation with 1–5 ml of water were quantified. Using Spearman’s correlation coefficient, we tested whether there was a relationship between the volume of fluid injected and the quantified variables. The delays of the motor responses of the pharyngeal and esophageal phases of swallowing were quantified from the beginning of CP inhibition, because the timing of this event was more accurately measured than other muscle responses. Timing of the occurrence for the other muscles was taken from the beginning of the EMG or manometric response. We tested whether the delays among responses of various muscles varied differently by comparing the coefficients of variation of the responses of GH, TH, CT, and E1-E using ANOVA with Tukey’s post hoc test comparing all muscles with each other. Because in this analysis we compared different muscles with each other, we decided to use only EMG data to minimize any variability caused by comparing responses using different techniques, i.e., EMG vs. manometry. In addition, EMG is inherently a more accurate muscle-recording technique for the analysis of timing characteristics of muscle responses. The effect of RLNP transection on the occurrence of esophageal peristalsis was tested using Fisher’s exact test. Differences in mean values due to RLNP transection were tested using Student’s t-test. In all cases, \( P < 0.05 \) was considered statistically significant.

### RESULTS

**Determination of the Relationship Between Bolus Size and Temporal Measures of the Esophageal Phase of Swallowing**

Incidence of the esophageal phase of swallowing is related to peripheral feedback. We found that injection of water into the nasopharynx stimulated the pharyngeal and esophageal phases of swallowing (Fig. 2). While the responses of the hyoid, laryngeal, and pharyngeal muscles during pharyngeal swallow occurred 100% of the time, regardless of the volume of the stimulus, the incidence (%occurrence) of the esophageal phase as measured by the EMG electrode was significantly \( (P < 0.05, \text{Spearman’s correlation coefficient}) \) related to the volume injected (Fig. 3). Moreover, the incidence of the esophageal phase at each level of the esophagus, measured by manometry, was significantly \( (P < 0.05, \text{Spearman’s correlation coefficient}) \) related to the volume of the stimulus (Fig. 3).

**Initiation of the esophageal phase of swallowing is significantly more variable than the pharyngeal phase.** The delays of muscle responses within the pharyngeal phase, i.e., between the beginning of CP EMG inhibition and the beginning of the EMG responses of the GH, TH, and CT and between the pharyngeal and esophageal phases of swallowing, i.e., between the beginning of CP EMG inhibition and the beginning of esophageal manometric responses at 1, 4, 7, and 10 cm and the beginning of the esophageal EMG response at E1, were not related to the volume of water injected (Fig. 3).

We observed that the delays to components of the esophageal phase seemed to be more variable than delays to components of the pharyngeal phase (Fig. 3); therefore, we examined this relationship statistically using coefficient of variation. Because there was no significant effect of volume of the stimulus on the delays of the muscle responses, we grouped measures of delays of all volumes within a muscle to increase \( n \), thereby decreasing the chance of statistical errors and increasing the reliability of the statistical results. We found that

Fig. 2. Variability of initiation of the esophageal phase of swallowing. A–C: effects of water injected into the nasopharynx on pharyngeal and esophageal phases of swallowing. Note large difference in delays to initiation of the esophageal phase after injection of 1 ml (A) vs. 4 ml (B) of water. Injection of 1 ml of water always activated the pharyngeal phase, but did not always activate the esophageal phase (C). Therefore, if initiation of the esophageal phase of swallowing was independent of peripheral feedback, then the high variability of occurrence, even to the point of lack of activation of the esophageal phase, would not occur. GH, geniohyoideus; TH, thyrohyoideus; CP, cricopharyngeus; CT, cricothyroideus; E1-E, EMG activity of the esophagus at 1 cm from the CP; E1-M and E4-M, motility of the esophagus at 1 and 4 cm from the CP, respectively.
the variability of the delays within the pharyngeal phase, i.e., the delay between CP EMG inhibition and the beginning of EMG responses of the GH, TH, and CT, compared with the delay between the pharyngeal and esophageal phases, i.e., between CP EMG inhibition and the beginning of the esophageal EMG response at 1 cm from the CP. For EMG recordings, while percent occurrences of motor responses of the pharyngeal phase occurred 100% of the time at all doses, response of the esophageal phase was related to the volume injected ($P < 0.05$, Spearman correlation coefficient), and sometimes no response occurred at lower volumes. Delays to responses were not related to the volume injected for any muscle. There were too few values obtained at 1 ml to quantify delays to E1. In bottom left, y-axes represent pharyngeal (left) and esophageal (right) phase responses. For manometric recordings, while percent occurrences of the esophageal phase at each level of the esophagus were related to the volume injected ($P < 0.05$, Spearman correlation coefficient), delays to activation were not. There were too few values obtained at 1 ml to quantify delays at all levels of the esophagus. In summary, timing of pharyngeal and esophageal phases and occurrence of the pharyngeal phase were not related to the swallow stimulus, but occurrence of the esophageal phase was related to the swallow stimulus. Therefore, while timing between the pharyngeal and esophageal phases of swallowing was not dependent on peripheral feedback, initiation of the esophageal phase was related to peripheral feedback. Values are means ± SE.

### Table 1. Variability of delays between pharyngeal and esophageal phases and within the pharyngeal phase of swallowing

<table>
<thead>
<tr>
<th>EMG Responses</th>
<th>GH</th>
<th>TH</th>
<th>CT</th>
<th>E1-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of variation</td>
<td>10.1 ± 2.0*</td>
<td>11.6 ± 1.8*</td>
<td>11.8 ± 1.6*</td>
<td>33.1 ± 7.7</td>
</tr>
</tbody>
</table>

Values (means ± SE) represent the coefficient of variation of time delay between cricopharyngeus (CP) electromyographic (EMG) activity inhibition and the beginning of the response of the CP muscle. *$P < 0.05$ (by ANOVA and Tukey’s test) vs. esophagus at 1 cm from CP using EMG electrodes (E1-E). No other comparisons differed significantly. Note that the coefficient of variation of delays between the pharyngeal phase and initiation of the esophageal phase, i.e., E1-E, was significantly greater than that of delays between initiation of muscles within the pharyngeal phase of swallowing, i.e., geniohyoideus (GH), thyrohyoideus (TH), and cricothyroideus (CT). If control of the esophageal phase of swallowing was similar to that of the pharyngeal phase of swallowing, one would not expect initiation of the esophageal phase to be ~3 times more variable than initiation of the pharyngeal phase.

### Role of Sensory Innervation of the Proximal Cervical Esophagus on Initiation of the Esophageal Phase of Swallowing

We found that RLNp transection eliminated or significantly reduced the incidence of the esophageal phase of swallowing at 1, 4, and 7 cm from the CP, depending on the volume of water used to activate the pharyngeal swallow (Fig. 4, Table 2). Because we found that the delays from CP EMG inhibition of the muscle responses in the pharyngeal and esophageal phases of swallowing were not related to the volume of the stimulus, we grouped responses of all volumes together for each muscle to quantify the effects of RLNp transection on these responses. We found that RLNp transection had no effect on the delays from CP EMG inhibition to the beginning of the muscle responses (Fig. 5) recorded with EMG or manometry.

### Effects of Peripheral Stimulation of Various Cervical Nerves on Esophageal Motility

We found that stimulation of the SLN (3 of 3 animals) toward the esophagus had no effect on esophageal motility (Fig. 6, Table 3), but stimulation of the PEN (3 of 3 animals) increased tone at 1 and 4 cm from the CP (Fig. 6, Table 3), and stimulation of the X (6 of 6 animals) increased esophageal tone at 7 and 10 cm from the CP (Fig. 6, Table 3). Stimulation of the PEN (1 of 3 animals) increased tone at 7 cm from the CP and
stimulation of the X (5 of 6 animals) increased tone at 4 cm from the CP (Table 3).

Effects of Centripetal Electrical Stimulation of the SLN Afferents on Esophageal Motility

We found that stimulation of the SLN (5 of 5 animals) toward the brain initiated esophageal peristalsis at 1 and 4 cm from the CP (Fig. 7, Table 3). SLN stimulation, at the parameters used, also activated the pharyngeal swallow (2 of 5 animals), but initiation of the esophageal phase of swallowing always (2 of 2 animals) occurred before initiation of the pharyngeal swallow. When initiation of swallowing occurred, it inhibited the ongoing esophageal peristalsis (Fig. 7). We found that stimulation of the RLNp toward the brain (7 of 7 animals) had no effect on proximal cervical esophageal motility (Fig. 7, Table 3).

DISCUSSION

The role of a central program in control of the timing sequence of activation of the esophageal striated muscle during the esophageal phase of swallowing is well accepted (14, 16). However, the mechanism of initiation of the esophageal phase of swallowing has been disputed for decades. Some studies (12, 13) suggest that the esophageal phase is initiated by a central pathway from the central program controlling the pharyngeal phase, whereas others (10, 11, 19, 20) suggest that the

Table 2. Contingency table analysis of effects of RLNp transection on occurrence of the esophageal phase of swallowing

<table>
<thead>
<tr>
<th>Volume of Water Injected, ml</th>
<th>1 cm</th>
<th>4 cm</th>
<th>7 cm</th>
<th>10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>2</td>
<td>5/3</td>
<td>0/8*</td>
<td>9/1</td>
<td>1/9*</td>
</tr>
<tr>
<td>3</td>
<td>6/2</td>
<td>0/8*</td>
<td>10/0</td>
<td>2/8*</td>
</tr>
<tr>
<td>4</td>
<td>7/1</td>
<td>0/8*</td>
<td>10/0</td>
<td>2/8*</td>
</tr>
<tr>
<td>5</td>
<td>8/0</td>
<td>2/6*</td>
<td>10/0</td>
<td>2/8*</td>
</tr>
</tbody>
</table>

Values represent numbers of animals where the stimulus was a success/failure at the location in the esophagus, i.e., 1, 4, 7, or 10 cm from the CP, before and after transection of the most proximal 1–2 cm of the recurrent laryngeal nerve (RLNp). *P < 0.05 (by Fisher’s exact test) vs. Before at each esophageal location and each volume. RLNp transection blocked or significantly reduced initiation of the esophageal phase of swallowing at 1, 4, and 7 cm from the CP. Therefore, the RLNp transmits information essential to initiation of the esophageal phase of swallowing.
esophageal phase is initiated by feedback from peripheral reflexes. Our studies have addressed this issue by observing the normal operation of the phases of swallowing and by examining the effects of eliminating the sensory innervation of the esophagus.

We found that initiation of the esophageal phase of swallowing is more labile in the more proximal regions of the esophagus. If initiation of the esophageal phase of swallowing is closely linked to initiation of the pharyngeal phase by a central neural pathway (16), then one would not expect initiation of the esophageal phase to be so labile. On the other hand, the percent occurrences of the responses of the proximal cervical esophagus were related to the bolus volume, suggesting that the link between the pharyngeal and esophageal phases of swallowing is due to peripheral reflexes. Therefore, both of these findings suggest that the link between the pharyngeal and esophageal phases of swallowing is dependent on peripheral reflexes, and not a central program.

We then found that the delay between the pharyngeal and esophageal phases of swallowing was significantly more variable than the delays within the pharyngeal phase. The primary role of a central pattern generator is to ensure a relatively constant delay between muscles of a complex function to ensure proper operation of that function, regardless of external conditions. The fact that the variabilities of the delays between all recorded muscle responses within the pharyngeal phase of swallowing were small and similar reflects the concept that initiation of these muscle responses was controlled by the same well-coordinated central program. In contrast, the high variability of the delay between the pharyngeal and esophageal phases of swallowing strongly indicates that the link between the pharyngeal and esophageal phases is subject to nonpreprogrammed factors, e.g., peripheral feedback.

Other studies have found that peripheral feedback is essential for peripheral expression of the esophageal phase of swallowing. That is, in the decerebrate cat (19), diversion of the bolus during a pharyngeal swallow, such that it never touched any part of the esophagus, completely prevented activation of the esophageal phase of swallowing. Other studies of bolus diversion in dogs and monkeys have been more complex, as

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Fig. 5. Lack of effect of RLNp transection on delays within and between phases of swallowing recorded using EMG. A: delays within the pharyngeal phase and between the pharyngeal and the esophageal phase recorded by EMG. B: delays between the pharyngeal and the esophageal phase of swallowing, where the esophageal phase was recorded by manometry. RLNp transection had no effect on these delays to any muscle within the pharyngeal phase or between the pharyngeal and the esophageal phase of swallowing. Therefore, information transmitted in the RLNp does not control timing within the pharyngeal phase or between the pharyngeal and the esophageal phase of swallowing. B and A, before and after RLNp transection, respectively. Values are means ± SE.
none had diverted the bolus from the entire esophagus. In two dog studies, investigators found that when they diverted the bolus from the upper (20) or mid (11) cervical esophagus, initiation of the esophageal phase of swallowing was totally (20) or almost totally (0.9% occurrence rate) (11) blocked. On the other hand, in monkeys (10), bolus diversion 2 cm below the esophagopharyngeal junction did not block the esophageal phase of swallowing. The results of studies in all three animal species, i.e., cat (19), dog (11, 20), and monkey (10), indicate that bolus activation of the esophagus is necessary for initiation of the esophageal phase of swallowing. Some species, i.e., dogs, may require more esophageal feedback than others, i.e., monkeys, but the esophageal phase is not initiated unless at least some part of the esophagus is stimulated by the bolus.

Although there have been no bolus diversion studies in humans, there are data in humans that support the conclusion gleaned from the bolus diversion studies described above. It has been found that the esophageal phase of swallowing is initiated in 96% of wet swallows (5, 9), but in only 62% (5) or 71% (9) of dry swallows. These data demonstrate that initiation of the esophageal phase of swallowing in humans depends on the nature of the bolus. If the central programs controlling the pharyngeal and esophageal phases of swallowing are linked by a central neural connection, one would expect that the esophageal phase would be initiated in 100% of swallows and that initiation of the esophageal phase would not be related to the bolus, but neither expectation occurs. Therefore, studies in humans also support the conclusion that the esophageal phase of swallowing is initiated by peripheral reflexes, and not a central program.

An argument against the conclusion above might be that since a dry swallow activated esophageal peristalsis, a bolus is not necessary for activation of the esophageal phase of swallowing. However, a dry swallow is not absent a bolus, as it contains saliva and air. We found in our prior study (19) that diverting the bolus of a dry swallow also blocked the esophageal phase of swallowing. Therefore, the bolus of a dry swallow is sufficient to stimulate esophageal receptors that activate the esophageal phase of swallowing, and studies of dry swallows cannot be used to exclude the essential role of sensory feedback from the esophagus in initiation of the esophageal phase of swallowing.

Table 3. Effect of nerve stimulation on esophageal motility

<table>
<thead>
<tr>
<th>Location in Esophagus</th>
<th>E1-M</th>
<th>E4</th>
<th>E7</th>
<th>E10</th>
</tr>
</thead>
<tbody>
<tr>
<td>X to esophagus</td>
<td>0/6</td>
<td>5/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>SLN to esophagus</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>PEN to esophagus</td>
<td>3/3</td>
<td>3/3</td>
<td>1/3</td>
<td>0/3</td>
</tr>
<tr>
<td>SLN to brain</td>
<td>5/5</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>RLNP to brain</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
</tbody>
</table>

Values represent numbers of animals in which a motility response was observed divided by numbers of animals tested at each esophageal location and each nerve stimulated. Locations of manometric responses were 1 (E1-M), 4 (E4), 7 (E7), and 10 (E10) cm from the CP. The vagus nerve (X) and pharyngoesophageal nerve (PEN) are motor nerves for the esophagus, the superior laryngeal nerve (SLN) is not. Therefore, since the RLNP is a branch of the SLN and the SLN provides no motor innervation to the esophagus, transection of the RLNP cannot reduce or eliminate motor innervation to the esophagus. Thus effects of transection of the RLNP (see Table 2) must be due to elimination of esophageal afferent information. Also, SLN, but not RLNP, stimulation to the brain activated esophageal peristalsis, before activating the pharyngeal phase of swallowing, in the proximal cervical esophagus. Therefore, afferent stimulation of the SLN can activate the cervical component of the esophageal phase of swallowing independent of the pharyngeal phase.
Perhaps our most significant finding was that transection of the RLNp blocked or significantly inhibited initiation of the esophageal phase of swallowing. Considering that we and others (29) have shown that the RLNp is purely sensory for the esophagus, this observation provides strong evidence for the essential role of peripheral reflexes, rather than a central program, in initiation of the esophageal phase of swallowing.

This effect of RLNp transection was more pronounced in more orad portions of the esophagus, reflecting the innervation pattern of the RLNp (30). Similarly, this effect was less pronounced with higher volumes of water, which probably reflects the ability of higher volumes of water to affect areas outside the innervation pattern of the RLNp, i.e., areas innervated by the X.

The concept that initiation of the esophageal phase of swallowing is generated by a central program is based on previous findings (12–16) that SLN stimulation in paralyzed animals activated premotor neurons associated with the pharyngeal, as well as esophageal, phase of swallowing. Given that no esophageal contractions could occur to stimulate afferent feedback, it was concluded that feedback from peripheral reflexes was not the initiator of the esophageal phase of swallowing. However, the SLN contains afferents from the esophagus (17, 21, 26, 30); therefore, electrical stimulation of the SLN would stimulate peripheral feedback from the esophagus. In addition, activation of premotor neurons does not guarantee activation of motor neurons or muscles. The esophageal premotor neuronal activity initiated by SLN stimulation was quite minimal, amounting to only a handful of action potentials (12); therefore, activation of esophageal peristalsis was unlikely. Moreover, in the current study we found that electrical stimulation of the SLN can activate the esophageal phase of swallowing independent of the pharyngeal phase. Therefore, while the central program that governs the esophageal phase of swallowing may be active in paralyzed animals after SLN stimulation, the esophageal phase of swallowing is not necessarily initiated by this central program.

The brain stem studies described above actually strongly support the conclusion that initiation of the esophageal phase of swallowing is dependent on feedback from peripheral reflexes. The finding that sensory feedback from the esophagus greatly enhanced the activation of esophageal phase premotor neurons (12–16) makes this activity much more likely to activate motor neurons and the esophageal phase of swallowing. Therefore, while the central program of the pharyngeal phase may activate the esophageal premotor neurons, this activation alone may not be sufficient to cause peripheral expression of the esophageal phase of swallowing.

![Fig. 7. Effects of centripetal stimulation of the SLN and RLNp toward the brain on esophageal motility. Stimulation of the SLN toward the brain activated esophageal peristalsis at 1 and 4 cm from the CP in 5 of 5 animals (see Table 2) prior to activation of swallowing. Esophageal pressure increases were inhibited when the pharyngeal swallow was activated (vertical arrow). Stimulation of the RLNp toward the brain had no effect on esophageal motility in 7 of 7 animals (see Table 2). Therefore, electrical stimulation of afferents in the SLN, but not the RLNp, initiates the esophageal phase of swallowing. Thus, in order for afferents of the RLNp to initiate the esophageal phase of swallowing, activation of pharyngeal/laryngeal afferents of the SLN may also be required. Electrical stimulation caused large noise artifacts on the EMG electrodes; therefore, no conclusions were drawn from these responses.](http://ajpgi.physiology.org/)

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Esophageal motor neurons have been recorded during swallowing activated by SLN stimulation (13), but in these studies peripheral feedback was not prevented, as the animals were not paralyzed. Therefore, these studies do not exclude the possibility that this esophageal motor neuron activity was initiated by peripheral feedback, rather than a central pathway from the pharyngeal phase central pattern generator.

Although our studies do not support the concept that a central program initiates the esophageal phase of swallowing, they do support the concept that the central program of the esophageal phase controls the timing sequence of esophageal peristalsis. That is, we found that the delays between peristaltic responses were not dependent on peripheral feedback and must have been preprogrammed.

Our studies of the effects of electrical stimulation of afferent fibers may have revealed a new concept in the mechanism of initiation of the esophageal phase of swallowing. We found that while transection of the RLNp blocked initiation of the esophageal phase in the esophagus, centripetal electrical stimulation of the RLNp did not activate the esophageal phase of swallowing. On the other hand, centripetal electrical stimulation of the SLN, which carries afferent pathways from receptors in the esophagus, larynx, and pharynx, activated the esophageal phase of swallowing in the proximal cervical esophagus independent of the pharyngeal phase. Similar results were obtained in studies using anesthetized sheep (26). Therefore, while initiation of the esophageal phase of swallowing in the proximal cervical esophagus requires direct stimulation of the esophagus, this response also depends on afferent signaling from the pharynx or larynx.

The necessity of activating the pharynx or larynx during swallowing for the esophageal phase of swallowing to occur has significant physiological advantages. Secondary peristalsis, no matter where it is initiated, always occurs orad of the initiating site (2, 3, 23, 27), which prevents retrograde flow. If the initiating site were in the proximal cervical esophagus, there would be no room for orad activation within the esophagus. Therefore, the esophageal phase would occur at the site of the bolus and exert a high bolus pressure in the orad, as well as caudal, direction, which might overwhelm the antireflux barrier of the upper esophageal sphincter. Therefore, a bolus in the proximal cervical esophagus is best cleared by a swallow, and not secondary peristalsis.

Not only is a bolus in the proximal cervical esophagus best cleared by activation of a swallow, rather than secondary peristalsis, prior studies in animals (17) and humans (6) found that the proximal cervical esophagus is insensitive to activation of secondary peristalsis. Therefore, while peripheral feedback is necessary to activate the esophageal phase of swallowing, this can only occur if pharyngeal or laryngeal stimulation also occurred, as during swallowing. While stimulation of the pharynx or larynx is important for allowing esophageal stimulation to initiate the esophageal phase of swallowing, no studies have reported that stimulation of the larynx or pharynx can initiate the esophageal phase of swallowing. In fact, the opposite is true. Stimulation of the pharynx has been shown to inhibit progression of the esophageal phase (18, 28), and the esophageal phase is blocked during rapid swallowing in the process of deglutitive inhibition (29). Therefore, we hypothesize that the role of laryngeal or pharyngeal afferents during swallowing is not to initiate the esophageal phase but, rather, to allow esophageal stimulation to initiate the esophageal phase. Further studies are needed to confirm this hypothesis.

Numerous esophageal motility disorders have been classified in humans (1), and some include various forms of failed peristalsis associated with swallowing. It is possible that defects in the afferent innervation of the proximal cervical esophagus mediated by the RLNp may account for some of these abnormalities, and further studies are needed to examine this issue.

In summary (Fig. 8), multiple studies in humans and animals indicate that the peripheral reflexes through the bolus significantly affect initiation of the esophageal phase of swallowing. The occurrence of the esophageal phase during swallowing is related to the type and size of the bolus. Not only is the esophageal phase dependent on the bolus, it can be activated independent of the pharyngeal phase. Timing of initiation of the esophageal phase is much more variable than timing of initiation of muscle responses within the pharyngeal phase. Most importantly, however, our current and prior studies have found that activation of peripheral reflexes is essential for initiation of the esophageal phase of swallowing. The only studies used to conclude that the esophageal phase of swallowing is initiated by a central program more closely support the conclusion that the esophageal phase of swallowing is initiated by activation of peripheral reflexes, rather than a central program.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
AUTHOR CONTRIBUTIONS

I.M.L. is responsible for conception and design of the research; I.M.L. and B.K.M. performed the experiments; I.M.L. and A.B. analyzed the data; I.M.L. and A.B. interpreted the results of the experiments; I.M.L. prepared the figures; I.M.L. drafted the manuscript; I.M.L., A.B., and R.S. edited and revised the manuscript; I.M.L., B.K.M., A.B., and R.S. approved the final version of the manuscript.

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