Modulation of Laryngeal Responses to Superior Laryngeal Nerve Stimulation by Volitional Swallowing in Awake Humans

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Barkmeier, Julie M., Steve Bielamowicz, Naoya Takeda, and Christy L. Ludlow. Modulation of laryngeal responses to superior laryngeal nerve stimulation by volitional swallowing in awake humans. J. Neurophysiol. 83: 1264–1272, 2000. Laryngeal sensorimotor closure reflexes are important for the protection of the airway and prevent the entry of foreign substances into the trachea and lungs. The purpose of this study was to determine how such reflexes might be modulated during volitional swallowing in awake humans, when persons are at risk of entry of food or liquids into the airway. The frequency and the amplitude of laryngeal adductor responses evoked by electrical stimulation of the internal branch of the superior laryngeal nerve (ISLN) were studied during different phases of volitional swallowing. Subjects swallowed water on command while electrical stimuli were presented to the ISLN at various intervals from 500 ms to 5 s following the command. Laryngeal adductor responses to unilateral ISLN stimulation were recorded bilaterally in the thyroarytenoid muscles using hooked wire electrodes. Early ipsilateral R1 responses occurred at 17 ms, and later bilateral R2 began around 65 ms. The muscle responses to stimuli occurring during expiration without swallowing were quantified as control trials. Responses to stimulation presented before swallowing, during the swallow, within 3 s after swallowing, and between 3 and 5 s after a swallow were measured. The frequency and amplitude of three responses (ipsilateral R1 and bilateral R2) relative to the control responses were compared across the different phases relative to the occurrence of swallowing. Results demonstrated that a reduction occurred in both the frequency and amplitude of the later bilateral R2 laryngeal responses to electrical stimulation for up to 3 s after swallowing (P = 0.005). The amplitude and frequency of ipsilateral R1 laryngeal responses, however, did not show a significant main effect following the swallow (P = 0.28), although there was a significant time by measure interaction (P = 0.006) related to reduced R1 response amplitude up to 3 s after swallowing (P = 0.021). Therefore, the more rapid and shorter unilateral R1 responses continued to provide some, albeit reduced, laryngeal protective functions after swallowing, whereas the later bilateral R2 responses were suppressed both in occurrence and amplitude for up to 3 s after swallowing. The results suggest that R2 laryngeal adductor responses are suppressed following swallowing when residues may remain in the laryngeal vestibule putting persons at increased risk for the entry of foreign substances into the airway.

INTRODUCTION

The pharyngeal phase of swallowing is largely under brain stem control and protects the airway during swallowing. This phase elevates the larynx beneath the tongue base and closes the larynx at three levels, including the true vocal folds, the ventricular folds, and the aryepiglottic folds. Additional protection of the airway is offered through gag and cough reflexes when substances inadvertently enter the larynx and/or airway (Karlsson et al. 1988; Widdicombe 1980, 1995). These protective reflexes are induced by stimulation of afferents contained in the internal branch of the superior laryngeal nerve. Laryngeal closure responses to afferent stimulation can be studied by electrically stimulating the internal branch of the superior laryngeal nerve (ISLN) in both animals and humans (Ludlow et al. 1992; Sasaki and Suzuki 1976). In humans, thyroarytenoid (TA) muscle responses occur after stimulation of the ISLN. These include both an early ipsilateral TA response, R1, which begins ~16–20 s after the stimulus, and later R2 responses that begin between 60 and 70 s after the stimulus simultaneously on both sides of the larynx (Ludlow et al. 1992). These R1 and R2 responses were unrelated in their amplitude and frequency with increased intensity of ISLN stimulation (Yamashita et al. 1997). This suggests that, although both responses are elicited by electrical stimulation of the ISLN, they may involve different neural reflex pathways and/or mechanisms of control.

The later bilateral R2 responses are affected by conditioning to a greater degree than R1 responses. During paired ISLN stimulation, the first electrical stimulus serves as a conditioning stimulus and will modify responses to the second stimulus, the test stimulus (Ludlow et al. 1995). The amount of response suppression induced by a conditioning stimulus can be quantified when responses to the test stimulus are reduced. The period of suppression following the initial presentation of a stimulus can be determined by altering the interval between the conditioning and test stimuli. In humans, notable R2 suppression occurred with stimulus pairs at interstimulus intervals of <1 s (Ludlow et al. 1995). That is, R2 responses appeared reduced in frequency and amplitude using the conditioning paradigm. In cats, afferent responses become suppressed with an interstimulus interval <400 ms (Sessle 1973a,b). This finding was related to presynaptic suppression of neurons in the tractus solitarius in the medulla. Others (Sessle and Storey 1972) also studied the effects of mechanical stimulation to the periodontal and perioral mucosa on superior laryngeal nerve (SLN) primary afferent excitation in the nucleus tractus solitarius (NTS). They demonstrated that trigeminal afferent excitation from the periodontal and perioral mucosal areas resulted in presynaptic suppression of electrically stimulated ISLN afferent signals to the NTS. These findings suggest that excitation of laryngeal sensorimotor patterns may be suppressed during mastication to lessen gag and cough reflexes triggered in the oropharynx by mechanoreceptors. These laryngeal adductor reflexes may become uncontrolled resulting in laryngo-
spasm under certain conditions such as prolonged and intense laryngeal stimulation. The laryngeal adductor response may also relate to the laryngeal component of protective mechanisms involved in coughing (Gestreau et al. 1997).

Laryngeal sensorimotor responses may normally protect the airway from the entry of materials that could interfere with air exchange. During swallowing, substances pass over and around the laryngeal structures as a necessary sequence of nutritional intake (Logemann et al. 1992). However, protective responses such as coughing are not normally elicited during swallowing, although mechano- and chemosensory stimuli are present, which could otherwise elicit these responses. This may be related to either the laryngeal mechanoreceptors being slowly adapting or to central modulation of the laryngeal adductor response pathway. Studies of the laryngeal mechanoreceptor responses when recorded from the SLN (Davis and Nail 1987) or presynaptically in the NTS (Esaki et al. 1997) suggest that a significant proportion are rapidly adapting.

The laryngeal adductor response pathway in the cat involves the afferent neurons located in the nodose ganglion, the NTS, the lateral tegmental field of the reticular system, and the nucleus ambiguus (Gestreau et al. 1997; Tanaka et al. 1995, 1996). The R2 pathway is relatively unknown, however, because it rarely occurs in animals under anesthesia (Sasaki and Suzuki 1976). Occasional late R2 responses were observed on the side contralateral to stimulation in the cat and may have disappeared following sectioning at the level of the superior colliculus (Mochida 1990).

The purpose of this study was to evaluate the possibility of central suppression of laryngeal adductor responses during swallowing. The possible roles of adaptive changes in the laryngeal mechanoreceptors were bypassed by stimulating the ISLN electrically. This allowed us to study the central modulation of the laryngeal reflex responses during different phases of swallowing in awake human subjects.

**METHODS**

**Subjects**

The present investigation studied laryngeal adductor reflex responses relative to the onset and offset of the swallow in four male and five female awake healthy volunteers between the ages of 25 and 57 yr (mean = 41 yr). The subjects were administered informed consent to participate in a protocol approved by the Internal Review Board of the National Institute of Neurological Disorders and Stroke and then examined by an otolaryngologist and found to have normal laryngeal structure and function.

Subjects also completed a medical history questionnaire and a physical examination. Only subjects with a normal larynx and voicing patterns, and without a history of swallowing or neurological abnormalities, were included for study. All subjects had been cautioned regarding the use of anticoagulants 24 h before onset of the study.

**Electrode placement and recording procedures**

Electrode recordings were obtained from the TA, thyrohyoid, mylohyoid complex, and the superior constrictor muscles for the purpose of determining the onset and offset of the pharyngeal swallow (Perlman et al. 1989, 1999). Electrodes were placed into the ipsilateral cricothyroid muscle, which is innervated by the external branch of the SLN. Our purpose was to stimulate only the ISLN and not the external branch that innervates the cricothyroid muscle. When cricothyroid muscle responses occurred around 2 ms, the stimulating electrodes were readjusted so that only a reflex response was observed in the TA muscles. Signals recorded from the TA muscles bilaterally were used to measure reflex laryngeal muscle responses to electrical stimulation of the ISLN (Ludlow et al. 1992).

Before electromyographic (EMG) recordings, 2-V peak-to-peak square-wave calibration signals were recorded for each EMG channel on a TEAC multiple channel VHS FM data recorder. Subjects were electrically grounded and placed in a supine position with the neck extended. To reduce discomfort during electrode insertion over the sites of electrode placement, 2% lidocaine hydrochloride (Xylocaine) with epinephrine (1:100,000) was injected subcutaneously over the cricothyroid membrane.

A bipolar needle electrode (27 gauge) was used to locate each of the laryngeal and extralaryngeal muscles following standard technique (Hirano and Ohala 1969) before placement of bipolar hooked wire electrodes. Increased activation during sustained phonation and effort closure verified TA muscle electrode placement. The electrode was moved to an anterior and superior position when activation of the muscle was characterized by onset and offset bursts for phonation indicating sampling from the lateral cricoarytenoid muscle. A gradual increase and decrease in muscle activation as voice pitch increased and decreased during a pitch glide task verified cricothyroid muscle placement. The cricothyroid electrode was readjusted if muscle activation increased when the subject actively flexed their neck and continued breathing, which indicated placement in a strap muscle. Thyrohyoid muscle recording verification included increased muscle activation during neck flexion and swallowing. Mylohyoid complex recordings were verified by increased activation during tongue extrusion and at swallow onset (Perlman et al. 1999; Schultz et al. 1994). Correct placement into the superior constrictor muscle was verified by increased activation at the onset of a swallow (Perlman et al. 1989).

Once satisfactory activation patterns were obtained using the bipolar needle electrode probe for a muscle, bipolar insulated hooked wire electrodes (each bared for 1 mm at the tip) were placed and verified using a 27-gauge, 30-mm carrier needle.

Two monopolar needle electrodes for electrical stimulation were inserted superficial to the thyrohyoid membrane on either side of the right ISLN. The rostral anode electrode and the caudal cathode electrode were usually 0.5–1.5 cm apart and 1–3.5 cm in depth, depending on a subject’s neck anatomy. A DISA 15E07 current stimulator was used to deliver single 1-ms rectangular current pulses. The optimal location was determined where minimal electrical stimulation to the ISLN (1–3 mA) resulted in maximum R1 muscle responses in the ipsilateral TA. Then stimulating needle electrodes were replaced with bipolar hooked wire electrodes similar to those used for the muscle recordings. A third hooked wire ground electrode was placed in subcutaneous tissue between the point of ISLN stimulation and the EMG recording electrodes close to the midline of the superior border of the thyroid cartilage to reduce the stimulation artifact in the EMG recordings during nerve stimulation.

The current level was started at zero and increased until the R1 response in the ipsilateral TA muscle reached a supramaximal level. Six baseline trials of ISLN stimulation were presented at rest during expiration and separated by at least 20 s between trials. Another trial was presented 30 s later if a subject swallowed during a baseline trial. In a few cases, the highest stimulation level that could be tolerated by the subject below 5 mA was selected if supramaximal levels were not clearly attained. The stimulus range was between 3 and 5 mA and remained the same throughout the study.

The R1 muscle response amplitude was checked periodically at least 30 s after a swallow to determine whether the R1 response was similar to baseline levels or had changed, which would indicate movement of the stimulating hooked wire electrodes. This occurred in a few instances in some subjects. In those instances, a new set of baseline tokens of ISLN stimulation without swallowing were pre-
sent and used as control baseline trials for subsequent experimental trials. This new set of baseline trials was used during the signal processing and analysis stage.

All EMG channels were band-pass filtered between 100 and 5,000 Hz, amplified, and recorded along with the stimulus trigger on a TEAC multiple channel FM tape recorder. Respiratory movements were transduced using Respirtrace bands placed on the chest and abdomen and their summation recorded along with the EMG signals. Thyroid cartilage movement was monitored using a piezoelectric movement transducer placed on the overlying skin to indicate the pharyngeal phase of the swallow (Sedory-Holzer and Ludlow 1996). Heart rate was monitored with a three-lead electrocardiogram (EKG), as a safety measure, throughout the study.

A 2-ml bolus of water via syringe was placed in the subject’s mouth in the supine position before the command to swallow. The subject held the water in their mouth until a cue was given for them to swallow. Delays in the time of presentation of the ISLN stimulus relative to the command to swallow were programmed with a Master 8-pulse generator. The pulse generator triggered the DISA current stimulator at 0, 500-ms, 1-s, 2-s, and 5-s delays after the swallow command. Two of the nine subjects underwent the reverse order in time delays between the command and the ISLN stimulus. The ISLN stimulation delays were selected to occur at different approximate time periods after the command to swallow: at 500 ms occurring just before the onset of the swallow; at 1 s, occurring during the swallow; at 2 s, occurring within the first 3 s postswallow; and at 5 s, occurring between 3 and 5 s postswallow.

Sets of six trials each were presented at each of the five stimulation delays (0, 500 ms, 1 s, 2 s, and 5 s) in addition to baseline/control sets at the beginning, middle, and end of the study. The muscle responses during the baseline/control trials were used for comparison with responses elicited during the experimental swallowing trials. A new set of baseline/controls were conducted at the same stimulus level or amplitude, beginning around 65 ms (Fig. 1).

Sets of six trials each were presented at each of the five stimulation delays (0, 500 ms, 1 s, 2 s, and 5 s) with baseline/control sets at the middle and end of the upright study.

Data analysis

The ISLN stimulation trigger, EMG recordings, respiratory, piezoelectric, and calibration signals were digitized off-line at 5,000 samples per second with anti-aliasing low-pass filtering at 2,000 Hz. The respiratory signal was sampled at 500 Hz with anti-aliasing at 100 Hz. Linear interpolation from the calibration signals was used to convert the EMG signals into microvolts. Nonrectified data were averaged for each EMG channel over 10 s of quiet respiration and the mean value subtracted to correct each EMG channel for DC offset. The signals were corrected for DC offset, rectified, and the minimum activation level between motor-unit firings during quiet respiration was subtracted for the EMG channel to correct for differences in electrode impedance. This final correction was usually <1 μV.

Visual inspection identified muscle responses in the ipsilateral and contralateral TA EMG signals within 200 ms after each trigger. The trigger pulse was used for synchronized display of each muscle response following the stimulus trigger to assist in response identification. After the stimulus artifact had returned to baseline, the initial deflection of the EMG response, usually around 17 ms after the stimulus, was identified as the R1 onset latency. Response offset was marked when the EMG signal returned to baseline and remained stable for several milliseconds. Similarly, R2 responses were marked bilaterally, beginning around 65 ms (Fig. 1).

Automated signal-processing algorithms were used to compute the following using the onsets and offsets labeled for each ipsilateral R1 and the bilateral R2 responses: 1) response latency, 2) response duration, and 3) the total area under the curve (microvolts multiplied times seconds), as a measure of response magnitude. The mean level of rectified TA activity during a 20-ms baseline period before onset of the electrical stimulus was then used to compute the baseline activity for the duration of each response. This baseline mean was multiplied by the response duration and subtracted from the total response area under the curve to correct for differences in response amplitude due to changes in overall muscle activity independent from the response. The resulting response magnitudes were then determined for the experimental swallow trials and the control trials without swallow. The mean percent of the control response amplitude for an experimental response set was determined by dividing the mean experimental response magnitude by the mean control response magnitude and multiplying by 100. Thus the mean percent of the control response was computed for the ipsilateral R1 and the ipsilateral and bilateral R2

![Fig. 1. Set of 6 consecutive R1 and R2 ipsilateral thyroarytenoid muscle responses to electrical stimulation of the superior laryngeal nerve. The identification of R1 and R2 response onsets and offsets are identified by a cross (+).](http://jn.physiology.org/)
responses. A repeated ANOVA within subjects was computed to determine whether significant differences were found in mean response magnitudes (as a percent of control trial responses) when stimuli were presented at four time intervals following the command to swallow (500 ms, 1 s, 2 s, and 5 s).

For each response, the time of its occurrence relative to swallowing onset was also determined. The onset and duration of the swallow was measured from laryngeal movement signals obtained from a piezoelectric movement transducer. These were further confirmed by examining the correspondence of the movement transducer with the activation onset of the TA muscles for each swallow (Fig. 2). As a result, each ISLN stimulation was classified into one of four phases as to when the stimulation occurred relative to swallowing: before swallow onset (phase A) during the swallow (phase B), during the first 3 s postswallow (phase C), and 3–5 s postswallow (phase D). The percent response occurrence within a phase was determined for each response type by dividing the frequency of a response type by the total number of stimuli administered during that phase relative to swallowing for a subject. Although the numbers of ISLN stimulations falling within each of the phases differed across subjects, percent occurrences of responses could be computed for each phase in all subjects, except one. Repeated ANOVAs were used to compare the response frequencies relative to the time of the swallow for the ipsilateral R1 and for the ipsilateral and contralateral R2 responses. One multivariate repeated ANOVA was computed to compare ipsilateral R1 response amplitudes and frequencies relative to the time of swallowing and the interaction between the time relative to swallowing and the measures of response amplitude and frequency. Another multivariate repeated ANOVA was computed to compare the effects of time relative to the time of swallowing for the measures of response amplitude and frequency for both the ipsilateral and contralateral R2 responses. The analysis also examined the interactions between time relative to swallowing with both the measures and the muscle response sides.

Because two ANOVAs were computed, one for the ipsilateral R1, and a second combining the ipsilateral and contralateral R2s, a Bonferroni correction in the criterion P value was used (0.05/2 = 0.025).

Significant findings from the multivariate repeated ANOVA testing underwent post hoc testing using the C matrix test. C matrix testing can be used to designate means or combinations of means that are of interest when a study does not use equal time intervals. Because the time intervals tested in this investigation were unequal, the C matrix test was the best post hoc comparison to use for the purpose of identifying and describing mean differences among response amplitudes and frequencies at different stimulus time intervals. A criterion P value of 0.05 was used, which was Bonferroni adjusted by the C matrix post hoc testing procedure.

RESULTS

Responses to ISLN stimulation were only found in the TA muscles; no reflex responses were seen in the superior constrictor, the thyrohyoid, or the mylohyoid muscles. When direct muscle action potentials were seen in the cricothyroid muscle, the electrodes stimulating the SLN were readjusted to include a more medial area less likely to invoke responses in the external SLN. Stimulation of the ISLN produced the R1 response in the ipsilateral TA at ~17 ms and R2 responses in both the ipsilateral and the contralateral TA muscles at ~65 ms (Fig. 1). These responses were similar to those seen previously using electrical stimulation of the ISLN (Deleyiannis et al. 1999; Ludlow et al. 1992, 1995; Yamashita et al. 1997). The group means ± SD of the relative magnitudes of early R1 and later R2 responses across subjects for each phase relative to the time of the swallow are presented in Table 1. Table 2 provides the percent frequency of R1 and R2 responses during each phase relative to the time of swallowing. Multivariate repeated ANOVAs contrasting response amplitude within subjects across the different times following the swallowing command, included before the swallow, up to 3 s after the swallow and between 3 and 5 s after the swallow only. The responses

<table>
<thead>
<tr>
<th>TABLE 1. Average response magnitude relative to time of swallow</th>
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<tr>
<td>Response Type</td>
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<td></td>
</tr>
<tr>
<td>Ipsi R1</td>
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<tr>
<td>Ipsi R2</td>
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<tr>
<td>Contra R2</td>
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</tbody>
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Values are means ± SD and are expressed as a percentage of the comparison control trial magnitude.
occuring during the swallow were not tested due to the excessive and variable amount of average EMG background activity measured during this phase corresponding with the forceful closure of the larynx during swallowing. The subtraction of the baseline activity during this time would have artificially reduced the response amplitudes. However, when measuring responses, some correction had to be made for the increased level of background TA activity during swallowing, which contributed to response amplitude. Therefore it was decided not to compare response amplitudes during a swallow with before or after a swallow.

The ipsilateral R1 response multivariate repeated ANOVA did not demonstrate a significant main effect of time relative to swallow ($F = 1.558; P = 0.276$), although there was a significant interaction between the effect of time relative to swallow with the type of measure ($F = 11.637; P = 0.006$).

C matrix testing comparing the response amplitudes before swallow with up to 3 s after swallow demonstrated a significant decrease in R1 amplitude ($F = 8.155; P = 0.021$). No significant differences were found in R1 response amplitude between before swallowing with between 3 and 5 s after swallowing ($F = 1.289; P = 0.289$) or in R1 response frequency from before the swallow to up to 3 s after the swallow ($F = 0.252; P = 0.629$) or between 3 and 5 s after a swallow ($F = 0.047; P = 0.834$).

A multivariate repeated ANOVA comparison for the R2 responses demonstrated a significant main effect of the time relative to swallow on response amplitude and frequency ($F = 12.543; P = 0.005$) with no significant interactions between the main effect of time with either the type of measures ($F = 2.729; P = 0.133$), or the muscle response side ($F = 0.935; P = 0.437$), and no significant three-way interaction between time, measure, or side ($F = 0.581; P = 0.584$).

C matrix testing demonstrated that the R2 response amplitudes were significantly different from before the swallow up to 3 s after the swallow ($F = 17.895; P = 0.003$) but not between before the swallow and 3–5 s after the swallow ($F = 1.148; P = 0.315$). Similarly, R2 response frequency was significantly different from before swallow up to 3 s after the swallow ($F = 19.438; P = 0.002$) but not between before the swallow and 3–5 s after the swallow ($F = 0.732; P = 0.417$; Fig. 3). The pattern of subjects’ responses for ipsilateral and contralateral R2 responses exhibited a reduction in magnitude as shown in Fig. 3, B and C. Subjects demonstrated a relative drop in R2 response magnitude for electrical stimuli occurring up to 3 s beyond completion of the swallow (phase C), in comparison with stimuli occurring before swallow onset (phase A). All but two of the subjects exhibited a drop in R2 relative magnitude after the swallow command (phase C).

The subjects’ relative frequencies of R2 responses dropped during phases B (during the swallow) and C (during the 1st 3 s after the swallow) compared with phases A (before the swallow) and D (between 3 and 5 s after the swallow) as shown in Fig. 4, B and C. Figure 4A, on the other hand, shows no systematic change in the frequency of the R1 responses at any time relative to swallowing.

Figure 5 shows the baseline EMG magnitude for the right and left TA muscles during the different phases studied. As seen here, an increase in the baseline EMG magnitude occurred during the swallow, on average, followed by a drop in activity level during phases C and D bilaterally in the TA muscles. The electrical stimulus occurred during the swallow (phase B),

### Table 2. Percent frequency of responses relative to the time of occurrence of swallow

<table>
<thead>
<tr>
<th>Response Type</th>
<th>Pre</th>
<th>During</th>
<th>0–3 after</th>
<th>3–5 after</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsilateral R1</td>
<td>100 ± 0</td>
<td>63.9 ± 35</td>
<td>66.7 ± 50</td>
<td>87.0 ± 33</td>
</tr>
<tr>
<td>Ipsilateral R2</td>
<td>72.5 ± 29</td>
<td>19.4 ± 22</td>
<td>23.3 ± 35</td>
<td>66.7 ± 42</td>
</tr>
<tr>
<td>Contralateral R2</td>
<td>70.2 ± 33</td>
<td>17.4 ± 14</td>
<td>22.0 ± 34</td>
<td>51.9 ± 45</td>
</tr>
</tbody>
</table>

Values are means ± SD.

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**FIG. 3.** Percent of control response amplitude of laryngeal adductor responses to electrical stimulation of the superior laryngeal nerve presented at different times relative to the occurrence of swallowing: before swallow onset (phase A); during the 1st 3 s postswallow (phase C); and 3–5 s postswallow (phase D). A: R1 responses in the thyroarytenoid muscle ipsilateral to the side of electrical stimulation. B: ipsilateral R2 response amplitudes. C: contralateral R2 response amplitudes. Each line connects the mean response amplitude for a subject during different times relative to swallowing.
whereas the stimuli presented during phases C and D, respec-
tively, occurred after the swallow was completed. The signif-
icant reductions in response amplitude up to 3 s after the
command to swallow (phase C) were not due to greater base-
line muscle activity being subtracted from the response as can
be seen in Fig. 5. Such an effect would have only occurred
during phase B when the larynx was maximally adducted
during the swallow.

The hooked wire placement for ISLN stimulation remained
in place in only two of the subjects after they were repositioned
to the upright position. Therefore no statistical comparisons
could be made between laryngeal R1 and R2 response fre-
quency and amplitude between the two positions. Instead the
individual data of the mean amplitude and mean frequency of
occurrence of the laryngeal responses at different times before,
during, or after the swallow in each of these two subjects in the
supine and upright positions are plotted in Fig. 6. No system-
atic differences in R1 or R2 response amplitude or frequency
between the two positions were found in these two subjects.

**DISCUSSION**

This study demonstrated changes both in the relative fre-
quency and magnitude of R2 laryngeal responses to electrical
stimulation of the ISLN for several seconds following the
completion of a swallow.

When the subjects initiated a swallow, a change in the
sensitivity of laryngeal afferents may have occurred because of
laryngeal mechanoreceptor adaptation to continuous stimula-
tion ongoing in the pharyngeal and laryngeal regions (Esaki et
al. 1997). Such adaptation might allow bolus material to pass
through the hypopharynx into the esophagus without triggering
coughing and gagging. However, the adductor laryngeal re-
sponse in this study was elicited by electrical stimulation to the

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**FIG. 4.** Percent frequency of occurrence of laryngeal adductor responses to electrical stimulation of the superior laryngeal nerve presented at different times relative to the occurrence of swallowing: before swallow onset (phase A); during the swallow (phase B); during the 1st 3 s postswallow (phase C); and 3–5 s postswallow (phase D). A: R1 responses in the thyroarytenoid muscle ipsilateral to the side of electrical stimulation. B: ipsilateral R2 response amplitudes. C: contralateral R2 response amplitudes. Each line connects the mean response frequency for a subject during different times relative to swallowing.

**FIG. 5.** Mean amplitude of muscle activity in microvolts before electrical stimulation in the thyroarytenoid ipsilateral (A) and contralateral (B) to electrical stimulation of the internal branch of the superior laryngeal nerve presented at different times relative to the occurrence of swallowing: before swallow onset (phase A); during the swallow (phase B); during the 1st 3 s postswallow (phase C); and 3–5 s postswallow (phase D).
ISLN. Thus, any adaptive changes occurring in the laryngeal mechanoreceptors were bypassed here by direct nerve stimulation. The changes in the muscle reflex responses, therefore, were due to interneuronal modulation in the CNS.

The results demonstrated a suppression of laryngeal sensorimotor R2 long-latency responses up to 3 s after a swallow. In addition, the relative magnitude of the R2 long-latency responses were significantly reduced for up to 3 s (phase C) after the swallow compared with the early phase before the swallow (phase A). The R1 response frequency, however, was not affected during or after the completion of a swallow, and the R1 amplitude was only reduced up to 3 s after the swallow. The differential effects on the R1 and R2 responses found in this study further support our previous studies demonstrating an independence of these two responses. In one study, increasing stimulus intensity produced a linear increase in R1 response amplitude, whereas the R2 responses did not change and were unrelated to R1 responses (Yamashita et al. 1997). In two studies, response conditioning was examined in normal subjects and patients with different forms of laryngeal dystonia. Response conditioning affected the R2 responses but not the R1 responses (Deleyiannis et al. 1999; Ludlow et al. 1995). In these studies as well as this one, then, the R2 responses were modulated to a greater degree by interneuronal activity.

In the cat, R1 responses have been found to involve brainstem pathways including the NTS, the lateral segmental field, and the nucleus ambiguus (Gestreau et al. 1997; Tanaka et al. 1995, 1996). The NTS and nucleus ambiguus regions of the brain stem are also considered primary components of the swallowing centers in the dorsal region of the medulla (Jean 1984a,b; Sessle and Henry 1989; Sugimoto et al. 1998; Umezaki et al. 1998). However, the unchanged R1 response frequencies across swallowing phases in this study suggest that the mechanisms by which the R2 responses were suppressed did not alter the R1 responses.

The suppression of R2 responses may be in regions of the CNS other than those involving the R1 laryngeal response pathway. Specifically, these regions may be involved in the integrative control of swallowing. The areas of the CNS in the human that may be associated with the sensorimotor modification of the pharynx are the thalamus, basal ganglia, and cortical areas (Hamdy and Rothwell 1998). Studies in primates have found that the lateral precentral cortex and anterolateral frontal cortex are typically involved in the control of voitional swallowing (Martin et al. 1997; Narita et al. 1999). The role of multiple sites within the cortex in changing the timing and duration of muscle activity during swallowing has also been demonstrated (Martin et al. 1999). Martin et al. (1997) related the firing patterns of neuronal pools within the primary motor cortex and cortical masticatory areas to tongue protrusion and

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**FIG. 6.** Top 3 graphs: percent of control response amplitude of laryngeal adductor responses to electrical stimulation of the superior laryngeal nerve presented at different times relative to the occurrence of swallowing: before swallow onset (phase A); during the 1st 3 s postswallow (phase C); and 3–5 s postswallow (phase D) in the supine (—) and upright (—) positions in subject 1 (squares) and subject 2 (circles). Bottom 3 graphs: the percent frequency of occurrence of laryngeal adductor responses to electrical stimulation of the superior laryngeal nerve presented at different times relative to the occurrence of swallowing: before swallow onset (phase A); during the swallow (phase B); during the 1st 3 s postswallow (phase C); and 3–5 s postswallow (phase D). Left: R1 responses in the thyroarytenoid muscle ipsilateral to the side of electrical stimulation. Middle: ipsilateral R2 response amplitudes. Right: contralateral R2 response amplitudes. Each line connects the mean response for a subject during different times relative to swallowing in one position.
swallowing activities in two monkeys. Their findings demonstrated participation of the primary motor cortex in tongue-related activity for swallowing. Within the pool of neurons studied, differential activation was noted relative to experimental tasks such as tongue protrusion, licking, chewing, and activation of the genioglossus before and during swallow.

Narita et al. (1999) used a cold block-induced inactivation of the primary motor cortex and cortical masticatory areas in two monkeys to relate the alteration in neuronal firing to the activation of the genioglossus, masseter, anterior digastric, and thyrohyoid muscles. This investigation demonstrated the significance of the lateral pericentral cortex in deglutition. Under cold block-induced inactivation, the initiation of the swallow was significantly delayed. In addition, the amplitude was reduced and the duration prolonged in each of the muscles except the genioglossus. Overall, although the pattern of muscle onset was not affected, the initiation timing of swallowing was slowed by inactivation of the cortical regions of interest.

Clinically, swallowing can be disturbed by pathology at many different levels of the CNS. Lateral medullary infarcts, as in Wallenberg’s syndrome, can cause devastating effects on swallowing partially because of vocal fold paralysis resulting from damage to the descending tracts to the nucleus ambiguus (Hamdy and Rothwell 1998; Horner et al. 1991). Swallowing motor control deficits, in the absence of lower motor neuron involvement, occur after cerebrovascular accidents in the middle cerebral artery, particularly on the right side (Daniels et al. 1996; Hamdy and Rothwell 1998; Hamdy et al. 1996, 1997).

Many neurodegenerative diseases affecting the basal ganglia can also interfere with the volitional control of swallowing in the absence of lower motor neuron deficits (Logeman 1988; Robbins et al. 1986). One or more of these regions in the CNS may modulate the R2 responses. Further, the cortical control invoked during the volitional swallowing task may have suppressed the generation of the R2 long-latency responses.

The central mechanisms involved in the evocation and modulation of swallowing may play a role in the prolonged suppression of the late bilateral R2 adductor responses. This suppression was for up to 3 s after the completion of a swallow. During the time period following a swallow, any residual material in the pharynx could enter the larynx if subsequent swallows do not clear it. Our results suggest that the more rapid R1 response continues to occur even when the later bilateral R2 responses suppress during this time period. The rapid R1 response may be part of the cough reflex (Gestreau et al. 1997), which remains active during the swallow, while suppression of the later bilateral R2 responses occurs. Therefore rapid protective mechanisms such as the R1 sensorimotor responses will still be triggered by entry of material into the laryngeal vestibule and penetration to the level of the vocal folds. Should the R2 response relate to the protective adductory laryngeal response, its suppression after the swallow may result in a reduced strength of the laryngeal closure response.

The differential effects of swallowing on the R1 and R2 responses suggest that these two reflexes involve functionally separate pathways. The task of volitional swallowing to command did not reduce the R1 response frequency which has a well known pathway in the medulla involving the interstitial subnucleus of the NTS, the lateral tegmental field and the nucleus ambiguus (Gestreau et al. 1997; Tanaka et al. 1995, 1996). Fictive swallowing has been found to modify the firing of neurons in the pre-Bötzinger complex involved in the rhythmogenesis for respiration (Nakazawa et al. 1999). These authors found that neurons identified as augmenting or decrementing expiration, or decrementing inspiration, were suppressed during swallowing. They also found that the same neurons were inhibited following a single electrical stimulus to the SLN.

It is possible that the inhibitory effects of swallowing on the respiratory rhythm in the medulla were also involved in suppression of R2 laryngeal muscle responses. In awake humans, respiratory suppression, or apnea, occurs during swallowing (Klahn and Perlman 1999; Martin et al. 1994; Preiksaitis et al. 1992). The duration of this respiratory apnea was measured while swallowing 5 ml of water (Klahn and Perlman 1999). From the time of swallow onset, the cessation of respiration lasted 750 ms, and the subsequent expiratory phase continued between 1.6 and 1.8 s in women and men, respectively, before the resumption of normal respiration. The period of bilateral R2 suppression in this study, for up to 3 s after onset, covers a similar period to that of respiratory modification relative to the swallow. Swallowing mechanisms, therefore, may modulate both respiratory control as well as laryngeal responses to sensory stimuli following swallowing. Further investigation is necessary to determine the possible connection between the roles of the laryngeal R1 and R2 responses with respiratory control related to the swallow.

Limitations of the current study include the large age range in the small number of subjects, the ability to contrast position effects in only two of the subjects, the inability to isolate muscle recordings in human subjects, and the use of only one bolus size. Only nine subjects were studied; however, the procedures were relatively invasive and lengthy, making subject recruitment difficult. We were able to complete the full study, comparing the supine and upright positions, in only two of the nine subjects, although the original aim of the study was to examine the effects of position on the modulation of the sensorimotor responses. In the other subjects, readjusting the head affected the position of the stimulating electrodes, making the comparison invalid. No systematic effects of body position on the modulation of sensory responses after the swallow were found. Further investigation is warranted, however, given the small numbers of subjects over a wide age range involved in addressing this question. There is no assurance that adjacent muscle activity was not included in the muscle recordings because of the size and proximity of the head and neck muscles, although care was taken to use well-known tasks to verify the location of the recording electrodes in each of the muscles. For example, the mylohyoid muscle is part of a complex of submental muscles and cannot be differentiated from adjacent muscles such as the geniohyoid, which is activated at the same time. Similarly, the thyrohyoid cannot be separated from the sternothyroid, and the superior constrictor is contiguous with other pharyngeal constrictors.

Additional investigation is also needed concerning the size and viscosity of the bolus used in this study. The 2-ml water bolus in this study approximates that of a saliva swallow. Previous work addressing bolus size and muscle patterns during swallowing reported earlier and prolonged submental EMG activity with saliva than with larger water bolus sizes (Perlman et al. 1999). However, the previous investigation did not compare a saliva swallow to one using 2 ml water. Nonetheless, the
question remains unanswered as to whether bolus size affects the patterns of laryngeal reflex suppression reported in this study. Finally, because bolus size affects the duration of apnea during swallowing (Preiksaitis and Mills 1996), bolus size may alter the suppression of the laryngeal long-latency responses, either directly or indirectly, during swallowing. Such changes in laryngeal sensorimotor responses during different bolus sizes may play a role in the protection of the airway during normal eating when the size of bolus is less controlled.

The authors thank Dr. Paul Smith, Chair, Dept. of Statistics, University of Maryland, College Park, for advice on statistical analysis of the data. Present address and address for reprint requests: J. M. Barkmeier, Dept. of Speech and Hearing Sciences, The University of Arizona, PO Box 210071, Tucson, AZ 85710.

Received 18 June 1999; accepted in final form 1 November 1999.

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