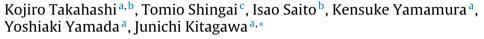
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# Facilitation of the swallowing reflex with bilateral afferent input from the superior laryngeal nerve



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#### HIGHLIGHTS

• The superior laryngeal nerve (SLN) plays a major role in the swallowing reflex.

The latency of swallow decreases during bilateral stimulation of the SLN.

• Bilateral afferent input from the SLN facilitates the swallowing reflex.

## ARTICLE INFO

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# ABSTRACT

To determine the cooperative effect of laryngeal afferent signals on the swallowing reflex, we examined whether afferent signals originating from the left and right superior laryngeal nerve (SLN) modulates elicitation of the swallowing reflex in urethane-anesthetized rats. Mylohyoid electromyographic activity was recorded to quantify the swallowing reflex. The onset latency of the swallowing reflex and the time intervals between successive swallows were used to quantify and compare the effects of unilateral and bilateral electrical stimulations of the SLN. The mean latency of the first swallow and the mean time interval between swallows evoked with low frequency stimulation were both significantly different between unilateral and bilateral stimulations of the SLN. These findings suggest that facilitatory effect of afferent signals originating from the SLN bilaterally increase the motoneuronal activity in the medullary swallowing center and enhance the swallowing reflex.

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## 1. Introduction

The larynx is highly reflexogenic [1] and is especially important for eliciting the swallowing reflex. The superior laryngeal nerve (SLN) innervates the larynx and plays a major role in initiating the swallowing reflex [2–8]. Previous animal studies have used electrical stimulation of the SLN to evoke the swallowing reflex [7–13].

The swallowing process is divided into oral, pharyngeal, and esophageal stages, depending on the location of the food bolus [14]. The speed at which the food bolus moves to the oropharynx and larynx depends on whether the food is a solid or liquid. Chemical or mechanical stimulations of the pharyngeal and laryngeal mucosae can evoke the swallowing reflex [6,11,15–20]. This suggests that interactions between the afferent signals originating from the pharynx and larynx play a major role in the swallowing reflex. Mechanical stimulation of pharyngeal regions innervated by the pharyngeal branch of the glossopharyngeal nerve (GPN-ph) can readily evoke the swallowing reflex [11,15–18]. We previously used electrical stimulation to demonstrate that the GPN-ph plays a major role in initiating the swallowing reflex from the pharynx [11]. In addition, we demonstrated that afferent signals from the GPN-ph and the SLN could spatially summate in the medullary swallowing center and evoke the swallowing reflex [21]. However, the mechanisms through which afferents originating from the left and right laryngeal mucosae interact, and how these inputs help to evoke the swallowing reflex, are unknown. Here we evaluate the cooperative effect of afferent signals originating bilaterally from the laryngeal mucosae on the swallowing reflex. Specifically, we examined the onset latency of the swallowing reflex and the interval between swallows evoked with unilateral and bilateral electrical stimulations of the SLN.

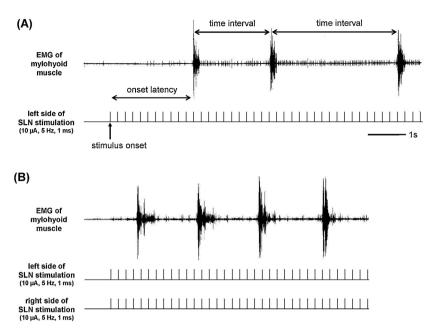






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**Fig. 1.** Representative electromyographic (EMG) activity recorded from the mylohyoid muscle during swallowing. (A): successive swallows evoked with unilateral electrical stimulation of the superior laryngeal nerve (SLN). (B): successive swallows evoked with bilateral electrical stimulation of the SLN. Electrical stimulation was applied at 5 Hz, 10 μA, and 1.0 ms in both conditions.

#### 2. Materials and methods

The experimental protocols were approved by the Intramural Animal Care and Veterinary Science Committee of Niigata University.

Experiments were conducted using 20 male Wistar rats weighing 200–400 g. The animals were anesthetized with urethane (1.0 g/kg, intraperitoneal) and laid in the supine position in a stereotaxic frame. The body temperature was maintained at 37 °C using a heating pad. A longitudinal midline incision was made on the ventral surface of the neck. The trachea was cannulated to maintain respiration. Swallowing was identified by electromyographic (EMG) activity recorded from the mylohyoid muscle and by visual observation of laryngeal movement. Thus, bipolar enamel-coated stainless steel wire electrodes (5 mm in diameter) were inserted into the left mylohyoid muscle to record EMG activity. The mylohyoid muscle was examined because it is recognized as the "obligate muscle" involved in swallowing movements [10,11,22].

The SLN was exposed bilaterally through blunt dissection of the sternothyroid muscle, which was performed using a scissor. The left and right SLN were dissected from the surrounding tissue, and bipolar platinum wire electrodes were fitted onto the central cut end of each SLN for electrical stimulation.

The SLN was stimulated either bilaterally or unilaterally with a rectangular pulse (frequency: 5, 10–70 Hz, intensity: 10  $\mu$ A, pulse duration: 1.0 ms) to examine the cooperative effect of afferent signals originating from both the SLNs. Bilateral stimulation was accomplished by simultaneously stimulating the left and right SLN. The parameters for unilateral stimulation of the SLN are described in our previous study [11].

The onset latency for eliciting the first swallow was defined as the time between the onset of electrical stimulation and the onset of mylohyoid EMG activity during the first swallow. We then compared the onset latencies of the first swallow evoked with bilateral and unilateral stimulations of the SLN. In addition, the interval between the first and third swallows was measured. The time interval divided by 2 was considered the mean value of the time intervals (mean time interval of swallows). The mean time intervals between swallows were compared between unilateral and bilateral stimulations of the SLN.

Statistical analysis was performed using an analysis of variance (ANOVA) followed by the Newman–Keuls test. The results are presented as the mean  $\pm$ SD. Differences were considered significant at p < 0.05.

## 3. Results

Representative examples of the swallowing reflex evoked with unilateral and bilateral electrical stimulations of the SLN are shown in Fig. 1. Multiple swallows were evoked with both unilateral (Fig. 1A) and bilateral (Fig. 1B) stimulations. With unilateral stimulation, the latency of the first swallow was 2.21 s, whereas the mean interval between swallows was 2.71 s (Fig. 1A). With bilateral stimulation (Fig. 1B), the onset latency of the first swallow was 0.72 s, whereas the mean interval between swallows was 1.81 s (Fig. 1B). The number of swallows was greater following bilateral than for following unilateral stimulation. (Fig. 1A,B). The onset latency of the swallowing reflex was significantly shorter following bilateral stimulation than with unilateral stimulation.

The mean latency of the first swallow and the mean interval between swallows varied with the frequency of electrical stimulation. The relationship between the stimulus frequency and both the latency of the first swallow and the mean interval between swallows were determined by applying unilateral and bilateral stimulation to the SLN at  $10 \,\mu$ A, 5,  $10-70 \,\text{Hz}$  and  $1.0 \,\text{ms}$ . The relationships between stimulus intensity and first swallow latency or swallow intervals shown in Figs. 2 and 3 were obtained from 20 rats in each graph.

The mean latency of the first swallow evoked by unilateral stimulation of the SLN decreased significantly as the stimulation frequency was increased up to 30 Hz (Fig. 2). In contrast, the mean latency did not change with bilateral stimulation of the SLN. However, the mean latency of the first swallow differed significantly between unilateral and bilateral stimulations across the range of 5, 10–20 Hz (Fig. 2).

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