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Research report

Differential response properties of peripherally and cortically evoked swallows by electrical stimulation in anesthetized rats

Takanori Tsujimura[a,*], Kojun Tsuji[a], Jin Magara[a], Shogo Sakai[a], Taku Suzuki[a], Yuki Nakamura[a], Kayoko Nozawa-Inoue[b], Makoto Inoue[a]

[a] Division of Dysphagia Rehabilitation, Niigata University Graduate School of Medical and Dental Sciences, Chuo-ku, Niigata 951-8514, Japan
[b] Division of Oral Anatomy, Niigata University Graduate School of Medical and Dental Sciences, Chuo-ku, Niigata 951-8514, Japan

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ABSTRACT

We compared onset latency, motor-response patterns, and the effect of electrical stimulation of the cortical masticatory area between peripherally and cortically evoked swallows by electrical stimulation in anesthetized rats. The number of swallows and the motor patterns were determined using electromyographic recordings from the thyrohyoid, digastric, and masseter muscles. The onset latency of the first swallow evoked by electrical stimulation of the cortical swallowing area (Cx) was significantly longer than that evoked by stimulation of the superior laryngeal nerve (SLN). The duration of thyrohyoid burst activity associated with SLN-evoked swallows was significantly longer than that associated with either Cx-evoked or spontaneous swallows. Combining Cx with SLN stimulation increased the number of swallows at low levels of SLN stimulation. Finally, A-area (the orofacial motor cortex) stimulation inhibited Cx-evoked swallows significantly more than it inhibited SLN-evoked swallows. These findings suggest that peripherally and cortically evoked swallows have different response properties and are affected differently by the mastication network.

1. Introduction

Swallowing is considered an essential action for life as it provides two vital functions: alimentation and protection of the upper respiratory tract. Basic swallowing movements may be programmed by the central pattern generator (CPG) for swallowing, which is located in the lower brain stem (Jean, 2001). In humans (Dziewas et al., 2003; Michou and Hamdy, 2009; Shingai et al., 1989) and animals (Kitagawa et al., 2002; Sumi, 1969), the swallowing CPG can be activated through cortical input as well as sensory input from the pharynx and larynx.

In humans, a number of spatially and functionally distinct cortical regions are involved in swallowing, including activation of the insular cortex during voluntary swallowing (Martin et al., 2001). The importance of the insula is shown by clinical studies in which swallows were evoked in subjects with epilepsy via insular stimulation (Penfield and Faulk, 1955; Soros et al., 2011), and from a patient who developed dysphagia after acute perisylvian infarcts (Foix–Chavany–Marie opercular syndrome) involving the bilateral insular cortex (Singh et al., 2011). Although these reports strongly suggest a critical role of the insular cortex in swallowing, to our knowledge, few studies have systematically investigated this function (Jezzini et al., 2012).

Two major cortical masticatory regions have been reported in rats, an anterior area (A-area) and a posterior area (P-area) (Zhang and Sasamoto, 1990). The P-area is located in the insula, and we previously found that electrically stimulating this region induced swallowing with rhythmic jaw movements (RJMs) in anesthetized rats (Tsujimura et al., 2012a). In the current study, we successfully initiated swallowing by electrically stimulating the cortical swallowing area (Cx) within the insular cortex. Although peripheral and central inputs are capable of inducing swallowing, few studies have investigated how these two types of swallows are functionally different. Sumi studied peripherally and cortically evoked swallows from the same animal, and also showed facilitation of swallowing initiation with the combination of peripheral and cortical stimulation (Sumi, 1969). However, to our knowledge, no report has quantitatively compared peripherally and cortically evoked swallows. Thus, the first aim of the current study was to determine

Abbreviations: CPG, central pattern generator; Cx, cortical swallowing area; RJMs, rhythmic jaw movements; SLN, superior laryngeal nerve.

* Corresponding author. Division of Dysphagia Rehabilitation, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Gakkocho-dori, Chuo-ku, Niigata 951-8514, Japan. Fax: +81 25 227 2998.
E-mail address: tsujimura@dent.niigata-u.ac.jp (T. Tsujimura).

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the differences between swallows induced by peripheral electrical stimulation of the superior laryngeal nerve (SLN) versus those induced by cortical electrical stimulation of Cx.

Initiation of the swallowing reflex is affected by peripheral input from mechanical, gustatory, thermal, and noxious receptors (Chee et al., 2005; Hamdy et al., 2003; Tsujimura et al., 2009, 2011, 2013). When chewing, bolus processing in the oral and pharyngeal cavity always precedes swallowing (Hiiemae and Palmer, 1999; Palmer et al., 1992), suggesting that initiation of swallowing may be inhibited by chewing. Experimentally, during cortically (A-area)-evoked RJMs that resemble chewing, SLN-evoked swallowing was inhibited in anesthetized rats (Tsujimura et al., 2012a). Although we reported that SLN-evoked swallowing is inhibited by A-area stimulation, the initiation of swallowing during chewing in awake humans occurs in the hypopharynx (Saitoh et al., 2007), which is mainly innervated by the SLN. Thus, we hypothesized that SLN-evoked swallowing is less inhibited by A-area stimulation than cortically-evoked swallows. The second aim of this study was to investigate the difference in the effect of A-area stimulation between SLN and Cx-evoked swallows.

2. Materials and methods

2.1. Preparation

This study was reviewed and approved by the Niigata University Intramural Animal Care and Use Committee (82-2). Experiments were performed on 26 male Sprague-Dawley rats weighing between 250 and 350 g. Animals were anesthetized with urethane (1.3 g/kg, i.p.), and supplemented with urethane whenever necessary to maintain anesthesia at a level that resulted in total absence of the corneal reflex. Rectal temperature was maintained at 37–38 °C by a thermostatically controlled heating pad. A midline incision was made along the ventral aspects from the pogonion to the caudal portion of the neck. The trachea and right femoral vein were cannulated to allow for respiration and intravenous administration of drugs, respectively. After the physiological experiment was completed, animals were sacrificed with an overdose administration of urethane (2.0 g/kg, i.v.).

2.1.1. Electromyographic recording

Bipolar enamel-coated copper wire electrodes (0.18 mm in diameter and 2 mm in inter-polar distance) were inserted into the left thyrohyoid (Thy), anterior belly of the digastric (Dig), and masseter (Mas) muscles for electromyographic (EMG) recording.

2.1.2. Initiation of swallowing

For peripherally evoked swallowing, two enamel-coated silver wire electrodes (0.2 mm in diameter) were placed bilaterally on the SLN and were fixed by silicon material. For cortically evoked swallowing, the cortical surface was exposed after the head was fixed in a stereotaxic holder, and a bipolar concentric electrode (inner diameter, 0.25 mm; outer diameter, 0.6 mm) was inserted vertically into the cerebral cortex.

Swallowing was evoked by electrical stimulation delivered to either the SLN (200-μs pulse; 5–50 Hz) or Cx (500-μs pulse; 5–100 Hz) located at 1.5–2.0 mm anterior and 4.6–5.0 mm lateral to bregma, and 4.3–5.0 mm below the cortical surface. According to our previous study, swallowing was identified by both the Dig and Thy EMG bursts and visual confirmation of the laryngeal elevation (Tsuji et al., 2015). The stimulation threshold for eliciting swallowing was determined as the minimum stimulus intensity needed to evoke a swallow at least once during either the SLN or Cx stimulation for 10 s. The current intensity was determined as 0.8, 1.0, or 1.2 times the stimulation threshold (T) for each (see below) because stimulation of sites neighboring the Cx often evoked RJMs, as did stimulation of the Cx at greater than 1.5 × T. Spontaneous swallows, defined as a swallow without any preceding stimulation, were also recorded during the stimulation sessions.

At the end of the Cx-stimulation experiments, the brain was removed, serial sections (50-μm-thick) were cut, and the stimulating sites were identified histologically.

2.1.3. Initiation of RJMs

To evoke RJMs, the A-area (30° behind the vertical, 4.0–5.3 mm anterior and 3.0–3.3 mm lateral to bregma, 3.0–4.7 mm below the cortical surface) was electrically stimulated unilaterally for 10 s (500-μs pulse; 30 Hz) using a bipolar concentric electrode (inner diameter, 0.15 mm; outer diameter, 0.4 mm) (Tsujimura et al., 2012b). RJMs were identified as rhythmic bursts observed in EMGs from the Dig and Mas muscles and visual confirmation of mandibular movements. The current intensity was determined as the threshold for eliciting RJMs.

2.2. Data collection and analysis

The interval between recordings was at least 2 min in all experiments. EMG signals were amplified (AM-601G, Nihon Kohden, Tokyo, Japan), digitized, stored on a computer hard disk at sampling rate of 10 kHz, and analyzed using the Spike2 analysis package (Cambridge Electronic Design, Cambridge, UK). Results are presented as mean ± SEM. The effect of A-area stimulation on SLN- and Cx-evoked swallows was analyzed using non-parametric Mann–Whitney *U* test. The Wilcoxon rank-sum test was used to compare the first-swallow onset latency between SLN and Cx stimulation conditions. Differences among multiple groups were assessed using one-way ANOVA or Kruskal–Wallis one-way ANOVA with a post hoc Tukey test after normality and equality tests. Differences were considered statistically significant at $p < 0.05$.

2.2.1. Effect of stimulus frequency on swallow initiation

To determine the threshold of swallow initiation, the SLN and Cx were stimulated at 30 Hz and 10 Hz, respectively. Stimulation was applied at 5 Hz, 10 Hz, 20 Hz, 30 Hz, and 50 Hz to the SLN and at 5 Hz, 10 Hz, 20 Hz, 30 Hz, 50 Hz, and 100 Hz to the Cx. Stimulation intensity was set at 1.2 × T for all trials. The number of swallows was counted and the onset latency of the first swallow was calculated. This onset latency was defined as the time interval between the start of stimulation and the peak of the first Thy EMG burst.

2.2.2. Differences in response properties across three swallowing conditions

Activity patterns of the Dig and Thy muscles were compared across three conditions: SLN-evoked, Cx-evoked, and spontaneous swallowing. Data for spontaneous swallowing were recorded during the stimulation sessions when swallows were detected without any preceding stimulation. Stimulation intensity was set at 1 × T in all cases, and the frequency was set at 30 Hz for the SLN and 10 Hz for the Cx. We calculated the onset latency of the first swallow and the onset and offset-lag times. These were defined as the time between Dig and Thy onset EMG bursts and the time between Dig and Thy offset EMG bursts, respectively. In addition, we calculated the duration of Dig and Thy EMG bursts. The duration of EMG bursts was calculated as the time duration over the mean + 2SD of background activity recorded from stable 5 s periods at rest.

2.2.3. Effect of suturing the SLN on Cx-evoked swallows

To eliminate the contamination of SLN activity by Cx stimulation on swallow initiation, Cx stimulation was performed after bilateral tight sutures of the SLN nerve in two animals, which prevents neural