Brain Research 1694 (2018) 19–28

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

Effect of peripherally and cortically evoked swallows on jaw reflex responses in anesthetized rabbits

Taku Suzuki, Midori Yoshihara, Shogo Sakai, Kojun Tsuji, Kouta Nagoya, Jin Magara, Takanori Tsujimura, Makoto Inoue *

Division of Dysphagia Rehabilitation, Niigata University Graduate School of Medical and Dental Sciences, Chuo-ku, Niigata 951-8514, Japan

ARTICLE INFO

Article history:
Received 3 October 2017
Received in revised form 3 March 2018
Accepted 1 May 2018
Available online 3 May 2018

Keywords:
Swallow
Jaw-opening reflex
Jaw-closing reflex
Superior laryngeal nerve
Cortical swallowing area
Rabbit

ABSTRACT

This study aimed to investigate whether the jaw-opening (JOR) and jaw-closing reflexes (JCR) are modulated during not only peripherally, but also centrally, evoked swallowing. Experiments were carried out on 24 adult male Japanese white rabbits. JORs were evoked by trigeminal stimulation at 1 Hz for 30 s. In the middle 10 s, either the superior laryngeal nerve (SLN) or cortical swallowing area (Cx) was simultaneously stimulated to evoke swallowing. The peak-to-peak JOR amplitude was reduced during the middle and late 10-s periods (i.e., during and after SLN or Cx stimulation), and the reduction was dependent on the current intensity of SLN/Cx stimulation: greater SLN/Cx stimulus current resulted in greater JOR inhibition. The reduction rate was significantly greater during Cx stimulation than during SLN stimulation. The amplitude returned to baseline 2 min after 10-s SLN/Cx stimulation. The effect of co-stimulation of SLN and Cx was significantly greater than that of SLN stimulation alone. There were no significant differences in any parameters of the JCR between conditions. These results clearly showed that JOR responses were significantly suppressed, not only during peripherally evoked swallowing but also during centrally evoked swallowing, and that the inhibitory effect is likely to be larger during centrally compared with peripherally evoked swallowing. The functional implications of these results are discussed.

1. Introduction

Ingestion is the early stage of nutrition in most mammals. It is widely accepted that masticatory movements, including chewing and swallowing, are programmed by the central nervous system, including a central pattern generator (CPG) in the brainstem (Jean, 2001; Miller, 1982; Nakamura and Katakura, 1995).

Swallowing can be triggered by either peripheral or central inputs (Jean, 2001). For the former, pharyngeal and laryngeal stimulation can readily evoke the swallowing reflex. Electrical stimulation of the superior laryngeal nerve (SLN), which contains the pharyngeal/laryngeal sensory nerve, is one of the most common methods for activating the swallowing CPG in animals (Jean, 2001) and in humans (Aida et al., 2015; Tsukano et al., 2012). Brain imaging studies have shown that some cortical loci are involved in the voluntary swallowing process in humans, including the sensorimotor cortex, primary sensory cortex, inferior parietal lobe, insula, and anterior cingulate cortex, although there is some variability between studies (see (Humbert and Robbins, 2007; Soros et al., 2009)). Although a number of studies have corroborated neurophysiological data in animals (Martin and Sessle, 1993; Martin et al., 1997; Martin et al., 1999; Narita et al., 1999) and humans (Hiraoka, 2004), some contradictory results have been reported. For example, some studies reported laterality of sensorimotor cortical activation (Dziewas et al., 2003; Martin et al., 2004; Teismann et al., 2009) while others revealed bilateral activation (Hamdy et al., 1999b; Zald and Pardo, 1999). Furthermore, the location of insula activation has varied between studies, with some studies reporting left insula (Dziewas et al., 2003), right insula (Martin et al., 2001), anterior insula (Hamdy et al., 1999a), or posterior insula (Suzuki et al., 2003) activation. This discrepancy may be due to differences in the swallowing tasks used (voluntary or reflexive swallowing; bolus or saliva swallowing) and/or the demographics of the participants.

Abbreviations: CPG, central pattern generator; Cx, cortical swallowing area; Dig, digastric muscle; EMG, electromyography; IAN, inferior alveolar nerve; JCR, jaw-closing reflex; JOR, jaw-opening reflex; Mas, masseter muscle; MesV, mesencephalic trigeminal nucleus; SLN, superior laryngeal nerve; Thy, thyrohyoid muscle.

* Corresponding author at: Division of Dysphagia Rehabilitation, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Gakkocho-dori, Chuo-ku, Niigata 951-8514, Japan.

E-mail address: inoue@dent.niigata-u.ac.jp (M. Inoue).

https://doi.org/10.1016/j.brainres.2018.05.002
0006-8993/

Recently, we succeeded in initiating swallowing by electrical stimulation of the swallowing cortical area (Cx) within the insular cortex in anesthetized rats (Tsujimura et al., 2016). In this study, we found that the onset latency of the first swallow evoked by insular stimulation was significantly longer than that evoked by stimulation of the SLN. These results might be expected because initiating centrally evoked swallowing in natural situations involves activation of not only the insular cortex but also other areas, as described above.

It has been reported that the jaw-opening reflex (JOR), one of the elementary jaw reflexes evoked by trigeminal stimulation (Lund et al., 1981; Lund et al., 1983), is suppressed during chewing. The finding that paralysis does not change such modulation, in which the excitability of the JOR pathway is modulated during chewing, strongly suggests that these processes are not dependent on the sensory feedback system (Lund et al., 1983).

Our previous studies revealed that the JOR is suppressed not only during chewing but also during swallowing. Yamada et al. (Yamada et al., 2013) reported that JOR evoked by innocuous intra-oral stimulation was suppressed during natural chewing and swallowing in conscious animals. Fukuhara et al. (Fukuhara et al., 2011) investigated the effects of swallowing responses evoked by electrical stimulation of the SLN on JORs in anesthetized animals. The authors found that JORs evoked by low-threshold trigeminal afferents were significantly inhibited during and after SLN stimulation. This suggested that activation of the swallowing-related neural network, but not the swallowing movements, is involved in the inhibition of JORs. Such processes may be required to prevent undesirable jaw movements caused by weak stimulation during functions such as chewing and swallowing.

The present study aimed to investigate whether JOR responses are modulated not only during peripherally evoked swallowing but also during centrally evoked swallowing. In addition, changes in the opposing jaw reflex (i.e., jaw-closing reflex; JCR) during swallowing, were also investigated. We hypothesized that JORs would be inhibited not only by peripheral inputs but also central inputs to evoke swallowing, and that the stimulus intensity applied to the central and peripheral regions to evoke swallowing would be similarly related to the reduction of JORs.

2. Results

2.1. Baseline data

The inferior alveolar nerve (IAN) stimulus threshold to evoke the JOR in the digastric (Dig) muscle ranged from 0.05 to 0.30 mA (0.12 ± 0.07 mA, n = 30) and that of the mesencephalic trigeminal nucleus (MesV) to evoke the JCR in the masseter (Mas) muscle ranged from 0.02 to 2.00 mA (0.53 ± 0.49 mA, n = 14). The onset latency of the JOR ranged from 6.42 to 9.64 ms (7.62 ± 0.77 ms, n = 30) and that of the JCR ranged from 1.71 to 2.18 ms (1.96 ± 0.13 ms, n = 13).

The SLN stimulus threshold ranged from 0.02 to 0.60 mA (0.10 ± 0.12 mA, n = 31) and that of Cx ranged from 0.10 to 2.00 mA (1.03 ± 0.63 mA, n = 29). The mean number of swallows evoked by SLN stimulation increased and onset latency of the first swallow decreased with increasing stimulus intensity (Fig. 1). This was not the case for Cx stimulation; there was no difference in the number of swallows and the onset latency of the first swallow with Cx stimulation between 1.0 and 1.4 times (T) the threshold for eliciting the swallowing reflex at least once for 10 s (Fig. 1).

2.2. Effect of SLN/Cx stimulation on JORs

An example JOR recording is shown in Fig. 2. JOR responses, in terms of peak-to-peak amplitude, were inhibited during and immediately after swallowing evoked by SLN and Cx stimulation. The mean JOR amplitude was significantly smaller in the middle and late 10-s periods (i.e., during and after SLN/Cx stimulation) compared with the early 10-s period (i.e., before SLN/Cx stimulation) (Fig. 3). The inhibitory effect on the JORs was dependent on the stimulus intensity, such that greater SLN/Cx stimulus intensity was associated with greater inhibition. There was no difference in the reduction rate of JOR amplitude between ipsilateral and contralateral SLN/Cx stimulation and the latency among the stimulus conditions of SLN/Cx (data not shown).

Time-dependent changes in JOR responses were analyzed (Fig. 4). The JOR amplitude gradually decreased during SLN/Cx stimulation and continued to decrease after stimulation, returning to baseline level (i.e., before SLN/Cx stimulation) 2 min after SLN/Cx stimulation.

To evaluate how the occurrence of swallowing is related to the inhibition of JOR responses and to compare SLN and Cx stimulation, we calculated the reduction rate of JOR amplitude per swallow (Fig. 5). The rate was significantly greater during Cx stimulation at 1.0 T than during SLN stimulation at 1.0 T.

2.3. Effect of co-stimulation of SLN and Cx on JORs

JOR responses were strongly inhibited during co-stimulation of SLN and Cx. JOR amplitude was significantly smaller during co-stimulation of SLN and Cx than during SLN (4.0 T) stimulation alone, but not significantly smaller than that during Cx (1.0 T) stimulation alone (Fig. 6).

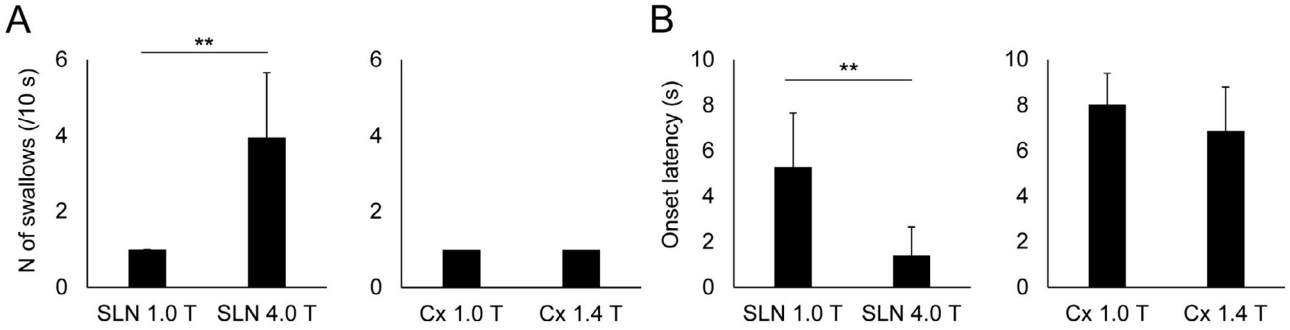

Fig. 1. The number of swallows and onset latency of the first swallow during SLN/Cx stimulation. A: The number of swallows during 1.0 T and 4.0 T SLN stimulation was 1.0 ± 0.0 and 4.0 ± 1.7 (n = 22 for each group), respectively, and that during 1.0 and 1.4 T Cx stimulation was 1.0 ± 0.0 and 1.0 ± 0.0 (n = 17 for each group), respectively. B: The onset latency of the first swallow during 1.0 T and 4.0 T SLN stimulation was 5.3 ± 2.4 s and 1.4 ± 1.3 s (n = 22 for each group), respectively, and that during 1.0 and 1.4 T Cx stimulation was 8.0 ± 1.4 s and 6.9 ± 1.9 s (n = 17 for each group), respectively. During 0.8 T SLN/Cx stimulation, no swallows were evoked in any of the animals. $^{**}p < 0.01$.

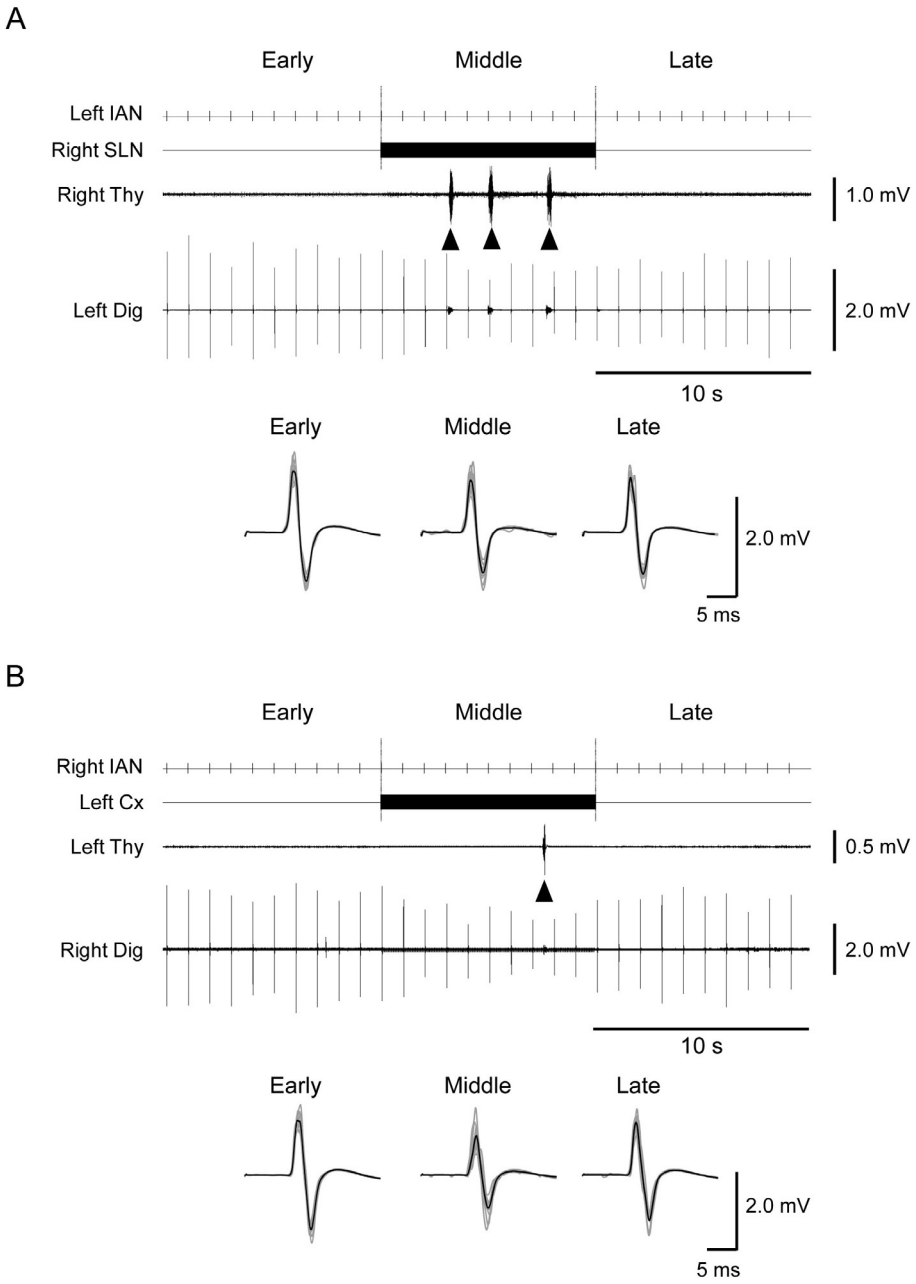

Fig. 2. Example of changes in JOR responses during SLN and Cx stimulations. Data obtained from the same animal are shown. JORs were evoked by IAN stimulation at 1 Hz for 30 s. Three swallows were evoked during SLN stimulation at 4.0 T the threshold for evoking swallowing (i.e., in the middle 10 s) (A) and one swallow was evoked during Cx stimulation at 1.4 T the threshold for evoking swallowing (B). Averaged waveforms of JOR with eight (Early) or ten (Middle, Late) superimposed responses are shown on the bottom of each trace. Thy, thyrohyoid muscle.

2.4. Effect of SLN/Cx stimulation on JCRs

In contrast to the effect of SLN/Cx stimulation on JORs, JCRs were unaffected by SLN/Cx stimulation (Fig. 7). The results revealed no significant differences in the peak-to-peak amplitude of the JCR responses (Table 1), as well as the latency (data not shown).

2.5. Effect of IAN/MesV stimulation on SLN/Cx evoked swallows

The effect of the trigeminal inputs on swallowing initiation was evaluated by comparing the number of swallows with and without IAN/MesV stimulation. The number of swallows evoked by 4.0 T SLN stimulation with and without ipsilateral IAN stimulation was 4.4 ± 1.0 and 4.5 ± 0.7 (n = 12 in each group), respectively, and that with and without ipsilateral MesV stimulation was 2.6 ± 1.1 (n = 6) and 3.3 ± 1.0 (n = 12), respectively. The number of swallows evoked by 1.4 T Cx stimulation with and without ipsilateral IAN stimulation was 1.1 ± 0.1 and 1.0 ± 0.0 (n = 7 in each group), respectively, and that with and without ipsilateral MesV stimulation was 1.0 ± 0.0 (n = 5) and 1.0 ± 0.0 (n = 10), respectively. There was no difference in the number of swallows between the conditions, with and without IAN/MesV stimulation.

2.6. Histology

Finally, the stimulus sites for the Cx and MesV were identified histologically. The former was located within or near the insular cortex (Fig. 8) and the latter was within the MesV.

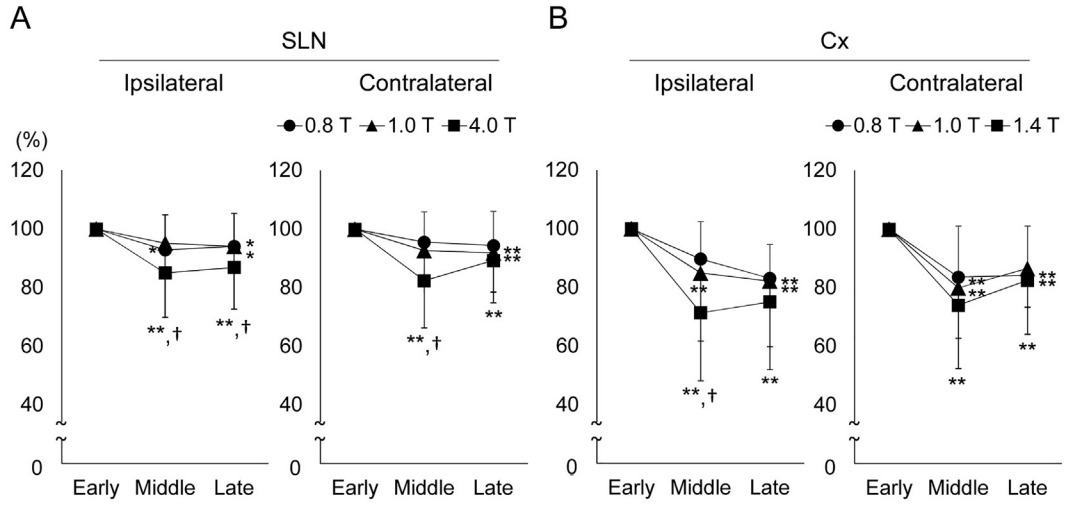

Fig. 3. Modulation of peak-to-peak amplitude of JOR during SLN (A)/Cx (B) stimulation. Values were normalized to that in the early 10-s period. A: Normalized JOR amplitude in the middle and late stages was 92.9 ± 8.2% (n = 26) in the middle 10 s and 94.1 ± 8.3% (n = 26) in the late 10 s for ipsilateral SLN stimulation at 0.8 T, 95.1 ± 9.7% (n = 26) in the middle 10 s and 94.1 ± 11.3% (n = 26) in the late 10 s for ipsilateral SLN stimulation at 1.0 T and 85.0 ± 15.2% (n = 26) in the middle 10 s and 86.9 ± 14.2% (n = 26) in the late 10 s for ipsilateral SLN stimulation at 4.0 T, and was 95.6 ± 10.3% (n = 26) in the middle 10 s and 94.4 ± 11.6% (n = 26) in the late 10 s for contralateral SLN stimulation at 0.8 T, 92.7 ± 11.8% (n = 26) in the middle 10 s and 91.9 ± 13.4% (n = 26) in the late 10 s for contralateral SLN stimulation at 1.0 T and 82.4 ± 16.1% (n = 26) in the middle 10 s and 89.3 ± 14.4% in the late 10 s for contralateral SLN stimulation at 4.0 T. B: The JOR amplitude in the middle and late stages was 89.7 ± 12.7% (n = 15) in the middle 10 s and 83.1 ± 11.5% (n = 15) in the late 10 s for ipsilateral Cx stimulation at 0.8 T, 84.9 ± 23.2% (n = 15) in the middle 10 s and 82.1 ± 22.4% (n = 15) in the late 10 s for ipsilateral Cx stimulation at 1.0 T and 71.3 ± 23.2% (n = 15) in the middle 10 s and 75.0 ± 23.1% (n = 15) in the late 10 s for ipsilateral Cx stimulation at 1.4 T, and was 83.6 ± 17.4% in the middle 10 s and 84.3 ± 16.8% (n = 15) in the late 10 s for contralateral Cx stimulation at 0.8 T, 79.9 ± 17.2% (n = 15) in the middle 10 s and 86.6 ± 13.3% (n = 15) in the late 10 s for contralateral Cx stimulation at 1.0 T and 74.0 ± 21.6% (n = 15) in the middle 10 s and 82.5 ± 18.4% (n = 15) in the late 10 s for contralateral Cx stimulation at 1.4 T. The mean JOR amplitude evoked by unilateral IAN stimulation was reduced in the middle and late 10-s periods with both ipsilateral and contralateral SLN/Cx stimulation. Greater SLN/Cx stimulation was associated with more inhibition of JOR amplitude, particularly in the middle 10 s. $^{*}p < 0.05$ vs Early, $^{**}p < 0.01$ vs early, $^{\dagger}p < 0.05$ vs 0.8 T and 1.0 T.

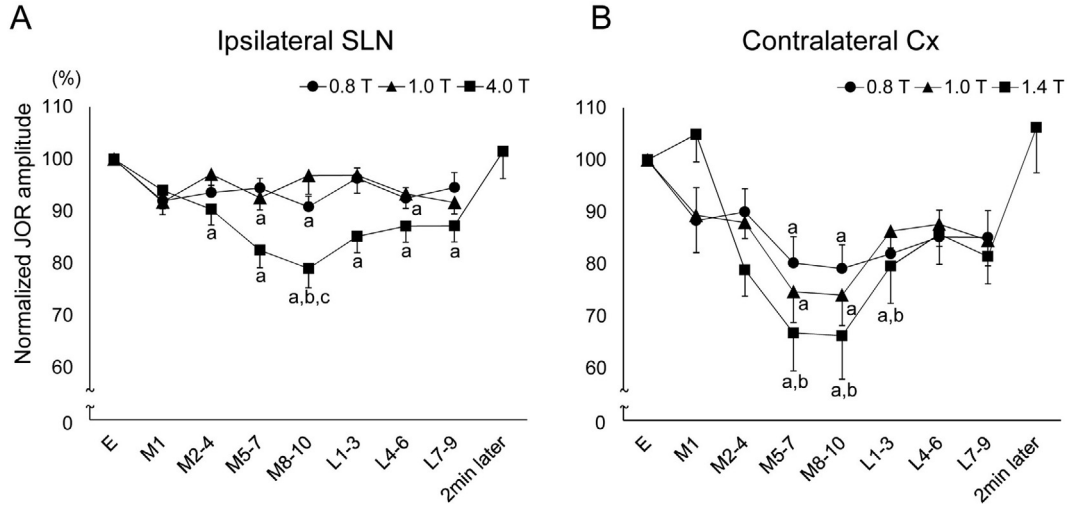

Fig. 4. Time course of modulation of the peak-to-peak amplitude of JOR. Values were normalized to those in the early (E) 10-s period (i.e., before SLN/Cx stimulation). The mean amplitudes of JORs evoked by unilateral IAN stimulation are shown during ipsilateral SLN (n = 26) (A) and contralateral Cx (B) stimulation (n = 15), respectively. M1, M2-4, M5-7, and M8-10 indicate the first, second to fourth, fifth to seventh, and eighth to tenth stimulus points in the middle 10-s period, respectively. L1-3, L4-6, and L7-9 indicate the first to third, fourth to sixth, and seventh to ninth stimulus points in the late 10-s period, respectively. It should be noted that the JOR amplitude gradually decreased during SLN/Cx stimulation, which continued after stimulation, and returned to baseline levels (i.e., before SLN/Cx stimulation) 2 min after SLN/Cx stimulation (n = 16 for SLN stimulation, n = 14 for Cx stimulation). $^{a}p < 0.05$ vs Early, $^{b}p < 0.05$ vs M1, $^{c}p < 0.05$ vs M2-4. See text for details.

3. Discussion

In this study, we examined how peripherally and centrally evoked swallowing affects jaw reflex responses in the anesthetized rabbit. The JOR was evoked at 1 Hz for 30 s, and in the middle 10 s, swallows were evoked by either SLN or Cx stimulation. The number of swallows increased and the onset latency of the first swallow decreased with increasing current intensity in the SLN while there was no difference in the number of swallows and the onset of the first swallow evoked by Cx stimulation between 1.0 T and 1.4 T. The JOR amplitude was inhibited during the middle and late 10-s periods, and the reduction rate was dependent on the current intensity of SLN/Cx stimulation; greater SLN/Cx stimulus current was associated with greater inhibition of JORs. There was no difference in JOR amplitude between ipsilateral and contralateral SLN/Cx stimulation. The amplitude returned to baseline at 2 min after 10-s SLN/Cx stimulation. The reduction rate was greater during Cx stimulation than during SLN stimulation. The effect of co-stimulation of

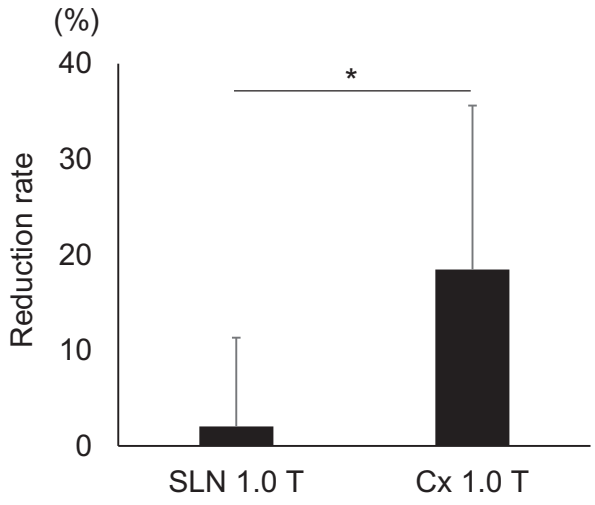

Fig. 5. Reduction of the JOR amplitude. The reduction of the JOR during 1.0 T SLN stimulation with ipsilateral IAN was 2.0 ± 9.3% and that during 1.0 T Cx stimulation with contralateral IAN was 18.5 ± 17.2% (n = 12). The inhibition was significantly greater during Cx stimulation than during SLN stimulation. $^{*}p < 0.05$.

SLN at 4.0 T and Cx at 1.0 T was greater than SLN stimulation at 4.0 T alone. Trigeminal inputs to evoke JOR/JCR did not affect swallowing initiation. Finally, there was no significant difference in the onset latency of JORs and JCRs in all cases. Possible mechanisms of these modulations are discussed below.

3.1. Function of the digastric muscle in the rabbit

We previously examined the effects of peripherally evoked swallows on JOR responses in the rabbit (Fukuhara et al., 2011; Yamada et al., 2013). Functionally, the Dig is regarded as a jaw-opening mechanism, like the mylohyoid muscle (Weijs and Dantuma, 1981). The Dig is also a hyoid elevator, and the border of the anterior and posterior bellies of the Dig are thought to be attached to the hyoid and contribute to elevation of the hyothyroid complex during swallowing. However, in rabbits, only the anterior belly of the Dig is present and its sources of innervation are rich in the trigeminal nucleus and less rich in the facial nucleus (Baisden et al., 1985). In addition, the Dig is not activated during swallowing (Naganuma et al., 2001; Yamada et al., 2013). We were, therefore, able to observe how JOR responses in the Dig are modulated during swallowing. In the current study, regardless of the occurrence of swallowing, the JOR was inhibited during SLN/Cx stimulation. Although the animal was not paralyzed in this study, we suggest that the inhibition of JOR observed in the Dig may be related to the modulation of swallow-related central neural excitability.

3.2. Cortically evoked swallowing

In the current study, electrical Cx stimulation stably evoked swallowing and most stimulus sites were identified within the insular cortex. Previous studies reported that cortical stimulation can be used to initiate swallowing in non-human animals. Sumi (Sumi, 1969) succeeded in initiating chewing and swallowing by electrical stimulation of the cortex in the anesthetized rabbit. In that study, most swallowing responses were accompanied by rhythmic jaw movements, and stimulation of only seven points of the 231 points explored produced swallowing alone. This suggests that the cortical organization involved in swallowing initiation overlaps with the cortical masticatory area. We suspect that the stimulus site differed between the current study and that of Sumi's study, although these details were not described in the previous study (Sumi, 1969). In the present study, the insular cortex was stimulated to evoke swallowing. The depth of the tip of the electrode was between 8.0 and 12.0 mm below the cortical surface, which was slightly deeper than the cortical masticatory area, which is reported to be located between 5.0 and 8.0 mm below the cortical surface (Ariyasinghe et al., 2004). Martin et al. (Martin et al., 1999) reported that swallowing alone was evoked by continuous electrical stimulation of the deep cortical area including the cortical masticatory area and face-primary motor cortex (MI) in awake monkeys. The authors reported that swallowing was more frequently evoked together with other responses, such as rhythmic jaw movements. Both Sumi and Martin et al. suggested that the Cx area that evokes swallows lies within a narrower area, while rhythmic jaw movements can readily be evoked (Martin et al., 1999; Sumi, 1969). In our preliminary experiments, rhythmic jaw movements were often observed when Cx was stimulated at 2.0 T or greater. In addition, the level of general anesthesia may affect swallowing initiation more strongly than chewing initiation, since swallowing is more susceptible to anesthesia compared with chewing. This is why the Cx stimulation intensity in the

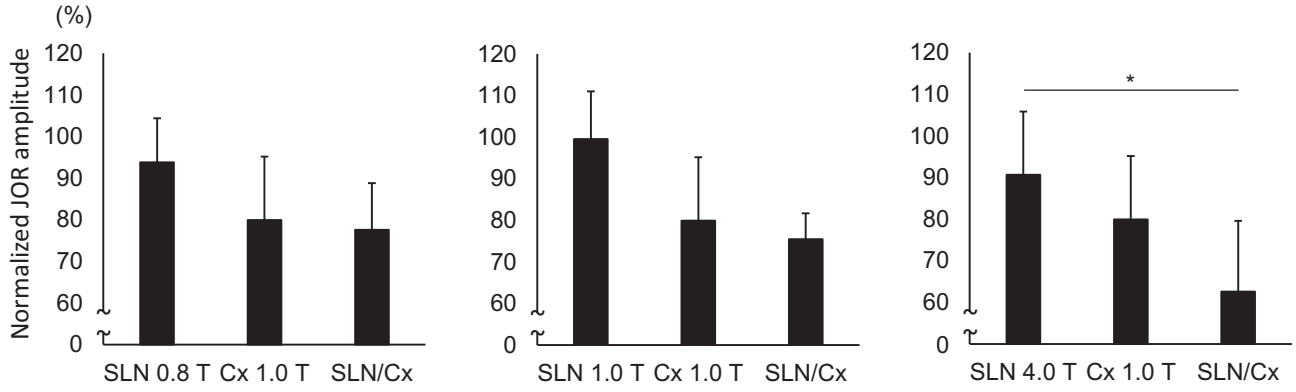

Fig. 6. Effect of co-stimulation of SLN and Cx on JOR responses. The effect on the JOR response was compared among SLN alone (0.8 T, 1.0 T, or 4.0 T), Cx (1.0 T) alone, and both SLN and Cx stimulation together (n = 6 for each group). Normalized JOR amplitude was 93.8 ± 10.6% for SLN stimulation at 0.8 T, 79.9 ± 15.2% for Cx stimulation at 1.0 T and 77.5 ± 11.2% for SLN stimulation at 0.8 T and Cx stimulation at 1.0 T (left), was 99.5 ± 11.5% for SLN stimulation at 1.0 T, 79.9 ± 15.2% for Cx stimulation at 1.0 T and 75.4 ± 6.2% for SLN stimulation at 1.0 T and Cx stimulation at 1.0 T (middle), and was 90.7 ± 15.2% for SLN stimulation at 4.0 T, 79.9 ± 15.2% for Cx stimulation at 1.0 T and 62.6 ± 17.0% for SLN stimulation at 4.0 T and Cx stimulation at 1.0 T (right). Although the order of decreased JOR amplitude was both SLN and Cx, Cx alone, then SLN alone in all cases, a significant difference was only found between 4.0 T SLN alone and 4.0 T SLN and 1.4 T Cx co-stimulation conditions. $^{*}p < 0.05$.

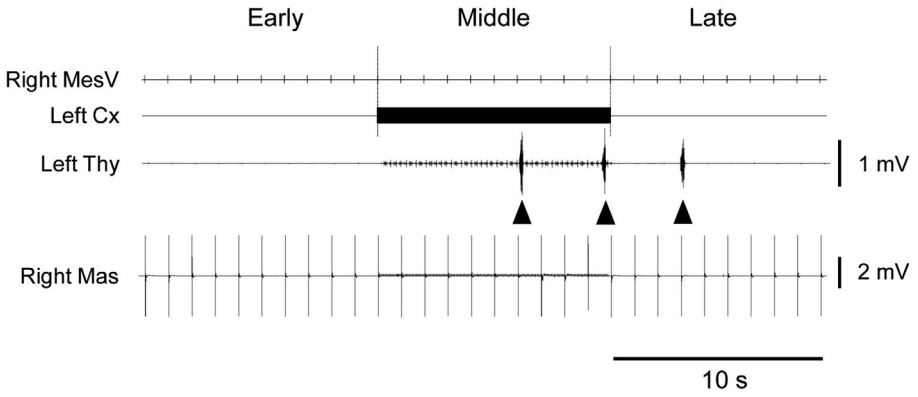

Fig. 7. Example of changes in JCR responses during Cx stimulation. The whole recording period is shown. JCRs were evoked by MesV stimulation at 1 Hz for 30 s. The stimulus intensity of the Cx was set at 1.4 T the threshold for evoking swallowing. In the middle and late 10-s periods, three swallows were evoked (shown by arrowheads). Thy, thyrohyoid muscle.

Table 1
Effect of SLN/Cx stimulation on normalized peak-to-peak amplitude of JCR (%).

	SLN (n = 6)				Cx (n = 5)			
	Ipsilateral		Contralateral		Ipsilateral		Contralateral	
	Middle	Late	Middle	Late	Middle	Late	Middle	Late
0.8 T	101.5 ± 7.0	96.0 ± 14.7	100.1 ± 7.3	96.8 ± 6.3	101.9 ± 2.7	101.1 ± 3.9	101.6 ± 4.6	100.2 ± 3.9
1.0 T	102.2 ± 7.8	98.3 ± 9.0	100.9 ± 3.5	100.3 ± 5.7	102.5 ± 7.8	102.9 ± 10.9	100.0 ± 3.4	98.5 ± 5.6
4.0 T	98.4 ± 6.0	100.8 ± 18.8	101.7 ± 5.0	101.8 ± 5.4				
1.4 T					98.1 ± 13.5	93.6 ± 19.9	99.5 ± 9.6	98.6 ± 10.8

All data are normalized to the peak-to-peak amplitude of JCR in the early 10 s (i.e., before SLN/Cx stimulation) in each animal.

current study was increased to a maximum of 1.4 T, which was substantially lower than that of SLN stimulation (4.0 T).

3.3. Effect of SLN/Cx stimulation on jaw reflex responses

In the current study, inhibition of JOR responses (i.e., a reduction of peak-to-peak amplitude) was observed not only during SLN stimulation but also Cx stimulation. Some of the current results are consistent with our previous study (Fukuhara et al., 2011); JOR responses were inhibited before the occurrence of the first swallow during SLN stimulation and the reduction of JOR responses progressed during SLN stimulation and persisted immediately after the offset of SLN stimulation. Doty (Doty, 1968) and Kajii et al. (Kajii et al., 2002) showed that swallowing reflexes persist upon discontinuation of peripheral stimulation. This may also be the case in SLN/Cx stimulation, in that SLN/Cx stimulation might inhibit JOR responses via the swallowing neural network and the after-effects on JOR responses might be attributable to sustained excitation of the swallowing neural network.

Another possible explanation for the reduction of JOR responses may be attributed to the activation of nociceptive neural network, in accord with previous studies suggesting that jaw reflexes are modulated by pain sensation (Graven-Nielsen et al., 2000; Hansen et al., 1999; Kurose et al., 2005; Mason et al., 2002; Mason et al., 2007; Svensson et al., 1998; Svensson et al., 1999; Svensson et al., 2000; Svensson et al., 2001; Svensson et al., 2003; Wang et al., 1999; Wang et al., 2000; Wang et al., 2001; Wang and Svensson, 2001; Zubrzycka et al., 2005). We previously reported that pain sensation in the masticatory muscles inhibited JOR responses (Kurose et al., 2005). In that study, the effects on JORs evoked with either noxious or innocuous inputs were evaluated. The results revealed that inhibition lasted for more than 30 min, although the effect on JORs evoked with noxious inputs was more prominent than that on JORs evoked with innocuous inputs. In the current study, the peak-to-peak amplitude returned to baseline within 2 min after the SLN/Cx stimulation. Based on these findings, we can conclude that short-term changes in JOR responses during SLN/Cx stimulation were not caused by the activation of a pain-related neural network but the activation of a swallowing-related neural network.

There was no difference in the reduction of JOR amplitude between the ipsilateral and contralateral stimulation sides. Regardless of whether the full activation of the swallowing CPG is required to initiate swallowing, increases in the activity of the swallowing-related neural network, including indirect projections from the NTS to the trigeminal nucleus, might be involved in suppression of the JOR pathway by the swallowing CPG.

It is currently unclear how the swallowing CPG modulates JOR responses. Numerous studies have reported that JORs evoked by

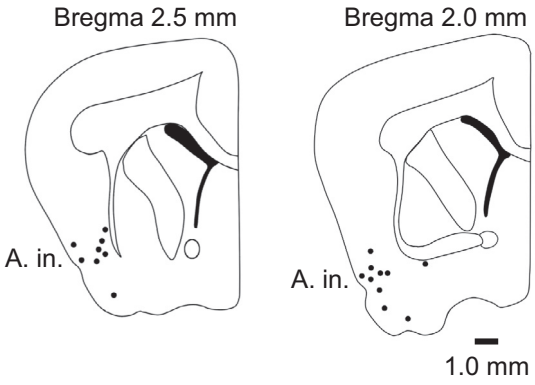

Fig. 8. Diagrams illustrating the stimulus sites for the Cx. The stimulus sites were identified in the area insularis (A. in.) and neighboring areas. The stimulus sites for both hemispheres are shown on the left side (n = 18).

trigeminal stimulation are tonically and phasically suppressed during chewing (Lund and Rossignol, 1981; Lund et al., 1983; Yamada et al., 2013). In this condition (i.e., during chewing), mastication-triggered or phase-linked modulation has been observed in the terminals of primary afferents (Kurasawa et al., 1988), and in the neuronal activity of the trigeminal nucleus (Olsson and Landgren, 1980; Olsson et al., 1988; Westberg et al., 2001), depending on the cycle phase. Further studies should be performed to clarify the underlying mechanisms of the inhibition of JOR responses during SLN/Cx stimulation.

There was no effect of SLN/Cx stimulation on JCR responses including the peak-to-peak amplitude and onset latency. Although the functional involvement of swallowing in jaw closing has not been clarified, it is likely that the JCR pathway is unaffected by SLN/Cx stimulation or swallowing.

3.4. Difference between SLN and Cx stimulation effects on JOR response

In the current study, we found that inhibitory effects were significantly greater during Cx stimulation than during SLN stimulation, although the number of swallows evoked was similar between them. If the activation of the swallowing CPG truly causes modulation of the JOR pathway, why was the reduction rate of JOR responses unrelated to the number of swallows evoked?

Central projection of afferent fibers of the SLN has been described extensively in previous studies (Furusawa et al., 1996; Hanamori and Smith, 1986; Hanamori and Smith, 1989; Pascual-Font et al., 2011; Sweazey and Bradley, 1986). Furusawa et al. (Furusawa et al., 1996) reported that SLN containing afferent fibers densely projected to the interstitial subnucleus of the nucleus of the solitary tract (NTSis) only on the ipsilateral side in the rabbit. Anatomical studies suggested that NTSis neurons may be important for initiating swallows. Based on tracing experiments, the solitary interneurons in the NTSis, intermediate NTS, ventral NTS, and ventromedial NTS have been proposed to contribute to initiation of swallowing because these neurons project to swallow-related pharyngeal motoneurons (Bieger and Hopkins, 1987; Cunningham and Sawchenko, 2000; Lang et al., 2004).

Regarding central inputs from the insular cortex to the NTS, direct projections have been reported to be bilateral with an ipsilateral predominance in the mouse (Shipley, 1982) and rat (Willett et al., 1986; Yasui et al., 1991) or with a contralateral predominance in the rat (Saper, 1982; Terreberry and Neafsey, 1983) and hamster (Whitehead et al., 2000). Saper (Saper, 1982) suggested that there are two descending pathways from the insular cortex to the NTS; one descending via the ipsilateral pyramidal tract and decussated and terminated in the contralateral NTS, and another that follows Probst's tract from the parabrachial nuclei to the NTS bilaterally. Although previous studies have produced conflicting findings regarding the projection side, it has been established that anterograde labelling is heaviest in rostral regions of the NTS. The NTSis commences just caudal to the obex and persists until the accessory trigeminal nucleus, caudal to the main mass of the motor trigeminal nucleus, suggesting the longest and most rostral representative of NTS. It should be noted that central inputs have been shown to be bilateral and projection sites mainly contain NTSis, which receive rich inputs from the afferent fibers of the SLN. It is possible that bilateral inputs from the insular cortex may be more effective at inhibiting JOR responses than ipsilateral peripheral inputs. Alternatively, the cortical masticatory area may be activated during Cx stimulation. It was previously reported that activation of the cortical masticatory area followed by rhythmic jaw movements or natural chewing strongly inhibited the JOR, as mentioned above. Thus, it is possible that stimulus current spread over the cortical masticatory area and therefore modulated JOR responses.

3.5. Functional implications

Once the swallowing CPG is excited, the upper and lower lips and jaw are closed and the tongue is elevated to propel the food bolus or liquid into the pharynx. In freely behaving animals, when bolus swallowing is initiated during natural chewing, the jaw closing movements are not interrupted. At this point, any unnecessary or weak sensory transmission from the oral cavity to evoke the JOR needs to be inhibited more strongly during chewing than at rest, to transport the bolus. Our previous results also suggest that the reduction rate of JOR amplitude is greater during chewing than that at rest (Fukuhara et al., 2011; Yamada et al., 2013). Although we do not have any direct evidence, the insular cortex may be involved in the strong inhibition of JOR responses to propel the bolus in the oral and pharyngeal cavities smoothly during swallowing when the animal chews food. Further, because we found that inhibitory effects on the JORs were relatively weak in the present study, it is not likely that persistent effects after SLN/Cx stimulation interrupt subsequent ingestive behaviors.

4. Experimental procedure

4.1. Animals

Experiments were carried out on 24 adult male Japanese white rabbits weighing between 2.0 and 2.5 kg, following the Guide for the Care and Use of Laboratory Animals (NIH Publication #86-23, revised 1996). The study was reviewed and approved by the Niigata University Intramural Animal Care and Use Committee (79-7).

4.2. Surgical preparations

Animals were anesthetized with urethane (1.0 g/kg body weight) administered intravenously via the marginal ear vein, supplemented with urethane whenever necessary to maintain anesthesia at a level at which neither corneal reflex nor spontaneous eye movements occurred. Physiological saline was administered continuously (10 ml/kg/h). The trachea was cannulated and heart rate and arterial pressure were continuously monitored. Rectal temperature was maintained at 38–39 °C with a thermostatically controlled heating pad. To eliminate the influence of saliva, the bilateral submandibular and infraorbital glands were removed and the parotid ducts were ligated.

The experimental setup for recording and stimulation is shown in Fig. 9. Bipolar urethane-coated copper wire electrodes (0.18 mm in diameter and 2.0 mm in inter-polar distance) were inserted bilaterally into the Dig, Mas, and thyrohyoid (Thy) muscles for electromyographic (EMG) recordings. To evoke the JOR and swallowing reflex, bipolar urethane-coated silver wire electrodes (0.2 mm in diameter and 2.0 mm in inter-polar distance) were inserted into the mandibular canal to stimulate the IAN, and placed on the SLN, respectively.

After the head of the animal had been fixed on a stereotaxic frame in which lambda was 1.5 mm lower than bregma according to previous studies (Inoue et al., 2002; Tsujimura et al., 2012), a concentric electrode (0.25 mm in inner diameter and 0.6 mm in outer diameter) was inserted vertically into the Cx (2.0–2.5 mm anterior and 5.5–6.5 mm lateral to bregma, and 8.0–12.0 mm below the cortical surface) to evoke central swallows. To evoke the JCR, a monopolar parylene-coated tungsten electrode (1.0 MΩ in impedance; 0.13 mm in diameter) was inserted into the MesV. The electrode was angled at 20° behind the vertical and located at 2.0 mm posterior and 2.0 mm lateral to lambda. Using this electrode to record, the location of the tip of the electrode was confirmed by observing the responses of afferents arising from

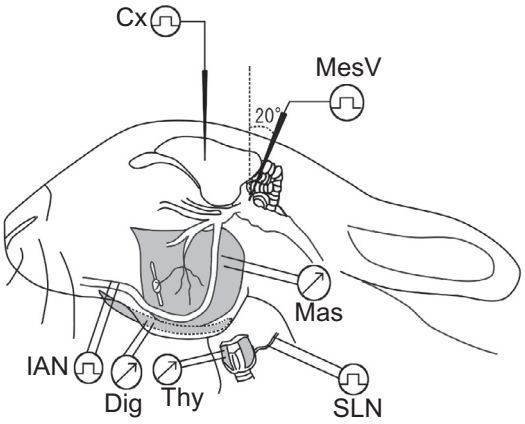

Fig. 9. Experimental setup. Stimulating electrodes were fixed in the IAN and MesV to evoke JORs and JCRs, respectively and in the SLN and Cx to evoke the peripherally and centrally evoked swallows, respectively. Recording electrodes were inserted bilaterally into the Dig, Mas, and Thy muscles.

the muscle spindles in the Mas when the jaw was passively opened.

4.3. Data collection

In one session, the JOR was evoked in the Dig using IAN stimulation (single pulse, 0.2 ms pulse duration) at 1 Hz for 30 s. The stimulus intensity was 2.0 T the threshold for eliciting the JOR.

In the middle 10 s, either the SLN or Cx was simultaneously stimulated (train pulses at 30 Hz, 0.2 ms pulse duration for SLN and 0.5 ms pulse duration for Cx) on either the ipsilateral or contralateral side of IAN stimulation to evoke swallows as a conditioning stimulation. Swallowing was identified by visual observation of the laryngeal elevation and EMG bursts of the Thy muscle. The threshold of stimulus intensity was determined as 1.0 T when swallowing was induced at least once for 10 s. The current intensity of SLN was set at 0.8, 1.0 or 4.0 T and that of Cx was set at 0.8, 1.0 or 1.4 T. The order of the stimulus intensity and side (ipsilateral or contralateral side of IAN) were randomized and the time interval between sessions was 2 min.

To evaluate the effect of inputs from the both SLN and Cx on JOR responses, the SLN and Cx were simultaneously stimulated in the middle 10 s of the recording session. In this session, the stimulus side for SLN stimulation was ipsilateral to the IAN stimulation side and the stimulus intensity was changed from 0.8 T to 4.0 T for SLN, and fixed at 1.0 T for Cx.

Another recording session was performed to evaluate changes in JCRs in the Mas evoked by MesV stimulation (single pulse, 0.2 ms pulse duration). The experimental protocol was the same as that for JORs, although we did not investigate the effect of co-stimulation of both SLN and Cx on JCRs.

Finally, we evaluated the influence of IAN/MesV stimulation on SLN/Cx-evoked swallow initiation. Either the ipsilateral SLN or contralateral Cx was stimulated for 10 s with or without IAN or MesV stimulation. Stimulus intensity was fixed at 4.0 T for the SLN and 1.4 T for the Cx.

4.4. Data analysis

EMG signals were amplified using an AM-601G (Nihon Kohden, Tokyo, Japan) with the time constant (0.03 s) and low-pass (10 kHz). They were digitized on a personal computer at a sampling rate of 10 kHz and analyzed using the Spike2 analysis package (Cambridge Electronic Design, Cambridge, UK).

We suspected that the number of swallows and onset latency of the first swallow would be dependent on the stimulus intensity of SLN/Cx. Those values were compared between 1.0 T and 4.0 T SLN stimulation and between 1.0 T and 1.4 T Cx stimulation using paired *t*-tests.

In each 10-s period (early, middle, and late 10 s) of one session, the mean peak-to-peak amplitude and onset latency of the JOR/JCR were calculated. The mean peak-to-peak amplitude was normalized to that in the early 10-s period. In the early 10-s period, the first two reflex responses were excluded because they were not stable at the very early stage.

The normalized amplitudes and latencies of the JOR and JCR were analyzed using two-way repeated measures ANOVA with period (early, middle, and late) and stimulus intensity as factors. Post hoc analysis using Tukey's test was performed if significant interactions were found between the factors. Differences in responses between the stimulus sides and between sole and simultaneous stimulations were assessed using paired *t*-tests and one-way repeated measures ANOVA with a post hoc Tukey's test, respectively.

To clarify time-dependent changes in JOR responses in one session, JORs in the middle 10 s were sub-divided into the first, second to fourth, fifth to seventh, and eighth to tenth responses and those in the last 10 s were divided into the first to third, fourth to sixth, and seventh to ninth responses. The mean peak-to-peak amplitude and onset latency of the JOR responses of each sub-period as well as those at 2 min after a 10-s conditioning stimulation were compared among the periods using one-way repeated measures ANOVA with a post hoc Tukey's test or Kruskal-Wallis one-way ANOVA on ranks with Dunn's test.

Our previous study suggested that the stimulus intensity or number of swallows was related to the inhibition rate of JOR amplitude (Fukuhara et al., 2011). To compare the inhibition rate on JOR responses between SLN and Cx stimulation, the reduction rate of JOR amplitude per swallow was compared between SLN and Cx stimulation at 1.0 T using Student's *t*-test.

To evaluate the influence of IAN/MesV stimulation on SLN/Cx-evoked swallowing initiation, the number of swallows evoked by SLN or Cx stimulation was compared between with and without simultaneous IAN or MesV stimulation using Student's *t*-tests.

Statistical analysis was performed using Sigmaplot software (Sigmaplot 12.0, Systat Software Inc., CA, USA). The results are presented as mean ± SD. Differences were considered statistically significant at $p < 0.05$.

4.5. Histology

After the physiological experiment was completed, animals were sacrificed with an overdose administration of urethane (2.0 g/kg, i.v.). The brains were removed and serial sections (50 μm thick) were cut and stained with cresyl violet. The stimulus sites were then histologically identified.

Declaration of interest

The authors declare that they have no conflicts of interest.

Acknowledgement

We thank Wendy Brooks, PhD, from Edanz Group (www.edanzediting.com/ac) for English editing of a draft of this manuscript.

Funding

This work was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (JSPS KAKENHI Grant number JP16H05522 to M.I.).

Author contributions

Conceptualization; TS, MY, SS, KT, KN, JM, TT, MI
Methodology; TS, MY, SS, KT, MI
Software; TS, MI
Validation; JM, TT, MI
Formal analysis; TS, MI
Investigation; TS, MY, SS, KT, KN, TT, MI
Resources; TS, TT, MI
Data curation; TS TT, MI
Writing; TS, TT, MI
Review; TT, JM, MI
Visualization; TS, MI
Supervision; JM, TT, MI
Administration; MI
Funding acquisition; MI.

References

Aida, S., Takeishi, R., Magara, J., Watanabe, M., Ito, K., Nakamura, Y., Tsujimura, T., Hayashi, H., Inoue, M., 2015. Peripheral and central control of swallowing initiation in healthy humans. Physiol. Behav. 151, 401–411.
Ariyasinghe, S., Inoue, M., Yamamura, K., Harasawa, Y., Kurose, M., Yamada, Y., 2004. Coordination of jaw and extrinsic tongue muscle activity during rhythmic jaw movements in anesthetized rabbits. Brain Res. in press.
Baisden, R.H., Woodruff, M.L., Whittington, D.L., Benson, A.E., 1985. The motor innervation of the single-bellied digastric muscle in the rabbit: a retrograde horseradish peroxidase study. Neurosci. Lett. 56, 129–136.
Bieger, D., Hopkins, D.A., 1987. Viscerotopic representation of the upper alimentary tract in the medulla oblongata in the rat: the nucleus ambiguus. J. Comp. Neurol. 262, 546–562.
Cunningham Jr., E.T., Sawchenko, P.E., 2000. Dorsal medullary pathways subserving oromotor reflexes in the rat: implications for the central neural control of swallowing. J. Comp. Neurol. 417, 448–466.
Doty, R.W., 1968. Neural organization of deglutition. In: Handbook of Physiology. The Alimentary Canal. Am Physiol Soc, Washington D. C., pp. 1861–1902.
Dziewas, R., Soros, P., Ishii, R., Chau, W., Henningsen, H., Ringelstein, E.B., Knecht, S., Pantev, C., 2003. Neuroimaging evidence for cortical involvement in the preparation and in the act of swallowing. Neuroimage 20, 135–144.
Fukuhara, T., Tsujimura, T., Kajii, Y., Yamamura, K., Inoue, M., 2011. Effects of electrical stimulation of the superior laryngeal nerve on the jaw-opening reflex. Brain Res. 1391, 44–53.
Furusawa, K., Yasuda, K., Okuda, D., Tanaka, M., Yamaoka, M., 1996. Central distribution and peripheral functional properties of afferent and efferent components of the superior laryngeal nerve: morphological and electrophysiological studies in the rat. J. Comp. Neurol. 375, 147–156.
Graven-Nielsen, T., Svensson, P., McMillan, A.S., Arendt-Nielsen, L., 2000. Quantitative analysis of reflex inhibition in single motor units in human masseter muscle: effects of stimulus intensity. Muscle Nerve 23, 259–266.
Hamdy, S., Mikulis, D.J., Crawley, A., Xue, S., Lau, H., Henry, S., Diamant, N.E., 1999a. Cortical activation during human volitional swallowing: an event-related fMRI study. Am. J. Physiol. 277, G219–G225.
Hamdy, S., Rothwell, J.C., Brooks, D.J., Bailey, D., Aziz, Q., Thompson, D.G., Mikulis, D. J., Crawley, A., Xue, S., Lau, H., Henry, S., Diamant, N.E., 1999b. Identification of the cerebral loci processing human swallowing with H2(15)O PET activation. J. Neurophysiol. 81, 1917–1926.
Hanamori, T., Smith, D.V., 1986. Central projections of the hamster superior laryngeal nerve. Brain Res. Bull. 16, 271–279.
Hanamori, T., Smith, D.V., 1989. Gustatory innervation in the rabbit: central distribution of sensory and motor components of the chorda tympani, glossopharyngeal, and superior laryngeal nerves. J. Comp. Neurol. 282, 1–14.
Hansen, P.O., Svensson, P., Arendt-Nielsen, L., Jensen, T.S., 1999. Relation between perceived stimulus intensity and exteroceptive reflex responses in the human masseter muscles. Clin. Neurophysiol. 110, 1290–1296.
Hiraoka, K., 2004. Movement-related cortical potentials associated with saliva and water bolus swallowing. Dysphagia 19, 155–159.
Humbert, I.A., Robbins, J., 2007. Normal swallowing and functional magnetic resonance imaging: a systematic review. Dysphagia 22, 266–275.
Inoue, M., Nozawa-Inoue, K., Donga, R., Yamada, Y., 2002. Convergence of selected inputs from sensory afferents to trigeminal premotor neurons with possible projections to masseter motoneurons in the rabbit. Brain Res. 957, 183–191.
Jean, A., 2001. Brain stem control of swallowing: neuronal network and cellular mechanisms. Physiol. Rev. 81, 929–969.
Kajii, Y., Shingai, T., Kitagawa, J., Takahashi, Y., Taguchi, Y., Noda, T., Yamada, Y., 2002. Sour taste stimulation facilitates reflex swallowing from the pharynx and larynx in the rat. Physiol. Behav. 77, 321–325.
Kurasawa, I., Hirose, Y., Sunada, T., Nakamura, Y., 1988. Phase-linked modulation of excitability of presynaptic terminals of low-threshold afferent fibers in the inferior alveolar nerve during cortically induced fictive mastication in the guinea pig. Brain Res. 446, 113–120.
Kurose, M., Yamamura, K., Noguchi, M., Inoue, M., Ootaki, S., Yamada, Y., 2005. Modulation of jaw reflexes induced by noxious stimulation to the muscle in anesthetized rats. Brain Res. 1041, 72–86.
Lang, I.M., Dean, C., Medda, B.K., Aslam, M., Shaker, R., 2004. Differential activation of medullary vagal nuclei during different phases of swallowing in the cat. Brain Res. 1014, 145–163.
Lund, J.P., Rossignol, S., 1981. Modulation of the amplitude of the digastric jaw opening reflex during the masticatory cycle. Neuroscience 6, 95–98.
Lund, J.P., Rossignol, S., Murakami, T., 1981. Interactions between the jaw-opening reflex and mastication. Can. J. Physiol. Pharmacol. 59, 683–690.
Lund, J.P., Enomoto, S., Hayashi, H., Hiraba, K., Katoh, M., Nakamura, Y., Sahara, Y., Taira, M., 1983. Phase-linked variations in the amplitude of the digastric nerve jaw-opening reflex response during fictive mastication in the rabbit. Can. J. Physiol. Pharmacol. 61, 1122–1128.
Martin, R.E., Sessle, B.J., 1993. The role of the cerebral cortex in swallowing. Dysphagia 8, 195–202.
Martin, R.E., Murray, G.M., Kemppainen, P., Masuda, Y., Sessle, B.J., 1997. Functional properties of neurons in the primate tongue primary motor cortex during swallowing. J. Neurophysiol. 78, 1516–1530.
Martin, R.E., Kemppainen, P., Masuda, Y., Yao, D., Murray, G.M., Sessle, B.J., 1999. Features of cortically evoked swallowing in the awake primate (Macaca fascicularis). J. Neurophysiol. 82, 1529–1541.
Martin, R.E., Goodyear, B.G., Gati, J.S., Menon, R.S., 2001. Cerebral cortical representation of automatic and volitional swallowing in humans. J. Neurophysiol. 85, 938–950.
Martin, R.E., MacIntosh, B.J., Smith, R.C., Barr, A.M., Stevens, T.K., Gati, J.S., Menon, R. S., 2004. Cerebral areas processing swallowing and tongue movement are overlapping but distinct: a functional magnetic resonance imaging study. J. Neurophysiol. 92, 2428–2443.
Mason, A.G., Scott, B.J., van der Glas, H.W., Linden, R.W., Cadden, S.W., 2002. Remote noxious stimuli modulate jaw reflexes evoked by activation of periodontal ligament mechanoreceptors in man. Exp. Physiol. 87, 699–706.
Mason, A.G., Newton, J.P., Cadden, S.W., 2007. Modulation of an inhibitory jaw reflex by remote noxious stimulation: effects of spatial conditioning factors. Eur. J. Oral Sci. 115, 371–377.
Miller, A.J., 1982. Deglutition. Physiol. Rev. 62, 129–184.
Naganuma, K., Inoue, M., Yamamura, K., Hanada, K., Yamada, Y., 2001. Tongue and jaw muscle activities during chewing and swallowing in freely behaving rabbits. Brain Res. 915, 185–194.
Nakamura, Y., Katakura, N., 1995. Generation of masticatory rhythm in the brainstem. Neurosci. Res. 23, 1–19.
Narita, N., Yamamura, K., Yao, D., Martin, R.E., Sessle, B.J., 1999. Effects of functional disruption of lateral pericentral cerebral cortex on primate swallowing. Brain Res. 824, 140–145.
Olsson, K.A., Landgren, S., 1980. Facilitation and inhibition of jaw reflexes evoked by electrical stimulation of the cat's cerebral cortex. Exp. Brain Res. 39, 149–164.
Olsson, K.A., Lund, J.P., Valiquette, C., Veilleux, D., 1988. Activity during mastication of periodontal mechanosensitive neurons of the trigeminal subnucleus oralis of the rabbit. J. Neurophysiol. 59, 341–357.
Pascual-Font, A., Hernandez-Morato, I., McHanwell, S., Vazquez, T., Maranillo, E., Sanudo, J., Valderrama-Canales, F.J., 2011. The central projections of the laryngeal nerves in the rat. J. Anat. 219, 217–228.
Saper, C.B., 1982. Convergence of autonomic and limbic connections in the insular cortex of the rat. J. Comp. Neurol. 210, 163–173.
Shipley, M.T., 1982. Insular cortex projection to the nucleus of the solitary tract and brainstem visceromotor regions in the mouse. Brain Res. Bull. 8, 139–148.
Soros, P., Inamoto, Y., Martin, R.E., 2009. Functional brain imaging of swallowing: an activation likelihood estimation meta-analysis. Hum. Brain Mapp. 30, 2426–2439.
Sumi, T., 1969. Some properties of cortically-evoked swallowing and chewing in rabbits. Brain Res. 15, 107–120.
Suzuki, M., Asada, Y., Ito, J., Hayashi, K., Inoue, H., Kitano, H., 2003. Activation of cerebellum and basal ganglia on volitional swallowing detected by functional magnetic resonance imaging. Dysphagia 18, 71–77.
Svensson, P., De Laat, A., Graven-Nielsen, T., Arendt-Nielsen, L., 1998. Experimental jaw-muscle pain does not change heteronymous H-reflexes in the human temporalis muscle. Exp. Brain Res. 121, 311–318.
Svensson, P., McMillan, A.S., Graven-Nielsen, T., Wang, K., Arendt-Nielsen, L., 1999. Modulation of an inhibitory reflex in single motor units in human masseter by tonic painful stimulation. Pain 83, 441–446.
Svensson, P., Miles, T.S., Graven-Nielsen, T., Arendt-Nielsen, L., 2000. Modulation of stretch-evoked reflexes in single motor units in human masseter muscle by experimental pain. Exp. Brain Res. 132, 65–71.

Svensson, P., Macaluso, G.M., De Laat, A., Wang, K., 2001. Effects of local and remote muscle pain on human jaw reflexes evoked by fast stretches at different clenching levels. Exp. Brain Res. 139, 495–502.
Svensson, P., Wang, K., Arendt-Nielsen, L., 2003. Effect of muscle relaxants on experimental jaw-muscle pain and jaw-stretch reflexes: a double-blind and placebo-controlled trial. Eur. J. Pain 7, 449–456.
Sweazey, R.D., Bradley, R.M., 1986. Central connections of the lingual-tonsillar branch of the glossopharyngeal nerve and the superior laryngeal nerve in lamb. J. Comp. Neurol. 245, 471–482.
Teismann, I.K., Dziewas, R., Steinstraeter, O., Pantev, C., 2009. Time-dependent hemispheric shift of the cortical control of volitional swallowing. Hum. Brain Mapp. 30, 92–100.
Terreberry, R.R., Neafsey, E.J., 1983. Rat medial frontal cortex: a visceral motor region with a direct projection to the solitary nucleus. Brain Res. 278, 245–249.
Tsujimura, T., Yamada, A., Nakamura, Y., Fukuhara, T., Yamamura, K., Inoue, M., 2012. The digastric muscle is less involved in pharyngeal swallowing in rabbits. Dysphagia 27, 271–276.
Tsujimura, T., Tsuji, K., Magara, J., Sakai, S., Suzuki, T., Nakamura, Y., Nozawa-Inoue, K., Inoue, M., 2016. Differential response properties of peripherally and cortically evoked swallows by electrical stimulation in anesthetized rats. Brain Res. Bull. 122, 12–18.
Tsukano, H., Taniguchi, H., Hori, K., Tsujimura, T., Nakamura, Y., Inoue, M., 2012. Individual-dependent effects of pharyngeal electrical stimulation on swallowing in healthy humans. Physiol. Behav. 106, 218–223.
Wang, K., Svensson, P., Arendt-Nielsen, L., 1999. Modulation of exteroceptive suppression periods in human jaw-closing muscles by local and remote experimental muscle pain. Pain 82, 253–262.
Wang, K., Svensson, P., Arendt-Nielsen, L., 2000. Effect of tonic muscle pain on short-latency jaw-stretch reflexes in humans. Pain 88, 189–197.
Wang, K., Arendt-Nielsen, L., Svensson, P., 2001. Excitatory actions of experimental muscle pain on early and late components of human jaw stretch reflexes. Arch. Oral Biol. 46, 433–442.
Wang, K., Svensson, P., 2001. Influence of methodological parameters on human jaw-stretch reflexes. Eur. J. Oral Sci. 109, 86–94.
Weijs, W.A., Dantuma, R., 1981. Functional anatomy of the masticatory apparatus in the rabbit. Netherlands J. Zool. 31, 99–147.
Westberg, K.G., Scott, G., Olsson, K.A., Lund, J.P., 2001. Discharge patterns of neurons in the medial pontobulbar reticular formation during fictive mastication in the rabbit. Eur. J. Neurosci. 14, 1709–1718.
Whitehead, M.C., Bergula, A., Holliday, K., 2000. Forebrain projections to the rostral nucleus of the solitary tract in the hamster. J. Comp. Neurol. 422, 429–447.
Willett, C.J., Gwyn, D.G., Rutherford, J.G., Leslie, R.A., 1986. Cortical projections to the nucleus of the tractus solitarius: an HRP study in the cat. Brain Res. Bull. 16, 497–505.
Yamada, A., Kajii, Y., Sakai, S., Tsujimura, T., Nakamura, Y., Ariyasinghe, S., Magara, J., Inoue, M., 2013. Effects of chewing and swallowing behavior on jaw opening reflex responses in freely feeding rabbits. Neurosci. Lett. 535, 73–77.
Yasui, Y., Itoh, K., Kaneko, T., Shigemoto, R., Mizuno, N., 1991. Topographical projections from the cerebral cortex to the nucleus of the solitary tract in the cat. Exp. Brain Res. 85, 75–84.
Zald, D.H., Pardo, J.V., 1999. The functional neuroanatomy of voluntary swallowing. Ann. Neurol. 46, 281–286.
Zubrzycka, M., Fichna, J., Janecka, A., 2005. Inhibition of trigemino-hypoglossal reflex in rats by oxytocin is mediated by mu and kappa opioid receptors. Brain Res. 1035, 67–72.