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坂詰　智仁

Stimulation of the center of the lateral reticular nucleus suppresses the swallowing reflex in the rats

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Abstract

Morphological studies have demonstrated that the lateral reticular nucleus (LRt) receives fibers projected from sites that are related to control of the swallowing reflex. Although the LRt may therefore be related to control of the swallowing reflex, the functional role of the LRt in the swallowing reflex remains unknown. The present study examined whether the swallowing reflex is modulated by stimulation of the LRt. These experiments were performed on rats anesthetized by urethane. The swallowing reflex was evoked by repetitive electrical stimulation of the superior laryngeal nerve (SLN) and was identified by electromyographic activities from the mylohyoid muscle. Electrical stimulation was applied to the LRt or glutamate was injected into the LRt. The number of swallows was reduced, and the latency of the onset of the first swallow was increased during electrical stimulation near the middle of the rostrocaudal direction of the LRt. The number of swallows was reduced, and the latency of onset of the first swallow increased after microinjection of glutamate near the rostrocaudal center of the LRt. The present study suggests that the LRt is involved in control of the swallowing reflex.

Keywords: swallowing reflex; superior laryngeal nerve; lateral reticular nucleus; electrical stimulation; glutamate injection, rats

1. Introduction

The swallowing central pattern generator (CPG) induces swallowing and forms movement patterns. The swallowing CPG consists of dorsal and ventral swallowing groups. The former is the solitary tract nucleus (NTS) and the reticular formation around it. The latter is the lateral reticular formation (LRF) near the nucleus ambiguus (Fig. 1) [1]. The neural pathways of swallowing in the brainstem have not been fully elucidated.

The lateral reticular nucleus (LRt) is located in the medulla oblongata. Morphological studies have demonstrated that the LRt receives projections from the contralateral side of the red nucleus (RN) [2], the paratrigeminal nucleus [3], the nucleus raphe magnus [4], and the periaqueductal grey (PAG) [5]. The LRt projects to the PAG [6], the lateral vestibular nucleus (LVN) [7]. Our previous study demonstrated that the swallowing reflex was suppressed by stimulation of the RN [8]. It found that the paratrigeminal nucleus was involved in the suppression of the swallowing reflex by noxious stimulation of the orofacial region [9]. Stimulation of the PAG or the nucleus raphe magnus suppressed the swallowing reflex (Fig. 1) [10, 11].

Dysphagia is one of the symptoms caused by spinocerebellar degeneration [12]. Spinocerebellar ataxias type 3 and type 4 are due to spinocerebellar degeneration. The neuropathological findings in spinocerebellar ataxias type 3 and type 4 are of degeneration in the NTS, the LRt, the RN, the PAG, the LVN, and the nucleus raphe magnus [12, 13].

According to the above studies, it appears that the LRt is involved in the control of swallowing. The purpose of this study was to investigate whether the swallowing reflex is modulated by stimulation of the LRt.

2. Material and methods

Twenty-nine male Sprague-Dawley rats aged 8−9 weeks and weighing 281-364 g were used. This study was approved by the Laboratory Animal Committee of The Nippon Dental University School of Life Dentistry at Niigata (approval number 186) and was performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The rats were anesthetized with urethane (1.3 g/kg, i.p.). Supplementary urethane (0.2 g/kg, i.p.) was also administered when the withdrawal reflex was evoked by noxious stimulation of the hindlimb. The cervical trachea was cannulated to secure the airway. Rectal temperature was controlled to approximately 37 °C by a body temperature control device (ATC-402, UNIQUE MEDICAL Co. Ltd., Tokyo, Japan). The heart and respiratory rates were recorded automatically by the body temperature control device via a vibration sensor in the heating pad. During the experiment, the heart and respiratory rates were recorded to verify the condition of the animals, maintaining a heart rate of 250-450 beats/min and a respiratory rate of 70-115 breaths/min [14].

A midline incision was made along the ventral aspects from the mandible to the rostral portion of the neck. The anterior belly of the digastric muscle was exposed. To record the

electromyograms (EMGs), pairs of Teflon-coated silver wires (diameter 0.1 mm, exposed tip 2.0 mm) were inserted bilaterally using a 26-gauge needle into the mylohyoid muscle (Myl) running beneath the anterior belly of the digastric muscle. The sternohyoid muscle on the hyoid bone side was cut and was inverted to expose the superior laryngeal nerve (SLN). Bipolar stimulating silver-wire electrodes (diameter 0.1 mm) were hooked on both sides of the SLN. The swallowing reflex was induced by electrical stimulation (0.2 ms duration, 30 Hz, 10 s) of the SLN and identified by visual observation of the laryngeal elevation and by the EMG burst in the Myl muscle. The stimulus parameters used were based on those applied in a previous study of the swallowing reflex [15]. The SLN threshold for evoking the swallowing reflex was determined as the minimum stimulus intensity that evoked swallowing at least once with a 10-s stimulus. The SLN stimulus intensity was set at 1.2 times the threshold for evoking the swallowing reflex.

After the head of the rat was fixed in a stereotaxic frame, part of the occipital bone was removed using a forceps and a dental drill to expose the surface of the cerebellum. The caudal half of the cerebellum was removed by suction to expose the medulla. A bipolar concentric electrode (outer diameter 200 μm) (TK213-091, UNIQUE MEDICAL Co. Ltd.) was set stereotaxically 1.0-1.3 mm rostral from the obex and 1.6-1.9 mm lateral from the obex, and it was inserted 3.5-3.6 mm from the surface of the medulla toward both sides of the LRt in 12 rats. First, the left or right side SLN alone was stimulated (pre-control). Next, one minute later, the SLN and LRt were stimulated at the same time to investigate the

effect of LRt stimulation on the swallowing reflex. Repetitive electrical stimulation (0.2 ms duration, 30 Hz, 100 μA, for 10 s) was applied to the LRt or around the LRt ipsilateral to the SLN stimulation. The stimulus intensity of the LRt was started from 200 μA and was decreased by 50 μA. In three rats, swallowing was not induced when the intensity of the LRt stimulus was 200 or 150 μA. In addition, when the intensity of the LRt stimulus was 50 μA, the number of swallows was almost the same as in pre-control. Therefore, the stimulus intensity for the LRt was set to be 100 μA. Finally, one minute later, the SLN alone was stimulated again (post-control) same as pre-control to confirm that whether the number of swallows returned to level of pre-control. The process from the pre-control to the post-control was implemented twice for one rat, and the number of swallows and the onset latency of the first swallow were averaged to determine the value for one rat.

In chemical stimulation experiments, a stainless pipe (TK212-010b, Unique Medical Co. Ltd.), attached to a microelectrode, was filled with sodium L-glutamate dissolved in 0.9% physiological saline (0.1 mM). A stainless pipe and a 1-μl Hamilton syringe were attached with a polyethylene tube, and they were inserted stereotaxically as in the electrical stimulation experiments toward the right LRt. Because it was technically difficult to insert pipes on both sides of the LRt, the pipes were inserted only on the right side of the LRt. Right SLN stimulation (0.2 ms duration, 30 Hz, for 10 s) was performed in 17 rats as a control. Next, 20 seconds later, glutamate was injected (0.1 μl, injection duration 90 s) into the LRt. The swallowing reflexes were recorded at 0, 2, 5, 10, 15, 20, 25, 30, 35, and 40

min after the injection was finished.

The EMG of the Myl muscle was amplified through a biophysical amplifier with the aid of a high-cut filter (1 kHz) and a low-cut filter (10 Hz) and then stored on a computer disk. The EMG recording data were analyzed using the Spike2 analysis package (Cambridge Electronic Design, Cambridge, UK) at a sampling rate of 2 kHz. In each recording session, the number of swallows, the onset latency of the first swallow (the time from the onset of the SLN stimulus to the peak of the EMG burst), the duration of EMG bursts, and the peak-to-peak amplitude of the EMG were measured. The duration of EMG bursts was calculated as the time duration over the mean + 2SD of background activity recorded from the stable 3 s prior to SLN stimulation. The effects of electrical stimulation of the LRt were assessed by the Wilcoxon signed-rank test with Bonferroni correction, followed by Friedman's test as a post hoc test. The Wilcoxon signed-rank test was used to analyze the effects of glutamate injection into the LRt. Differences were considered significant at the $P < 0.05$ level.

When the swallowing recordings were complete, the animals were given a lethal dose of urethane. After euthanasia of the animals, a passing negative direct current of 20 μA for 90 s was applied through the electrode to create electrolytic lesions. The brainstem was taken out of the head and fixed in 10% buffered formalin solution for 3-5 days. Serial coronal sections were cut (60-μm-thick), followed by Nissl staining. The stimulation and injection sites were verified using a standard atlas [16].

3. Results

The threshold for evoking the swallowing reflex by stimulating the SLN was 11-300 μA (92.4 ± 81.0 μA, mean ± SD, n = 31). In some cases, the threshold of the SLN for evoking the swallowing reflex was increased during recording of the swallowing reflex. The SLN alone was stimulated as a post-control following the SLN and LRt being stimulated at the same time to confirm that the suppression of the swallowing reflex was caused by the LRt stimulation and not by the increase in the SLN threshold. When the number of swallows in the post-control was lower than during electrical stimulation of the LRt, or when the number of swallows continued to decrease and did not return to the condition prior to injection 40 min after injection of glutamate into the LRt, no data were collected, because there is a possibility that the reduction in the number of swallows during the LRt stimulation is due to a continuously increasing SLN threshold. When the number of swallows in the post-control was lower than during electrical stimulation of the area outside the LRt, or when the number of swallows continued to decrease after injection of glutamate into the area outside the LRt, no data were collected.

3.1. Stimulation sites

The histological locations of electrical stimulation and of glutamate microinjection sites are shown in Fig. 2 and Fig. 3. The electrical stimulation sites that showed suppressive

effects on the swallowing reflex were mainly located near the middle of the LRt in the rostrocaudal direction and the dorsal border of the LRt (open circles); regions dorsal or ventral to the LRt were not effective (filled circles) (Fig. 2A). The effective sites for glutamate microinjection were located near the middle of the LRt in the rostrocaudal direction (open circles), whereas the regions dorsal or ventral to the LRt were not effective (filled circles) (Fig. 2B).

3.2. Electrical stimulation

The swallowing reflex was suppressed by the LRt stimulation (Fig. 4A). The number of swallows was significantly reduced ($P = 0.005$) during LRt stimulation compared to that with no stimulation (Fig. 4B, left). No significant difference was found in the number of swallows between the pre-control and the post-control. The onset latency of the first swallow was significantly altered ($P = 0.009$) during LRt stimulation (Fig. 4B, right). No significant difference was found in the onset latency of the first swallow between the pre-control and the post-control. Individual data and stimulation sites are shown in Table 1. The suppressive effect tended to be stronger at the rostral stimulation sites than at the caudal stimulation sites. No significant difference was found in the inter-swallow interval between the pre-control and the post-control. The duration of Myl EMG bursts was not significantly different during LRt stimulation compared to that with no stimulation (Fig. 4C, left). The peak-to-peak amplitude of the Myl EMG was not changed by stimulation of

the LRt (Fig. 4C, right).

3.3. Chemical stimulation

The swallowing reflex was also suppressed after glutamate injection into the LRt (Fig. 5A). The number of swallows was significantly lower at 0 ($P = 0.018$), 2 ($P = 0.009$), 5 ($P = 0.009$), 10 ($P = 0.022$), 20 ($P = 0.015$), and 25 min ($P = 0.030$) after injection than before the injection (Fig. 5B, left). Of 8 rats, the swallowing reflexes were not evoked from 2 to 15 min after injection in one rat. Accordingly, the latency of the onset of the first swallow could not be measured in this rat. The onset latency of the first swallow was also significantly longer ($P = 0.046$) at 10 min after injection (Fig. 5B, right). Individual data and stimulation sites are shown in Table 2. The suppressive effect tended to be stronger at the central rostral stimulation site than at the dorsal or ventral sites.

3.4 Stimulation exterior to the LRt

The swallowing reflex was not suppressed or facilitated by electrical stimulation outside the LRt (n=8). No significant difference was found in the number of swallows or in the onset latency of the first swallow with and without stimulation exterior to the LRt (Fig. 6A).

No significant difference was found in the number of swallows before and after glutamate injection into the exterior of the LRt ($n = 9$). The onset latency of the first

swallow was significantly longer at 2 min (P = 0.033) after injection into the exterior of the LRt (Fig. 6B).

4. Discussion

4.1. Methodological considerations

The swallowing reflex was suppressed by electrical and chemical stimulation of the area near the center of the LRt. Electrical stimuli of the dorsolateral LRt, however, would be predicted to affect the rubrospinal tract by current spread (-14.28 and -14.40 mm from the bregma; Fig. 2A). The rubrospinal tract descends from the red nucleus to the spinal cord [17]. Our previous study showed that the swallowing reflex was suppressed by stimulation of the red nucleus [8]. There is a possibility that the suppression of the swallowing reflex by electrical stimulation of the dorsolateral LRt is caused by stimulation of the rubrospinal tract. Microinjection of glutamate into the LRt suppressed the swallowing reflex in the present study. These results suggest that suppression of the swallowing reflex by electrical stimulation of the LRt is caused by excitation of the cell bodies in the LRt, rather than excitation of the rubrospinal tract.

When the intermediate reticular formation (IRt) was stimulated electrically (-14.16 mm from the bregma; Fig. 2A), the swallowing reflex was not modulated. The previous study demonstrated that the neurons in the IRt are involved in swallow pattern generation [18]. These neurons are present in the rostral IRt located dorsally to the facial nucleus. Since the

stimulation site in this study was the caudal IRt, the swallowing reflex may not be affected.

4.2. Neural mechanism of the reduction in the number of swallows

The number of swallows was significantly lower during stimulation of the area near the center of the LRt in the present study. To the best of our knowledge, there are no reports that the LRt projects directly to the LRF above the nucleus ambiguus. The LRt projects to the lateral (dentate) cerebellar nucleus (Lat) [6, 7], and the Lat projects to the LRF [19]. A physiological study demonstrated that the number of swallows was decreased by microinjection of a $GABA_A$ agonist into the LRF [20]. GABAergic inputs from the Lat by excitation of the LRt may reduce activation in the LRF. The LRF receives input from the NTS and sends commands to the swallowing-related cranial motor nerves bilaterally [21]. The excitation of the LRt may therefore inhibit the activity of the LRF via the Lat and reduce the number of swallows.

4.3. Neural mechanism of the extension of onset latency

In the present study, the onset latency of the first swallow was significantly longer with stimulation near the center of the LRt. Nevertheless, a direct inhibitory projection from the LRt to the NTS has not been reported. We assume that the following neuronal pathways are involved in the extension of onset latency by stimulation of the LRt (Fig. 7). The first is the neural pathway from the LRt to the NTS via the LVN and the parabrachial

nucleus (PBN). The LRt projects to the LVN [6, 7], and the LVN projects to the PBN [22]. The PBN has an inhibitory projection to the NTS [23]. The SLN-induced swallowing reflex was suppressed by stimulation of the ventrolateral part of the PBN [24]. Moreover, lesions in the PBN facilitated spontaneous swallowing [25].

The second is the neural pathway from the LRt to the NTS via the LVN and the PAG, or via the PAG without the LVN. The LVN projects to the PAG [26]. There is a direct projection from the LRt to the PAG as the other pathway [27]. The PAG projects to the NTS [28]. Stimulation of the PAG suppresses the swallowing reflex [11].

The third is the neural pathway from the LRt to the NTS via the vestibular nuclei. The LVN has reciprocal connections with the medial vestibular nucleus (MVN) [29]. There is a glutamatergic and GABAergic projection from the MVN to the NTS in the rat [30]. The GABAergic projection from the MVN to the NTS may be activated by stimulation of the LRt.

The fourth is the neural pathway from the LRt to the NTS via the LVN and the spinal trigeminal nucleus. The LVN projects to the spinal trigeminal nucleus, oral part (Vo) [31]. The Vo has intranuclear projections to the spinal trigeminal nucleus, caudal part (Vc) [32]. In addition, the Vc projects to the NTS [33].

4.5. Differences in the suppressive effect depending on stimulation site

The suppressive effect tended to be stronger at the rostral stimulation sites than at the

caudal stimulation sites (Table 1 and Table 2). Our previous study demonstrated that the swallowing reflex was suppressed by stimulation of the RN [8]. The RN projected to the rostral two-thirds of the LRt in the rat [2]. Differences in the morphological projection patterns may be related to differences in suppressive effects.

4.5. Relation to respiration

The rostral ventral respiratory group (RVRG) is dorsal to the LRt [17]. The LRt and the RVRG contain both inspiratory and expiratory neurons [34]. There is a deep relationship between swallowing and respiration [19, 35]. The swallowing reflex was not modulated by electrical and chemical stimulation of the dorsal sites to the LRt in the present study (Fig. 2). Therefore, suppression of the swallowing reflex appears to be induced by excitation of the LRt and is not influenced by respiration.

4.6. Limitations of this study

The swallowing reflex was suppressed within 5 min after glutamate injection into the LRt in 7 cases. However, the number of swallows decreased at 20 min after injection into the deepest of the LRt (diamond symbols in Fig. 5B). In addition, the number of swallows decreased at 25 min after injection into the ventrolateral to the LRt (cross symbols in Fig. 6B). These differences might be attributed to the spread of glutamate injections in and

around the LRt. Thus, further experiments using dyes are required to evaluate the spread of the injected solution.

Studies on stimulation of the vestibular and trigeminal nuclei are required to further investigate the effects of LRt stimulation on the swallowing system. Two electrodes cannot be inserted into the LRt and the vestibular nucleus or the spinal trigeminal nucleus, because they are close to each other. A new stimulation method must be devised.

4.7. Conclusion

In the present study, the number of swallows was decreased, and the onset latency of the first swallow was increased by electrical and chemical stimulation of the LRt. These results suggest that the LRt is involved in the control of swallowing. It is thought that the LRt indirectly inhibits the dorsal and ventral swallowing groups in the swallowing CPG through four neural pathways.

CRediT authorship contribution statement

Tomohito Sakazume: Data curation, Investigation, Methodology, Formal analysis, Writing – original draft. Yoshihide Satoh: Conceptualization, Data curation, Methodology, Formal analysis, Funding acquisition, Project administration, Writing – original draft. Shogo Ohkoshi: Validation, Writing – original draft.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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Figure legends

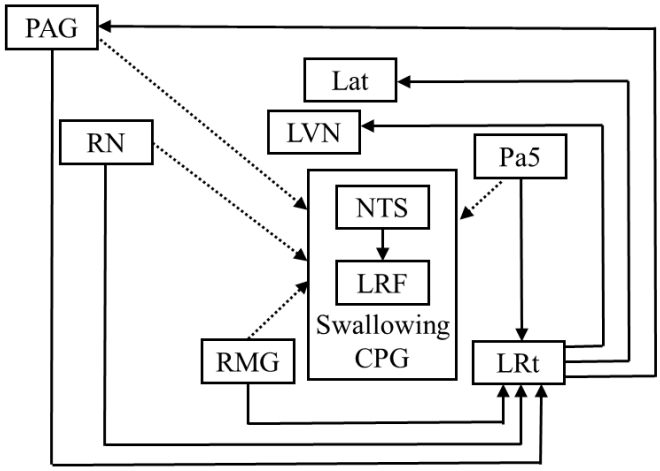
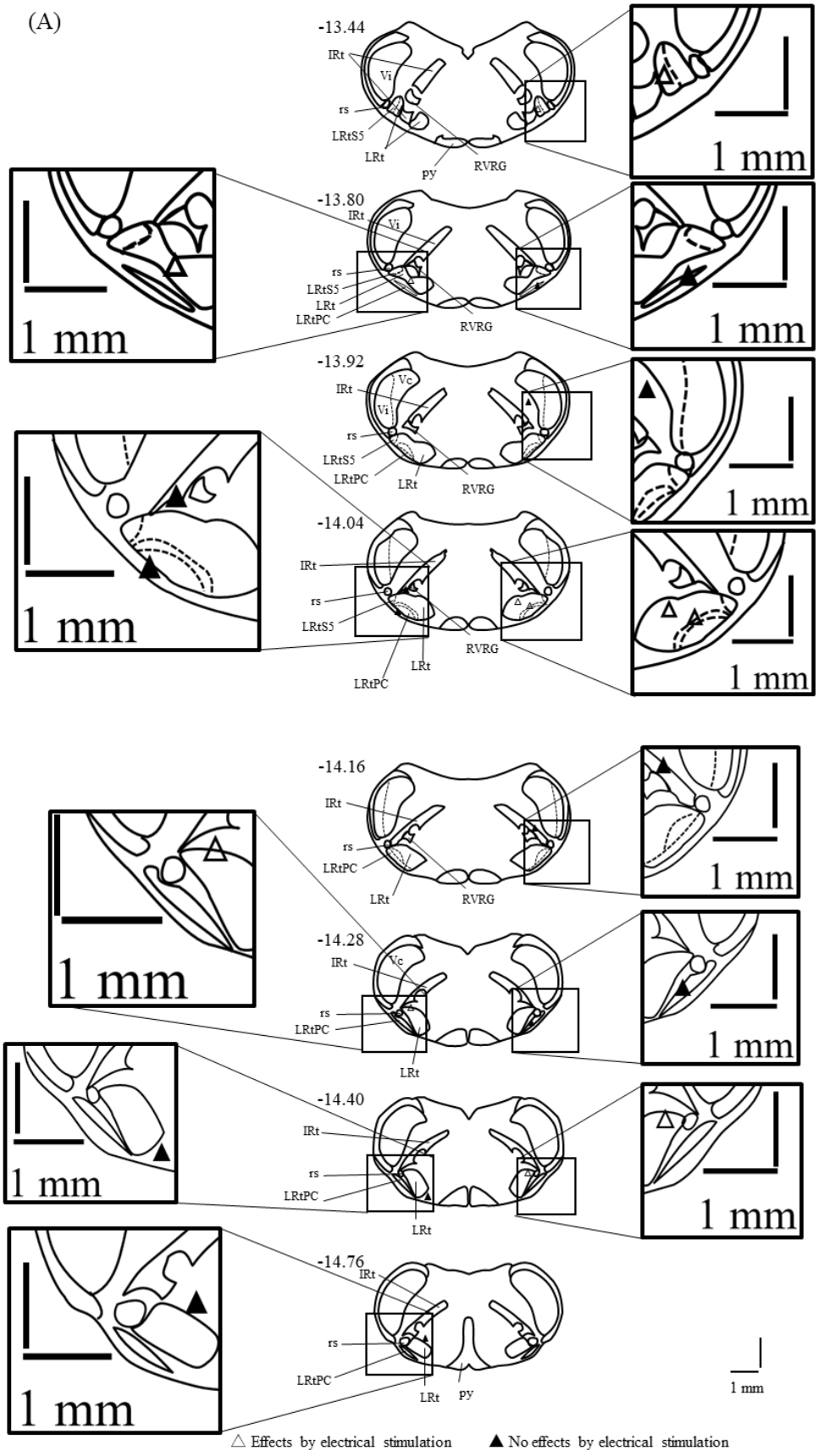


Fig. 1. Neural components of the swallowing central pattern generator (CPG) and anatomical connections to the LRt. Each site shows the approximate position in the sagittal plane. The left side is rostral, and the right side is caudal. Dashed lines indicate the inhibitory direct or indirect projections to the swallowing CPG. Abbreviations: NTS, nucleus of the solitary tract; Lat, lateral (dentate) cerebellar nucleus; LRF, the lateral reticular formation; LRt, lateral reticular nucleus; LVN, lateral vestibular nucleus; PAG, periaqueductal grey; Pa5, paratrigeminal nucleus; RMG, nucleus raphe magnus; RN, red nucleus.


(A)
-13.44
IRt
Vi
rs
LRtS5
LRt
py
RVRG
-13.80
IRt
Vi
rs
LRtS5
LRt
LRtPC
RVRG
-13.92
IRt
Vc
Vi
rs
LRtS5
LRtPC
LRt
RVRG
-14.04
IRt
rs
LRtS5
RVRG
LRt
LRtPC
-14.16
IRt
rs
LRtPC
LRt
RVRG
-14.28
IRt
Vc
rs
LRtPC
LRt
-14.40
IRt
rs
LRtPC
LRt
-14.76
IRt
rs
LRtPC
LRt
py
1 mm
△ Effects by electrical stimulation
▲ No effects by electrical stimulation

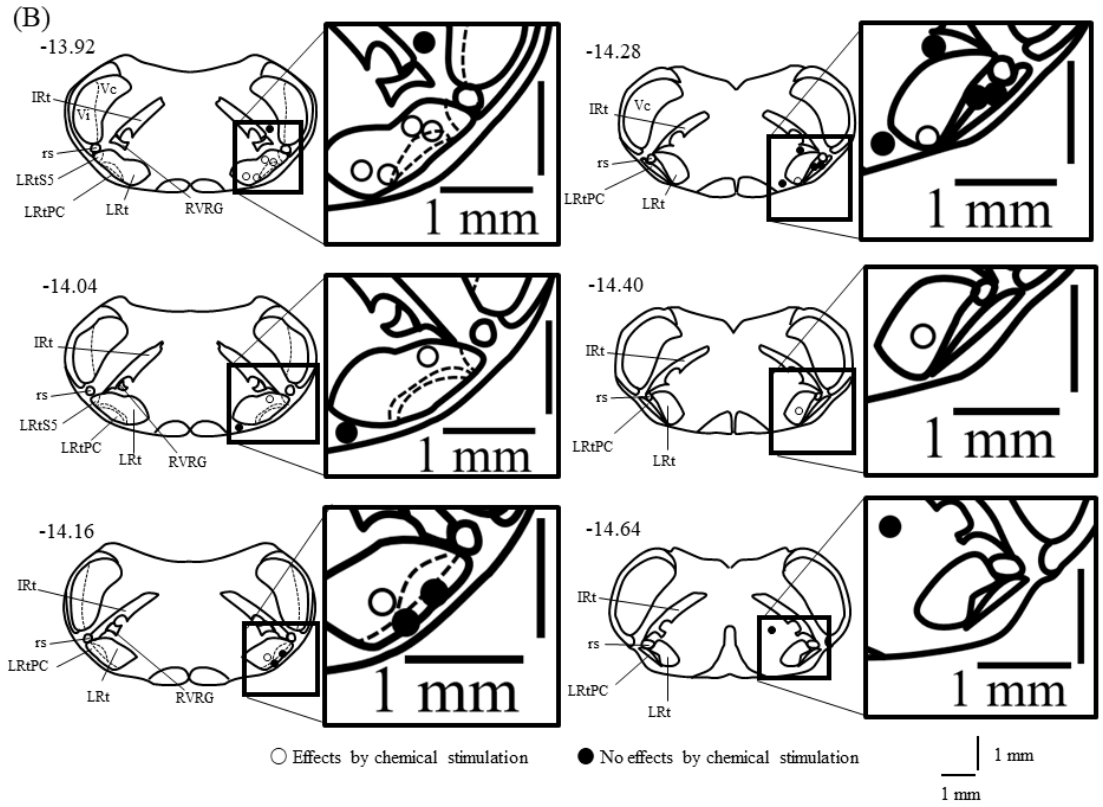


Fig. 2. The sites of electrical stimulation (A) and glutamate administration (B) shown on coronal histological sections. The number of each section represents the level from the bregma in mm; (–) means caudal to the bregma. The right direction of each drawing is the right side of the animal. The boxed figures on the right in Fig. 2B show magnified views of the boxed portion of each figure. Abbreviations: IRt, intermediate reticular nucleus; Vi, spinal trigeminal nucleus, interpolar part; Vc, spinal trigeminal nucleus, caudal part; LRt, lateral reticular nucleus; LRtS5, lateral reticular nucleus, subtrigeminal part; LRtPC, lateral reticular nucleus, parvicellular part; rs, rubrospinal tract; py, pyramidal tract; RVRG, rostral ventral respiratory group.

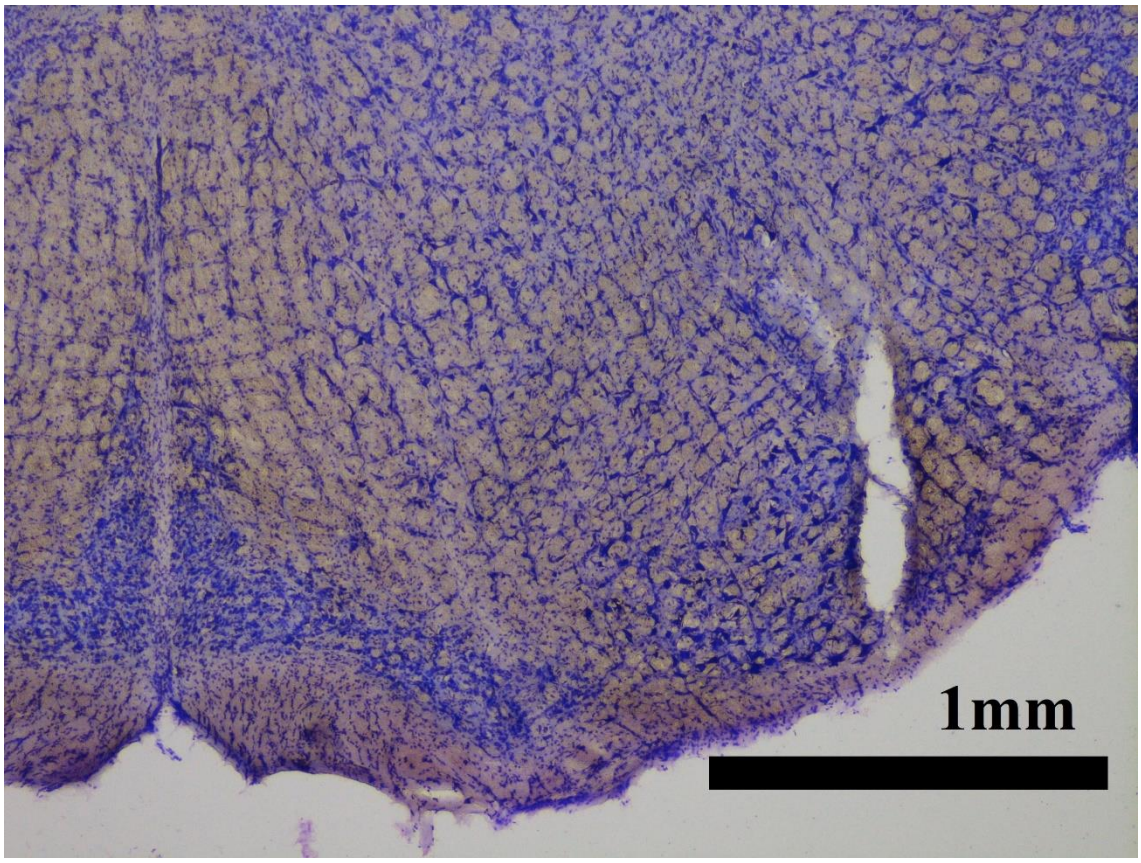
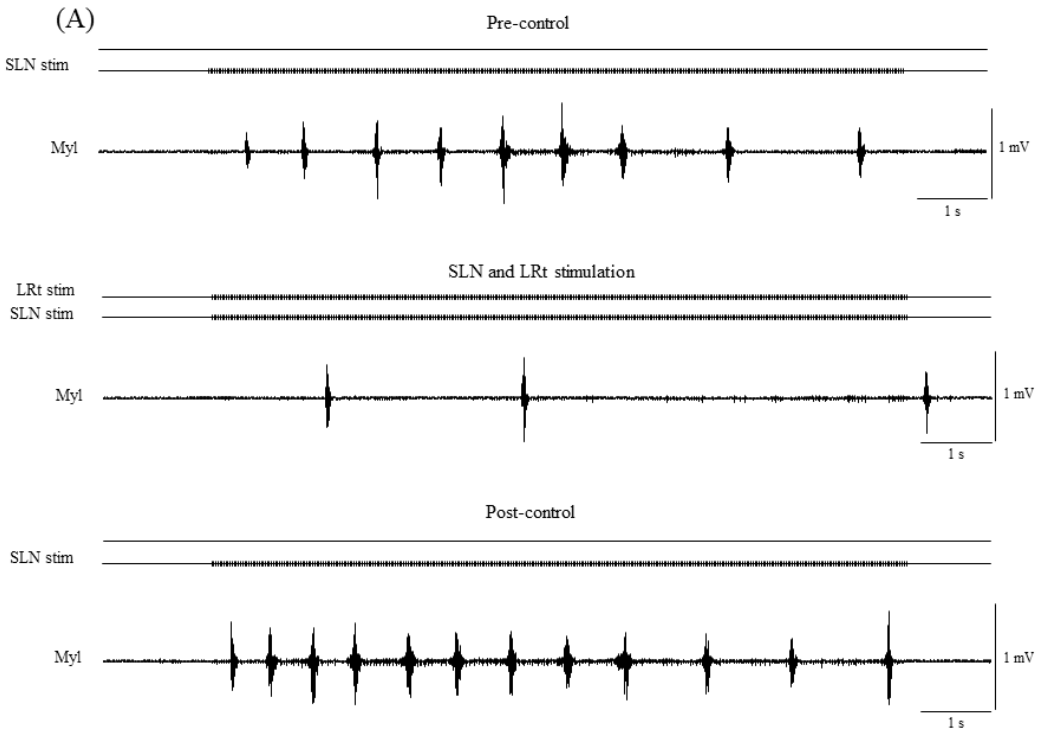


Fig. 3. Photomicrograph of a transverse section through the medulla, showing the site of glutamate injection on the right side. The solid line shows the position of the LRt. This section is −13.92 mm caudal to the bregma. Scale bar = 1 mm.


(A)
Pre-control
SLN stim
Myl
1 mV
1 s
SLN and LRt stimulation
LRt stim
SLN stim
Myl
1 mV
1 s
Post-control
SLN stim
Myl
1 mV
1 s

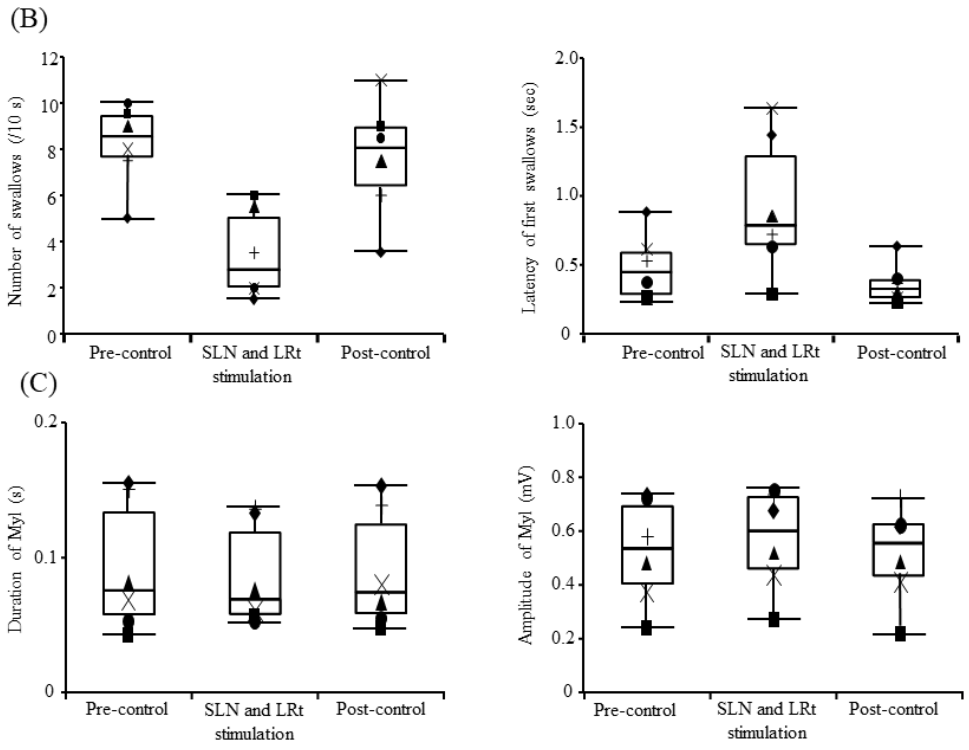
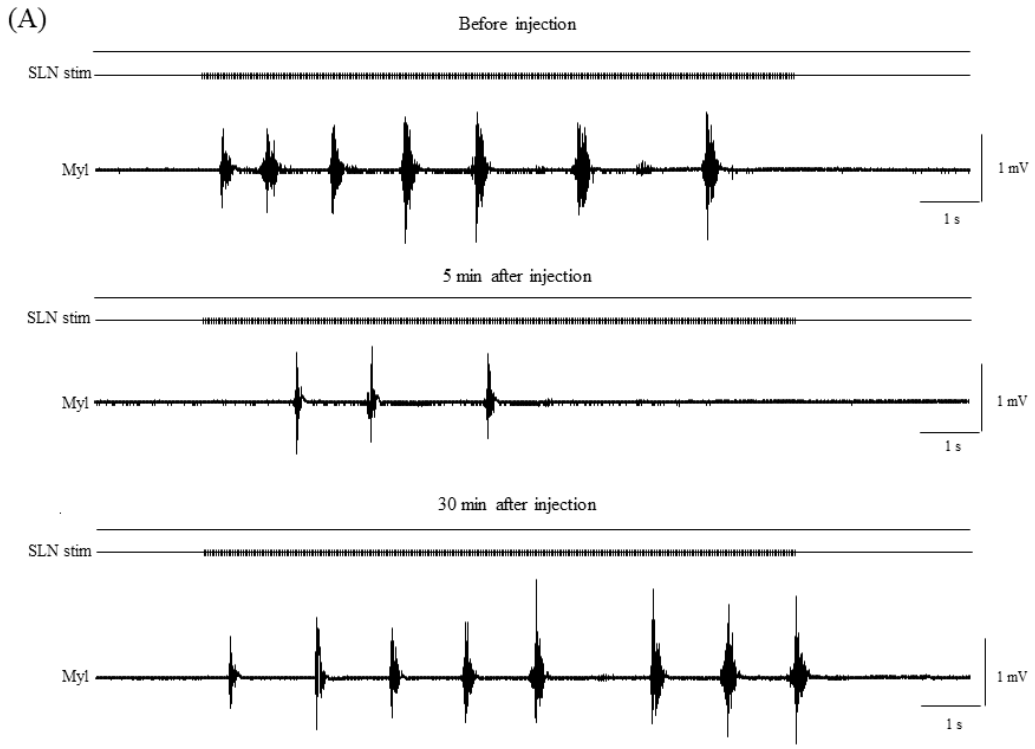

(B)
Number of swallows (/10 s)
Pre-control
SLN and LRt stimulation
Post-control
Latency of first swallows (sec)
Pre-control
SLN and LRt stimulation
Post-control
(C)
Duration of Myl (s)
Pre-control
SLN and LRt stimulation
Post-control
Amplitude of Myl (mV)
Pre-control
SLN and LRt stimulation
Post-control

Fig. 4. (A) Examples of the swallowing reflex by electrical stimulation of the SLN alone (upper and lower traces) and by simultaneous electrical stimulation of the SLN and the LRt (middle traces). (B) Reduction in the number of swallows (left) and extension of the onset latency of the first swallow (right) by electrical stimulation of the LRt (n = 6). Each symbol represents data from individual animals. (C) Effects on the duration (left) and the amplitude (right) of the Myl EMG by electrical stimulation of the LRt (n = 6).



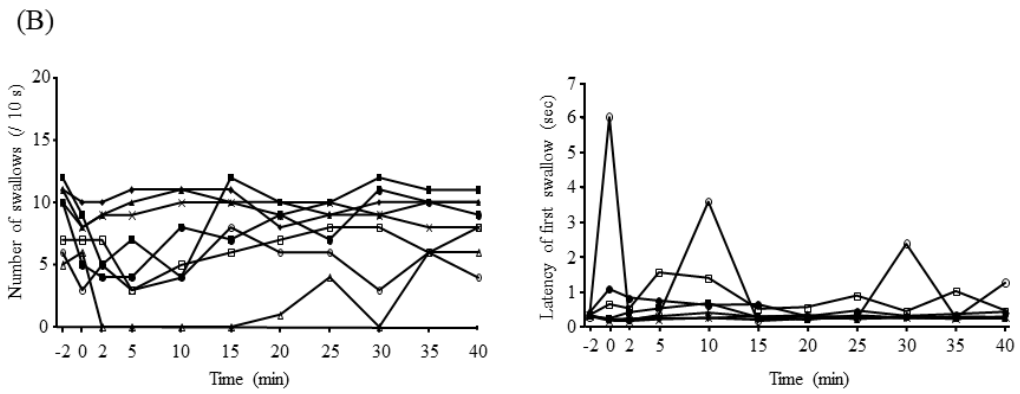


Fig. 5. (A) Examples of the swallowing reflex by electrical stimulation of the right SLN before (upper traces), 5 minutes after (middle traces), and 30 minutes (lower traces) after injection of glutamate into the right LRt. (B) Reduction in the number of swallows (left, n = 8) and extension of the onset latency of the first swallow (right, n = 7) after injection of glutamate into the LRt. Other details are shown in Fig. 4.

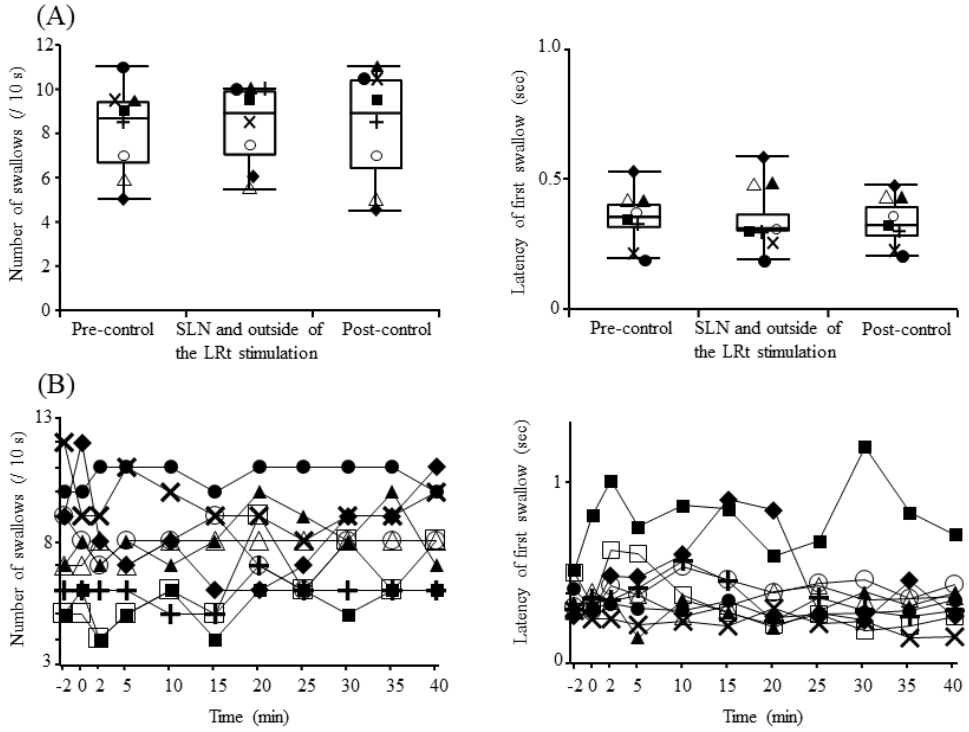


Fig. 6. (A) The number of swallows (left) and the onset latency of the first swallow (right) by electrical stimulation outside the LRt (n = 8). (B) The number of swallows (left) and the onset latency of the first swallow (right) by microinjection of glutamate to the exterior of the LRt (n = 9). Other details are shown in Fig. 4.

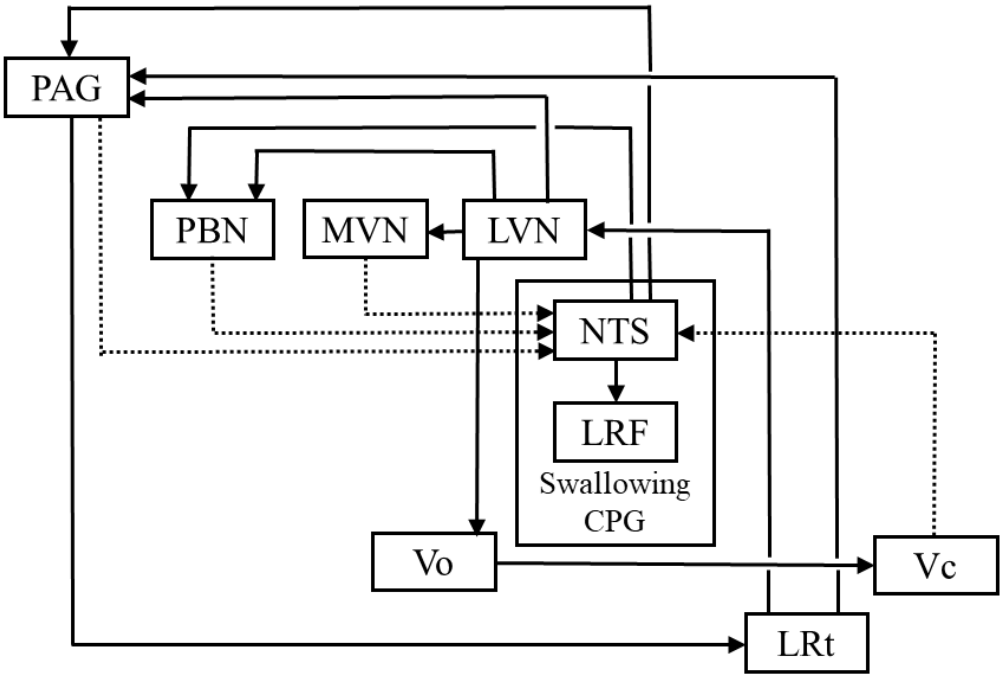


Fig. 7. Neural organization of increasing the onset latency by stimulation of the LRt. Each site shows the approximate position in the sagittal plane. Solid and dashed lines indicate the excitatory and inhibitory projections, respectively. Abbreviations: MVN, medial vestibular nucleus; Vo, spinal trigeminal nucleus, oral part; Vc, spinal trigeminal nucleus, caudal part; PBN, parabrachial nucleus. Other details are shown in Fig. 1.

Symbol	Stimulation site
●	-14.04 lateral
■	-14.40
▲	-14.28
◆	-13.80
+	-14.04 medial
×	-13.44

Table 1. Data and electrical stimulation sites. The symbols are identical to those of Fig. 4B and Fig. 4C.

Symbol	Stimulation site
●	-13.92 medial
■	-14.04
▲	-14.40
◆	-14.28
□	-13.92 ventral
×	-13.92 dorsal
○	-13.92 lateral
△	-14.16

Table 2. Data and microinjection sites. The symbols are identical to those of Fig. 5B.