ラットにおける巨大細胞網様核刺激による嚥下反射の変調

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Modulation of the swallowing reflex by stimulation of the gigantocellular reticular nucleus in the rat

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Abstract

Objectives: The gigantocellular reticular nucleus (Gi) projects to the solitary tract nucleus (NTS) and the lateral reticular formation (LRF) above the nucleus ambiguus. The swallowing central pattern generator comprises the NTS and the LRF. The present study examined whether stimulation of the Gi affects the swallowing reflex.

Methods: Experiments were performed on urethane-anesthetized rats. The swallowing reflex was evoked by repetitive electrical stimulation of the superior laryngeal nerve and was recorded on an electromyogram from the mylohyoid muscle. The Gi was stimulated electrically. In addition, glutamate was injected into the Gi. The Friedman's test, followed by the Wilcoxon signed-rank test with Bonferroni correction, were used to assess the effects of electrical stimulation of the Gi. The Wilcoxon signed-rank test was used to assess the effects of glutamate injection into the Gi. Differences were considered significant at the $P < 0.05$ level.

Results: The number of swallows was significantly increased or decreased by electrical

stimulation of the Gi or after injection of glutamate into the Gi. In both electrical stimulation of the Gi and injection of glutamate into the Gi, the onset latency of the first swallow was prolonged when the number of swallows was decreased but showed no change when the number of swallows was increased.

Conclusions: The present results suggest that the Gi is involved in the control of swallowing.

Keywords: swallowing reflex; gigantocellular reticular nucleus; superior laryngeal nerve; electrical stimulation; glutamate injection

1. Introduction

The swallowing reflex is evoked and patterned by the swallowing central pattern generator (CPG) in the medulla [1-3]. The swallowing CPG consists of the dorsal swallowing group (DSG) and the ventral swallowing group (VSG). The DSG is composed of the nucleus of the solitary tract (NTS) and the reticular formation around it. The VSG comprises the lateral reticular formation (LRF) above the nucleus ambiguus.

According to morphological studies, the gigantocellular reticular nucleus (Gi) receives projection fibers from the pedunculopontine tegmental nucleus (PTg) [4,5] and projects bilaterally to the NTS and the LRF [6, 7]. Our previous study demonstrated that the swallowing reflex was suppressed by stimulation of the PTg [8]. Swallowing frequency

was increased transiently after N-methyl-D-aspartic acid (NMDA) injections into the Gi [9].

The aim of the present study was to examine whether stimulation of the Gi affects the swallowing reflex.

2. Materials and methods

Thirty-six male Sprague-Dawley rats (303-378g) were used. Female rats were excluded because their physical condition may change due to the estrous cycle. The rats were anesthetized with urethane (1.3 g/kg, i.p.). Additional urethane (0.2 g/kg, i.p.) was injected during animal surgery when the withdrawal reflex was provoked by noxious stimulation of the hindlimb. During the experiment, rectal temperature was controlled to roughly 37 ℃ by a body temperature control device (ATC-402, UNIQUE MEDICAL Co. Ltd., Tokyo, Japan). Heart and respiratory rates were recorded automatically by the body temperature control device via a vibration sensor in the heating pad. Heart rate was maintained at 250–450 beats/min and respiratory rate was maintained at 70–115 breaths/min, and both were recorded during the experiment to verify the condition of the animals [10].

A midline incision was made from the mandibular mental part to the rostral portion of the neck to expose the anterior belly of the digastric muscle. To record the electromyogram (EMG), pairs of Teflon-coated silver wires (diameter 0.1 mm, exposed tip 2.0 mm) were inserted using a needle into the left mylohyoid muscle (Myl) running beneath the anterior

belly of the digastric muscle. The left and/or right superior laryngeal nerve (SLN) was suspended by bipolar stimulating electrodes of silver wires (diameter 0.1 mm), and the swallowing reflex was induced by electrical stimulation (0.2 ms duration, 30 Hz, for 10 s). The SLN stimulation parameters were set based on parameters used in previous studies [8, 11-15]. The swallowing reflex was identified by visual observation of laryngeal movements and by an EMG burst of the Myl muscle. The SLN threshold for inducing the swallowing reflex was defined as the minimum stimulus intensity that induced swallowing at least once during 10 s stimulation of the SLN. The intensity was 1.2 times the threshold for inducing the swallowing reflex.

After the head of the rat was fixed by stereotaxic apparatus, part of the parietal bone was removed using a dental drill to expose the surface of the cerebellum. In the electrical stimulation experiments, to stimulate the Gi, a bipolar concentric electrode (outer diameter 200 μm) (TK213-091, UNIQUE MEDICAL Co. Ltd.) was inserted toward the Gi in the 18 rats. The left SLN alone was stimulated as a pre-control. One minute later, the SLN and the left Gi or around the left Gi were stimulated simultaneously to explore the effect of Gi stimulation on the swallowing reflex. Repetitive electrical stimulation (duration 0.2 ms, 30 Hz, 50-150 μA, for 10 s) was applied to the Gi or around the Gi. The Gi stimulation parameters were set based on parameters used in previous studies [8, 14, 15]. The intensity of the stimulus to the Gi was increased every 50 μA between 50 μA and 150 μA. The Gi stimulation was set as the lowest intensity that modulated the number of swallows

compared to pre-control. After one minute, the SLN alone was stimulated again (post-control) to confirm that there was no significant difference between the pre-control and the post-control in the number of swallows. This protocol was performed twice in each of the rats from the pre-control to the post-control. For each rat, the number of swallows and the onset latency of the first swallow were defined as the mean values of the two sets.

In the chemical stimulation experiments, a stainless pipe, attached to a microelectrode (TK212-010b, Unique Medical Co. Ltd.), was inserted into the left Gi or around the Gi. The stainless pipe and a 1 μl Hamilton syringe were connected with a polyethylene tube, and filled with sodium L-glutamate dissolved in saline (0.1 mM). The glutamate concentration was selected based on the concentration used in previous studies [8, 14-16]. The left or right SLN was stimulated electrically (0.2 ms duration, 30 Hz, for 10 s) in 18 rats as a control. The right SLN was stimulated if the swallowing reflex was not evoked by stimulation of the left SLN. Twenty seconds after finishing the recording of the swallowing reflex, the glutamate was injected (0.1 μl, injection duration 90 s) into the Gi or around the Gi. Swallowing reflexes were then recorded at 0, 2 and 5 min, and at 5 min intervals thereafter up to 180 min.

In some rats, except for the 36 rats, the threshold of the SLN was increased during recording of the swallowing reflex. In the case of a significant difference in the number of swallows between the pre-control and post-control, or when the number of swallows continued to decrease and did not return to the condition prior to injection by 180 min after

injection of glutamate into the Gi, the data were excluded from further analysis because of the possibility that the reduction in the number of swallows during the Gi stimulation could have been caused by a continuously increasing SLN threshold.

EMG of the Myl muscle was amplified (filter bandwidth, 10 Hz to1 kHz) and stored on a computer with 2 kHz sampling rate via an A/D converter. The data were analyzed using the Spike2 analysis package (Cambridge Electronic Design, Cambridge, UK). The number of swallows and the onset latency of the first swallow (the time from the oncoming SLN stimulus to the peak of the EMG burst), the duration of Myl EMG bursts, and peak-to-peak amplitude in each EMG recording session were measured. The duration of Myl EMG bursts was calculated as the time duration over the mean + 2SD of background activity following a stable 3 s period immediately before the onset of the SLN stimulation. The effects of electrical stimulation of the Gi were assessed by the Friedman's test, followed by the Wilcoxon signed-rank test with Bonferroni correction as a post hoc test. The effects of glutamate injection into the Gi were assessed by the Wilcoxon signed-rank test. Differences were considered significant at the $P < 0.05$ level.

After the experiment, a lethal dose of urethane was injected into each rat. Electrolytic lesions were created by passing a negative direct current of 20 μA through the electrode for 150 s. The brainstem was removed and fixed in 10% buffered formalin solution for 4–7 days. Serial coronal sections were cut (60 μm thick) and dyed by Nissl staining using 0.1% cresyl echt violet. The stimulation and injection sites were verified using a standard

atlas [17].

3. Results

The threshold for evoking the swallowing reflex by stimulating the SLN was 7–220 μA (62.5 ± 45.6 μA, mean ± SD, n = 36).

3.1 Electrical stimulation

The swallowing reflex was consistently facilitated (n = 6, Fig. 1) or suppressed (n = 6, Fig. 2) by electrical stimulation of the Gi. There was no mixed effect of both facilitation and suppression. An increasing in the Gi stimulus intensity did not alter the effect of the swallowing reflex.

In the case of facilitation of the swallowing reflex, the number of swallows was significantly increased (P = 0.010) during the Gi stimulation compared with the pre- and post-controls (Fig. 1B, left). The number of swallows was 7.7 ± 1.1 (mean ± standard error of the mean) in the pre-control, 10.5 ± 1.1 during the Gi stimulation, and 7.3 ± 1.1 in the post-control. There was no change in the onset latency of the first swallow (P = 0.933) during the Gi stimulation (Fig. 1B, right). The onset latency of the first swallow was 0.44 ± 0.10 s in the pre-control, 0.46 ± 0.10 s during the Gi stimulation, and 0.39 ± 0.07 s in the post-control.

In the case of suppression of the swallowing reflex, the number of swallows was

significantly decreased (P = 0.009) during the Gi stimulation compared with the pre- and post-controls (Fig. 2B, left). The number of swallows was 6.9 ± 0.8 in the pre-control, 3.7 ± 1.2 during the Gi stimulation, and 7.3 ± 0.9 in the post-control. The onset latency of the first swallow was significantly longer (P = 0.009) during the Gi stimulation (Fig. 2B, right). The onset latency of the first swallow was 0.64 ± 0.08 s in the pre-control, 2.49 ± 1.36 s during the Gi stimulation, and 0.60 ± 0.05 s in the post-control.

In both facilitation and suppression, there was no modulation of either the duration of Myl EMG bursts or the peak-to-peak amplitude of Myl EMG during the Gi stimulation (Fig. 3A, 3B).

3.2 Chemical stimulation

The swallowing reflex was also consistently facilitated (n = 6, Fig. 4) or suppressed (n = 6, Fig. 5) after injection of glutamate into the Gi. Although the time at which the modulation of the swallowing reflex occurred differed between animals, there was no mixed effect of both facilitation and suppression.

In the case of facilitation of the swallowing reflex, the number of swallows was significantly increased at 10-30, 40, 70, and 75 min (P = 0.015-0.040) after injection compared with before injection (Fig. 4B, upper). The onset latency of the first swallow was not modulated after injection into the Gi compared with before the injection (Fig. 4B, lower).

In the case of suppression of the swallowing reflex, the number of swallows was significantly decreased at 10-65, 80, 100-140, and 155-170 min (P = 0.015-0.040) after injection into the Gi compared with before injection (Fig. 5B, upper). The onset latency of the first swallow was significantly longer at 15, 40-50, and 180 min (P = 0.015-0.038) after injection into the Gi compared with before injection (Fig. 5B, lower).

3.3 Stimulation around the Gi

Even with a stimulus intensity of 150 μA around the Gi, there was no suppression or facilitation of the swallowing reflex. There was no significant difference in the number of swallows, onset latency of the first swallow, duration of Myl EMG bursts, and peak-to-peak amplitude of Myl EMG with and without stimulation around the Gi (n = 6, Fig. 6A).

The number of swallows was significantly decreased at 130, 150, 155, 165, and 170 min (P = 0.022-0.047) after glutamate injection around the Gi compared with before injection. Three of the six rats had two or more fewer swallows than before the injection during these periods. However, there was no significant difference in the onset latency of the first swallow before and after injection around the Gi (n = 6, Fig. 6B).

3.4 Stimulation sites

Figure 7 shows the histological locations of the electrical stimulation and of the glutamate microinjection sites. The electrical stimulation sites that showed facilitation or

suppression of the swallowing reflex were located within the Gi, and those that showed no modulation of the swallowing reflex were located in the dorsal, lateral, medial, or ventral to the Gi.

The microinjection sites that showed facilitation or suppression of the swallowing reflex were located in the central, dorsal, ventral, or medial regions of the Gi, and those that showed no modulation of the swallowing reflex were located in the dorsal, medial, or ventral to the Gi.

The effective sites of the swallowing reflex for electrical stimulation and glutamate injection were intermingled in the Gi.

4. Discussion

4.1 Methodological considerations

A previous study indicated that the stimulus current spread had a radius of 0.63 mm at stimulus intensity of 150 μA and duration of 0.25 ms with monopolar electrodes, and the current spread was less with concentric electrodes than with monopolar electrodes [18]. In the present study, concentric electrodes were used, the stimulus intensity of the Gi was 50-150 μA, and the stimulus duration was 0.2 ms. The radius of the current spread was therefore smaller, in the range of 0.2 to 0.4 mm. Electrical stimulation of the dorsal or dorsolateral Gi would affect the dorsal and dorsolateral border of the Gi, and could not have affected the medial or ventral parts of the NTS. Based on the findings of previous

studies [19-21], we assumed in the present study that the glutamate excited neurons up to a radius of about 0.1 mm from the injection site. Therefore, we believe that glutamate injection into the dorsolateral Gi appears to be restricted to the Gi, and does not affect the NTS. It has been reported that microinjections of glutamate into the NTS resulted in the initiation of deglutition [22]. In the present study, the swallowing reflex was not evoked without SLN stimulation. These results suggest that modulation of the swallowing reflex by electrical and chemical stimulation of the Gi is caused by excitation of the cell bodies in the Gi, rather than by excitation of the NTS by current spread or by spread of glutamate.

In the chemical stimulation experiments, the right SLN was stimulated if the swallowing reflex was not evoked by stimulation of the left SLN. The NTS receives sensory inputs from the SLN bilaterally and swallowing is a symmetrical movement [23]. Moreover, the Gi projects bilaterally to the NTS and the LRF [6, 7]. Accordingly, stimulation of the right SLN does not affect modulation of the swallowing reflex by stimulation of the Gi.

4.2 Effects of swallowing reflex by stimulation of the Gi

In the present study, the sites at which the swallowing reflex was facilitated or suppressed were located throughout the Gi, and were intermingled within the Gi. The stimulus intensities needed to elicit facilitative or suppressive effects of the swallowing reflex were not uniform within the Gi. In other words, the stimulus intensity was not lower in the central Gi and was not higher in the peripheral Gi (Fig. 7). A previous study has

reported a transient increase in swallowing frequency after NMDA injections into the central, dorsal, ventral, and lateral regions of the Gi [9], which is in agreement with some of the present results.

In our previous studies, the swallowing reflex was suppressed by electrical stimulation (100-200 μA) or glutamate injection (0.1 μl) into several areas in the brainstem. The number of swallows was significantly decreased at 0 or 2 min after injection [8, 14, 15]. The reason for this immediate effect is thought to be that only suppression occurred in these studies. In contrast, stimulus intensity of the Gi was 50-150 μA, and the facilitatory or suppressive effect appeared at 10 min after glutamate injection in the present study. Because the stimulus intensity was weaker than in our previous studies, it is likely that the area that was stimulated electrically was narrower, and that the neurons associated with control of swallowing were clustered within the Gi. As neurons that facilitate and suppress the swallowing reflex are intermingled and may be clustered in the Gi, the same number of both neuron types would have been excited within 10 min. Accordingly, the effect may not have appeared within 10 min. It is assumed that the glutamate excited neurons up to a radius of about 0.1 mm at 10 min later. Thus, when the effect is facilitation, the number of excited neurons that facilitate the swallowing reflex may be greater than the number of excited neurons that suppress it, and vice versa.

4.3 Neural mechanism for increase in number of swallows

There was no change in the onset latency of the first swallow when the number of swallows increased by stimulation of the Gi. The DSG receives sensory inputs from the pharyngeal and laryngeal regions and initiates the swallowing reflex, whereas the VSG receives inputs from the DSG and sends outputs to motoneurons related to swallowing [1]. This result suggests that when the number of swallows was increased, the initiation of the swallowing reflex was not affected, and the VSG was excited or disinhibited by stimulation of the Gi rather than via the DSG. The Gi projects directly to the LRF [6, 7], and the Gi neurons express mRNA encoding for enzymes that are associated with glutamatergic neuronal signaling [24]. It is therefore possible that glutamatergic neurons in the Gi may activate the VSG directly, and increase the number of swallows.

A physiological study reported a transient increase in swallowing frequency after NMDA injections into the vestibular nuclei (VN) [9]. VN glutamatergic neurons project to the Gi [25]. In the facilitation of the swallowing reflex, the Gi might receive excitatory inputs from the VN.

4.4 Neural mechanism for reduction in number of swallows

The onset latency of the first swallow was extended when the number of swallows was decreased in response to stimulation of the Gi. This result suggests that the DSG and the VSG were inhibited or disfacilitated by stimulation of the Gi. The Gi projects directly to the NTS [6] and to the LRF [6, 7]. The Gi neurons express mRNA encoding for enzymes

that are associated with GABAergic neuronal signaling [24]. It has been shown that injection of muscimol (GABA agonist) into the NTS inhibits the pharyngeal phase of swallowing and esophageal peristalsis [26]. It is therefore possible that GABAergic neurons in the Gi may inhibit the DSG and the VSG directly, decreasing the number of swallows and extending the onset latency of the first swallow.

Our previous study showed that the number of swallows was decreased and the onset latency of the first swallow was extended by stimulation of the PTg [8]. It has been suggested that there is no direct projection from the PTg to the NTS [27]. The PTg projects to the Gi [4,5]. It is likely, therefore, that the Gi is involved in suppression of the swallowing reflex by stimulation of the PTg. The Gi might receive excitatory inputs from the PTg.

4.5 Functional significance of the Gi on the swallowing reflex

Rhythmic masticatory-related jaw movements as well as swallowing have been evoked by repetitive electrical stimulation of the cerebral cortex [28-32], or the central amygdaloid nucleus [33]. It has been demonstrated that the orofacial motor cortex (A-area) in rats projects to the Gi [34], and the central amygdaloid nucleus projects to the PTg [35]. It was suggested that the Gi is part of the central rhythm generator for mastication [36]. The SLN-induced swallowing reflex is suppressed during rhythmic jaw movements induced by stimulation of the A-area [37] or the central amygdaloid nucleus [33]. Accordingly,

suppression of the swallowing reflex by stimulation of the Gi may contribute to suppression of the swallowing reflex during mastication.

The VN and the Gi are involved in the control of head movements [38, 39]. It was reported that head flexion resulted in easier swallowing [40], and that head extension assists oropharyngeal transport of a tablet [41]. It is therefore possible that facilitation of the swallowing reflex by stimulation of the Gi may be related to control of swallowing and head movements.

4.6 Limitations

In the present study, only male rats were used, and the EMG was recorded from only the Myl muscle. In future experiments, female rats should also be used to confirm the influence of gender on the swallowing reflex, and the EMG should be recorded from several muscles that contribute to swallowing for more accurate identification of the swallowing reflex.

The number of swallows was significantly decreased at 130, 150, 155, 165, and 170 min after microinjection into around the Gi (Fig. 6B). These results might be due to elevation of the SLN threshold for evoking the swallowing reflex caused by drying out of the SLN or from body fluids penetrating between the SLN and the electrode. It is necessary to devise techniques that permit the SLN threshold to be maintained for up to 200 min.

5. Conclusions

In the present study, the number of swallows was increased or decreased, and the onset latency of the first swallow was extended when the number of swallows was decreased by electrical and chemical stimulation of the Gi. These results suggest that the Gi is involved in the control of swallowing through excitation of the VSG and inhibition of the VSG and DSG in the swallowing CPG.

Ethical approval

This study was sanctioned by the Laboratory Animal Committee of The Nippon Dental University School of Life Dentistry at Niigata (approval number 186) and conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Conflicts of interest

The authors have no conflicts of interest.

Author contributions

Arisa Murakawa: Data curation, Investigation, Methodology, Formal analysis, Writing - original draft. Yoshihide Satoh: Conceptualization, Data curation, Methodology, Formal analysis, Funding acquisition, Project administration, Writing - original draft.

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Declaration of Generative AI and Ai-assisted technologies in the writing process

The authors do not use generative AI and Ai-assisted technologies.

References

[1] Jean A. Brain stem control of swallowing: neuronal network and cellular mechanisms. Physiol. Rev. 2001; 81: 929-69.

[2] Kessler JP, Jean A. Identification of the medullary swallowing regions in the rat. Exp Brain Res. 1985; 57: 256-63.

[3] Chiao GZ, Larson CR, Yajima Y, Ko P, Kahrilas PJ. Neuronal activity in nucleus ambiguous during deglutition and vocalization in conscious monkeys. Exp. Brain. Res. 1994; 100: 29-38.

[4] Grofova I, Keane S. Descending brainstem projections of the pedunculopontine tegmental nucleus in the rat. Anat. Embryol. 1991; 184: 275-90.

[5] Martinez-Gonzalez C, van Andel J, Bolam JP, Mena-Segovia J. Divergent motor projections from the pedunculopontine nucleus are differentially regulated in Parkinsonism. Brain. Struct. Funct. 2014; 219: 1451-62.

[6] Jones BE, Yang T-Z. The efferent projections from the reticular formation and the locus

coeruleus studied by anterograde and retrograde axonal transport in the rat. J. Comp. Neurol. 1985; 242: 56-92.

[7] Ohtake T. Ascending projections from the gigantocellular reticular and dorsal paragigantocellular nuclei of the medulla oblongata in the rat: an anterograde PHA-L tracing study. Neurosci. Res. 1992; 14: 96-116.

[8] Satoh Y, Tsuji K. Suppressive effect of the swallowing reflex by stimulation of the pedunculopontine tegmental nucleus. Neurosci. Res. 2021; 169: 40-7.

[9] Feroah TR, Forster HV, Fuentes CG, Wenninger J, Martino P et al. Contributions from rostral medullary nuclei to coordination of swallowing and breathing in awake goats. J. Appl. Physiol. 2002; 93: 581-91.

[10] Wolfenshon S, Lloyd M. Handbook of Laboratory Animal Management and Welfare. 2nd ed. London: Blackwell Science; 1998.

[11] Kitagawa J, Nakagawa K, Hasegawa M, Iwakami T, Shingai T, Yamada Y, Iwata K. Facilitation of reflex swallowing from the pharynx and larynx. J Oral Sci. 2009; 51(2): 167-171.

[12] Kobashi M, Shimatani Y, Fujita M, Mitoh Y, Yoshida R, Matsuo R. The effects of mutual interaction of orexin-A and glucagon-like peptide-1 on reflex swallowing induced by SLN afferents in rats. Int J Mol Sci. 2020;21(12):4422.

[13] Sugiyama N, Nishiyama E, Nishikawa Y, Sasamura T, Nakade S, Okawa K, Nagasawa T, Yuki A. A novel animal model of dysphagia following stroke. Dysphagia. 2014; 29(1):

61-67.

[14] Satoh Y, Tsuji K, Tsujimura T, Ishizuka K, Inoue M. Suppression of the swallowing reflex by stimulation of the red nucleus. Brain Res Bull. 2015; 116: 25-33.

[15] Sakazume T, Satoh Y, Ohkoshi S. Stimulation of the center of the lateral reticular nucleus suppresses the swallowing reflex in rats. Neurosci Lett. 2023; 794: 136998.

[16] Satoh Y, Ishizuka K, Murakami T. Facilitation of the jaw reflexes by stimulation of the red nucleus in the rat. Brain Res. 2001; 978: 51-58.

[17] Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 7th ed. San Diego: Academic Press; 2013.

[18] Bagshaw EV, Evans MH. Measurement of current spread from microelectrodes when stimulating within the nervous system. Exp. Brain Res. 1976; 25: 391-400.

[19] Goodchild AK, Dampney RAL, Bandler RA. A method for evoking physiological responses by stimulation of cell bodies, but not axons of passage, within localized regions of the central nervous system. J. Neurosci. Methods 1982; 6: 351-63.

[20] Randich A, Roose MG, Gebhart GF. Characterization of antinociception produced by glutamate microinjection in the nucleus tractus solitarius and the nucleus reticularis ventralis. J. Neurosci. 1988; 8: 4675-84.

[21] Chiang CY, Dostrovsky JO, Sessle BJ. Periaqueductal gray matter and nucleus raphe magnus involvement in anterior pretectal nucleus-induced inhibition of jaw-opening reflex in rats. Brain Res. 1991; 544: 71-8.

[22] Kessler JP, Jean A. Evidence that activation of N-methyl-D-aspartate (NMDA) and non-NMDA receptors within the nucleus tractus solitarii triggers swallowing. Eur J Pharmacol. 1991; 201: 59-67.

[23] Sugimoto T, Umezaka T, Takagi S, Narikawa K, Shin T. Crossing inputs of the superior laryngeal nerve afferents to medullary swallowing-related neurons in the cat. Neurosci. Res. 1998; 30: 235-45.

[24] Martin EM, Devidze N, Shelley DN, Westberg L, Fontaine C et al. Molecular and neuroanatomical characterization of single neurons in the mouse medullary gigantocellular reticular nucleus. J. Comp. Neurol 2011; 519: 2574-93.

[25] Shi X, Wei H, Chen Z, Wang J, Qu W et al. Whole-brain monosynaptic inputs and outputs of glutamatergic neurons of the vestibular nuclei complex in mice. Hear. Res. 2021; 401: 108159.

[26] Wang YT, Bieger D. Role of solitarial GABAergic mechanisms in control of swallowing. Am. J. Physiol. 1991; 261: 639-46.

[27] Rye DB, Lee HJ, Saper CB, Wainer BH. Medullary and spinal efferents of the pedunculopontine tegmental nucleus and adjacent mesopontine tegmentum in the rat. J. Comp. Neurol. 1988; 269: 315-41.

[28] Hiraba H, Sato T. Cortical control of mastication in the cat: properties of mastication-related neurons in motor and masticatory cortices, Somatosens Mot Res. 2004; 21: 217-27.

[29] Huang CS, Hiraba H, Murray GM, Sessle BJ. Topographical distribution and

functional properties of cortically induced rhythmical jaw movements in the monkey (Macaca fascicularis),. J. Neurophysiol. 1989; 61: 635-50.

[30] Martin RE, Kemppainen P, Masuda Y, Yao D, Murray GM, Sessle BJ. Features of cortically evoked swallowing in the awake primate (Macaca fascicularis), J. Neurophysiol. 1999; 82: 1529-41.

[31] Satoh Y, Ishizuka K, Murakami T. Changes in cortically induced rhythmic jaw movements after lesioning of the red nucleus in rats, Brain Res. 2007; 1165: 60-70.

[32] Sumi T. Some properties of cortically-evoked swallowing and chewing in rabbits, Brain Res. 1969; 15: 107-20.

[33] Satoh Y, Tsuji K. Suppression of the swallowing reflex during rhythmic jaw movements induced by repetitive electrical stimulation of the dorsomedial part of the central amygdaloid nucleus in rats. Life (Basel) 2020; 10: 190.

[34] Zhang GX, Sasamoto K. Projections of two separate cortical areas for rhythmical jaw movements in the rat. Brain Res. Bull. 1990; 24: 221-30.

[35] Semba K, Fibiger HC. Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: a retro- and antero-grade transport and immunohistochemical study. J. Comp. Neurol. 1992; 323: 387-410.

[36] Nozaki S, Iriki A, Nakamura Y. Localization of central rhythm generator involved in cortically induced rhythmical masticatory jaw-opening movement in the guinea pig. J. Neurophysiol. 1986; 55: 806-25.

[37] Tsujimura T, Tsuji K, Ariyasinghe S, Fukuhara T, Yamada A et al. Differential involvement of two cortical masticatory areas in modulation of the swallowing reflex in rats. Neurosci. Lett. 2012; 528: 159-64.

[38] Vidal P-P, Sans A. Vestibular system. In; Paxinos G, editor. The Rat Nervous System. 3rd ed. San Diego: Academic Press; 2004. p. 965–96.

[39] Cowie RJ, Robinson DL. Subcortical contributions to head movements in macaques. I. Contrasting effects of electrical stimulation of a medial pontomedullary region and the superior colliculus. J. Neurophysiol 1994; 72: 2648-64.

[40] Sakuma T, Kida I. Relationship between ease of swallowing and deglutition-related muscle activity in various postures. J Oral Rehabil. 2010; 37: 583–9.

[41] Sugiyama S, Iida T, Morimoto Y, Yamazaki Y, Mikuzuki L et al. Effects of tablet size and head posture on drug swallowing: A preliminary examination using endoscopy in healthy subjects. Acta Med. Okayama. 2021; 75: 495-503.

Figure legends

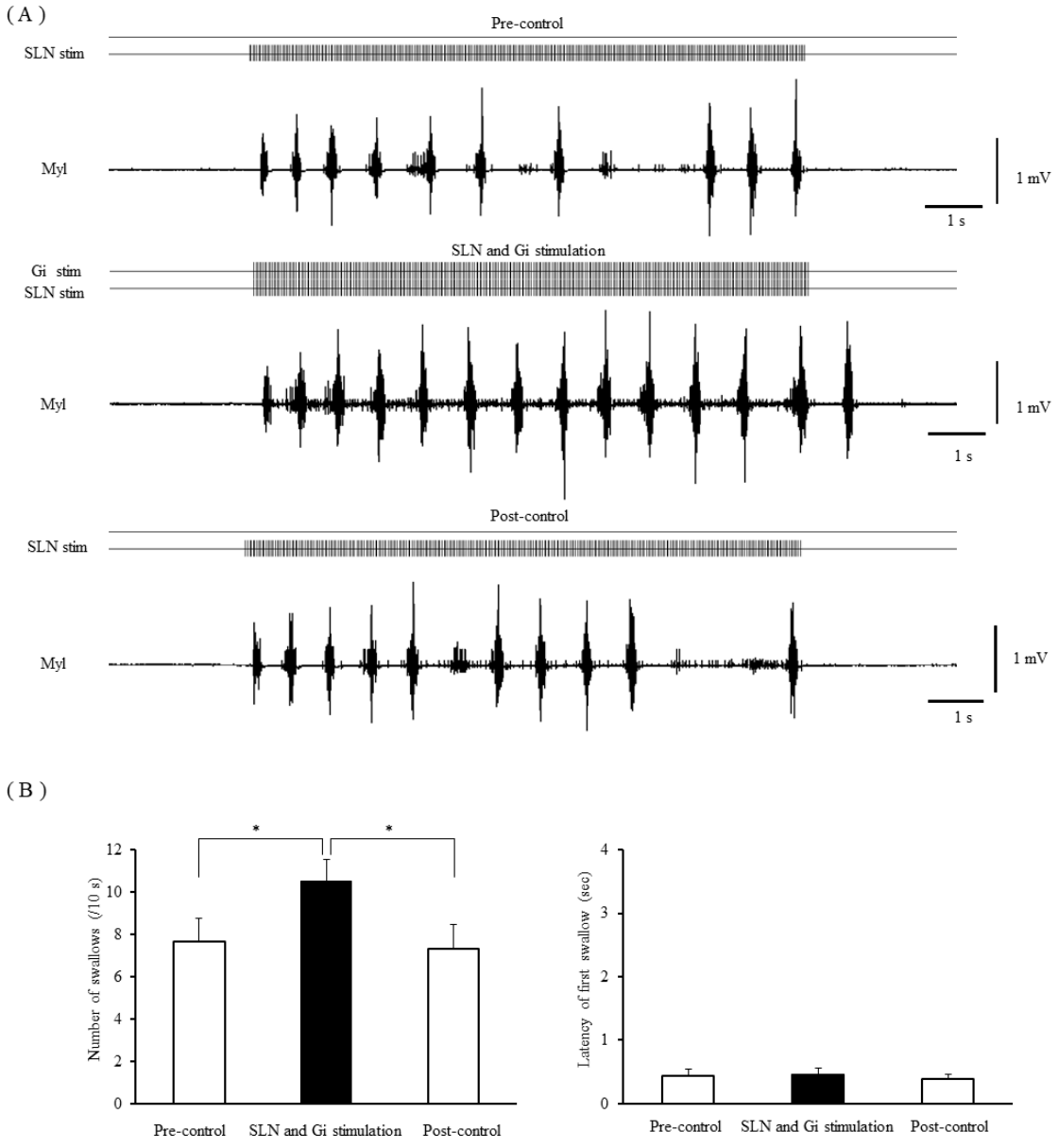

Fig. 1. (A) Examples of facilitation of the swallowing reflex by electrical stimulation of the SLN alone (upper and lower figures) and by simultaneous electrical stimulation of the SLN and the Gi (middle figure). (B) Modulation of the number of swallows (left) and of latency (right) by electrical stimulation of the Gi (n = 6). Vertical bars are ± standard error of the mean. Asterisks indicate significant differences compared with the control ($P < 0.05$).

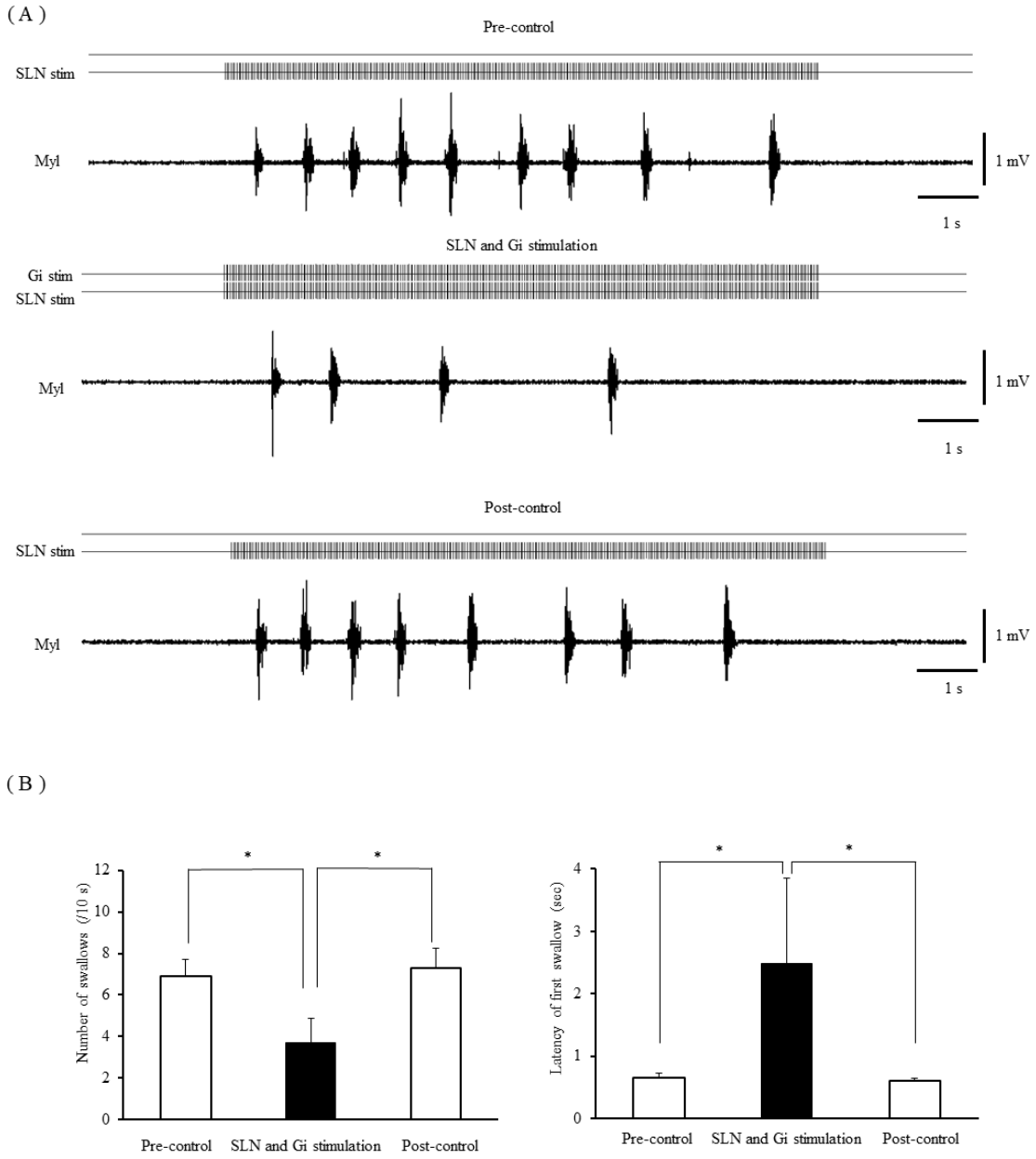

Fig. 2. (A) Examples of suppression of the swallowing reflex by electrical stimulation of the SLN alone (upper and lower figures) and by simultaneous electrical stimulation of the SLN and the Gi (middle figure). (B) Modulation of the number of swallows (left) and of latency (right) by electrical stimulation of the Gi (n = 6). Other details are as for Fig. 1.

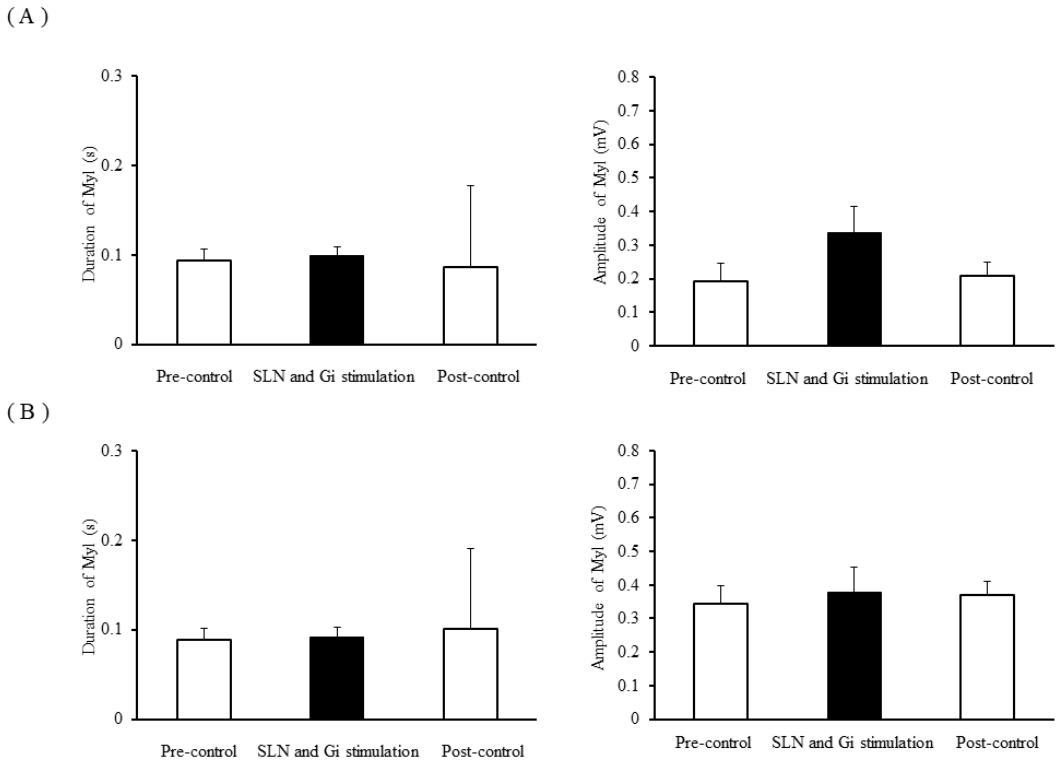

Fig. 3. Duration of Myl EMG (left) and the amplitude of Myl EMG (right) by electrical stimulation of the Gi. (A) The case of facilitation (n = 6). (B) The case of suppression (n = 6). Other details are as for Fig. 1.

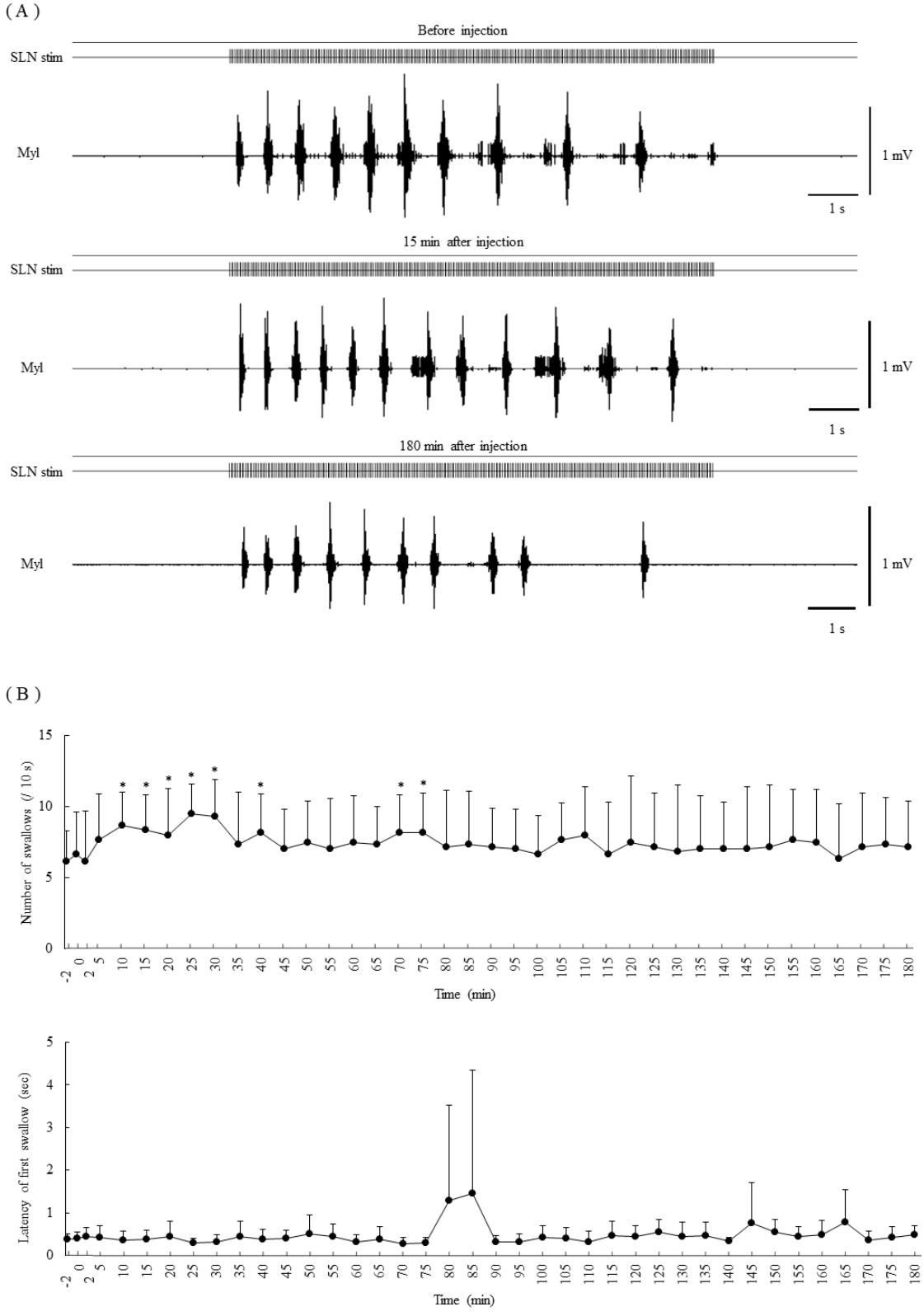

Fig. 4. (A) Examples of facilitation of the swallowing reflex by injection of glutamate into

the Gi. (B) The number of swallows (upper figure) and latency (lower figure) when the number of swallows increased after injection of glutamate into the Gi (n = 6). Vertical bars are ± standard deviation. Asterisks show significant differences compared with the control ($P < 0.05$).

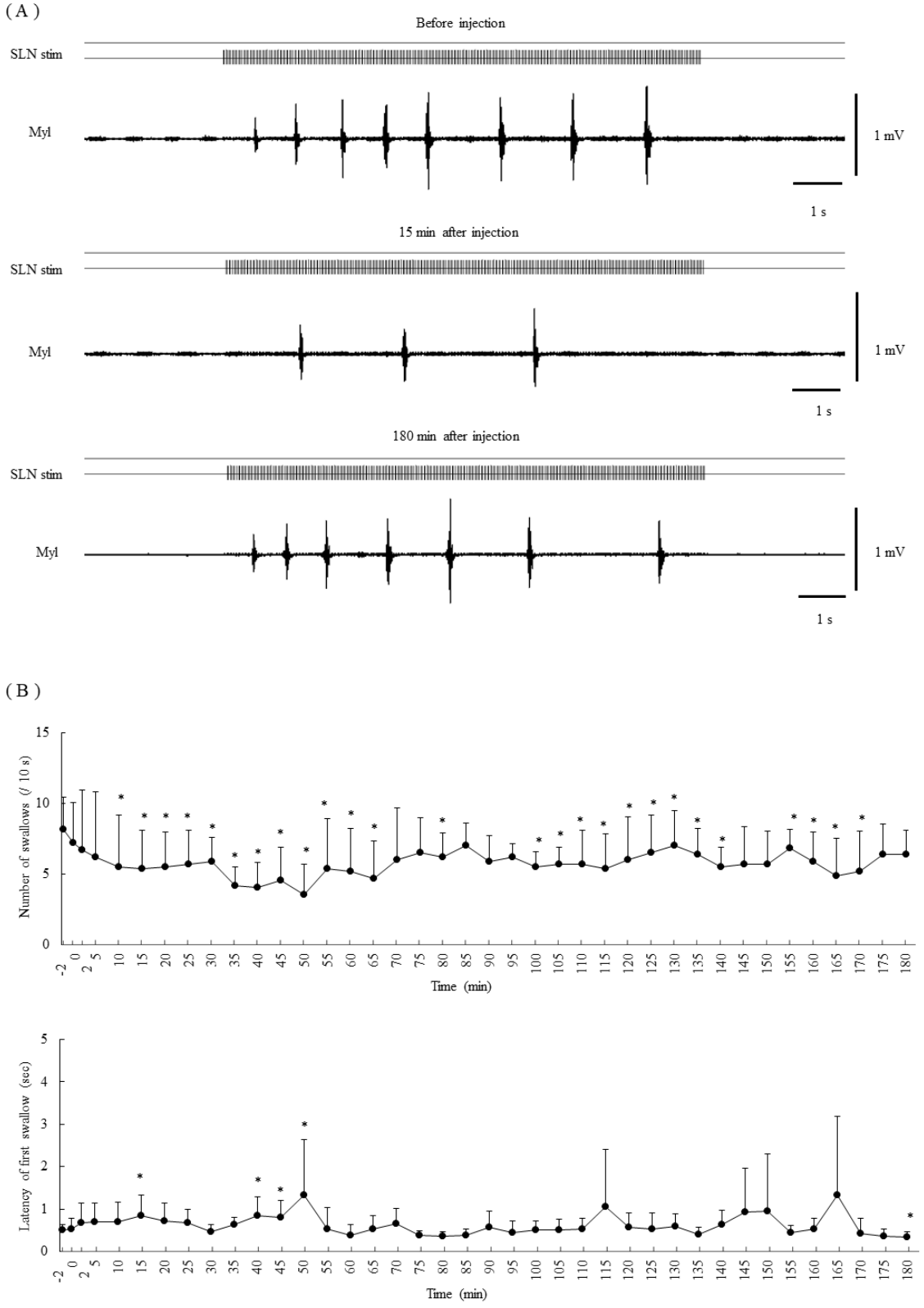

Fig. 5. (A) Examples of suppression of the swallowing reflex by injection of glutamate

into the Gi. (B) The number of swallows (upper figure) and latency (lower figure) when the number of swallows decreased after injection of glutamate into the Gi (n = 6). Other details are as for Fig. 4.

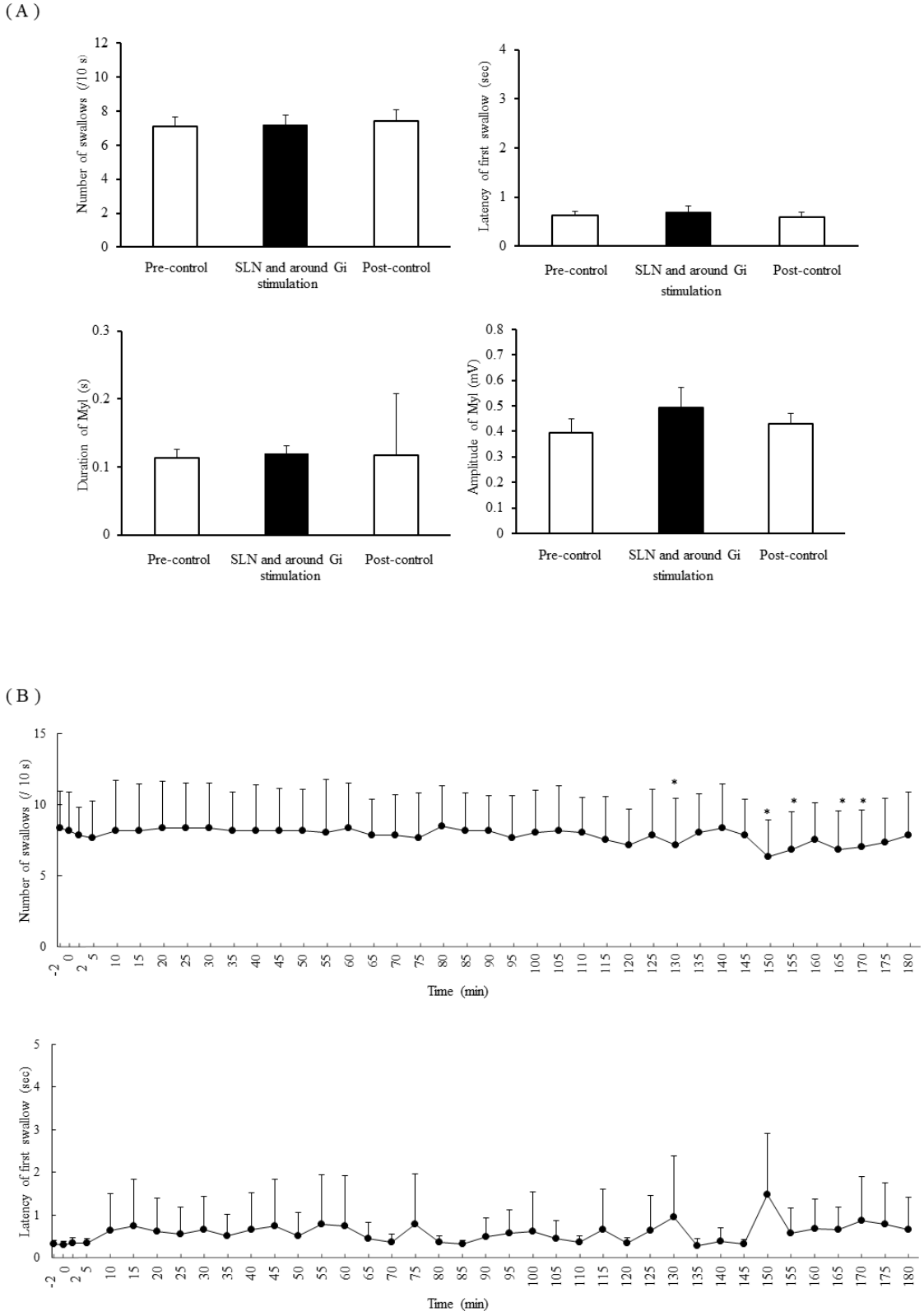

Fig. 6. (A) The number of swallows (upper left), latency (upper right), duration of Myl

EMG (lower left), and amplitude of Myl EMG (lower right) by electrical stimulation around the Gi (n= 6). Other details are as for Fig. 1. (B) The number of swallows (upper figure) and latency (lower figure) when glutamate was injected around the Gi (n= 6). Other details are as for Fig. 4.

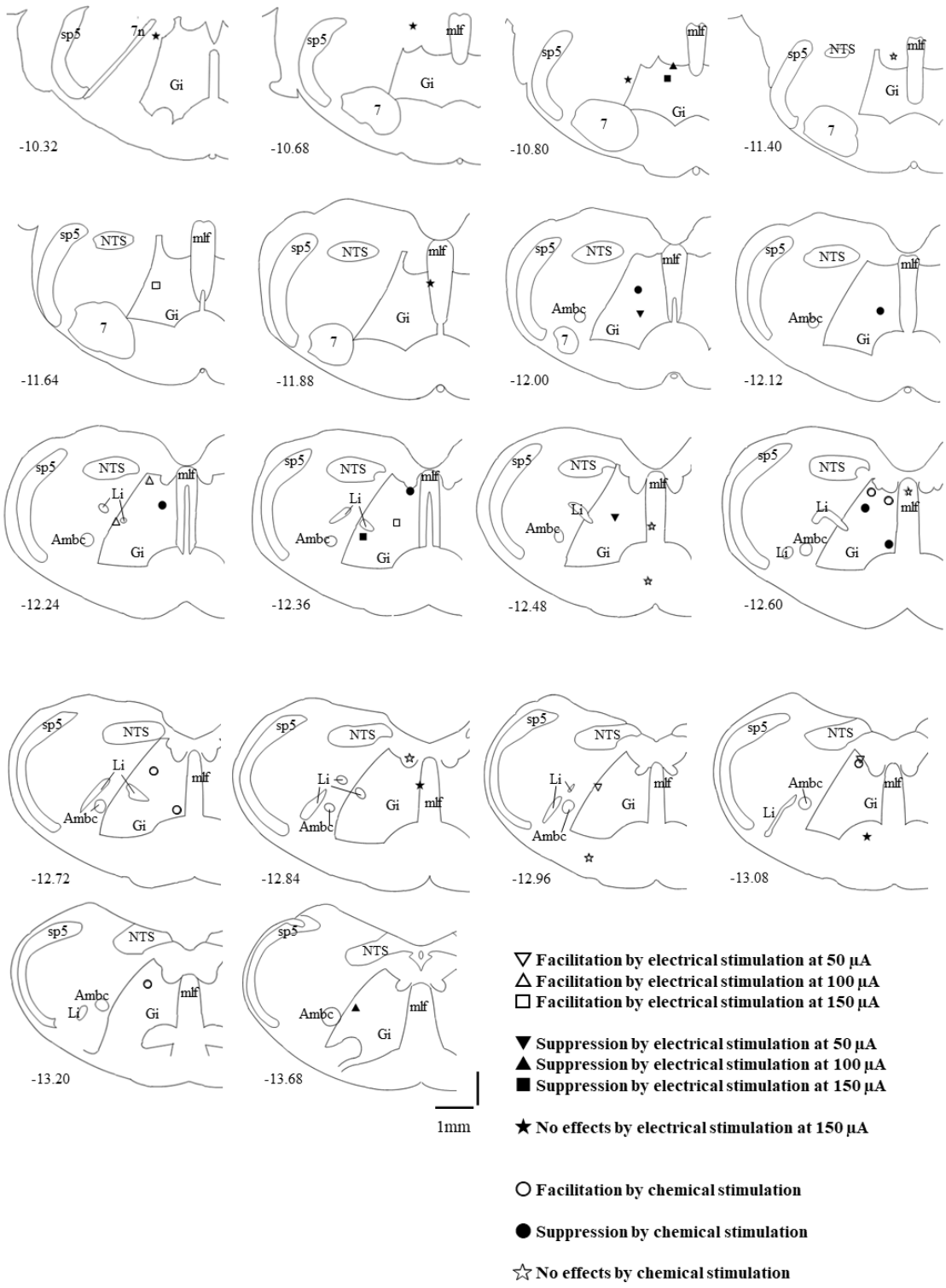

Fig. 7. Sites of electrical and chemical stimulation shown on coronal histological sections

of the brainstem. The number on the lower left of each section designates the level from the bregma in mm; (-) indicates caudal to bregma. Abbreviations: Gi, gigantocellular reticular nucleus; NTS, nucleus of the solitary tract; Ambc, ambiguus nucleus, compact part; mlf, medial longitudinal fasciculus; Li, linear nucleus of the medulla; 7n, facial nerve; 7, facial nucleus; sp5, spinal trigeminal tract.