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Swallowing-related neurons in perfused rats

Laryngeal afferent modulation of swallowing interneurons in the dorsal medulla in perfused rats

RUNNING HEAD: Afferent modulation of swallowing neurons

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GRANTS and Conflict of Interests

This work was supported by a Grant-in-Aid for Scientific Research (C) (Grant Number 18K09353). MD was supported by a NHMRC project grant (GNT1165529). The authors have no conflict of interest to declare.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1002/lary.28284](https://doi.org/10.1002/lary.28284)

Meeting information

Combined otolaryngology spring meetings (American Broncho-Esophagological Association) held in Austin, TX, USA on May 1, 2019

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Authorship

ICMJE standards:

1. Have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data.
2. Been involved in drafting the manuscript or revising it critically for important intellectual content.
3. Given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.
4. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ABSTRACT

Objectives: The purpose of this study was to investigate the influence of laryngeal afferent inputs on brainstem circuits that mediate and transmit swallowing activity to the orofacial musculature.

Methods: Experiments were performed on 19 arterially-perfused juvenile rats. The activities of swallowing interneurons in relation to their respective motor outputs in the hypoglossal and vagus nerves were assessed during fictive swallowing with or without concurrent laryngeal sensory stimulation at intensities of 20, 40, and 60 μ A.

Results: The hypoglossal nerve activity was gradually enhanced with increasing intensity of the sensory stimulation, while the vagus nerve activity was not altered. The activities of various interneurons were modulated by the laryngeal stimulation, but more than 50% of the recorded neurons were inhibited by the stimulation. Some interneurons demonstrated no obvious change in their discharge rates with laryngeal sensory stimulation during fictive swallowing.

Conclusion: Laryngeal afferent inputs partially modulated the swallowing motor activity via enhanced or suppressed activities of the swallowing interneurons, while the essential motor pattern underlying the pharyngeal stage of swallowing remained basically unchanged. Thus, the output patterns of the complex sequential movements of swallowing could be basically predetermined and further adjusted according to sensory

information related to the properties of the ingested food by a swallowing central pattern generator.

KEY WORDS: Swallowing interneuron, Perfused brainstem preparation, Rat, Sensory feedback

Level of Evidence: NA

INTRODUCTION

The motor sequence of swallowing, particularly the pharyngeal stage of swallowing, is centrally controlled by ponto-medullary brainstem networks¹⁻⁵. The kernel circuitry of swallowing pattern generation is located in the medulla oblongata and is referred to as the swallowing central pattern generator (sw-CPG). The sw-CPG can be subdivided into two main compartments, the dorsal swallowing group (DSG) that may control the initiation and the sequential output pattern of swallowing and the ventral swallowing group that may drive the motoneurons to perform the complex swallowing movements¹⁻³. Previous studies have proposed that the neurons of the DSG are the kernel for the mediation and generation of the sequential swallowing motor pattern in mammals^{1-4,6} in absence of sensory afferent signals^{1,2,7}. Nevertheless, several lines of evidence have supported the hypothesis that sensory inputs from the alimentary tract are involved in, not only the initiation of reflex swallowing, but also in the fine-motor adjustments of sw-CPG activities during an ongoing swallowing sequence^{1,8}. Indeed, researchers have proposed that the magnitude of swallowing-related muscle contraction and relaxation during the pharyngeal and esophageal stage of swallowing may depend upon the specific sensory feedback related to the properties of a specific food bolus^{1,9-15}.

Previous studies of neuronal characteristics of swallowing interneurons have revealed that most of the interneurons that control the pharyngeal stage of swallowing are activated by pharyngeal or laryngeal afferent signals^{1,2,16-18}, while pharyngeal

sensory stimulation can also inhibit sw-CPG interneurons that are involved in esophageal peristalsis during swallowing⁸. That latter could imply that inhibitory sensory feedback during swallowing may help to adjust sw-CPG activity and, in turn the swallowing motor sequence to aid successful pharyngeal-esophageal bolus transit without aspiration. However, other investigators have reported that the output patterns of swallowing-related muscle and nerve activities are not appreciably modulated via laryngeal afferent stimulation¹⁹.

To assess these contradictory findings with regard to the role of sensory feedback mechanisms during ongoing swallowing, we investigated whether or not sensory signals applied during pharyngeal swallow may influence the activity of sw-CPG interneurons. To do so, we recorded sensory evoked modulation and compared the neuronal effects with the ongoing fictive swallowing determined by a distinct activity pattern of swallowing-related motor nerves in a *in situ* perfused brainstem preparation of rat.

METHODS

All experimental procedures of the present study were approved by the local University Committee for the Use of Animals in Research, and performed following the principles for the Care and Use of Animals of the Physiological Society in Japan.

General surgical procedures

Data were collected from 19 Wistar rats (Shimizu Laboratory Supplies, Kyoto, Japan) of either sex, weighing 30 to 54 g (postnatal days 14-23). Animals were anesthetized using isoflurane (4% for induction, 1.5-2.5% for maintenance) vaporized in O₂. The depth of anesthesia was maintained to prevent any spontaneous or reflex movements. Under deep anesthesia, a transverse transection of the lower body was made at a level distal to the diaphragm. Subsequently a pre-collicular decerebration was then performed with their upper body immersed in cold artificial cerebrospinal fluid (aCSF) saturated with carbogen (95% O₂ and 5% CO₂). The preparations were then placed in a stereotaxic frame attached to a perfusion chamber secured by ear bars and cannulated by a catheter into the descending aorta. The systemic circulation was perfused with aCSF (NaCl 125 mM, KCl 3 mM, KH₂PO₄ 1.25 mM, CaCl 2.5 mM, MgSO₄ 1.25 mM, NaHCO₃ 25 mM, D-glucose 10 mM and 1.25% Ficoll) bubbled with carbogen^{6,20}. The perfusate was continuously circulated with a peristaltic pump set within a range of 14 to 23 mL/min depending on the individual preparation. The perfusate was filtered and maintained at 31°C by a temperature controller. The perfusate leaking from various cut blood vessels was recirculated to the reservoir bottle for re-oxygenation and re-perfusion.

Before a recording session was initiated, the perfusion of preparation was fine-adjusted until a stable fictive eupneic-like motor pattern, characterized by an augmenting ramp-like pattern of the phrenic nerve activity (PNA) and biphasic pattern of

the vagus nerve activity (VNA), emerged. All nerves including hypoglossal nerve activity (HNA) were recorded using suction electrodes^{20,21}, as well as for the stimulation of the superior laryngeal nerve (SLN). The animals were paralyzed with vecuronium bromide (Fuji Pharma, Tokyo, Japan; initial injection of 1.5 mg/kg, additional injections of 0.75 mg/kg when necessary) into the perfusate.

Electrophysiological procedures

For single extracellular cell recording we used glass micropipette filled with 2 M NaCl saturated with Fast Green (tip impedance 5-15 MΩ). Electrodes were aimed 0 to 1.5 mm to the midline, and between 0.5mm caudal and 1.8 mm rostral to the calamus scriptorius (opening of the 4th ventricle). The laryngeal stimulation can be predominantly transmitted to the nucleus tractus solitarius (NTS) through the SLN as visceral afferent signals and then conveyed to the higher center, albeit a part of sensory inputs to the larynx could be conveyed to the somatosensory area in the cortex²²⁻²⁴. Fictive swallowing was elicited by stimulation of the SLN (pulse duration, 0.2 ms; frequency, 20 Hz) unilaterally that triggered distinct bursting activity in either the VNA or HNA^{2,3,6,25,26}.

After locating a single cell unit in the DSG that responded to single pulse SLN stimulation, we elicited fictive swallowing by short trains of SLN stimuli (typically 3-5 pulses) prior to the initiation of the swallowing-related nerve activities only for the induction of swallowing so that we can diminish the laryngeal sensory inputs during the

period of pharyngeal swallow. The fictive swallowing elicited by this stimulus condition could be comparable to unconsciously reflexive swallowing triggered by the saliva, from the point of view of laryngeal afferent feedback during actual swallowing. Subsequently, repetitive SLN stimulations (approximately 10 sec) were applied at intensities of 20, 40, and 60 μ A to elicit sequential swallowing^{5,18,27,28}, the pattern of which is likely to be similar to that in humans^{5,29}. The output activities of both the interneurons and the swallowing-related nerves have no influence on pharyngeal and laryngeal sensory afferents during swallowing in the absence of concurrent SLN stimulation, since the sensory signals caused by the bolus transit as well as swallowing movement itself are eliminated under the condition of fictive swallowing. The intensity levels were therefore set at approximately threshold level and substantial current levels (approximately two or three times threshold), in order to estimate the effect of the intensity of laryngeal sensory information on neuronal activities without nociceptive responses. The same protocol was applied for any unit regardless whether swallowing-related unit was orthodromically activated by SLN stimulation. Action potentials of the swallowing interneurons were amplified (MEZ-8301; Nihon Kohden, Tokyo, Japan), and the cell activity was sampled at 20,000 Hz using a Power 1401 mk 2 data collection system and Spike2 version 6 software (Cambridge Electronic Design, Cambridge, UK). The nerve activities were also amplified and filtered with a band pass of 100-10,000 Hz and recorded at a sampling rate of 5,000 Hz. The nerve signals were integrated with a 1-ms

time constant and full-wave rectified. The detailed surgical and recording procedures were described in a recent study to perform recordings from the swallowing-related neurons in the dorsal medulla⁶. All other basic experimental procedures of perfused brainstem preparation were in accordance with previous reports^{20,30}.

Histological procedures and statistical analyses

The brainstem was removed and fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, and subsequently cut transversely into consecutive sections (50- μ m thickness) using a freezing microtome. The tissue sections were stained using neutral red and photographed using a digital camera (DP22; Olympus, Tokyo, Japan) mounted on an upright microscope (BX51; Olympus). Recording sites were plotted on the semi-schematic drawings of the caudal brainstem using Adobe Illustrator software (Adobe Systems, San Jose, CA). The recording sites were then referenced to their positions regarding stereotaxic coordinates with reference to the location of electrolytic lesions, the electrode track positions relative to the obex, and depths from the dorsal surface.

Data Analysis

The spontaneous firing rates of sampled neurons were calculated during spontaneous activity as well as during swallowing. The latencies of orthodromic stimulation of the SLN were measured as time from stimulus onset to an evoked action

potential. The peak amplitude and duration of the swallowing-related activities of the VNA and HNA were calculated from cycle triggered averages of several measurements (typically 3-4 swallows). We then compared the amplitudes and durations of these nerve activities (with concomitant repetitive SLN stimulation during the swallowing-related nerve bursts) to those without SLN stimulation. We evaluated the response patterns of the interneurons in the early part of the sequential swallowing (typically 3 or 4 swallows) to prevent additional effects of adaptation during the course of stimulation. Stimulus artifacts of the SLN stimulation at approximately 0.6 ms after each stimulus were cropped, so that the neuronal activity could be concealed during this period of artifact cancelation. Data were statistically analyzed using Prism 8 software (GraphPad Software, San Diego, CA), and were represented as mean $\pm$ one standard error. Significant level was set at $P < 0.05$.

RESULTS

The respiratory rate of all analyzed preparations was 16.9 ± 1.06 cycle/min, and thus in accordance with various reports using the in situ perfused brainstem preparation.

SLN-evoked swallowing motor patterns

The average threshold for SLN stimulus intensity that evoked fictive swallowing was 22.6 ± 0.86 μ A. The integrated activities of the swallowing-related nerves without

concurrent SLN stimulation and with repetitive stimulation of the SLN at 20-, 40-, and 60- μ A stimulation intensities were 1.11 ± 0.13 , 1.21 ± 0.15 , 1.02 ± 0.11 , and 1.04 ± 0.12 (μ V $\cdot$ s) for VNA, and 0.47 ± 0.10 , 0.54 ± 0.12 , 0.51 ± 0.10 , and 0.58 ± 0.11 (μ V $\cdot$ s) for HNA respectively. The integrated activity of the swallowing-related HNA appears to gradually increase in response to concomitant increase at 60- μ A SLN-stimulus intensity (Wilcoxon matched-pairs signed $p = 0.0073$; **Fig. 1**).

The durations of swallowing-related VNA and HNA during control stimulation without additional SLN stimulation were 0.46 ± 0.02 s and 0.37 ± 0.02 s, respectively.

Concomitant SLN stimulation did not change the duration of either VNA or HNA at stimulus intensities of 20 μ A (0.46 ± 0.03 and 0.36 ± 0.02 s for VNA and HNA), 40 μ A (0.45 ± 0.02 and 0.38 ± 0.02 s), or 60 μ A (0.45 ± 0.02 and 0.39 ± 0.02 s). Nevertheless, HNA showed a mild trend to increase discharge duration in response to increasing current intensity of concomitant SLN stimulation (see **Fig. 1**).

Single-unit recordings of the 27 neurons were activated during the pharyngeal stage of swallowing, and were henceforth termed swallowing interneurons (**Table 1**)^{1-3,8}. Of these 27, 15 neurons (55%) displayed respiratory-related activity during ongoing fictive breathing ($n=8$ expiratory-modulated and $n=7$ inspiratory modulated neurons).

Consequently, the remaining 12 neurons (45%) did not show respiratory modulation and were characterized by low frequency tonic activity (1.8 ± 0.66 spikes/s). Since the signal-to-noise ratio of HNA was too low to identify the swallowing-related burst during

the sessions for recording 6 units, the analyses of HNA in these sessions were not conducted.

SLN-evoked response of neurons in the dorso-caudal medulla oblongata

Eleven units orthodromically responded to ipsilateral SLN stimulation with a latency of 5.95 ± 1.42 ms on average (range, 1.8-15.5 ms). A unit was deemed to receive monosynaptic input from the SLN stimulation, when the latency was less than 3 ms and its variability was less than 0.3 ms^{2,18,31,32}. According to these criteria, five neurons (19%) were monosynaptically activated by the SLN stimulation.

The mean firing rate of all neurons triggered by fictive swallowing was 30.3 ± 5.02 spikes/s during control. These swallowing-related firing frequencies were overall not altered by additional SLN stimulation at intensities of 20, 40, and 60 μ A (31.5 ± 5.70 , 29.2 ± 4.60 , and 29.4 ± 4.10 spikes/s, respectively). The general response of swallowing interneurons to additional SLN stimulation pattern can be subdivided into three distinct classes: (i) SLN-excited neurons, which increased >20% in their firing rates during ongoing fictive swallowing (**Fig. 2**), (ii) SLN-inhibited neurons, which decreased >20% in their firing rates during ongoing fictive swallowing (**Fig. 3**), and (iii) neurons which did not change their activity during SLN stimulation (**Fig. 4**). In the present study, 30% of the recorded neurons were excited by SLN stimulation (3 non-respiratory, 2 expiratory, and 3 inspiratory units), whereas 44% were the inhibited by

SLN stimulation (8 non-respiratory, 2 expiratory, and 2 inspiratory units). Finally, the remaining 26% of neurons showed no change in their discharge activity during SLN stimulation (1 non-respiratory, 4 expiratory, and 2 inspiratory units) (**Table 1**). In Fig. 2, the effects of SLN stimulation deemed to be more evident when higher intensities were applied. As indicated in Fig. 3, whereas the activity of the inhibited interneurons during the initial swallowing tended to gradually decrease with increasing the current strength, this inhibitory effect was clearly observed in the following sequential swallows.

All neurons tested for their synaptic modulation during additional sensory feedback during ongoing swallowing activity were anatomically located 1.04 ± 0.02 mm lateral to the midline, 0.18 ± 0.08 mm rostral to the calamus scriptorius, and 0.58 ± 0.05 mm to the dorsal surface (**Fig. 5**). The sensory modulated neurons were distributed randomly within the NTS and adjacent reticular formation (two-way analysis of variance [ANOVA]). However, neurons that were unresponsive to orthodromic SLN stimulation were located more ventrally compared to responsive neurons (Mann Whitney test; $p = 0.0037$).

DISCUSSION

The findings in the present study suggest that although the amplitude of swallowing related hypoglossal nerve activity revealed a modest modulation in amplitude in response to SLN-mediated sensory activation during a fictive food bolus transit during

the pharyngeal stage of swallowing, overall the spatiotemporal motor pattern of the pharyngeal swallowing was not altered by concomitant superior laryngeal stimulation.

At the single neuron level our study shows that robust numbers of swallowing interneurons responded to concomitant SLN-stimulation (74%) whereas a subset of the interneurons did not show any modulation of their activity. These findings imply that ongoing activity of sw-CPG, at least in part, can be reconfigured and modulated during the bolus transit at the pharyngeal stage of swallowing; however, this reconfiguration may provide only a limited contribution to the final output motor patterns contrary to other motor CPGs such as the respiratory and locomotion CPGs^{33–36}. Consequently, the motor program of sequential swallowing is likely to be essentially pre-determined, regardless of food properties, and slightly modulated by sensory feedback from bolus transit, resulting in providing properly coordinated movements according to various rheological characteristics of food.

The results of previous studies have shown that the majority of the interneurons controlling the pharyngeal stage of swallowing are orthodromically activated by either the SLN or glossopharyngeal nerve^{1,3,6,17,18}, suggesting that sensory signals (produced by the bolus transit through the pharyngeal cavity) may modulate the sw-CPG to aid an efficient food bolus transfer into the esophagus. We speculate that swallowing interneurons that were excited by concomitant SLN-stimulation indeed could be mainly associated with the sensory feedback regulation of pharyngeal swallow rather than pre-

determined sequential output signals. This assumption is supported by motion kinetics of swallowing analyzed by either videofluoroscopy, manometry, or electromyography in human subjects, which revealed that swallowing movement can be modulated by the volume and the physical properties of the bolus^{9,10,12,14,15}. This feedback mechanism in turn can alter the strength of the contraction of tongue, suprahyoid, and infrahyoid muscles, as well as the duration of the relaxation of the upper esophageal sphincter (UES)^{9,10,12,14,15}.

Interestingly, many swallowing interneurons received sensory evoked synaptic inhibition during ongoing pharyngeal swallowing. The physiological properties of these neurons are not well known. Jean et al. (1984) have postulated that during the esophageal stage of swallowing, the activity of the interneurons that control the proximal portion of the esophagus is increased by the distension related to the bolus transfer⁸. Contrary, simultaneous activities of interneurons that regulate the more distal portion of the esophageal peristalsis appear to be suppressed⁸. Jean and colleagues have also proposed that the pharyngeal pressure produced by both the muscle contractions itself and bolus properties, such as bolus volume and viscosity, can inhibit the activity of esophageal interneurons. This inhibition was thought to originate from pharyngeal interneurons⁸. The interneurons that govern swallowing-related activity of the alimentary tract in the rostral areas of the dorsal sw-CPG may inhibit neurons in the more caudal area. Consequently, interneurons that receive sensory inhibition might not contribute to

the mediation of pharyngeal constriction, but could be rather involved in the relaxation of muscles, such as UES and the lower esophagus during the pharyngeal stage of swallowing. This might explain the variability of UES pressure in relation to the bolus volume^{12,15}.

However, previous experiments using adult cats demonstrated that activity patterns of swallowing-related muscles during the pharyngeal stage of spontaneous swallowing were identical to those induced by sensory stimulation of the SLN and concluded that laryngeal sensory signals may be dispensable for maintaining or modulating the swallowing pattern¹⁹. This observation is in part consistent with our data. Indeed, the results of the present study indicate that the fictive swallowing was not basically altered by sensory feedback from the larynx possibly attributable to the fixed ongoing activity of subsets of swallowing interneurons. One possible explanation for this phenomenon is that rheological characteristics may affect the actual structural pressure in the pharynx, even when the output intensity of the pharyngeal constrictor has not been essentially altered. Data obtained in this study using juvenile rats also implicate that movement of the tongue base and laryngeal elevation may be weakly modulated by bolus related sensory information. However, further studies are required to further define mechanisms of the pharyngeal bolus transition.

A potential limitation in interpreting the present data is that the effects of laryngeal sensory stimulation in the present study might be underestimated since laryngeal

afferent fibers were stimulated unilaterally. It is therefore feasible to speculate that the activity of the swallowing interneurons as well as the motor activity of the hypoglossal nerve may respond more robustly to the laryngeal sensory stimulation induced by actual food intake in intact animals.

Conclusion

We analyzed the influence of the laryngeal afferent inputs on the activity of swallowing interneurons, as well as the peripheral motor pattern of swallowing. Although the hypoglossal nerve activity was slightly mediated by sensory inputs from the larynx during ongoing swallowing, the spatiotemporal motor pattern related to pharyngeal swallow was not markedly modulated by sensory feedback arising from the larynx. Interestingly however, the response patterns of the interneurons to the laryngeal stimulation were heterogeneous during swallowing, suggesting that the sw-CPG fundamentally produces pre-determined motor output patterns of swallowing and provides further adjustments to the swallowing movement for optimal bolus transit during the pharyngeal stage of swallowing.

REFERENCES

1. Jean A. Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol Rev* 2001; **81**(2): 929-969.
2. Umezaki T, Matsuse T, Shin T. Medullary swallowing-related neurons in the anesthetized cat. *Neuroreport* 1998; **9**(8): 1793-1798.
3. Sugiyama Y, Shiba K, Nakazawa K, et al. Axonal projections of medullary swallowing neurons in guinea pigs. *J Comp Neurol* 2011; **519**(11): 2193-2211.
4. Kessler JP, Jean A. Identification of the medullary swallowing regions in the rat. *Exp Brain Res* 1985; **57**(2): 256-263.
5. Bautista TG, Dutschmann M. Ponto-medullary nuclei involved in the generation of sequential pharyngeal swallowing and concomitant protective laryngeal adduction in situ. *J Physiol* 2014; **592**(12): 2605-2623.
6. Hashimoto K, Sugiyama Y, Fuse S, et al. Activity of swallowing-related neurons in the medulla in the perfused brainstem preparation in rats. *Laryngoscope* 2019; **129**(2): E72-79.

7. Sugiyama Y, Shiba K, Mukudai S, et al. Role of the retrotrapezoid nucleus/parafacial respiratory group in coughing and swallowing in guinea pigs. *J Neurophysiol* 2015; **114**(3): 1792-1805.
8. Jean, A. Control of the central swallowing program by inputs from the peripheral receptors. A review. *J Auton Nerv Syst* 1984; **10**(3-4): 225-233.
9. Inagaki D, Miyaoka Y, Ashida I, et al. Influence of food properties and body position on swallowing-related muscle activity amplitude. *J Oral Rehabil* 2009; **36**(3): 176-183.
10. Reimers-Neils L, Logemann J, Larson C. Viscosity effects on EMG activity in normal swallow. *Dysphagia* 1994; **9**(2): 101-106.
11. Steele CM, Van Lieshout PH. Influence of bolus consistency on lingual behaviors in sequential swallowing. *Dysphagia* 2004; **19**(3): 192-206.
12. Ryu JS, Park D, Oh Y, et al. The Effects of Bolus Volume and Texture on Pharyngeal Pressure Events Using High-resolution Manometry and Its Comparison with Videofluoroscopic Swallowing Study. *J Neurogastroenterol Motil* 2016; **22**(2): 231-239.
13. Perlman AL, Schultz JG, VanDaele DJ. Effects of age, gender, bolus volume, and bolus viscosity on oropharyngeal pressure during swallowing. *J Appl Physiol* 1985; **75**(1): 33-37.

14. Dodds W, Man K, Cook I, et al. Influence of bolus volume on swallow-induced hyoid movement in normal subjects. *AJR Am J Roentgenol* 1988; **150**(6): 1307-1309.
15. Dantas RO, Kern MK, Massey BT, et al. Effect of swallowed bolus variables on oral and pharyngeal phases of swallowing. *Am J Physiol Liver Physiol* 1990; **258**: G675-G681.
16. Ootani S, Umezaki T, Shin T, Murata Y. Convergence of afferents from the SLN and GPN in cat medullary swallowing neurons. *Brain Res Bull* 1995; **37**(4): 397-404.
17. Ezure K, Oku Y, Tanaka I. Location and axonal projection of one type of swallowing interneurons in cat medulla. *Brain Res* 1993; **632**(1-2): 216-224.
18. Saito Y, Ezure K, Tanaka I. Swallowing-related activities of respiratory and non-respiratory neurons in the nucleus of solitary tract in the rat. *J Physiol* 2002; **540**(Pt3): 1047-1060.
19. Umezaki T, Shiba K, Zheng Y, et al. Upper airway motor outputs during vomiting versus swallowing in the decerebrate cat. *Brain Res* 1998; **781**(1-2): 25-36.
20. Dutschmann M, Mörschel M, Rybak IA, Dick TE. Learning to breathe: Control of the inspiratory-expiratory phase transition shifts from sensory- to central- dominated during postnatal development in rats. *J Physiol* 2009; **587**(Pt20): 4931-4948.

21. Dutschmann M, Herbert H. The Kölliker-Fuse nucleus gates the postinspiratory phase of the respiratory cycle to control inspiratory off-switch and upper airway resistance in rat. *Eur J Neurosci* 2006; **24**(4): 1071-1084.
22. Altschuler SM, Bao X, Bieger D, et al. Viscerotopic Representation of the Upper Alimentary Tract in the Rat: Sensory Ganglia and Nuclei of the Solitary and Spinal Trigeminal Tracts. *J. Comp. Neurol* 1989; **283**: 248-268.
23. Fukuyama T, Umezaki T, Shin T. Origin of laryngeal sensory-evoked potentials (LSEPs) in the cat. *Brain Res. Bull* 1993; **31**: 381-392.
24. Miyaji H, Hironaga N, Umezaki T, et al. Neuromagnetic detection of the laryngeal area: Sensory-evoked fields to air-puff stimulation. *Neuroimage* 2014; **88**: 162-169.
25. Nishino T, Honda Y, Kohchi T, Shirahata M, Yonezawa T. Effects of increasing depth of anaesthesia on phrenic nerve and hypoglossal nerve activity during the swallowing reflex in cats. *Br J Anaesth* 1985; **57**(2): 208-213.
26. Shiba K, Satoh I, Kobayashi N, Hayashi F. Multifunctional laryngeal motoneurons: an intracellular study in the cat. *J Neurosci* 1999; **19**(7): 2717-2727.
27. Oku Y, Tanaka I, Ezure K. Activity of bulbar respiratory neurons during fictive coughing and swallowing in the decerebrate cat. *J Physiol* 1994; **480**(Pt2): 309-324.

28. Gestreau C, Milano S, Bianchi AL, Grélot L. Activity of dorsal respiratory group inspiratory neurons during laryngeal-induced fictive coughing and swallowing in decerebrate cats. *Exp Brain Res* 1996; **108**(2): 247-256.
29. Aydogdu I, Tanriverdi Z, Ertekin C. Dysfunction of bulbar central pattern generator in ALS patients with dysphagia during sequential deglutition. *Clin. Neurophysiol* 2011; **122**(6): 1219–1228.
30. Paton JFR. The ventral medullary respiratory network of the mature mouse studied in a working heart-brainstem preparation. *J Physiol* 1996; **493**(Pt3): 819-831.
31. Bellingham MC, Lipski J. Morphology and electrophysiology of superior laryngeal nerve afferents and postsynaptic neurons in the medulla oblongata of the cat. *Neuroscience* 1992; **48**(1): 205-216.
32. Berry MS, Pentreath VW. Criteria for distinguishing between monosynaptic and polysynaptic transmission. *Brain Res* 1976; **105**(1): 1-20.
33. Feldman JL, Del Negro CA. Looking for inspiration: new perspectives on respiratory rhythm. *Nat Rev Neurosci* 2006; **7**(3):232-242.
34. Pearson KG. Proprioceptive regulation of locomotion. *Curr Opin Neurobiol* 1995; **5**(6):786–791.

35. Guertin PA. Central pattern generator for locomotion: Anatomical, physiological, and pathophysiological considerations. *Front Neurol* 2013; **3**,1–15.
36. Dutschmann M, Dick TE. Pontine mechanisms of respiratory control. *Compr Physiol* 2012; **2**(4),2443–2469.

Figure and table Legends

Figure 1. Changes in the amplitude and duration of swallowing-related nerve activities during concomitant laryngeal stimulation. A representative pattern of vagus (VNA) and hypoglossal (HNA) nerve activities during fictive swallowing are depicted in panel **A**. While VNA did not show apparent changes in the bursting duration or peak amplitude in response to laryngeal afferent stimulation (20 Hz) at intensities of 20, 40, and 60 μ A, the swallowing-related HNA was gradually enhanced by increasing current intensities. *Closed triangles* indicate superior laryngeal nerve stimuli (SLN stim). Lower panels show the changes of the integrated activity and duration of the VNA (**B** and **C**) and HNA (**D** and **E**) during control and concomitant laryngeal stimulation. The vertical thick lines indicate the amplitude scales (μ V). PNA, the phrenic nerve activity.

Figure 2. Firing pattern of a swallowing interneuron that receives laryngeal sensory excitation during ongoing fictive swallowing. This neuron was activated during the period of the vagus and hypoglossal burst induced by the SLN stimulation (40 μ A) without concurrent laryngeal afferent stimulation (**A**). Five orthodromic spikes evoked by a single shock stimulus of the SLN are superimposed in **B**. The swallowing-related activities of the neuron with concurrent stimulation of the SLN during swallowing at the intensity of 20, 40, and 60 μ A are displayed in **C**, **D**, and **E**, respectively. The

activity during swallowing increased in response to the laryngeal sensory stimulation at 40- and 60- μ A stimulus current. Fictive swallowing (sw) is indicated by arrowheads. The unit and nerve activities in the rectangular box in **A**, **C**, **D**, and **E** are extended to longer time scale and displayed in the right panel. Inst. Freq., instantaneous frequency.

Abbreviations are the same as in Fig. 1.

Figure 3. Firing pattern of a swallowing interneuron that receives laryngeal sensory inhibition during ongoing fictive swallowing. The swallowing-related activity of a neuron recorded without concurrent electrical stimulation of the SLN during the period of the swallowing-related nerve bursts (elicited by repetitive SLN stimulation at 40 μ A prior to swallowing initiation) (**A**), was suppressed by the SLN stimulation at 40- (**B**, **C**) and 60- (**D**) μ A current intensities. This neuron was not orthodromically activated by the SLN stimulation. The unit and nerve activities in the rectangular box in each panel are extracted in the right panel with an extended time-scale. Arrowheads and abbreviations are the same as in Figs. 1 and 2.

Figure 4. Firing pattern of a swallowing interneuron that remains unaffected by laryngeal sensory input during ongoing fictive swallowing. Activity of the neuron during swallowing without simultaneous SLN stimulation is shown in **A**. Firing frequency of this unit during swallowing was not substantially altered (<20%) by the concurrent

stimulation of the SLN at the 20- (**B**), 40- (**C**), and 60- (**D**) μ A intensities compared with that when stimulating the SLN (40 μ A) only for induction of swallowing (**A**). The orthodromic response was not recorded by a single or by multiple shocks of the SLN stimulation at the intensity of 60 μ A. The unit and nerve activities in the rectangular box in each panel are extracted in the right panel with an extended time-scale. Arrowheads and abbreviations are the same as in Figs. 1 and 2.

Figure 5. Anatomical distribution of the swallowing interneurons. Locations of the swallowing interneurons recorded in the medulla oblongata are plotted at transverse (**A**) and horizontal (**B**) planes. *Circles*: Neurons that increased in the neuronal activity in response to the SLN stimulation during swallowing (*Excited*). *Triangles*: Neurons suppressed by the stimulation (*Inhibited*). *Squares*: Neurons that showed no apparent changes in swallowing-related activity by concurrent stimulation of the SLN (*unaffected*). *Closed symbols*: Neurons that displayed respiratory modulation. *Open symbols*: non-respiratory neurons. *Black symbols*: Neurons that were orthodromically activated by the SLN stimulation. *Gray symbols*: Neurons that were not activated by the SLN stimuli. The rostrocaudal levels from the central canal opening to the 4th ventricle are displayed at the upper right corner in each panel in **A**. AP, area postrema; Cu, cuneate nucleus; Gr, gracile nucleus; IO, inferior olive; sol, solitary tract; NA, nucleus ambiguus; py,

pyramidal tract; Sp5, spinal trigeminal nerve; 10N, dorsal motor nucleus of the vagus; 12N, hypoglossal nucleus.

Table 1. Response pattern of swallowing interneurons to the laryngeal afferent inputs during ongoing pharyngeal swallowing.

(+) Neurons orthodromically activated by superior laryngeal nerve (SLN) stimulation.

(-) Neurons that did not respond to the stimulation.

Three types of response patterns of neurons to the SLN stimulation during fictive swallowing: Excited (firing increased during swallowing with concurrent laryngeal afferent inputs), Inhibited (swallowing-related activity decreased in response to the SLN stimulation), and Unaffected (showed <20% changes in firing rates). Data are presented as means $\pm$ SEM.

Table 1. Response pattern of swallowing interneurons to the laryngeal afferent inputs during ongoing pharyngeal swallowing.

Neuron type	Orthodromic response to the SLN			Neuronal activity		
			Latency (ms)	Excited	Inhibited	Unaffected

Non respiratory (N=12)	(+)	5	6.1 ± 3.0	3	2	0
	(-)	7		0	6	1
Respiratory (N=15)						
Expirartory (N=8)	(+)	2	13.5 ± 2.0	0	0	2
	(-)	6		2	2	2
Inspiratory (N=7)	(+)	4	2.0 ± 0.1	3	0	1
	(-)	3		0	2	1
Total		27		8	12	7

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lary_28284_laryngeal afferent modulation fig4 rev final.eps

lary_28284_laryngeal afferent modulation fig5.eps

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Title:

Laryngeal afferent modulation of swallowing interneurons in the dorsal medulla in perfused rats

Date:

2019-09-09

Citation:

Fuse, S., Sugiyama, Y., Hashimoto, K., Umezaki, T., Oku, Y., Dutschmann, M. & Hirano, S. (2019). Laryngeal afferent modulation of swallowing interneurons in the dorsal medulla in perfused rats. LARYNGOSCOPE, 130 (8), pp.1885-1893. <https://doi.org/10.1002/lary.28284>.

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