Influences of GABAergic inhibition in the dorsal medulla on contralateral swallowing neurons in rats

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Influences of GABAergic inhibition in the dorsal medulla on contralateral swallowing neurons in rats

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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: We aimed to examine the effect of unilateral inhibition of the medullary dorsal swallowing networks on the activities of swallowing-related cranial motor nerves and swallowing interneurons.

Methods: In 25 juvenile rats, we recorded bilateral vagal nerve activity (VNA) as well as unilateral phrenic and hypoglossal activity (HNA) during fictive swallowing elicited by electrical stimulation of the superior laryngeal nerve during control and following microinjection of the GABA agonist muscimol into the caudal dorsal medulla oblongata in a perfused brainstem preparation. In 20 animals, swallowing interneurons contralateral to the muscimol injection side were simultaneously recorded extracellularly and their firing rates were analyzed during swallowing.

Results: Integrated VNA and HNA ipsilateral to the injection side decreased to 49.0 ± 16.6 and 32.3 ± 17.9%, respectively. However, the contralateral VNA showed little change after muscimol injection. Following local inhibition 10 out of 20 swallowing interneurons tested showed either increased or decreased of their respective firing discharge during evoked-swallowing, while no significant changes in activity were observed in the remaining 10 neurons.

Conclusion: Bilaterally organized neuronal networks in the dorsal medulla that govern the
pattern generation of swallowing mediate the ipsilateral motor outputs and modulate the contralateral activity of swallowing interneurons via mutual interconnectivity that underpin the bilateral coordination of oropharyngeal swallowing movements.

Keywords: Swallowing interneuron, Swallowing central pattern generator, Perfused brainstem preparation, Rats

Level of Evidence: NA
2 Introduction

The oropharyngeal stage of swallowing consists of a series of sequential contraction of laryngeal and pharyngeal muscles that transfer fluids or food boluses into the pharynx is controlled by neuronal networks in the brainstem that are referred to as the swallowing central pattern generator (Sw-CPG)\textsuperscript{1-5}. This neuronal circuitry is thought to accurately synchronize the timing and patterning of the contraction of bilaterally organized laryngeal muscles to allow for fluid or food transport to the esophagus without aspiration\textsuperscript{1,6}. This synchronization and patterning of the swallowing motor pattern can be disrupted by peripheral or central nervous dysfunction associated with such as brain infarction or neurological degeneration\textsuperscript{7-10}. For example, the lateral medullary stroke may cause disturbed function of the unilateral Sw-CPG in the nucleus tractus solitarius (NTS) and the associated vagal and hypoglossal motor nuclei that can result in decline in swallowing function of laryngeal muscles unilateral to infarction site\textsuperscript{9,11}. However, while clinically highly relevant, the organization of bilateral synaptic interaction that underlie the generation and coordination of synchronized oropharyngeal swallowing are not well determined in particular at the cellular level. Previous studies have reported on the importance of the mutual interaction of bilateral swallowing networks\textsuperscript{12,13}. Specifically, Sugimoto et al.\textsuperscript{12} have shown that the motor activity of pharyngeal swallow on the
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contralateral side of the superior laryngeal nerve (SLN) stimulation was markedly attenuated by cutting nerve fibers that cross the midline of the medulla. Furthermore, studies also demonstrated that the decrease or elimination of bilateral interconnections of the Sw-CPG can lead to the deterioration of swallowing following unilateral SLN stimulation. These observations are compatible with the hypothesis that crossing signals between the bilaterally organized networks of Sw-CPG are indispensable for motor regulation of swallowing.

The Sw-CPG is considered to consist of dorsal and ventral swallowing groups (DSG and VSG, respectively) which may have a sparse anatomical overlap\textsuperscript{1–4,14}. The motor pattern of swallowing is primarily governed by the specific neuronal populations which are activated during swallowing (i.e., swallowing interneurons) in the DSG\textsuperscript{1–4,15}. Contrary to the notion that the pre-motor neurons in the VSG drive the final motor output of swallowing, several investigators have showed evidence that in particular laryngeal (vagal) pre-motor neurons are also located within the DSG circuits\textsuperscript{16,17}.

To investigate the putative bilateral organization of the control circuits for swallowing, we examined whether the unilateral inhibition of the DSG can trigger an ipsilateral attenuation of the swallowing-activity recorded from vagal hypoglossal nerves at the same site. In addition, we simultaneously evaluate whether or not unilateral DSG inhibition will modulate the activity of swallowing interneurons at the contralateral DSG. To do so, we recorded the activities of the swallowing interneurons as well as the swallowing-related nerves before and
1 after microinjection of the γ-amino-butyric acid (GABA) A receptor agonist muscimol into
2 the dorsal medulla (e.g. DSG) in an arterially-perfused brainstem preparation in rats, as a
3 standard method to investigate the neurophysiology of swallowing\textsuperscript{18–22}.

5 Materials and Methods

6 All of the procedures used in the present study were approved by the local Universal
7 Committee for the Use of Animals in Research and confirmed to the Physiological Society of
8 Japan Principles for the Care and Use of Animals.

9 Experiments were conducted on 25 juvenile Wistar rats (Shimizu Laboratory Supplies,
10 Kyoto, Japan) of either sex weighing 32–52 g (postnatal days 15–25).

12 The perfused brainstem preparation: Surgical procedures

13 The full experimental procedures for the perfused brainstem preparation including
14 extracellular recordings of brainstem neurons as well as the recordings of various respiratory
15 motor nerves was described in full detail previously\textsuperscript{23–26}.

16 Animals were deeply anesthetized with isoflurane (4% for induction, 1.5–2.5% for
17 maintenance) vaporized in O\textsubscript{2}. The whole body was transected below the diaphragm and then
18 the upper body was transferred into cold artificial cerebrospinal fluid (aCSF, 5°C) bubbled
19 with a carbogenic gas mixture (95% O\textsubscript{2} and 5% CO\textsubscript{2}). A pre-collicular decerebration was
attempted immediately after the transection, and cerebellectomy was also performed to facilitate insertion of glass micropipettes into the medulla. The lungs and heart were removed. A catheter was inserted into the descending aorta and was used to perfuse the animal with aCSF (NaCl 125 mM, KCl 3 mM, KH$_2$PO$_4$ 1.25 mM, CaCl$_2$ 2.5 mM, MgSO$_4$ 1.25 mM, NaHCO$_3$ 25 mM, D-glucose 10 mM, and 1.25% Ficoll) with carbogen at 31°C. The systemic circulation of the perfusate was adjusted for individual preparation ranging from 16 to 24 mL/min. The animals were positioned into the recording chamber connected with a stereotaxic frame. The cervical vagus, hypoglossal, phrenic nerves were isolated, and the distal ends of these nerves were placed on the suction electrodes for nerve recordings. The SLN was also dissected and fixed into the suction electrode for electrical stimulation. The animals were paralyzed with vecuronium bromide (Fuji Pharma, Tokyo, Japan; initial dose of 1.3 mg/kg, additional injections of 0.65 mg/kg, when necessary).

Upon initiating the recording sessions, we confirmed that the animals were stabilized into the eupnea-like respiratory motor pattern characterized by a burst-like activity of the phrenic nerve and a post-inspiratory activity of the vagus nerve.

**Recording of nerves and neurons**

Single unit extracellular neurons recordings were conducted using glass micropipettes filled with 2M NaCl saturated with Fast Green (tip impedance 8–15 MΩ) at locations -1.0 to 0.7 mm rostral to the opening of the 4th ventricle (henceforth referred to calamus scriptorius),
and 0.5 to 1.5 mm lateral to the midline.

2 Experimental protocols

Repetitive electrical stimulations of the SLN to evoke fictive swallowing and orthodromic potential for the extracellular single unit recordings were performed using either a 20 Hz stimulus train (typically 3–5 trains) or single or multiple pulse stimuli (typically one or two pulses) at a pulse duration of 0.2 ms and 40-μA intensity. Fictive swallowing was identified by burst-like patterns of the vagus (VNA) and hypoglossal (HNA) nerve activities. After identification of brainstem neurons that responded to the ipsilateral SLN stimuli, we recorded the spontaneous unit activity for approximately one minute and then determined whether the unit fired in relation to the SLN-evoked swallowing. After identification of such swallowing interneurons, a glass-pipette attached to the fluorocarbon polymer microtube connected to a 0.5-μL Hamilton syringe filled with the GABA<sub>A</sub> agonist muscimol (5 mM, dissolved in phosphate-buffered saline saturated with Direct Blue 1, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) was inserted into the medulla on the contralateral side of the neuronal recording at the following coordinates: 0.8–1.6 mm lateral to the midline, -1.0–0.2 mm rostral to the calamus, and 0.4–0.8 mm ventral to the dorsal surface. The spontaneous and swallowing-related discharge patterns of single unit were compared before and after 50-nL muscimol injections. The exact location of the injected area was confirmed histologically. The changes in the swallow-related VNA and HNA ipsilateral to
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the injection side were also analyzed between before and after the injection. Please note that
the ipsilateral HNA could not be analyzed in two preparations because of a weak amplitude
of the neural discharge.

In 12 of 25 preparations, recordings of the contralateral VNA allowed for the evaluation of
inhibitory effects of muscimol on contralateral swallowing activity in vagal motor output,
however HNA contralateral to the injection side was not recorded in the present study.

At the end of the recording session, a negative DC current (8–12 µA) for 10–20 mins was
delivered through the recording electrode in order to mark the recording sites.

Data treatment and analyses

The nerve and interneuronal activities were amplified and filtered with a band pass of
100–10,000 Hz (MEZ-8301; Nihon Kohden, Tokyo, Japan). These data were processed with
an AD converter (Power 1401 mk 2 data collection system) and fed into Spike2 version 7
software (Cambridge Electronic Design, Cambridge, UK) in a computer at a sampling rates
of 5,000 and 20,000 Hz for the nerve and unit activities, respectively. The burst amplitudes of
full-wave rectified and integrated (0.1 s time constant) VNA and HNA were calculated. The
VNA and HNA burst durations were also analyzed and smoothed at a short 1 ms time
constant to avoid inaccurate measurement of burst duration as seen with longer time
constants (e.g. 100ms).

Since the data were not normally distributed, we used non-parametric statistical analyses
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1 such as the Mann Whitney U test or Wilcoxon signed rank test in Prism 8 software

2 (GraphPad Software, San Diego, CA). All parameters are expressed mean ± standard

3 deviation.

Histological verification of injections and cell recording sites

The brainstem was fixed with 4% paraformaldehyde in 0.1M phosphate buffer and cut

transversely into 50-µm thick sections. Consecutive sections were counter stained using

neutral red and the injection or recording sites were photographed using a digital camera

mounted on a microscope and were processed with the assembling system (PTGui-Pro; New

House Internet Services BV, Rotterdam, the Netherlands).

The recording sites were identified according to the stereotaxic coordinates and location of

dye deposition or electrolytic lesion, and were mapped onto camera lucida drawings of

the sections using Adobe Illustrator (Adobe Systems, San Jose, CA). The injection area was

also determined and analyzed with reference to the dye spread.

Result

Unilateral muscimol injections triggered no significant changes on the baseline respiratory

frequency (21.0 ± 7.62 cycles/min PNA bursts before vs 21.2 ± 8.19 PNA bursts after

muscimol) indicating that general drive of the respiratory network was not altered. Thus, we
do not report specific changes in the breathing pattern in the present study although in some cases changes in the respiratory motor pattern were observed \textsuperscript{30,31}.

**Activity patterns of swallowing-related VNA and HNA before and after muscimol injection**

The mean duration of swallowing activity in the ipsilateral VNA, HNA, and contralateral VNA prior to muscimol injections were $0.48 \pm 0.08$ s (range $0.38 – 0.68$), $0.42 \pm 0.05$ s (range $0.34 – 0.54$), and $0.50 \pm 0.09$ s (range $0.36 – 0.62$). The amplitudes of the ipsilateral VNA, HNA, and contralateral VNA during swallowing were $1.76 \pm 0.98$, $1.87 \pm 1.00$, and $1.11 \pm 0.54 \mu$A.

Several minutes after muscimol injection into the contralateral medulla the durations of swallowing related ipsilateral VNA and HNA showed overall only a significant change in duration compared to control (Fig. 1). However, as illustrated in Fig. 1 the amplitudes of the VNA and HNA recorded ipsilateral to the injection side were significantly reduced to $49.0 \pm 16.6\%$ ($0.91 \pm 0.70 \mu$A) and $32.3 \pm 17.9\%$ ($0.58 \pm 0.45 \mu$A). Contrary the contralateral VNA showed not significant effect albeit a minor reduction of the mean discharge amplitude to $88.7 \pm 18.6\%$ ($0.99 \pm 0.55 \mu$A). The strength of decrease in discharge amplitudes of the ipsilateral VNA and HNA was heterogenous. Thus, the effects of muscimol injections were grouped into four categories (Fig. 2): an amplitude decrease (1) to less than 30%, (2) to
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30-50%, (3) to 50-70%, and (4) to >70%. However, no obvious correlation was found between the localization of the injection sites and the strength of decrease amplitudes of VNA or HNA as indicated by the contour plot (Figs 2C and F).

Finally, the latencies from the onset of the SLN stimulation to initiation of the swallowing-related VNA before and after the injection did not show a significant change (0.16 ± 0.07 and 0.18 ± 0.08 s).

Activity of swallowing interneurons before and after muscimol injection into the dorsal brainstem.

A total of 20 recorded neurons included 13 non-respiratory and 7 respiratory-modulated neurons in the dorsal brainstem fired in synchrony with SLN-evoked swallowing bursts in the VNA. The spontaneous firing rates of non-respiratory units (0.08 ± 0.16 spikes/s) were significantly lower compared with those of respiratory neurons (4.90 ± 3.43 spikes/s), as confirmed by Mann Whitney test ($p < 0.0001$) (Table 1). Orthodromic activation after SLN stimulation was observed in 10 (50%) units (5 non-respiratory and 5 respiratory neurons), with the latency of 6.91 ± 4.94 ms (range, 2.32–17.0 ms). The discharge frequencies of non-respiratory units (70.8 ± 55.2 spikes/s) during swallowing were higher compared to respiratory units (35.8 ± 26.1 spikes/s), however due to large range of firing frequencies no statistical significance was detected (Mann Whitney test, $p = 0.1574$) (Table 1). Fifty percent of the sampled neurons (10/20) were altered after muscimol microinjection into contralateral...
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side of the dorsal medulla. Four (40%) neurons were showed disinhibition and increased their
firing rates (Fig. 3), while six (60%) neurons showed inhibition and decreased discharge
frequencies (Fig. 4). The remaining 10 neurons exhibited less than 20% changes in their
firing rates, which were defined as no change units (Fig. 5). The average spontaneous firing
rate after the injection was $2.78 \pm 4.31$ spikes/s ($n = 20$) and did not change in comparison to
control values. (Wilcoxon matched-pairs signed rank test, $p = 0.2334$).

The recorded neurons were located $1.00 \pm 0.08$ mm lateral to the midline, $-0.13 \pm 0.21$ mm
rostral at the level of the apex of the calamus scriptorius and at a depth of $0.97 \pm 0.19$ mm
ventral to the dorsal surface of the brainstem. According to the anatomical reconstruction of
the recording sites, all neurons were identified ventral to the solitary tract and 75% of neurons
were located in the medullary reticular formation (RF) ventral to nucleus of solitary tract.
Finally, no topographical organization of the location of different neuron types was detected
(Fig. 6).

Discussion

We investigated the effect of GABA-receptor agonist in the NTS and adjacent structures
on activities of swallowing interneurons and the swallowing motor pattern identified by
cranial nerve recordings. Our findings indicate that the neural control of SLN-evoked
swallowing may be substantially mediated via a neuronal circuitry in the ipsilateral dorsal
medulla. In addition, single unit recordings revealed that the swallowing interneurons receive
excitatory or inhibitory modulatory inputs from the contralateral dorsal medulla. The latter
suggests that mutual interconnections between bilateral neuronal networks that generate
swallowing motor pattern are indispensable for the bilateral coordination of swallowing
movements. These data show for the first time that mutual synaptic interaction across
bilateral dorsal medullary swallowing networks is necessary to generate the oropharyngeal
stage of swallowing.

Changes in the motor patterns of swallowing following unilateral inhibition of the
dorsal swallowing group (DSG).

Data in the present study showed that a microinjection of a GABA\textsubscript{A} agonist into the NTS
and adjacent RF attenuated the VNA and HNA ipsilateral to the injection site. In particular,
the amplitudes of the VNA and HNA were reduced by approximately 50\% and 70\%,
respectively. The differences in change in motor nerve discharge amplitudes may be
attributed to the heterogenous distributions of hypoglossal and laryngeal (vagal) pre-motor
neurons in relation to the injection site. Previous histological and electrophysiological
evidences have indicated indeed that the vagal pre-motor neurons are located in the DSG
including the NTS and adjacent RF as well as the ventrolateral RF including the VSG and the
respiratory neuron groups 3,16,17,32–34, and anterograde and retrograde tracing studies showed
that hypoglossal pre-motor neurons were preferentially found in the dorsal medulla overlapping with the DSG. As indicated in Fig. 2, the effects of the muscimol microinjection on the swallowing-related nerve activities could vary depending on the inhibited area. Thus, in accordance with previous publications our study supports the notion that the neuronal networks of swallowing interneurons are widely distributed in the NTS and the RF which in turn, directly or indirectly control the oropharyngeal motor pattern of swallowing.

The latencies as well as the burst durations of the bilaterally recorded VNA were not significantly altered after unilateral inhibition of the DSG. The latter suggests that patterning of the vagal motor output largely depends on distributed network components of the Sw-CPG. As already mentioned above, the networks implemented in the generation of the swallowing pattern are likely to be distributed across the dorsal and ventral RF and the NTS.

Indeed previous works have shown that more aspects of the RF can regulate oropharyngeal swallowing and thus could have compensated for synaptic inhibition of the DSG.

Mutual synaptic interaction of swallowing interneurons across the bilateral compartments of the DSG.

To our knowledge, this study is the first to analyze the modulation of afferent signals via inputs from the contralateral Sw-CPG under reduced experimental conditions where
swallowing motor pattern generation can be executed without sensory feedback regulation \cite{25,40,41}.

The data provided by single neuron recordings show excitatory or inhibitory modulation of swallowing interneuron discharge arising from the contralateral DSG. Swallowing interneurons that displayed a decrease in firing rate after GABAergic inhibition of the contralateral dorsal medulla are likely to normally receive excitatory inputs from the contralateral DSG, in contrast to the neurons that show increased spiking should normally receive inhibitory inputs from the contralateral DSG. Sixty-five percent of the swallowing interneurons showed no respiratory modulation with no or very low spontaneous firing rate, which seem likely to be a strong candidate for the swallow-specific interneurons \cite{2,5,25,26}.

These observations further highlight the importance of interconnections and interaction between bilateral swallowing neuronal networks.

The muscimol evoked unilateral inhibition of the DSG had no effect on the discharge of 50\% of interneurons recorded. Nevertheless this observation and the previous observation that muscimol injection only decreased the amplitudes of swallowing motor burst in VNA HNA ipsilateral to injection site while the contralateral site remained unaffected further suggests that the primary generation of swallowing motor pattern basically remains operational without modulatory inputs form the contralateral DSG. However, the role of modulation of the swallowing motor pattern via synaptic interconnectivity between the left
and right DSG\textsuperscript{3,12,42} needs to be further explored with SLN stimulation that evoke sequential
swallowing.

Conclusion

The bilaterally organized neuronal networks of swallowing interneurons in the DSG can
drive the swallowing motor outputs in the vagal hypoglossal nerves with a clear ipsilateral
dominance. However, the coordination and synchronization of bilateral swallowing motor
sequence targeting bilateral laryngeal muscles may be modulated via mutual synaptic
interactions between neurons of the left and right DSG. Further investigations are required to
determine the afferent and efferent connections of bilaterally organized swallowing
interneurons.
References


8. Umezaki T, Adachi K, Matsubara N, Lee Y. Tracheoesophageal diversion and puncture


8. Dutschmann M, Mörschel M, Rybak IA, Dick TE. Learning to breathe: Control of the

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1  Neuroscience. 2006;140(2):577-95.

2  38. Umezaki T, Sugiyama Y, Fuse S, et al. Supportive effect of interferential current
3  stimulation on susceptibility of swallowing in guinea pigs. Exp Brain Res.  
4  2018;236(10):2661-76.

6  nucleus retroambiguus region on the pharyngeal phase of swallowing. Respir Physiol

8  40. Jean A. Control of the central swallowing program by inputs from the peripheral


12 42. Beart PM, Summers RJ, Stephenson JA, Christie MJ. Excitatory amino acid
13  projections to the nucleus of the solitary tract in the rat: a retrograde transport study utilizing
Figure and table legends

**Figure 1**: Fictive swallowing motor patterns in the activities of ipsilateral/contralateral vagus nerves (VNA) and ipsilateral hypoglossal nerve (HNA) in relation to ongoing respiration recorded in phrenic nerve activity (PNA) before and after microinjection of the GABA$_A$ agonist muscimol into the dorsal medulla (A). The lower traces of each nerve show the integrated activity of each nerve signal (time constant, 0.1 s). The robust decreases in the amplitudes of ipsilateral VNA and HNA were observed after synaptic inhibition of the dorsal medulla. The vertical lines indicate the amplitude scale bar (10 µV). Panel B illustrates the location of muscimol injection as a gray area in a representative transverse section of the medulla oblongata at the level of 0.05mm rostral to the opening of the 4th ventricle (calamus scriptorius). The group data for the muscimol evoked changes of the amplitudes of swallowing related bursts in VNA and HNA (smoothed with 0.1 s time constant) are shown in (C). Abbreviation: SLN stim, superior laryngeal nerve stimulation; sw, swallowing; Cu, cuneate nucleus; IO, inferior olive; NA, nucleus ambiguus; py, pyramidal tract; s, solitary tract; Sp5, spinal trigeminal nucleus; 10N, dorsal motor nucleus of vagus; 12N, hypoglossal nucleus. Ipsi, ipsilateral to the injection site; contra, contralateral to the injection site.

**Figure 2**: Schematic illustration of the rostro-caudal topography of muscimol injection sites. The effects strength (see below) of inhibition of swallowing burst amplitude of VNA...
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are shown in panels A – C and for HNA in panels D – F. The center of the injection sites for each animal is plotted in transverse (A and D) and horizontal (B and E) planes. *Stars* indicate the injection sites where muscimol caused a decrease in the discharge amplitude to < 30% (amplitude reduction by > 70%); *circles* delineate the sites where a reduction of the burst amplitudes to 30–50% were observed; *triangles* mark the injection sites where burst amplitudes were reduced to 50 and 70%; finally *squares* show injection sites with >70% amplitude. The 3D contour plot of percent changes of VNA and HNA burst amplitudes with rostrocaudal and mediolateral coordinates in relation to the calamus. Gr, gracile nucleus.

Abbreviations see Fig. 1.

**Figure 3:** Firing pattern of a swallowing-interneuron which increases its discharge frequency during swallowing following muscimol microinjection. Please note that this specific neuron fired only in the earlier period of fictive swallowing prior to muscimol (A).

After the injection, the swallowing-related unit activity increased even when the VNA and HNA amplitudes were decreased during swallowing (B). Boxes in the left panel of A and B show the neuron spiking in the expanded time axis. Five orthodromic spikes from the SLN stimulation are superimposed in (C). Schematic illustration of the recording site (dot) and injection area (gray area) in relation to the calamus are shown in D and E. Abbreviations: Inst. Freq., instantaneous frequency the abbreviations for specific brain nuclei are the same as Figs. 1 and 2.
Figure 4: Firing pattern of a swallowing interneuron that exhibited a decrease in the firing rate during swallowing after muscimol injection. Prior to muscimol injection, this neuron showed a decrementing firing pattern during evoked swallowing (A). The discharge frequency activity of this neuron decreased after the injection of muscimol (B). Orthodromic responses to the SLN stimulation are shown in C. The recording site and the injection area are illustrated in D and E, respectively. Anatomical abbreviations are the same as Fig. 1. AP, area postrema.

Figure 5: Activity of a swallowing-interneuron that showed no apparent change in firing pattern after muscimol injection. This neuron spiked during evoked swallowing but spontaneous firing was not observed under eupneic condition. In addition, the neuron showed no orthodromic activity after SLN stimulation (A). Firing pattern of this unit was unchanged after muscimol injection. (B). The Location of the neuron and the injection area are depicted in panels C and D. Abbreviations are the same as Fig. 4.

Figure 6: Summary of the anatomical distribution of swallowing interneurons and their response characteristics to the dorsal medullary inhibition. Locations of units recorded are plotted in transverse (A) and horizontal (B) planes. Neurons which showed increased spiking activities after muscimol injection are delineated by circles, while neurons that decreased discharge frequency after muscimol injection are indicated by triangles. Squares mark neurons whose firing rates were not altered (less than 20% change) after inhibition. Black
symbols indicate non-respiratory neurons, while gray symbols indicate neurons that had respiratory modulation in relation to ongoing respiration. Abbreviations are the same as Fig. 4.

Table 1: Neuronal characteristics of swallowing interneurons due to the GABAergic inhibition of contralateral dorsal medulla. Increased, neurons that increased in firing rate after GABAergic inhibition of contralateral dorsal medulla; decreased, neurons whose firing rate decreased after the inhibition; no change, neurons that exhibited less than 20% changes in firing rate due to the inhibition. Numbers in parentheses are the numbers of neurons. NTS, nucleus tractus solitarius; SLN, superior laryngeal nerve; RF, reticular formation. A single asterisk (*) indicates significance at $p < 0.0001$. The Laryngoscope
**A**

**Pre injection**

- **VNA (ipsi)**
  - Waveform:
    - Pre injection: 
    - Post injection: 

- **VNA (contra)**
  - Waveform:
    - Pre injection: 
    - Post injection: 

- **HNA (ipsi)**
  - Waveform:
    - Pre injection: 
    - Post injection: 

- **HNA (contra)**
  - Waveform:
    - Pre injection: 
    - Post injection: 

- **PNA**
  - Waveform:
    - Pre injection: 
    - Post injection: 

- **ʃ VNA**
  - SW: 

- **ʃ VNA**
  - SW: 

- **ʃ HNA**
  - SW: 

- **ʃ PNA**
  - SW: 

**Post injection**

- **VNA (ipsi)**
  - Waveform:
    - Pre injection: 
    - Post injection: 

- **VNA (contra)**
  - Waveform:
    - Pre injection: 
    - Post injection: 

- **HNA (ipsi)**
  - Waveform:
    - Pre injection: 
    - Post injection: 

- **HNA (contra)**
  - Waveform:
    - Pre injection: 
    - Post injection: 

- **PNA**
  - Waveform:
    - Pre injection: 
    - Post injection: 

**B**

- Diagram showing anatomical structures.

**C**

- **VNA (ipsi)**
  - Graph showing comparison between Pre and Post injection.

- **VNA (contra)**
  - Graph showing comparison between Pre and Post injection.

- **HNA (ipsi)**
  - Graph showing comparison between Pre and Post injection.

**Legend:**

- **ʃ** denotes integration.
- **SLN stim** denotes stimulation of the superior laryngeal nerve.
- **1 mm** denotes the scale of the diagram.
- **0.05** denotes a significance level.
- **n.s.** denotes nonsignificant results.
- ***** denotes significant results.
<table>
<thead>
<tr>
<th>Neuron type</th>
<th>Non-respiratory</th>
<th>Respiratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Decreased</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>No change</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

**Diagram**

- **A**: Neuron type distribution across different regions labeled with markers (10N, Sp5, Cu, AP, Gr, 12N).
- **B**: Graph showing neuronal distribution in ML and AP axes with markers indicating changes in neuronal activity.

**Legend**

- **ML**: Medial-Lateral axis
- **AP**: Anterior-Posterior axis

**Scale**: 1mm
Table 1: Neuronal characteristics of swallowing interneurons due to the GABAergic inhibition of contralateral dorsal medulla.

<table>
<thead>
<tr>
<th>Neuron</th>
<th>Spontaneous firing rate (spikes/s)</th>
<th>Firing rate during swallowing (spikes/s)</th>
<th>Orthodromic latency from SLN stimulation (ms)</th>
<th>Changes in firing rate due to GABAergic inhibition (%)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increased</td>
<td>Decreased</td>
<td>No change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-respiratory (n = 13)</td>
<td>0.08 ± 0.16*</td>
<td>70.8 ± 55.2</td>
<td>7.25 ± 4.24</td>
<td>177.6 ± 60.2 (2)</td>
<td>NTS (4), RF (9)</td>
</tr>
<tr>
<td>Respiratory (n = 7)</td>
<td>4.90 ± 3.43</td>
<td>35.8 ± 26.1</td>
<td>6.58 ± 6.05</td>
<td>178.4 ± 19.6 (2)</td>
<td>NTS (1), RF (6)</td>
</tr>
<tr>
<td>E (n = 4)</td>
<td>3.22 ± 1.54</td>
<td>19.8 ± 15.8</td>
<td>3.22 ± 1.54</td>
<td>192.2 (1)</td>
<td>NTS (1), RF (3)</td>
</tr>
<tr>
<td>I (n = 3)</td>
<td>4.61 ± 4.44</td>
<td>57.1 ± 21.9</td>
<td>11.6 ± 7.57</td>
<td>164.6 (1)</td>
<td>RF (3)</td>
</tr>
<tr>
<td>Total (n = 20)</td>
<td>1.76 ± 3.05</td>
<td>58.8 ± 49.4</td>
<td>6.91 ± 4.94</td>
<td>178.0 ± 36.6 (4)</td>
<td>NTS (5), RF (15)</td>
</tr>
</tbody>
</table>

Table 1: Neuronal characteristics of swallowing interneurons due to the GABAergic inhibition of contralateral dorsal medulla. Increased, neurons that increased in firing rate after GABAergic inhibition of contralateral dorsal medulla; decreased, neurons whose firing rate decreased after the inhibition; no change, neurons that exhibited less than 20% changes in firing rate due to the inhibition. Numbers in parentheses are the numbers of neurons. NTS, nucleus tractus solitarius; SLN, superior laryngeal nerve; RF, reticular formation. A single asterisk (*) indicates significance at \( p < 0.0001 \).
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Kinoshita, S; Sugiyama, Y; Hashimoto, K; Fuse, S; Mukudai, S; Umezaki, T; Dutschmann, M; Hirano, S

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