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Original Reports

Hashimoto et al.

Swallowing-Related Neurons in Perfused Rats

Activity of Swallowing-Related Neurons in the Medulla in the Perfused Brainstem Preparation in Rats

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This Manuscript was received on March 31, 2018, revised on May 17, 2018, and accepted for publication on May 29, 2018.

Presented at the Combined Otolaryngology Spring Meetings, National Harbor, Maryland, U.S.A., April 18–22, 2018.

K.H. and Y.S. contributed equally to this work and are co–first authors.

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.27401](https://doi.org/10.1002/lary.27401)

Each author participated sufficiently in the work to take public responsibility for appropriate portions of the content. K.H. and S.F. made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; gave final approval of the version to be published; and 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. Y.S. made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; was involved in drafting the manuscript or revising it critically for important intellectual content; gave final approval of the version to be published; and 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. T.U., Y.O., and S.H. gave final approval of the version to be published and 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. M.D. was involved in drafting the manuscript or revising it critically for important intellectual content; gave final approval of the version to be published; and 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.<zaq;1>

This work was supported by a <GS>Grant-in-Aid for Young Scientists (B) </GS> (grant number <GN>15K20220</GN>).

The authors have no other funding, financial relationships, or conflicts of interest to disclose.

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Objectives/Hypothesis: We aimed to investigate and validate the cellular activity patterns and the potential topographical organization of neurons of the medullary swallowing pattern generator (Sw-CPG). We used the perfused brainstem preparation as an innovative experimental model that allows for stable neuronal recording in the brainstem.

Study Design: Animal model.

Methods: Experiments were conducted in 14 juvenile Wistar rats. The activities of the phrenic, vagus, and hypoglossal nerves were recorded at baseline, and fictive swallowing was elicited by stimulation of the superior laryngeal nerve. Extracellular action potentials of 72 swallowing-related neurons were recorded in the Sw-CPG of the dorsal medulla oblongata.

Results: Neurons could be classified into three types: sensory relay, and neurons that were excited or inhibited during fictive swallowing. Approximately one-third of the neurons likely received monosynaptic input from the laryngeal afferents. One-third of neurons recorded showed respiratory-related activity, most of which exhibited inspiratory modulation. The neurons were widely distributed in the nucleus tractus solitarius and reticular formation.

Conclusions: The perfused brainstem preparation of rat fully preserves the Sw-CPG. The recorded cellular activities and general topographical organization of swallowing neurons are in accordance with previous in vivo studies. Thus, the perfused brainstem preparation is an ideal experimental model to advance the understanding of neuronal mechanisms underlying swallowing.

Key Words: Swallowing, central pattern generator, perfused brainstem preparation, rat.

Level of Evidence: NA

<H1>INTRODUCTION</H1>

The pharyngeal stage of swallowing is produced by complex network interaction within the

central pattern generator of swallowing (Sw-CPG) in the dorsal and ventral medulla oblongata.^{1–}

³ The Sw-CPG provides the spatiotemporal coordination of a series of sequential contractions of the oral, pharyngeal, and laryngeal musculatures that allow food transport through the pharynx and across the upper esophageal sphincter without entering the airway.

The Sw-CPG can be separated into two main components: the dorsal swallowing group (DSG), which is located in the nucleus tractus solitarius (NTS) and around the medullary reticular formation (RF), and the ventral swallowing group (VSG), which is located near the nucleus ambiguus.¹ The DSG may govern the reflex control of swallowing and the generation of rhythmic activities that drive complex sequential oropharyngeal motor patterns. Neurons of the VSG may provide the direct command to the motoneurons to mediate contractions of the lingual, pharyngeal, and laryngeal muscles.^{1,4–7}

Previous studies that investigated mechanisms of swallowing pattern generation have been performed predominantly in higher mammalian species such as monkeys, sheep, and cats.^{1,4,7–9} A recent publication has shown that the perfused brainstem preparation of rats could be a valuable model to study swallow pattern generation and coordination of swallowing and breathing.¹⁰ In the present study, we aimed to extracellularly record swallowing-related neurons (SRNs) in the dorsal medulla oblongata. The perfused brainstem preparation is an ideal model for such studies because absent or diminished pulsatile cardiac output allow for stable cellular recording. The perfused brainstem preparation is an established experimental model for stable recording of brainstem neurons.^{11–13} Although some investigators have denoted the brainstem neuronal activity in this preparation in rodents,^{11,13} the activity of the SRNs was not investigated. Previous studies using in vivo experiments have investigated the activities, locations, and interconnections of the SRNs in the medulla.^{1–7,14} Nevertheless, the neuronal mechanisms of swallowing pattern generation are still not fully understood. Thus, we recorded

the neuronal activity of the SRNs in the NTS and RF in the perfused brainstem preparation to assess whether this experimental approach is suitable to substitute time consuming and technically highly demanding in vivo experiments using decerebrate, paralyzed, artificially ventilated animals.

<H1>MATERIALS AND METHODS</H1>

Experiments were performed on 14 purpose-bred Wistar rats (Shimizu Laboratory Supplies, Kyoto, Japan) of either sex, weighing 40 to 60 g (postnatal days 16–29). All experimental procedures were conducted according to the principles for the Care and Use of Animals of the Physiological Society in Japan. The animal ethics were reviewed and approved by the local University Committee for the Use of Animals in Research.

<H2>Surgical Procedures</H2>

Each animal was initially anesthetized with isoflurane (4% for induction, 1.5%–2.5% for maintenance) vaporized in O₂, sufficient to prevent spontaneous or reflexive movements. After deep anesthesia, the body of each animal was cut transversely at the level below the diaphragm. Immediately after transection, the upper body of the animal was immersed in cold artificial cerebrospinal fluid (aCSF) saturated with carbogen (95% O₂ and 5% CO₂) at 5°C. The animal was then decerebrated at the precollicular level and was cerebellectomized. The lungs were removed. The heart was also removed subsequent to ligation of the aortic bow to prevent backflow of oxygenated aCSF. The animal was placed in a recording chamber, and a catheter was inserted through the descending aorta exposed near the thoracic spinal column. Subsequently, the animal was mounted in a stereotaxic apparatus, to allow for reproducible location of recording sites. The perfusate (NaCl 125 mM, KCl 3 mM, KH₂PO₄ 1.25 mM, CaCl 2.5

mM, MgSO₄ 1.25 mM, NaHCO₃ 25 mM, D-glucose 10 and 1.25% Ficoll) bubbled with a gas mixture of 95% O₂ and 5% CO₂ was continuously circulated through the catheter using a peristaltic pump (Minipuls 3 Peristaltic Pump; ADInstruments, Bella Vista, NSW, Australia). The osmolarity and the pH were maintained at 300 ± 10 mOsm and 7.35 ± 0.05 , respectively. Before injection into the descending aorta, the perfusate was filtered and passed through bubble traps, and its temperature was stabilized at 31°C by a temperature controller (HCT-10; ALA Scientific Instruments Inc., Farmingdale, NY). Perfusate that was leaking from cut blood vessels of the preparation was collected from the recording chamber for reoxygenation and recirculation using the roller pump.

The left phrenic (PHR) nerve was isolated and cut at the distal end just above the diaphragm. In the neck area, the vagus nerve (VN), the hypoglossal (HyG) nerve, and the superior laryngeal nerve (SLN) were also isolated and cut distally. The suction electrodes were positioned and attached to the distal end of the PHR, the VN, and the HyG nerves for recording, and to the SLN for stimulation. Flow rates were carefully adjusted and fine-tuned within the 16 to 22 mL/min range for any individual preparation. Before experimental procedures were initiated, we established an augmenting ramp-like bursting pattern of the PHR and decrementing activity of the VN during the postinspiratory in every preparation. This activity pattern is considered to reflect the baseline eupnea-like<zaq;2> respiratory motor pattern of the perfused brainstem preparation.^{12,15} The animal was then paralyzed by injection of vecuronium bromide (Fuji Pharma, Tokyo, Japan; initial injection of 1.5 mg/kg, additional injections of 0.75 mg/kg when necessary) into the reservoir bottle.

All surgical procedures for perfused brainstem preparation were fundamentally the same as those reported in the previous studies.^{12,13} A schematic representation of the experimental settings used in the present study is shown in Figure 1. To elicit fictive swallowing identified by

bursting activity of the VN and the HyG and that can be distinguished from fictive coughing regarding activity patterns of these nerves,^{3,16–18} we delivered electrical stimulation through the suction electrode via the SLN unilaterally at the proper intensity sufficient for the induction of swallowing (pulse duration, 0.2 ms; frequency, 20 Hz; intensity, 20–50 μ A).¹⁹ At the end of the experiment, the animal was perfused with 100 mL of physiological saline followed by 100 mL of 4% paraformaldehyde in 0.1 M phosphate buffer for subsequent histological verification of the recording sites.

<H2>Electrophysiological Recordings</H2>

Extracellular recordings were performed using a glass micropipette filled with 2 M NaCl with 2% Fast Green (tip impedance 8–15 M Ω). The NTS and RF recording sites were at the following coordinates: –0.6 to 1.7 mm rostral to the level of central canal opening to the fourth ventricle, 0.5 to 1.6 mm from the midline, and 0.2 to 1.8 mm to the dorsal surface of the medulla.

Activity of neurons was amplified (MEZ-8301; Nihon Kohden, Tokyo, Japan) and 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 phrenic, vagus, and hypoglossal activities were amplified, filtered with a band pass of 100 to 10,000 Hz, integrated with a 1-ms time constant, full-wave rectified, and sampled at 2,000 Hz. Spike detection and sorting feature of the Spike2 was used for the delineation of spikes from the unit activity.

When encountering a unit that responded to SLN stimulation (single or multiple stimulations of 0.2-ms duration), we recorded the spontaneous activity to assess whether the unit discharged in relation to the respiratory cycles. Subsequently, we recorded the activity of the unit during fictive swallowing. In addition, when a spontaneously active neuron that was unresponsive to

orthodromic SLN stimulation, we also examined the unit's activity during swallowing. An electrolytic lesion or an iontophoretic deposition of dye was made at a recording site near the end of the recording session.

<H2>Histological and Data Analysis Procedures</H2>

The brainstem was cut transversely into 50- μ m-thick consecutive sections using freezing microtome. Tissue sections were stained with neutral red. The sections were photographed by a charge-coupled device digital camera (DP22; Olympus, Tokyo, Japan) mounted on an upright microscope (BX51; Olympus) and assembled with PTGui-Pro photostitching software (New House Internet Services BV, Rotterdam, the Netherlands). Camera lucida drawings of the sections were made using Adobe Illustrator software (Adobe Systems, San Jose, CA). The recording sites were reconstructed according to the relative positions of electrode tracks, and microelectrode depths indicated by electrolytic lesions or dye depositions and mapped onto schematic drawings of dorsal medulla oblongata.

The recorded cells were analyzed for their spontaneous and evoked firing rate. The spike latencies of the neurons that responded to the SLN stimulation were measured. Statistical analyses were performed using Prism 6 software (GraphPad Software, San Diego, CA). Pooled data were represented as mean $\pm$ one standard error. A confidence interval of $P < .05$ was considered significant.

<H1>RESULTS</H1>

The activity of 72 neurons was recorded in the present study. Neurons were classified into three different types regarding activity patterns during SLN-evoked fictive swallowing: 1)

sensory-relay neurons (42/72, 58%) that orthodromically responded to SLN stimulation but did not show swallow-related activity changes, 2) excited SRNs (ESRNs) (18/72, 25%) that were activated during swallowing, and 3) inhibited SRNs (ISRNs) (12/72, 17%), whose activity was suppressed according to the swallow-related burst of the VN and the HyG (Fig. 2, Table I). Twenty-five of the 72 (35%) neurons displayed respiratory discharge patterns (16 sensory-relay neurons, four ESRNs, and five ISRNs). The majority of the recorded respiratory units showed inspiratory modulation ($n = 19$, 11 sensory-relay neurons, three ESRNs, and five ISRNs), whereas the only expiratory unit was characterized as ESRN. Five remaining sensory-relay neurons discharged at the inspiratory–expiratory phase transition during eupnea.

The spontaneous firing rate of all units was 5.39 ± 1.22 spike/s on average (Table I). The nonrespiratory neurons had significantly ($P < .0001$, Mann-Whitney test) lower discharge rates (1.02 ± 0.36 spike/s on average) compared to respiratory neurons (13.6 ± 2.81 spike/s on average). In more detail, 11 (15%) neurons (eight sensory-relay neurons, one ESRN, and two ISRNs) were silent during respiration (Fig. 2A,D). Sixty-three neurons (88%) were activated orthodromically from single to four-train stimulation of the ipsilateral SLN, whereas the remaining nine ESRNs did not exhibit any response to the stimulation at one to seven trains per set. The mean latency to orthodromic spikes of the sensory-relay neurons, ESRNs, and ISRNs were 3.29 ± 0.21 ms (range, 1.07–7.69 ms), 5.75 ± 1.85 ms (range, 1.87–19.3 ms), and 5.88 ± 0.68 ms (range, 2.71–10.0 ms), respectively (Table I). The sensory-relay neurons showed significantly shorter latencies compared to the other types of neurons (one-way analysis of variance [ANOVA], Dunn's multiple comparisons test). Several lines of evidence have suggested that the presumably monosynaptic input to NTS neurons resulting from the laryngeal afferent stimulus could be evoked within <3 ms in latency and with <0.3 ms of variability.^{2,14,20,21} In the present study, approximately half ($n = 19$) of the sensory-relay neurons and one-fifth ($n = 3$) of the ESRNs met these criteria. A Pearson correlation coefficient of 0.491 was obtained between

the mean latency of the SRNs from the SLN stimulation and the distance to the dorsal surface of the medulla.

The locations of the neurons in the NTS and RF whose firing patterns were analyzed during respiration and swallowing are shown in Figure 3. The sensory-relay neurons were located on average at 1.18 ± 0.03 mm lateral to the midline, 0.16 ± 0.06 mm rostral to the central canal opening to the fourth ventricle, and 0.58 ± 0.02 mm ventral to the dorsal medulla. The ESRNs were distributed at 1.01 ± 0.07 mm to the midline, 0.26 ± 0.13 mm rostral to the caudal most level of the fourth ventricle, and 0.78 ± 0.09 mm ventral to the dorsal surface of the medulla. The ISRNs were distributed on average at 1.09 ± 0.04 mm to the midline, 0.20 ± 0.13 mm rostral to the caudal most level of the fourth ventricle, and 0.55 ± 0.07 mm to the dorsal surface. The majority of neurons were widespread within the NTS and around the RF, showing no significant topographical organization of specific cell types (two-way ANOVA). Otherwise, the neurons that showed no orthodromically evoked activity from the SLN stimulation were located more ventrally compared to those that responded to the stimulation as confirmed by a two-way ANOVA combined with Sidak's multiple comparisons test.

The histological reconstruction of recording sites revealed that the sensory-relay neurons were located in the dorsal (NTSd) ($n = 3$), dorsolateral (NTSdl) ($n = 3$), intermediate (NTSint) ($n = 5$), interstitial (NTSis) ($n = 17$), ventral (NTSv) ($n = 2$), ventrolateral (NTSvl) ($n = 9$), and medial (NTSm) ($n = 1$) subnuclei, whereas the ESRNs were distributed in the NTSd ($n = 2$), NTSdl ($n = 2$), NTSint ($n = 2$), NTSis ($n = 3$), NTSv ($n = 2$), and NTSvl ($n = 1$).²² The ISRNs were located in the central (NTScen) ($n = 1$), NTSd ($n = 4$), NTSint ($n = 1$), NTSis ($n = 2$), NTSv ($n = 2$), and NTSvl ($n = 1$). Nine neurons, including two sensory-relay neurons, six ESRNs, and one ISRN, were sparsely distributed in the RF.

<H1>DISCUSSION</H1>

This study is the first to describe the firing patterns of the SRNs in the NTS and RF, in the perfused brainstem preparation of rat. The present study also showed that the activity patterns and anatomical locations of recorded cells are in general accordance with reports using anesthetized or decerebrate preparations.^{1-3,14} Thus, no apparent differences of neuronal activities underlying swallowing pattern generation were detected in the perfused brainstem preparation compared to traditional experimental models. In addition, this study also suggested that the various visceral afferent signals, including the pulmonary adapting receptors,²³ could not significantly interfere with the core network mechanisms that produce pharyngeal swallowing. Consequently, the perfused preparation that favors stable long-term recordings of brainstem neurons can facilitate the analysis of the synaptic and intrinsic membrane properties of neurons in the Sw-CPG. In summary, the perfused brainstem preparation is an excellent experimental model that will allow for the quantitative electrophysiological and neuropharmacological investigation of synaptic mechanisms underlying swallowing pattern generation in the NTS.

Previous studies have demonstrated various types of firing patterns of SRNs in relation to swallow-related nerve burst.^{2-4,14} In the present study, the activity of the individual neurons associated with swallowing was heterogeneous in terms of activation latency, discharge frequency, and patterns (Fig. 2C,D,F). The observed variability may act as a source of precisely timed excitatory or inhibitory premotor inputs to orofacial motoneurons, resulting in the sequential peristaltic movements of the upper alimentary tract.^{1,17,24-26}

Approximately one-third of neurons recorded had respiratory-related activity, mostly inspiratory. The histological verification of recording sites showed that approximately one-third

of the respiratory-related neurons were located within the region of the dorsal respiratory group (DRG).^{27–31} Saito et al.¹⁴ documented that swallow-related inspiratory neurons of the DRG in rats are likely to be propriobulbar neurons. Other investigators have also reported that various DRG inspiratory neurons have swallowing-related activity, and thus are contributing to swallowing pattern generation.^{29–31} On the other hand, some DRG neurons could connect to the NTS sw-CPG neurons, to the RF adjacent to the nucleus ambiguus, or to the RF near the hypoglossal nucleus,³² where relevant cranial premotor neurons are located.^{4,7,33,34} We suggest that respiratory-related SRNs may participate in the generation of swallowing, via the interaction with proprioceptive swallowing interneurons.³

Although the vast majority of the neurons were orthodromically activated by SLN stimulation, the neurons that had presumptive monosynaptic input from the SLN were relatively lower in number compared to those reported in an earlier study using rats.¹⁴ One possibility is that approximately half of the sensory-relay neurons and three-fourths of the ESRNs and ISRNs were distributed outside the interstitial and intermediate subnuclei of the NTS, the primary termination field of laryngeal afferents.^{22,35} Therefore, many neurons recorded in the present study may have received second- or higher-order synaptic input from the SLN. Nevertheless, the data obtained in the present study are consistent with the previous notion that ventrally located SRNs have a tendency to have a long latency to SLN stimulation, indicating the existence of a polysynaptic pathway described elsewhere.^{1,4,36}

The majority of the SRNs were broadly distributed within the NTS and adjacent RF, whereas some ESRNs in the RF were located apart from either the DSG or the VSG. Several studies as well as our own findings support the hypothesis that the DSG could overlap, at least in part, with the VSG. However, further studies are needed to prove this hypothesis.^{2,3}

Neurons that are activated after the oropharyngeal swallowing burst of the vagus and hypoglossal nerve were not identified in the present study. Thus, we did not identify neurons that could be involved in the mediation of the esophageal stage of swallowing, although previous reports showed that the esophageal peristaltic contractions during swallowing could be controlled by the neurons in the NTS^{3,14,33}. Additional studies are necessary to further investigate the mechanism underlying the neuronal circuitry involved in the esophageal stage of swallowing.

<H1>CONCLUSION</H1>

This is the first report to record activity of the SRNs in the NTS and RF with the perfused brainstem preparation that allows stable neuronal recording in rats. The neuronal characteristics and cellular distribution of perfused rats were similar to those in other mammals using the traditional in vivo approaches. Therefore, the perfused preparation is a valuable tool to advance the understanding and operational principles of the Sw-CPG.

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<3>Fig. 1. Schematic illustration of the experimental approach to record swallowing-related neurons in the medulla oblongata using perfused brainstem preparation. A decerebrate paralyzed juvenile rat preparation was placed in the recording chamber, fixed into a stereotaxic frame. Subsequently, the preparation was perfused with aerated (carbogen) artificial cerebrospinal fluid (aCSF) through a double lumen catheter. The aCSF was warmed to 31°C using a temperature controller before perfusing the descending aorta. The flow rate of the perfusate was optimized by a peristaltic pump to oxygenate the brainstem to allow for the generation of eupnea-like respiratory network function. The latter was assessed by recordings of the activity of the phrenic (PHR), vagus (VN), and hypoglossal (HyG) nerves recorded using suction electrodes. The tissue particles in the perfusate were filtered, and the air bubbles that could potentially trigger embolism were removed by a bubble trap. The effluent perfusate was returned to the reservoir bottle using a roller pump for recirculation and reoxygenation. The distal end of the superior laryngeal nerve (SLN) was connected to the suction electrode for electrical stimulation. The horizontal position of the dorsal surface of the medulla was maintained by perpendicular movement of the recording chamber. A glass microelectrode was

attached to the three-dimensional manipulator mounted on the stereotaxic frame to facilitate precise penetration into the brainstem. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

Fig. 2. Examples of the firing patterns of the swallowing-related neurons during fictive respiration and swallowing. Activity of a neuron that responded orthodromically to the superior laryngeal nerve (SLN) stimulation during both eupnea and swallowing (sensory-relay neuron) is shown in (A) and (B). This neuron did not discharge spontaneously during eupnea. The firing pattern of a neuron that was activated during swallowing (excited swallowing-related neurons [ESRN]) without receiving orthodromic inputs from the SLN is shown in (C). This neuron fired at the onset of the swallow-related activities of the vagus (VN) and hypoglossal (HyG) nerves. Another example of activity of an ESRN is displayed in panels (D) and (E). This neuron was not spontaneously active during eupneic conditions but was activated during swallow-related nerve bursting. The firing patterns of an ESRN with respiratory-related activity are depicted in panels (F) and (G). This neuron exhibited inspiratory-modulated activity during respiration and started firing, which led to the initiation of the swallow-related nerve activity. The activity of a neuron that ceased firing during swallowing (inhibited SRNs [ISRN]) is indicated in panels (H) and (I). The orthodromic responses by electrical stimulation of the SLN (five superimposed) are shown in panels (B), (E), (G), and (I). The arrowheads indicate fictive swallowing. Thick lines indicate repetitive stimulation of the SLN, whereas the arrows illustrate single shock stimulation of the SLN. E = expiratory phase; I = inspiratory phase; Inst. freq. = instantaneous frequency; PHR = phrenic; sw = swallowing.

Fig. 3. Semischematic drawing of the nucleus tractus solitarius that illustrates the anatomical locations of the swallowing-related neurons. The neurons are plotted at transverse planes (A), horizontal planes (B), as well as in the three-dimensional data plot (C). Landmarks for

histological reconstructions refer to the level of the transition from the central canal to the fourth ventricle (AP), lateral to the midline (ML), and the dorsal surface of the medulla (Depth). The sensory-relay neurons, excited swallowing-related neurons (ESRN), and inhibited swallowing-related neurons (ISRN) are indicated by squares, circles, and triangles, respectively. Open symbols indicate neurons that responded to the superior laryngeal nerve (SLN) stimulation, whereas filled symbols designate neurons that were not orthodromically activated from the SLN. Black symbols indicate units that did not exhibit respiratory-related activity, whereas grey symbols designate respiratory neurons. Numbers at the upper right corner in panel (A) indicate the rostrocaudal level (in millimeters) between the brainstem section and the central canal opening to the fourth ventricle (calamus). 10N = dorsal motor nucleus of the vagus; 12N = hypoglossal nucleus; AP = area postrema; Cu = cuneate nucleus; Gr = gracile nucleus; IO = inferior olive; sol = solitary tract; MVe = medial vestibular nucleus; NA = nucleus ambiguus; py = pyramidal tract; SpVe = spinal vestibular nucleus; Sp5 = spinal trigeminal nerve.

TABLE I.

Spontaneous Firing Rates, Latencies of Orthodromic Responses to SLN Stimulation, and Cellular Locations of the Swallowing-Related Neurons Within Specific Subnuclei of the NTS.

Neuron Type	Total No.	Spontaneous Spike Rate (Spike/s)	Orthodromic Response to the SLN			Location (NTS Subnuclei)
			No. (+)	Latency (ms)	No (-)	
Sensory-relay neuron	42	4.81 ± 1.21	42	3.29 ± 0.21	0	d, dl, int, is, m, v, vl
Respiratory	16	11.1 ± 2.41	16	2.76 ± 0.14	0	dl, int, is, v, vl
Nonrespiratory	26	0.93 ± 0.39	26	3.61 ± 0.31	0	d, dl, int, is, m, v, vl
ESRN	18	4.55 ± 1.72	9	5.75 ± 1.85	9	d, dl, int, is, v, vl
Respiratory	4	14.8 ± 3.95	3	2.29 ± 0.33	1	dl, is, v
Nonrespiratory	14	1.63 ± 0.99	6	7.49 ± 2.53	8	d, dl, int, is, v, vl
ISRN	12	8.67 ± 5.53	12	5.88 ± 0.68	0	cen, d, int, is, v, vl
Respiratory	5	20.6 ± 11.9	5	5.90 ± 1.26	0	d, v, vl
Nonrespiratory	7	0.16 ± 0.07	7	5.86 ± 0.84	0	cen, d, int, is, v
Total	72	5.39 ± 1.22	63	4.13 ± 0.35	9	
Respiratory	25	13.6 ± 2.81	24	3.36 ± 0.38	1	
Nonrespiratory	47	1.02 ± 0.36	39	4.61 ± 0.50	8	

Neurons recorded were classified into three types: sensory-relay neurons, ESRNs, and ISRNs. Amongst these three types, the neurons of each type were subdivided into respiratory-related and non-respiratory-related units. The numbers of different types recorded neurons are indicated. The neurons that were orthodromically activated by the SLN stimulation and unresponsive neurons are denoted by (+) and (-), respectively. All data are presented as mean ± standard error of the mean.

cen = central; d = dorsal; dl = dorsolateral; ESRN = excited swallowing-related neurons; int = intermediate; is = interstitial; ISRN = inhibited swallowing-related neurons; m = medial; NTS = nucleus tractus solitarius; SLN = superior laryngeal nerve; v = ventral; vl = ventrolateral.

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Title:

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Date:

2019-02-01

Citation:

Hashimoto, K., Sugiyama, Y., Fuse, S., Umezaki, T., Oku, Y., Dutschmann, M. & Hirano, S. (2019). Activity of swallowing-related neurons in the medulla in the perfused brainstem preparation in rats. LARYNGOSCOPE, 129 (2), pp.E72-E79. <https://doi.org/10.1002/lary.27401>.

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