Swallowing reflex and brain stem neurons activated by superior laryngeal nerve stimulation in the mouse

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Swallowing reflex and brain stem neurons activated by superior laryngeal nerve stimulation in the mouse. Am J Physiol Gastrointest Liver Physiol 280: G191–G200, 2001.—The purpose of the present study was to identify vagal subnuclei that participate in reflex swallowing in response to electrical stimulation of the left superior laryngeal nerve (SLN). SLN stimulation at 10 Hz evoked primary peristalsis, including oropharyngeal and esophageal peristalsis, and LES relaxation. It also induced c-fos expression in interneurons in the interstitial (SolI), intermediate (SolIM), central (SolCe), dorsomedial (SolDM) and commissural (SolC) solitary subnuclei. Neurons in parvicellular reticular nucleus (PCRt) and area postrema (AP) and motoneurons in the semicompact (NAsc), loose (NAI), and compact (NAc) formations of the nucleus ambiguus and both rostral (DMvr) and caudal (DMvc) parts of the dorsal motor nucleus of vagus were also activated. The activated neurons represent all neurons concerned with afferent SLN-mediated reflexes, including the swallowing-related neurons. SLN stimulation at 5 Hz elicited oropharyngeal and LES but not esophageal responses and evoked c-fos expression in neurons in SolI, SolIM, SolDM, PCRt, AP, NAsc, NAI, and DMvc but not in SolCe, NAc, or DMvr. These data are consistent with the role of SolI, SolIM, SolDM, NAc, NAI, and DMvc circuit in oropharyngeal peristalsis and LES relaxation and SolCe, NAc, DMvc, and DMvr in esophageal peristalsis and LES responses.

c-fos expression; solitary subnuclei; nucleus ambiguus; dorsal motor nucleus of vagus; swallowing program generator

Swallow-induced primary peristalsis is a centrally programmed sequence of oropharyngeal and esophageal contractions that is orchestrated by a so-called swallowing program generator (SPG) in the brain stem (10, 13, 14, 20). The SPG neurons receive input from peripheral oropharyngeal afferents and central cortical projections, which are responsible for activating reflex swallowing (10, 13). The premotor neurons send commands to motoneurons that execute the motor peristaltic sequence.

It has been shown that distinct components of the SPG are responsible for oropharyngeal and esophageal phases of swallowing (10, 13, 14). The oropharyngeal swallowing is evoked by oropharyngeal afferents carried primarily in the superior laryngeal nerves (SLN) that project onto the second-order interneurons in interstitial solitary subnuclei (SolI) and intermediate solitary subnuclei (SolIM) (16, 24). Studies (3) using pseudorabies virus as a transneuronal tracer have shown that these subnuclei also contain premotor neurons for motoneurons in semicompact formation of the nucleus ambiguus (NAsc), loose formation of the nucleus ambiguus (NAI), and other cranial nerve nuclei that innervate the oropharyngeal striated muscles. Interneurons in SolI and SolIM also project onto central solitary subnuclei (SolCe) interneurons (8). SolCe neurons are premotor neurons for motoneurons in the compact formation of the nucleus ambiguus (NAc) that innervate the esophageal striated muscle (4, 15). During swallow-evoked primary peristalsis, the oropharyngeal and esophageal components of SPG act in concert. However, the oropharyngeal and esophageal phases may be dissociated (10). Oropharyngeal activity without esophageal peristalsis may occur when SolI and SolIM interneurons fail to activate SolCe interneurons. On the other hand, secondary peristalsis (esophageal peristalsis alone) is elicited when SolCe interneurons are activated by afferents arising from the esophagus (17).

The above conclusions apply primarily to the activity in the striated muscle of the oropharnyx and esophagus (6, 8, 13, 20). The preganglionic motoneurons for the smooth muscle portion of the esophagus and the lower esophageal sphincter (LES) are located in the dorsal motor nucleus of vagus (DMV) (1, 9, 28, 29, 30); however, location of their premotor neurons is unclear (2, 27).

We investigated the swallowing responses elicited by SLN stimulation at various frequencies in mice. Electrical stimulation of the SLN at 10 Hz produced a primary peristalsis, 5 Hz produced oropharyngeal contractions and LES relaxation without esophageal peristalsis, and 1 Hz produced no response. We also examined the location of putative swallow-related neurons in the brain stem that may be involved in these reflexes. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
activities by identifying the c-fos-activated neurons at different SLN stimulus frequencies in separate animals. c-fos is an early response gene that is expressed in transsynaptically activated neurons. Identification of c-fos expression may serve as an important technique to identify neural circuits in reflex activities (11, 22, 26, 34).

MATERIAL AND METHODS

Twenty-eight adult male Swiss Webster mice (Taconic, Germantown, NY) weighing 20–40 g were used. The procedures were approved by the Animal Committee of the Brockton/West Roxbury Veterans Affairs Medical Center.

Esophageal manometry. Esophageal manometry was performed using a custom-designed catheter assembly (Dentsleeve, Parkside, SA, Australia). The assembly consisted of three silicon catheters (ID, 0.3 mm; OD, 0.6 mm) that were glued together. The catheter was 6.5 cm long and had an outside diameter of 1.2 mm. Each catheter had a 0.3-mm side opening; the side openings were located 2 mm apart. The catheters were filled and continuously perfused with bubble-free preboiled water at a rate of 7 μl/min using a pressurized nitrogen chamber. The catheters provided a low-compliance system and were connected to flow through pressure transducers that were connected to ETH 400 Bridge amplifiers (CB Science, Dover, NH). The output of pressure signals was displayed and recorded on MacLab software (AD Instruments, Castle Hills, NSW, Australia).

The mice were anesthetized with a subcutaneous injection of pentobarbital sodium (60 mg/kg) and placed on a heating pad. The catheter assembly was introduced into the esophagus through a gastroscope. The catheters were then withdrawn gradually in 0.5- to 1-mm increments to scan for the high-pressure zone of the LES. The three catheter openings were positioned in the LES and at 2 and 4 mm above the LES. Esophageal and LES pressure changes in response to a spontaneous swallow and electrical stimulation of the SLN were then recorded. The occurrence of artifactual LES relaxation-associated axial movement of the LES was excluded by careful visual monitoring. Pharyngeal swallowing activity was identified by visually observing the motion of the exposed laryngopharyngeal area in the neck. The pharyngeal pressures were not recorded for technical reasons.

A midline neck incision was then made, and the left SLN was exposed. In the mouse, the SLN arises from the vagus below the nodose ganglion and lies dorsal to the carotid artery. The SLN was isolated and severed at the point where it enters the laryngeal musculature. The central end of the SLN was placed on a bipolar (silver-platinum) hook electrode and covered with warm mineral oil and stimulated using a Grass stimulator (model S11; Quincy, MA). Current spread and covered with warm mineral oil and stimulated using a Grass stimulator (model S11; Quincy, MA). Current spread and covered with warm mineral oil and stimulated using a Grass stimulator (model S11; Quincy, MA). Current spread

The electrical stimuli used to induce c-fos expression were similar to those used for the manometric studies, except that the 9-s stimuli were repeated with a stimulus free interval of 10 s for a period of 30 min. Repeated stimuli with 10-s stimulus-free intervals were applied to avoid decay of the swallowing responses with continuous stimuli (33). At the end of the experiment, the mice were killed with a subcutaneous injection of pentobarbital sodium (100 mg/kg) and perfused transcardially with 60 ml PBS (0.9% NaCl in 0.01 M sodium phosphate buffer, pH 7.0) followed by 60 ml of 4% formaldehyde (4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.0). The brain stem with attached upper cervical spinal cord was removed and postfixed in the same fixative overnight at 4°C. The fixative was then removed with three washes in PBS, and the tissue was stored in PBS containing 1% sodium azide plus 30% sucrose for 24 h at 4°C before being sectioned. Frozen coronal serial sections of 25-μm thickness were cut and collected directly on silane-coated slides (Sigma Chemical, St. Louis, MO) so that the orientation of the sections was always the same. The sections were then processed for histochemical and immunohistochemical staining.

The first of every three sections was stained with cresyl violet for anatomic landmark studies. The section was washed in water and stained with 1% cresyl violet (Fisher Scientific, Pittsburgh, PA) containing 0.25% acetic acid for 5 min. After being rinsed in water, the section was differentiated in 50% ethanol for 5 min and rinsed again in water. The section was then mounted with the mounting medium (Sigma Chemical).

The second section was immunostained for choline acetyltransferase (ChAT) and c-fos. The section was incubated in a mixture of primary antisera that contained a rabbit anti-c-fos (1:3,000, Oncogene Research, Cambridge, MA) and a goat anti-ChAT (1:100, Chemicon International, Temecula, CA) for 16–24 h. The unbound antibodies were removed by three washes in PBS. The section was then incubated in a biotinylated donkey anti-rabbit IgG (1:200, Jackson ImmunoResearch, West Grove, PA) and a biotinylated donkey anti-sheep IgG (1:500, Jackson ImmunoResearch) for 2 h and Vectastain avidin-biotin complex kit (Vector Laboratory, Burlingame, CA) for another 2 h. Immunoreactivity was revealed using a peroxidase substrate kit (diaminobenzidine, DAB; Vector Laboratory). We distinguished c-fos and ChAT immunoreactivities by the location of the immunoreactive products. c-fos stained the cell nucleus, whereas ChAT stained the cell cytoplasm. ChAT immunoreactivity can be revealed by a donkey anti-sheep IgG even though the primary antibody is a goat anti-ChAT (30).

The third section was immunostained for c-fos and neuronal nitric oxide synthase (nNOS). The section was incubated in a mixture of a rabbit anti-c-fos and a rabbit anti-nNOS (1:400, Santa Cruz Biotechnology, Santa Cruz, CA), and the immunoreactivity was revealed by DAB reaction as described above. Again, c-fos and nNOS immunoreactivities were also distinguished by the location of immunoreactive products. c-fos stained the cell nucleus, whereas nNOS stained the cell cytoplasm.

All the preparations were examined with a Zeiss Axioskop microscope. Images were captured on a Hamamatsu camera with Openlab software.

C-fos-positive neurons per coronal sections that showed the maximum number of activated neurons were quantitated semiquantitatively and scored as follows: −, for none; +, for less than 5; ++, for between 5 and 10; ++++, for between 10 and 50; and ++++, for greater than 50 c-fos-positive neurons.

In the present study, we used posterior limits of area postrema (pAP) as the reference point for describing brain stem levels in craniocaudal orientation. AP neurons are a group of small cresyl violet-stained neurons with a subpopulation of ChAT-positive neurons. The craniocaudal extent of the AP in the mouse is ~500 μm. Obex was not used as a reference point, because the level of obex is defined differently by different investigators.

The solitary subnuclei (Sol) were identified by their relative location to anatomic landmarks, such as the fourth ventricle (IV), AP, solitary tract (SolT), and DMV, and corre-
lated with the cytoarchitectural characteristics as defined by cresyl violet staining and nNOS staining.

From a coronal perspective, Sol is broadly divided into a smaller lateral and a larger medial subdivision based on their position in relation to the SolT. The lateral subdivision is further subdivided into four subnuclei: dorsolateral (SolDL), ventrolateral (SolVL), ventral (SolV), and SolI. The medial subdivision is further subdivided into seven subnuclei: SolIM, SolCe, medial (SolM), commissural (SolC), dorsomedial (SolDM), gelatinosus (SolG), and rostrocentral (SolRCe). SolCe can be easily distinguished from the other Sol subnuclei as it stands out as a distinct group of compactly packed nNOS-immunoreactive neurons. SolG is recognized by its acellular gelatinous appearance due to the preponderance of the unmyelinated fibers. In the mouse, parvicellular reticular nucleus (PCRt) is a group of scattered cells that lies just ventral to the Sol and extends further ventrally to NA. In the craniocaudal orientation, PCRt extends $-600 \mu m$ rostrally from the anterior AP (aAP).

In the mouse, DMV extended from 1,250 $\mu m$ rostral to and 500 $\mu m$ caudal to the pAP. It was arbitrarily divided into rostral DMV (DMVR) and caudal DMV (DMVC). Immunohistochemical staining with ChAT or nNOS revealed that a subpopulation of DMV neurons were ChAT- or nNOS-immunoreactive neurons.

The NA is located in ventral regions of the medulla and in the mouse extends throughout its craniocaudal extent of the medulla. It is easily recognized by the cholinergic nature of its neurons. Similar to that described in the rat (7), in the mouse the dorsal division of NA is composed of three rostrocaudally aligned subdivisions, including NAc, NAsc, and NAi formations, respectively. The ventral division of the NA corresponds to the external formation that extends along the entire length of the medulla.

**RESULTS**

**Motor response of esophagus and LES to SLN stimulation.** As shown in Fig. 1, during a spontaneous swallowing manometric recording showed a propagating pressure wave along the esophageal body and relaxation followed by an aftercontraction of the LES. Pharyngeal swallowing activity that was associated with esophageal manometric response was observed by visual inspection of the exposed pharynx. Electrical stimulation of the central end of the SLN induced esophageal pressure changes in the esophagus and LES with frequency-dependent responses. Electrical stimulation of the SLN at 1 Hz did not induce any obvious pressure changes in either the esophageal body or the LES. Electrical stimulation at 5 Hz induced pharyngeal response and a slight decrease in pressures in the LES, but no esophageal response. SLN stimulation at 10 Hz evoked a pharyngeal as well as an esophageal response, including relaxation followed by an aftercontraction of the LES. Each 9-s stimuli typically evoked two swallowing responses.

**c-fos expression in vagal subnuclei with SLN stimulation.** Successful studies were obtained in 4 or 5 animals in each group receiving SLN stimulation at 1, 5, and 10 Hz. Electrical stimulation of the left SLN induced c-fos expression in neurons that were located over a wide area along the entire rostrocaudal extent of the vagal nuclei. c-fos was expressed bilaterally with activated neurons being more numerous on the ipsilateral than the contralateral side.

In the solitary nucleus, c-fos expression was found in several different solitary subnuclei (see Figs. 2, 3, and

![Fig. 1. Examples of esophageal and lower esophageal sphincter (LES) pressure responses to spontaneous swallowing and superior laryngeal nerve (SLN) stimulation at 1, 5, and 10 Hz. A spontaneous swallow is characterized by an esophageal peristaltic contraction associated with LES relaxation and aftercontraction. SLN stimulation at 5 Hz induced an LES relaxation without esophageal contraction. SLN stimulation at 10 Hz for 9 s elicited 2 episodes of propulsive contractions of the esophageal body and relaxation of the LES followed by an aftercontraction at 10-Hz stimulation. Stimuli were square wave pulses of 0.5 ms.](image-url)
7 and Table 1). In SolI, c-fos immunoreactivity was seen with all three stimulus frequencies examined. The number of c-fos-positive neurons increased as the stimulation frequency increased (Fig. 2, A–C, and Table 1). In SolIM, c-fos-positive neurons were observed throughout its rostrocaudal extent, but they were most numerous at a level just rostral to the aAP (500–600 μm rostral to pAP). The number of c-fos-positive neurons also increased as the stimulus frequency increased (Fig. 2, D–F, and Table 1). Sparse c-fos-reactive neurons were also seen in SolDM and SolCe. In SolCe, c-fos-positive neurons were observed with 10-Hz SLN stimulation but not with 1- or 5-Hz SLN stimulation (Fig. 3). In SolM, few c-fos-positive cells were seen at 1 Hz, and their number did not change with higher stimulus frequencies. We have interpreted these observations as indicating a nonspecific response.

In SolC, c-fos-positive neurons were seen with all the stimulus frequencies throughout the rostrocaudal extent of SolC. The number progressively increased in...
Table 1. c-fos-Positive neurons in vagal subnuclei with SLN stimulation

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−, Not detected; +, the highest number of positive cells on a single section was <5; ++, the highest number of positive cells on a single section was between 5 and 10; ++++, the highest number of positive cells on a single section was between 10 and 50; +++++, the highest number of positive cells on a single section was >50. SLN, superior laryngeal nerve; Sol, solitary subnucleus; SolI, interstitial Sol; SolIM, intermediate Sol; SolM, medial Sol; SolCe, central Sol; SolDM, dorsomedial Sol; SolC, commissural Sol; AP, area postrema; PCRt, parvicellular reticular nucleus; DMV, dorsal motor nucleus of vagus. DMVr, rostral DMV; DMVc, caudal DMV; NA, nucleus ambiguus; NAsc, semicompact formation of NA; NAc, compact formation of NA.

The number of neurons expressing c-fos with SLN stimulation is summarized in Table 1. The c-fos expres-
The brain stem circuit involved in swallowing has been investigated using a variety of techniques (2, 5, 6, 14). These include studies (24, 31) of swallowing responses after microlesions or microstimulation of well-defined medullary regions, electrical activity in the identified neurons in response to afferent stimuli that elicit reflex swallowing, and morphological tracer studies (2–4, 9, 18, 19) that show connectivity of neurons in the circuit. Together, they have produced important information on the neural circuit concerned with reflex swallowing. The above studies have been carried out in a number of different animal species. Although no studies have been reported in the mouse, available studies show that despite some obvious species differences, the general organization of the swallowing circuit across animal species is fairly similar.

The present studies using esophageal manometry and c-fos expression in brain stem nuclei provide information on functional anatomy of the medullary neurons that may constitute the swallowing-related neurons in the mouse. Moreover, by using the different stimulus parameter of the SLN to elicit different esophageal and LES responses, these studies also help identify the medullary neurons that may be involved in different components of swallowing responses. Although identification of the brain stem swallowing circuit using c-fos expression as a marker has not been described before, this technique has been used widely to identify neurons involved in a variety of reflexes (11, 22, 26, 34). The results of c-fos expression studies require careful interpretation and correlation with results of studies using different techniques. This is because c-fos expression may not directly correlate with a reflex response. For example, c-fos is not expressed in neurons with inhibitory links to SLN input. Even in neurons with an excitatory link to SLN input, c-fos expression is most marked in the first-order neurons, and it decreases progressively in second- and third-order neurons. Stimulation of the SLN afferents elicits a variety of swallow-related and other reflexes, including gagging, retching, coughing, laryngeal spasm, bronchoconstriction, and apnea. These reflexes use overlapping neural circuits. However, the type of reflex evoked by the SLN stimulation depends on the stimulus parameters used (5, 10, 25). In the mouse, SLN stimulation at 10 Hz elicited a full swallowing response, consisting of oropharyngeal and esophageal components including LES relaxation. Stimulation at 5 Hz produced only oropharyngeal and LES relaxation without evoking an esophageal response. Much stronger SLN stimulation (>10 Hz) produced apnea. Therefore we did not use stimuli with frequencies >10 Hz to avoid activation of other SLN-mediated reflexes. Even so, it is possible that some of the c-fos-expressing neurons observed in this study may be concerned with functions other than reflex swallowing.

Although fiber tracing or electrophysiological studies dealing with reflex swallowing are not available in the...
mouse, such data in other animal species provide good correlation with c-fos data in the mouse. Studies (5, 12, 18, 19, 24) have shown that SLN afferents project onto a large number of solitary subnuclei, such as SolVL, SolII, and SolIM, that are concerned with respiratory protective reflexes, including swallowing. In the mouse, neurons in all these areas expressed c-fos in response to SLN stimulation. In the rat, studies (2, 3, 8) using pseudorabies virus as a neurotracer have shown that SolI and SolIM contain premotor neurons for the NAsc and NAl that house motoneurons for pharyngeal muscles. In the mouse, we found that electrical stimulation of the SLN evoked c-fos expression in interneurons in SolI and SolIM and in motoneurons in NAsc and NAl. SolI and SolIM neurons have also been shown to project onto SolCe (8) that contain premotor neurons for NAc motoneurons innervating the esophageal striated muscle (4). SLN stimulation at 5 Hz produced only the oropharyngeal phase of swallowing, and stimulation at 10 Hz produced a full swallowing response. Therefore, in the mouse, interneurons in SolI and SolIM and motoneurons in NAsc and NAI may constitute the brain stem circuit for oropharyngeal swallowing. Interneurons in SolII, Sol IM, and SolCe and motoneurons in NAsc, NAI, and NAc may be responsible for the full swallowing reflex. These results are consistent with the results of microstimulation and electrophysiological studies (10, 16) showing that the dorsal part of the SPG that includes the region of the SolT nucleus is responsible for the coordination of the oropharyngeal phase as well as the complete swallowing sequence.

Studies (4, 6, 17) using extracellular unit recordings, microstimulation, and morphological circuit tracing have also shown that the SolCe is the main site of termination of esophageal afferents. In our studies, SLN stimulation at 10 Hz but not at 5 Hz showed c-fos-expressing neurons in SolCe, NAc, and esophageal body contractions. Moreover, the number of activated neurons even at 10 Hz was small. The reason for this low level of activated neurons is not clear; however, it may be related at least in part to the fact that SolCe interneurons represent second- or third-order neurons in the swallowing circuit. These observations suggest that the neural circuit responsible for the esophageal phase of the peristalsis requires higher-intensity SLN simulation than that for the pharyngeal phase of the peristalsis and are consistent with the view that SolCe-NAc may form the brain stem circuit for esophageal peristalsis (4, 8, 17).

The SPG was originally mapped into two regions (13, 14). The dorsal region is constituted by Sol and adjoining PCRt and a ventral region encompassing NA and the adjoining PCRt. These studies (13, 14) and others

Fig. 5. c-fos expression in rostral DMV (DMVR) with 5- and 10-Hz SLN stimulation. Coronal sections processed for c-fos (nuclear staining) and ChAT (cytoplasm staining) double immunostaining. The location of DMVR can be recognized as a group of ChAT-positive neurons. In A (low power) and A’ (high power), a lack of c-fos-immunoreactive neurons is shown in DMVR with 5-Hz stimulation. In B (low power) and B’ (high power), a c-fos-positive neuron is shown (arrow) in the DMVR with 10-Hz stimulation. A and B: scale bars, 100 µm. A’ and B’: scale bars, 50 µm.
Fig. 6. *c-fos* expression in caudal DMV (DMVc) with different frequency SLN stimulation. Coronal sections were processed for *c-fos* (nuclear staining) and ChAT (cytoplasm staining) double immunostaining. The location of DMVc can be recognized as a group of ChAT-positive neurons. Neurons expressing *c-fos* (arrowheads) were found with stimulation at all 3 frequencies shown. A–C: scale bars, 100 μm. A’–C’: scale bars, 50 μm.

Fig. 7. Diagrammatic representation of subnuclei of vagal complex showing *c-fos*-active neurons (shaded area) with 10-Hz SLN stimulation. The level of 0 represents the posterior border of the AP. The darkness of the shade represents the density of *c-fos*-positive neurons in vagal subnuclei, with the darkest being the densest. Note that SLN stimulation at 10 Hz that evoked a full swallowing motor response also induced *c-fos* in neurons in SolI, SolIM, SolCe, NAc, NAsc, DMVc, and DMVr. SolDL, dorsolateral Sol; SolVL, ventrolateral Sol; SolV, ventral Sol; SolRCe, rostrocentral Sol; SolG, gelatinous Sol; aAP, anterior AP; pAP, posterior AP.
using microelectrode recordings and chemostimulation techniques have suggested that PCRt may play an important role in reflex swallowing. In the present study, we found that SLN stimulation evoked c-fos expression in PCRt neurons, which is consistent with the view of a possible role for PCRt neurons in reflex swallowing (13, 14, 31, 32).

The LES in all animal species, including the mouse, and the distal esophagus in many species are composed of smooth muscles that receive preganglionic motor innervation from DMV motoneurons (1, 9, 27–29). Several studies (1, 28) have suggested that DMVr and DMVc neurons provide excitatory and inhibitory inputs, respectively, to the smooth muscle of the distal esophagus and LES. In the present study, 10-Hz SLN puts, respectively, to the smooth muscle of the distal DMVc neurons provide excitatory and inhibitory  

Electrical spike potentials in neurons (26). Therefore, it is possible that SLN stimulation at 1 Hz activates these neurons without producing a motor response. It has been shown that SLN stimulation that does not evoke swallowing activity can evoke electrical activity in the swallowing neurons (16). The frequency-dependent motor responses and c-fos expression in response to SLN stimulation further support the validity of the results with SLN stimulation.

In summary, these studies show that SLN stimulation evokes swallowing and peristalsis and c-fos expression in interneurons constituting the putative SPG and motoneurons in the brain stem. These findings are consistent with the view that SolI, SolIM, NAsc, and NAI neurons are responsible for oropharyngeal swallowing and that SolCe and NAc neurons form the circuit for esophageal striated muscle peristalsis. Both these circuits are involved in primary peristalsis. Further studies are needed to more precisely determine the roles of PCRt in reflex swallowing and define links that are responsible for bilateral integration of the two hemi SPGs. Studies are also needed to test whether SolDM, rather than SolCe or SolIM, house premotor neurons for the DMVc neurons that mediate LES relaxation during reflex swallowing.

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