

Proprioceptive Representation of the Levator Veli Palatini Muscle in the Solitary Nucleus of the Rat

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The levator veli palatini muscle is innervated by motoneurons of the glossopharyngeal nerve, which are located within the ambiguus nucleus; however, little is known about the afferent fibers of this muscle. A horseradish peroxidase study was conducted in rats following injection into the levator veli palatini muscle branch to reveal the location and the distribution of dendrites of the afferent fibers of the muscle. Terminal labels were densely distributed in the lateral region of the solitary nucleus, which receives afferents of the glossopharyngeal nerve, ipsilateral and contralateral to the injection site. The relationship of the levator veli palatini muscle with respiration was suggested by the localization of labeled terminals at sites where the afferents from the respiratory organs project densely, and by the demonstrated proprioceptive role of the afferent fibers passing through the muscle spindles contained in the levator veli palatini muscle.

KEY WORDS: *ambiguus nucleus, glossopharyngeal nerve, horseradish peroxidase-wheat germ agglutinin, levator veli palatini muscle, respiratory motoneuron, solitary nucleus*

The levator veli palatini muscle (LVPm) has a very important role in the complex functions regulating velopharyngeal mechanisms in cleft palate patients. The topographic distribution of the sensory neurons and motoneurons of the glossopharyngeal nerve has been extensively studied by horseradish peroxidase (HRP) methods in cats. The solitary nucleus is known to receive projections from the glossopharyngeal nerve, and the ambiguus nucleus is known to contain cells originating in the glossopharyngeal motor nerve. Van Loveren et al. (1983), for example, demonstrated that HRP-labeled cells, following injection into the LVPm, were located in the rostral ambiguus nucleus, both ipsilateral to and contralateral to the injection site, and in the retrofacial nucleus ipsilateral to the injection site. This suggested that identification of the LVPm motoneurons within the ambiguus nucleus does not exclude innervation by the glossopharyngeal nerve. We found that the LVPm movement ceases on severing the levator veli palatini muscle branch of the glossopharyngeal nerve (Br.LVP) (Furusawa et al., 1991), and that the LVPm has muscle spin-

dles in the rat (Yamaoka et al., 1992). However, there is no information on the distribution and location of dendrites receiving afferent fibers of the Br.LVP. This information would lead to a comprehension of the difference in neuromuscular velopharyngeal function during various activities and feedback therapy for velopharyngeal incompetence using the physiologic properties of the LVPm. In this study, we attempted to delineate the innervation of the LVPm by the glossopharyngeal nerve, based on the assessment of the relationship between the Br.LVP and the specific regions of the projection to the solitary nucleus and the ambiguus nucleus. The possibility that the glossopharyngeal nerve may have feedback effects in blowing and speech is also discussed.

For a complete list of abbreviations used throughout this article, refer to Table 1.

METHODS

Fifteen male Wistar rats weighing between 200 and 250 g were given an intraperitoneal injection of ketamine HCl 50 to 100mg/kg and then placed on a table in the supine position. An operating microscope with glass-fiber illumination and fine microsurgical instruments were used for the experiments. A skin incision was made from the mental protuberance to the tympanic bulla along the inner side of the border of the mandible either unilaterally or bilaterally. The posterior belly of the digastric muscle was cut at its upper insertions near the tympanic bulla and pulled downward. The ventromedial approach from the inner aspect of

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TABLE 1 Abbreviations

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| Amb = ambiguus nucleus |
| AP = area postrema |
| Br.Ling = lingual-tonsillar branch of the glossopharyngeal nerve |
| Br.LVP = levator veli palatini muscle branch of the glossopharyngeal nerve |
| Br.Ph-IX = pharyngeal branch of the glossopharyngeal nerve |
| Br.Ph-X = pharyngeal branch of the vagus nerve |
| Br.Sin = carotid sinus branch of the glossopharyngeal nerve |
| cc = central canal |
| HRP-WGA = horseradish peroxidase-wheat germ agglutinin |
| io = inferior olive |
| LVPm = levator velum palatini muscle |
| Plex-Ph = pharyngeal plexus |
| Pr = pyramidal tract |
| RAmb = retroambiguus nucleus |
| Sol = solitary nucleus |
| sol = solitary tract |
| Vc = trigeminal nucleus caudalis |
| Vi = trigeminal nucleus interpolaris |
| Vtr = spinal trigeminal tract |
| 7 = facial nucleus |
| 10 = dorsal motor vagus nucleus |
| 12 = hypoglossal nucleus |
| IX = glossopharyngeal nerve |
| X = vagus nerve |

the mandible medial to the internal pterygoid muscle exposed the levator veli palatini muscle, which arises from the inferior surface of the carotid canal, runs anteromedially, and enters the midline of the soft palate radially. All trunks of the glossopharyngeal, vagus, and hypoglossal nerves were identified between the LVPm and the base of the skull. The levator veli palatini muscle branch of the glossopharyngeal nerve (Br.LVP), the pharyngeal branch of the glossopharyngeal nerve (Br.Ph-IX), or the pharyngeal branch of the vagus nerve (Br.Ph-X) was elevated in three separate groups of five rats each. The central cut ends of these branches were suctioned with a glass micropipette with a tip diameter of 50 μ m filled with 10% horseradish peroxidase-wheat germ agglutinin (HRP-WGA; TOYOBO) (Fig. 1). The end of the nerve was soaked for 90 to 120 minutes, and the wounds were closed in layers. After 48 hours, the rats were anesthetized with sodium pentobarbital 80 mg/kg IP and perfused transcardially with 200 mL of heparinized physiologic saline followed by 500 mL of 1.25% glutaraldehyde solution and 1% paraformaldehyde (pH 7.4) in 0.1M phosphate buffer. The brain stem from the area of the inferior colliculus to the upper cervical cord was removed and refrigerated for 48 hours in 30% sucrose solution. Serial transverse sections of the brain stem were cut at 30 μ m on a freezing microtome and reacted for HRP-WGA with tetramethylbenzidine. Two sets of alternate sections were mounted on gelatin-coated glass slides: the sections in one set were covered with coverslides unstained, and those in the other set stained with neutral red. The sections were examined under a light microscope using both bright- and dark-field illuminations. A camera lucida apparatus (BH-DA-LB, Olympus) was then used for tracing.

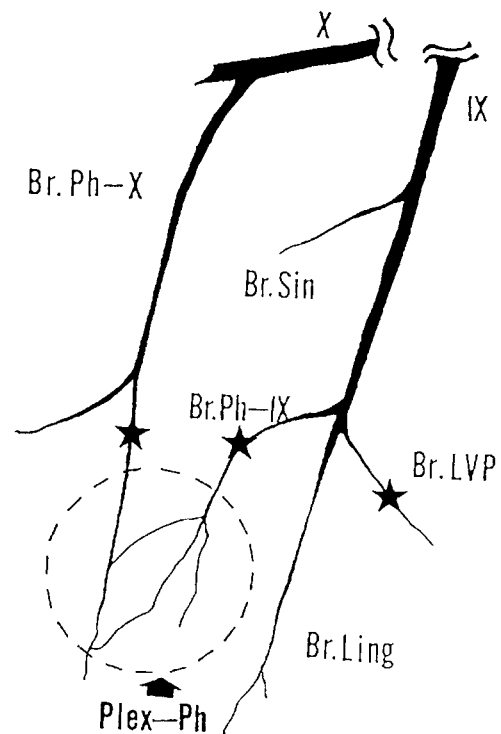


FIGURE 1 Diagram showing the position of the central cut end of the Br.LVP, Br.Ph-IX, and Br.Ph-X, where HRP-WGA was injected. Abbreviations as follows: IX = glossopharyngeal nerve, Br.Sin = carotid sinus branch of the glossopharyngeal nerve, Br.LVP = levator veli palatini muscle branch of the glossopharyngeal nerve, Br.Ph-IX = pharyngeal branch of the glossopharyngeal nerve, Br.Ling = lingual-tonsillar branch of the glossopharyngeal nerve, X = vagus nerve; Br.Ph-X = pharyngeal branch of the vagus nerve, Plex-Ph = pharyngeal plexus.

RESULTS

Application of Horseradish Peroxidase-Wheat Germ Agglutinin to the Levator Veli Palatini Muscle Branch of the Glossopharyngeal Nerve (Figs. 2A-D and 3)

In all of the five rats that were injected in the Br.LVP with HRP-WGA, labeled cells and terminals were observed. As shown in the example in Figures 2A and B and in the camera lucida drawing in Figure 3, the HRP-labeled cells were noted within the ambiguus nucleus ipsilateral to the injection site, rostrocaudally from the slightly rostral level of the obex to the intermediate region. As shown in the example in Figures 2C and D and in the camera lucida drawing in Figure 3, the HRP-labeled terminals were localized to the lateral region of the solitary nucleus bilateral to the injection site at the level between the rostral region of the hypoglossal nucleus and the caudal region of the inferior olive.

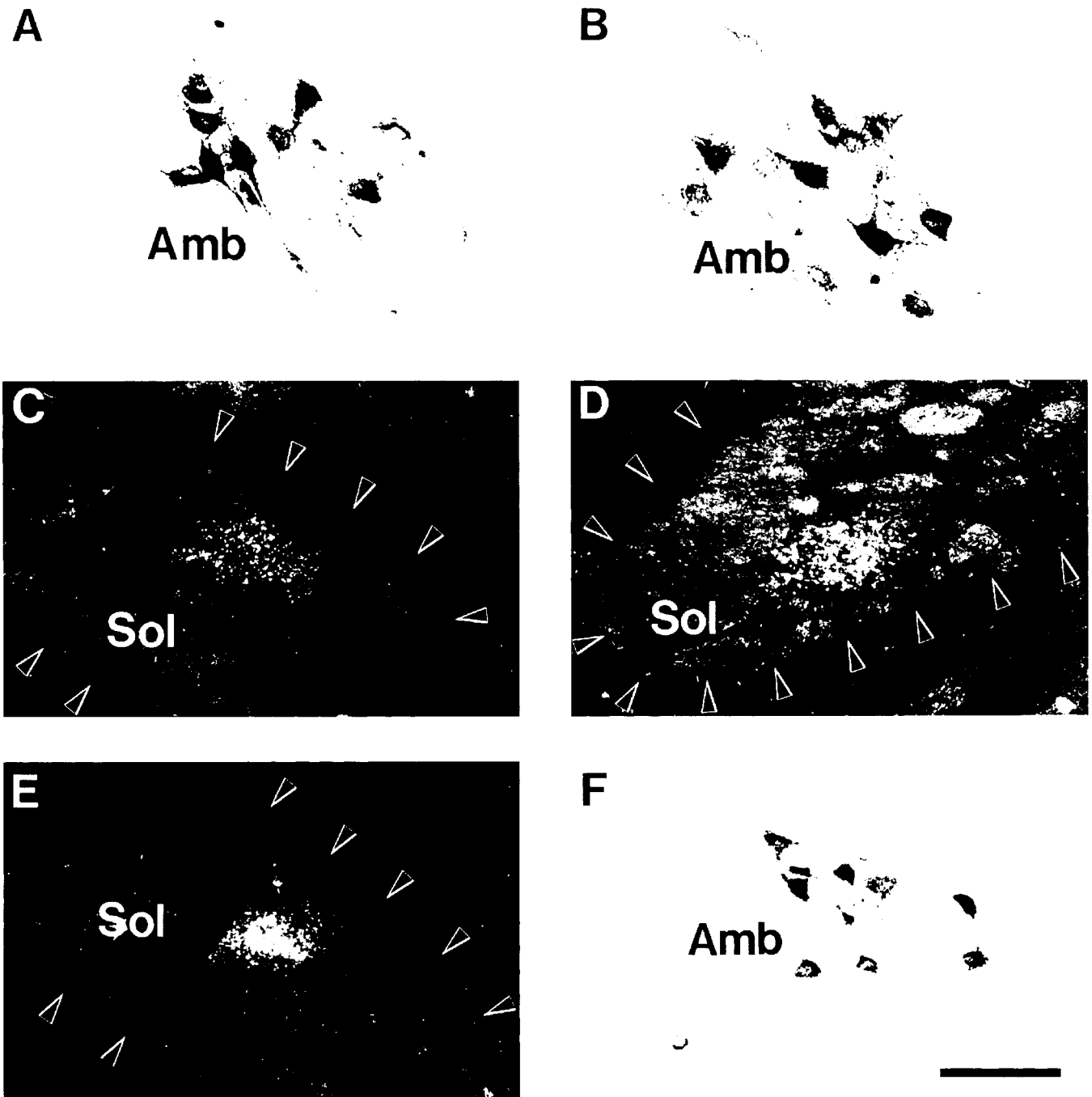


FIGURE 2A and B: Bright-field photomicrographs of motoneurons illustrating retrogradely labeled neurons after injection of HRP-WGA from the Br. LVP in the ambiguus nucleus (Amb) ipsilateral to the injection site. The neurons lie at the slightly caudal level rostrocaudally. **C and D:** Dark-field micrographs of the solitary nucleus (Sol) branch terminals are distributed in the level between the rostral region of the hypoglossal nucleus and the caudal level of the inferior olive after injection of HRP-WGA from the Br.LVP. Labeling is deposited ipsilaterally (C) and contralaterally(D). **E:** Dark-field micrograph of HRP-labeled terminals in the solitary nucleus ipsilateral to the injection site following injection from the Br.Ph-IX. No labeled cells were located in the Amb. **F:** Bright-field photomicrograph of cells in the ambiguus nucleus following injection from the Br.Ph-X. No labeled terminals were observed in the solitary nucleus (Sol). The inner region surrounded by arrows is the solitary nucleus. Section B and F are counterstained with neutral red. Scale bar = 100 μ m. Abbreviations are as follows: Amb = ambiguus nucleus, Br.LVP = levator veli palatini muscle branch of the glossopharyngeal nerve, Br.Ph-IX = pharyngeal branch of the glossopharyngeal nerve, Br.Ph-X = pharyngeal branch of the vagus nerve, Sol = solitary nucleus.

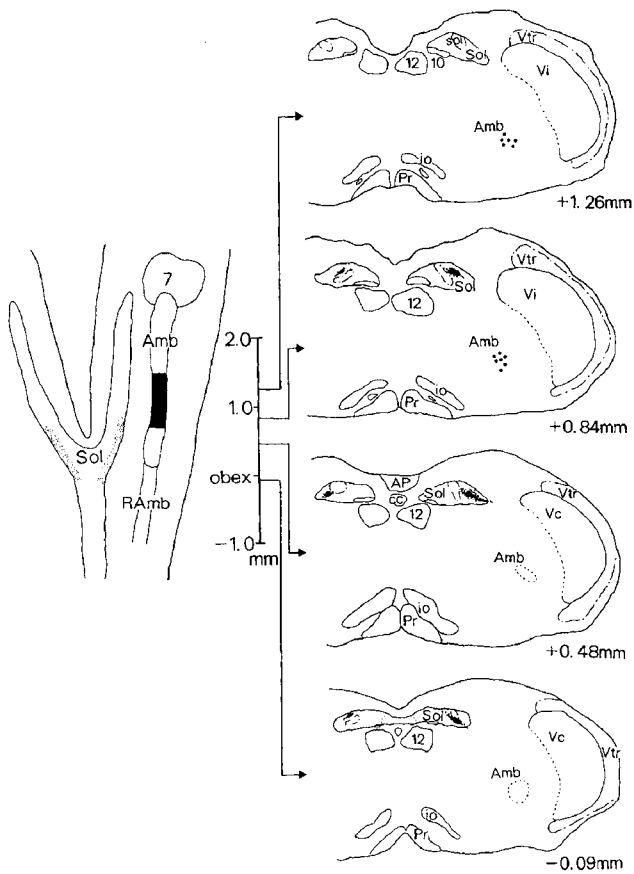


FIGURE 3 Locations of the labeled cells in the Amb and labeled terminals in the Sol seen on illustration of the dorsal view of the medulla (left) and camera lucida drawings at the coronal sections of the medulla (right) after HRP-WGA was injected from the Br.LVP. The numbers to the right of the drawings represent the distance rostral or caudal to the obex. Black dots represent fiber terminals, filled circle represent cell bodies. Abbreviations are as follows: Amb = ambiguus nucleus, AP = area postrema, cc = central canal, Br.LVP = levator veli palatini muscle branch of the glossopharyngeal nerve, io = inferior olive, Pr = pyramidal tract, R.Amb = retroambiguus nucleus, Sol = solitary nucleus, sol = solitary tract, Vc = trigeminal nucleus caudalis, Vi = trigeminal nucleus interpolaris, Vtr = spinal trigeminal tract, 7 = facial nucleus, 10 = dorsal motor vagus nucleus, 12 = hypoglossal nucleus.

Application of Horseradish Peroxidase-Wheat Germ Agglutinin to the Pharyngeal Branch of the Glossopharyngeal Nerve (Figs. 2E and 4)

In all of the five rats injected in the Br.Ph-IX with HRP-WGA, labeled terminals were observed. As shown in the example in Figure 2E and in the camera lucida drawing in Figure 4, the HRP-labeled terminals were seen in both the medial and lateral regions of the solitary nucleus ipsilateral to the injection site at the rostral level, and only in the lateral region of the solitary nucleus ipsilateral to the injection site at the caudal level. These labeled terminals were located rostrocaudally between the rostral region of the hypoglossal nucleus and the caudal limit of the inferior olive (see Fig. 4).

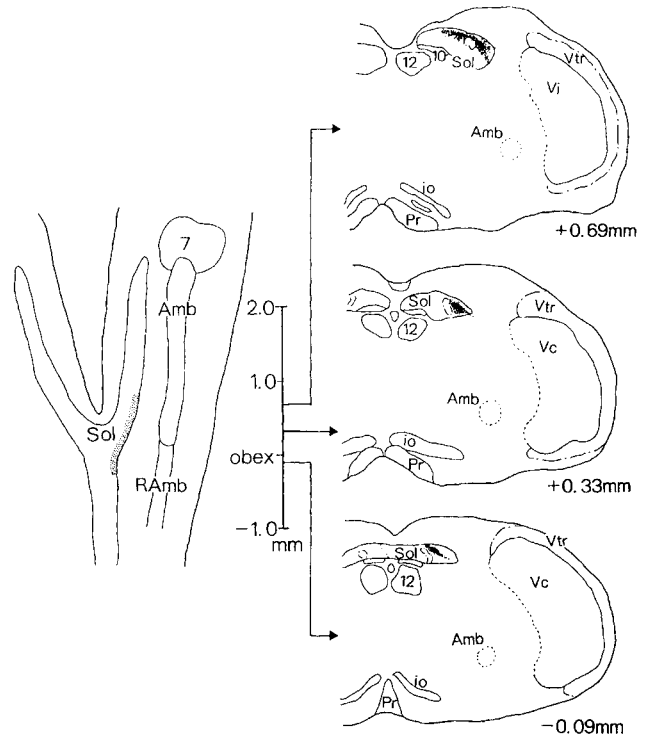


FIGURE 4 Locations of labeled terminals in the Sol on illustration of the dorsal view of the medulla (left) and camera lucida drawings at the coronal sections of the medulla (right) after HRP-WGA was injected from the Br.Ph-IX. The numbers to the right of the drawings represent the distance rostral or caudal to the obex. Black dots represent fiber terminals. Abbreviations are as follows: Amb = ambiguus nucleus, AP = area postrema, cc = central canal, Br.Ph-IX = pharyngeal branch of the glossopharyngeal nerve, io = inferior olive, Pr = pyramidal tract, R.Amb = retroambiguus nucleus, Sol = solitary nucleus, sol = solitary tract, Vc = trigeminal nucleus caudalis, Vi = trigeminal nucleus interpolaris, Vtr = spinal trigeminal tract, 7 = facial nucleus, 10 = dorsal motor vagus nucleus, 12 = hypoglossal nucleus.

Application of Horseradish Peroxidase-Wheat Germ Agglutinin to the Pharyngeal Branch of the Vagus Nerve (Figs. 2F and 5)

Five rats were injected in the Br.Ph-X with HRP-WGA. In all of the rats, the labeled terminals of the Br.Ph-X were not distributed in the solitary nucleus, but rather labeled cells were observed in the ambiguus nucleus ipsilateral to the injection site. As shown in the example in Figure 2F and in the camera lucida drawing in Figure 5, the labeling distribution was more caudalward than that of the labeling following application of HRP-WGA to the Br.LVP, extending from slightly below the obex to slightly below the intermediate region.

DISCUSSION

The motoneurons for the LVPm are observed in the rostral part of the ambiguus nucleus (Miyazaki, 1982), and the location of the motoneurons supplying the LVPm has been

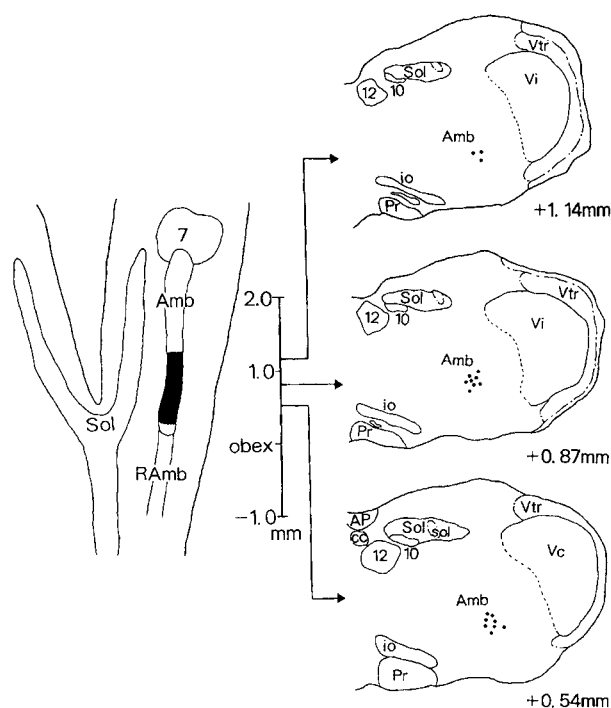


FIGURE 5 Locations of the labeled cells in the ambiguus nucleus on illustration of the dorsal view of the medulla (left) and camera lucida drawings at the coronal sections of the medulla (right) after HRP-WGA was injected from the Br.Ph-X. Filled circle represent cell bodies. Abbreviations are as follows: Amb = ambiguus nucleus, AP = area postrema, cc = central canal, Br.Ph-X = pharyngeal branch of the vagus nerve, io = inferior olive, Pr = pyramidal tract, RAmb = retroambiguus nucleus, Sol = solitary nucleus, sol = solitary tract, Vc = trigeminal nucleus caudalis, Vi = trigeminal nucleus interpolaris, Vtr = spinal trigeminal tract, 7 = facial nucleus, 10 = dorsal motor vagus nucleus, 12 = hypoglossal nucleus.

proposed to be the ambiguus nucleus (Van Loveren et al., 1983). The motoneurons innervating the muscles of the upper airway are concentrated within the ambiguus nucleus (Davis and Nail, 1984; Bieger and Hopkins, 1987; Grélot et al., 1989). The LVPm is physiologically involved in respiratory control in the rat (Furusawa et al., 1992). The motoneurons innervating the LVPm are known to discharge during inspiration (Furusawa et al., 1991). Inspiration neurons were found to be distributed in the ambiguus nucleus by synaptic potentials at the projection site following the neuron's extracellular spike (Ezure et al., 1988; Zheng et al., 1991). Some of these inspiration neurons may be the neurons found in the present study. Bianchi et al. (1995) described that the ambiguus nucleus includes motoneurons driving some pharyngeal muscles are not part of the respiratory central pattern generator. Thus, glossopharyngeal motoneurons innervating the LVPm, which are one of three functional classes of neurons in the ventral respiratory group, may be controlled by efferent projections from the ambiguus nucleus.

Since most reports other than those of Furusawa et al. (1992) and Yamaoka et al. (1992) have indicated that muscle spindles are not found in the LVPm, the question of the location

of afferent terminals is controversial. The demonstration of afferent terminals in the solitary nucleus is consistent with the presence of spindles in the muscle and is physiologically important for several reasons. For instance, the nucleus of the solitary tract is a principal visceral sensory center in the brain stem. It receives input from receptors located in such structures as the pharynx, larynx, and respiratory tract (Tanaka et al., 1987). Most afferents of the pharyngeal branch of the glossopharyngeal nerve enter the ipsilateral nucleus of the solitary tract (Nomura and Mizuno, 1982; Grélot et al., 1989) and terminate in the solitary nucleus (Nomura and Mizuno, 1982). The LVPm is innervated by the glossopharyngeal nerve (Furusawa et al., 1991). Hence, the Br.LVP must possess terminal boutons within the solitary nucleus in which the glossopharyngeal nerve has dendrites. In our study, dendritic segments covered with boutons arising from the Br.LVP were found ipsilaterally and contralaterally. These findings may be related to the specific anatomic feature of the suspension from the bilateral petrous portions of the LVPm, which has muscle spindles. Moreover, Furusawa et al. (1991) found that the duration and the interval of the synchronized bursts of the Br.LVP were unchanged by LVPm stretch, suggesting that the afferent input from the proprioceptors of the LVPm did not exert any influence on the medullary respiratory rhythm generator. In addition, the implications of our findings in relation to the pharyngeal plexus, which could be separated into the glossopharyngeal nerve and the vagus nerve, should be noted. The pharyngeal branch of the glossopharyngeal nerve (Br.Ph-IX) was not confirmed to contain any efferent fibers. After the application of HRP-WGA to the pharyngeal branch of the vagus nerve (Br.Ph-X), labeled neurons were seen ipsilaterally in the ambiguus nuclear complex.

There are data suggesting that swallowing is accompanied by neurologic function different from that in speech. Complete velopharyngeal closure in swallowing is seen radiographically and endoscopically, whereas incompetent closure is seen in speech in normal individuals and in cleft-palate patients regardless of whether their LVPm has been restored or not. The sphincteric closure on swallowing is quite distinct from the palatal-flap-valve action in speech, and the former is an action consistently stronger than the latter (Calnan, 1953; Moll, 1964; Yamaoka et al., 1972; Matsuya et al., 1974; Shprintzen et al., 1974). The features of blowing are very similar to those of speech (Yamaoka et al., 1972; Matsuya et al., 1974; Shprintzen et al., 1974). Moreover, the velopharyngeal closure employed in swallowing does not demonstrate sufficiency to serve as adequate velopharyngeal closure in speech (Peterson, 1973; Starr, 1993). Complex behavioral manifestations in velopharyngeal closure may be due to segregation of neuromuscular units, and these activities are likely to be mediated by the Br.Ph-X in swallowing and by the efferents contained in the Br.LVP in blowing and speech. The labeling distribution in the ambiguus nucleus following application of HRP-WGA to the Br.Ph-X was consistently more caudalward than that after injection from the Br.LVP, and labeling was seen below the obex. This suggests that the Br.Ph-X has a functional role other

than that involved in the upper-airway patency, which is related to the ambiguous nucleus at the level rostral to the obex (Davis and Nail, 1984; Bieger and Hopkins, 1987; Grélot et al., 1989).

The activation of afferents may convert the rhythmic and synchronized contractions of the LVPm involved in the voluntary functions in breathing, blowing, and speech production through mediation by forebrain control. Additional mechanisms may be responsible for the modulation as a sensory-motor unit, and proprioceptive afferents may have a functional role in the determination of the appropriate respiratory outputs for maintenance of homeostasis under spontaneous, continuously active conditions. Although our findings (Furusawa et al., 1991) pertain to respiratory-related activity during anesthesia in the rat, nonanesthetized functions would also be triggered by afferent inputs. Therefore, the precise comprehension of the afferent and efferent connections of the Br.LVP is of importance in the understanding of the dynamic cooperation between automatic rhythmic function and voluntary function associated with feedback during the learning of speech.

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