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The distribution of respiration-related and swallowing-related motoneurons innervating the rat genioglossus muscle

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Abstract

The present study was an attempt to identify the location of genioglossal respiratory and swallowing motoneuron cell bodies within the hypoglossal (XII) nucleus using both electrophysiological and morphological studies. The genioglossus muscle is innervated by the genioglossal branch of the medial XII nerve. At the entrance to the muscle, the genioglossal branch divides in the directions of the mandible and tongue. Five of five rats displayed both respiratory-related and swallowing-related bursts in the medial XII branch towards the mandible. All five rats also displayed swallowing-related bursts in the medial XII branch towards the tongue. In addition, horseradish peroxidase conjugated to wheatgerm agglutinin (HRP:WGA) was injected into the proximal cut ends of each branch. When HRP:WGA was injected into the branch in the direction of the mandible, HRP-labeled cells were detected in the lateral region of the ventromedial subnucleus in the XII nucleus, extending from 0.7 to 1.2 mm rostral to the obex. On the other hand, after injection into the branch in the direction of the mandible, HRP-labeled cells were detected in the ventromedial subnucleus of the XII nucleus, extending from 0.3 to 1.2 mm rostral to the obex. These results provide evidence that genioglossal respiration-related and swallowing-related motoneurons are located in different portions within the ventromedial subnucleus of the XII nucleus.

Key words: *inspiration, upper airway, tongue, medulla*

Introduction

The location of genioglossal motoneuron cell bodies within the hypoglossal (XII) nucleus has been studied in the rat (Krammer *et al.*, 1979; Uemura-Sumi *et al.*, 1988; Altschuler *et al.*, 1994; Aldes, 1995), cat (Uemura *et al.*, 1979; Miyazaki *et al.*, 1981), dog (Chibuzo and Cummings, 1982; Uemura-Sumi *et al.*, 1988), rabbit (Uemura-Sumi *et al.*, 1988), frog (Matesz *et al.*, 1999), and monkey (Uemura-Sumi *et al.*, 1981; Sokoloff and Deacon, 1992). These studies found that the genioglossal motoneurons are located within the XII nucleus. The rat genioglossal motoneurons are located in the lateral region of the ventromedial subnucleus in the hypoglossal nucleus (Uemura-Sumi *et al.*, 1988; Altschuler *et al.*, 1994; Aldes, 1995), and are active during inspiration (Smith *et al.*, 1991; Funk *et al.*, 1994; Parkis *et al.*, 1995; Selvaratnam *et al.*, 1998). The genioglossus muscle plays an important role in maintaining upper airway patency during inspiration (Remmers *et al.*, 1978; Mathew *et al.*, 1982; Plowman *et al.*, 1990). On the other hand, the activities of the genioglossus muscle and motoneurons increase corresponding to swallowing (Tomomune and Takata, 1988; Travers and Jackson, 1992).

However, the location of the respiration-related and swallowing-related motoneuron cell bodies within the XII nucleus is not clear. The aim of this study was to identify the location of genioglossal respiration-related and swallowing-related motoneuron cell bodies within the XII nucleus using both electrophysiological and horseradish peroxidase (HRP) tracing techniques.

Materials and methods

General procedures

The study was carried out on 15 Wistar rats (200–250 g), which were anesthetized with an intraperitoneal injection of ketamine (0.05–0.1 mg/g) without tracheal cannulation. Each rat was placed on a table in the supine position, and the body temperature of the animal was maintained at 37–38°C using a heating pad. An operating microscope was used for the surgical preparation. A unilateral saggital skin incision was made in the intermandibular space to the sternoclavicular bone. The anterior belly of the digastric muscles was retracted in a ventromedial direction. The XII nerve trunk was visualized by this procedure. At the level of the hyoid bone, the rat XII nerve divides into the medial and the lateral branches, and then the medial branch gives off the branch innervating the genioglossus muscle (Genioglossal branch). This branch divides into two branches running in the direction of the mandible and the direction of the tongue, as shown schematically in Figure 1.

Electrophysiological study

Five male Wistar rats were used (236 ± 14 g). Animals were maintained under anesthesia with a stable heart rate and a spontaneous respiratory rate. For efferent discharge recording, the proximal cut end of each branch was placed on a bipolar tungsten hook electrode (resistance > 2 MΩ). Respiration was monitored

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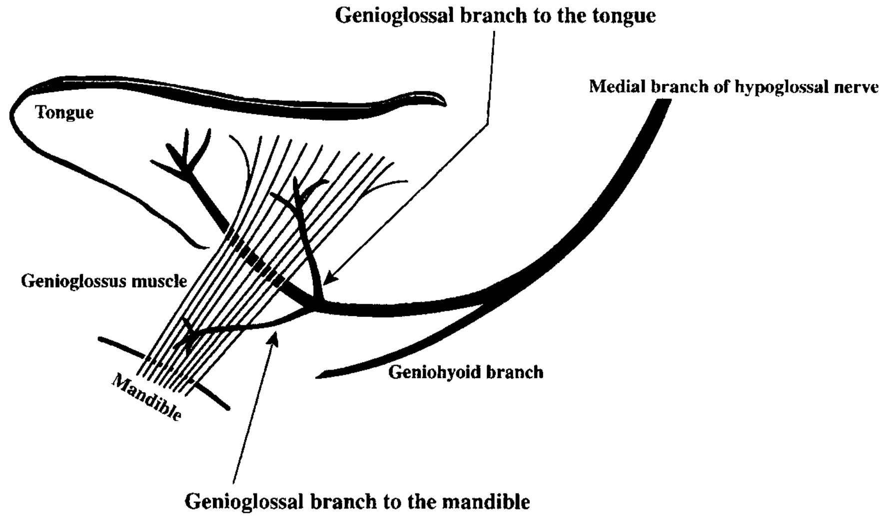

FIGURE 1. Schematic drawing of the genioglossal branch of the XII nerve.

by bipolar electromyographic (EMG) electrodes placed in the external intercostal muscle. Swallowing was monitored by bipolar EMG electrodes placed in the mylohyoid muscle. The amplitudes of the neural discharges and EMG activities were amplified by a high-input impedance preamplifier and main amplifier (AVH-10, Nihon Kohden, Tokyo). The impulses were displayed on an oscilloscope (VC-11, Nihon Kohden); these data were then stored using a magnetic tape recorder at a speed of 38 cm/s (MR-30, TEAC).

Morphological study

Ten male Wistar rats were used (223 ± 12 g). The genioglossal branch of XII nerve was exposed under a surgical microscope. One of the two branches of the genioglossal branch (one branch each in five rats) was dissected free immediately distal to its entry into the genioglossal muscle. The proximal cut end of the branch was suctioned into a glass micropipette (tip diameter = 30–50 μm) filled with 10% HRP conjugated to wheatgerm agglutinin (HRP:WGA; Toyobo) in 0.3 M KCl and 0.05 M Tris buffer at pH 7.6, and left for 90 min (Yasuda *et al.*, 1995; Furusawa *et al.*, 1996a, b). After the HRP:WGA was washed out of the central cut ends of the nerve, the wound was closed. At 48 h after surgery, the ascending aorta was perfused with heparinized physiological saline (200 ml), followed by 400–500 ml of a solution of 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and finally by 200–300 ml of cold (4°C) 10% sucrose in 0.1 M phosphate buffer. The brainstem and upper cervical cord were removed as one piece and stored for 24–48 h at 4°C in phosphate buffer containing 30% sucrose.

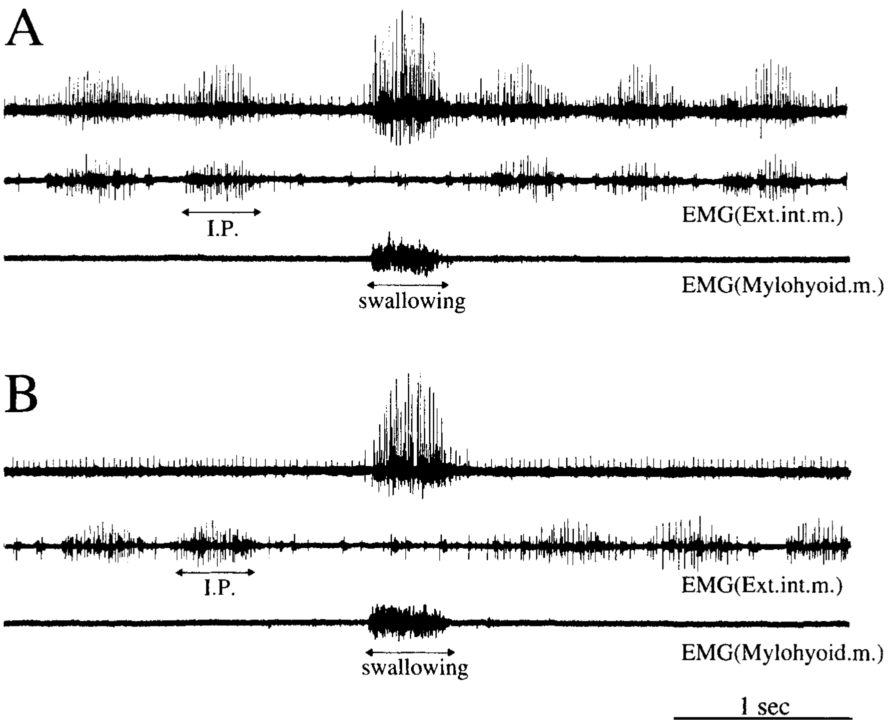

FIGURE 2. The upper panel (A) shows the typical efferent discharges from the proximal cut end of the branch in the direction of the mandible which was recorded with the electromyographic (EMG) of the external intercostal muscle (Ext. int. m.) and mylohyoid muscle. These discharges exhibited both concentrated burst activity synchronized with both inspiration and swallowing. The lower panel (B) shows the typical efferent discharges from the proximal cut end of the branch in the direction of the tongue, which was recorded with the EMG of the Ext. int. m. and mylohyoid muscle. These discharges exhibited burst activity synchronized with swallowing.

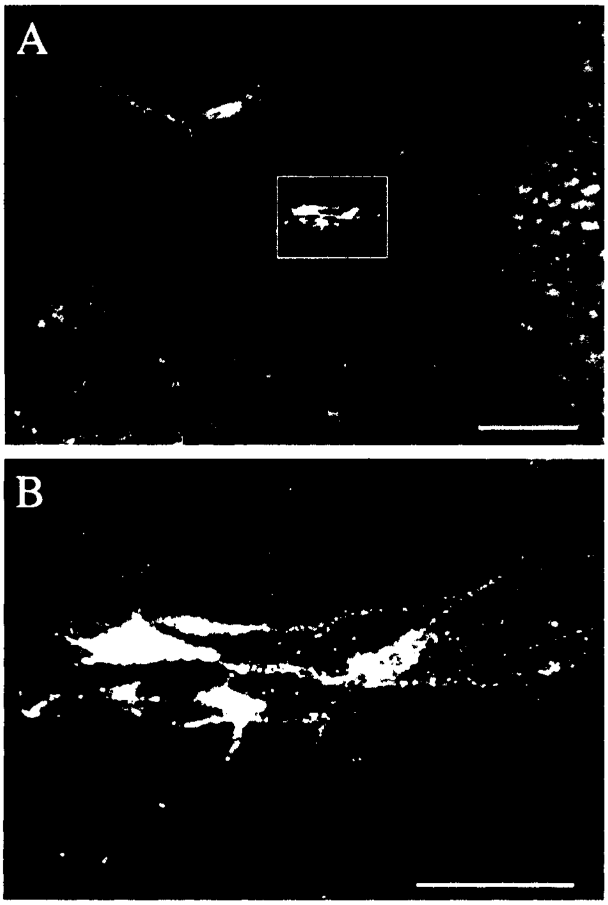

FIGURE 3. Darkfield photomicrograph of labeled cells localized within the rostral portion of the ventromedial subnucleus in the XII nucleus following application of HRP:WGA to the branch in the direction of the mandible. Scale bars = 100 μm in A, 50 μm in B.

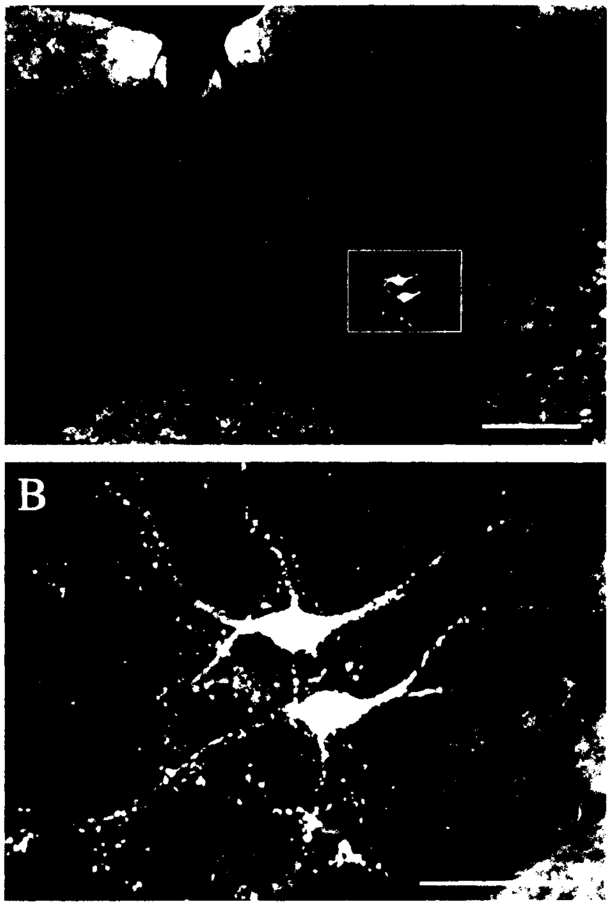

FIGURE 4. Darkfield photomicrograph of labeled cells localized within the ventromedial subnucleus in the XII nucleus following application of HRP:WGA to the branch in the direction of the tongue. Scale bars = 100 μm in A, 50 μm in B.

Tetramethylbenzidine was used as the chromogen in the histochemical procedure for detection of HRP activity (Mesulam, 1982). The sections were mounted on chrome-alum gelatin-coated glass slides, and alternate sections were counterstained with 1% neutral red. The sections were examined under a light microscope using both bright- and darkfield illuminations. A camera lucida (BH-DA-LB, Olympus) was then used for tracing. In the description of our findings, the obex was used as a point of reference, and was defined as the caudal end of the area postrema (Hamilton and Norgren, 1984). The outline of the labeled soma in each counterstained transverse section was traced by using the camera lucida apparatus. The soma area and average somal diameter ($\bar{D} = 2\sqrt{\text{area}/\pi}$; Jacquin *et al.*, 1983; Aldes, 1990; Furusawa *et al.*, 1996a) were calculated by using a computer graphics tablet and an Apple computer (Power Macintosh G3, software package; NIH image, National Institutes of Health).

Results

Neuronal activities from the nerve supply to the genioglossal muscle

Efferent discharges from the proximal cut end of the branch ran in the direction of the mandible, and EMG activities of the external intercostal muscle and mylohyoid muscle were recorded. The neural discharges were found to be synchronized with both the external intercostal muscle and mylohyoid muscle EMG activities (Fig. 2A). On the other hand, efferent discharge from the proximal cut end of the branch, running in the direction of the tongue, exhibited irregular bursts synchronized not with respiration phase but with swallowing (Fig. 2B).

Central distribution of the motoneurons innervating the genioglossus muscle

HRP-labeled cells were observed in all five rats injected with HRP:WGA in the branch in the direction of the mandible (Fig. 3). These cells were localized in the lateral region of the ventromedial subnucleus in the XII nucleus ipsilateral to the injection site, rostrocaudally extending from approximately 0.7 to 1.2 mm rostral to the obex (Fig. 5). A total of 55 labeled cells were observed in counterstained sections from five rats. These cells, of which the average somal diameter was 24.1 ± 2.4 μm, were multipolar (Fig. 6A).

On the other hand, HRP-labeled cells were observed in all five rats injected with HRP:WGA in the branch to the direction of the tongue (Fig. 4). These cells were localized in a more medial site than the preceding labeled cells in the lateral region of the ventromedial subnucleus in the XII nucleus ipsi-

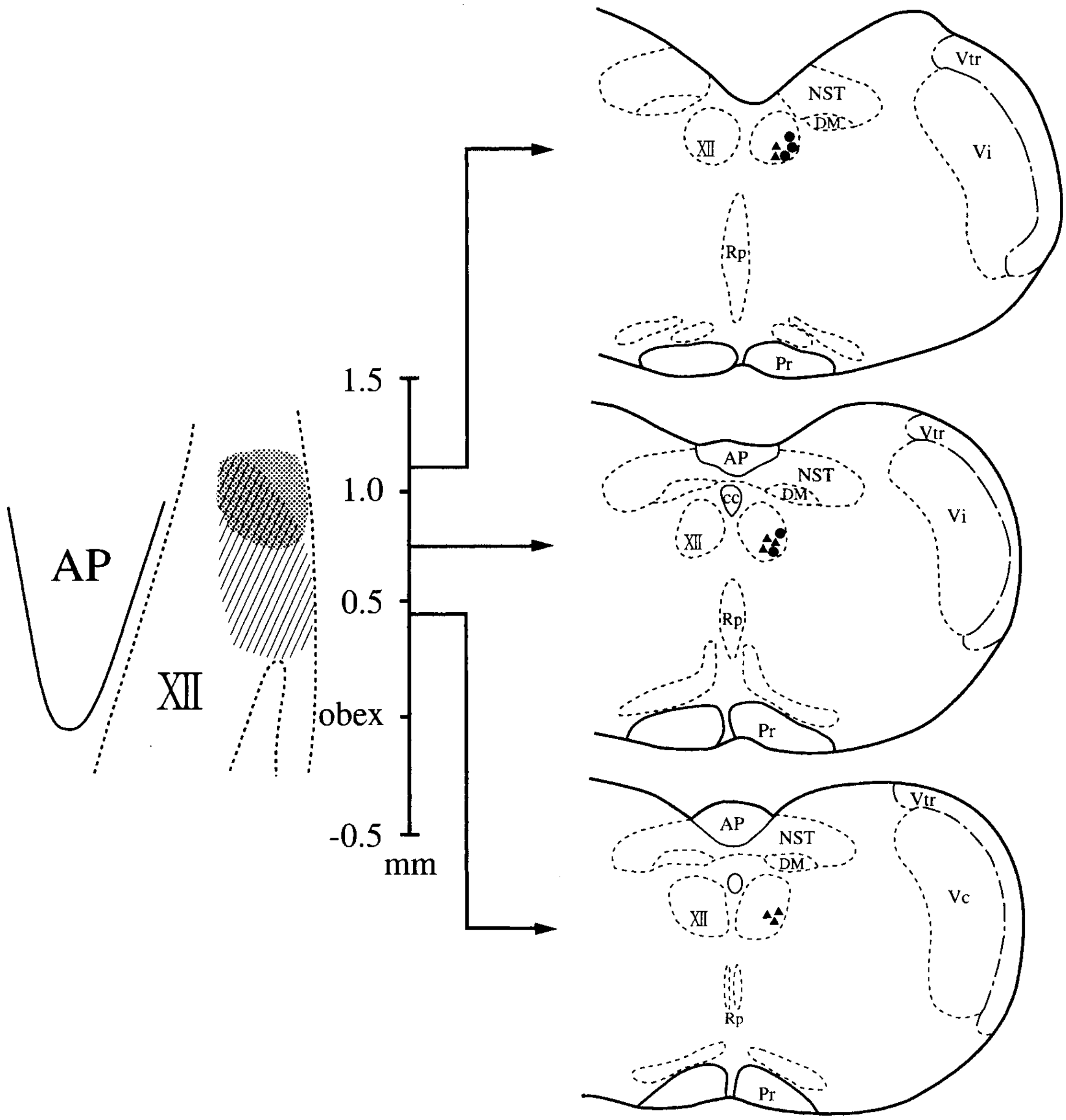

FIGURE 5. Location of labeled cells within the ventromedial subnucleus of the XII nucleus shown in an illustration of the dorsal view of the medulla (left) and coronal sections of the medulla (right) after HRP:WGA was injected into each branch. Filled circle and dots indicate individual labeled cells and area after injection to the branch running in the direction of the mandible. Filled triangle and slants indicate individual labeled cells and area after injection to the branch running in the direction of the tongue. The numbers to the right of the drawings represent the distance in mm rostral or caudal to the obex.

lateral to the injection site, rostrocaudally extending from approximately 0.3 to 1.2 mm rostral to the obex (Fig. 5). A total of 52 labeled cells were observed in counterstained sections from five rats. These cells, of which the average somal diameter was 22.4 ± 3.4 μm, were multipolar (Fig. 6B). There was a significant difference between the somal diameters of the preceding labeled cells ($p < 0.001$ level, Welch's *t*-test).

Discussion

The tongue coordinates movements that assist in respiration (for maintenance the upper airway patency), swallowing, phonation, and mastication (Lowe, 1981, 1984). Muscle responsibilities for tongue movements derive their innervations from the XII nucleus and include, for retraction, the hyoglossus and styloglossus, for protrusion, the genioglossus and geniohyoid, and for changes in tongue form, the intrinsic tongue muscle (Odutola, 1976; Lowe, 1981, 1984). The location of these muscle motoneurons within the XII nucleus has already been investigated in several kind of animals: rat (Krammer *et al.*, 1979; Uemura-Sumi *et al.*, 1988; Altschuler *et al.*, 1994; Aldes, 1995), cat (Uemura *et al.*, 1979), dog (Chibuzo and Cummings, 1982; Uemura-Sumi *et al.*, 1988), rabbit (Uemura-Sumi *et al.*, 1988), frog (Matesz *et al.*, 1999), and monkey (Uemura-Sumi *et al.*, 1981; Sokoloff and Deacon, 1992). In the rat, the XII nucleus consists of a distinct dorsal subnucleus, ventromedial subnucleus, and ventrolateral sub-

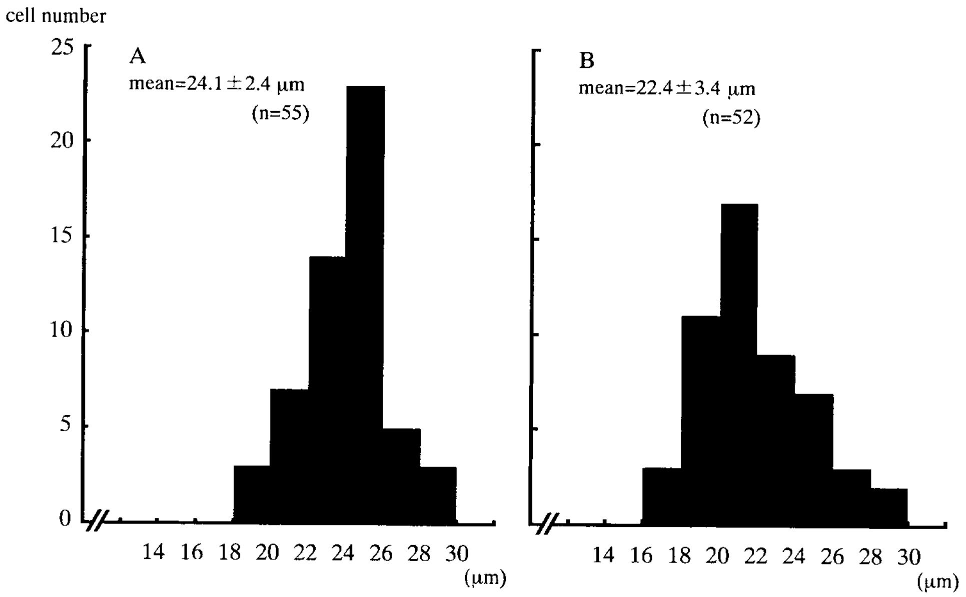

FIGURE 6. Morphometric analysis results for labeled cells following application of HRP:WGA to the respiration branch or swallowing branch. Soma diameters (D = 2□ area/π) are plotted against cell numbers for each structure. (A) Injection to the branch running in the direction of the mandible. (B) Injection to the branch running in the direction of the tongue.

nucleus (Krammer *et al.*, 1979; Altschuler *et al.*, 1994; Aldes, 1995). The rat genioglossal motoneurons are located in the lateral region of the ventromedial subnucleus in the XII nucleus (Uemura-Sumi *et al.*, 1988; Altschuler *et al.*, 1994; Aldes, 1995). In our results, the branch in the direction of the mandible was not only related to the respiration but also swallowing, while the branch in the direction of the tongue was related to swallowing. Our results confirm that almost all of the respiration-related motoneurons are located more rostrally than the swallowing-related motoneurons of the genioglossal motoneuron pool.

The motoneurons labeled following application of HRP:WGA in the branch in the direction of the tongue were located lateral to the ventromedial subnucleus in the XII nucleus. The caudal end of this labeled motoneuron area was adjacent to the geniohyoid muscle motoneuron area. It has been amply demonstrated that the geniohyoid motoneurons are localized in the ventrolateral subnucleus of the XII nucleus (Krammer *et al.*, 1979; Uemura-Sumi *et al.*, 1988; Altschuler *et al.*, 1994; Aldes, 1995), and the geniohyoid muscle is active during swallowing (Travers and Jackson, 1992; Tanaka, 1998). Our results and these observations are compatible with XII motoneurons being somatotopically organized according to function in the rat (Lewis *et al.*, 1971).

The labeled motoneurons might include other motoneurons except for respiration-related and swallowing-related motoneurons. The soma size of labeled motoneurons following application of HRP:WGA to the branch in the direction of the mandible was greater than that of labeled motoneurons following application to the branch in the direction of the tongue. It is known that the dendritic arbor extension is proportionally related to the soma size. Several investigators have suggested that the respiratory-related XII motoneurons receive various inputs from respiratory rhythm generator neurons and other non-respiratory inputs.

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