

# The Distribution of Afferent Neurons in the Trigeminal Mesencephalic Nucleus and the Central Projection of Afferent Fibers of the Mylohyoid Nerve in the Rat

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**Abstract** Horseradish peroxidase conjugated to wheatgerm agglutinin (HRP:WGA) was injected into the proximal cut ends of three branches of the mylohyoid nerve in rats: the branch to the mylohyoid muscle (BrMh), the branch to the anterior belly of the digastric muscle (BrDg), and the cutaneous branch (BrCu). HRP-labeled cells were detected in the ipsilateral caudal portion of the trigeminal mesencephalic nucleus (Vmes) and the ipsilateral ventromedial division of the trigeminal motor nucleus, except when HRP:WGA was applied to the BrCu. Morphologically, all labeled Vmes cells were of the pseudounipolar type.

Projections of the primary afferents of the BrMh were observed in the ipsilateral trigeminal nucleus caudalis, the upper cervical dorsal horns of laminae I–III, and the dorsolateral reticular formation (Rf), whereas the primary afferents of the BrDg terminated in the ipsilateral trigeminal nucleus principalis and Rf. These observations suggest that the role of the afferent inputs of the mylohyoid muscle differs from that of those of the anterior belly of the digastric muscle in terms of several functions associated with jaw-closing and infrahyoid muscles.

**Key words** mylohyoid nerve, muscle spindle, trigeminal mesencephalic nucleus, HRP:WGA, rat

In the rat, the mylohyoid nerve gives off branches to the mylohyoid muscle (BrMh) and the anterior belly of the digastric muscle (BrDg), and then divides into two other branches extending between these two muscles: a cutaneous branch (BrCu) and a mandibular transverse muscle branch (Yamaoka et al., 1992; Furusawa et al., 1993). The suprahyoid muscles, including the mylohyoid muscle, were formerly believed to be devoid of muscle spindles (Szentagothai, 1948; Blom, 1960; Dubner et al., 1978). However, the presence of muscle spindles in the mylohyoid muscle in the rat has been demonstrated more recently by neurophysiological and histological methods (Yamaoka et al., 1992; Furusawa et al., 1994).

The trigeminal mesencephalic nucleus (Vmes) is known to include primary afferent neurons of the jaw-closing muscle spindles and periodontal ligament receptors. In an early study, a few Vmes cells showed positive reactions after separate injection of horseradish peroxidase (HRP) into either the anterior belly of the digastric muscle or the mylohyoid muscle in the cat (Alvarado-Mallart et al., 1975).

Furthermore, Jacquin et al. (1983a) demonstrated a small number of labeled cells located in the Vmes, following application of HRP to the mylohyoid nerve. On the basis of these studies' results and our previous findings, we speculated that Vmes neurons innervating muscle spindles are present in the mylohyoid nerve. However, there is no information in the literature regarding the distribution of the Vmes neurons in the mylohyoid nerve. Previous HRP studies in the rat and in the cat (Jacquin et al., 1983b; Shigenaga et al., 1988) demonstrated the central projections of the primary afferent neurons of the mylohyoid nerve, but the distribution of labeled terminals from the branches of the mylohyoid nerve has not previously been clarified. The purposes of the present study were to determine the distribution of Vmes neurons innervating the anterior belly of the digastric muscle and mylohyoid muscle, as well as that of the central projections of the afferent fibers of each branch of the mylohyoid nerve.

## MATERIALS AND METHODS

Sixteen male Wistar rats weighing 200 to 250 g were used in the present study. Each rat was anesthetized with an

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intraperitoneal injection of ketamine (50–100 mg/kg) and then placed on a table in the supine position. Under a surgical microscope, a vertical incision was made over the masseter muscle, exposing the mylohyoid nerve. One of three branches of this nerve (the BrMh in six rats, the BrDg in five, and the BrCu in five) was dissected free immediately distal to its insertion into the muscle or skin. The proximal cut end of the branch was suctioned into a glass micropipette (diameter of the tip = 100–150  $\mu\text{m}$ ) 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 90–120 min. After the HRP:WGA was washed out of the central ends of the nerve, the wound was closed and the rat was allowed to recover from the anesthesia under inhalation of 80% oxygen. At 48 hr after the 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 between the superior colliculus and the upper cervical cord was removed and stored for 24–48 hr at 4°C in phosphate buffer containing 30% sucrose. It was then cut into serial transverse frozen sections 30  $\mu\text{m}$  thick. 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 distribution of labeled boutons and cells was plotted with the aid of darkfield or brightfield illumination.

## RESULTS

The 11 rats in which HRP:WGA was applied to the BrMh or BrDg showed evidence of anterograde and retrograde labeling in the brainstem; labeled-cell clusters were observed in the anterior belly of the digastric muscle and the mylohyoid motoneuron pool, and corresponded to the dorsomedial and the ventrolateral subdivisions, respectively, in the ventromedial division of the trigeminal motor nucleus. Findings for Vmes are described below. Data for one rat (injections to BrMh) were excluded, because we observed labeled neurons in the dorsolateral division (jaw-closing muscle motoneuron pool) of the trigeminal motor nucleus, which we attributed to leakage of HRP:WGA tracer from the injection site.

### *HRP-Labeled Cells in the Trigeminal Mesencephalic Nucleus (Figs. 1 and 2, Table 1)*

In each of the five BrMh rats, from two to seven HRP-labeled cells were concentrated in an area of the ipsilateral Vmes extending from 0.78 to 1.5 mm rostral to the caudal

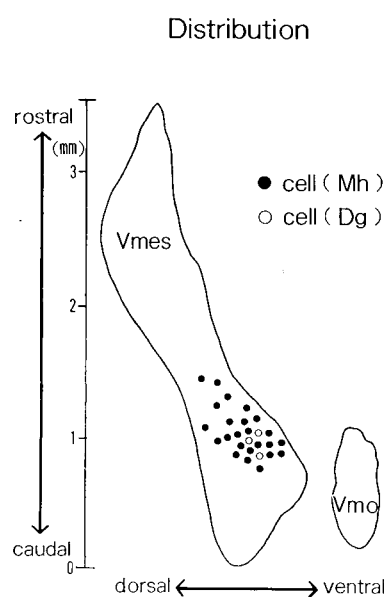


FIGURE 1. Schematic parasagittal drawing of the distribution of labeled cells in all BrMh and BrDg experiments in which horizontal sections in the Vmes were assessed. Filled circles, labeled cells following application of HRP:WGA to the BrMh; open circles, labeled cells following application of HRP:WGA to the BrDg. Vmo, trigeminal motor nucleus.

pole of the nucleus. Only one or two HRP-labeled cells were counted in two of the five BrDg rats; these cells were located in an area of the Vmes extending from 0.87 to 1.08 mm rostral to the caudal pole of the nucleus. All labeled Vmes cells were pseudounipolar cells (Figs 2A and 2B).

### *Central Projections of Primary Afferent Fibers (Figs. 2–5)*

Projections of the primary afferents of the BrMh were observed in the ipsilateral regions from the rostrocaudal midlevel of the trigeminal nucleus caudalis (Vc) to the rostral parts of the C<sub>2</sub> segment (Figs. 2C and 3), and in the dorsolateral reticular formation (Rf) (Fig. 2D). In the Vc, this branch terminated in lamina I from the rostrocaudal midlevel of the nucleus to the C<sub>1</sub> segments, and in laminae I–III between C<sub>1</sub> and the caudal C<sub>2</sub> segments. Small patches of labeled terminals were found in the dorsomedial region of the Rf at the level between the caudal portion of Vi and the caudal portion of Vc.

Afferent fibers of the BrDg terminated in the ipsilateral rostrocaudal midlevel of the trigeminal nucleus principalis (Vp) and in the Rf (Figs. 2E and 4). In the Vp, labeled terminals were located in the most lateral region of the nucleus. In the Rf, small patches of the labeled terminals were located in about the same region in which those of the BrMh were found. Labeled terminals could be observed in the Rf when the labeled cells in the Vmes were examined following application of HRP:WGA to the BrDg.

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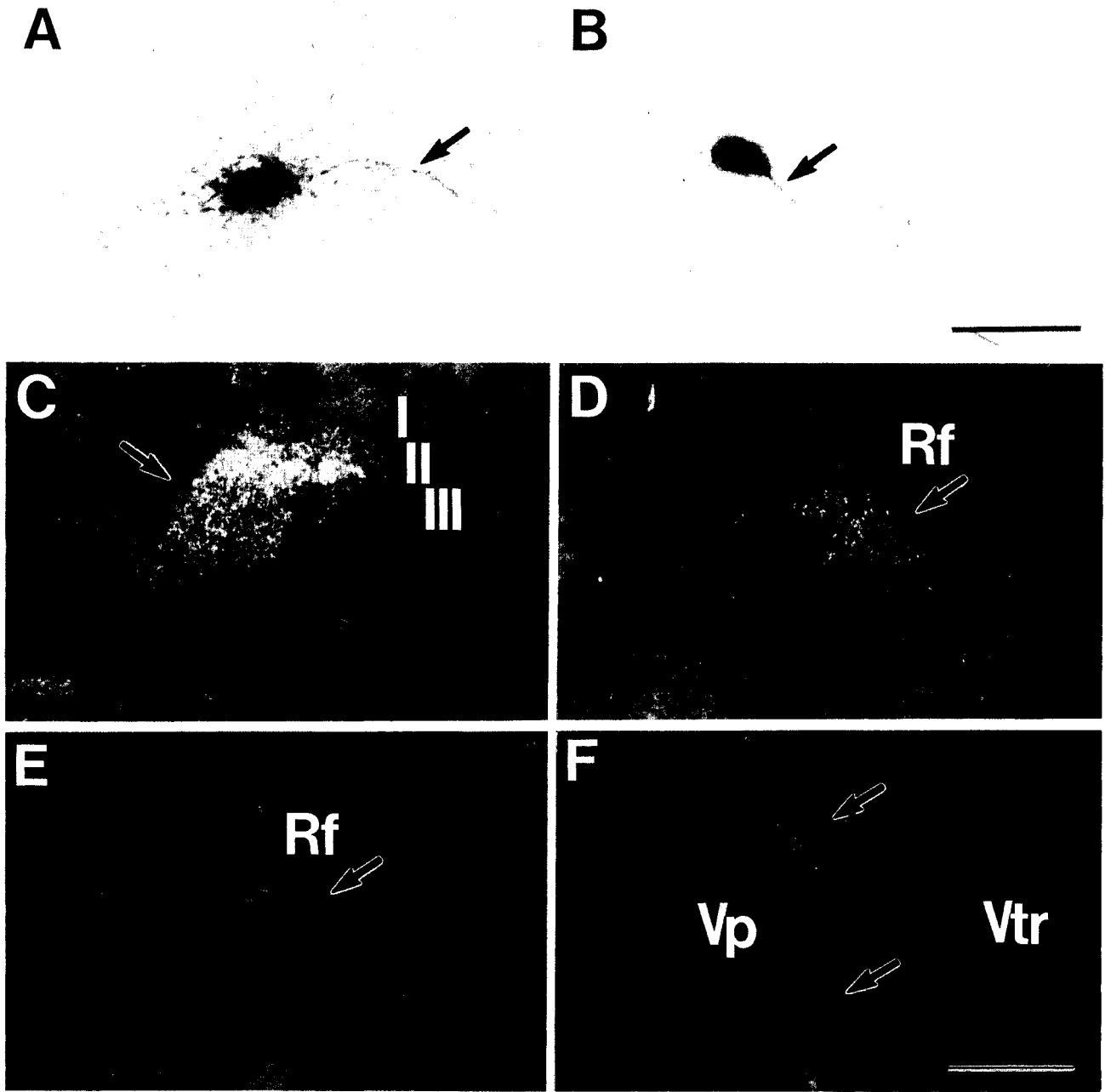


FIGURE 2. Brightfield (A, B) and darkfield (C–F) photomicrographs showing examples of the HRP-labeled cells and terminals. A and B show typical labeled Vmes cells following application of HRP:WGA to the BrMh. Each arrow indicates a stem axon. C, D, and E show typical labeled terminals in the upper cervical dorsal horn, Rf, and Rf, respectively, following application of HRP:WGA to the BrMh, to the BrMh, and to the BrDg, respectively. F shows typical labeled terminals in the Vp following application of HRP:WGA to the BrCu. Vtr, spinal trigeminal tract. Calibration bar in B (applies also to A): 50  $\mu$ m. Calibration bar in F (applies also to C–E): 100  $\mu$ m.

Afferent fibers of the BrCu terminated throughout the ipsilateral rostrocaudal level of the Vp (Fig. 2F), in the rostral trigeminal nucleus oralis (Vo), in the caudal Vi, and in the regions from the rostrocaudal midlevel of the Vc to the rostral parts of the C<sub>2</sub> segment (Fig. 5). In the Vp, dense patches of labeled terminals were observed in the most dorsolateral region of the nucleus. In the Vo, small

patches of labeled terminals were located in the most lateral region of the nucleus; in the trigeminal nucleus interpolaris (Vi), labeled terminals were also located in the most lateral region of the nucleus. In the Vc, this branch terminated from the rostrocaudal midlevel of the Vc to the rostral C<sub>2</sub> segments, and in laminae I–III it terminated between the C<sub>1</sub> and rostral C<sub>2</sub> segments.

TABLE 1. Numbers of HRP-Labeled Cells in the Vmes Following HRP:WGA Application to BrMh and BrDg

Rat no.	Numbers of cells
Mh-1	2
Mh-2	7
Mh-3	7
Mh-4	4
Mh-5	4
Dg-1	1
Dg-2	0
Dg-3	2
Dg-4	0
Dg-5	0

## DISCUSSION

Following the application of HRP to the afferent fibers of the deep temporalis, masseter, and medial pterygoid muscles, HRP-labeled cells have been detected in the trigeminal ganglion and the Vmes (Gottlieb et al., 1984; Shigenaga et al., 1988). On the other hand, following the application of HRP to the afferent fibers of the suprahyoid muscles (such as the digastric and mylohyoid fibers) in the cat, HRP-labeled cells were seen only in the trigeminal ganglion (Gottlieb et al., 1984; Shigenaga et al., 1988). However, in our study HRP-labeled Vmes cells were observed ipsilaterally in the caudal portions of the Vmes following application of HRP:WGA to the BrMh and BrDg. Furthermore, the results of other studies in which HRP was used in cats (Alvarado-Mallart et al., 1975) and in rats (Jacquin et al., 1983a) provide

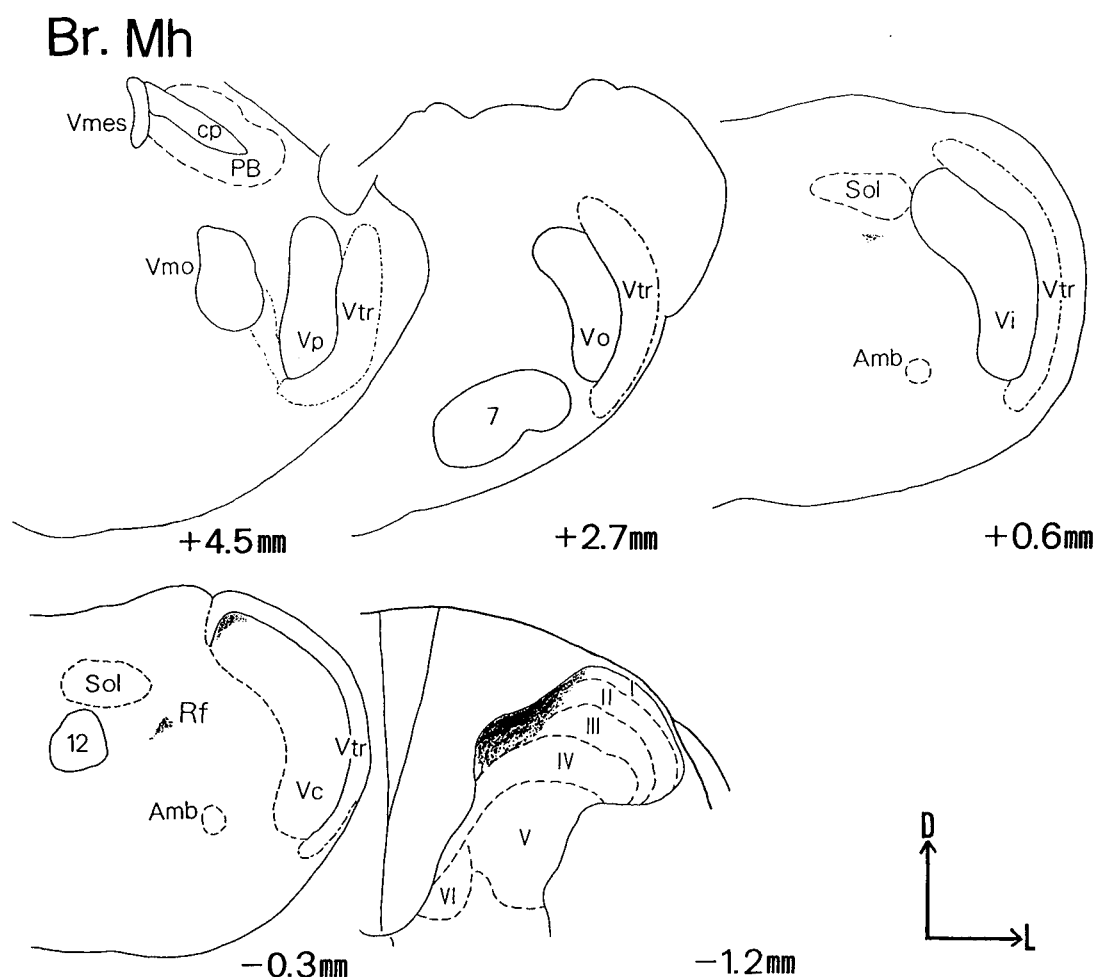


FIGURE 3. Schematic drawings illustrating the distribution of HRP-labeled terminals at different levels of the brainstem and upper cervical cord after HRP:WGA application to the BrMh. The numbers to the right of the drawings represent the distance in millimeters rostral or caudal to the obex. D, dorsal; L, lateral; Amb, nucleus ambiguus; cp, cerebellar peduncle; PB, parabrachial nucleus; Sol, nucleus solitarius; 7, facial nucleus; 12, hypoglossal nucleus. Other abbreviations as in Figures 1-2 and text.

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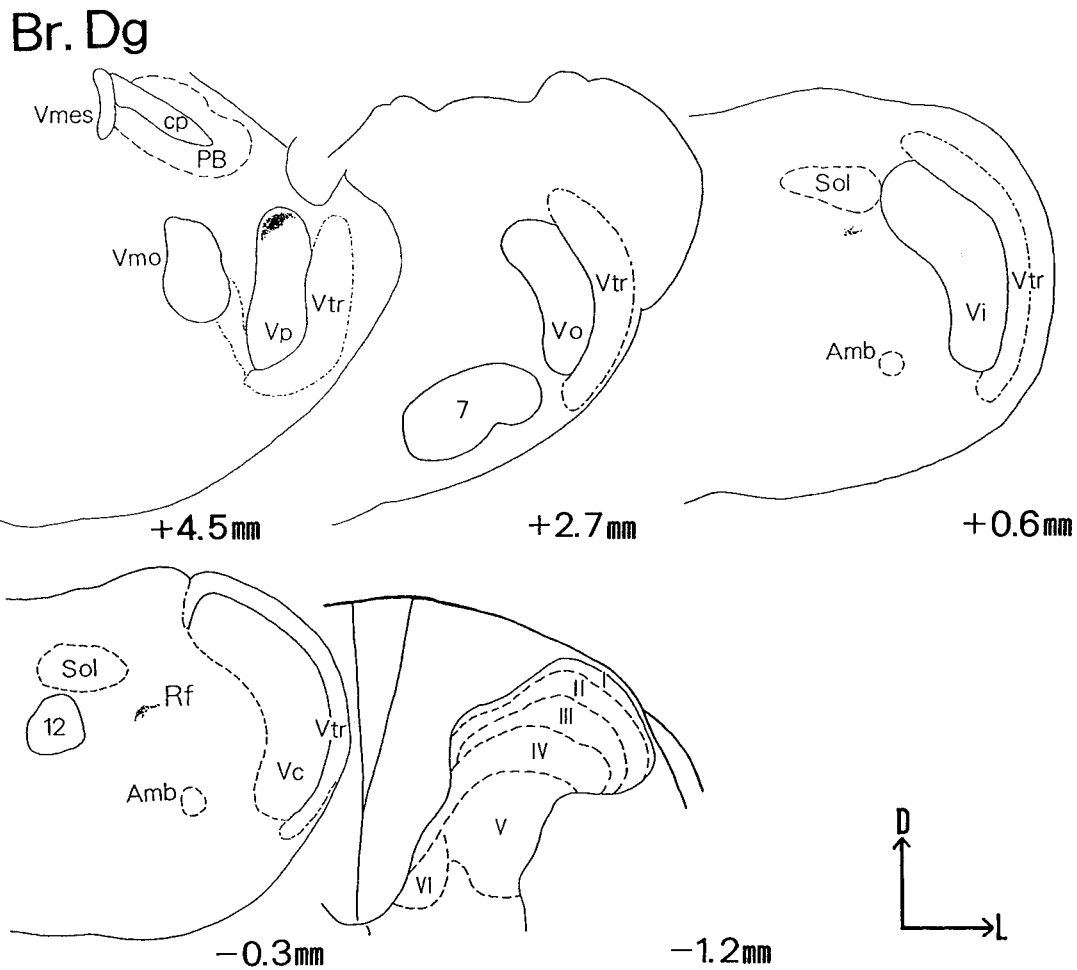


FIGURE 4. Schematic drawings illustrating the distribution of HRP-labeled terminals at different levels of the brainstem and upper cervical cord after HRP:WGA application to the BrDg. Conventions and abbreviations as in Figure 3.

evidence that a few neurons in the Vmes innervate the suprahyoid muscle. These conflicting results may be attributed to the paucity of muscle spindles, and presumably also to species-specific differences or to differences in experimental methods, but the results of all studies suggest the possible existence of muscle spindles in the mylohyoid muscle and/or the anterior belly of the digastricus muscle. The organization of the HRP-labeled Vmes neurons for BrMh in the present study was similar to that for BrDg. Labeled cells extended from the level of the middle to the first third of the caudal portion. Vmes neurons innervating the jaw-closing muscles are located in the rostral half of the Vmes, and Vmes neurons innervating the periodontal ligament are located in the caudal half of the Vmes (Alvarado-Mallart et al., 1975; Jacquin et al., 1983a; Gottlieb et al., 1984; Rokx and van Willigen, 1988; Shigenaga et al., 1988, 1989, 1990). Results of earlier investigations and our own results support the view that functional differences among the Vmes neurons innervating the muscles or periodontal ligament are

associated with differences in the location of the somata in the Vmes.

Recent HRP studies provide evidence that about 40% of the Vmes neurons innervating the jaw-closing muscles in the adult cat are multipolar cells (Shigenaga et al., 1988), and in an intracellular HRP labeling study in the adult rat, 13% of the Vmes neurons innervating the jaw-closing muscles were found to be multipolar cells (Luo et al., 1991). Furthermore, in some electron-microscopic studies, synaptic contacts were seen upon HRP-labeled dendritic profiles of Vmes neurons (Witkovsky and Roberts, 1976; Nomura et al., 1985). The area around the Vmes is thought to receive inputs from many afferent neurons of peripheral receptive fields, including the oral mucosa and skin (Hayashi and Tabata, 1989). We found in the present study that all labeled Vmes cells innervating the suprahyoid muscles, such as the mylohyoid muscle and the anterior belly of the digastricus muscle, were cells of the pseudounipolar type.

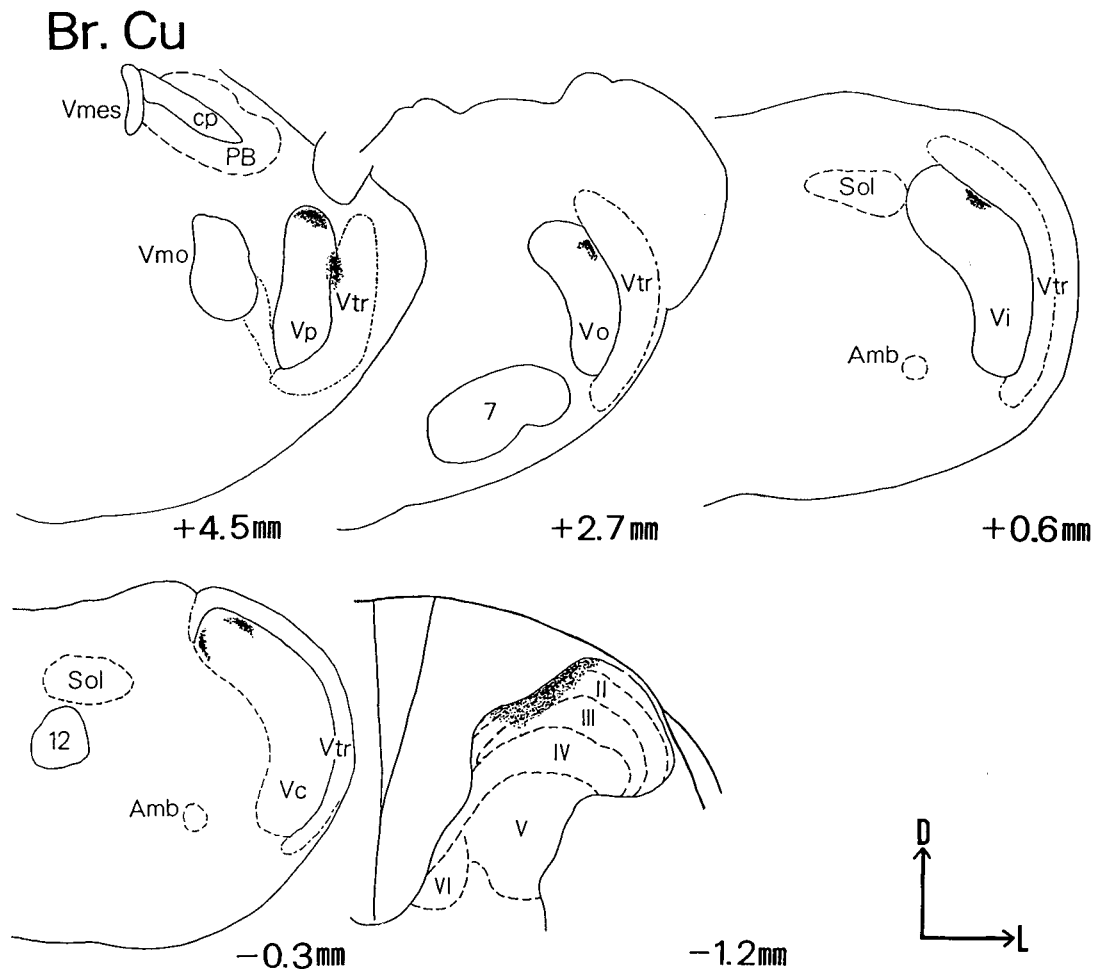


FIGURE 5. Schematic drawings illustrating the distribution of HRP-labeled terminals at different levels of the brainstem and upper cervical cord after HRP:WGA application to the BrCu. Conventions and abbreviations as in Figure 3.

The present study also demonstrated that the primary afferent neurons innervating the mylohyoid muscle, the anterior belly of the digastric muscle, and the skin over the lower jaw project to the trigeminal sensory nuclear complex, the upper cervical dorsal horn, and the Rf. Our results are consistent with those of an HRP tracing study (Jacquin et al., 1983b), except for the observation of the projections of labeled terminals at the Rf when HRP:WGA was applied to the BrMh and BrDg. These discrepant results may be attributed to differences in methods, in that we injected HRP:WGA into each of three branches of the mylohyoid nerve (BrMh, BrDg, and BrCu). The labeled terminals detected in the Rf may derive from the Vmes, since the trajectory to the Rf is similar to that of Probst's tract (Corbin, 1942; Matesz, 1981; Shigenaga et al., 1989, 1990; Yoshida et al., 1994). Nociceptive-specific neurons and wide-dynamic-range neurons in the trigeminal ganglion terminate in laminae I–II and V–VI in the Vc (Amano et al., 1986) and in laminae I–II in the upper cervical cord (Christensen

and Perl, 1970; Shigenaga et al., 1988). Our data demonstrate that the main terminal fields of the BrMh and BrCu afferent fibers are in lamina I in the Vc and the upper cervical dorsal horn. These findings suggest that the labeled terminals in the Vc and upper cervical cord may be derived from the somata in the trigeminal ganglion and may be related to cutaneous and muscle pain (Shigenaga et al., 1988; Takemura et al., 1993). On the other hand, terminals labeled when HRP:WGA was applied to the BrDg were not observed in the Vc or upper cervical cord. This might be attributable to the functional difference between the mylohyoid muscle and the anterior belly of the digastric muscle. It has been amply demonstrated by electrophysiological methods that the afferent activities of the proprioceptors are modulated by respiration, and a reflex arc has been found between the mylohyoid muscle and the sternohyoid muscle in the rat (Van de Graaff et al., 1984; St. John and Bledsoe, 1985; Van Lunteren et al., 1987; Furusawa et al., 1994). Specifically, the mylohyoid muscle has not only a jaw-

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opening function but also a relationship to the respiratory system, in contrast with the anterior belly of the digastric muscle, which plays a part chiefly in jaw opening.

### REFERENCES

- ALVARADO-MALLART, M. R., C. BATINI, C. BUISSERET-DELMAS, and J. COVISIER (1975) Trigeminal representations of the masticatory and extraocular proprioceptors as revealed by horseradish peroxidase retrograde transport. *Exp. Brain Res.* 23: 167–179.
- AMANO, N., J. W. HU, and B. J. SESSLE (1986) Responses of neurons in feline trigeminal subnucleus caudalis (medullary dorsal horn) to cutaneous, intraoral, and muscle afferent stimuli. *J. Neurophysiol.* 55: 227–243.
- BLOM, S. (1960) Afferent influences on tongue muscle activity. *Acta Physiol. Scand.* 49: 1–97.
- CHRISTENSEN, B. N., and E. R. PERL (1970) Spinal neurons specifically excited by noxious or thermal stimuli: Marginal zone of the dorsal horn. *J. Neurophysiol.* 33: 293–307.
- CORBIN, K. B. (1942) Probst's tract in the cat. *J. Comp. Neurol.* 77: 455–467.
- DUBNER, R., B. J. SESSLE, and A. T. STOREY (1978) *The Neural Basis of Oral and Facial Function: Peripheral Components of Motor Control*, Plenum Press, New York.
- FURUSAWA, K., M. YAMAOKA, K. FUJIMOTO, and T. KUMAI (1994) Role of proprioceptor in the mylohyoid muscle. *Brain Res. Bull.* 35: 233–236.
- FURUSAWA, K., M. YAMAOKA, K. IGUCHI, and T. KUMAI (1993) Tactile-evoked response of sensory fibers in buccal and submandibular regions of the rat. *Somatosens. Res.* 10: 291–295.
- GOTTLIEB, S., A. TAYLOR, and M. A. BOSLEY (1984) The distribution of afferent neurons in the mesencephalic nucleus of the fifth nerve in the cat. *J. Comp. Neurol.* 228: 273–283.
- HAYASHI, H., and T. TABATA (1989) Physiological properties of sensory trigeminal neurons projecting to mesencephalic parabrachial area in the cat. *J. Neurophysiol.* 52: 1153–1160.
- JACQUIN, M. F., R. W. RHOADES, H. L. ENFIEJIAN, and M. D. EGGER (1983a) Organization and morphology of masticatory neurons in the rat: A retrograde HRP study. *J. Comp. Neurol.* 218: 239–256.
- JACQUIN, M. F., K. SEMBA, M. D. EGGER, and R. W. RHOADES (1983b) Organization of HRP-labeled trigeminal mandibular primary afferent neurons in the rat. *J. Comp. Neurol.* 215: 397–420.
- LUO, P. F., B. R. WANG, Z. Z. PENG, and J. S. LI (1991) Morphological characteristics and terminating patterns of masseteric neurons of the mesencephalic trigeminal nucleus in the rat: An intracellular horseradish peroxidase labeling study. *J. Comp. Neurol.* 303: 286–299.
- MATESZ, C. (1981) Peripheral and central distribution of fibers of the mesencephalic trigeminal root in the rat. *Neurosci. Lett.* 27: 13–17.
- MESULAM, M.-M. (1982) *Tracing Neural Connections with Horseradish Peroxidase: Principles of Horseradish Peroxidase Neurohistochemistry and Their Applications for Tracing Neural Pathway—Axonal Transport, Enzyme Histochemistry and Light Microscopic Analysis*, Wiley, New York.
- NOMURA, S., A. KONISHI, K. ITOH, T. SUGIMOTO, T. YASUI, A. MITANI, and N. MIZUNO (1985) Multipolar neurons and axodendritic synapses in the mesencephalic trigeminal nucleus of the cat. *Neurosci. Lett.* 55: 337–342.
- ROKX, J. T. M., and J. D. VAN WILLIGEN (1988) Organization of neuronal clusters in the mesencephalic trigeminal nucleus of the rat: Fluorescent tracing of temporals and masseteric primary afferents. *Neurosci. Lett.* 86: 21–26.
- SHIGENAGA, Y., K. DOE, S. SUEMUNE, Y. MITSUHIRO, K. TSURU, K. OTANI, Y. SHIRANA, M. HOSOI, A. YOSHIDA, and K. KAGAWA (1989) Physiological morphological characteristics of periodontal mesencephalic trigeminal neurons in the cat: Intra-axonal staining with HRP. *Brain Res.* 505: 91–110.
- SHIGENAGA, Y., Y. MITSUHIRO, Y. SHIRANA, and H. TSURU (1990) Two types of jaw-muscle spindle afferents in the cat as demonstrated by intra-axonal staining with HRP. *Brain Res.* 514: 219–237.
- SHIGENAGA, Y., Y. SERA, T. NISHIMORI, S. SUEMUNE, M. NISHIMURA, A. YOSHIDA, and K. TSURU (1988) The central projection of masticatory afferent fibers to the trigeminal sensory nuclear complex and upper cervical spinal cord. *J. Comp. Neurol.* 268: 489–507.
- ST. JOHN, W. M., and T. A. BLEDSOE (1985) Comparison of respiratory-related trigeminal, hypoglossal and phrenic activities. *Resp. Physiol.* 62: 61–78.
- SZENTAGOTHAI, J. (1948) Anatomical considerations of monosynaptic reflex arcs. *J. Neurophysiol.* 11: 445–454.
- TAKEMURA, M., Y. NAGASE, A. YOSHIDA, K. YASUDA, S. KITAMURA, Y. SHIGENAGA, and S. MATANO (1993) The central projections of the monkey tooth pulp afferent neurons. *Somatosens. Mot. Res.* 10: 217–227.
- VAN DE GRAAFF, W. B., S. B. GOTTFRIED, J. MITRA, E. VAN LUNTEREN, N. S. CHERNIACK, and K. P. STROHL (1984) Respiratory function of hyoid muscle and hyoid arch. *J. Appl. Physiol.* 57: 187–204.
- VAN LUNTEREN, E., M. A. HAXHIU, and N. S. CHERNIACK (1987) Mechanical function of hyoid muscle during spontaneous breathing in cats. *J. Appl. Physiol.* 62: 582–590.
- WITKOVSKY, P., and B. L. ROBERTS (1976) Electron microscopic observations of the mesencephalic nucleus of the fifth nerve in the selachian brain. *Proc. Roy. Soc. Lond.* 5: 643–660.
- YAMAOKA, M., K. FURUSAWA, K. FUJIMOTO, K. IGUCHI, and T. KUMAI (1992) Muscle spindle in the mylohyoid muscle of rats. *Int. J. Oral Maxillofac. Surg.* 21: 309–312.
- YOSHIDA, A., K. YASUDA, J. O. DOSTROVSKY, Y. C. BAE, M. TAKEMURA, Y. SHIGENAGA, and B. J. SESSLE (1994) Two major types of premotoneurons in the feline trigeminal nucleus oralis as demonstrated by intracellular staining with horseradish peroxidase. *J. Comp. Neurol.* 347: 495–514.