〔Original〕 松本歯学 26：31～35，2000

key words：Trigeminal nerve — Synapses — Electron microscopy — Mylohyoid muscle — Trigeminal motor nucleus

Synaptic Connections of Mylohyoid Primary Afferent Terminals Made on Jaw-Opening Muscle Motoneurons in the Rat

KOUICHI YASUDA, KIYOFUMI FURUSAWA, YOKO NAKAYAMA, MIKIKO TANAKA, DAIZO OKUDA, MIZUHO TANAKA and MINORU YAMAOKA

Oral and Maxillofacial Surgery, Matsumoto Dental University School of Dentistry
(Chief: Prof. M. Yamaoka)

Summary

Horseradish peroxidase conjugated with wheat germ agglutinin (HRP-WGA) was injected into the muscle branch of the mylohyoid nerve in rats. HRP-labeled neuronal cell bodies were observed ipsilaterally in the caudal portion of the trigeminal mesencephalic nucleus and the ventromedial division of the trigeminal motor nucleus (Vmo.vm). Electron microscope observations were carried out on sections through Vmo.vm containing HRP-labeled cell bodies. Synaptic contacts were found between HRP-labeled axon terminals and HRP-labeled nerve cells. The results suggested that mylohyoid muscle spindle afferents are synaptic contacts on the mylohyoid motoneurons.

It is well known that the trigeminal mesencephalic nucleus (Vmes) contain primary afferent neurons innervating the periodontal ligament receptors and muscle spindles of the jaw-closing muscles and the both primary afferents made synaptic contacts with jaw-closing motoneurons. Although it has been reported the cat jaw-opening muscles, including the mylohyoid muscle, were formerly believed to be devoid of muscle spindles[6], in resent studies[4,16] the presence of muscle spindles in the rat mylohyoid muscle has been demonstrated electrophysiologically and histologically. In these studies, a few neurons in the Vmes were labeled after injection of horseradish peroxidase (HRP) into the mylohyoid muscle or the mylohyoid muscle branch of the mylohyoid nerve in the cat[1] and rat[7,17]. These HRP-labeled cells may come from primary afferent fibers innervating the mylohyoid muscle spindle[17].

Recent studies have revealed light or electron-microscopic morphological features of the synaptic contacts made between the jaw-closing muscle spindle afferents and their motoneurons[2,8,15]. However, there has been no discussion on the existence of synaptic terminals from mylohyoid muscle spindle afferents in the ventromedial division of the trigeminal motor nucleus (Vmo.vm ; jaw-opening motoneuron pool)[10]. Our experiments attempted to clarify the ultrastructure of the synaptic contacts made between primary afferents supplying the mylohyoid muscle and their motoneurons.

The experiments were conducted in three male Wistar rats weighing 200 to 250g. They were anes-

(submitted February 25, 2000 ; accepted April 19, 2000)

thetized with an intraperitoneal injection of ketamine (0.05–1.0mg/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℃ using a heating pad. Under a surgical microscope, the mylohyoid muscle branch of the mylohyoid nerve was exposed. The mylohyoid muscle branch was dissected free immediately distal to its insertion into the muscle. The proximal cut end of the branch was suctioned into a glass micropipette (tip diameter ; 100–150μm) filled with 10% HRP conjugated with wheat germ agglutinin (HRP–WGA ; Toyobo) in 0.3 M KCl and 0.05 M Tris buffer at pH7.6, and left for 90–120 min.[5]. The HRP–WGA solution was then washed away from the tissue surrounding the nerve, and the wound was closed. At 48hr after surgery, the animal was perfused through the ascending aorta with heparinized physiological saline (50–100ml), followed by 400–500ml of a solution of 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer(pH7.2).The brainstem between the superior colliculus and the upper cervical cord was removed. Serial transverse sections 60 μm thick were cut with a Vibratome, collected, and reacted with tetramethylbenzidine (TMB) according to the method of Mesulam[9]. The wet sections were examined with a light microscope to select sections of the Vmo.vm containing HRP–labeled cells. The best section per rat was treated with a 1% O_sO_4 solution in 0.1 M phosphate buffer (pH6.0) at room temperature for 45 minutes[3, 14]. After the osmication sections were rapidly dehydrated, infiltrated with Epon, and flat–embedded. The embedded sections were then viewed with the light microscope, and the Vmo.vm containing labeled cells was trimmed out and cut on an ultramicrotome. Contrast was provided by treatment with uranyl acetate followed by lead citrate. The ultrathin sections were studied with a JEOL JEM–1200EX II electron microscope.

The three rats in which HRP–WGA was applied to the mylohyoid muscle branch of the mylohyoid nerve showed evidence of anterograde and retrograde labeling in the brain stem ; the labeled–cell clusters were seen in the ipsilateral Vmes (Fig.1) and Vmo.vm. In each of the three rats, from three to five HRP–labeled cells were concentrated in an area of the ipsilateral Vmes extending 0.80mm to 1.44mm from the caudal to rostral pole of the nucleus. As the labeled motoneurons were located within the Vmo.vm, the leakage of HRP–WGA tracer from the injection site was denied. Ten to twelve HRP–labeled Vmo.vm neurons were observed within four or five wet serial sections per rat, and we selected the best section per rat. The electron microscopic observations were based on study of a thousand or more ultrathin serial sections. Twelve synaptic contacts were found between the HRP–labeled terminals and the HRP–labeled motoneurons. The HRP–labeled terminals were found to make contact with the soma (Fig.2 A, B) and dendrite (Fig.3). Many synaptic contacts were observed between the terminals and the motoneurons near the neuronal cell body.

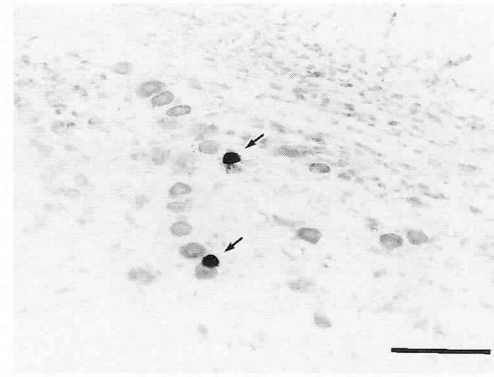

Fig.1 : Light micrographs of HRP–labeled cells (arrows) in the Vmes. It was counterstained with 1% neutral red. Scale bar,100μm

Primary afferents arising from the Vmes, which innervate periodontal ligament receptors or jaw–closing muscle spindles[8, 11, 12], rarely project to the jaw–opening motoneuron pool[13]. It is generally accepted that among trigeminal primary afferents only Vmes neurons innervating muscle spindle and

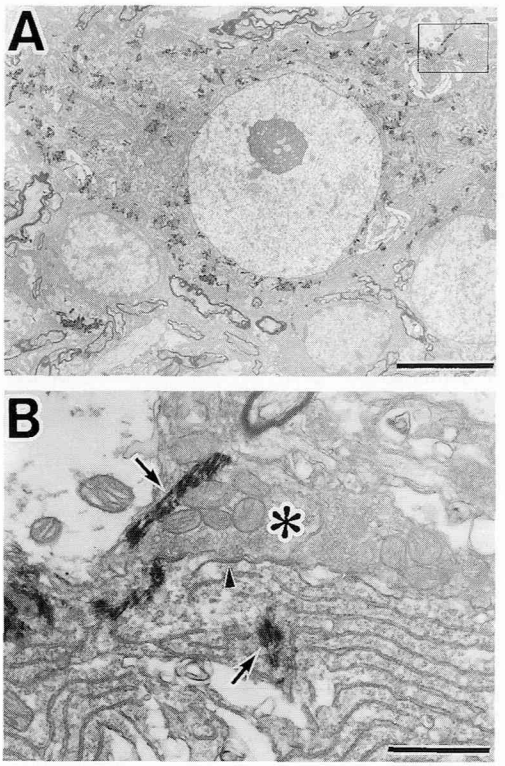

Fig.2 : Electron micrograph of an HRP-labeled terminal (asterisk) contacting the HRP-labeled soma. B shows a higher-power view of the rectangular area in A. Arrowhead indicates synaptic contacts. Arrows indicate reaction products. Scale bars,10μm in A, 1μm in B.

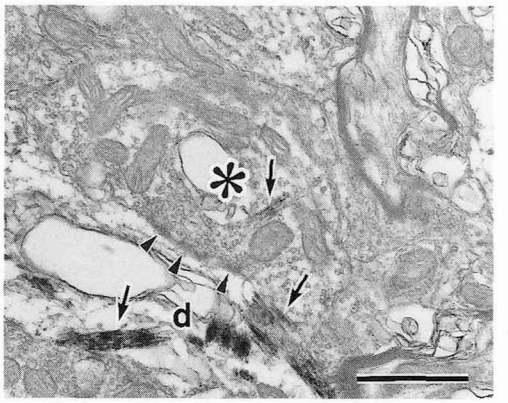

Fig.3 : Electron micrographs of HRP-labeled terminal (asterisk) contacting the HRP-labeled dendrite (d). Arrowheads indicate synaptic contacts. Arrows indicate reaction products. Scale bar, 1μm.

periodontal ligament project directly to the trigeminal motor nucleus. In our observations, synaptic contacts were found between HRP-labeled terminals and HRP-labeled motoneurons in Vmo.vm. It thus becomes apparent that mylohyoid motoneurons receive direct input from Vmes primary afferents that may innervate the mylohyoid muscle spindle.

It is generally believed that synapses distributed widely upon the motoneuronal somatic and dendritec surface, including the distal parts of dendritic tree. Our results also demonstrated that axon terminals from presumed mylohyoid muscle spindle afferents made synapses upon dendrites and the soma. Recent studies have indicated that jaw-closing muscle spindle afferents of the cat can be classified into two groups (group Ia and group II)[13, 15]. The groups Ia and II afferents send their collaterals into the jaw-closing motoneuron pool of the trigeminal motor nucleus and the supratrigeminal nucleus whereas the proportion of terminal distribution in the supratrigeminal nucleus is higher for group II than group Ia. Futher it has been demonstrated group II afferent terminals make synaptic contacts on more distal dendrites of the motoneurons than do group Ia afferent terminals. Our recent study suggested that a large number of mylohyoid muscle spindle afferents were group Ia, because the mylohyoid primary afferents did not project to the supratrigeminal nucleus[17]. The present study also indicated a large number of synaptic contacts made between group Ia afferents and α-motoneurons near the neuronal cell body.

References

1) Alvarado-Mallart MR, Batini C, Buisseret-Delmas C and Corvisier J (1975) Trigeminal representations of the masticator and extraocular proprioceptors as revealed by horseradish peroxi-

dase retrograde transport. Exp Brain Res **23** : 167–79.

2) Bae YC, Nakagawa S, Yasuda K, Yabuta NH, Yoshida A, Pil PK, Moritani M, Chen K, Nagase Y, Takemura M and Shigenaga Y (1996) Electron microscopic observation of jaw–muscle spindle and periodontal afferent terminals in the trigeminal motor and supratrigeminal nuclei in the cat. J Comp Neurol **374** : 421–35.
3) Carson KA and Mesulam MM (1982) Electron microscopic demonstration of neural connections using horseradish peroxidase : a comparison of the tetramethylbenzidine procedure with seven other histochemical methods. J. Histochem. Cytochem **30** : 425–35.
4) Furusawa K, Yamaoka M, Fujimoto K and Kumai T (1994) Role of proprioceptor in the mylohyoid muscle. Brain Res Bull **35** : 233–6.
5) Furusawa K, Yasuda K, Okuda D, Tanaka M and Yamaoka M (1996) Central distribution and peripheral functional properties of afferent and efferent components of the superior laryngeal nerve : morphological and electrophysiological studies in the rat. J Comp Neurol **375** : 147–56.
6) Gottlieb S, Taylor A and Bosley MA (1984) The distribution of afferent neurons in the mesencephalic nucleus of the fifth nerve in the cat. J Comp Neurol **228** : 273–83.
7) Jacquin MF, Rhoades RW, Enfiejian HL and Egger MD (1983) Organization and morphology of masticatory neurons in the rat : a retrograde HRP study. J Comp Neurol **218** : 239–56.
8) Luo PF, Wang BR and Li JS (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** : 286–99.
9) Mesulam MM and Brushart TM (1979) Transganglionic and anterograde transport of horseradish peroxidase across dorsal root ganglia : A tetramethylbenzidine method for tracing central sensory connections of muscles and peripheral nerve. Neuroscience **4** : 1107–17.
10) Mizuno N, Konishi A and Sato M (1975) Localization of masticatory motoneurons in the cat and rat by means of retrograde axonal transport of horseradish peroxidase. J Comp Neurol **164** : 105–16.
11) Shigenaga Y, Mitsuhiro Y, Yoshida A, Cao CQ and Tsuru H (1988) Morphology of single mesencephalic trigeminal neurons innervating masseter muscle of the cat. Brain Res **445** : 392–9.
12) Shigenaga Y, Yoshida A, Mitsuhiro Y, Doe K and Suemune S (1988) Morphology of single mesencephalic trigeminal neurons innervating periodontal ligament of the cat. Brain Res **448** : 331–8.
13) Shigenaga Y, Mitsuhiro Y, Shiraha Y and Tsuru H (1990) Two types of jaw–muscle spindle afferents in the cat as demonstrated by intra–axonal staining with HRP. Brain Res **514** : 219–37.
14) Stürmer C, Bielenberg K and Spatz WB (1981) Electron microscopical identification of 3, 3', 5, 5'–tetramethylbenzidine–reacted horseradish peroxidase after retrograde axoplasmic transport. Neurocsi Lett **23** : 1–5.
15) Yabuta NH, Yasuda K, Nagase Y, Yoshida A, Fukunishi Y and Shigenaga Y (1996) Light microscopic observations of the contacts made between two spindle afferent types and alpha–motoneurons in the cat. J Comp Neurol **374** : 436–50.
16) Yamaoka M, Furusawa K, Fujimoto K, Iguchi K and Kumai T (1992) Muscle spindles in the mylohyoid muscle of rats. Int J Oral Maxillofac Surg **21** : 309–12.
17) Yasuda K, Furusawa K, Tanaka M and Yamaoka M (1995) The distribution of afferent neurons in trigeminal mesencephalic nucleus and the central projection of afferent fibers of the mylohyoid nerve in the rat. Somatosens Mot Res **12** : 309–15.

和文抄録：顎舌骨筋運動ニューロンと一次求心線維のシナプス接合について

安田浩一，古澤清文，中山洋子，田中三貴子，奥田大造，田中瑞穂，山岡　稔（松本歯大・口外）

ラットの顎舌骨筋に分布する神経枝に horseradish peroxidase conjugated with wheat germ agglutinin（HRP-WGA）を注入し，HRP 標識神経細胞の局在および微細構造について検討した．光学顕微鏡下で HRP 標識神経細胞は三叉神経中脳路核の尾側部と三叉神経運動核腹側内側部（Vmo.vm）に認められた．さらに HRP 標識神経細胞を含む Vmo.vm を電子顕微鏡で観察したところ，HRP に標識された軸索終末と神経細胞とのシナプス接合が観察された．これらの結果より，三叉神経中脳路核に起始細胞を有する顎舌骨筋筋紡錘求心線維は顎舌骨筋運動ニューロンに単シナプス性に入力することが示唆された．