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Termination sites of afferent fibers from a single tooth pulp in the Japanese monkey (*Macaca fuscata*)

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INTRODUCTION

Recent anatomical studies have shown central termination of afferent fibers from the tooth pulp in the rat[1,2], cat[3-5], dog[6] and rhesus monkey[7]. These studies indicated that afferents from the tooth pulp project to the entire extents of the brain stem trigeminal sensory nuclei. Among these nuclei, the subnucleus caudalis of the spinal trigeminal nucleus (Vc) has been traditionally considered to play the role of a relay nucleus for trigeminal nociceptive information[8,9]. However, these anatomical data were obtained by a tracing technique that relied on injections into the pulp of a number of teeth.

On the other hand, the afferent fibers from the tooth pulp have also been considered to be involved in relaying nociceptive sensation[10,11]. A number of electrophysiologial studies revealed the central termination areas of these afferent fibers and indicated that they terminate in the subnucleus caudalis (Vc)[12-16], the subnucleus interporalis (Vi)[17], the subnucleus oralis (Vo) of the spinal trigeminal nucleus (Vsp)[18-20] and the principal sensory nucleus of the trigeminal nerve (Vp)[18,21,22]. Also, recent experimental studies in monkeys have demonstrated that the trigeminal tractotomy at the level of the lower medulla (obex) eliminates nociceptive information[23].

Therefore the present study was undertaken to clarify the central projection courses and sites of termination of afferent fibers arising from a single tooth pulp in the trigeminal sensory nuclear complex. To achieve this we used retrograde and anterograde tracing techniques in the Japanese monkey.

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MATERIALS AND METHODS

Sixteen Japanese monkeys of both sexes weighing 3.9-12.0 kg were used. They were anesthetized with an intramuscular injection of ketamine hydrochloride (4-5 mg/kg body weight), followed by an intraperitoneal injection of sodium pentobarbital (25-30 mg/kg body weight). Supplementary doses of sodium pentobarbital were given to maintain a stable level of anesthesia during the surgical operation.

In these monkeys, a small preparation was made into either the tooth of the upper first incisor (four cases), the upper second incisor (one case), the upper canine (two cases), upper first molar (two cases), the upper, second molar (two cases), the upper, third molar (one case), the lower first incisors (four cases), the lower canine (two cases), or the lower, second molar (five cases), with a dental drill. After bleeding had stopped, each pulp was injected with 2.0-6.0 μl of a sterile saline solution containing 30% horseradish peroxidase (HRP; Toyobo, Grade 1-C) and 5% wheat germ agglutinin conjugated HRP (WGA-HRP; Toyobo, salt free) using a glass micropipette with a tip diameter of 30-100 μm. The micropipette was then connected by a short length of polyethylene tube to a 10 μl Hamilton microsyringe.

After a survival period of 24-48 h, the animals were reanesthetized and perfused through the ascending aorta for 30 min with 9% formalin in 0.1 M phosphate buffer, (pH 7.2-7.3), followed by 10% sucrose in the same buffer. The brain stems were removed and saturated with 30% sucrose in the same buffer at 4°C. The brain stems were cut transversely into serial sections on a freezing microtome set at 60 μm, and then processed for histochemical demonstration of HRP[24].

After the reaction, the alternating sections were mounted on glass slides coated with chrome alum gela-

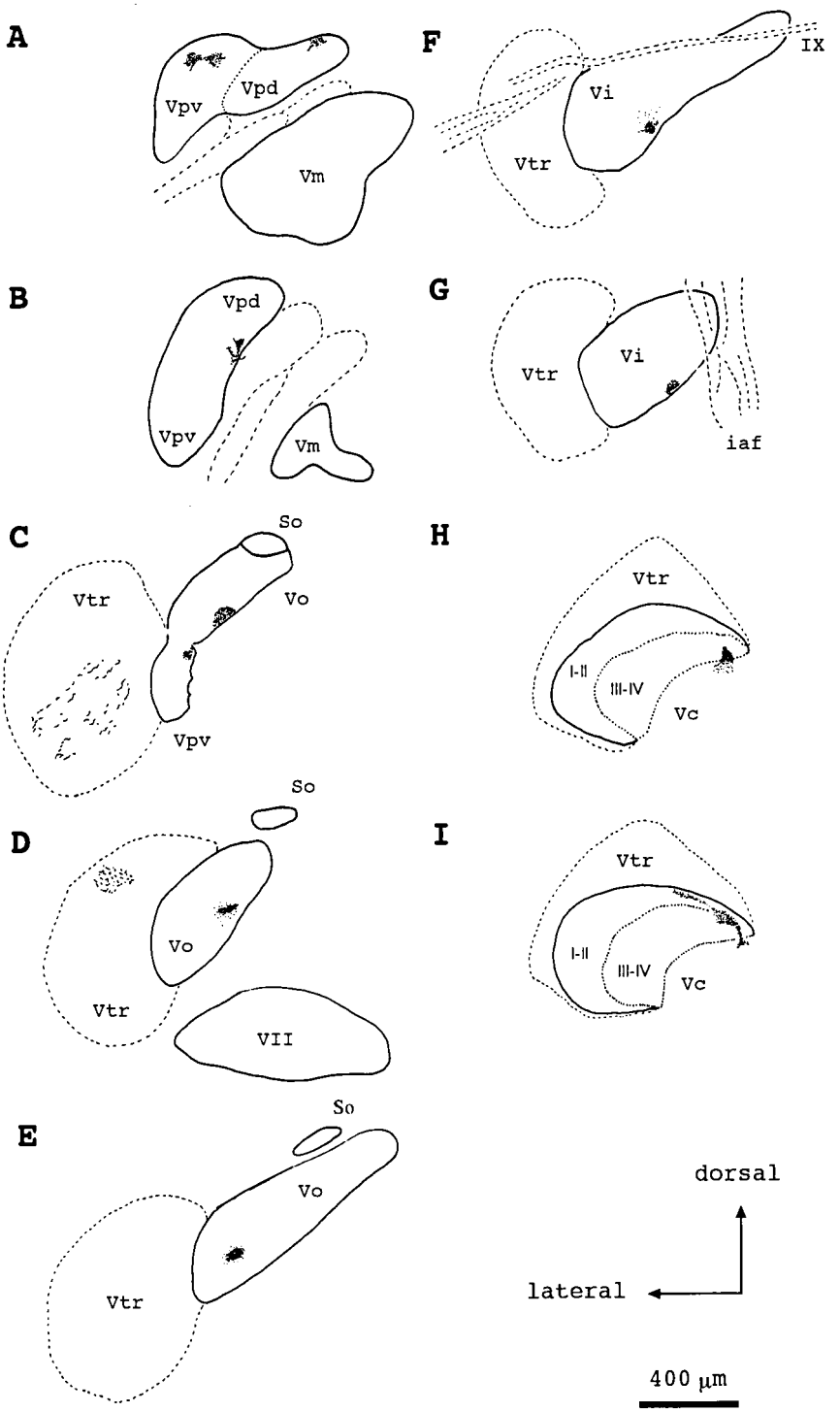

Fig. 1 Camera lucida drawings showing HRP-labeled axons and axon terminal after injection of the single tooth pulp of a lower, first incisor. iaf; the internal arcuate fibers, So; the nucleus of the solitary tract, Vc; the subnucleus caudalis of the spinal trigeminal nucleus (Vsp), Vi; the subnucleus interpolaris of Vsp, Vm; the trigeminal motor nucleus, Vo; the subnucleus oralis of Vsp, Vpd; dorsal division of the principal sensory nucleus of the trigeminal nucleus (Vp), Vpv; ventral division of Vp, Vtr; the spinal trigeminal tract. Scale bar = 400 μm.

tin[25]. One group of sections was counterstained with 1% neutral red, and observed under a microscope with light- and dark-field illumination. Another group of sections was not counterstained in order to allow observation of the distribution of labeled axons and their terminal under a camera lucida apparatus.

RESULTS

The same nomenclature of previous studies of the monkey and cat trigeminal nuclei was adopted in this study[26]. No labeled fibers and cells in the mesencephalic trigeminal nucleus (Vmes) were seen in most of the animals, and this absence of labeling was interpreted as evidence that there was no leakage of the HRP solution as a tracer into the periodontal ligament. The data from five animals that showed labeling of the cells in the Vmes was omitted from the study. In each case, labeled fibers and their terminal were observed in the sensory trigeminal nuclei, which included the most rostral part of the subnucleus caudalis (Vc) of the spinal trigeminal nucleus (Vsp), the subnucleus interporalis (Vi) and the subnucleus oralis (Vo) of Vsp, the dorsal division (Vpd) and the ventral division (Vpv) of the principal sensory nucleus of the trigeminal nerve (Vp).

Labeling in Vc was restricted to the most rostral part of this nucleus, corresponding to a region where the confine of the nucleus and its lamination were not so apparent (Figs. 1H, 1I). The labeling was largely confined to lamina I-II and III-IV of the dorsal part of Vc. In lamina V, ~~the~~ terminal labeling was occasionally encountered (Figs. 1H, 1I).

Labeling in Vi was weak compared to that observed in Vp and Vo, and was rostrocaudally discontinuous (Fig. 1F, 1G). The terminal labeling from the upper and lower teeth was located in the medial borders of Vi and the neighboring reticular formation, with the fields from the upper teeth lying dorsal to those from the lower teeth.

Terminal fields in Vo were localized in the ventromedial border of Vo (Fig. 1C). Projections from the upper and lower tooth pulp were arranged in a dorsoventral direction in Vo (Figs. 1C–1E, 3). In no case was labeling of the caudal part of Vo observed in this study.

In the rostral part of Vp, the labeled terminal field of the upper, lower, incisal and molar pulp afferent fibers

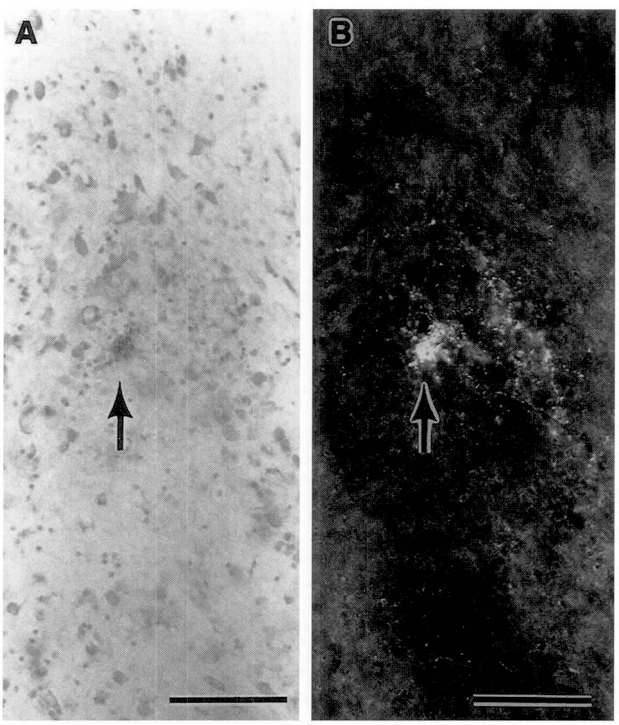

Fig. 2 HRP-labeled axon terminal in the principal sensory nucleus of the trigeminal nerve with bright-field (A) and dark-field (B) illumination. Scale bar = 100 μm.

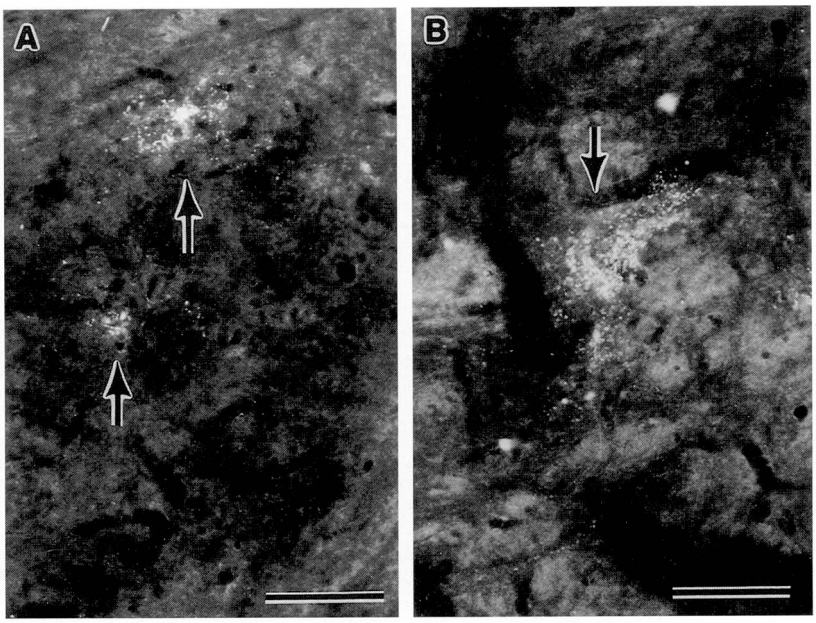

Fig. 3 HRP-labeled axon terminal in a oral part (A) and a caudal part (B) of the subnucleus oralis of the spinal trigeminal nucleus. Scale bar = 100 μm.

were seen in the restricted areas of Vpd and Vpv. They were observed preferentially in the marginal zone (Figs. 1A–B, 2). No apparent differences in the termination patterns between the four groups of teeth were observed at this level. In more caudal sections, the terminal field moved to a ventral portion in Vpv. Within Vpv the terminal fields often aggregated to form a smaller terminal field. In Vpv, the terminal fields from the lower tooth pulp were seen in a dorsal part of Vpv that that lay dorsal to the terminal fields from the upper teeth. Within the terminal fields from the lower teeth, projections from the incisors and molars were organized in a lateral to medial sequence. In the caudal part of Vpv that extended ventrally beneath the rostral part of Vo, there was no labeling. In the middle part of Vpv labeling shifted to a slightly dorsal position that was located rostrocaudally. This labeling merged with that of the Vo in the more caudal sections (Fig. 1C).

DISCUSSION

Central projections of pulpal afferents have been studied in the rat[1, 2], cat[3–5, 27], dog[6], and rhesus monkey[7] to elucidate the primary relay nuclei of trigeminal nociceptive afferent input. Tooth pulp afferents have been considered to function as a source of pure nociceptive information, since excitation of these afferent fibers causes a sensation of pain when the intensity of the stimulus is sufficiently high[10, 11].

In the present study, terminal labeling from a single tooth pulp in Vpv and Vpd was confirmed in the Japanese monkey. In the rat tooth, pulp afferents from the mandibular teeth projected only to the dorsal area of the Vp. In another study, afferent fibers from the upper molars were found to terminate not only within the dorsomedial part, but also within the lateral part of Vp in the rat[1]. The reason for these discrepancies is presently unclear but may reflect peculiarities of the species employed in the studies. In Vpd and Vpv, no somatotopic organization of the fibers was found, although labeling was preferentially localized in the marginal regions of Vpd and Vpv. In this nucleus , it has been reported that there is overlap between the termination sites of different species[2, 4, 6].

It was found that the Vo of the Japanese monkey received the heaviest number of projections from the tooth pulp. This finding is consistent with similar results obtained with the rat, cat and dog[2, 4, 6, 7]. Pulpal projections were encountered in the ventromedial areas of the Vo.

The Vi in the Japanese monkey received fewer projections from the tooth pulp than did Vp and Vo. This pat-

tern is reminiscent of that seen in other animals[1,4,6]. The pattern is much more apparent in rhesus monkey[7] and the Japanese monkey. The topographic arrangement of the upper and lower teeth in the monkey Vi is different from that seen in the rat, cat, and dog, whereas the topographies of the incisor and molars are similar to those of the rat, cat, and dog[1,4,6]. Again, these differences may reflect species differences. Terminal fields which receive pulpal projections in the monkey may correspond to the dorsal cap and intermediate regions identified in the rat[28].

Very small projections of the tooth pulp afferents *were* identified in the Vc and this projection was found to be limited to the rostralmost levels of the Vc. These features are inconsistent with the results obtained from other animal species[1,4,6]. The terminal fields of the monkey's pulpal afferents in the Vc were located in the dorsomedial part of laminae I–II and III–IV.

The functional significance of the heavy number of projections from tooth pulps to the Vo shown here and in other studies is not clear[1–4,6,7]. The Vo has major connections with the neighboring reticular formation and with the trigeminal motor and hypoglossal nuclei[29–30]. Anatomical and physiological studies indicated that Vo cells contribute little to the trigeminothalamic pathways[31–33]. These data suggested that afferent inputs of the tooth pulp to the Vo may relay stimuli to cells that give rise to ascending projections of motivation-affective consequences[34] and serving reflex motor and autonomic functions[10,20,35], rather than neurons serving in sensory discrimination[19]. It is clear that impulses that are relayed to the Vp and Vi reach the somatosensory cortex via the ventroposterior medial nucleus of the thalamus[11,14,16,19,21,31,32,36]. Thus, Vp and Vi cells receiving tooth pulp afferents may be implicated in the discriminative function of dental nociceptions.

CONCLUSION

Retrograde and anterograde transport of wheatgerm agglutinin conjugated horseradish peroxidase (WGA-HRP) injected into a single tooth pulp was used to study the patterns, distributions and topographic arrangement of primary afferent fibers and termini in the Japanese monkey (*Macaca fuscata*). HRP-labeled afferent fibers innervating the lower and upper teeth were found to project ipsilaterally to lamina I–II and/or III–IV of the most rostral levels of the Vc, Vi and Vo, and to restricted areas of Vp, Vpd and Vpv. The labeled fibers and terminal of the tooth pulp afferents formed a rostrocaudal column from the midlevel of Vp to the rostral level of Vc. The labeling in Vp and Vo was dense, but the column of terminal was interrupted frequently. The upper and lower teeth were represented in Vp, Vo, Vi and Vc as a dorsoventral and lateromedial sequence. Topographic distribution was also a little recognized as projections of afferent fibers of tooth pulp. The present results indicate a few projection from pulpal afferents Vc in the Japanese monkey. It appears that the topographic arrangement of projections from the tooth pulp, the termination sites and the density of the termini in the trigeminal sensory nuclei differ significantly depending on the animal species.

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REFERENCES

1) Marfurt, C. F. and Turner, D. F.: The dental projection of tooth pulp afferent neurons in the rat as determined by the transganglionic transport of horseradish peroxidase, J. Comp. Neurol., **223**, 535–547, 1984
2) Takemura, M., Sugimoto, T. and Shigenaga, Y.: Difference in central projection of primary afferents innervating facial and intraoral structures in the rat. Exp. Neurol., **111**, 324–331, 1991
3) Arvidsson, J., and Gobel, S.: An HRP study of the central projections of primary trigeminal neurons which innervate tooth pulps in the cat. Brain Res., **210**, 1–16, 1981
4) Shigenaga, Y., Suemune, S., Nishimura, M., Nishimori, T., Sato, H., Ishidori, H., Yoshida, A., Tsuru, K., Tsuiki, Y., Dateoka, Y., Nasution, I. D. and Hosoi, M., Topographic representation of lower and upper teeth within the trigeminal sensory nuclei of adult cat as demonstrated by the transganglionic transport of horseradish peroxidase, J. Comp. Neurol., **251**, 299–316, 1986
5) Shigenaga, Y., Nishimura, M., Suemune, S., Nishimori, T., Doe, K. and Tsuru, H.: Somatotopic organization of tooth pulp primary afferent neurons in the cat, Brain Res., **477**, 66–89, 1989
6) Ishidori, H., Nishimori, T., Shigenaga, Y., Suemune, S. and Dateoka, Y., Sera, M. and Nagasaka, N.: Representation of upper and lower primary teeth in the trigeminal sensory nuclear complex in the

young dog, Brain Res., **370**, 153–158
7) Takemura, M., Nagase, Y., Yoshida, A., Yasuda, K., Kitamura, S., Shigenaga, Y. and Matano, S.: The central projections of the monkey tooth pulp afferent neurons, Somatosens. Mot. Res., **10**, 217–227, 1993
8) Gerard, M. W.: Afferent impulses of the trigeminal nerve. Arch. Neurol. Psychiat. **9**, 306–338, 1923
9) Kunc, A.: Significant factors pertaining to the results of trigeminal tractotomy. In Trigeminal Neuralgia, R. Hassler and A. E. Walker eds., pp. 90–98, W. B. Saunders, Philadelphia, 1970
10) Dubner, R., Sessle, B. J. and Storey, A. T.: The basis of oral and facial function, Plenum Press, New York, 1987
11) Sessle, B. J.: Is the tooth pulp a "pure" source of noxious input? In Advances in Pain Research and Therapy, Vol. 3., J. J. Bonica, J. S. Liebeskind and D. York, 1979
12) Nord, S. G. and Young, R. F.: Projection of tooth pulp afferents to the cat trigeminal nucleus caudalis, Brain Res., **90**, 195–204, 1975
13) Yokota, T.: Excitation of units in marginal rim of trigeminal subnucleus caudalis elicited by tooth pulp stimulation, Brain Res., **95**, 154–158, 1975
14) Yokota, T.: Two types of tooth pulp units in the bulbar reticular formation, Brain Res., **104**, 325–329, 1976
15) Dostrovsky, J.O.: An electrophysiological study of canine, premolar and molar tooth pulp afferents and their convergence on medullary trigeminal neurons, Pain, **19**, 1–12, 1984
16) Amano, N., Hu, J. W., Sessle, B. J.: Responses of neurons in feline trigeminal subnucleus caudalis (medullary dorsal horn) to cutaneous, intraoral, and muscle afferent stimuli, J. Neurophysiol., **55**, 227–243, 1986
17) Hayashi, H., Sumino, R. and Sessle, B. J.: Functional organization of trigeminal subnucleus interpolaris: Nociceptive and innocuous afferent inputs, projections to thalamus, cerebellum and spinal cord and descending modulation from periaqueductal gray, J. Neurophysiol., **51**, 890–905
18) Greenwood, M. W.: Afferent impulses of the trigeminal nerve, Arch. Oral Biol. **18**, 771–785, 1973
19) Tamarova, Z. A., Shaporalov, A. I. and Vyklicky, L.: Projection of tooth pulp afferents in the brain stem of rhesus monkey, Brain Res., **64**, 442–445, 1973
20) Hu, J. W. and Sessle, B. J.: Comparison of response of cutaneous nociception and nonnociceptive brain stem neurons in trigeminal subnucleus caudalis (medullary dorsal horn) and subnucleus oralis to natural and electrical stimulation of tooth pulp. J. Neurophysiol., **52**, 39–53, 1984
21) Sessle, B. J. and Greenwood, L. F.: Inputs to trigeminal brain stem neurons from facial, oral, tooth pulp and pharyngolaryngeal tissues: I. Responses to innocuous and noxious stimuli. Brain Res., **117**, 211–226, 1976
22) Azerad, J., Woda, A., Albe-Fessarad, D: Physiological properties of neurons in different parts of the cat trigeminal sensory complex. Brain Res., **246**, 7–21, 1982
23) Young, R. F.: Effect of trigeminal tractotomy on dental sensation in humans. J. Neurosurg., **56**, 812–818, 1982
24) Mesulam, M.-M. and Brushart, T. M.: Transganglionic and anterograde transport of horseradish peroxidase across dorsal root ganglia: A tetramethylbenzidine method for tracing central sensory connections of muscles and peripheral nerves, Neurosci., **4**, 1107–1117, 1979
25) Pappas, P. W.: The use of a chrome alum-gelatin (subbing) solution as a general adhesive for paraffin sections, Stain Technol., 46, 121-124, 1971 trigeminal motor nucleus, J. Comp. Neurol., **215**, 290–300, 1983
26) Takahashi, O.: Distribution of trigeminospinal neurons. A retrograde HRP study in the macaque monkey and the cat. J. Hiroshima Univ. Dent. Soc., **19**, 14–32, 1987
27) Westrum, L. E., Canfield, R. C. and O'Connor, T. A.: Each canine tooth projects to all brain stem trigeminal nuclei in cat, Exp. Neurol., **74**, 787–799, 1981
28) Phelan, K. and Falls, W.: The interstitial system of the spinal trigeminal tract in the rat: Anatomical evidence for morphology and functional heterogeneity, Somatosens.
29) Mizuno, N., Yasui, Y., Nomura, S., Itoh, A., Konishi, M., Takada, M. and Kudo, M.: A light and electron microscopic study of premotor neurons for the Mot. Res., 6, 333-366, 198927) Travers, J. B. and Norgren, R.: Afferent projections to the oral motor nuclei in the rat. J. Comp. Neurol., **220**, 280–298, 1983
30) Takada, M., Itoh, K., Yasui, Y., Mitani, A., Nomura, S. and Mizuno, N.: Distribution of premotor neurons for the hypoglossal nucleus in the cat. Neurosci. Lett., **52**, 141–146, 1984
31) Shigenaga, Y., Yoshida, A., Mitsuhiro, Y., Tsuru, K. and Doe, K.: Morphological and functional properties of trigeminal nucleus oralis neurons projecting to the trigeminal motor nucleus of the cat. Brain Res., **461**, 143–149, 1988
32) Burton, H. and Craig, A. D. Jr.: Distribution of trigeminothalamic projection cells in cat and monkey, Brain Res., **161**, 515–521, 1979

33) Fukushima, T. and Kerr, F. W. L.: Organization of trigeminothalamic tracts and other thalamic afferent systems of the brainstem in the rat: Presence of gelatinosa neurons with thalamic connections, J. Comp. Neurol., **183**, 169–184, 1979

34) Melzack, R. and Casey, K. L.: Sensory, motivational and central control determinants of pain. In The Skin Senses, D. R. Kenshalo, K.D., pp. 423–443, Charles C. Thomas, Springfield, IL, 1968

35) Matthews, B., Baxter, J. and Watts, S.: Sensory and reflex responses to tooth pulp stimulation I man, Brain Res., **113**, 83–94, 1976

36) Shigenaga, Y., Nakatani, Z., Nishimori, T., Suemune, S., Kuroda, R. and Matano, S.: The cells of origin of cat trigeminothalamic projections, alive especially in the caudal medulla, Brain Res., **277**, 201–222, 1983