Experimental Studies of Afferent Fibers in the Hypoglossal Nerve in the Cat: A Scanning Electron Microscopic Observation on the Lingual Mucosa Following Transection of the Nerve, and a Degeneration Study with Silver Impregnation Methods

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Summary. Following transection of the nerves innervating the tongue, changes in the lingual mucosa of the cat were observed using a scanning electron microscope. Transection of the lingual nerve induced ipsilaterally marked changes in the lingual mucosa; ulcerous changes occurred within 48 hrs after transection of the nerve. In contrast, even 30 days after transection of the hypoglossal nerve, no changes in the lingual mucosa were noted despite marked atrophy of the tongue musculature ipsilateral to the lesion. Therefore, the hypoglossal afferents do not seem to be of primary importance for nociception and coordinate movements of the tongue.

An attempt was also made to locate the sensory ganglion of the hypoglossal afferents and the distribution of these fibers in the brain stem by means of the silver impregnation methods. Following intracranial rhizotomy of the hypoglossal nerve, no convincing findings could be obtained indicating termination in the brain stem of fibers running through the hypoglossal roots. Since the afferent fibers in the hypoglossal nerve could reach the brain stem via the roots of the vagus nerve after coursing through the anastomoses between the hypoglossal and vagus nerves, the following experiments were performed. Following intracranial transection of the roots of the vagus nerve, two groups of degenerated fibers were found in the brain stem; fibers in the solitary tract and those in the trigeminal system. The former were distributed to the entire extent of the ipsilateral solitary nucleus, to the commissural nucleus, and to the contralateral solitary nucleus at the level of the commissura infima; the latter were found to terminate in the interpolar subnucleus of the spinal trigeminal nucleus and/or the juxtagingival reticular formation. Following nodosectomy only the components in the solitary tract were degenerated. On the basis of the present and previous findings, it was inferred that at least the majority of the afferent fibers in the hypoglossal nerve reach the brain stem through the vagus roots, and that these fibers, belonging probably to the somatic system, have their ganglion cells in the jugular ganglion and terminate in the spinal trigeminal nucleus and/or the juxtagingival reticular formation.

Afferent fibers in the hypoglossal nerve have been the subject of many studies, particularly in connection with proprioceptive innervation of the tongue (cf. Blom, 1960; Hosokawa, 1961; Adatia and Gehringer, 1971). The existence of afferent fibers in the hypoglossal nerve of the cat appears to be currently established (Green and Negishi, 1963; Sauerland and Mizuno, 1968; Lindquist and Martensson, 1969; Hanson and Widén, 1970; Nakamura et al., 1970; Zapata and Torrealba, 1971), but the nature of these fibers and the routes that they take into the central nervous system are still controversial.
The present study was carried out in an attempt to compare the possible role which the afferent fibers in the hypoglossal nerve may play in the maintenance of the lingual mucosa with that performed by the lingual nerve, and further, the location of the sensory ganglion cells of the afferent fibers in the hypoglossal nerve and the terminal areas of these fibers in the brain stem were also investigated.

Material and Methods

Scanning electron microscopy

Eleven young adult cats were used. Animals were anesthetized with sodium pentobarbital (40mg/kg i.p.). In 4 of them branches of the hypoglossal nerve on one side were dissected and severed at the point of their entrance into the extrinsic and intrinsic tongue musculature. In 5 cats the lingual nerve on one side was transected at the medial side of the body of the mandible. In the other 2 cats, the superior cervical ganglion on one side was removed. All animals were treated postoperatively with antibiotics (mycillin).

After survival periods of 24 hrs to 45 days the cats were anesthetized with an overdose of pentobarbital, and then perfused through the ascending aorta with 500ml of 2.5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.2), containing 7.5% sucrose. The tongue was then carefully removed and placed in a large volume of normal saline. Tissue blocks about 5×5mm large and 2mm thick were prepared from the apex, lateral border and body of the tongue, and washed by a gentle stream of normal saline. Tissue blocks taken from the side where the nerve was intact were utilized in each animal as controls. Subsequently the tissue blocks were fixed for 24 to 48 hrs in the same fluid as used for perfusion, and then postfixed for 1.5 to 2 hrs in a 1% osmium tetroxide solution in the phosphate buffer (0.1 M, pH 7.2). After being washed with a fresh solution of the same buffer, the specimens were dehydrated in graded series of acetone, and then allowed to air dry.

The specimens were subjected to double coating with carbon and gold, and studied in a JSM-U3 scanning electron microscope (Japan Electron Optics Laboratory Co., Ltd.), using a beam accelerating voltage of 5, 10, 15 or 25 kV.

The remaining parts of the tongue, especially those adjacent to the areas from where the tissue blocks were taken for scanning electron microscopy, were further fixed in 10% formalin. Subsequently paraffin-sections were prepared and stained with hematoxylin-eosin or azan for light microscopy.

Degeneration study

Six young adult cats were used. All surgical procedures were performed under deep general anesthesia produced by sodium pentobarbital (40mg/kg i.p.). In 2 cats intracranial transection of the rootlets of the hypoglossal nerve was carried out, using a parapharyngeal approach. The longus colli and capitis muscles were divided and the origins of these muscles were curetted away; the longitudinal ligament was stripped from the rim of the foramen magnum. Sufficient bone was removed from the midline of the basis cranii to expose the dura overlying the olivary eminence. After coagulating visible veins, the dura was incised and the hypoglossal rootlets were exposed. The hypoglossal rootlets on one side were sectioned using a dissecting microscope. In 2 cats rhizotomy of the vagus nerve was performed intradurally.
through a small occipital craniotomy. The exposed cerebellum was lifted gently and
held up by the introduction of small cotton pledgets to allow a view of the rootlets.
The rootlets on one side were cut, using a small, hooked knife, under a dissecting
microscope. In the other 2 cats the nodose ganglion on one side was exposed and
removed along with a section of nerve peripheral to the ganglion. All these animals
received antibiotics (mycillin) postoperatively.

After survival periods of 5 to 7 days they were sacrificed by means of intravital
perfusion technique. After appropriate fixation periods, transverse sections of 30 μ
thickness were prepared according to the NAUTA-GYGAX (1954), NAUTA (1957) or FINK-
HEIMER (1967) procedure. Details pertaining to perfusion, fixation, serial sectioning
and counterstaining were in all respects similar to those described elsewhere (MIZUNO,
1966, 1670; MIZUNO et al., 1967, 1973a, b).

Findings

Scanning electron microscopy

The dorsal surface of the pars libera of the tongue was entirely covered by
numerous filiform papillae and dome-shaped fungiform papillae which were scattered
singly among the former (Fig. 1). The filiform papillae, which curved generally
backward above the surface of the tongue, were variable in size and shape. The fili-
form papillae on the tongue apex were small and looked soft in character. The fili-
form papillae distributed in the lateral portions of the lingual body were medium-sized
and sharply pointed (Fig. 1). The large filiform papillae were located in the regions
near the midline of the lingual body. The largest ones were in the intermolar region.

![Fig. 1. The dorsal surface of a lateral portion of the lingual body. Four fungiform papillae are seen among medium-sized filiform papillae with sharply-pointed tips. ×70](image)
The large filiform papillae just posterior to the tongue apex were frequently accompanied by a spinous patch. The tips of the papillae on these patches were not sharply...

Fig. 2. Large filiform papillae with club-shaped tips are seen on the spinous patches. $\times 70$

Fig. 3. The under surface of the pars libera of the tongue. $\times 1,150$
pointed (Fig. 2). On the surface of the filiform papillae a tortoise shell-like or scale-like arrangement of keratinized epithelial cells was seen (Fig. 2). The fungiform papillae were encountered more frequently in the lingual apex than in the tongue body. The fungiform papillae in the apex were generally smaller than those in the body. The lingual areas close to the midline were devoid of the fungiform papillae. The mucous membrane on the under surface of the tongue was rather smooth except at the tip, where a few fungiform and filiform papillae existed. A squamous arrangement of the keratinized epithelial cells was also seen on the under surface of the tongue (Fig. 3).

Fig. 4. Increased exfoliation on the under surface of the tongue apex, 36 hrs after transection of the lingual nerve. Erosive region is seen at the right upper portion of the figure; the area at the left lower portion of the figure is almost intact. ×345

Transection of the lingual nerve induced rapidly marked changes of the lingual mucosa on the side ipsilateral to the lesion. Following the first feeding after recovery from general anesthesia, slight changes of the filiform papillae were already noted in the tongue apex and/or the lateral border of the lingual body. In these areas tips of the cuticular spines of many filiform papillae were broken and the tendency of exfoliation was increased. Increased tendency of exfoliation was also observed on the under surface of the tongue (Fig. 4). In the regions where the cuticular spines of the filiform papillae had dropped off, the remaining spinous patches showed exaggerated exfoliation (Fig. 5). The extent and grade of these changes rapidly developed, and ulcerous changes firstly appeared in the apex and the lateral border of the pars libera of the tongue within 48 hrs. After complete removal of the epithelium the differences between the dermal cores of the filiform and fungiform papillae could not be discerned (Fig. 6). These changes in the lingual mucosa did not cross over to
the contralateral side of the tongue at least within the first 7 days.

**Fig. 5.** Increased exfoliation on the surface of the spinous patches, 48 hrs after transection of the lingual nerve. The cuticular spines have dropped off. ×345

**Fig. 6.** Ulcerous region on the dorsal surface of the lingual body, 60 hrs after transection of the lingual nerve. ×1,150
Following transection of the hypoglossal nerve, both the extrinsic and intrinsic tongue musculature showed a high degree of atrophy within 4 weeks on the side ipsilateral to the lesion. Notwithstanding these marked changes in the musculature, no special changes in the mucosa were found either light microscopically and scanning electron microscopically (Fig. 7).

Fig. 7. Photomicrograph of a transverse section of the tongue. In spite of a high degree of atrophy on the left side, the lingual mucosa remains intact 30 days after transection of the left hypoglossal nerve trunk. Azan stain. ×70

No changes in the lingual mucosa were noted within 45 days after removal of the superior cervical ganglion.

Degeneration study

In the cats with unilateral hypoglossal rhizotomy, occasional argyrophilic particles were seen bilaterally in the corticospinal tract, predorsal fascicle, inferior olivary nucleus and ventral reticular nucleus. Since the operative procedures were rather complicated in these animals, it appeared hazardous to consider these particles

Fig. 8. Degenerated nerve fascicles of the vagus nerve, skirting the dorsal margin of the spinal trigeminal tract (T) and nucleus (N). Nauta impregnation. ×160
indicative of the degeneration of hypoglossal afferents coursing through the transected hypoglossal rootlets.

Following intracranial sectioning of the rootlets of the vagus nerve, degenerated fiber fascicles were seen to pierce the spinocerebellar tracts, to run in a dorsomedial direction skirting the dorsal border of the spinal trigeminal tract and nucleus (Fig. 8) and to join the solitary tract (Fig. 9). Arriving at the solitary tract, the fibers appeared to bifurcate into an ascending and a descending component and to terminate in the entire solitary nucleus (Fig. 9). The smaller ascending component reached the level of the cranial pole of the inferior olive, and the larger component descended into the rostral levels of the first cervical segment of the spinal cord, where the solitary

![Figure 9](image9.png)

**Fig. 9.** Degenerated nerve fascicles in the solitary tract (S) and preterminal fibers in the solitary nucleus (arrows). Nauta impregnation. ×80

![Figure 10](image10.png)

**Fig. 10.** Degenerated nerve fascicles, separating from the solitary tract (S) and coursing toward the contralateral side via the commissura infima of Haller (I). C central canal. Nauta-Gygax impregnation. ×80
tract disappeared at the base of the nucleus cuneatus. At the level of the commissura infima of Haller a considerable number of degenerated fibers crossed the commissure (Fig. 10) and terminated in the contralateral solitary nucleus at the level of the commissure. On the way to the contralateral solitary nucleus many fibers ran through the commissural nucleus of Cajal ipsilaterally and contralaterally. It is highly probable that some of these fibers terminated in the commissural nucleus, although discrimination between fibers of passage and terminal fibers was rather difficult in this area. In addition to the components in the solitary tract a small number of degenerated fibers separated from the main fascicle on the way to the solitary tract and entered into the dorsal portions of the spinal trigeminal tract and nucleus at the level of the interpolar subnucleus of the spinal trigeminal nucleus. Although a few fine degenerated fibers were occasionally observed around the cells located within or around the dorsal portions of the interpolar subnucleus (Fig. 11), the components in the spinal trigeminal tract could not be traced further because of their paucity.

Following nodosectomy the resulting degeneration was mostly confined to the solitary tract and nucleus, although a few degenerated fibers were rarely observed within the spinal trigeminal tract. Thus, the unequivocal termination was found ipsilaterally in the almost entire extent of the solitary nucleus, bilaterally in the commissural nucleus, and contralaterally in the solitary nucleus at the level of the commissura infima.

**Discussion**

Stimulation of the cut central end of the hypoglossal nerve has been reported to induce several effects; a rise in blood pressure (Tarkhan, 1936, cat, dog; Downman, 1939, cat; Tarkhan and Abou-el-Naga, 1947, dog; Hanson and Widen, 1970, cat), reflex responses in the nictitating membrane (Acheson et al., 1936, cat), dilatation of
the pupil (Downman, 1939, cat; Weddell et al., 1940, rat; Hanson and Widén, 1970, cat), postsynaptic potentials in the hypoglossal motoneurons (Green and Negishi, 1963, cat; Porter, 1965, 1966, cat), a response in the recurrent laryngeal nerve and intrinsic laryngeal muscles (Sauerland and Mizuno, 1968, cat; Lindquist and Martinsson, 1969, cat), a response in thoracic and renal sympathetic nerves (Whitwam et al., 1969, dog), evoked potentials in the cerebral cortex and the thalamus (Bowman and Combs, 1969a, b, monkey), reflex responses in the facial muscles (Lindquist and Martensson, 1969, cat; Hanson and Widén, 1970, cat) and the styloglossus muscle (Hanson and Widén, 1970, cat), and effects on the masseteric monosynaptic reflex (Nakamura et al., 1970, cat).

Several attempts have also been made to record afferent impulses in the proximal portion of the hypoglossal nerve trunk during various types of stimulation to the tongue. Sensory discharges were recorded by Cooper (1954, cat), Blom (1960, cat), Bowman and Combs (1968, monkey), Hanson and Widén (1970, cat) and Zapata and Torrealba (1971, cat), but negative results were also reported by Barron (1936, cat, rat, rabbit), Corbin and Harrison (1938, cat), Downman (1939, cat) and Porter (1966, cat).

Regarding the course through which these afferent impulses proceed to the brain stem, several proposals have been made; along the entire course of the hypoglossal nerve (Downman, 1939; Green and Negishi, 1963; Nakamura et al., 1970), through sympathetic fibers (Weddell et al., 1940), by way of the roots of the vagus nerve through the anastomoses established between the hypoglossal and vagus nerves (Downman, 1939; Tarkhan and Abou-El-Naga, 1947; Sauerland and Mizuno, 1968; Hanson and Widén, 1970; Zapata and Torrealba, 1971), via the peripheral connections between the terminal branches of the hypoglossal and lingual nerves (Blom, 1960), via the ansa hypoglossi and dorsal roots of the upper cervical segments of the spinal cord (Bowman and Combs, 1969a), or by current spread to the lingual nerve (Porter, 1965, 1966). Although extensive peripheral connections between the hypoglossal and lingual nerves were described (Fitzgerald and Law, 1958, cat, rabbit, pig, dog, man; Blom, 1960, cat), current spread to the lingual nerve appears to be well controlled in the experiments mentioned above. According to Weddell et al. (1940), sympathetic fibers run in the hypoglossal nerve to reach the blood vessels of the tongue in the rat, but the existence of such fibers were denied in the dog by Tarkhan and Abou-El-Naga (1947). Although hypoglossal afferents ascending from the upper cervical roots have been described in the hedgehog (Van der Sprenkel, 1924), monkey (Corbin et al., 1937; Corbin and Harrison, 1939) and rabbit (Yee et al., 1939; Boyd, 1941), the cervical roots do not contribute to the hypoglossal nerve fibers in the cat (Hinsey and Corbin, 1934; Corbin and Harrison, 1938; Downman, 1939) and dog (Tarkhan and Abou-el-Naga, 1947). On the other hand, the presence of sensory ganglion cells along the hypoglossal roots or trunk has been reported in the cat (Langworthy, 1924; Tarkhan and Abd-el-Malek, 1950), but the existence of such cells does not seem to be constant and, even where they exist, the cells appear to be small in number (cf. Woźniak and Young, 1968). Dault and Smith (1969) have asserted that the mesencephalic trigeminal nucleus gives rise to axons running directly into the hypoglossal nerve, though Mizuno and Sauerland (1970) could not trace such fibers experimentally from the mesencephalic trigeminal nucleus to the hypoglossal
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In the present study no unequivocal findings indicating the presence of afferent fibers in the hypoglossal roots were obtained. According to previous findings (Green and Negishi, 1963; Nakamura et al., 1970), however, the possibility of the presence of afferent fibers in the hypoglossal roots cannot be excluded. The existence of anastomoses between the hypoglossal and vagus nerves in the vicinity of the nodose ganglion has been demonstrated in man (cf. Woźniak and Young, 1968), the cat (Langworthy, 1924; Sauerland and Mizuno, 1968; Hanson and Widén, 1970; Zapata and Torrealba, 1971) and the dog (Tarkhan and Abou-el-Naga, 1947), though these were disputed by Hinsey and Corbin (1934) in the cat and by Boyd (1941) in the rabbit.

Afferent fibers in the hypoglossal nerve have been shown to respond to mechanical stimulation produced by stretching the tongue (Cooper, 1954; Blom, 1960; Hanson and Widén, 1970; Zapata and Torrealba, 1971), but not to respond to tactile stimuli applied to the surface of the tongue (Porter, 1966; Zapata and Torrealba, 1971). Although Langworthy (1924) described muscle spindles in the intrinsic tongue muscles of the cat, there appears to be general agreement that muscle spindles do not occur in the intrinsic and extrinsic musculature of the tongue of the cat (Sherrington, 1894; Cooper, 1953; Law, 1954; Blom, 1960). According to Lindquist and Mårtensson (1969), hypoglossal afferents belong to group III fibers and are not involved in the proprioceptive control of the tongue muscle, since their receptors do not respond to tongue muscle twitches, and since no myotatic reflex can be elicited. In the present study the lingual mucosa was maintained intact as long as 30 days after transection of the hypoglossal nerve. Therefore, even if the hypoglossal nerve may contain fibers concerning proprioception and/or nociception as suggested by Hanson and Widén (1970), the hypoglossal afferents of the cat were probably of secondary importance for protective mechanisms and coordinate movements of the tongue (cf. Sauerland and Mizuno, 1970).

By means of microelectrode recording from the area of the spinal trigeminal nucleus, Hanson and Widén (1970) recorded a repetitive unitary response to single shock stimulation of the proximal cut end of the hypoglossal nerve with 10 to 15 msec latency to the first discharge. In the present study 2 groups of degenerated fibers were observed in the vagus afferents following the rhizotomy of the vagus nerve; a larger group in the solitary tract and a smaller one in the spinal trigeminal system. The trigeminal components in the vagus afferents have been described in the cat (Windle, 1933; Ingram and Dawkins, 1945; Kerr, 1962), monkey (Taren, 1964; Rhoton et al., 1966), rabbit (Kimmel 1941) and rodents (Cajal, 1909; Aström, 1953; Torvik, 1956). In agreement with the findings of Aström (1953), Kerr (1962) and Rhoton et al. (1966), a few fibers of the trigeminal components were seen to terminate in the interpolar subnucleus of the spinal trigeminal nucleus, but termination in the substantia gelatinosa of the spinal trigeminal nucleus and in the dorsal horn of the cervical cord could not be confirmed. Rhoton et al. (1966) have also described the termination of the vagus afferents in the reticular formation medial to the spinal trigeminal nucleus following rhizotomy of the vagus nerve of the monkey. In the present study a few degenerated fibers were seen around the small and medium-sized cells located in the transitional areas between the spinal trigeminal nucleus and the juxtageminal reticular formation. The precise terminal points of these fibers, however, could not be determined, because the spinal trigeminal nucleus belongs to
the “noyau ouvert,” and because the dendrites of the cells of the reticular formation
also penetrate into the gray of the spinal trigeminal nucleus (Mannen, 1960, 1966).
On the other hand, the trigeminal components of the vagus afferents were not
degenerated following nodosectomy. These findings appear to suggest, as the results
of previous investigations (Allen, 1923; Dubois, 1929; Kerr, 1962; Cottle, 1964;
Rhotan et al., 1966), that the cells of the nodose ganglion belong to the visceral
afferent system and the jugular to the somatic system of the vagus nerve. If these
assumptions are correct, and if the hypoglossal afferents entering into the brain
stem through the rootlets of the vagus nerve belong to the somatic system, it is most likely
that the ganglion cells of the afferent fibers in the hypoglossal nerve are located in
the jugular ganglion, and that the fibers of these cells terminate in the spinal tri-
geminal nucleus and/or the juxtaganglionic reticular formation, where the inter-
neurons of the brain stem reflex pathways are located (cf. Mizuno, 1970; Mizuno and
Sauerland, 1970; Mizuno et al., 1972a).

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