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Nerve fiber analysis for the lingual nerve of the human adult subjects

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Abstract The neuro-motor control of the human tongue musculature had not been investigated in detail. This study identified first that the lingual nerve should play the neuro-motor control of some lingual muscles. Six en bloc samples (12 sides), including the tissues from the skull base to the hyoid bone, and three whole tongues were obtained from adult human cadavers. The former samples were used for the study of nerve fiber analysis of the lingual nerve with the aid of binocular stereomicroscope, and the latter samples were used for histological study by serial section method. On nerve fiber analysis of the lingual nerve from the trigeminal ganglion to the tongue musculature, we found that the motor-root of the trigeminal nerve gave off its supply to the lingual nerve and traveled into the lingual nerve, and branched to the superior and the inferior longitudinal muscles. On histological study, it was revealed that in the anterior part of the tongue the superior and the inferior longitudinal muscles surrounded the other lingual musculature and combined with the sub-mucosal connective tissues closely like the cutaneous muscle, for example, the facial muscles. The lingual nerve entered the inner side of the space between the genioglossus and the inferior longitudinal muscles with the lingual artery.

These findings suggested that the superior and the inferior longitudinal muscles should be innervated by the motor fibers traveled into the lingual nerve from the motor root of the trigeminal nerve, and do not originate from the myotome originating in occipital somites but branchial muscles.

Keywords Lingual nerve · Trigeminal nerve · Superior longitudinal lingual muscle · Inferior longitudinal lingual muscle · Nerve fiber analysis

Introduction

The tongue performs important roles for biological activity and the human being, for example, swallowing, mastication, tasting, speaking, expression (the click sound /tut/, putting out the tongue, etc.), etc. However, it was believed that the neuro-motor control of all the intrinsic muscles of the tongue was derived from myotomes originating in occipital somite and differentiated in situ, and was supplied by the hypoglossal nerve alone [10]. Saigusa et al. [13] revealed that the posterior part of the transverse lingual muscle connected with the superior pharyngeal constrictor muscle, form a ring muscle at the base of the tongue in the human tongue. Those findings suggested that the posterior part of the transverse lingual muscle might not be differentiated in situ as an intrinsic muscle of the tongue and supplied not by the hypoglossal nerve, but the other nerve. Considering the unique and complex capability of the human tongue, we could not conclude that only the hypoglossal nerve might carry out the neuro-motor control of all the tongue muscles.

In this study, we investigated by nerve fiber analyses and histological study whether or not the motor fibers of the trigeminal nerve could give off its supply to the lingual nerve and branch to the tongue musculature, especially the superior and inferior longitudinal lingual muscles. Based on our findings, the neuro-motor control and the origin of those two intrinsic muscles of the tongue are assessed.

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Material and method

All research was approved and conducted according to the institutional ethical committee guidelines at the Nippon Medical School.

Specimens

Six en bloc samples, including tissues from the skull base to the hyoid bone, and three whole tongues were obtained from human adult cadavers that had been fixed by a 10% formaldehyde solution for more than 3 months. The former samples were resected en bloc from the cervical vertebra and cranial bone. The latter samples were removed by total glossectomy by the subperiosteal pull-through technique.

Nerve fiber analysis of the lingual nerve

Twelve sides of six en bloc samples, including tissues from the skull base to the hyoid bone, were used for the studies of nerve fiber analysis of the lingual nerve. On the trigeminal ganglion, the motor root of the trigeminal nerve could be identified precisely. Then, by removing the perineurium minutely with the aid of binocular stereomicroscope, we traced the fiber of the motor root of the trigeminal nerve and investigated whether the fiber supplied to the lingual nerve or not. And, we investigated how the nerve fiber derived from the motor root of the trigeminal nerve was run along the lingual nerve and whether the nerve fiber branched to the intrinsic muscle of the tongue, when the lingual nerve received nerve fiber from the motor root of the trigeminal nerve.

Study of the superior and the inferior longitudinal lingual muscles

Three whole tongues were cut in half sagittally and embedded in paraffin. Each sample was then sectioned frontally in series into 30 μ m thick slices from the posterior border of the tongue to the tongue tip. Each sectioned specimen was stained with hematoxylin and eosin for light microscopy.

Results

Relationship between the lingual nerve and the motor root of the trigeminal nerve

In all specimens, the motor root of the trigeminal nerve supplied its fibers into the lingual nerve and traveled with sensory fibers of the lingual nerve (Table 1), and entered to the muscle layer consisting

Table 1 Patterns of supplying motor fiber from motor root of the trigeminal nerve and relationships between the lingual nerve and the hypoglossal and the mylohyoid nerves

No	Pattern of motor fiber branching from motor root	Extra-lingual anastomosis with hypoglossal nerve	Peripheral anastomosis with mylohyoid nerve.
1	TypeI	+	+
2	TypeI	+	-
3	TypeI	+	+
4	TypeI	+	+
5	TypeI	+	+
6	TypeI	-	+
7	TypeI	+	-
8	TypeI	+	-
9	TypeII	+	+
10	TypeII	-	-
11	TypeII	+	-
12	TypeIII	+	-

of the superior and the inferior longitudinal lingual muscles as branches to these muscles (Figs. 1–3). Finally, these fibers reached the sub-mucosal layers of the tongues (Fig. 4). A side-to-side correlation between both sides of the lingual nerves was not seen in all specimens. In eight specimens (subjects 1–8), the motor fiber from the motor root of the trigeminal nerve gave off its supply to the lingual nerve near the region where the motor root branched to the medial and the lateral pterygoid nerves (Figs. 1, 5a). In three specimens (subjects 9–11), the motor fiber from the motor root of the trigeminal nerve gave off its supply to the lingual nerve before the region where the motor root branched to the lateral and medial pterygoid nerves (Figs. 2, 5b). In one specimen (subject 12), once the motor fiber from the motor root of the trigeminal nerve gave off its supply, it traveled with the inferior alveolar nerve until the inferior alveolar nerve entered into the mandibular canal. Then the motor fiber left the inferior alveolar nerve and joined into the lingual nerve (Fig. 3, 5c).

Extra-lingual connections between the lingual and the hypoglossal nerves

Extra-lingual connections between the lingual and the hypoglossal nerves were found in ten specimens (83%) (Figs. 1c, 2c, 3c, Table 1). In these specimens, the hypoglossal nerve gave off its supply to the lingual nerve beyond the region where the hypoglossal nerve branched to the hyoglossal muscle, and connected beyond the submandibular ganglion of the lingual nerve. The branch of the hypoglossal nerve connected to the fibers of the lingual nerve of the inferior longitudinal muscle. In another two specimens, clear extra-lingual connections between the lingual and the hypoglossus nerves were not found, but the hypoglossal nerve supplied its fibers to the inferior longitudinal muscles near the fibers derived from the lingual nerves.

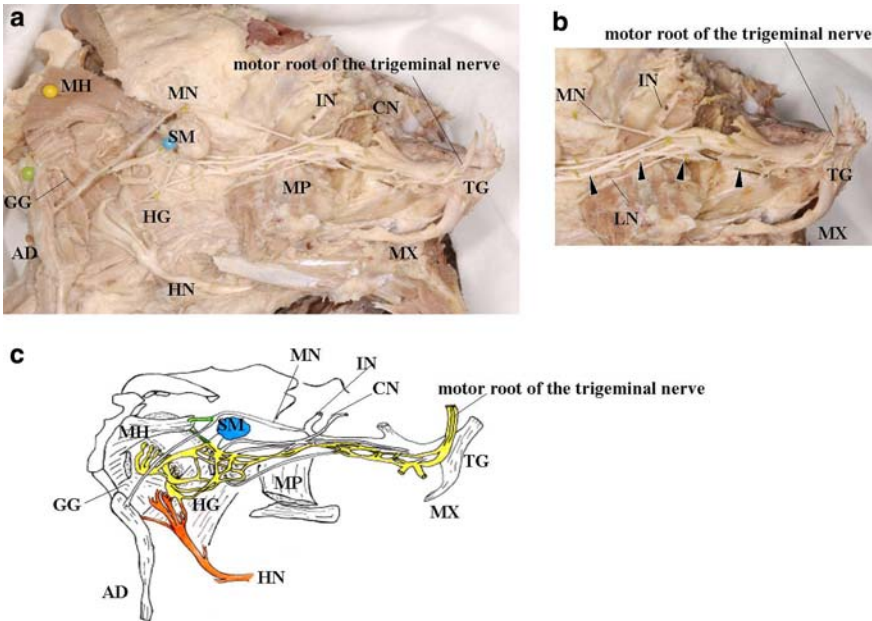


Fig. 1 Morphology of nerve fiber analysis of the lingual nerve in subject 1. Motor root of the trigeminal nerve gave off its supply to the lingual nerve near the region where motor root branched to the medial and the lateral pterygoid nerves. **a** total analysis of motor fiber that branched from the motor root of the trigeminal nerve into the lingual nerve. **b** closer view of the relationship between motor root of the trigeminal nerve and motor fiber of the lingual

nerve (*black arrows*) at the trigeminal ganglion. **c** schematic depiction of **a**. *AD*, anterior belly of digastric muscle; *CN*, chorda tympani; *GG*, genioglossus muscle; *HG*, hyoglossus muscle; *HN*, hypoglossal nerve; *IN*, inferior alveolar nerve; *LN*, lingual nerve; *MH*, mylohyoid muscle; *MN*, mylohyoid nerve; *MP*, medial pterygoid muscle; *MX*, maxillary nerve; *SM*, sub-mandibular gland; *TG*, trigeminal ganglion

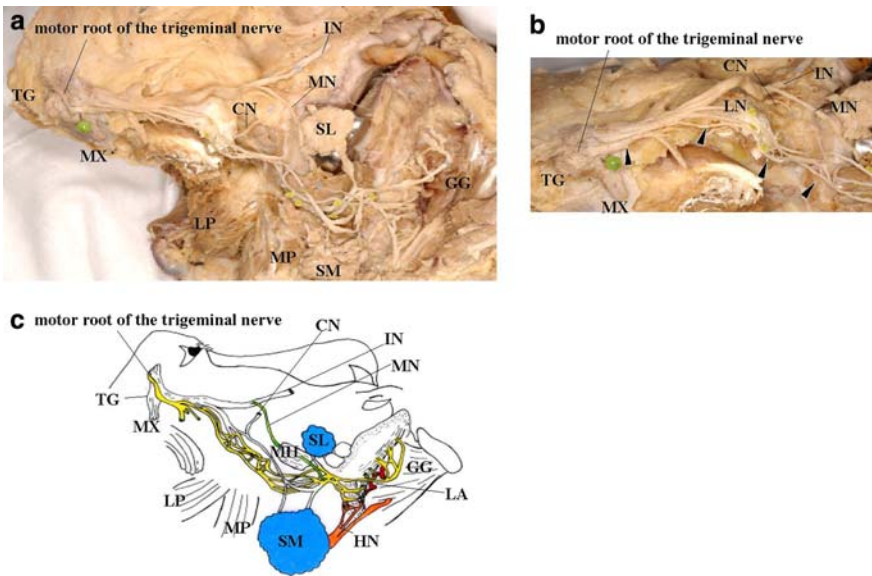


Fig. 2 Morphology of nerve fiber analysis of the lingual nerve in subject 9. Motor root of the trigeminal nerve gave off its supply to the lingual nerve near the region before where motor root branched to the medial and the lateral pterygoid nerves. **a** total analysis of motor fiber that branched from the motor root of the trigeminal nerve into the lingual nerve. **b** closer view of the relationship between the motor root of the trigeminal nerve and motor fiber of the lingual nerve (*black arrows*) at the trigeminal

ganglion. **c** schematic depiction of **a**. *AD*, anterior belly of digastric muscle; *CN*, chorda tympani; *GG*, genioglossus muscle; *HG*, hyoglossus muscle; *HN*, hypoglossal nerve; *LA*, lingual artery; *LN*, lingual nerve; *LP*, lateral pterygoid muscle; *MH*, mylohyoid muscle, *MN*, mylohyoid nerve; *MP*, medial pterygoid muscle; *MX*, maxillary nerve; *SL*, sublingual gland; *SM*, sub-mandibular gland; *TG*, trigeminal ganglion

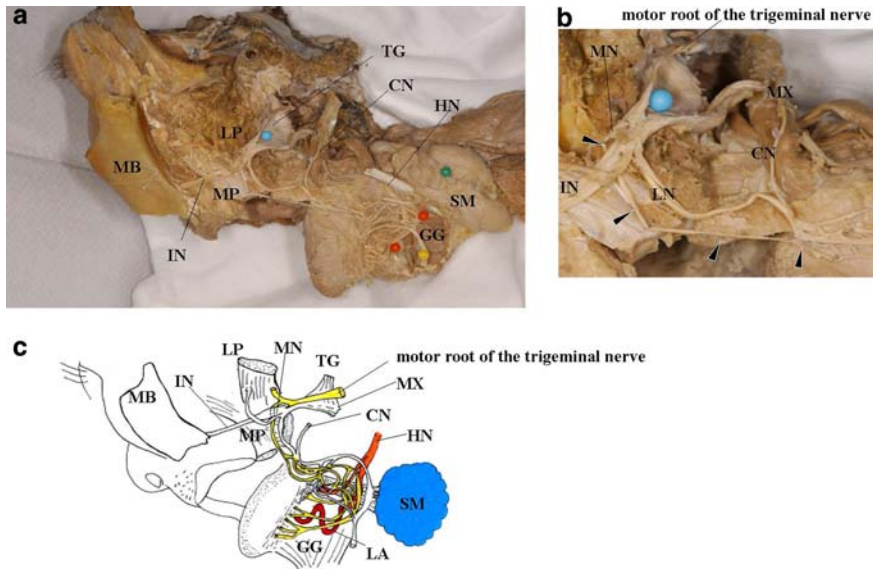


Fig. 3 Morphology of nerve fiber analysis of the lingual nerve in subject 12. Motor root of the trigeminal nerve gave off its supply and traveled with the mandibular nerve until the inferior alveolar nerve entered into the mandibular canal. Then, the motor fiber left the mandibular nerve and joined with the lingual nerve. **a** total analysis of motor fiber that branched from the motor root of the trigeminal nerve into the lingual nerve. **b** closer view of the relationship between the motor root of the trigeminal nerve and motor fiber of the lingual nerve (*black arrows*) at the trigeminal

ganglion. **c** schematic depiction of **a**. *AD*, anterior belly of digastric muscle; *CN*, chorda tympani; *GG*, genioglossus muscle; *HG*, hyoglossus muscle; *HN*, hypoglossal nerve; *IN*, inferior alveolar nerve; *LA*, lingual artery; *LN*, lingual nerve; *LP*, lateral pterygoid muscle; *MB*, mandibular bone; *MH*, mylohyoid muscle; *MN*, mylohyoid nerve; *MP*, medial pterygoid muscle; *MX*, maxillary nerve; *SL*, sublingual gland; *SM*, sub-mandibular gland; *TG*, trigeminal ganglion

Connection between the lingual and the mylohyoid nerves

Direct connections between the lingual and the mylohyoid nerves were found in six specimens (50%) (Table 1). In these specimens, the mylohyoid nerve gave off its supply to the lingual nerve beyond the sub-mandibular ganglion of the lingual nerve.

Total structures of the superior and inferior longitudinal muscles

The three samples in this study showed similar results. In the posterior portion of the tongue (Fig. 6a), the fiber of the superior longitudinal lingual muscle was found in the superior portion near the dorsum of the tongue and in the more medial portion than the styloglossus muscle. The fiber of the inferior longitudinal lingual muscle was found in the lesion between the hyoglossus and the genioglossus muscles under the styloglossus and the transverse lingual muscles. In the medial portion of the tongue (Fig. 6b), the lesion occupied by the fibers of both the superior and inferior longitudinal lingual muscles were enlarged on decreasing the lesion of the styloglossus and the hyoglossus muscles. In the anterior portion of the tongue (Fig. 6c), the fibers of both the superior and inferior longitudinal lingual muscles were joined to each other to surround the hyoglossus muscle. In the more anterior portion (Fig. 6d), the fibers of both the superior and the inferior longitudinal lingual muscles

surrounded the whole frontal section of the tongue and combined with the sub-mucosal connective tissues closely. In the tip of the tongue (Fig. 6e), there were inferior longitudinal, transverse, and vertical muscles on decreasing the fiber of the superior longitudinal muscle. The lingual nerve was found to enter with the lingual artery into the lesion between the genioglossus muscle and the inferior longitudinal muscles, and gave off supplies to the musculature consisting of the superior and the inferior longitudinal muscles (Fig. 6c, 6d). The hypoglossal nerve supplied to the genioglossus muscle (Fig. 6b). Figure 7 is a schematic depiction of coronal serial sections of the tongue. Figure 8 is a schematic representation of the fiber arrangements of the superior and the inferior longitudinal lingual muscles, and their relationships to the lingual artery, the lingual nerve, and the hypoglossal nerve.

Discussion

During mastication, the tongue moves exactly in cooperation with the masticatory movement. It was reported that the combination between the tongue and the mandible on masticatory movement was controlled by the central coordinating system of the brainstem [11, 12]. For speech production, the exact coordinating movement between the tongue and the mandible was also required [3, 4]. Clinically, for patients who disturbed the mobility of the tongue, compensatory excessive movements of the mandible without the patient's consciousness, the so

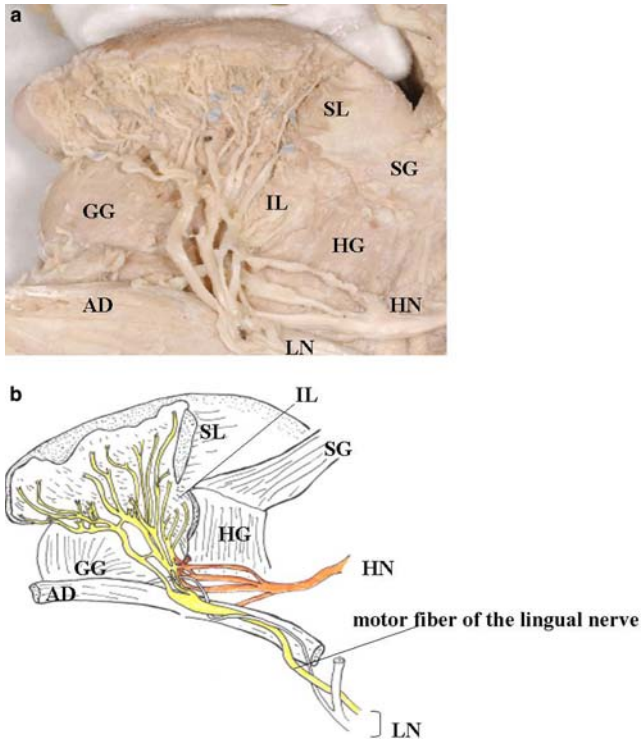


Fig. 4 Peripheral morphology of the lingual nerve. Motor fibers derived from the motor root of the trigeminal nerve travel into the lingual nerve and give off its supplies to the inferior and superior longitudinal muscles, and reach the sub-mucosal layer of the tongue. **a** Photograph of peripheral morphology of lingual nerve. **b** Schematic depiction of **a**. *AD*, anterior belly of digastric muscle; *GG*, genioglossus muscle; *HG*, hyoglossus muscle; *HN*, hypoglossal nerve; *IL*, inferior longitudinal muscle; *LN*, lingual nerve; *SG*, styloglossus muscle; *SL*, superior longitudinal muscle

called jaw dependency, were seen [3]. It was reported that oral anesthesia could not affect speech production [1]. From these findings, it could be considered that the close coordinating motor control between the tongue and the mandible should exist. In this study, there were certain new findings such as the following.

1. The motor root of the trigeminal nerve gave off its supply to the lingual nerve that had been believed to have only sensory fibers, but not motor fibers.
2. The motor fiber traveled into the lingual nerve and branched to the superior and the inferior longitudinal

muscles. In general, the muscle has its own motor nerve, and wherever the muscle migrates, it carried its own motor nerve with itself [7, 9, 10]. From this point of view, it could be considered that the superior and the inferior lingual muscles were innervated by the motor fiber of the trigeminal nerve, and those muscles had been derived from the branchial arch. All the other fibers of the motor root of the trigeminal nerve gave of their supplies to the masticatory muscles. It should be considered that these findings might contribute to the closely coordinating motor control between the tongue and the mandible. In 6 of the 12 specimens (50%), there were direct connections between the lingual and mylohyoid nerves. According to Kameda [5], the anastomosis between the lingual and the mylohyoid nerves was seen in 46.3% of the cases. This connection between the lingual and mylohyoid nerves could be useful for the coordinated complex movements between the tongue and the mandible.

Takemoto [14] reported and developed that the tongue musculature could be grouped into the “stem” and “core” for the inner musculature and the “cover” and “fringes” for the outer musculature. The inner musculature of the tongue is composed of the genioglossus, the transverse, and the vertical muscles. The outer musculature is composed of the superior and inferior longitudinal muscles as the “cover” and the hyoglossus and styloglossus muscles as the “fringes.” It could be considered that the inner and outer musculatures of the tongue not only had these roles, but also another neuro-motor control and origins from each other. In these studies, we found that the nerve fibers from the motor root of the trigeminal nerve traveled into the lingual nerve and branched to the superior and the inferior lingual muscles. From the coronal serial sections, we found that in the anterior portion of the tongue, the superior and the inferior longitudinal lingual muscles joined each other and surrounded the whole tongue and combined with the sub-mucosal connective tissues closely. It could be considered that these morphological features resembled the cutaneous muscles, for example the facial muscles. We found that the lingual nerve was found to enter with the lingual artery into the portion between the genioglossus muscle and the

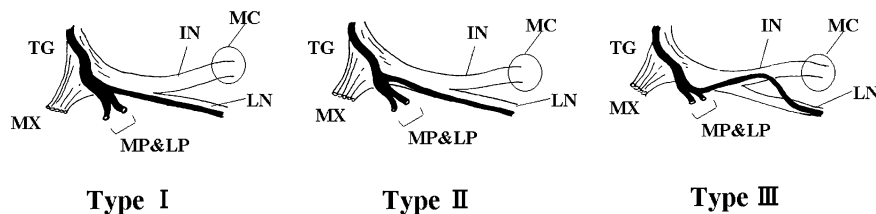


Fig. 5 Patterns of motor fiber branching to the lingual nerve from the motor root of the trigeminal nerve. Type 1: motor fiber branching to the lingual nerve near the medial and the lateral pterygoid nerves. Type 2: motor fiber branching to the lingual before the medial and lateral pterygoid nerves. Type 3: motor fiber

from the motor root traveled with the inferior alveolar nerve until the inferior alveolar nerve entered into the mandibular canal, and then joined with the lingual nerve. *IN*, inferior alveolar nerve; *LN*, lingual nerve; *MC*, mandibular canal; *MP & LP*, media and lateral pterygoid nerves; *MX*, maxillary nerve; *TG*, trigeminal ganglion

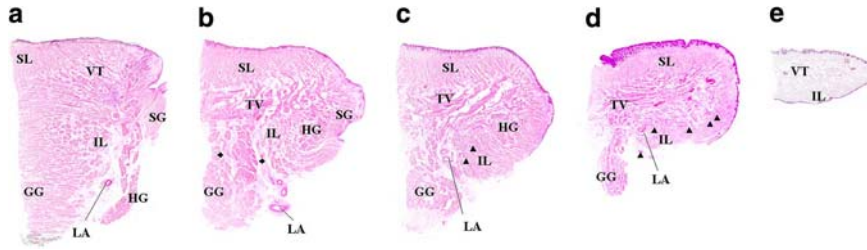


Fig. 6 Light micrographs of coronal sections at the five sectional planes (H & E). **a** posterior portion of the tongue. **b** medial portion of the tongue. **c** anterior portion of the tongue. **d** more anterior portion of the tongue, **e** the tip of the tongue. The *black rhombuses*

indicate the hypoglossal nerve. The *black triangles* indicate the lingual nerve. *GG*, genioglossus muscle; *HG*, hyoglossus muscle; *IL*, inferior longitudinal muscle; *LA*, lingual artery; *SG*, styloglossus muscle; *SL*, superior longitudinal muscle; *VT*, vertical muscle

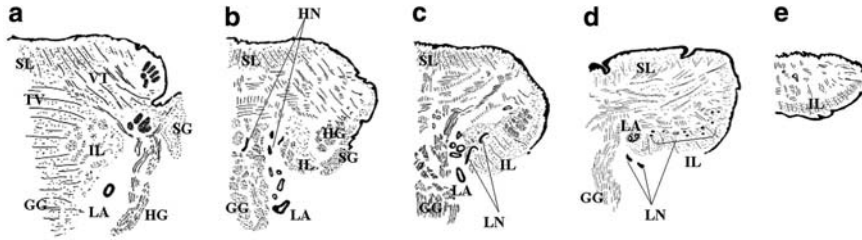


Fig. 7 Tongue musculature in coronal sections. **a** posterior portion of the tongue. **b** medial portion of the tongue. **c** anterior portion of the tongue. **d** more anterior portion of the tongue, **e** the tip of the tongue. *GG*, genioglossus muscle; *HG*, hyoglossus muscle; *HN*,

hypoglossal nerve; *IL*, inferior longitudinal muscle; *LA*, lingual artery; *LN*, lingual nerve; *SG*, styloglossus muscle; *SL*, superior longitudinal muscle; *VT*, vertical muscle

inferior longitudinal muscle, and gave off supplies to the musculature consisting of the inferior and the superior longitudinal muscles. It could be considered that the outer musculature supplied by the lingual nerve had a different origin from the inner musculature supplied by the hypoglossal nerve. Thus, from the coronal serial sections of the tongue, it should also be considered that the superior and the inferior longitudinal lingual muscles originated not from the myotomes in the occipital somite, but from the branchial musculature. Saigusa et al. [13] reported that the posterior part of the transverse lingual muscle might not be differentiated from the

myotome in occipital somite. From animal anatomic findings, Portman [8] described that the primordium of a bird's tongue had only motor fiber of the trigeminal nerve without sensory fiber. Marcus [6] reported that the tongue of a primeval fish (*Polypterus*) was composed of smooth muscle. Further investigation may be warranted to confirm "true" neuro-anatomic composition of the tongue musculature by embryological and morphological approaches.

It had been reported that the peripheral connections between the lingual and hypoglossal nerves were found in the extra-lingual and intra-lingual portions [2].

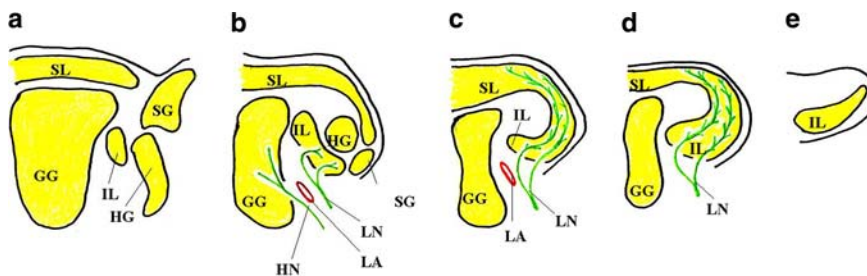


Fig. 8 Diagram showing the fiber arrangements of the superior and the inferior longitudinal muscles and their relationships with the lingual artery, the hypoglossal nerve, and the lingual nerve in coronal sections. Fibers of the superior longitudinal muscle become connected with the fibers of the inferior longitudinal muscle at the level of the anterior portion of the tongue. The lingual artery divides the genioglossus muscle from the inferior longitudinal muscle with the hypoglossal nerve (inner side) and the

lingual nerve (outer side). **a** posterior portion of the tongue. **b** medial portion of the tongue. **c** anterior portion of the tongue. **d** more anterior portion of the tongue. **e** the tip of the tongue. *GG*, genioglossus muscle; *HG*, hyoglossus muscle; *HN*, hypoglossal nerve; *IL*, inferior longitudinal muscle; *LA*, lingual artery; *LN*, lingual nerve; *SG*, styloglossus muscle; *SL*, superior longitudinal muscle

In these studies, extra-lingual connections between the lingual and the hypoglossal nerves were seen in 10 of the 12 specimens (83%). In ten specimens, the branch of the hypoglossal nerve connected to the fiber of the lingual nerve of the inferior longitudinal muscles. In another two specimens without extra-lingual anastomosis, the hypoglossal nerve supplied fibers to the inferior longitudinal muscles near the fiber from the lingual nerve. From our coronal serial-section study of the tongue musculature, the inferior longitudinal lingual muscle defined boundaries between the genioglossus and the other lingual muscles and “covered” the genioglossus muscle with the superior longitudinal muscle. It could be considered that these close relationships between the lingual and hypoglossal nerves and the neuro-motor control of their own lingual muscles would be helpful for coordinating movements of the inner and outer musculatures of the tongue.

Further anatomic and physiologic studies may be warranted for the “true” neuro-motor control and the functional mechanisms of the tongue musculature. We are sure that the new knowledge of the tongue musculature might be helpful to develop a useful therapeutic approach, for example, rehabilitation technique and surgery, for dysphagia patients and dysarthric patients in the near future.

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