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Human Tongue Neuroanatomy: Nerve Supply and Motor Endplates

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Abstract

The human tongue has a critical role in speech, swallowing, and respiration, however, its motor control is poorly understood. Fundamental gaps include detailed information on the course of the hypoglossal (XII) nerve within the tongue, the branches of the XII nerve within each tongue muscle, and the type and arrangement of motor endplates (MEP) within each muscle. In this study, five adult human tongues were processed with Sihler's stain, a whole-mount nerve staining technique, to map out the entire intra-lingual course of the XII nerve and its branches. An additional five specimens were microdissected into individual muscles and stained with acetylcholinesterase and silver staining to study their MEP morphology and banding patterns. Using these techniques the course of the entire XII nerve was mapped from the main nerve to the smallest intramuscular branches. It was found that the human tongue innervation is extremely dense and complex. Although the basic mammalian pattern of XII is conserved in humans, there are notable differences. In addition, many muscle fibers contained multiple *en grappe* MEP, suggesting that they are some variant of the highly specialized slow tonic muscle fiber type. The transverse muscle group that comprises the core of the tongue appears to have the most complex innervation and has the highest percentage of *en grappe* MEP. In summary, the innervation of the human tongue has specializations not reported in other mammalian tongues, including non-human primates. These specializations appear to allow for fine motor control of tongue shape.

Keywords

tongue; innervation; hypoglossal nerve; lingual nerve; motor endplates; Sihler's stain; acetylcholinesterase staining; silver stain; neuromuscular compartments; speech; swallowing; breathing; genioglossus muscle; superior longitudinal muscle; inferior longitudinal muscle; styloglossus muscle; hyoglossus muscle; vertical muscle; transverse muscle; palatoglossus muscle

INTRODUCTION

In humans, tongue movements are critical for speech, swallowing, and respiration (for review, see Hiiemae and Palmer, 2003); and tongue dysfunction could lead to dysarthria, dysphagia, and obstructive sleep apnea (OSA), respectively. Advances in the treatment of these disorders require a greater understanding of tongue biomechanics. However, little is known about how each of the tongue muscles contributes to the overall movement of the

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tongue. The biomechanics of any structure derives from its anatomy, and this is especially true for the tongue. The unique aspect of tongue biomechanics is that the tongue is a muscular hydrostat (Kier and Smith, 1985). Limb muscles like the biceps move simple mechanical levers in 2 dimensions, specifically bones rotating around a joint. In contrast, the tongue has no internal bones and a contracting tongue muscle can cause three dimensional changes in tongue shape. Humans have a uniquely robust ability to shape the tongue and this is a large part of speech articulation (Stone and Lundberg, 1996). This makes the structural details of tongue muscles and their innervation an important subject.

At present, some fundamental aspects of human tongue anatomy are unknown. A number of recent studies focus on determining fiber type and myosin heavy chain composition in individual human tongue muscles to better understand their contractile properties and functions (Stal et al., 2003; Sokloff et al., 2007, 2009). However, the most fundamental lack of knowledge relates to the gross anatomy of the tongue. Although the basic muscles that compose the tongue have been described (Abd-El-Malek, 1938), the motor innervation to these muscles has still not been described in detail. Moreover, a single muscle is often made up of separate bellies, also called neuromuscular compartments, that have separate terminal nerves and can have different activity than the rest of the same muscle (English and Weeks, 1984). As tongue muscles have very complex innervation with many terminal nerves entering single muscles, there is a huge amount of detail regarding tongue muscle function that is yet to be described.

The goal of this study was to address some of the most basic questions of tongue muscle anatomy, the distribution of the XII nerve supply inside the tongue and how this nerve supply is distributed within individual muscles. Although there are some studies in animal models, these can only be generalized to the human with caution. It is known that the humans are different from other mammals by having a curved pharynx and tongue, a radical anatomical change that is believed to allow for human speech (Fitch, 2000). Therefore, human tongue anatomy is unique and must be studied directly.

It is known that tongue is composed of extrinsic (genioglossus, styloglossus, hyoglossus, and palatoglossus) and intrinsic (superior and inferior longitudinal, transverse and vertical) muscles. It is generally described that the tongue muscles in mammals are innervated by the hypoglossal (XII) nerve which is divided into a lateral (l-XII) and a medial (m-XII) division (Abd-El-Malek, 1938; Weddell et al., 1940; Hellstrand, 1981). However, there are controversies regarding the distribution of the l-XII and m- XII nerve branches. For example, in humans the l-XII supplies hyoglossus, styloglossus, and inferior longitudinal muscles, whereas the m-XII innervates the genioglossus, superior longitudinal, transverse and vertical muscles (Abd-El-Malek, 1938). In the cat, however, the m-XII also supplies the hyoglossus and styloglossus muscles (Hellstrand, 1981). These conflicting results may be due to limitations of the microscopic dissection techniques used by these investigators, as it is impossible to demonstrate the entire nerve supply in complex organs such as the tongue. As a result, the peripheral organization of the nerve supply to the human tongue has never been described in detail.

A recent advance in the investigation of the innervation of skeletal muscles is the use of Sihler's stain, a wholemount nerve staining technique (for review, see Mu and Sanders, 2010). Sihler's stain renders a large specimen transparent while counterstaining all nerves. The Sihler's stain has some major advantages. 1) Precise branching of all nerves in a specimen can be seen in their normal *in vivo* relationships. Albeit very complex organs such as the tongue require some dissection due to their thickness. 2) The terminal branching within individual muscles can be seen in the same specimen. This level of detail is often beyond the capability of traditional microdissection. 3) Post mortem tissue can be used. This makes the technique uniquely valuable for studying human tissue. Although the best detail is seen in specimens fixed with formalin before or at the tme of death, specimens can be used for up to 7 days post mortem. The older the specimen is before fixation, the more degradation of fine terminal branching.

The Sihler's stain has recently been used to re-examine upper airway anatomy and has resulted in novel observations regarding the innervation of the human larynx (Wu and Sanders, 1992; Sanders et al., 1993a,b, 1994; Mu et al., 1994; Wu et al., 1994; Sanders and Mu, 1998; Mu and Sanders, 2009), and human pharynx (Mu and Sanders, 1996, 1998a, 2000a, 2001, 2007, 2008). Regarding tongue anatomy, the Sihler's stain has been used to study the sensory innervation of the human tongue (Zur et al., 2004; Doty et al., 2009) and the motor innervation of the canine (Mu and Sanders, 1999, 2000b) and rat (McClung and Goldberg, 2000) tongues. This report will be the first on the motor innervation of the human tongue.

New information was expected from Sihler's stained tongues regarding the the terminal nerve branching within individual muscles. However, as sensory and motor nerves look the same in Sihller's stained specimens, a complementary study was performed on the motor endplates (MEPs) of tongue muscles to confirm that the terminal branches were indeed motor. In limb and trunk muscles, each muscle fiber usually has one MEP which locates the mid-way between its origin and insertion. Thus, in muscles composed of myofibers with equal length, the MEPs form a band across the middle of muscle (Coers, 1959). Furthermore, very few muscles have a simple structure, many are composed of smaller neuromuscular compartments. As each of these compartments has its own MEP band, studying their number and location is a useful indicator of the neuromuscular compartments within a muscle (Galvas and Gonyea, 1980; Mu and Sanders, 1998a,b, 2000b). To date, the distribution and types of the MEPs in the majority of the tongue muscles have never been determined.

A secondary contribution of MEP staining is to study the type of MEPs present. Although the vast majority of limb skeletal muscles have a single *en plaque* type MEP, cranial muscles appear to have significant numbers of muscle fibers with multiple MEPs. For instance, some highly specialized cranial muscles including the extraocular (Kupfer, 1960), laryngeal (Rossi and Cortesina, 1965; Bendiksen et al., 1981), middle ear (Erulkar et al., 1964; Fernand and Hess, 1969), pharyngeal (Mu and Sanders, 2007), and tongue (Slaughter et al., 2005) muscles contain muscle fibers with multiple MEPs. Specifically, in the human tongue, some superior longitudinal muscle fibers have two MEPs (Slaughter et al., 2005).

Interestingly, different muscles of the same organ can have very different proportions of muscle fibers with multiple MEPs. For example, in the human larynx the muscle fibers with multiple MEPs account for 70–80% in the vocalis muscle, 50% in the cricothyroid and lateral cricoarytenoid muscles, respectively, and only 5% in the posterior cricoarytenoid muscle (Rossi and Cortesina, 1965). These observations suggest that the multiply innervated muscle fibers may have distinct contractile properties.

In addition to the banding patterns and the muscle fibers with either single or multiple MEPs, the forms of the MEPs (i.e., *en plaque* and *en grappe*) also indicate muscle fiber specializations. In this regard, *en grappe* MEPs have been reported to innervate slow-tonic muscle fibers that exist in certain specialized cranial muscles such as the extraocular (Hess and Pilar, 1963), vocalis (Han et al., 1999), and pharyngeal muscles (Mu and Sanders, 2007). This type of MEPs has been also identified in the superior longitudinal muscle of the human tongue (Slaughter et al., 2005). Since the MEP is the physiological interface between the motor neuron and the muscle fiber, the banding and type-distribution of the MEPs within a muscle also reflects muscle function.

The aim of this study was to improve our understanding of neural control of the human tongue by determining motor innervation patterns of individual tongue muscles.

MATERIALS AND METHODS

Ten adult human tongues (six males and four females; ages 51–84 yr; mean age, 62 yr) without neuromuscular disease were obtained from autopsies within 24 hr postmortem. Five specimens were processed with Sihler's stain to study the branching and distribution of the XII nerve within the tongue. The remaining five specimens were dissected and stained for acetylcholinesterase (AChE) and silver stain to investigate banding patterns and types of the MEPs in the tongue muscles.

Sihler's Stain

Five adult human tongue specimens were processed with Sihler's stain. The details regarding this technique have been given elsewhere (Mu and Sanders, 1999, 2008, 2009, 2010). Briefly, human tongue specimens were: 1) fixed for four weeks in 10% unneutralized formalin; 2) macerated for three weeks in 3% aqueous potassium hydroxide (KOH) solution (add 3 drops of 3% v/v hydrogen peroxide into 100 ml 3% w/v KOH solution for depigmentation) with several changes; 3) decalcified for two weeks in Sihler's solution I (one volume glacial acetic acid, one volume glycerin, and six volumes 1% w/v aqueous chloral hydrate) with several changes; 4) stained for four weeks in Sihler's solution II (one volume stock Ehrlich's hematoxylin, one volume glycerin, and six volumes 1% w/v aqueous chloral hydrate); 5) destained for three hours in Sihler's solution I; 6) immersed for one hour in 0.05% w/v lithium carbonate solution to darken the nerves; 7) cleared for three days in 50% v/v aqueous glycerin; and 8) preserved for 4 to 6 weeks in 100% glycerin with a few thymol crystals for transparency. The specimens were washed in running tap water for 1 hr between the first six steps.

The Sihler's stained specimens were preserved in 100% glycerin for 1–2 months and then dissected with the aid of a dissecting microscope (TYP 3555110; Wild, Heerbrugg, Switzerland) at a magnification of 10–30× by using microsurgical instruments after transillumination by a xenon light source (model 610; Karl Storz, Endoscopy-America, Culver City, CA). The nerve branches were traced from the XII nerve to their terminations within individual tongue muscles. During dissection, some soft tissue was removed for showing the nerve branching. After dissection, the nerve supply pattern of each muscle was recognized and photographed with a 35 mm camera (OM-4 Olympus, Tokyo, Japan) fitted with a 20, 38, or 50 mm macrolens. The numbers and branching points of the major nerve branches derived from the XII nerve were recorded.

Wholemount AChE Staining and Silver Stain

Five tongue specimens were prepared for either AChE staining or silver stain to determine the MEP bands, MEP types, and terminal innervation of the tongue muscle fibers. The individual tongue muscles were identified and removed from each specimen under a dissecting microscope. It should be pointed out that the superior longitudinal, transverse and vertical muscles were isolated using different methods. Specifically, the superior longitudinal muscle fascicles are organized into a single layer lying beneath the dorsal mucosa of the tongue. After removal of the mucosa, this muscle was visualized and dissected out. Since the transverse and vertical muscles are arranged in alternating layers within the tongue (Mu and Sanders, 1999), the whole tongue specimens were frozen and macro-sectioned in the frontal plane (~5 mm thick). Each of the slices was dissected to show a transverse muscle layer on one side and a vertical muscle layer on the other side. All the muscle samples were processed for wholemount AChE staining.

The details of the wholemount AChE staining have been described in our previous publications (Mu and Sanders, 1998a,b, 2000b; Ren and Mu, 2005). Briefly, the muscle samples were: 1) fixed in 10% phosphate-buffered formalin at room temperature for 2 hr; 2) washed in 0.1 M phosphate buffer at pH 7.4 and pH 6.0 for 15 min in each; 3) incubated in stock solution [cupric sulfate 150 mg, glycerin 190 mg, magnesium chloride 500 mg, maleic acid 900 mg, 4% sodium hydroxide 15 ml, 40% sodium sulfate (anhydrous) 85 ml, and acetylthiocholine iodide 100 mg] at pH 6.0 and 37°C for 2 hr; 4) rinsed in 40% sodium sulfate (anhydrous) for 15 min; 5) washed in distilled water (DW) for 15 min; 6) immersed in 20% potassium ferricyanide for 15 min; 7) washed in DW for 1 hr; and 8) transferred into 50% glycerin for preservation. The stained specimens were dissected under a dissecting microscope (TYP 3555110, Wild) after transillumination by a xenon light source (model 610, Karl Storz) and photographed with an OM-4 Olympus camera (Tokyo, Japan), using a 50, 38, or 20 mm lens.

After overall distribution of the MEPs within each muscle was demonstrated and photographed, the AChE stained muscle samples were impregnated with 10% silver nitrate at 37°C for 1 hr, rinsed in DW for 90 min, reduced in Bodian's developer for 5 min, and rinsed in DW for 2 hr (Oda, 1984). The silver-stained muscle samples allowed the types of the MEPs (*en plaque* or *en grappe*) and preterminal branching patterns of the innervating axons (single or multiple) to be determined (Tuffery, 1971; Ren and Mu, 2005; Mu and Sanders, 2007). Single muscle fibers were teased from some silver-stained samples by using microneedles under a dissecting microscope (TYP 3555110, Wild) with 50×. The teased single fibers were mounted, viewed on a Zeiss universal microscope (Axiophot-2), and photographed with a Spot-32 digital camera (Diagnostic Instruments; Keene, NH) to show the morphological characteristics and innervation patterns of the MEPs. In addition, the silver-stained transverse and vertical muscle layers were also used to show their terminal nerve branching and innervation patterns.

RESULTS

Branching and Distribution of the XII Nerve within the Human Tongue

Sihler's stain showed branching and distribution patterns of the nerves within the human tongue (Figs. 1 and 2). Individual tongue muscles and overall branching of the XII nerve as revealed by Sihler's stain are schematically illustrated in Figure 3. Anatomical locations and actions of the tongue muscles including the styloglossus (SG), hyoglossus (HG), genioglossus (GG), superior longitudinal (SL), inferior longitudinal (IL), transverse (T) and vertical (V) muscles are summarized in Table 1. The tongue muscles are enveloped by mucosa supplied by the glossopharyngeal (IX) and lingual (LN) nerves. The nerves supplying the lingual structures were organized in a nerve-dependent and muscle-specific manner as described below.

The examined adult human tongue specimens exhibited similar nerve branching and distribution patterns. Specifically, the XII nerve on each side entered the tongue at the ventrolateral aspect of the posterior tongue. After it reached the dorsal surface of the HG muscle, the XII usually gave off its first branch to innervate the geniohyoid (GH) muscle. It was then divided into l-XII and m-XII branches to supply different tongue muscles (Figs. 1–3).

Two types (single and multiple) of l-XII branching were observed. The l-XII was given off as a single branch with a very short main trunk (~ 2 mm) in 40% (4/10) of the samples (Fig. 1A-left). In this case, the l-XII was then divided into 3–5 first-order branches to innervate the longitudinally arranged tongue muscles [i.e., SL, SG, HG, and lateral IL (l-IL)]. In contrast, the remaining 60% (6/10) of the tongue specimens showed that the XII nerve gave off multiple l-XII branches at different points on the nerve main trunk to supply these muscles (Figs. 1A-right, 1B, 2, 3, and 5A).

After the l-XII branches arose from the XII nerve, the remaining m-XII turned medially at the anterior edge of hyoglossus muscle and began to run anteriorly. The posterior third of the m-XII provided its first-order branches to supply the GG, posterior T/V, and medial IL (m-IL) muscles (Figs. 2 and 3). At the junction between the posterior and middle portions of the tongue (i.e., at the level of the vallate papillae), the m-XII immediately split off a bundle of first- and second-order branches, forming a dense plexus which supplied nerve branches to the T and V muscle fiber layers in the anterior two thirds of the tongue (Figs. 1, 2A and 3). During its course, the m-XII gave off approximately 40–50 first-order branches at different levels.

Overall, the XII nerve in adult humans gave off approximately 50–60 primary nerve branches along its whole length within the tongue. In general, the XII branched in the following order from the root to the apex of the tongue: GH, SL, SG, HG, l-IL, horizontal GG, oblique GG, posterior T/V, m-IL, and anterior T/V (Figs. 2A and 3).

Entrance of the XII nerve branches to each of the tongue muscles was examined. The first-order branches of the XII nerve to the longitudinally arranged muscles (i.e., SG, HG, IL, and SL) entered the muscles in the posterior tongue, whereas those to the muscles arranged in various directions (i.e., GG, T and V) entered the muscles in different ways (Fig. 3) as described below.

Intramuscular Nerve Supply Patterns and MEP Bands within the Human Tongue Muscles

The l-XII innervating muscles—Sihler's stain showed that the l-XII nerve branches supplied two extrinsic (i.e., SG and HG) and two intrinsic (i.e., SL and IL) muscles which are longitudinally arranged and span the whole length of the tongue. Therefore, the l-XII innervating muscles form the cover layer of this muscular organ.

Styloglossus and hyoglossus muscles: The SG muscle arises from the anterolateral aspect of the styloid process, passes downwards and forwards, and enters the tongue dorsolaterally. The HG muscle is quadrilateral in shape and arises from the greater cornu and the front of the body of the hyoid bone. It runs vertically up to enter the side of the tongue between the laterally located SG and the medially positioned IL. The SG and HG muscles compose the posterior lateral surface of the tongue, they converge and intermix at the level of the tongue base. Then the combined muscle passes anteriorly and spans the whole length of the tongue. From the tongue base to tongue tip the SG/HG combination composes the ventrolateral surface of the tongue just beneath the mucosa (Fig. 3).

Sihler's stain showed that the primary nerve branches of the l-XII entered the SG and HG in the posterior tongue where some of them split off fine terminal branches to innervate the muscles, whereas others continued to run anteriorly along the muscle fascicles (Figs. 1, 2A, 3, and 5A). The region innervated by the terminal branches in the posterior tongue was consistent with the MEP band in each muscle as revealed by wholemount AChE staining (Fig. 4).

Superior longitudinal muscle: The SL muscle is a flat muscular sheet that covers the dorsal (superior) surface of the tongue. It is just beneath and is intimately attached to the dorsal tongue mucosa (Figs. 3, 5A and B). Within this sheet muscle fascicles are arranged longitudinally (Fig. 5A), however, these fascicles do not course the entire length of the tongue. Instead they are composed of groups of short muscle fibers which have their own terminal nerve branch and MEB band. These fascicles interweave throughout the length of the SL merging and splitting at short intervals. The muscle fascicles close to the midline are much larger than the laterally located ones (Fig. 5C).

Sihler's stain showed that the first branch of the l-XII usually supplied the SL muscle (Figs. 2, 3 and 5). This primary branch was further divided into several secondary and numerous tertiary branches to supply the individual muscle fascicles (Figs. 5A–D). The nerve branch(es) on each muscle fascicle broke out terminal twigs to innervate SL muscle fibers. We found that branches from the lingual nerve also supplied the SL muscle fascicles and communicated with the XII nerve (Figs. 5A–D). On each muscle fascicle, multiple MEP bands were observed (Fig. 5E). These MEP bands presumably correspond to the terminal nerve branches seen in the Sihler's stained specimens. These findings suggest that the SL muscle is composed of neuromuscular compartments (NMCs) which are arranged in-series.

Inferior longitudinal muscle: The IL muscle is cylindrical in shape and situated ventrally between the HG and the GG muscles. It extends from the base to the tip of the tongue (Figs. 3). We found that this muscle was composed of two bellies or NMCs arranged in parallel, specifically a lateral (l-IL) and medial (m-IL) (Fig. 2).

Sihler's stain showed that the l-IL was supplied by the l-XII, whereas the m-IL received its innervation from the m-XII (Figs. 2 and 3). The IL appears to be the only muscle that receives its motor innervation from both the m-XII and the l-XII. We found that the lingual nerve also supplied innervation to the IL muscle and communicated with the XII nerve (Fig. 2).

The m-XII innervating muscles—Sihler's stain demonstrated that the m-XII provided motor nerve supply to an extrinsic (i.e., GG) and three intrinsic (i.e., m-IL, T and V) muscles which are arranged in different directions within the tongue (Fig. 3). As mentioned above, the m-IL was innervated by the m-XII. The muscles innervated by the m-XII, especially the T and V muscles, constitute the core of the tongue and have the most complex innervation.

Genioglossus muscle: The GG is a fan-like midline muscle which arises from a short tendon attached to the superior genial tubercle behind the mandibular symphysis, above the origin of geniohyoid muscle. Grossly it is shaped like a wedge with the sharp anterior edge corresponding to the frenulum. It was found to be composed of two NMCs, horizontal (GGh) and oblique (GGo). The GGh fibers inserted into the tongue base and hyoid bone, whereas the GGo fibers inserted into the tongue body (Figs. 3 and 6A).

The nerve branches to the GG entered the muscle at the junction between extra- and intra-lingual portions of the muscle (Figs. 3, 6A, B). Sihler's stain demonstrated that the GG compartments were innervated by separate primary nerve branches which arose from different points on the m-XII (Fig. 6B). The intramuscular neural organization patterns of the two compartments were found to be separate and significantly different. The primary nerve branch innervating the GGh gave off 2–3 second-order branches which traveled parallel to the muscle fibers. In contrast, five to six first-order branches supplying the GGo formed a plexus which was situated at the junction between extra- and intra-lingual portions of the muscle (Figs. 3 and 6B).

Wholemount AChE staining showed that GGo had a single MEP band, whereas GGh had two MEP bands (Figs. 4A, 6C, D). The locations of the MEP bans correspond to the terminal nerve branches seen in the Sihler's stained specimens.

Transverse and vertical muscles: The T and V muscles are the core of the tongue. Both muscles are organized into numerous alternating muscle fiber layers arranged perpendicular to each other. The T and V muscle fiber layers were identifiable in the tongue specimens which were processed with Sihler's stain and AChE stained frontal sections (~5 mm thick) (Fig. 7). The T muscle fiber layers connected the median septum to the lateral margin of the tongue. In contrast, the V muscle fiber layers attached both the dorsal and ventral mucosa of the tongue.

Sihler's stained specimens showed that the m-XII passed anteriorly and gave off many first-order nerve branches at different levels which further split off numerous secondary ones to supply the T and V muscle layers (Fig. 7C). Since the T and V muscles were arranged in alternating muscle fiber layers from the base to the apex of the tongue, they received their nerve supply from different levels along the course of the m-XII (Figs. 3, 7C). Combined AChE and silver stain showed that each of the V muscle layers was usually supplied by a secondary branch which ran across the muscle fibers. The secondary branch was generally subdivided into a number of twigs to supply individual muscle fascicles (Fig. 7D). Each of the V muscle fiber layers was found to have a single MEP band which was located in the middle of the muscle fibers (Fig. 7E). Similarly, each T muscle fiber layer was innervated by distinct nerve branches which traveled along the muscle fibers (Fig. 7F). In general, each T muscle fiber layer had two MEP bands (Fig. 7G).

The MEP Types and Their Distribution in the Tongue Muscles

Higher power view of the single muscle fibers teased from the silver-stained muscle fascicles showed that both *en plaque* and *en grappe* types of MEPs were present in the tongue muscles. Both types of MEPs differed considerably in size, shape and distribution pattern in the tongue muscles. In general, the *en plaque* MEP had a single preterminal branch (Fig. 8A), whereas the *en grappe* MEP was characterized by having multiple preterminal branches (Fig. 8B). On average, the *en plaque* MEPs (68% of the total MEPs examined) were much more than the *en grappe* MEPs (32% of the total MEPs examined). The *en grappe* MEPs were concentrated mainly in the T muscle (~40% of the *en grappe* MEP population). In addition, some of the SL, T and GGh muscle fibers were multiply innervated (Fig. 8C), suggesting that they may be slow tonic muscle fibers.

DISCUSSION

Overview of Principal Findings

To our knowledge, this is the first report to demonstrate the details regarding the motor nerve supply and MEP distribution patterns of the human tongue muscles using Sihler's stain, wholemount AChE staining, and silver stain. Several key findings were obtained from this study as described below.

First, using Sihler's stain we presented a complete nerve map of the entire human tongue. The data showed that the XII nerve gave off a single l-XII with a very short main trunk (~2 mm) in four specimens (40%). The short main trunk of the l-XII was then divided into 3 to 5 first-order branches to innervate the SL, SG, HG, and l-IL muscles. In the remaining six specimens (60%), the XII nerve gave off multiple l-XII branches which were derived from different points on the main trunk of the XII nerve.

Second, the m-XII innervated the GG, m-IL, T and V muscle. At the junction between the posterior and middle portions of the tongue, the m-XII split off numerous first- and second-order branches to form a dense plexus which provided nerve supply to the T and V muscle fiber layers in the anterior two thirds of the tongue. A second plexus formed by the m-XII was also identified in the GGo.

Third, most of the tongue muscles including the GG, SL, IL, and T/V muscles were found to be composed of NMCs. Specifically, two NMCs in the GG (i.e., GGh and GGo) and IL (i.e., l-IL and m-IL) and multiple NMCs in the SL and T/V muscles were delineated. The NMCs in the GG and IL were two main groups arranged in parallel, whereas there were many NMCs in each SL muscle fascicle arranged in series, while different fascicles were also arranged parallel to each other. The alternating muscle fiber layers in the T/V muscles were also considered as a special organization pattern of the NMCs.

Fourth, the IL was found to be a unique muscle which received its innervation from the l-XII and m-XII, as well as from the lingual nerve. The lingual nerve also supplied the SL muscle and communicated with the XII nerve. There were numerous communications between the lingual and XII nerves in the tongue, especially in the SL muscle.

Finally, the banding patterns and types of the MEPs within the tongue muscles were determined. The SL had multiple MEP bands, whereas GGh and T muscles had two MEP bands. The oblique part of the GG, the SG and the HG muscle all had a wide single MEP band, much like most skeletal muscles. The details regarding the peripheral neural organization patterns of the human tongue are critical for better understanding the motor control of the tongue movements and for guiding some clinical procedures dealing with the nerves and MEP bands to treat tongue-related upper airway disorders.

Specialized Neural Organization of the Human Tongue

At present, Sihler's stain has been applied to the rat (McClung and Goldberg, 2000), canine (Mu and Sanders, 1999), and human (present study) tongues. Similarities and dissimilarities in the branching and distribution patterns of the XII were found in these species.

Sihler's stain demonstrated species difference in the divisions of the XII nerve. Although the l-XII and m-XII exist in the rat (McClung and Goldberg, 2000), canine (Mu and Sanders, 1999), and human (present study) tongues and support classical description (Abd-El-Malek, 1938), the number of branches derived from the l-XII or m-XII in the human is much higher than that in other species. For example, the rat XII is clearly separated into a l-XII and a m-XII, each of which gives off branches to supply individual tongue muscles (McClung and Goldberg, 2000). In contrast, the l-XII in the human (present study) consists of multiple nerve branches. Clearly, neuromuscular innervation patterns are conserved throughout evolution, however, the human has lost the classical division into gross main medial and lateral branches, instead the lateral second-order branches come separately off the main XII, although they are all close together in a group.

Studies also showed species difference in the distribution of the l-XII and m-XII. Previous anatomical dissections showed that the m-XII in the cat also supplies branches to the SG and HG (Hellstrand, 1981). Although Sihler's stain demonstrated that the l-XII innervates the SG and HG, whereas the m-XII supplies the GG, transverse and vertical muscles in the rat (McClung and Goldberg, 2000), dog (Mu and Sanders, 1999), and human (present study), innervation of the SL and IL varies with species studied. Specifically, the IL and SL are innervated by the l-XII in the rat (McClung and Goldberg, 2000), whereas the canine IL and SL are supplied by the l-XII and m-XII, respectively (Mu and Sanders, 1999). In the human, however, the IL receives its nerve supply from the l-XII, m-XII, and lingual nerve, while the SL receives its innervation from the l-XII and lingual nerve (present study).

Another gross difference between humans and all other species is the complexity and density of innervation, this can be grossly appreciated by just a glance at any human specimen compared to the dog (Mu and Sanders, 1999) or rat (McClung and Goldberg, 2000). The highest density seems to involve the transverse/vertical group. The specializations of the neural organization of the human tongue are most likely related to human speech. The articulation of speech requires more control over tongue movement.

Physiological and Clinical Implications

The roles played by the tongue in respiration, swallowing, and phonation have been demonstrated in both humans and animals (Hrycyshyn and Basmajian, 1972; Lowe and Sessle, 1973; Stone and Lundberg, 1996; Thexton et al., 1998). It is generally accepted that activation of the intrinsic tongue muscles alters the tongue shape, whereas activation of the extrinsic tongue muscles is associated with the position and movement of the tongue (Standring et al., 2005). However, little is known about how each of the tongue muscles contributes to these motor tasks.

Demonstration of the NMCs within the lingual muscles could broaden our knowledge regarding the tongue functions. The NMCs within a muscle can be defined according to the anatomical and histochemical criteria (i.e., regional differences in muscle fiber and tendon arrangement, nerve supply pattern, endplate banding, and fiber-type distribution) (for review, see Windhorst et al., 1989; English et al., 1993; Mu and Sanders, 2010). Of these parameters, intramuscular nerve supply is the most important indicator for determining whether a muscle is functionally compartmentalized. Distinct regions within a muscle supplied by different primary nerve branches are considered to be controlled separately by the nervous system (Windhorst et al., 1989; English et al., 1993). In addition, distribution of the MEPs in each muscle is another indicator for delineating NMCs (Galvas and Gonyea, 1980; Mu and Sanders, 1998a,b, 2000b). Our studies have demonstrated that a number of upper airway muscles (Sanders et al., 1994; Mu and Sanders, 1998a,b, 1999, 2000b, 2001, 2007, 2008, 2009) including the tongue (present study) are composed of NMCs. The regional activation of the tongue muscles (Napadow et al., 1999) suggests that the NMCs within the tongue muscles are independently controlled to shape and regulate the various motions of the tongue. This would help explain how a small number of tongue muscles can cause a large number of different tongue shapes and movements.

Advanced knowledge on the human tongue innervation also has clinical significance. Dysfunction of the tongue has been widely recognized as a risk factor leading to some upper airway disorders such as OSA, oro-pharyngeal dysphagia, and dysarthria. Details about the tongue innervation are essential for a better understanding of the motor control of the tongue functions and for development of novel therapies aiming at the nerve and/or MEPs to treat tongue-related upper airway disorders. For example, electrical stimulation of either the whole XII nerve or its major branches has been used to treat patients with OSA (Eisele et al., 1997; Schwartz et al., 2001; Oliven et al., 2003). Although electrical stimulation of the XII nerve has been shown to be effective as a therapeutic treatment for OSA patients, some patients exhibit little or no improvement of ventilation (Schwartz et al., 2001; Oliven et al., 2003). This suggests that correct identification of the stimulated nerve and/or nerve branches, appropriate stimulation protocols, and optimal electrode design may affect the experimental results. Details regarding the nerve supply patterns and the compartmentalized structure of the tongue would be of great benefit for the surgeons to perform nerve stimulation and develop novel therapeutic approaches to treat OSA.

MEP band is a potential site for clinical manipulations. For instance, intramuscular injection of botulinum toxin has been used for treatment of tongue protrusion dystonia (Charles et al., 1997). Botulinum toxin inhibits acethylcholine release from nerve endings (Kao et al., 1976), thereby inducing muscular paresis. The botulinum toxin injection is most effective if injections are made within the MEP band. Therefore, the data on the MEPs in each of the tongue muscles are helpful for guiding procedures requiring the precise localization of the MEP band(s).

Future Research Directions

As the nerves and muscles in the tongue are arranged in a complex manner, considerable uncertainties and controversies remain to be clarified. For example, the nature of the lingual nerve (LN) supplying the tongue muscles needs to be determined. It is generally accepted that the LN is a sensory nerve which mediates sensation of the anterior two thirds of the tongue. However, Saigusa et al., (2006) microdissected formalin-fixed human cadavers and showed that the motor root of the trigeminal nerve gave off motor fibers which traveled into the LN and branched to the SL and IL muscles. In this study, Sihler's stain showed that the posterior branch of the LN supplied the IL muscle and communicated with the XII nerve. In the Sihler's stained SL, the LN branches were also identified to supply the muscle fascicles and frequently communicated with the XII nerve branches. In addition, various neural connections between the LN and XII nerves have been identified (Zur et al., 2004; Saigusa et al., 2006; present study). Taken together, these findings suggest that the LN may contain motor axons whatever they come from the trigeminal nerve as reported by Saigusa et al., (2006) or from other sources. Therefore, nerve histological techniques (Mu and Sanders, 2007) and retrograde neural tracing are needed to determine the nature of the LN supplying the IL and SL tongue muscles.

Comparative data on the tongue innervation are helpful for guiding some clinical and experimental studies. For example, dogs, cats, rabbits, and rats have been widely employed in many of the studies assessing the effects of the XII nerve stimulation on pharyngeal patency (Schwartz et al., 1993; Eisele et al., 1995; Gilliam and Goldberg, 1995; Oliven et al., 1996; Yoo et al., 2004; Bellemare et al., 2005). In this regard, branching and distribution of the XII nerve are critical for correctly identifying the stimulated nerve branch and design optimal electrode suitable for a given animal model. In addition, retrograde tracer studies (Sokoloff and Deacon, 1992; Aldes, 1995; McClung and Goldberg, 2002) showed that the horizontal and oblique compartments within the GG muscles of the rat and monkey are innervated by separate groups of hypoglossal motoneurons. Similar experiments are expected to be performed on other compartmentalized tongue muscles for better understanding their motor control.

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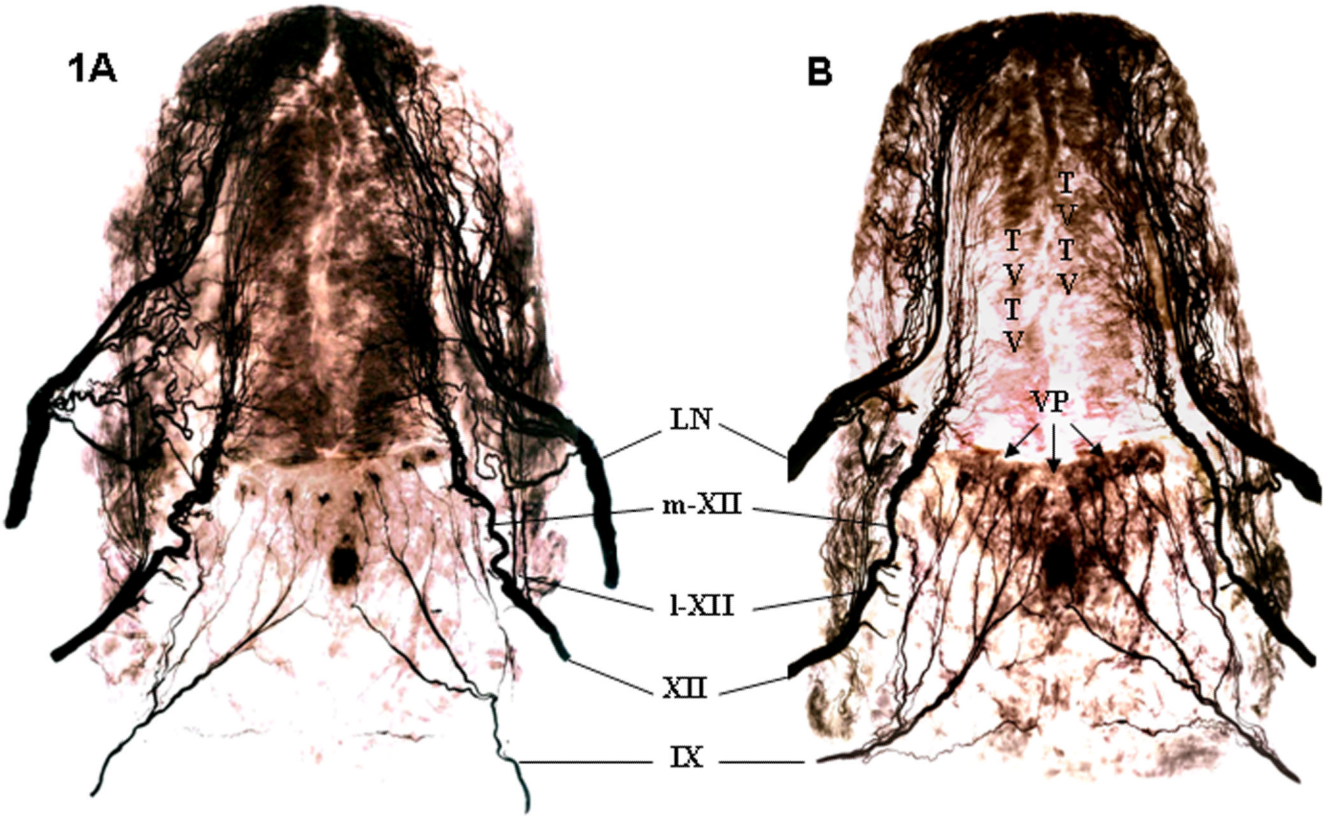

Fig. 1.
Entire nerve map of the adult human tongue. Sihler's stained specimens exhibited similar branching and distribution patterns of the nerves within the tongue. Note that some tongue muscles such as genioglossus (GG) and posterior transverse (T) and vertical (V) muscles were removed to track individual nerves. The hypoglossal nerve (XII) with its branches was located between the lingual (LN) and glossopharyngeal (IX) nerves in the posterior tongue. The diameters of the main trunks of the three nerves were ranked LN > XII > IX. The XII nerve gave off its lateral (l-XII) and medial (m-XII) branches. The l-XII was derived from the XII as a short single (left side in **A**) or multiple (right side in **A** and both sides in **B**) branches. Note that at the level of the vallate papillae (VP) the m-XII split off a bundle of branches to supply the T/V muscles in the anterior two-thirds of the tongue.

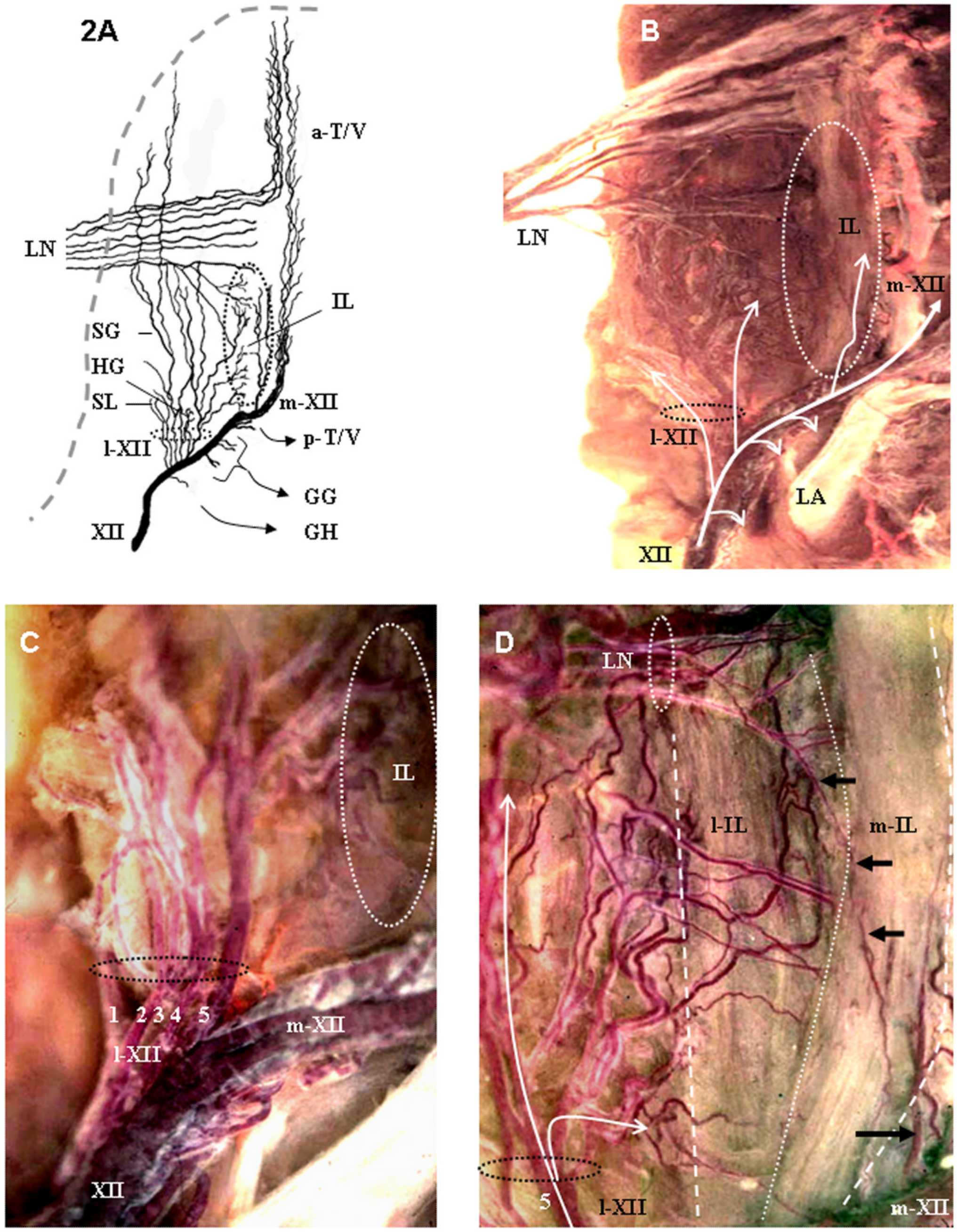

Fig. 2.

Hypoglossal nerve (XII), lateral (l-XII) and medial (m-XII) branches. **A**: Schematic drawing of nerve branching on the left hemi-tongue (ventral view) as shown in B. The branching of the l-XII (horizontal oval) and nerve supply pattern of the IL muscle (vertical oval) are illustrated. In this specimen, the lateral nerve branches arise as a group of small separate branches from the main XII nerve (horizontal oval). Five lateral branches innervate the superior longitudinal (SL), styloglossus (SG), hyoglossus (HG), and the lateral belly of the inferior longitudinal (IL) muscle. The continuation of the XII nerve after the lateral branches arise is the medial division of XII (m-XII). Small branches from the posterior part of the m-XII arise at intervals to innervate the genioglossus (GG), the posterior part of the transverse/

vertical (p-T/V) muscles, and the medial belly of the IL muscle. The main trunk of the XII continues anteriorly to innervate the anterior transverse/vertical (a-T/V) muscle group. Lingual nerve (LN) is divided into 6 to 7 main branches outside the tongue. Two or three anterior LN branches with numerous twigs travel anteriorly to the tongue tip. Three middle LN branches pass through the tongue muscles to reach the dorsal mucosa. The most posterior LN branch supplies innervation to the IL muscle and communicates with the l-XII. **B**: Sihler's stained left hemi-tongue (ventral view). Ventral view of the area of initial XII nerve branching in the posterior aspect of the human tongue. Sihler's stained nerves are difficult to see in this low-power view so they are marked by white arrows that closely correspond to the nerve branching seen in A. Note that after the XII nerve enters the tongue it gives off multiple l-XII branches. The earlier branches from the m-XII have been dissected away in this specimen. The dotted oval marks the IL muscle that is seen in more detail in D. LA, lingual artery. 3×. **C**: Magnification of the horizontal oval in B, showing the multiple branches of the l-XII. Note that the l-XII consists of five (1,2,3,4,5) first-order branches. These nerve branches supplied the longitudinally arranged tongue muscles as described in A. 12×. **D**: Close-up photograph of the region outlined in B and C (vertical oval), showing the innervation of the IL muscle. The IL muscle (vertical oval in A–C) is grossly separable into medial (m-IL) and lateral (l-IL) bellies: the outer boundaries of the whole muscle are marked by dashed lines while the boundary between the two bellies is marked by a dotted line. The m-IL has been retracted further medially to expose the nerve branches that pass between the two bellies. The fifth l-XII branch has multiple tertiary branches that innervate the l-IL, pass between the bellies, and anastomose with the lingual nerve branches (vertical oval on the top). The m-IL is innervated by distinct branch derived from the m-XII (large arrow). In addition, the posterior branch of the LN also supplies innervation to the IL (A, D; small arrows in D). 12×.

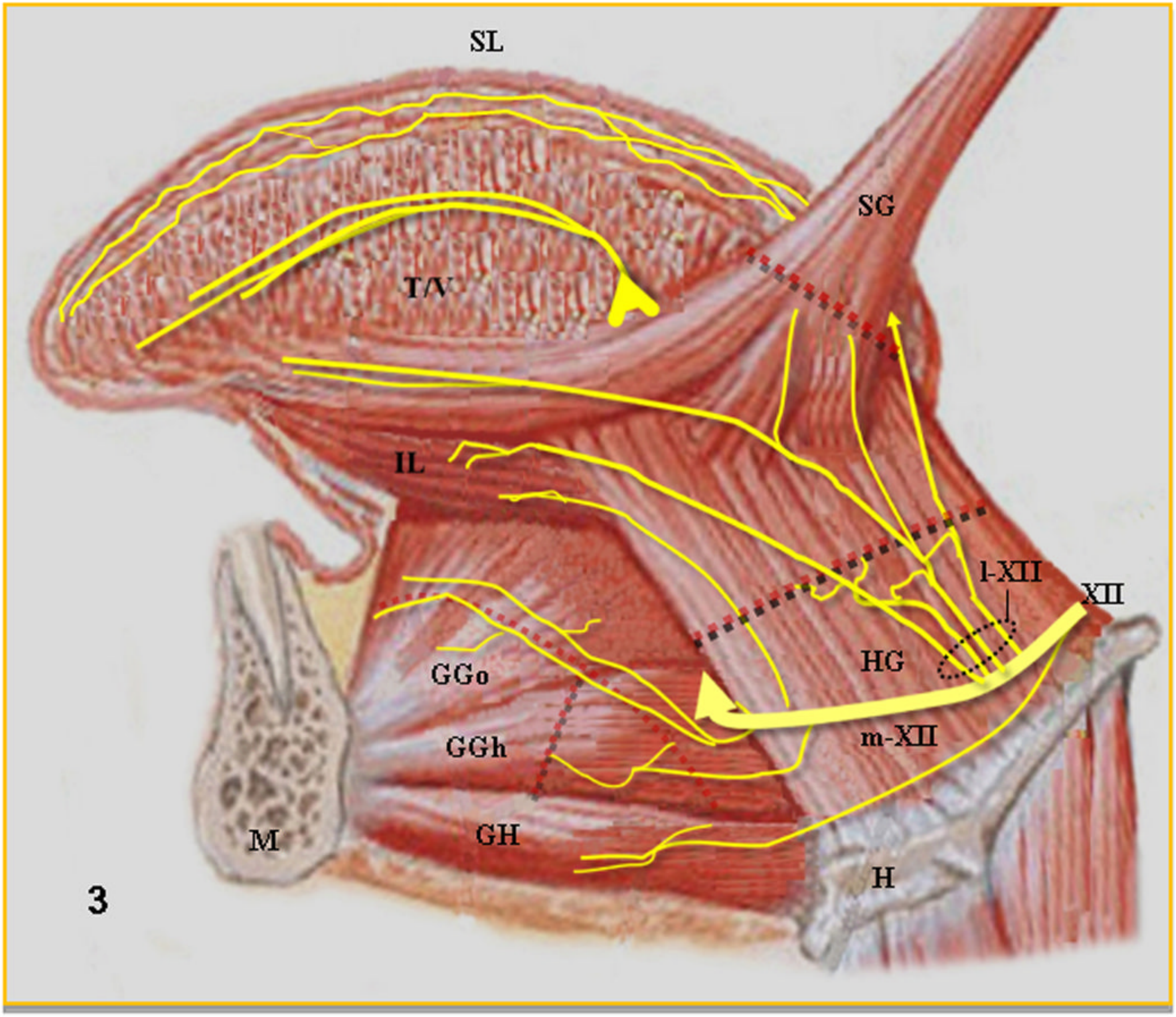

Fig. 3.
Schematic illustration of the tongue muscles and their motor innervation as revealed by Sihler's stain. Lateral view of a sagittally sectioned human tongue, showing the topographic locations of the tongue muscles and the branching and distribution of the XII nerve. Note that the XII nerve gives off four lateral branches (l-XII) to supply the superior longitudinal (SL), styloglossus (SG), hyoglossus (HG), and inferior longitudinal (IL) muscles, respectively. The continuation of the XII after the l-XII arises is its medial branch (m-XII). The m-XII gives off branches in the posterior tongue to supply the genioglossus (GG) and IL muscles. At the edge of the HG muscle, the m-XII turns medially and enters the GG at the boundary between oblique (GGo) and horizontal (GGh) compartments. It passes between the two layers of the GG and ascends up to the transverse and vertical (T/V) muscles from where it turns anteriorly. The dotted lines in the GG, HG and SG indicate the locations of the motor endplate bands in these muscles.

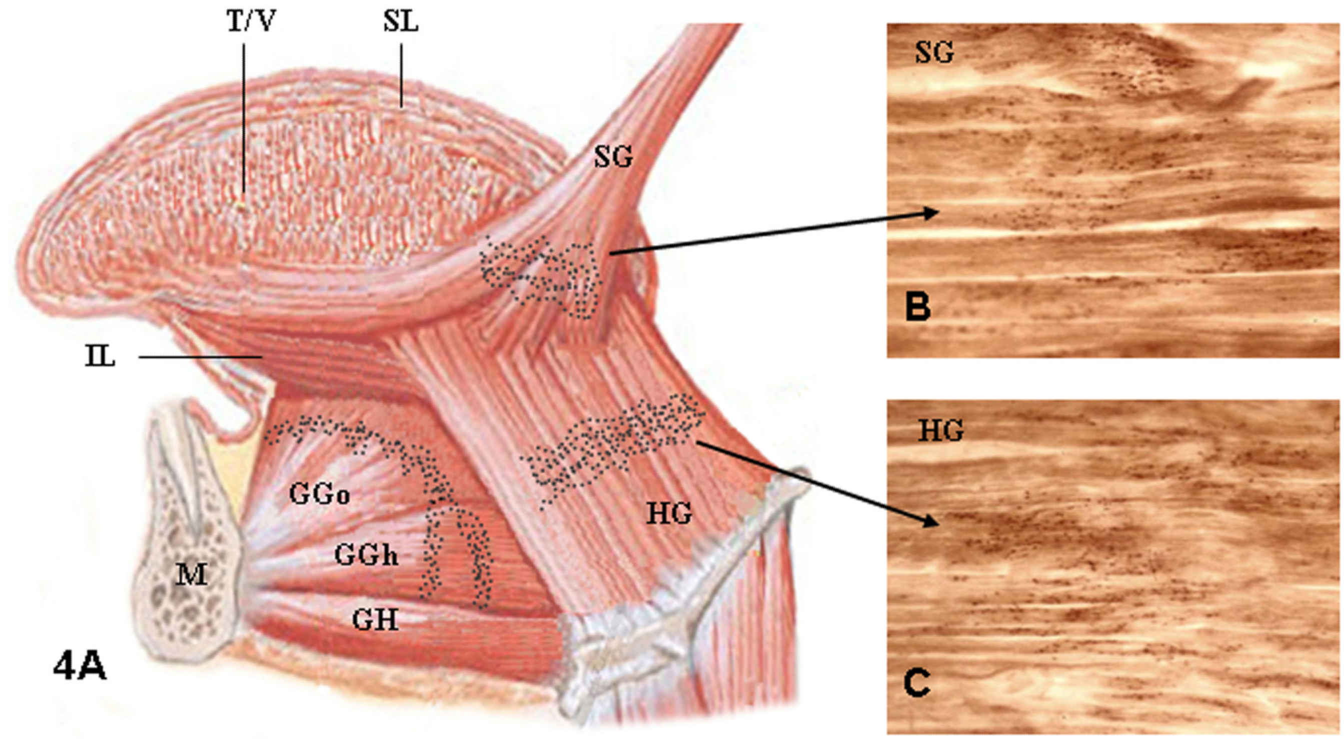

Fig. 4.

Motor endplate (MEP) bands in the human tongue muscles. **A**: Schematic of the human tongue (lateral view), showing the locations and patterns of the MEP bands in the styloglossus (SG), hyoglossus (HG), and genioglossus (GG) muscles as revealed by wholemount AChE staining. Note that the horizontal GG (GGh) has two endplate bands, whereas the oblique GG (GGo) has a single band. The MEP bands in the superior longitudinal (SL), transverse and vertical (T/V) muscles are shown in Figures 5 and 7, respectively. **B**: Wholemount AChE stained SG muscle. 9×. **C**: Wholemount AChE stained HG muscle. 9×. Note that either SG or HG has a single MEP band which is composed of numerous MEPs (black dots).

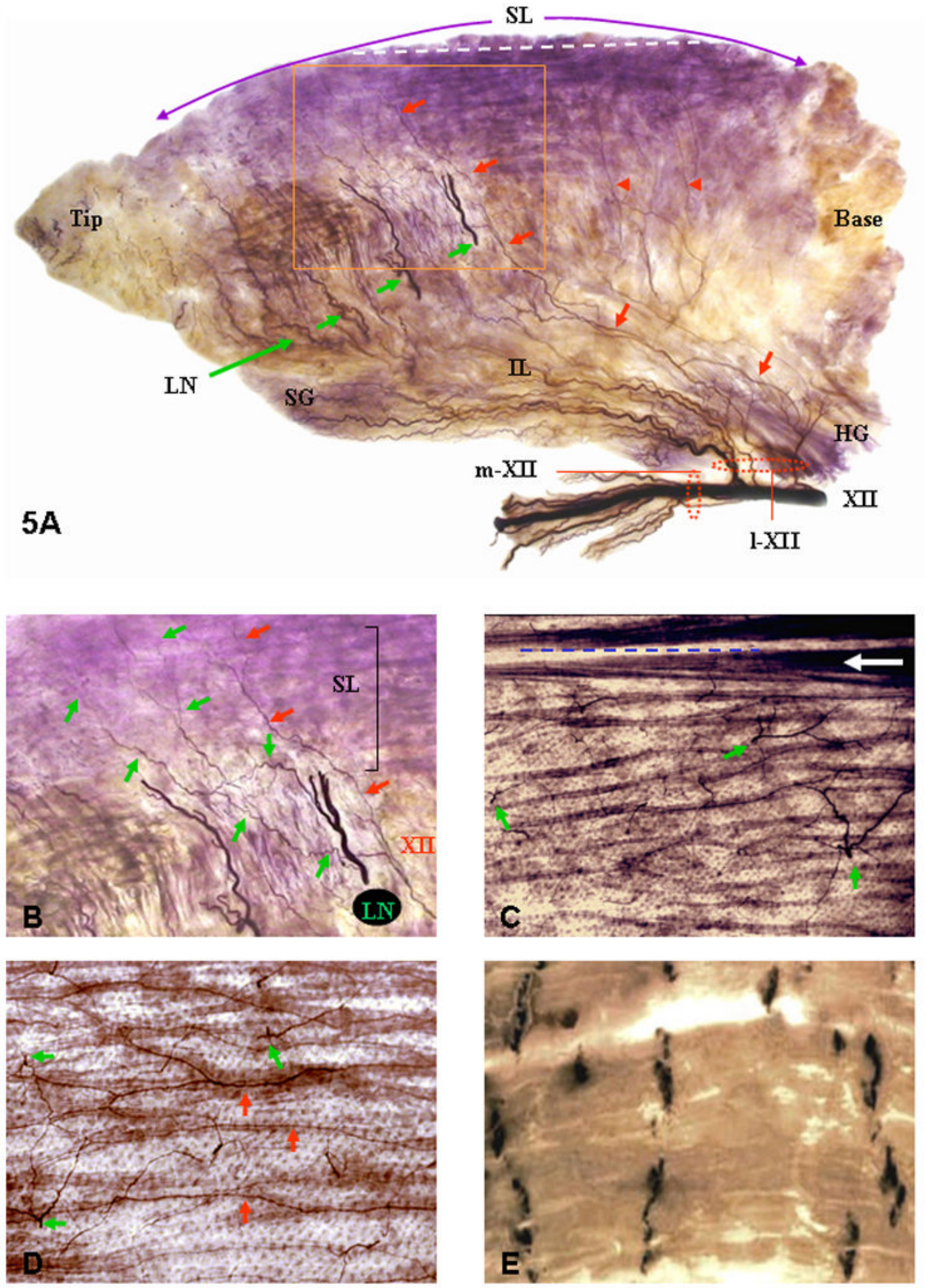

Fig. 5.

Innervation of the human superior longitudinal (SL) muscle. **A**: Lateral view of a Sihler's stained adult human tongue (left side) that has been microdissected to preserve the nerve branches from the main trunk of the XII nerve to the SL muscle. The red arrows track the path of a nerve branch from the main trunk of XII to its terminal branches within muscle fascicles of the SL. Note that the main trunk of the XII enters from lower right. Immediately after entering the tongue the lateral branches (l-XII) arise (horizontal oval). The XII then continues as the medial division (m-XII) (vertical oval). In this specimen, the first branch of the l-XII contributes innervation to the SL and hyoglossus (HG) muscles as well as a connection to the second lateral branch. Large nerve bundles arise from the third lateral branch to innervate the inferior longitudinal (IL) and the styloglossus (SG) muscles. Other nerves to the posterior SL are not seen through their whole course and are marked with red arrowheads. The branches of the lingual nerve (LN) are marked by green arrows. The white interrupted line indicates the midline of the tongue. 3×. **B**: Close-up photograph of enclosed region in A clearly shows that in addition to the XII the LN also contributes branches (green arrows) to supply the SL. 6×. **C**: Magnification of a Sihler's stained specimen, showing the arrangement of the SL muscle fascicles. Note that the fascicle (white arrow) close to the midline (interrupted line) on each side is much larger than others. Green arrows indicate the transected nerve stumps of the LN. 6×. **D**: Close-up photograph of same specimen shown in C. Note that each of the SL muscle fascicles is innervated by a distinct XII nerve branch (red arrows) which gives off terminal twigs during its course. Also note that the LN branches (green arrows) supply the SL muscle fascicles and communicate with the XII. 12×. **E**: Wholemount AChE stained SL muscle fascicles, showing that each muscle fascicle exhibited multiple MEP bands. 12×.

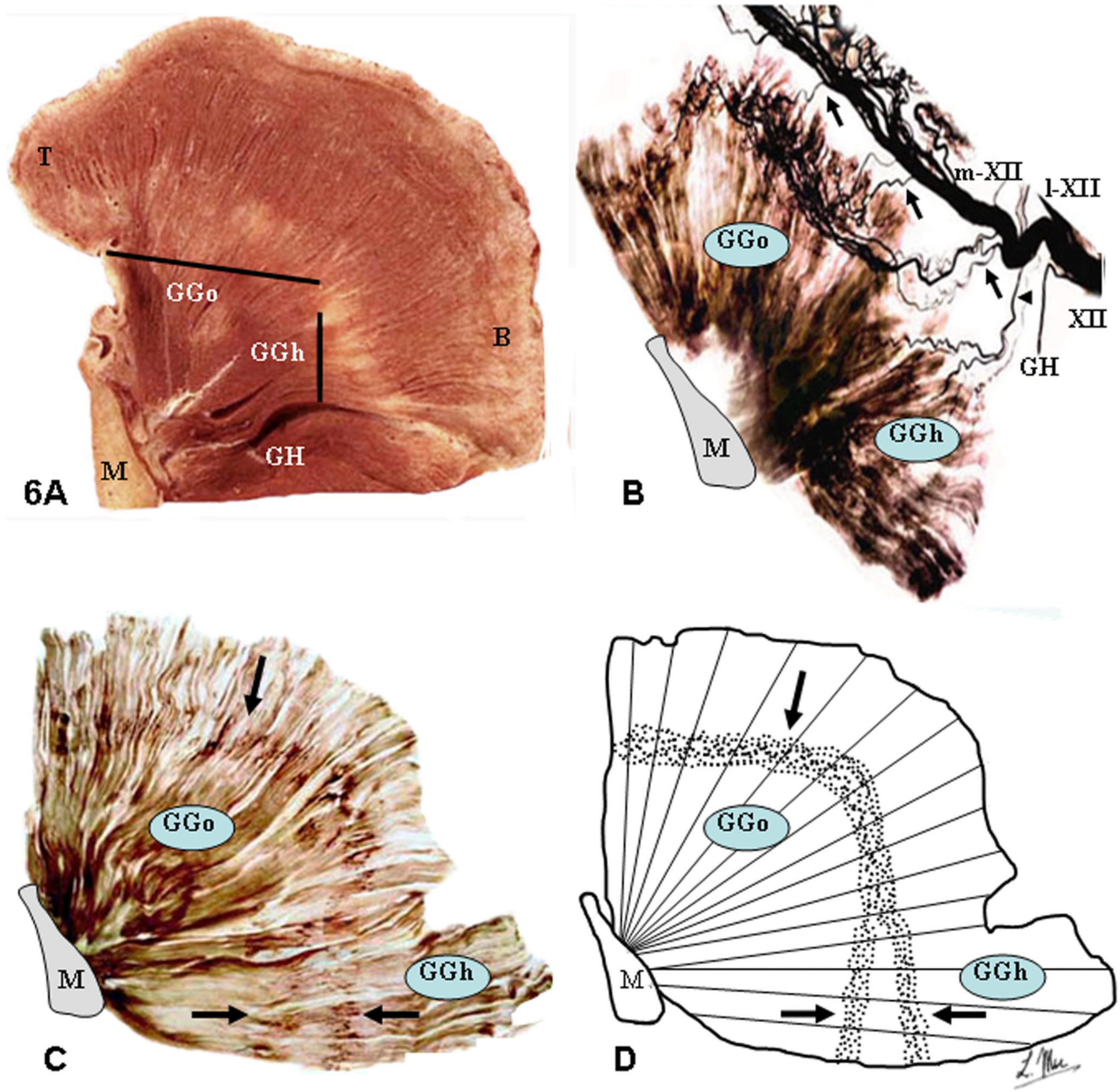

Fig. 6.

Neuromuscular compartments within the human genioglossus (GG) muscle. **A**: A midsagittal section of the tongue and mandible, illustrating the attachments of the GG muscle fibers and the locations of the horizontal (GGh) and oblique (GGo) compartments within the muscle. B, base of the tongue; GH, geniohyoid muscle; M, mandible; T, tip of the tongue. **B**: A Sihler's stained GG muscle, showing the nerve supply patterns of the GGh and GGo. Note that the GGh is supplied by a separate primary nerve branch (arrow head), whereas the GGo is innervated by several primary nerve branches (arrows) which form a dense nerve plexus. GH, geniohyoid branch; l-XII, lateral division of the XII nerve; M, mandible; m-XII, medial division of the XII nerve. **C**: A wholemount AChE stained GG muscle, showing the distribution of the motor endplates (MEPs). Note that the GGh has two

MEP bands (thick arrows), whereas the GGo has a single MEP band (thin arrow). **D**: Schematic of C, illustrating the MEP bands in the GG compartments.

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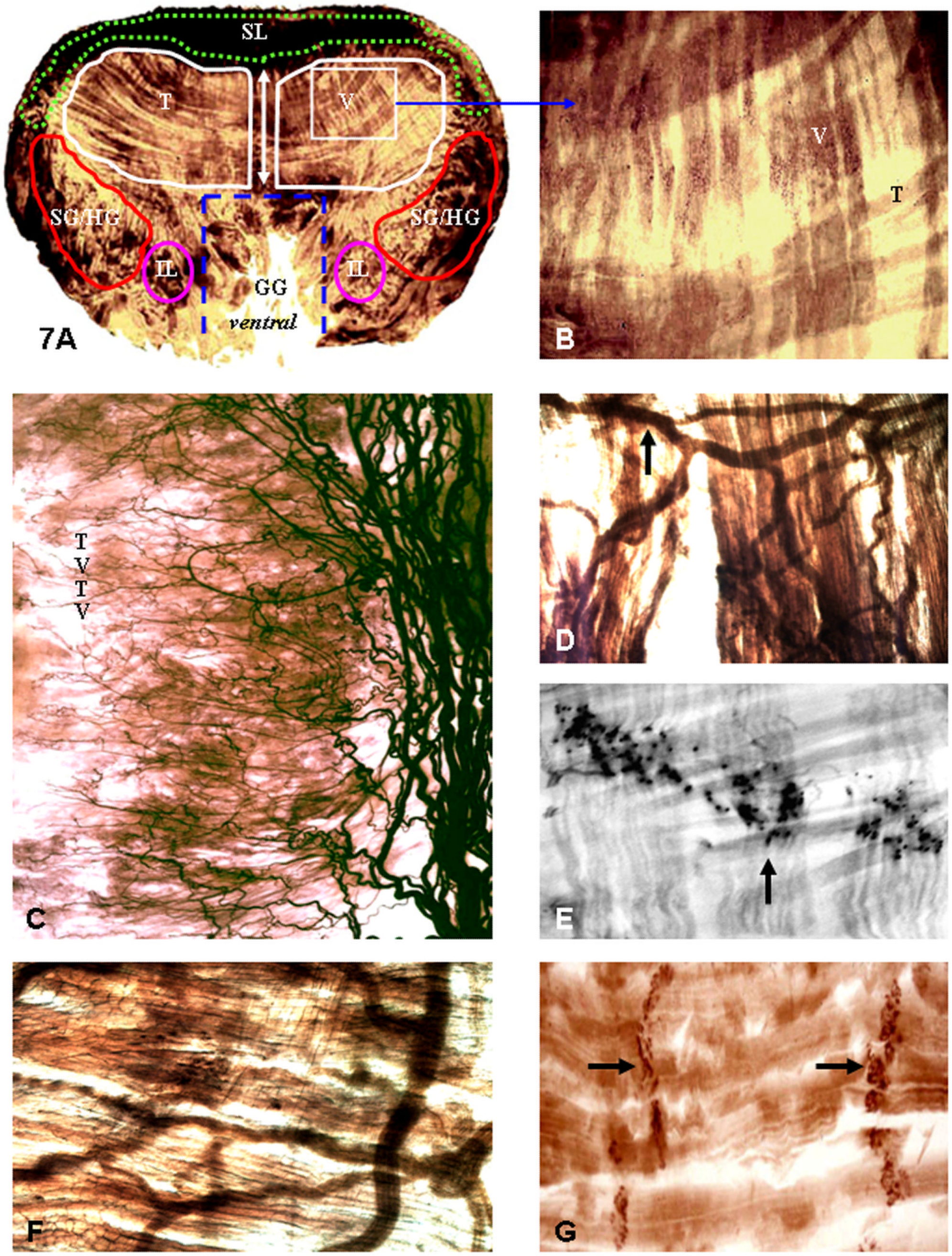

Fig. 7.

Neuromuscular organization of the transverse (T) and vertical (V) muscle fiber layers in the human middle tongue. **A**: A frontally sectioned and AChE stained meat slice from the middle tongue, illustrating the alternating fiber layers of the T and V muscles. The anatomical positions of the individual tongue muscles are outlined. The vertically placed arrows indicate the midline. Note that a T muscle fiber layer is shown on the left side, whereas a V muscle fiber layer can be seen on the right side. GG, genioglossus muscle; HG, hyoglossus muscle; IL, inferior longitudinal muscle; SG, styloglossus muscle; SL, superior longitudinal muscle. Note that most of the extra-lingual GG muscle as indicated by the interrupted lines has been removed. 2×. **B**: High-power magnification photograph of the squared region in A, showing a superficially located V muscle fiber layer and an underlying T muscle fiber layer. 8×. **C**: High-power view of Sihler's stained anterior T and V muscles in Fig. 1. Note that the medial division of the XII nerve is split off numerous primary branches (vertically oriented nerve branches shown on the right aspect of the photo) at this level, forming a dense plexus. The anterior T and V muscles are innervated by the nerve branches derived from this plexus. 12×. **D**: High-power view of an AChE and silver stained V muscle fiber layer. Note that a secondary nerve branch (arrow) which travels across the muscle fibers gives off several twigs to innervate different muscle fascicles. 16×. **E**: High-power view of an AChE stained V muscle fiber layer. Note that the V muscle fiber layer has a single motor endplate (MEP) band (arrow). 16×. **F**: High-power magnification of a T muscle fiber layer processed with AChE and silver stain, showing nerve supply pattern. 16×. **G**: High-power magnification of a T muscle fiber layer stained for AChE. Note that the T muscle fiber layer has two MEP bands (arrows). 12×.

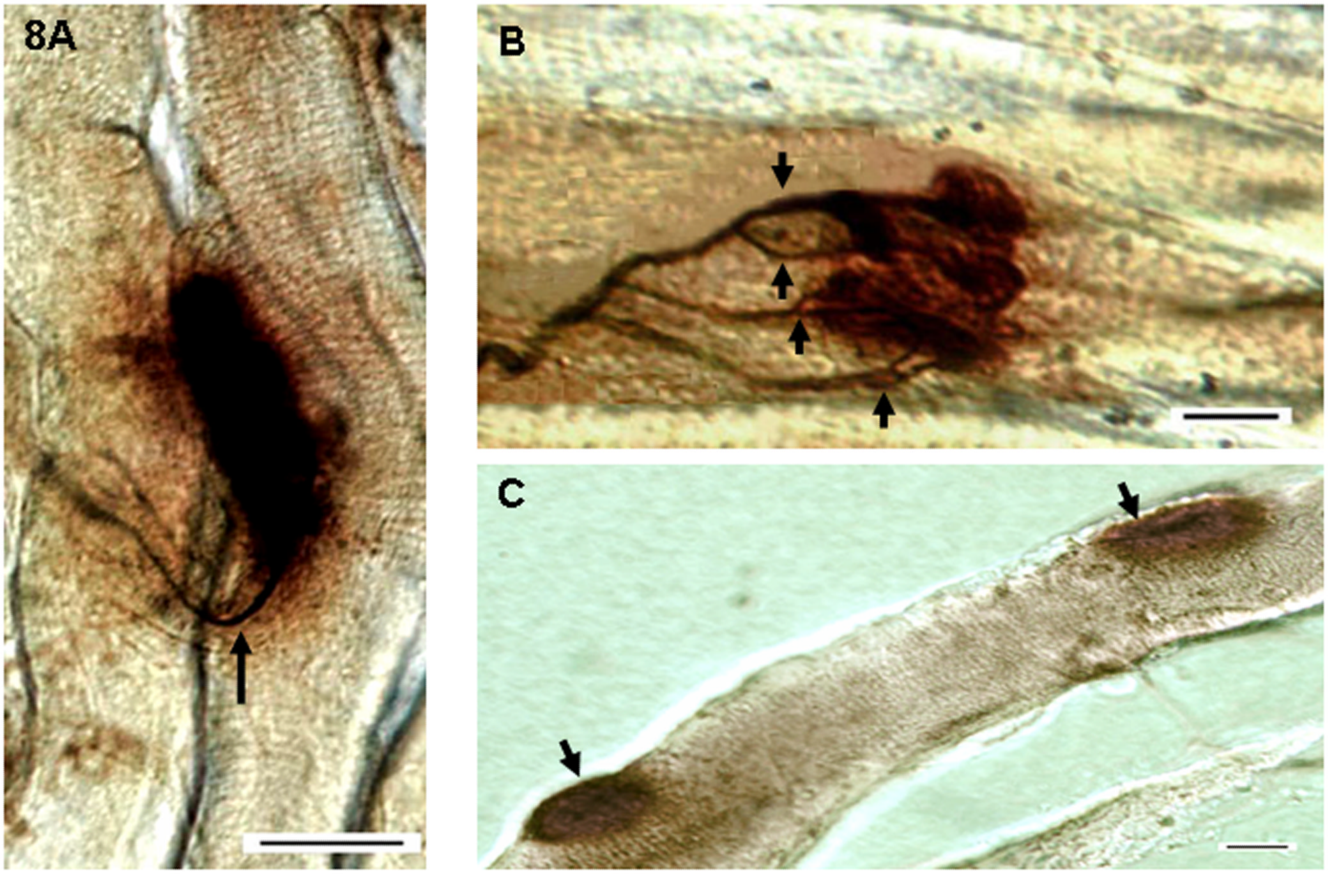

Fig. 8.
Photomicrographs of the single muscle fibers teased from the AChE and silver stained muscles in the human middle tongue, illustrating the types of the motor endplates (MEPs). Note that both *en plaque* and *en grappe* types of MEPs are present in the tongue muscles. **A**: An *en plaque* MEP with a single preterminal branch (arrow) on a vertical muscle fiber. **B**: An *en grappe* MEP with multiple preterminal branches (arrows) on a transverse muscle fiber. **C**: Two MEPs on a single muscle fiber teased from AChE stained horizontal genioglossus muscle. Bar = 50 µm.

TABLE 1

Motor Nerve, MEP Bands, and Mechanical Actions of the Human Tongue Muscles

Muscles	Attachments	Motor nerve	MEP band	Action
Extrinsic				
GGh	mandible → tongue base	m-XII	two	pulls the tongue base forward
GGo	mandible → tongue body	m-XII	one	pulls the tongue body downward
HG	hyoid bone → tongue side	l-XII	one	retracts and depresses the tongue
SG	styloid process → tongue side	l-XII	one	retracts and elevates the tongue
Intrinsic				
SL	tongue base → tongue tip (superior)	l-XII	multiple	shortens the tongue and curls the tip superiorly
m-IL	tongue base → tongue tip (inferior)	m-XII	–	shortens the tongue and curls the tip inferiorly
l-IL	the same as the m-IL	l-XII	–	the same as the m-IL
T	median septum → lateral margin	m-XII	two	narrows the tongue
V	dorsal mucosa → ventral mucosa	m-XII	one	flattens the tongue