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Microanatomy and Spatial Distribution of the Tongue Musculature of the Lesser Bamboo Rat (*Cannomys badius*)

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ABSTRACT. – The mammalian tongue consists of complex arrays of intertwining muscle fibers, the functions of which are based upon hydrostatic deformation and are directly responsible for food processing. The anatomy of tongue musculature has been well-described in the laboratory rat and mouse, representative of other rodents, but is lacking in wild species. Accordingly, this study presents a detailed description of the muscular structures of the lesser bamboo rat's tongue, a wild rodent distributed in Indochina. Primarily, serial coronal sections of three tongues were taken, stained, and histologically examined. The outlines and configurations of tongue muscles were observed and then used to construct three-dimensional (3D) visuals that facilitated evaluation of the interrelated anatomical orientation of muscles. Sagittal sections in one specimen reinforced the 3D depiction by providing details of the intricate myoarchitecture along the entire tongue length. As by-products of serial sectioning, the distributions of the lingual artery and the hypoglossal nerve were also traced at the posterior part of the tongue. The overall anatomy of the lesser bamboo rat's tongue is similar to that of the rat and mouse, but two principal noticeable differences among the three rodents were found in the anterior tongue: (i) the arrangement of the transversus muscle pertaining to longitudinal muscles and (ii) the end point of the genioglossus muscle. These orientations may be associated with movements of the tongue tip. Based on the anatomy of the tongue musculature in conjunction with its arterial and nerve supplies, types of tongue movements in the lesser bamboo rat are also proposed and explained in relation to food manipulation; enlightening the structure-function relationships for this complex muscle organ.

KEYWORDS: muscular hydrostat, rodent, tongue movement, tongue muscles, 3D reconstruction

INTRODUCTION

In mammals, tongue function is vital to food manipulation, grooming, and vocalization. This organ also contains taste receptor cells for gustatory perception. Mammalian tongues display a great morphological variability in relation to taxonomic characters and adaptations to various environmental factors, such as habitat, diet type, and feeding mechanism (e.g. Nonaka et al., 2008; Erdoğan et al., 2016; El-Bakary and Abumandour, 2017; Cizek et al., 2020). Extensive information has been reported about the microanatomy, distribution, and number of the dorsal lingual papillae. Rodents constitute the largest mammalian order in terms of the species number and diverse ecological niches. Not surprisingly, the order Rodentia shows remarkable variations in the structure and distribution of the lingual papillae (Jackowiak and Godynicki, 2005; Dinç et al., 2010; Kilinc et al., 2010; Ciena et al., 2013; Sadeghinezhad et al., 2018; Igbokwe and Mbajiorgu, 2019; Wannaprasert et al., 2020). There are also variations in the presence or absence of the median sulcus and the lingual prominence on the dorsal tongue surface (Goździewska-Harłajczuk, 2019; Wannaprasert et al., 2020). However, less attention has been paid overall to the analyses of the tongue musculature in mammals.

Mammalian tongues have been classified according to the amount of possible protrusion (Doran, 1975). Type I tongues are found in almost all mammals and usually protrude less than 50% of the resting tongue length. Type II tongues, found in pangolins and other myrmecophagous mammals, are extremely long and flexible, protruding up to 100% of the resting length. Type I tongues have a predominantly intraoral function during chewing, whereas Type II tongues are primarily used extraorally for food gathering. These functional differences can mainly be attributed to their complex muscle architecture. In classical terms, the tongue muscles are divided into two groups: the extrinsic and intrinsic muscles. The extrinsic muscles, genioglossus (GG), hyoglossus (HG), styloglossus (SG), and palatoglossus muscles, have one external attachment site to a bone and the insertion site within the tongue. The palatoglossus is sometimes not considered to be a tongue muscle but a palatal muscle (Fucci and Petrosino, 1981; Takemoto, 2001). The intrinsic muscles, transversus (T), verticalis (V), and superior and inferior longitudinalis (SL and IL) muscles, have both their origin and insertion within the tongue proper. Because of the tongue's structural complexity, its biomechanics is specialized and has been proposed to function under a muscular hydrostat model (Kier and Smith, 1985). That is, the mechanical function is more like a hydraulic system compared to the lever system used by most skeletal muscles. The connective tissue keeps the organ volume constant during muscle contractions and twists, resulting in various three-dimensional (3D) changes in the tongue shape.

Consequently, it is complicated and challenging to determine the structure-function relationships of the tongue.

With the use of varying two-dimensional and 3D imaging techniques, the tongue musculature has been described in several mammals (Doran and Baggett, 1972; Hellstrand, 1980; Mu and Sanders, 2000; Takemoto, 2008; Sanders and Mu, 2013), and this has revealed differences in the shape, size, and arrangement, particularly in the extrinsic muscles, between species. In rodents, detailed information on the tongue muscles is available only for lab rats and mice (McClung and Goldberg, 2000; Aoyagi et al., 2015), whereas other works on rats, mice, and guinea pigs have emphasized the extrinsic muscles and lack sufficient data on the intrinsic ones (Doran and Baggett, 1972; Yoshioka et al., 1979). Furthermore, the anatomy of tongue musculature in wild rodents is not currently available. Thus, the anatomy and actions of the tongue muscles representative of overall rodents are largely assumed by extrapolation from their anatomical arrangement in these laboratory rodent models.

The lesser bamboo rat *Cannomys badius* is the only species in the monotypic genus *Cannomys* within the family Spalacidae, which diverged early from other muroid lineages in the late Oligocene (Norris, 2017). It is a medium-sized rodent that is distributed across the Indochinese peninsula in a wide range of habitats from bamboo forests in the mountains to cultivated plantations and grassy areas (Lekagul and McNeely, 1988; Norris, 2017). Its skull, teeth, and feet are structurally adapted for excavating long complex tunnels of 6–56 m length and about 0.6 m depth from the soil surface (Lekagul and McNeely, 1988; Naksatit and Rojanadilok, 1988). At night, this rat forages aboveground for food and materials for nest reconstruction. It primarily subsists on plant roots and tubers growing within its tunnels and also grasses and fallen fruits near the burrow entrance (Naksatit and Rojanadilok, 1988; Norris, 2017). Sexual maturity is reached at one-year of age (Norris, 2017) and the life span can be over six years in captivity (Isarankura Na Ayudhya and Wannaprasert, 2020). Due to difficulties in habitat analysis and the need for animal tracking skills, biological data on the lesser bamboo rat are limited and studies are not easily accessible.

As mentioned above, most studies of the tongue in rodents have either described its external morphology or the microstructure of the lingual papillae. There are no detailed data on the tongue musculature in wild rodents, including the lesser bamboo rat. The purposes of the present study were to describe the complex anatomy of the lesser bamboo rat's tongue musculature and develop its simplified 3D model. Types of tongue movements in the lesser bamboo rat are deduced and considered in relation to its diet and feeding habit. Additionally, the arterial and nerve supplies of the tongue are of interest. The results are compared with previous reports on other mammalian species, particularly rodents; deepening our understanding of the structure-function relationships of tongue musculature in rodents.

MATERIALS AND METHODS

Four adult lesser bamboo rats (three females and one male) were used as the study material. The animals had an average body mass of 292.38 ± 42.70 g and skull length of 48.11 ± 3.03 mm. All were fresh corpses with intact tongues from local markets in central Thailand. After removal from the oral cavity, the gross and dimensional characteristics of the tongue were measured with a digital caliper (a precision of 0.01 mm). The mean and standard error (SE) of all measurements were calculated using the PAST v. 3.07 software (Hammer et al., 2001). To facilitate the anatomical description, the tongue was divided into three parts: the anterior, middle and posterior. The anterior was the free movable part in front of the frenulum. The middle part was the region between the frenulum and the lingual prominence, and the posterior part extended from the lingual prominence to the radix.

For histological processing, each tongue was fixed in a 10% neural buffered formalin solution for 48–72 h, subsequently dehydrated in a graded series of ethanol from 25% to 100% (v/v) and embedded in paraffin. The paraffin blocks were then cut into 5-µm-thick sections and stained with hematoxilin-eosin (H&E). Masson's trichrome stain was also used to help differentiate between collagenous connective tissue and muscular tissue.

For 3D image reconstruction of the tongue musculature, every fifth section of the serial coronal sections from each of three entire tongues (n =3) was selected. The glass slides were scanned after H&E staining using a slide scanner (Zeiss Axio Scan.Z1, USA). Scanned data sets of aligned images were uploaded into ImageJ 1.52s (USA) and concatenated to form a 3D volumetric data set. The contour and configuration of each tongue muscle were observed throughout the whole length of the tongue. All extrinsic and intrinsic muscles were included except for the palatoglossus, a tiny muscle that makes a minor contribution to tongue movement (Takemoto, 2008; Sanders and Mu, 2013) and is not generally mentioned in rodents. Collating data from several mammals was used to identify the anatomy of each muscle as accurately as possible. Additional observation of sagittal histological sections (n = 1) aided in analyzing

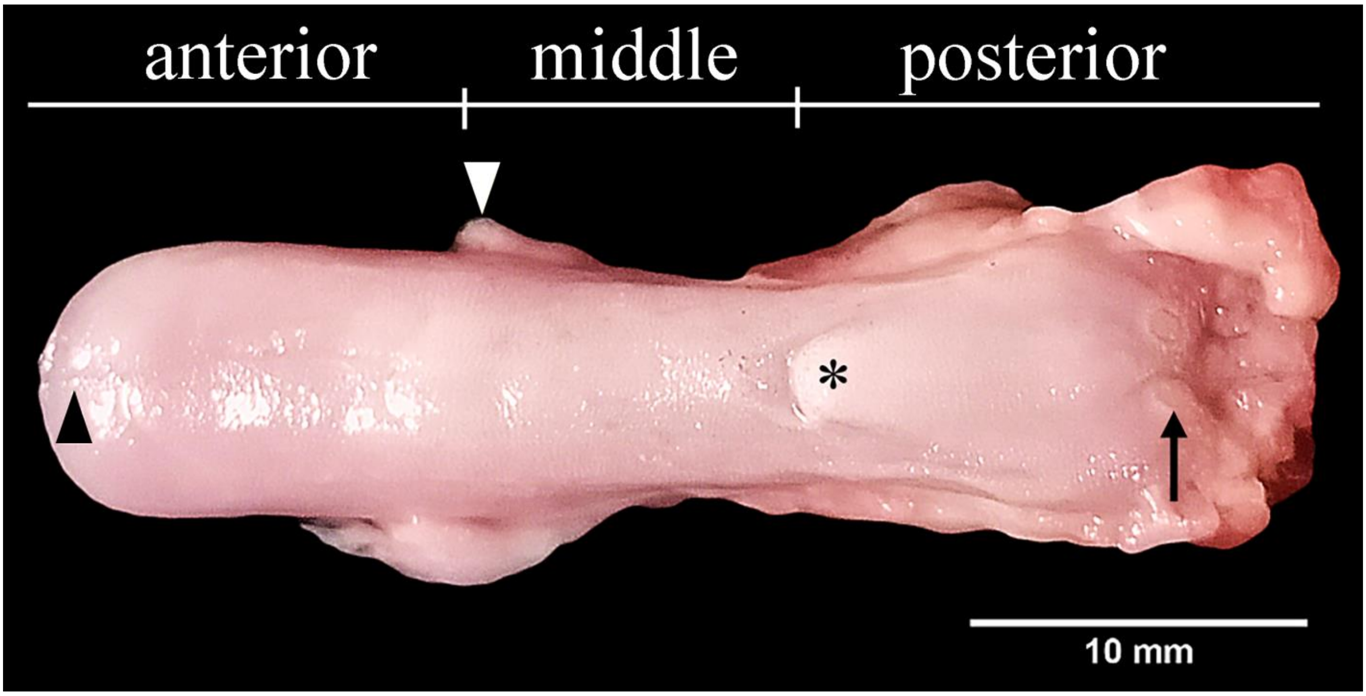

FIGURE 1. Macroscopic image of the lesser bamboo rat's tongue. The tongue was divided into the anterior, middle and posterior parts. Abbreviations: Arrow, vallate papilla; Asterisk, lingual prominence; Black arrowhead, median sulcus; White arrowhead, lingual frenulum.

the course of muscles and their relationships with each other. For 3D model construction based on the histological data, the ZBrush 4R8 software was initially used to sculpt a smooth, even surface of the tongue model. Next, the KeyShot 10 software was used to make 3D rendering, providing more accurate detailed appearances and real lighting of the model, followed by using the Adobe Photoshop CC 2020 for creating the muscle elements inside. Furthermore, the by-products of serial sectioning were an opening for roughly examining the branching and distribution of the lingual artery and hypoglossal nerve within the tongue.

RESULTS

Macroscopic features of the tongue

The lesser bamboo rat's tongue was elongated with a total length from the root to the apex of 35.76 ± 3.50 mm. The greatest width and thickness were found in the posterior part of the tongue at 9.49 ± 0.43 mm and 7.91 ± 1.07 mm, respectively. Two oval vallate papillae were located symmetrically near the radix (Fig. 1). The dorsal surface of the rounded apex had a median sulcus of 6.89 ± 0.47 mm length. The lingual frenulum anchored the tongue to the mouth floor at a distance of 10.18 ± 1.06 mm from the lingual apex.

Extrinsic tongue muscles

Genioglossus muscle (GG)

This muscle originated from the mandibular symphysis and consisted of two compartments, the horizontal and the oblique. The horizontal GG was the most posterior part of the GG and coursed posteriorly outside the tongue to insert into the tongue base and the hyoid bone. In a different manner, the oblique GG had a fan-like shape and inserted into the midline core of the tongue, flanked bilaterally by the HG and IL (Fig. 2A-C, 2G, 4B, 4C). The fascicles of the oblique GG were large posteriorly and became thinner in the middle tongue until absent after a slight extension into the tongue blade (Fig. 3F, 4F). A longitudinal path of adipose cell aggregates was observed among the GG fascicles in the midline under the intermolar prominence and the middle tongue (Fig. 2G, 3A). In the posterior tongue, the oblique GG constituted the majority of vertically-oriented fibers, crossing the T and SL layers to terminate in the lamina propria of the dorsal mucosa (Fig. 2F, 2G).

Hyoglossus muscle (HG)

The HG originated from the hyoid bone and ran into the inferolateral edge of the tongue base. The most posterior fascicles of the HG were obliquely-aligned fiber tracts with oval sections, located inferiorly to the GG and medially to the SG (Fig. 2A, 4B). The

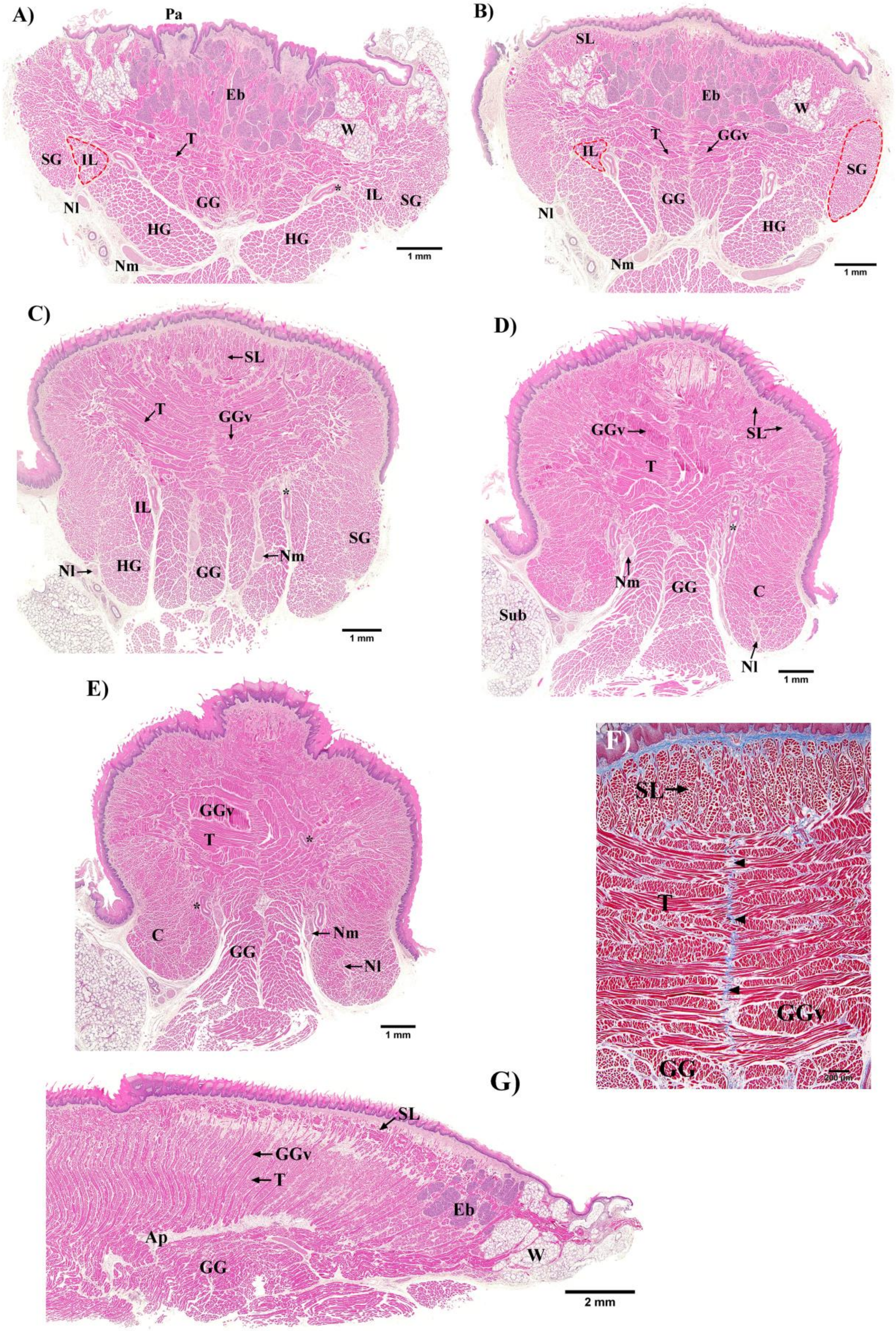

FIGURE 2. Sections through different regions of the posterior tongue of the lesser bamboo rat. Coronal sections [**(A)**–**(F)**] of: **(A)** Near the tongue radix, passing through the vallate papillae (Pa). **(B)** In front of the vallate papillae. **(C)** Proximal to the lingual prominence. **(D)** and **(E)** On the lingual prominence. **(F)** Masson's trichrome staining. The transversus muscle (T) forming alternating layers with the vertical fibers of GG (GGv). **(G)** Midsagittal longitudinal section of the posterior tongue. Abbreviations: Ap, adipose tissue; C, compound muscle; Eb, von Ebner's gland; GG, genioglossus muscle; HG, hyoglossus muscle; IL, inferior longitudinal muscle; Nl, lateral branch of the hypoglossal nerve; Nm, medial branch of the hypoglossal nerve; SG, styloglossus muscle; SL, superior longitudinal muscle; Sub, sublingual gland; W, Weber's gland; arrowheads, median fibrous septum; asterisk, lingual artery and its branches.

fascicles gradually leaned up, bordered by the GG and IL medially and by the SG laterally (Fig. 2C, 4C). At the level of the lingual prominence, the HG fascicles merged with the IL and SG to form the compound longitudinal muscle (Fig. 2D, 2E, 4D), coursing toward the anterior tongue.

Styloglossus muscle (SG)

The SG had its origin at the styloid process and ran downwards until reaching the lateral border of the tongue base. It was the most lateral of the tongue muscles, coursing superolateral to the obvious HG (Fig. 2A-C, 4B, 4C). The SG was relatively small and difficult to discern its border. At the intermolar prominence, the SG fibers were incorporated into the compound muscle, which in turn inserted into the anterior tongue.

Intrinsic tongue muscles

Superior longitudinal muscle (SL)

This muscle ran in a longitudinal direction from the posterior part of the tongue to the tongue tip beneath the lamina propria of the superolateral circumference. The SL was the only unpaired muscle of the tongue. Near the tongue radix, the arrangement of its fibers was sparse and less ordered between the vallate papillae and lingual salivary glands (von Ebner’s and Weber’s glands) (Fig. 2A, 2G, 4B). The SL thickened in the posterior tongue, except for the area under the lingual prominence, which was partly occupied by the connective tissue (Fig. 2D, 2E, 4D). The SL gradually became thinner in the middle tongue and appeared to intermingle with the compound muscle (IL, HG and SG) in the anterior tongue, forming a circumferential muscle zone surrounding the tongue core (Fig. 3B-D, 3F, 4F, 4G). When examined exhaustively, the SL consisted of abundant fascicles, between which fascicles of the V (of the GG) or T penetrated to reach the lamina propria of the tongue (Fig. 2G, 3E).

Inferior longitudinal muscle (IL)

The IL was a thin, poorly-demarcated muscle that was located lateral to the lingual arteries. The IL arose near the tongue root and ran anteriorly among the GG medially, the HG inferiorly and the SG laterally (Fig. 2B, 2C, 4B, 4C). At the level of the lingual prominence, the IL fibers were found to mingle with the HG and SG to form the compound muscle.

Vertical muscle (V)

The V was a component of the central muscles. One group of the V fibers was a continuation of the oblique GG in the medial region of the posterior and middle parts of the tongue (Fig. 2C-G, 3A, 4B-E). The other group arose more laterally from the connective tissue stroma, more visible from the distal part of the middle tongue (Fig. 3B, 4F, 4G). The V fibers projected upward and penetrated through the SL fascicles to insert into the lamina propria of the dorsal lingual surface.

Transversus muscle (T)

The T was present in the central core of the tongue, originated from the median fibrous septum. This muscle ran laterally and passed between the SL fascicles to insert into the lamina propria of the superolateral part of the tongue. In the posterior tongue, the T fibers formed well-organized alternating layers with the V fibers of GG (Fig. 2C, 2F, 4B-D). From the distal part of the middle tongue onwards, where the GG gradually disappeared, the T joined with the V fibers that originated from the connective tissue, forming a disorderly crisscross pattern at the tongue apex (Fig. 3D, 4G).

Arterial and nerve supplies

At the tongue radix, the paired lingual arteries were located lateral to the GG, above the HG and beneath Weber’s salivary glands (Fig. 2A). These arteries were considered as muscular arteries, characterized by several smooth muscle layers in the tunica media and a clearly defined internal elastic lamina in the tunica intima. Around the middle region of the lingual prominence, the lingual arteries gave off smaller branches, which ran further to supply the T and V in the core of the tongue (Fig. 2D, 2E), before ramifying laterally into other muscles along the tongue edge. The remaining arteries continued forwards lateral to the GG and medial to the compound muscle, accompanied by the hypoglossal nerve. The arteries proceeded to the middle tongue and later gave off branches in an irregular fashion within the anterior tongue.

Motor innervation for the tongue muscles was via the hypoglossal nerve on each side, which laid on the inferolateral aspect of the tongue radix. It was divided into medial and lateral branches to supply the different tongue muscles (Fig. 2A-E, 3A). The medial branch ran anteromedially beneath the HG and then moved upwards to penetrate the belly of the GG. It continued upwards and gave off branches into the core of the tongue to innervate the alternating sheets of the T and V fibers. Meanwhile, the smaller lateral division ran along the inferolateral edge of the tongue, adjoining the HG and SG. This division became the intramuscular course within the compound muscle and then continued deep to the body of the tongue to split off fine branches that supplied the SL.

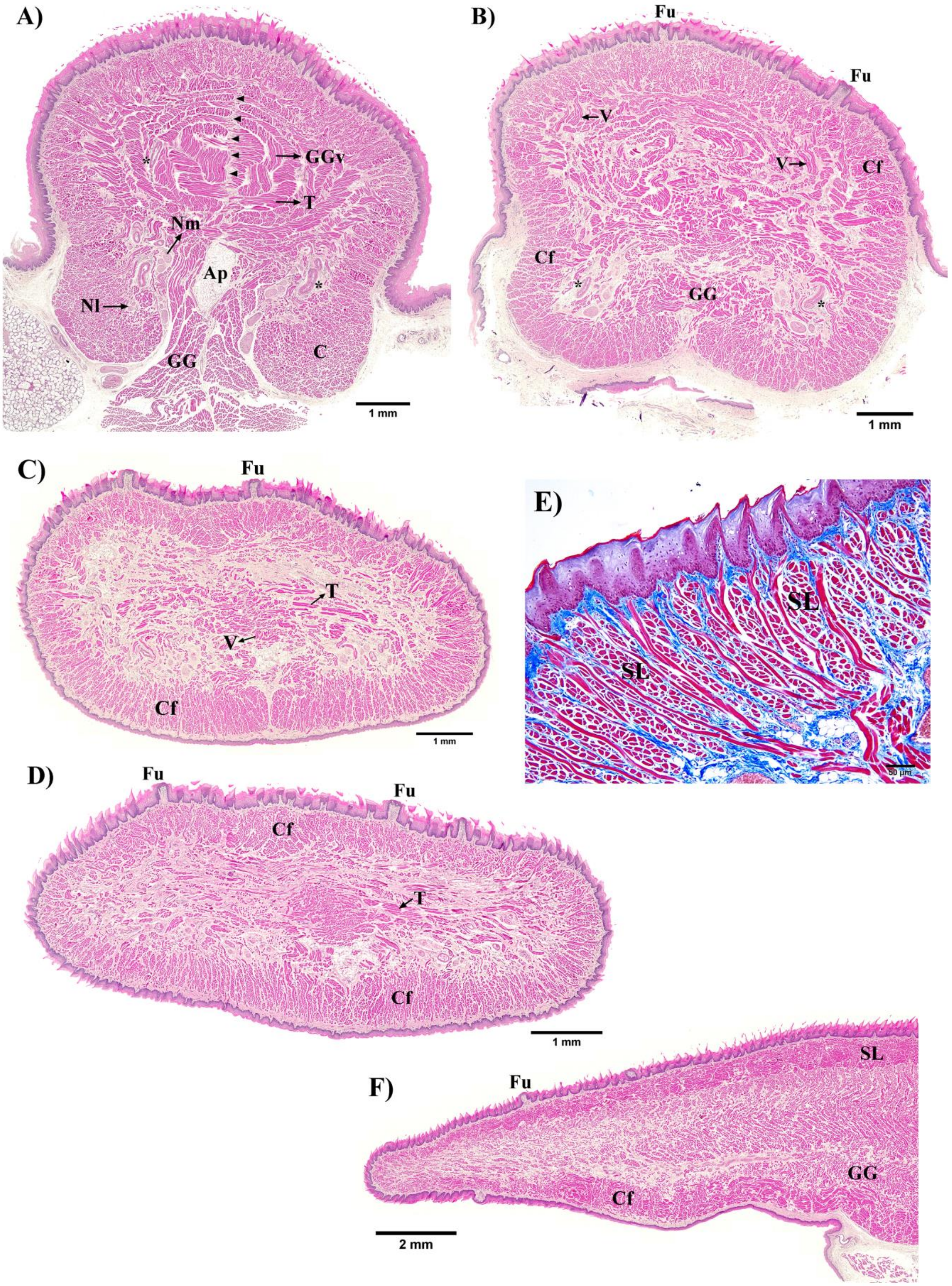

FIGURE 3. Sections through different regions of the middle and anterior tongue of the lesser bamboo rat. Coronal sections [**(A)**–**(E)**] of: **(A)** Middle part of the tongue. **(B)** Proximal part of the anterior tongue. **(C)** Distal part of the anterior tongue. **(D)** Tongue apex. **(E)** Masson's trichrome staining at the middle tongue. Fibers of other muscles penetrating between fascicles of the superior longitudinal muscle (SL) to reach the lamina propria. **(F)** Midsagittal longitudinal section of the anterior tongue. Abbreviations: Ap, adipose tissue; C, compound muscle; Cf, circumferential muscle; Fu, fungiform papillae; GG, genioglossus muscle; GGv, vertical fibers of the genioglossus; Nl, lateral branch of the hypoglossal nerve; Nm, medial branch of the hypoglossal nerve; T, transversus muscle; V, vertical muscle fibers; arrowheads, median fibrous septum; asterisk, lingual artery and its branches.

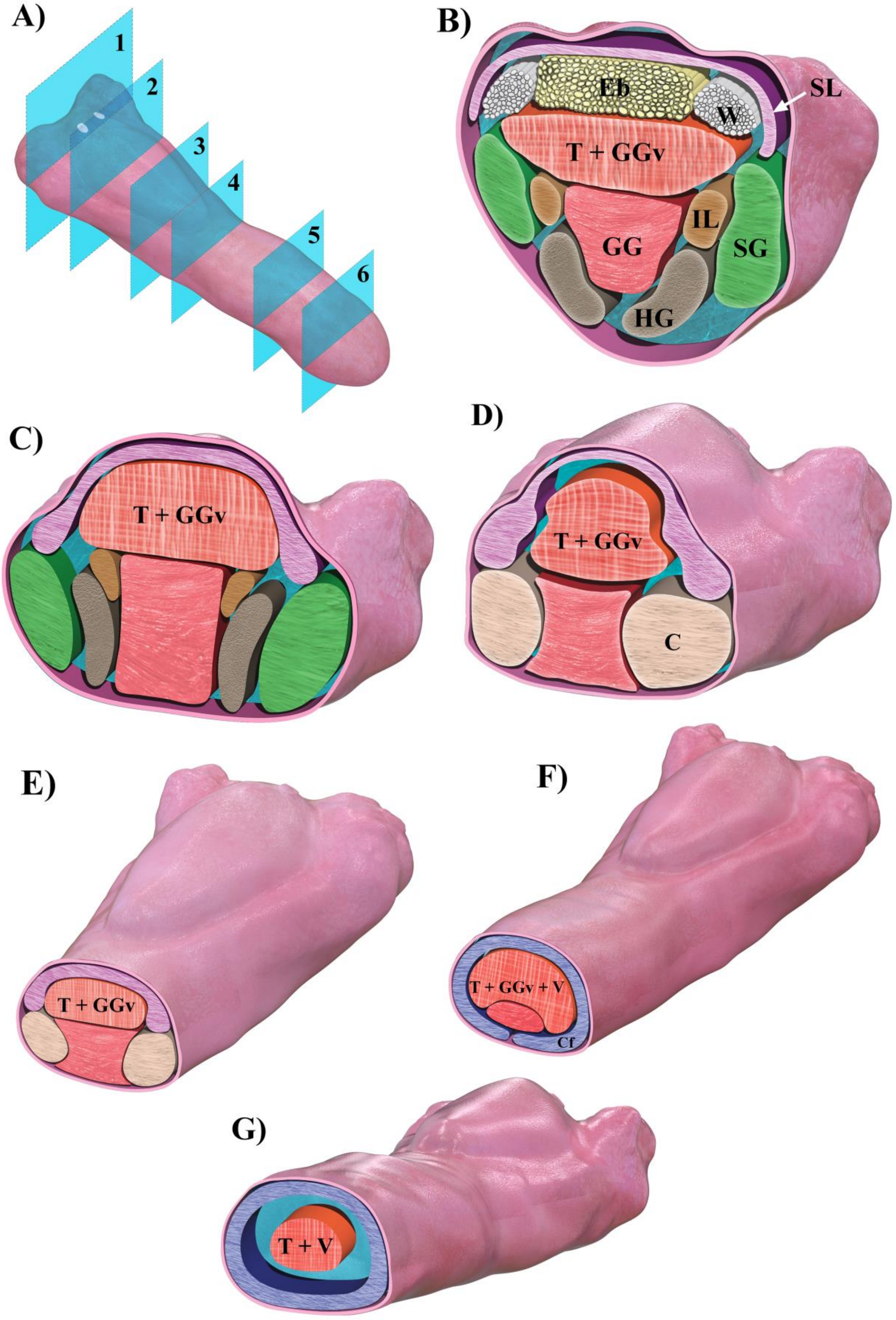

FIGURE 4. Simplified 3D model of the lesser bamboo rat's tongue and its musculature. **(A)** Six coronal planes for showing the positions of the extrinsic and intrinsic muscles. **(B)** The section of the plane 1 near the vallate papillae. **(C)** The section of the plane 2 at the posterior tongue. **(D)** The section of the plane 3 at the proximal part of the lingual prominence. **(E)** The section of the plane 4 at the middle tongue. **(F)** The section of the plane 5 at the proximal part of the anterior tongue. **(G)** The section of the plane 6 near the tongue apex. Abbreviations: C, compound muscle (light brown); Cf, circumferential muscle (blue); Eb, von Ebner's gland (yellow); GG, genioglossus muscle (red); GGv, vertical fibers of the genioglossus; HG, hyoglossus muscle (gray); IL, inferior longitudinal muscle (dark brown); SG, styloglossus muscle (green); SL, superior longitudinal muscle (purple); T, transversus muscle; V, vertical muscle fibers; W, Weber's gland (white). Cyan color represents the connective tissue.

DISCUSSION

Tongue muscles and motions

The mammalian tongue is mainly composed of intricate 3D arrays of muscle fibers. This muscular structure has no rigid elements, such as those that characterize the skeletal support systems in many animals, for example, exoskeletons in arthropods and bones in vertebrates. The tongue is proposed to be a muscular hydrostat organ, whose musculature itself not only creates motion, but also provides the skeletal support for that motion (Kier and Smith, 1985). The muscular hydrostat, like a hydrostatic skeleton found in many invertebrates, relies on the fact that water flow is essentially incompressible. Unlike the hydrostatic skeleton, where the musculature encloses a fluid-filled cavity, the tongue (muscular hydrostat) is almost entirely comprised of packed muscle masses. However, because muscles have a high water content (> 70%), water incompressibility is similarly effective in principle (Gilbert et al., 2007). Biomechanically, this causes the tongue to keep its volume constant during muscle contractions. Therefore, a decrease in one dimension will cause a compensatory increase in at least one other dimension.

A constant morphological characteristic of all muscular hydrostats in nature is the presence of their constituent muscle fibers oriented in two directions: perpendicular and parallel to the organ’s long axis (Gilbert et al., 2007). The fibers perpendicular to the long axis may be organized in a transverse, vertical, circular, or radial fashion. The transverse and vertical arrangements (T and V) are usually found in the mammalian tongue and display alternate layers within the core of the tongue. According to the muscular hydrostat theory, contractions of these muscles reduce the diameter of the tongue and simultaneously elongate it while keeping a constant volume. The fibers parallel to the long axis are arranged in longitudinal bundles that correspond to the SL and IL fibers. They act antagonistically against the perpendicular fibers to shorten and thicken the tongue. Bending of the tongue, either laterally or vertically, is produced by two synergistic contractions, unilateral contraction of the longitudinal sheath and regional bidirectional contraction of the T and V fibers (Wedeen et al., 2001; Gilbert et al., 2007; Stavness et al., 2012). The unilateral contraction results in surface shortening only, whereas the T and V contractions, which maintain a constant diameter by increasing the shear resistance, result in tongue bending (Gilbert et al., 2007).

The myoarchitecture of the mammalian tongue is organized into intrinsic and extrinsic fiber populations. By definition, intrinsic muscles are wholly contained within the tongue at approximately orthogonal directions, without any bony attachments. In contrast, extrinsic muscles have one end tethered to the surrounding bones and the other inserting into the tongue proper from several directions. The hydrostat concept as untethered intrinsic actuators has formerly been applied to the tongue, negating extrinsic influences. However, this idea is no longer tenable. It is now posited that a mechanism for isovolumetric deformation in the hydrostat theory is not fostered through contractions of intrinsic muscles alone. No single set of tongue movements (retraction, protrusion, or articulation) is attributed to only the intrinsic group. Extrinsic muscles, in fact, appear to merge seamlessly with the intrinsic fibers, anatomically indistinct since the point of their insertion into the tongue proper. Therefore, the tongue should appropriately be considered not as a set of discrete muscles but rather as a continuous array of interdigitating fibers with varying orientations and geometries. Both intrinsic and extrinsic muscles work synergistically to produce myriad changes in both the tongue position and its shape by virtue of the tissue’s hydrostatic properties (Gilbert et al., 2007; Zaidi et al., 2013). Extrinsic muscles may impose large-scale gross deformations and the intrinsic ones play a role in the aid to its fine-tuning (Gilbert et al., 2007; Zaidi et al., 2013). The coordinated activity of both muscle types also optimizes the speed and flexibility of the tongue (Gilbert et al., 2007).

This study presented the tongue muscle anatomy and generated 3D graphical depictions of the lesser bamboo rat’s tongue. Based on the arrangement and orientation of each muscle from the original serial sections in the coronal and sagittal planes and from the 3D model, movements of the lesser bamboo rat’s tongue were herein proposed. As used for descriptions of several mammals’ tongues, the movement parameters are dependent on the muscular hydrostat concept. To avoid confusion from some inconsistent definitions of the tongue movements in the literature, the terminology according to Sanders and Mu (2013) is used.

Retraction. It is the posterior movement of the tongue that usually results from combining movement of the tongue base posteriorly (retrusion) with shortening of the tongue body. Retrusion of the tongue is mainly performed by contraction of the extrinsic HG and SG, both of which run parallel to the longitudinal axis of the tongue. It is noteworthy that the HG and SG combined with the IL ventrally at the level of the intermolar prominence and may function independently or in series to retract the tongue (Smith, 1989; McClung and Goldberg, 2000). The same may occur in the anterior tongue, where the compound muscle (HG, SG and IL) intermingles with the SL.

Shortening of the tongue body can be accomplished by the SL and IL acting in concert, through relaxation of the T and V. As the tongue volume remains constant, shortening causes the tongue to thicken (increase in diameter). This configuration is useful for enhanced mechanical digestion by pushing food to grind with the palatine rugae. A large amount of the connective and adipose tissue found beneath the lingual prominence can also help bear the high grinding force produced during the digestion. Based on studies regarding myosin heavy chain (MyHC) isoform expression in the mouse and the rat, the vast majority of SL and IL fibers were positive for MyHC-2b, the isoform showing the greatest contraction speed and force but fatigue quickly (Abe et al., 2002; Maejima et al., 2005; Fogarty and Sieck, 2021). The MyHC-2b fibers are suited to generate fast but short-lasting bursts of activities, such as chewing and vocalizing (Zaidi et al., 2013). Therefore, the SL and IL fibers may be related to the increased speed of tongue movement for a high masticatory cycle (Abe et al., 2002; Maejima et al., 2005), and its large force to bolster mastication in rodents.

Protrusion. This is when the tongue moves anteriorly. The movement is composed of moving the tongue base forward (protrusion) and elongating the tongue body. Protrusion of the tongue base is likely due to the contraction of the horizontal GG and the posterior fascicles of the oblique GG. An additional action performed by the horizontal GG may be involved in respiration. The horizontal GG was inserted into the base of the tongue and the hyoid bone, so its contraction during inspiration would move the hyoid and the tongue base anteriorly, thus dilating the pharyngeal airway (Mu and Sanders, 2000; Sanders and Mu, 2013). Also, prior studies in the rat have reported that the GG is comprised of a high proportion of MyHC-1 and -2a fibers, displaying extended contractions with a high fatigue resistance (Sutlive et al., 2000; Fogarty and Sieck, 2021). The GG was suggested to be responsible for slow and long sustained activities like that required for maintaining the airway patent (John et al., 2005; Kidder et al., 2014).

Elongation is probably caused by the muscles oriented in the coronal plane within the core of the tongue, specifically the T and V. The T was predominant from the posterior part of the tongue and proceeded to clearly intermingle with the V. The combined action of these muscles reduces the diameter of the tongue and elongates it because the constant volume tongue is compressed. Tongue movements outside the mouth for licking, lapping, grooming, and panting can be accomplished by combining protrusion with elongation and tongue tip bending.

Depression and elevation. These actions are mainly controlled by the extrinsic muscles. Contraction of the V fibers of the oblique GG serves to depress the middle and posterior regions of the tongue along the midline. Additionally, the action of the HG leads to depression of the lateral edge of the tongue base. With the role of antagonist activity on the GG, the SG contributes to elevation of the posterior tongue laterally. Previous physiological studies revealed that grooved depression of the tongue in the midline and raising of its lateral margins were present during the sequential stages of deglutition (Napadow et al., 1999; Kayalioglu et al., 2007). It is possible that the GG and SG are involved and important in lingual deformation during deglutition.

Movements of the tongue tip. The tongue tip is capable of changing shape in several dimensions, such as bending upwards (dorsiflexion) and bending downwards (ventroflexion). Bending the tongue tip requires the muscular hydrostatic action achieved by unilateral contraction of the longitudinal sheath and regional contraction of the T and V. In the lesser bamboo rat, it corresponds to the circumferential muscle zone (HG, SG, IL and SL) which encloses the T and V core. That longitudinal muscles surround the T and V was likewise previously observed in the mouse (Aoyagi et al., 2015). However, tongue bending of the lesser bamboo rat and the mouse differs from that of the lab rat, which is supposed to be mediated by the cooperation of the T and the inner SL and IL that are enveloped by the T (McClung and Goldberg, 2000).

For retraction of the tongue tip, Doran and Baggett (1972) noted in the rat and guinea pig that none of the GG fibers reached into the tongue tip, and concluded that the action of the GG was not to retract the tongue tip. In the mouse, the GG showed varying degrees of extension into the anterior tongue (Yoshioka et al., 1979; Aoyagi et al., 2015). Yoshioka et al. (1979) considered that the GG could retract the tongue tip because its anterior fibers reached to half the length of the tongue blade. In the present study, the GG fibers of the lesser bamboo rat just extended slightly into the tongue blade and, therefore, seem to not retract the tongue tip.

Muscle fibers at the tongue tip of the lesser bamboo rat showed a crisscross fashion of T and V fibers. These core muscles were circumferentially surrounded by a relatively thin layer of longitudinal muscle bundles compared to the rest of the tongue. This layout may generate a flexible movement in many directions at the expense of the contraction force. With supporting findings on the presence of its numerous taste buds (Wannaprasert et al., 2020), the tongue tip of the lesser bamboo rat, and other rodents possibly, is structurally adapted for elaborate activities without the need for strength, e.g. licking on food for sampling and testing tastant stimuli.

Arterial and nerve supplies of the tongue

The present study provides the first anatomical description of blood and nerve supplies of the lesser bamboo rat's tongue. The blood supply to the tongue is primarily received from the lingual artery, arising from the external carotid artery. Due to limitations of the histological technique used and the very complex branching of blood vessels, the arterial supply of the lesser bamboo rat's tongue cannot be traced thoroughly and only the arterial distribution in the posterior tongue is roughly estimated. Little difference in the vascular supply of the tongue has been reported among some carnivores, artiodactyls, primates, rodents, and rabbits (Doran and Bagget, 1971; Hellekant, 1972). It was deduced that observations obtained from one of these species are valid in another. To be as accurate as possible, the course of arteries in the lesser bamboo rat's tongue is collated with the detailed information already reported in the rat and rabbit, as closely related orders (Nakajima et al., 1982; Shimizu, 1986; O'Reilly and Fitzgerald, 1990). As its location was sandwiched between the GG and HG and beneath Weber's salivary glands, a muscular artery on each side of the lesser bamboo rat's tongue radix was identified as the lingual artery. It gave off the branch passing into the core of the tongue before giving off small twigs to the lateral border of the tongue, which is possibly characteristic of the hyoid branch. The remaining artery ran forward alongside the hypoglossal nerve between the GG and the compound muscle. This artery, having a later approach to the middle and anterior tongue, may be defined as the deep lingual branch. Other vessels branching beyond cannot be differentiated.

The hypoglossal nerve in mammals is the primary motor nerve that innervates all tongue muscles except the vagal innervated palatoglossus. It is generally noted that the hypoglossal nerve contains two major divisions, the lateral and medial divisions, showing species difference in their distribution patterns. For example, the dog, cat, rat, and human have been employed in detailed examinations of the branching patterns of the nerve's two divisions (Hellstrand, 1981; Mu and Sanders, 1999; McClung and Goldberg, 2000; Mu and Sanders, 2010). In all four species, the medial branch supplies the GG, T and V, whereas the lateral branch innervates the SG, HG and IL. In a different manner, the medial branch also supplies the SG and HG in the cat, and the IL in human. The SL receives its innervation from the medial branch in the dog and cat, or from the lateral branch in the rat and human. According to the results of the present study, the hypoglossal nerve branching in the lesser bamboo rat is similar to that of the rat. Interestingly, it seems that the lateral branch in these rodents provides the nerve supply to the muscle group that forms the cover layer of the tongue (SG, HG, IL and SL), while the medial branch supplies the muscles that constitute the inner or the core of the organ (i.e., GG, T and V). Furthermore, from a functional point of view, the lateral division innervates the muscles working for retraction and the medial one innervates the muscles serving in protrusion. The idea regarding the "protrusor" and "retractor" subdivisions of the hypoglossal complex have been defined, primarily based on studies in the rat (Aldes, 1995; Dobbins and Feldman, 1995; McClung and Goldberg, 1999; McClung and Goldberg, 2000). Thus, the present study, describing the hypoglossal nerve branching in the lesser bamboo rat's tongue, supports this functional organization of the hypoglossal nerve innervating the tongue muscles in rodents.

CONCLUSIONS

The present study provides the first detailed information on the musculature and arterial and nerve supplies of the lesser bamboo rat's tongue. The overall anatomy of its tongue is similar to that observed in laboratory rodents. However, among rodents, the tongue body showed variations in aspects of (i) the position of the T fibers that either encircled longitudinal muscles or lay at the tongue core, and (ii) the end point of the GG in the anterior tongue. These orientations possibly result in different motions of the tongue tip, a relatively pliable part dealing with both extra- and intra-oral activities. Study of the tongue musculature together with arterial supply and innervation facilitates a better understanding of realistic tongue movements in food manipulation, serves as a useful indicator of the animal's feeding habits, and may also provide significant information for taxonomic and phylogenetic purposes.

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