The microstructure of lingual papillae in the Egyptian fruit bat (Rousettus aegyptiacus) as observed by light microscopy and scanning electron microscopy

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Summary. The microstructure of lingual papillae on the dorsal surface of the tongue of adult Egyptian fruit bats was examined by light microscopy (LM) and scanning electron microscopy (SEM). This elongated tongue with a rounded apex is approximately 3 cm long—including the 1.7 cm length of the anterior free part of the tongue—which facilitates considerable freedom of movement. The surface of the tongue has four types of lingual papillae: two types of mechanical papillae—filiform and conical papillae, and two types of gustatory papillae—fungiform and vallate papillae. Most numerous are filiform papillae with well developed keratinized processes represented by four morphological subtypes—small, giant, elongated, and bifid papillae. Our observations showed the small and giant filiform papillae to be present in the anterior part of the tongue and tilted to the back of the tongue. In the posterior part of the tongue, the filiform papillae with elongated processes were arranged on each side of the tongue and oriented perpendicularly to the median line of tongue. This arrangement of filiform papillae is considered to be useful for the efficient uptake of semiliquid food as it can be collected toward the median line of the tongue. Gustatory fungiform papillae were distributed among filiform papillae on the border of the apex and the anterior part of the body of the tongue and also on the posterior part of the tongue, while three vallate papillae surrounded by conical papillae were found on the root of the tongue. There were also taste buds along the ducts of the posterior lingual glands in the posterior-lateral part of the tongue. These morphological features are discussed in relation to adaptation to food uptake in the Egyptian fruit bat.

Introduction

Previous studies of the dorsal surface of the tongue in mammals have indicated a wide variation in the distribution and types of lingual papillae in relation to the type and manner of food uptake. Earlier anatomical and microscopic observations of bat tongues have primarily focused on insectivorous species from the families of Vespertilionidae, Rhinolophidae, Molossidae, and Hipposiderida as well as on piscivorous species from the Noctilionidae family (Azzali et al., 1991; Pastor et al., 1993; Sharma et al., 1999; Gregorin, 2003, Elizalde-Arellano et al., 2004). With regard to fruit- and nectar-eating bats, the microstructure of the tongue has been investigated in the large flying fox (Pteropus vampyrus), short-nosed fruit bat (Cynopterus brachyotis), and the species belonging to Phyllostomidae (Emura et al., 2001, 2002; Winter and von Helversen, 2003; Kobayashi et al., 2004). These findings showed the occurrence of two types of mechanical papillae (i.e. filiform and conical papillae) for food manipulation and two types of gustatory papillae (i.e. fungiform and vallate papillae) for taste perception.

The Egyptian fruit bat is a species belonging to the Pteropodidae family whose diet consists of fruit, fruit juice, and flower nectar. These animals also serve important pollinators and seed propagators (Heffner et al., 1999). The oral cavity in the nectar-,
flower- and fruit-eating bat species has several characteristic anatomical features for adaptation to the uptake of liquid food, the comminution of soft food parts, the passage of collected food, and the swallowing of food towards the pharynx (Greet and de Vree, 1984; Starck, 1995). In the dentition of fruit-eating bats, the characteristic traits include pronounced canines for cutting the surface of fruits and flattened postcanines for crushing food particles (Howell and Hodgin, 1976; Starck, 1995; Winter and von Helversen, 2003). As reported by Starck (1995) fruit-eating bats also have muscular cheeks which facilitate the sucking out of nectar or fruit flesh.

The tongues in these bats are usually elongated and the anterior part is mobile. The dorsal surface of the tongue has numerous filiform papillae which differ by the size, shape and number of the keratinized processes depending on their location on the tongue. In some nectar-eating species protractile tongues are covered by mechanical papillae which form a brush on the apex of the tongue (Muchhala, 2006).

The aim of the presented study is to describe the morphology of the tongue in the Egyptian fruit bat by light microscopy (LM) and scanning electron microscopy (SEM).

Materials and Methods

Six tongues of adult Egyptian fruit bats, donated by the Zoological Garden in Poznań, were fixed in 10% neutralized formalin. For LM, samples were collected from the apex of the tongue, the body of the tongue, and the root of the tongue, dehydrated in a graded series of ethanol (70–100%), and embedded in paraplast. Serial sections, about 4 μm thick, were stained with the Masson-Goldner method (Romeis, 1989). Morphometric data were obtained by means of a KS 400 computer morphometry system (ZEISS, Germany), and figures were obtained under an Axioscope 2 plus light microscope (ZEISS).

For the observation of the surface of lingual papillae by SEM, fixed tissues were dehydrated in a series of ethanol and acetone, and then critical point dried using CO2: (Critical Point Dryer K850, EMITECH, England). All the specimens were mounted on aluminium stubs covered with carbon tape, sputtered with gold (Sputter Coater S 150B, EDWARDS, England), and observed in an SEM Zeiss 435 VP (Germany) at accelerating voltage of 15 kV.

Results

Observations showed the tongue of the Egyptian fruit bat to be elongated with a rounded apex. It is about 3 cm long and 1 cm wide (Fig. 1). The thickness of the tongue gradually increases towards the pharynx, but there is no typical intermolar prominence of the tongue. The lingual frenulum is attached to the ventral part of the tongue at a distance of approximately 1.7 cm from the tongue apex.

Two types of mechanical lingual papillae (filiform and conical papillae) and two types of gustatory papillae (fungiform and vallate papillae) can be distinguished on the dorsal surface of the tongue. The shape and distribution of these lingual papillae are specific, depending on the region of the tongue. The ventral surface of the tongue is covered with a smooth mucosa with squamous non-keratinized epithelium whose thickness is about 82 μm at the apex and about 78.5 μm on the tongue body.

The apex of the tongue is covered mainly with small filiform papillae (Fig. 2a, b, c). These papillae are about 293 μm high, and have a base 145 μm wide. Each papilla has one major conical process which is covered with about 20 keratinized small processes—about 80 μm in length—on the anterior part. The thickness of the stratified squamous epithelium covering the surface of the papillae is about 109 μm and the keratinized layer is 14 to 58 μm thick. The fungiform papillae, with an
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Fig 2. Lingual papillae in the apex of the tongue of the Egyptian fruit bat. a: The fungiform papillae (arrows) are scattered, between small filiform papillae (sFi). gFi: giant filiform papillae with a trifurcated posterior process. SEM. b: Magnification of the small filiform papilla (sFi) and fungiform papilla (Fu). The white arrows show small anterior processes and the black arrow shows a posterior process of filiform papilla. SEM. c: Sagittal cross-section through the small filiform papillae. The connective tissue cores of papillae (C) cover the multilayered epithelium (Ep) with a thin keratin layer (arrows). Light micrograph; Masson-Goldner staining. d: Sagittal cross-section through fungiform papillae. Ep: epithelium. Arrows show the taste bud on the dorsal surface of papilla, arrowheads: thin layer of keratin on the surface of epithelium. Light micrograph, Masson-Goldner staining. Scale bars = 50 μm (a–c), 45 μm (d)

average height of 206.7 μm, are also scattered among these filiform papillae (Fig. 2a, b, d). The base of these papillae is narrow with a diameter of approximately 171 μm, while the upper part reaches 225 μm. The thickness of the surface covering the epithelium is 49–52 μm. This epithelium has 2 or 3 taste buds with a diameter of 25–30 μm (Fig. 2d).

Giant filiform papillae are present in the anterior part of the body of the tongue. They overlap each other and are arranged in group as a flat median band about 1 cm long and about 0.3 cm wide (Fig. 1, 2a, 3a). These papillae have a massive base which are covered with 13 keratinized anterior processes approximately 70 μm long on the anterior part consisting of one, trifurcated posterior process approximately 600 μm long (Fig. 3b, c). The average height of the giant filiform papillae is 412 μm, while the width of their base is approximately 286 μm. The thickness of the epithelium covering these papillae ranges from 82 to 141 μm, while the thickness of the superficial keratinized layer reaches up to 116 μm.
On both sides of the median band on the body of the tongue are small filiform papillae and fungiform papillae with structures similar to those found on the tongue apex (Fig. 3a).

In the posterior part of the tongue body—behind the giant filiform papillae—the filiform papillae become elongated and are arranged symmetrically along the longitudinal tongue axis (Fig. 1, 4a). The bases of these papillae are situated in the lateral edges of the tongue, while the long processes are tilted towards the median line of the tongue. Each filiform papilla consists of a major conical (but slightly serrated) process about 150 μm long, with 10 smaller filamentous processes (about 50 μm long) (Fig. 4b). The multilayered epithelium of these...
Fig. 5. a: A dorsal view of the surface of the root of the tongue of the Egyptian fruit bat. bFi: bifid filiform papillae, Co: conical papillae, V: vallate papillae, L: epiglottis, A: palatolaryngeal arch. b: A higher magnification of the bifid filiform papillae of the Egyptian fruit bat. On the surface of papillae appears a single cornified squama. c: The cross section through the vallate papillae of the Egyptian fruit bat. B: body of vallate papilla, Co: conical papillae surrounding the body of the papillae. Arrows show position of the taste buds, arrowheads show the excretory ducts of the lingual glands. Light micrograph; Masson-Goldner staining; Scale bar = 200 μm. d: A lateral view on the conical papillae on the left border of the root of the tongue of the Egyptian fruit bat. The arrow shows the single cornified cell desquamating from the surface of the epithelium. e: A cross-section through the conical papillae on the lateral border of the root of the tongue of the Egyptian fruit bat. The elongated connective tissue cores of papillae are covered by an epithelium with a thin keratin layer. Light micrograph, Masson-Goldner staining. Scale bar = 150 μm.
Fungiform papillae with an average height of 286 µm are present among these filiform papillae. The width of the upper part of these papillae ranges from 160 to 311 µm, while the lower part averages 136 µm wide. The thickness of the multilayered keratinized epithelium covering the fungiform papillae is similar to that on the tongue apex and is about 45 µm. These fungiform papillae usually have three taste buds about 32 µm in diameter on the dorsal surface.

Three types of lingual papillae can be distinguished on the root of the tongue: two types of mechanical papillae represented by the medially arranged bifid filiform papillae, and laterally located conical papillae and gustatory vallate papillae (Fig. 5a). The bifid filiform papilla have a wide base of approximately 150 µm which extends backward to two massive posterior processes with

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**Fig. 6.** Posterior part of the tongue of the Egyptian fruit bat. **a:** Lateral view of the surface of the posterior part of the tongue. bFi: bifid filiform papillae, Co: conical papillae, V: vallate papilla, arrows show openings of posterior lingual glands. SEM. **b:** Cross-section of the area with the opening (asterisk) and excretory duct of the posterior lingual glands of the Egyptian fruit bat. Arrow show the position of the taste bud in the epithelium of the excretory duct. GI: glands units, Co: conical papillae. Light micrograph, Masson-Goldner staining. Scale bar = 100 µm. **c:** A higher magnification of the multilayered epithelium of the lower part of the excretory duct of the posterior lingual glands of the Egyptian fruit bat. Arrows show taste buds. Light micrograph, Masson-Goldner staining. Scale bar = 30 µm.
sharp ends about 300 μm in length (Fig. 5a, b, 6a). The conical papilla on the other hand, are arranged on both sides of the root, and their single, sharpened processes are directed towards the median line of the tongue. They average 368 μm in length and about 220 μm in width at their base. The conical papillae are covered by a stratified epithelium about 36 μm thick, where the keratinized layer is on average 8 μm thick (Fig. 5f).

Three vallate papillae are present in the posterior part of the root of the tongue; they are arranged in the form of a triangle with the apex directed towards the pharynx (Fig. 1, 5a). Each vallate papilla is about 503 μm high. The width of the upper part of the vallate papillae is about 428 μm, while in the lower part it ranges from 290 to 340 μm. The vallate papillae is surrounded by a continuous circular groove approximately 470 μm deep and 100 μm wide, the opposite side of which is surrounded by a ring of partly fused conical papillae (Fig. 5c, d). The surface of the vallate papilla is covered with a non-keratinized epithelium approximately 55–60 μm thick (Fig. 5d). Taste buds about 30 μm in diameter are found in the epithelium on the lateral surface of the papillae as well as on the lateral surface of the ring around the vallate papilla (Fig. 5d).

Behind the posterior vallate papilla, the surface of the mucosa is smooth and also covered with the non-keratinized epithelium. Immediately in front of the pharyngopalatal arch on each side of the tongue, between the conical papillae, are 2–3 wide openings of lingual glands with diameters of 90–110 μm (Fig. 7a). In the stratified non-keratinized epithelium of glandular excretory ducts—both on the surface of the tongue and deeper—at secretory units of the glands in the muscular layer of the tongue are taste buds of approximately 30 μm in diameter (Fig. 6b, c). No glandular openings were found in the Egyptian fruit bat in the surface of the root of the tongue adjacent to the pharynx.

**Discussion**

The elongated tongue in the Egyptian fruit bat has a flat surface and free anterior part which facilitates the movement of the tongue while swiping the extracts of fruit pulp or licking nectar (Kunz and Jones, 2000). The issue of mobility of the tongue in the Egyptian fruit bat—the only Megachirottera species using echolocation—may be considered an important trait because it produces a series of clicks that help to locate objects along its path (Heffner et al., 1999). We could not observe such structures in mucosa on the surface of the tongue like a median sulcus or prominence in the central portion of the tongue as are often present in bats (Kobayashi and Shimamura, 1982; Emura et al., 2001; Gregorin, 2003).

Similar to other mammals, the mechanical filiform papillae with keratinized processes in bats tilted towards the back of the tongue predominate on the dorsal tongue surface. Previous SEM observations showed a considerable structural variation in filiform papillae in bat species. As reported by Greenbaum and Phillips (1974) and Emura et al. (2001, 2002) in flower- and fruit-eating bats, even 5 or 6 structural subtypes of these papillae can be distinguished.

In the present study, we confirmed the presence of three subtypes of mechanical filiform papillae in the Egyptian fruit bat—small, giant, and bifid filiform papillae—which have keratinized processes tilted posteriorly. Among them, giant filiform papillae are the most common forms of filiform papillae in bats.

We also showed the presence of two groups of symmetrically arranged filiform papillae with the elongated processes in the posterior part of the tongue in the Egyptian fruit bat. A similar pattern had been previously observed only in small nectar-eating and frugivorous marsupials—the feathertail glider (Acrobates pygmaeus) (Jackowiak and Godynicki, 2007). The appearance of such an arrangement of mechanical papillae in taxonomically different groups of mammals is a sign of convergence.

Functionally, the numerous filiform papillae increase the adhesion of the food to the surface of the tongue, resulting in efficient movement of the food towards the pharynx. Interestingly, the Egyptian fruit bat shows a unique distribution pattern of filiform papillae. All filiform papillae are oriented to the posterior in the anterior part of the tongue while elongated filiform papillae and conical papillae are tilted to the median line of the tongue in the posterior part of the tongue.

Owing to such an anisotropic arrangement of these mechanical papillae, the food particles probably move over the anterior part of the tongue and gather towards the median line in the posterior part of the tongue, and then on to the pharynx.

The distribution of mechanical papillae in the posterior part of the tongue in the Egyptian fruit bat seems to be slightly similar to the arrangement found in the short-nosed fruit bat (Cynopterus brachyotis). However, among the five morphological types of filiform papillae and conical papillae reported in this bat, have only the conical papillae on the root of the tongue had a transverse orientation. Emura et al. (2002) report in the frugivorous large flying fox that all types of filiform papillae in the posterior part of the tongue have their keratinized
processes tilted posteriorly.

The types and arrangement of gustatory papillae (i.e., fungiform and vallate papillae) in the Egyptian fruit bat are generally similar to those in other bats. However, the occurrence of fungiform papillae among the elongated filiform papillae in the posterior part of the tongue may contribute to the enhancement of taste perception of the food accumulated in this part before swallowing. The distribution of three vallate papillae in the form of a triangle in the Egyptian fruit bat is similar to frugivorous species (Emura et al., 2001, 2002), although the number of vallate papillae in bats varies; the long-nosed bat has four of them, whereas the bats from the Vespertilionidae, Rhinolophidae, Hipposideridae families and the European free-tailed bats have two vallate papillae, and the blood-drinking Desmodus rotundus has none (Greenbaum and Phillips, 1974). It should be noted that the arrangement of three vallate papillae is commonly observed in marsupials which are also an older mammalian order (Kubota et al., 1963; Krause and Cutts, 1982; Abe et al., 2001; Kobayashi et al., 2003).

To our knowledge there have been no reports on foliate papillae in bats although they are found in other mammals and are characterized by regular fissure-like furrows and abundant taste buds. However, our histological observations in the posterio-lateral part of the bat tongue showed the presence of taste buds located along the entire length of the efferent ducts of posterior lingual glands, and are characterized by regular fissure-like furrows and abundant taste buds. However, our histological observations in the posterio-lateral part of the bat tongue showed the presence of taste buds located along the entire length of the efferent ducts of posterior lingual glands.

In conclusion, we have described the surface structure of the tongue in Egyptian fruit bats, and showed the shape and distribution pattern of their mechanical filiform papillae and gustatory papillae, which probably indicate a specific adaptation to a frugivorous diet and the efficient uptake of semi-liquid food.

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