SEM Study on the Dorsal Lingual Surface of the Large Flying Fox, *Pteropus vampyrus*

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Summary: The dorsal lingual surface of the large flying foxes were examined by scanning electron microscopy. Filiform, fungiform and vallate papillae were observed. The filiform papillae were distributed over the entire dorsal surface of the tongue. The filiform papillae notably differed in morphology by their location on the tongue and could be classified into 6 types: 1) scale-like, 2) giant trifold, 3) small crown-like, 4) large crown-like, 5) long conical and 6) short conical papillae. The fungiform papillae were present rounded bodies on the anterior 2/3 of the tongue. The large flying foxes showed the triangular arrangement of the three vallate papillae, with the apex of the triangle directed posteriorly. These findings indicate that the tongue of the large flying bat is similar to that of the lesser dog-faced fruit bat.

Many works have been published on the three-dimensional structures of the lingual surfaces in various animals. In the order Chiroptera, there have been some scanning electron microscopy (SEM) studies of the tongues of bats (Greenbaum and Phillips, 1974; Howell and Hodgkin, 1976; Kobayashi and Shimamura, 1982; Pastor *et al.*, 1993; Emura *et al.*, 2001a). Such studies reveal variations in morphology and distribution of papillae on the dorsal lingual surface among animal species.

However, there is no study on the scanning electron microscopy of the tongue of the large flying fox. The purpose of this study is, therefore, to examine three-dimensionally the dorsal lingual surface of the large flying fox, in order to compare the results with those previous reports in other mammals.

Materials and Methods

Tongues from 4 male and 2 female large flying foxes (*Pteropus vampyrus*) of the order Chiroptera, weighing from 500 g to 1,000 g, were used in this study. The tongues were fixed in 10% formalin. The

tongues were washed in 0.1 M phosphate buffer and postfixed in a solution of 1% osmium tetroxide in the same buffer at room temperature for 2 h. Thereafter, the specimens were dehydrated through graded series of acetone, critical-point-dried and sputter-coated with gold before being examined under SEM (Hitachi S-3500N, Tokyo, Japan) at an accelerating voltage of 15 kV.

Results

Macroscopically, the tongues of the large flying foxes are relatively long and tapering. The tongues range in length from 6 to 7 cm (Fig. 1). Three vallate papillae arranged in an inverted V-shape are observed on the dorsum.

The filiform papillae are distributed over the entire dorsal surface. The filiform papillae differed in morphology by their location on the tongue and can be classified into 6 types. Type 1 is scale-like papilla, at the tip of the tongue (Fig. 2). Type 2 is giant trifold papilla, on the midportion of the anterior 1/3 (Fig. 3). Type 3 is small crown-like papilla, on both lateral regions of the anterior 1/3 of the

tongue (Fig. 3). The transitional form between the small crown-like and giant trifold papillae are observed on the border of those papillae (Fig. 3). Type 4 is large crown-like papilla, on the mid-portion of the posterior 1/3 (Fig. 4). Type 5 is long conical papilla, on both lateral regions of the posterior 1/3 (Fig. 5). Type 6 is short conical papilla, on the midportion of the posterior 1/3 (Fig. 6 and 6 inset). The filiform papillae are not observed in the region of the vallate papillae and lingual radix (Fig. 6).

There are fungiform papillae scattered among the filiform papillae, excepted for the regions of type 2 and 5 (Fig. 3). The fungiform papillae have no twigs which are observed in the filiform papillae of type 1, 3 and 4 (Fig. 3).

Fig. 1. Macrographs of the tongues of the large flying foxes. (a), (b), (c), (d) and (e) show the regions prepared for SEM observations. The tongues are relatively long and tapering.

Fig. 2. SEM micrograph of region (a). T1 = scale-like papillae. Juvenile bat.

Fig. 3. SEM micrograph of region (b). Showing the filiform papillae of 2 types and fungiform papillae (*). T2 = giant trifold papilla; T3 = small crown-like papilla; I = transitional forms between T2 and T3. Juvenile bat.

At the posterior end of the lingual body, the triangular arrangement of the three vallate papillae, with the apex of the triangle directed posteriorly, is observed and each vallate papilla is surrounded by a deep groove and pad (Fig. 6).

Discussion

The food habits of the bats are very diversified: insectivorous, fruit-eating, flower-eating, vampire and carnivorous feeding. These differences resulted in various adaptations in the number and the morphology of the teeth. The tongue was no exception and underwent many changes.

SEM studies of the lingual papillae have been performed in the order Chiroptera. In four species of long-nosed bats (Greenbaum and Phillips, 1974), Japanese long-fingered bat (Kobayashi and Shimamura, 1982), European common bat (Pastor *et al.*,

1993) and lesser dog-faced fruit bat (Emura *et al.*, 2001a) different types of the filiform papillae are distributed over restricted areas of the lingual dorsal surface. The filiform papillae of the long-nosed bat (flower-eating) are classified into 5 types (Greenbaum and Phillips, 1974), the Japanese long-fingered bat (insectivorous) into 6 types (Kobayashi and Shimamura, 1982), the European common bat (insectivorous) into 3 types (Pastor *et al.*, 1993) and the lesser dog-faced fruit bat (fruit-eating) into 5 types. The present study on large flying foxes indicated that the filiform papillae could be classified into 6 types. The filiform papillae similar to the giant trifold papillae (type 2) has been observed in the tongue of the lesser dog-faced fruit bat (Emura *et al.*, 2001a). Iwasaki and Sakata (1985) have reported that vallate papillae were encircled by filiform papillae in the posterior body of the beagle dog. The present study on the large flying fox indicated that the filiform papillae were not observed

Fig. 4. SEM micrograph of region (c). T4 = large crown-like papillae. Adult bat.

in the region of the vallate papillae and lingual radix. Similar results have been reported on the dorsal lingual surface of the lesser dog-faced fruit bat (Emura *et al.*, 2001a).

Greenbaum and Phillips (1974) have reported that in two species of bats (flower-eating) there are two large, bifid, horny papillae located next to each other along the midline of the tongue. Kobayashi and Shimamura (1982) have reported that in the Japanese long-fingered bats (insectivorous) the tongue has a lingual prominence in the posterior half. Emura *et al.* (2001a) have reported that in the lesser dog-faced fruit bat (fruit-eating) a concave aspect was observed on the central portion of the tongue. In the present study, the tongue of the large flying fox was morphologically similar to that of the lesser dog-faced fruit bat (fruit eating), but a concave aspect was not observed. The body weight of the large flying fox differs much from the lesser dog-faced fruit bat, however the food habits in two bats are very similar. Difference in food habit is

considered to result in adaptation in the morphology of the tongue.

In many animals, the fungiform papillae are scattered almost uniformly among the filiform papillae, mainly on the anterior part of the lingual body (Emura *et al.*, 1999a, b, c, 2000a, b, c). In this study, the fungiform papillae were scattered among the filiform papillae, excepted for the regions of type 2 and 5. This pattern of distribution of the fungiform papillae is different from that in many other animals.

The number of the vallate papillae has been reported in many vertebrates. There was a single vallate papilla on the tongue of the squirrel monkey (Iwasaki *et al.*, 1988). There were two vallate papillae in the Japanese long-fingered bat and nutria (Kobayashi and Shimamura, 1982; Emura *et al.*, 2001b) while the long-nosed bat had four (Greenbaum and Phillips, 1974). On the tongue of the bush dog, Emura *et al.* (2000c) observed five vallate papillae. Nine to twelve vallate papillae were found in

Fig. 5. SEM micrograph of region (d). T5 = long conical papillae. Adult bat.

the one-humped camel (Qayyum *et al.*, 1988). There were about 24 in the Japanese and Formosan serows (Funato *et al.*, 1985; Atoji *et al.*, 1998) and 60 in the black rhinoceros (Emura *et al.*, 2000b). Three vallate papillae were also found in the tongues of the marsupials (Kubota *et al.*, 1963; Krause and Cutts, 1982), primates (Kubota and Hayama, 1964; Emura *et al.*, 2002), rodents (Kubota and Togawa, 1966; Emura *et al.*, 1999b), lagomorphs (Kubota, 1966) and insectivores (Chunhabundit *et al.*, 1992). In this study, the large flying foxes showed the triangular arrangement of the three vallate papillae, with apex of the triangle directed posteriorly. Considering the number of the

vallate papillae on the tongue surface, it is possible to suggest that the number of the vallate papillae may be different among the species. However, additional study is required to clarify a relationship between the species and the number of the vallate papillae.

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Fig. 6. SEM micrograph of region (e). T6 = short conical papillae; V = vallate papillae. Juvenile bat. Inset = adult bat.

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