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Authors: Ojima, Koei, Takeda, Mamoru, Saiki, Chikako, and Matsumoto, Shigeji

Source: Zoological Science, 13(4) : 533-535

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.13.533

ZOOLOGICAL SCIENCE **13**: 533–535 (1996)

Angioarchitectural Classification of the Fungiform Papillae in the Cat Tongue

Koei Ojima* , Mamoru Takeda, Chikako Saiki and Shigeji Matsumoto

Department of Physiology, School of Dentistry at Tokyo, The Nippon Dental University, 1-9-20, Fujimi, Chiyoda-ku, Tokyo 102, Japan

ABSTRACT—Three-dimensional structures of the microvascular network of the fungiform papillae (FuP) of the cat tongue were observed by the corrosion cast method under a scanning electron microscope (SEM). FuP were found to be distributed sporadically among the different types of numerous filiform papillae (FiP). A single FuP consisted of the main process (MP) and the accessory process (AP) which were arranged at the anterior basal margin of the MP. FuP can be classified into four types (I~IV) according to the shape and size of the MP and the number of AP within each FuP. FuP-I to FuP-III contained a large, medium and small MP, showing the small fishnet-ball and oval-ball shapes, and these were surrounded at the anterior basal margin by AP inclining toward the anterior part of the tongue. FuP-IV contained only the small fishnet-ball-shaped MP.

INTRODUCTION

Many previous studies have focussed on the morphology of the lingual papillae on the dorsal surface of the cat tongue such as a whole morphological study of the FiP (Boshell *et al.*,1982) and a comparative study of the lingual papillae of cats and rabbits under SEM (Chamoro *et al.*,1987). Furthermore, there are also many studies in which the connective tissue cores of the lingual papillae were investigated by means of SEM (Iwasaki *et al.*,1987; Iwasaki, 1990,1993a, b; Kobayashi *et al.*,1988; Kobayashi and Iwasaki, 1988; Kobayashi, 1992). Recent SEM evidence has made it possible to establish a classification of FiP with microvascular cast specimen (MVCS) of the lingual papillae (Ojima and Lowe, 1995). However, these observations described only the morphological characteristics of the FuP in representative areas of the dorsal surface of the tongue (Chamoro *et al.*,1987; Kobayashi and Iwasaki, 1988; Kobayashi, 1992); little is known about the comparative morphology of the cat tongue with MVCS examined under SEM. The present study was conducted to obtain further information on the microvascular morphology of the FuP of the cat tongue which might be used for purposes of classification.

MATERIALS AND METHODS

Tongues from 38 Japanese crossbred domestic cats, *Felis catus*, were used in this study. Cats of both sexes (11 females and 27 males) were obtained from a commercial supplier and were adults of unknown age. Each cat weighed between 2.5 and 5.0 kg. They were killed with an over-dose of pentobarbital sodium (Nembutal) by intraperitoneal injection. Their tongues were immediately removed and both lingual arteries were cannulated and prepared for the macroscopical, stereomicroscopical and SEM observations.

For SEM observation, in general, after each tongue surface was rinsed with 0.9% physiological saline solution or Ringer's solution at 35~37°C, vascular perfusing with 0.9% physiological saline solution or Ringer's solution was done until all blood in the jugular veins had been replaced by the solution, then 5% glutaraldehyde solution in phosphate buffer (pH 7.4) was injected into both lingual arteries to fix the vessels and their tributaries.

Both lingual arteries were filled with synthetic resin (Mercox CL-2R or CL-2B, Dainippon Inki Chemical Co., Ltd., Tokyo, Japan) under manual pressure. The injected specimens of the tongue were fixed for 0.5 hr at room temperature and incubated for 0.5 hr at 55°C.

The specimens were then polymerized completely, immersed in 20% KOH or NaOH solution for over 3 hr at room temperature and then the soft tissues of the tongue were dissolved with those solutions. The finest macerated vascular samples were washed 2~3 times with warm water at 45~55°C and gently dried for 2~3 days at 55°C in an incubator.

The MVCS of the whole tongue were mounted on specimen stubs, sputter coated with gold-palladium and then examined with a S-4000 type scanning electron microscope (HITACHI,Tokyo, Japan) at an accelerating voltage of 5-10 kV.

RESULTS

Macroscopic examination revealed sporadic mushroom-shaped FuP that were scattered among the numerous, and different types of FiP were found to be distributed over the entire dorsal surface.

* To whom correspondence should be addressed.

The MVCS of the FuP at the anterior dorsal surface of the cat tongue consisted primarily of capillary loops, whose fine characteristic shape resembled a hairpin or horseshoe. These capillary loops entering the base of each FuP were arranged radially and geometrically from the middle third to the periphery of the tongue and contained many complex anastomoses. Because of their rich and complex capillary network, the FuP generally appeared as fishnet-balls at lower magnification.

In SEM observations at higher magnification, MVCS of the FuP of different types of shapes, sizes and distribution observed at the top, on both sides and at the dorsal center part of the anterior surface of the tongue. At the anterior top and on both sides in the anterior third, the capillary loops of FuP resembled the small fishnet-ball- or oval-ball-shaped of the papillae (FuP-IV) (Fig. 1C).

There were three types of FuP, small (FuP-III), medium (FuP-II) and large (FuP-I) from the periphery to the center part of the midline at the junction of the anterior and the medial thirds (Fig. 1A, B).

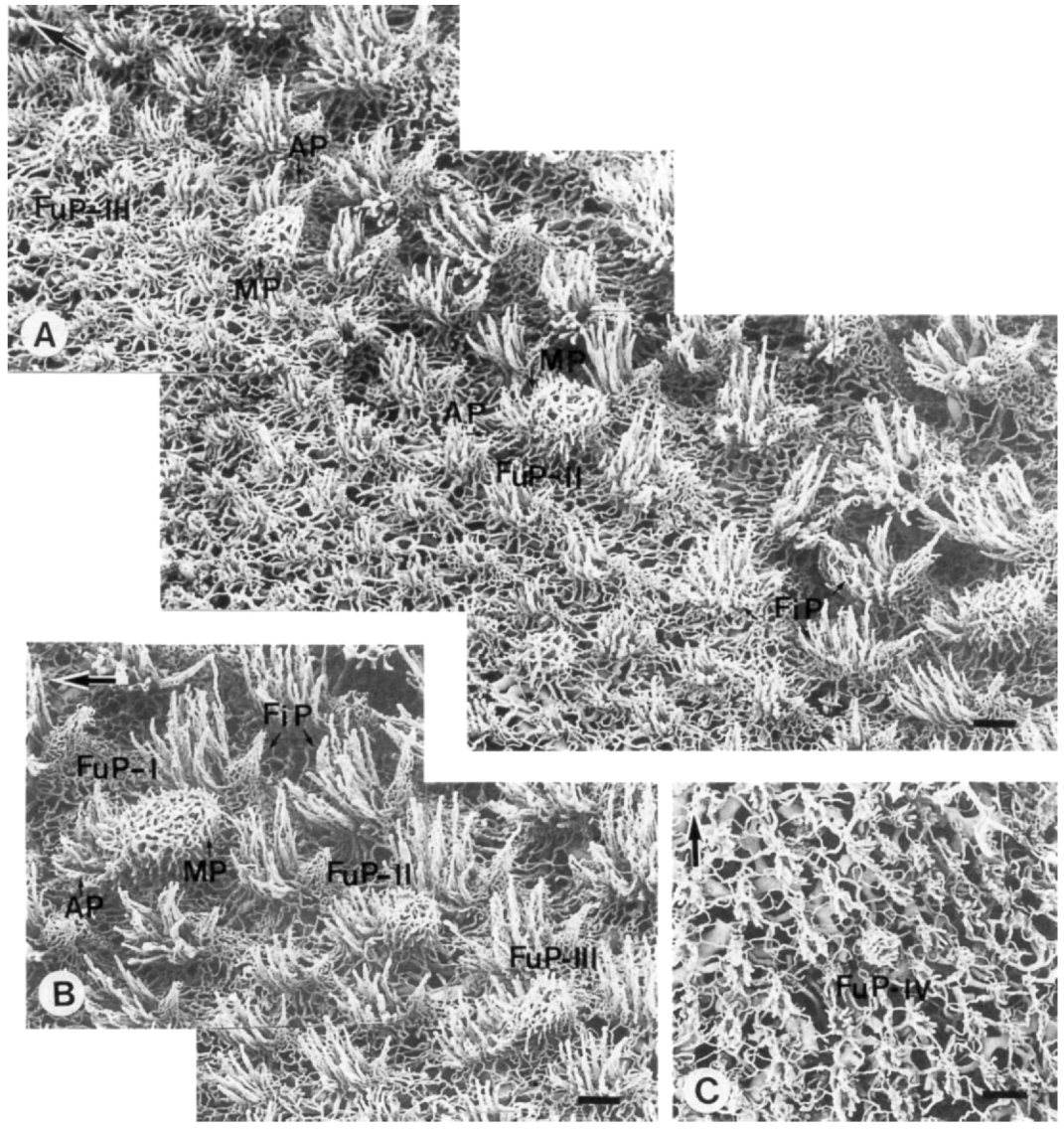

Fig. 1. (A) Upper view scanning electron micrograph of MVCS on the anterior dorsal surface of the left midportion of the cat tongue. The various types of filiform papillae (FiP) and fungiform papillae (FuP) were geometrically arranged from the periphery (lower left) to the central portion (upper right) and from the anterior (upper left) to the posterior portion (lower right). FuP consisted of a main process (MP) containing an oval-ball head inclined in the posterior direction and surrounded by many pairs of accessory process (AP) at the anterior basal margin. The FuP-II type, containing a medium-sized MP and AP consisting of 5~7 pairs, extended from the anterior portion and were inclined in the anterior direction and arranged in a circle on the basal margin. The FuP-III type contained a small-sized MP and the AP, consisting of 4~5 pairs, formed a pattern similar to that of the FuP-II type. (B) SEM upper view of MVCS on the dorsal surface from the anterior (left) side to the posterior (right) side on the midportion of the tongue. Three types of FuP-I (large), FuP-II (medium) and FuP-III (small) type are shown in this figure. (C) SEM upper view of MVCS on the anterior dorsal surface from the center (left side) to the periphery (right side) of the tongue. The capillary loop of FuP-IV type conforms to the small fishnet-ball head shown in C of this figure. The arrows in left upper corners in A, B, C of this figure indicate the anterior (apical) direction of the tongue. Bar = 200 μm.

Within each of FuP types I-III, a single FuP consisted of an oval-ball head (rugbyball-like) MP and a bundle of spine-like APs consisting of many pairs of processes that were arranged at the anterior basal part of the MP.

The FuP-I type was defined as large FuPs which contain a single MP and forms a large-sized oval-ball head (ca. 600 μm high, ca. 450 μm wide and ca. 300 μm thick) which was surrounded by 6~8 pairs of AP at the anterior basal part.

The FuP-II type was defined as medium-sized FuPs which contain a single MP and which forms a medium-sized oval-ball head (ca. 450 μm high, ca. 360 μm wide and ca. 240 μm thick) which was surrounded by 5 ~7 pairs AP at the anterior basal part.

The FuP-III type was defined as small FuPs contain which a single MP and which forms a small oval-ball sized head (ca. 300 μm high, ca. 240 μm wide and ca. 180 μm thick) which was surrounded by 4~5 pairs of AP at the anterior basal part.

DISCUSSION

It is known that the blood supply to the tongue is more plentiful than to most other tissues of the body (Hellekant, 1971), and the vascular structures of the tongue are the basic core of these tissues. The MVCS method (Ojima and Lowe, 1995; Ojima *et al.*, 1996a, b) provided an ideal, practical method for studying three-dimensional images of the microvascular network structure of the FuP of the cat.

Compared with previous vascular perfusion methods, this novel technique offers advantages in obtaining fine imprints of the walls of the small blood vessels of the FuP to make it possible to differentiate between arterioles and venules in terms of shape, size and anastomosis. In this study, the resin cast method used in previous studies (Kishi *et al.*,1990; Ojima and Lowe, 1995; Ojima *et al.*, 1996a, b) was modified and improved by injecting Mercox into the tongue vessels that had first been fixed twice with glutaraldehyde. In this way, we achieved better results in preserving the walls of blood vessels and preventing the extravasation of the injected Mercox. At the same time, we could observed the MVCS under better conditions.

There are many SEM studies of the lingual papillae on the dorsal surface of the cat tongue (Boshell *et al.*, 1982; Chamoro *et al.*, 1987; Iwasaki *et al.*, 1987; Iwasaki, 1990, 1993a, b; Kobayashi and Iwasaki, 1988; Kobayashi, 1992; Ojima and Lowe, 1995; Ojima *et al.*, 1996a, b). Only by means of Etec Autoscan SEM and transmission electron microscopy has it been possible to focus on variation in the shape, size and organization of FiP of the entire dorsal surface of the cat (Boshell *et al.*,1982), and classify the FiP by SEM (Ojima and Lowe, 1995). On the other hand, comparative studies of the lingual papillae of cats and rabbits have been performed using SEM (Chamoro *et al.*, 1987).

In the few descriptions of the lingual structure of the cat which are available in the literature, many fine structures of the FuP have been reported in the above-mentioned studies, but there are no studies on the angioarchitectural classification of the FuP by SEM. We, therefore, classified FuP-I~IV of FuP based on the MVCS analysis by SEM. As reported by Iwasaki *et al.* (1987), the transformation of FiP in the cat tongue occurs gradually from the lingual apex to the radix area. In this study, concerning the classification of the FuP, these findings were considered as a kind of transitional form. More detailed angioarchitectural observations are required to clarify the relationship between the shape, size and distribution of the FuP and their functions.

ACKNOWLEDGMENT

The authors are grateful to Mrs.Fusako Mitsuhashi, Dental Research Institute, School of Dentistry at Tokyo, for assistance with the SEM photography.

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(Received March 11, 1996 / Accepted April 23, 1996)