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FUNGIFORM PAPILLAE OF THE PIG AND THE WILD BOAR ANALYZED BY SCANNING ELECTRON MICROSCOPY

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Abstract

Fungiform papillae of wild boar and pig were studied by scanning electron microscopy (SEM). Four regions were defined on the tongue: rostral, medial and caudal thirds and lateral sides. Morphologically the fungiform papillae correspond with their denomination. Rostral and lateral tongue regions presented the largest average number of fungiform papillae. Taste pores opened onto the upper surface of the papillae and were easily identifiable by SEM. The total number of fungiform taste pores from both animals was the highest reported in the literature. The lateral papillae of wild boar and pig contained the largest average number of pores per papilla. This region must be important in taste sensitivity. Lateral and rostral papillae from both animals can provide a source of taste buds for study since each fungiform papilla presents numerous taste buds and these papillae are very abundant.

Key Words: Fungiform papillae, wild boar, pig, taste pores, morphological features, quantification, comparative analysis.

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Introduction

The upper surface of the mammalian tongue displays gustatory papillae which have taste buds and pores. Vallate and foliate papillae have a restricted location on the dorsal and dorsolateral surface, respectively, of the caudal third of the tongue, and they are present in small numbers. In contrast, fungiform papillae are present over the dorsal and lateral surfaces of the tongue in higher numbers than the other gustatory papillae. According to some general descriptions of swine lingual papillae, the fungiform papillae are located interposed among the filiform papillae of the lingual back and apex as well as on the lateral surface. The middorsum of the tongue (rudimentary "torus linguae") is devoid of fungiform papillae.

Scanning electron microscopy (SEM) provides three-dimensional images at high resolution and allows observation and morphological description of tongue papillae. Moreover, SEM studies can contribute to a more detailed understanding of the mechanism of taste (Arvidson *et al.*, 1988). There are numerous studies of the fungiform papillae by means of SEM on various animal species: horse and cow (Chamorro *et al.*, 1986), dog (Holland *et al.*, 1989), cat (Chamorro *et al.*, 1987), rabbit (Chamorro *et al.*, 1987; Nakashima *et al.*, 1990), rat (Oshima *et al.*, 1990), mouse (Iwasaki *et al.* 1987) and monkey (Arvidson, 1975; Iwasaki *et al.*, 1988).

The pig lingual surface morphology has also been described by means of SEM (Boshell *et al.*, 1979). In addition, Matravers *et al.* (1982) determined the surface features of several regions of porcine oral mucosa by SEM. These authors analyzed cellular characteristics such as cell shape, cell contacts and types of ridged surfaces. They used discriminant and cluster statistical analyses to determine whether differences in these features are related to differences in the degree of keratinization or specific characteristics of each mucosal region. Nevertheless, none of these authors analyzed the morphological features of fungiform papillae, which are the most wide spread gustatory papillae on the tongue surface. Thus, there is no information available on the morphological features and total number of fungiform papillae and their taste pores on the tongue of pig and wild boar.

The taste pore is the portion of the taste bud which opens onto the lingual surface (Kullaa-Mikkonen *et al.*, 1987) and it is the first anatomical structure involved in the gustatory process. Tips of taste receptors cells communicate with the fluid on the tongue surface through a narrow taste pore which is the only portion of the taste bud which can be identified without disruption of tongue surface (Miller and Reedy, 1990a). Differences in taste sensitivity between individual humans and animals may be related to differences in the number and distribution of their taste buds (Miller and Reedy, 1990a). Several studies have been carried out in which taste pores were counted in fungiform papillae by methods such as videomicroscopy (Miller and Reedy, 1988, 1990a). This is a method for quantifying taste buds in animals and man by counting their taste pores.

In this paper, we analyze morphological features of the wild boar and pig fungiform papillae by SEM with special attention to their taste pores. Thus, we have determined the number and size of fungiform papillae on the pig and wild boar tongues and counted the number of taste pores on papillae sampled from each lingual region by SEM, which is an easier method than serial histological sections. This is interesting for several reasons. On one hand, the swine is one of the better models for study of nutrition-related problems in man (Singh and Ireland, 1988). On the other hand, such a comparative analysis between pig and wild boar could suggest the role of this papillary type in taste in both animals; and, perhaps, it could show modifications as the result of domestication. Moreover, pig fungiform papillae are a source of taste buds for study, and may also serve as a model in several endocrinological and neurobiological investigations.

Materials and Methods

Tongues from 16 adult pigs and 14 wild boars were used in this study. The pig tongues were obtained immediately upon sacrifice from a local slaughter house. The wild boar tongues were collected from hunted animals and immediately transported in a refrigerated box to the laboratory. The entire tongues were rinsed with 0.1 M phosphate buffer (pH 7.4) and divided in rostral, medial, caudal and lateral regions according to the areas shown in Figure 1. Five to eight samples (1 cm^2) with fungiform papillae from each region were collected from each tongue.

The pieces were exposed to ultrasonic waves for several minutes and rewashed with 0.1 M phosphate buffer to remove the extraneous debris. The samples were fixed in 2% glutaraldehyde in the same buffer, pH 7.4, for 12 hours, post-fixed in 1% osmium tetroxide for 2 hours, dehydrated in ethanol and infiltrated with amyl acetate. Then they were dried by replacing amyl acetate with liquid CO_2 in a critical-point drying apparatus, mounted on aluminium stubs with conducting nickel paint and sputter-coated with gold palladium. The specimens were observed in a JEOL 35C SEM operated at 20 kV.

For analysis of the fungiform papillae in each tongue region, a minimum of 30 papillae from different animals were observed by SEM. On the upper surface of these papillae, SEM images show crater-like structures which can be considered as taste pores according to previous observations on fungiform papillae from other species (Davies *et al.*, 1979; Kullaa-Mikkonen and Sorvari, 1985). The following parameters (mean $\pm$ standard deviation) in each region were analyzed (Table 1): papillae number (PN), papilla mean surface area (S), number of pores per papilla (N) and numerical density of pores (Ns). S was obtained from the measurements of the major and minor diameters of each papilla considering their surface as an ellipsoid. Ns expresses the number of pores per mm^2, and it was calculated for each papilla as N/S. The mean values of different parameters were calculated and these values were analyzed by Student's t-test to find significant differences between distinct tongue regions and between both animals.

Results

Wild boar

Most fungiform papillae were present on the tongue edges, rostral portion and mid-portion of the lingual dorsum while the other group was located immediately rostral to vallate papillae. In order to carry out a comparative study between fungiform papillae from different lingual areas and animals we defined four regions: rostral, medial, caudal and lateral as described in Figure 1. The results concerning the parameters of fungiform papillae are summarized in Table 1 and the respective level of significance is shown in Table 2.

The lateral fungiform papillae were numerous (PN = 222.2) mainly in the caudal area of the lateral regions. They are mushroom-like and flattened in shape (Fig. 2a) and are surrounded by lingual mucosa without filiform papillae. These papillae have a circular shape and an approximate height of 250 μm. Their S is the highest of all wild boar fungiform papillae. In the lateral regions, the most caudally located papillae show the highest S. On the surface of the papilla, there are taste pores which appear as small holes similar to rounded craters of 10-15 μm in diameter (Fig. 2b) grouped near the central region of the papilla (Fig. 2a) and numerous (N = 26.2). Occasionally, several pores were connected by means of grooves (Fig. 2c). Also occasionally, two taste pores opened into the same crater-like structure (Fig. 2c). The lateral fungiform papillae contained the highest taste pores density (Ns = 49.6).

The rostral fungiform papillae are abundant (PN = 256), taller than the lateral ones, finger-like in shape and surrounded by filiform papillae. Their diameters and surface areas are significantly smaller than those of lateral papillae. The lowest number of taste pores (N = 1.9) and the smallest surface areas (23.3) were found in these papillae.

In the medial third, the fungiform papillae are mushroom-shaped, not flattened, and they are surrounded by abundant filiform papillae (Fig. 2d). They have a

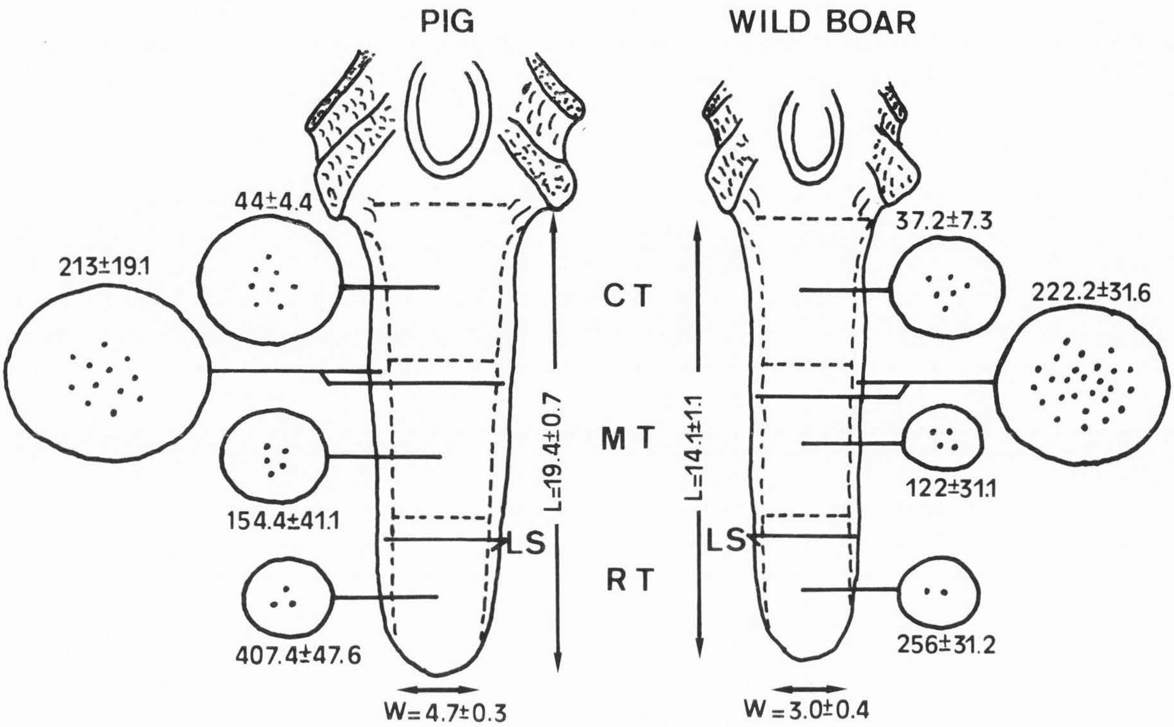


Figure 1. Schematic drawing of pig and wild boar tongue where the thirds of the lingual dorsum are denoted as follows: rostral (RT), medial (MT), and caudal (CT) as well as the lateral sides (LS) separated between them by broken line. For each region, the representative type of fungiform papilla is shown as an ellipse drawn according to major and minor mean diameters. Within each drawing of fungiform papilla, the mean number of taste pores is denoted as points. Near each drawing of fungiform papilla, the mean number ± standard deviation of papillae is indicated. Vertically and horizontally the mean length (L) and width (W) ± standard deviation of the tongue are indicated in cm.

greater height than lateral and rostral papillae (up to 300-400 μm). Medial fungiform papillae are less abundant (PN = 122) than rostral ones but their surface areas are similar (S = 0.10 and 0.11 respectively). N is higher than in the rostral papillae and lower than in the lateral papillae. Ns is very high and similar to the lateral region. Both rostral and medial non-flattened papillae showed taste pores on the upper ellipsoidal surface so all parameters were calculated in the same way as for flattened papillae.

A small group of caudal fungiform papillae (PN = 37.2) is located immediately rostral to the vallate ones. This papillary group is surrounded by fewer filiform papillae than medial and rostral fungiform papillae. Their morphology is intermediate between lateral and medial papillae. The S and N values of these papillae are also intermediate and the Ns is low, similar to the rostral papillae one.

At low magnification, the fungiform papillary surface was relatively smooth. At high magnification, it was observed smooth or with a pitted appearance.

Pig

The lateral fungiform papillae are circular and flattened with a height of only 250 μm (Fig. 3a), and some papillary bodies contain grooves. Lateral fungiform papillae have the highest mean surface area of all regions (S = 0.66). The number of fungiform papillae (PN) in the lateral region is second highest. Taste pores in these papillae have approximate diameters of 10-15 μm. These papillae are surrounded by a few mechanical papillae.

The rostral papillae are the most abundant (PN = 407.4) and the smallest (S = 0.13) fungiform papillae of the pig tongue. They are surrounded by filiform papillae and their surfaces show an abundance of desquamated cells. These papillae are taller than the lateral ones and they may present circular grooves on the external border. N is the smallest of the four groups of fungiform papillae although their Ns is similar (24.6).

The medial fungiform papillae are not located on the rudimentary torus linguae but they are in more rostral and lateral portions of this third. These papillae are surrounded by filiform papillae and they have small upper surface (0.19 mm^2) and more height than lateral papillae (Fig. 3b).

A small number of caudal fungiform papillae (PN = 44) are grouped rostral to the vallate papillae. These papillae may present a typical flattened morphology or

Table 1.- Papillae mean number (PN), papilla mean surface area in mm^2 (S), mean number of pores per papilla (N) and numerical density of pores (Ns = number of pores/mm^2 of papilla) of the fungiform papillae from each tongue regions of wild boar and pig. Standard deviation of each value is shown in brackets.

	PN	S	N	Ns
Wild boar				
Rostral	256.0 (31.2)	0.11 (0.10)	1.9 (1.5)	23.3 (18.8)
Medial	122.0 (31.1)	0.10 (0.07)	3.7 (2.7)	49.4 (43.0)
Caudal	37.2 (7.3)	0.21 (0.09)	5.6 (5.1)	23.9 (17.0)
Lateral	222.2 (31.6)	0.50 (0.20)	26.2 (17.6)	49.6 (20.8)
Pig				
Rostral	407.4 (47.6)	0.13 (0.05)	3.0 (1.3)	24.6 (11.3)
Medial	154.4 (41.1)	0.19 (0.06)	5.3 (3.3)	27.3 (14.5)
Caudal	44.0 (4.4)	0.37 (0.32)	7.9 (6.3)	22.3 (15.3)
Lateral	213.0 (19.1)	0.66 (0.34)	12.2 (5.9)	22.0 (12.9)

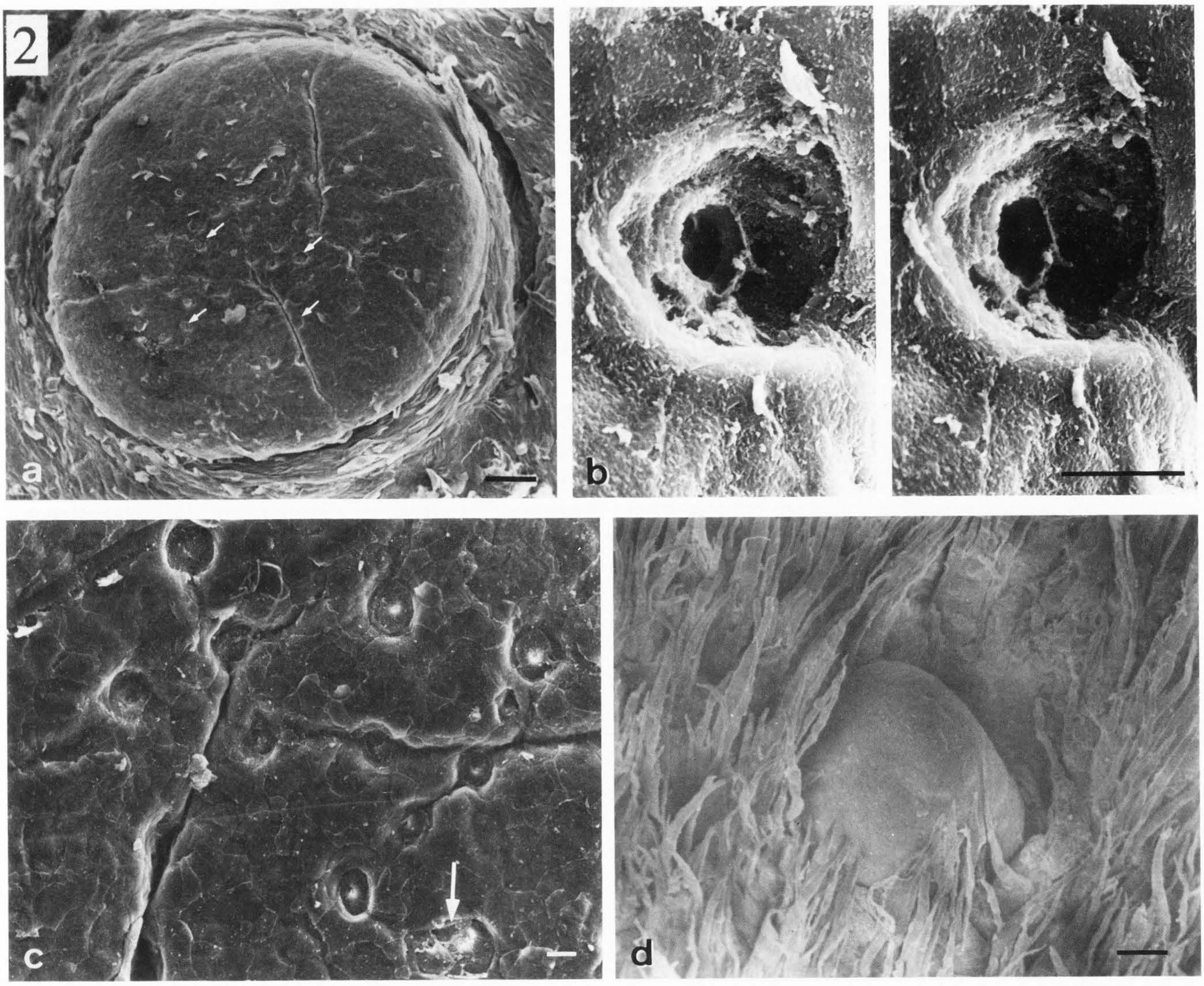
Table 2. Comparative statistical analysis of papillae mean number (PN), papilla mean surface area (S), mean number of pores per papilla (N), and numerical density of pores per papilla (Ns) between fungiform papillae from different tongue regions of wild boar and pig. * $P < 0.5$; ** $P < 0.01$; *** $P < 0.001$.

	Wild boar Medial				Wild boar Caudal				Wild boar Lateral			
	PN	S	N	Ns	PN	S	N	Ns	PN	S	N	Ns
Wild boar												
Rostral	***	-	**	**	***	***	**	-	-	***	***	***
Medial					***	***	-	*	***	***	***	-
Caudal									***	***	***	**

	Pig Rostral				Pig Medial				Pig Caudal				Pig Lateral			
	PN	S	N	Ns	PN	S	N	Ns	PN	S	N	Ns	PN	S	N	Ns
Wild boar																
Rostral	***	-	**	-	**	***	***	-	***	***	***	-	*	***	***	-
Medial	***	-	-	*	-	***	-	*	***	***	*	*	***	***	***	*
Caudal	***	***	*	-	***	-	-	-	-	**	-	-	***	***	***	-
Lateral	***	***	***	***	*	***	***	***	***	-	***	***	-	**	***	**
Pig																
Rostral					***	***	**	-	***	***	***	-	***	***	***	-
Medial									***	***	-	-	*	***	***	-
Caudal													***	***	**	-

Figure 2. **a**. A flattened fungiform papilla of wild boar showing numerous taste pores (arrows). This papilla arises from the lateral tongue region and is surrounded by mucosa without filiform papillae. Scale bar = 100 μm. **b**. Stereopair of a fungiform taste pore. A practically smooth surface surrounds this pore. Scale bar = 10 μm. **c**. Surface of a fungiform papilla. Some grooves connect taste pores. Two taste pores are seen opening in the same crater-like structure (arrow). The cell boundaries of the polygonal epithelial cells are visible. Scale bar =10 μm. **d**: Fungiform papilla of the medial region surrounded by abundant filiform papillae. Scale bar = 100 μm.

Figure 3. **a**. Lateral fungiform papilla of the pig with several taste pores (arrows). This papilla was located near the upper lingual surface. Some filiform papillae can be seen. Scale bar = 100 μm. **b**. Medial fungiform papilla of the pig with several taste pores (arrows). Abundant filiform papillae surround this papilla which is higher than lateral fungiform papillae. Scale bar = 100 μm.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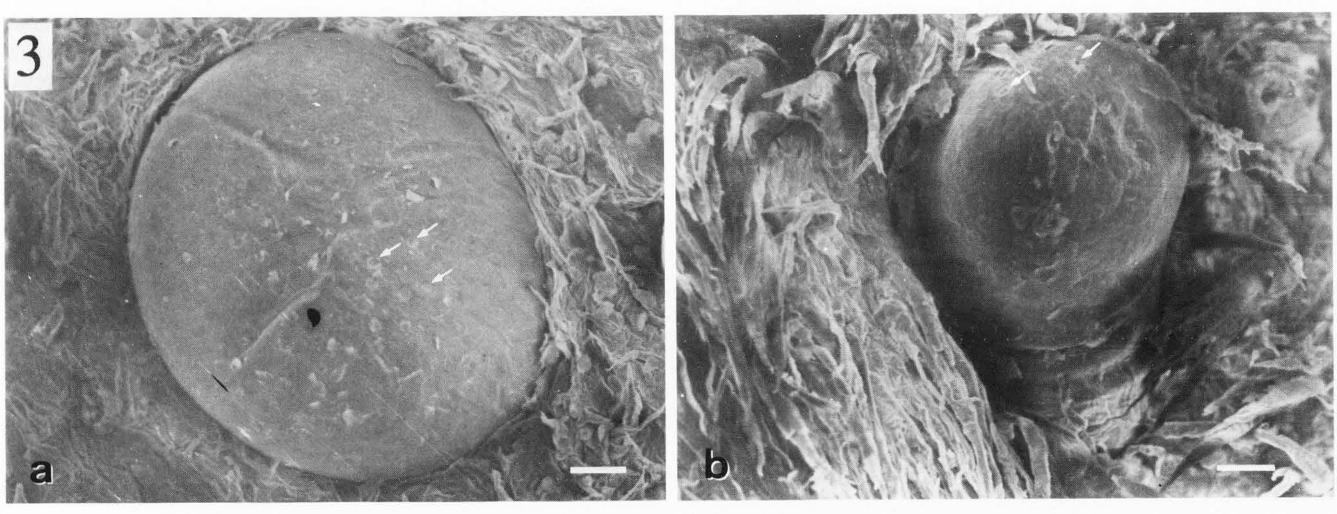
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a
b

a conical-like aspect. Conical-like fungiform papillae are 500 to 700 μm in height. Although the S and N values of the caudal fungiform papillae are intermediate between rostral and lateral papillae, the Ns is very similar in the three regions. The surface of squamous epithelial cells of fungiform papillae from all regions shows a pitted appearance.

Discussion

The non-circumscribed occurrence of fungiform papillae on the upper tongue surface and some differences between papillae from different lingual regions may create certain problems in comparative studies. For this reason, we have studied separately four different groups of fungiform papillae: rostral, medial, caudal and lateral. In studies of the variation in human fungiform taste bud densities among regions and subjects, Miller (1986) took samples from two tongue regions: tip and midlateral; he selected these two regions because of documented differences in their taste thresholds. avies *et al.* (1979) analyzed taste bud distribution on cow tongue fungiform papillae from three areas: tip, midportion and posterior portion. These later areas agree respectively with rostral, medial and caudal regions considered by us in the present work. In the lateral sides of the wild boar and pig tongue, numerous fungiform papillae are clearly seen so that we have considered these sides as another region according to Dasgupta *et al.* (1990) who describe bovine fungiform papillae from lateral and dorsal tongue regions.

Fungiform papillae of pig and wild boar are morphologically similar. The mushroom shape is relatively like the ones described by us for other species (horse: Chamorro *et al.*, 1986; cat and rabbit: Chamorro *et al.*, 1987) but fungiform papillae from other species were described with spherical shapes (cow: Chamorro *et al.*, 1986; human: Kullaa-Mikkonen and Sorvari, 1985). However, the lateral papillae of pig and wild boar are flattened in shape whereas those papillae located onto rostral and medial lingual surfaces are taller.

In the rostral and lateral regions of the tongue, most (75%) of the whole fungiform papillae are present in both animals. The caudal fungiform papillae represent only 5% and the medial papillae are the remaining 20%. Our results showed that there are approximately 637 and 818 fungiform papillae on the tongue of wild boar and pig, respectively. The total fungiform papillae numbers of other species appear to vary widely: 187 (Miller and Preslar, 1975) or 113-116 (Mistretta and Baum, 1984) in the rat, 195 in the man (Cheng and Robinson, 1989), 207-232 in the cow (Davies *et al.*, 1979), 250 in the cat (Robinson and Winkles, 1990). In any case, pig and wild boar tongue present the highest number of fungiform papillae.

The tongue tip is the region where fungiform papillae are most abundant, perhaps because, animals having such a concentration of papillae would have an evolutionary advantage; that is, such animals would be able to sample small amounts of food with the extended tongue prior to swallowing (Miller, 1989).

Pigs and wild boars are very similar but show some differences on the pores and fungiform papillae number as well as on the distribution of both lingual surface parameters which could be related to their different food diet. As for taste pores, their location near the central papillary region was observed in pig as well as in wild boar. The presence of communicating grooves between several pores was seen in wild boar and pig (Fig. 2c) but their origin and significance are unclear.

SEM images allow us to analyze various parameters of taste pores. In serial sections taken of the whole tongue, Robinson and Winkles (1990) found instances in which it was difficult to distinguish between fungiform and filiform papillae. According to these authors, such misinterpretation would give rise to the presence of fungiform papillae without taste buds, and this led them to excise the fungiform papillae individually under the operating microscope. Examination of papillae using serial sections taken at right angles to the tongue surface also appeared likely to give an underestimate of taste bud numbers due to counting difficulties with closely adjacent taste buds. In the present work, it is demonstrated that these problems are avoided using SEM which is a valid method for counting fungiform taste pores, that is to say, taste buds.

A schematic representation of the principal parameters from the tongue of pig and wild boar is shown in Figure 1. The lateral papillae of wild boar and pig present the highest N in all cases (26.2 and 12.2, respectively; see Tables 1 and 2). This is very similar to the results showed by Dasgupta *et al.* (1990) for cow tongue. These data may be related with the fact that the lateral region also has the largest papillae. In this sense, Davies *et al.* (1979) pointed out that the largest fungiform papillae of the bovine tongue contained more taste buds. Multiple taste buds have been reported in several species as monkey (Bradley *et al.*, 1985) or cow (Davies *et al.*, 1979), whereas others present only a single taste bud as hamster (Miller and Smith, 1984). Both wild boar and pig fungiform papillae show numerous taste pores and for this reason these animals may be used in studies of taste buds of domestic and wild species.

The N of the pig rostral region (3) is similar to the one of the human tongue tip region (3.37 ± 1.8; Miller, 1991). Nevertheless, in the medial region, the N of the human tongue (2.57 ± 1.6) is lower than in the pig tongue (5.3), although the medial region defined by Miller (1986) is more equivalent to the lateral one defined by us (N = 12.2).

On the other hand, two lingual regions from each animal present fungiform papillae without taste pores: rostral (16.7%) and caudal (12%) in wild boar and medial (2%) and caudal (10.7%) in pig. These percentages are much smaller than 60% for papillae without taste buds present in man (Arvidson and Friberg, 1980) and similar to the 8.8% showed by Miller and Reedy (1990b).

Regarding the Ns, two regions of the wild boar tongue contain the highest values: medial (49.4) and lateral (49.6) which differ statistically from other regions (Table 2). The pig tongue Ns does not show significant differences between the four regions.

Taste intensity in normal human subjects is proportional to the number of fungiform papillae which are stimulated (Smith, 1971). Similarly, the number of taste qualities elicited by stimulation of individual fungiform papillae is greater for papillae with multiple taste buds (Arvidson and Friberg, 1980). In the same sense, human subjects with higher taste bud densities in fungiform papillae show taste perceptions more intense for several tastes than subjects with lower taste bud densities (Miller, 1991). Comparing these data from human to wild boar, the lateral and rostral tongue regions (which have the highest PN, although considering percentages of papillae without taste pores) may produce higher taste intensity and the lateral papillae (with an elevated N) will give more taste qualities. Bearing in mind the same assumption, the major taste intensity in pig may be located in rostral (PN = 407.4) and lateral (PN = 213) regions. As for the taste qualities elicited, the pig lateral papillae show an N of 12.2 versus 3 in rostral ones.

The total number of taste pores per tongue region (T) can be calculated as PN x N. The highest T in both animals (5822) are found in the lateral region of wild boar tongue (rostral T = 486, medial T = 451 and caudal T = 208). In the pig tongue the highest T values are present in the lateral (2599) and rostral (1222) regions (medial T = 818 and caudal T = 348). In this sense, the surface of each one of the four tongue regions analyzed by us is similar. Thus, the regions with higher values may be important in taste sensitivity since behavioral evidence supports the conclusion that taste sensitivity is proportional to taste bud density in humans (Miller, 1986; Miller and Bartoushuk, 1991). Moreover, differences in the regional numerical density of the taste pores may lead to variations in taste sensitivity as has been pointed out in humans by Miller (1986). A possible functional interpretation for this regional distribution of taste sensitivity in the fungiform papillae of wild boar and pig would be that the rostral and lateral tongue regions are involved in two initial phases of the digestive process, which has been discussed above. These results have relevance to studies on variations of taste sensitivity among regions of the tongue.

The sum of T from each tongue region is 1.4 times higher in the wild boar fungiform papillae than in the pig ones. However, the total number of tongue fungiform papillae, which has been calculated from papillae counted under the stereomicroscope, showed that in pig this number is 1.29 times as high as in wild boar (818.8 ± 62.2 and 635.4 ± 40.7, respectively). In view of these results, it seems that wild boar fungiform papillae (wild animal) have a larger taste capacity than those of the pig (domesticated animal). In both animals, the total number of fungiform taste pores estimated by us (wild boar: 6968, pig: 4987) is higher than in man (according to an estimate of 1600 by Miller, 1986).

The high pore density of lateral fungiform papillae of wild boar provides a basis for employing these papillae as a source of taste buds for their study in this wild species. The high number of pores per papilla in pig lateral papillae indicate that these papillae can be used for taste bud studies.

In both animals, the epithelium of fungiform papillae was keratinized showing a pitted appearance at high magnification which is similar to that described by Kullaa-Mikkonen and Sorvari (1985) for human fungiform papillae. According to these authors and Kullaa-Mikkonen (1987), because of the contact of the upper surface of fungiform papillae with food, the epithelium becomes keratinized as a reaction to the environmental stress.

References

Arvidson K (1975). Scanning electron microscopy of fungiform papillae on the tongue of man and monkey. Acta Otolaryngol. **81**: 496-502.

Arvidson K, Friberg V (1980). Human taste: response and taste bud number in fungiform papillae. Science **209**: 807-808.

Arvidson K, Grafström RC, Pemer A (1988). Scanning electron microscopy of oral mucosa *in vivo* and *in vitro*: a review. Scanning Microsc. **2**: 385-396.

Boshell JL, Wilborn WH, Singh BB (1979). Surface morphology and bacterial flora of the dorsum of the pig tongue. Scanning Electron Microsc. **1979**; III: 363-368.

Bradley RM, Stedman HM, Mistretta CM. (1985). Age does not affect numbers of taste buds and papillae in adult rhesus monkeys. Anat. Rec. **212**: 246-249.

Chamorro CA, Paz P, Sandoval J, Fernández JG (1986). Comparative scanning electron-microscopic study of the lingual papillae in two species of domestic mammals (*Equus caballus* and *Bos taurus*). 1. Gustatory papillae. Acta Anat. **125**: 83-87.

Chamorro CA, Sandoval J, Fernández JG, Fernández M, Paz P (1987). Compared studies of the lingual papillae of the cat (*Felis catus*) and the rabbit (*Oryctolagus cuniculus*) by scanning electron microscopy. Anat. Histol. Embryol. **16**: 37-47.

Cheng LLH, Robinson PP (1989). The distribution of fungiform papillae and taste buds on the human tongue. J. Dent. Res. **68**: 597 (abstract).

Davies RO, Kare MR, Cagan RH (1979). Distribution of taste buds on fungiform and circumvallate papillae of bovine tongue. Anat. Rec. **195**: 443-446.

Dasgupta K, Singh A, Ireland P (1990). Taste bud density in circumvallate and fungiform papillae of the bovine tongue. Histol. Histopath. **5**: 169-172.

Holland VF, Zampighi GA, Simon SA (1989). Morphology of fungiform in canine lingual epithelium: location of intercellular junctions in the epithelium. J. Comp. Neurol. **279**: 13-27.

Iwasaki S, Miyata K, Kobayashi K. (1987) The surface structure of the dorsal epithelium of tongue in the mouse. Acta Anat. Nippon. **62**: 69-76.

Iwasaki S, Miyata K, Kobayashi K (1988). Scanning-electron-microscopic study of the dorsal lingual surface of the squirrel monkey. Acta Anat. **132**: 225-229.

Kullaa-Mikkonen A. (1987). Scanning electron microscopy in oral mucosal research: A review. Scanning Microsc. **1**: 1145-1155.

Kullaa-Mikkonen A, Sorvari TE. (1985). A scanning electron microscopic study of the dorsal surface of the human tongue. Acta Anat. **123**: 114-120.

Kullaa-Mikkonen A, Koponen A, Seilonen A. (1987). Quantitative study of human fungiform papillae and taste buds: variation with aging and in different morphological forms of the tongue. Gerodontics **3**: 131-135.

Matravers JM, Heaney TG, Appleton J. (1982). Computer analysis of the surface ultrastructural features of porcine oral mucosa. Archs. Oral Biol. **27**: 481-485.

Miller IJ Jr. (1986). Variation in human fungiform taste bud densities among regions and subjects. Anat. Rec. **216**: 474-482.

Miller IJ Jr (1989). Variation in human taste bud density as a function of age. In: Nutrition and the Chemical Senses in Aging: Recent Advances and Current Research Needs. Ann. NY Acad. Sci. **561**: 307-319.

Miller IJ Jr (1991). Taste preference and taste bud prevalence among inbred mice. In: Chemical Senses, Vol. 3. Wysocki CJ, Wysocki MR (eds.), Marcel Dekker, New York, pp. 263-266.

Miller IJ Jr, Preslar AJ (1975). Spacial distribution of rat fungiform papillae. Anat. Rec. **181**: 678-684.

Miller IJ Jr, Smith DV (1984). Quantitative taste bud distribution in the hamster. Physiol. Behav. **32**: 275-286.

Miller IJ Jr, Reedy FE Jr (1988). Human taste pore quantification with videomicroscopy. Chem. Senses **13**: 719 (abstract).

Miller IJ Jr, Reedy FE Jr (1990a). Quantification of fungiform papillae and taste pores in living human subjects. Chem. Senses **15**: 281-294.

Miller IJ Jr, Reedy FE Jr (1990b). Variations in human taste bud density and taste intensity perception. Physiol. Behav. **47**: 1213-1219.

Miller IJ Jr, Bartoushuk LM (1991). Taste perception, taste bud distribution, and spatial relationships. In: Smell and Taste in Health and Disease. Getchell TV, Doty RL, Bartoshuk LM, Snow JB (eds.), Raven Press, New York, pp. 205-233.

Mistretta CM, Baum BJ. (1984) Quantitative study of taste buds in fungiform and circumvallate papillae of young and aged rats. J. Anat. **138**: 323-332.

Nakashima T, Toyoshima K, Shimamura A, Yamada N (1990). Morphological changes of taste buds and fungiform papillae following long-term neurectomy. Brain Res. **533**: 321-323.

Ohshima H, Yoshida S, Kobayashi S (1990). Blood vascular architecture of the rat lingual papillae with special reference to their relations to the connective tissue papillae and surface structures: a light and scanning electron microscope study. Acta Anat. **137**: 213-221.

Robinson PP, Winkles PA (1990). Quantitative study of fungiform papillae and taste buds on the cat's tongue. Anat. Rec. **225**: 108-111.

Singh A, Ireland W (1988). Stereology of the porcine taste buds. Anat. Histol. Embryol. **17**: 376.

Smith DV (1971). Taste intensity as a function of areas and concentration: differentiation between compounds. J. Exp. Psychol. **87**: 163-171.

Discussion with Reviewers

S.I. Iwasaki: There are few data concerning the animals used, such as individual age, body-weight or tongue length. Are they juvenile or adult? These data are important for judging whether there is no difference of taste pore density between juvenile and adult animals.
Authors: All animals used were adults. Pigs were approximately one year old and wild boars more than one year old. Please see Figure 1 for tongue dimensions.

S.I. Iwasaki: Please comment on the significane of fungiform papillae without taste pores: e.g., mechanical papillae, taste pore-lost papillae, etc.
Authors: Arvidson (1979) found no significant change in the number of taste buds per fungiform papilla in humans as a function of age. The number of taste buds per papilla does not alter with age in rhesus monkeys (Bradley *et al.*, 1985). Nevertheless, I.J. Miller Jr. (unpublished data) has found that the number of taste pores in fungiform papillae of living animals and humans changes over time. Some seem to divide, while others disappear. On the other hand, taste buds in rat (Ganchrow and Ganchrow, 1989) and rabbit fungiform papillae (Nakashima *et al.*, 1990) are critically neurally dependent. The total denervation of the gustatory papillae results in a disappearance of taste buds but there is also an atrophy of the papillae. For this reason, the pathological denervation does not seem to be the motive for the presence of papillae without taste pores observed by us, since morphological alterations of these papillae are not present. According to Bradley *et al.* (1985) there is no adequate explanation for the absence of the gustatory sense organ from a substantial number of gustatory papillae. The mechanical role that they would play is not relevant. Thus, it is possible to think, according to Miller, that the number of taste pores changes over time.

Y. Ohta: Why is the value of N in the wild boar quite high (26.2)?
Authors: Both pigs and wild boars show a really high value of N in the lateral papillae. In the wild boars, this is the highest N value, which seems to be one more indication of their capacity to distinguish between taste qualities. This important affirmation is supported by the

high number (5822) of pores in the lateral regions of the wild boar tongue.

Y. Ohta: Please expand on the interrelation between high N and high Ns.
Authors: Since the relation between N and Ns depends on the area of the papillary surface, high N and high Ns do not have to be found simultaneously. However, in the case of the wild boar lateral papillae the three values are very high which *a priori* shows a high taste capacity in this region.

K. Arvidson: How many hours elapsed between biopsies/autopsies and fixation of tongues of the pigs or the wild boars?
Authors: The time elapsed between the extraction of the pigs' tongues and their fixation was two or three hours. During this time tongues were kept refrigerated. Wild boar tongues were fixed between four and six hours after sacrifice.

K. Arvidson: Why should pig fungiform taste buds be a better model for studying endocrinological and neurobiological questions than domestic laboratory animals?
Authors: Pig fungiform papillae may serve as a model in endocrinological and neurobiological investigations related to man because the pig is one of the best models for the study of the nutrition-related problems in man due to the similarities between these species. Moreover, the presence of almost 5000 taste buds in the fungiform papillae offers a quantitatively important source of supply. For comparison we would like to point out that the rat has a total of about 1265 taste buds of which about the 15% are located in the fungiform papillae, and that the hamster has 723 (18% in the fungiform papillae) (Miller, 1984). In any case, pigs are not a better model because of the obvious disadvantages in handling them, but they can be an option for some studies.

K. Arvidson: Are the grooves on the upper surface of the papillae a real structure or an artefact?
Authors: It is possible that some of the grooves are due to shrinkage caused by drying, but others, e.g., the ones in Figure 2c, seem real structures with a very peculiar arrangement and trajectory, connecting several taste pores.

I.J. Miller: What is meant by "domestication" from a genetic point of view?
Authors: Domestication implies an increase in consanguinity and hence an increase in homozygosis and a minor genetic variability. The domesticated animal receive a routine alimentation, and consequently, it lacks the selective capacity which, from the genetic point of view, could favor individuals with a higher taste capacity. In the pig, the selective criteria and those emphasized genetically are related to aspects different from the taste capacity (like prolificacy, weight, meat quality, etc.).

In the wild boar the natural selection itself increases the variability and the individuals with higher taste capacity, those that were able to select a better quality food by tasting, would have higher survival and reproduction rates.

I.J. Miller: How similar (or different) are the two varieties of swine by other markers?
Authors: The similarities are obvious, both animals being included in the same species. Regarding the differences, the pig pelvic limbs are much bigger than the thoracic limbs, while in the wild boar there is a inverse relationship, with predominant thoracic limbs as well as the anterior third. The whole body of the wild boar is covered by strong hairs, with bristles up to 12 cm in length disposed over the chine, impeding the view of its skin. Moreover, the wild boar has a very long snout and, in males, the fangs are also quite large.

I.J. Miller: How great are the variations in taste bud distribution among different varieties of domesticated swine? Did all of the domesticated animals come from the same variety?
Authors: We have not compared in this work the variation in taste bud distribution among different varieties of domesticated swine, but we have indeed found clear differences among a wild variety and the domesticated ones. All domesticated pigs originate from the *Sus scrofa* (Linné), at least in the European breeds (Grassé, 1955). If your question is concerning the animals used in this work, the pigs were commercial hybrids, especially the Pen-ar-lan type.

I.J. Miller: What factors influence the number of papillae and taste buds that animals have? Do both genetic and environmental factors interact to influence the numbers of taste buds and papillae that animals have?
Authors: Obviously there are genetic and environmental factors influencing the papillae and taste bud number of animals, which may depend on age, innervation, etc. You (Miller, 1989) found a wide variation in the taste bud densities of human cadaver tongues, which may be related to variations in taste sensitivity, which are probably normal in a human population and may be attributable to genetics, stress, and nutritional status. Similar factors may be considered in this work, bearing in mind that all factors are mediated by the wild or domesticated condition of these animals.

Additional References

Arvidson K (1979). Location and variation in number of taste buds in human fungiform papillae. Scand. J. Dent. Res. **87**: 435-442.

Bradley RM, Stedman HM, Mistretta M (1985). A quantitative study of lingual taste buds and papillae in the aging Rhesus monkey tongue. In: Behavior and Pathology of Aging in Rhesus Monkeys. Alan R. Liss, Inc., New York, pp. 187-189.

Ganchrow JR, Ganchrow D (1989). Long-term effects of gustatory neurectomy on fungiform papillae in the young rat. Anat. Rec. **225**: 224-231.

Grassé PP (1955). Traité de Zoologie. Mammifères. Tome XVII, Premier fascicule, (Handbook of Zoology. Mammals. Vol. XVII, Part 1) pp. 527.

Miller IJ Jr (1984). Quantitative taste buds distribution in the hamster. Physiol. Behav. **32**: 275-285.

Nakashima T, Toyoshima K, Shimamura A, Yamada N (1990). Morphological changes of taste buds and fungiform papillae following long-term neurectomy. Brain Res. **533**: 321-323.