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Light and Scanning Electron Microscopic Investigation of Postnatal Development of Vallate Papillae in the White Laboratory Mice*

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Abstract: The aim of this study was to investigate postnatal changes that might occur on vallate papilla of laboratory mice by using light and scanning electron microscopy (SEM). Eight groups were formed for 1, 15, 30, 60, 90, 120, 150 and 180 days old mice and 8 mice were used in each group. Micrometric measurements of vallate papilla which was located median line on dorsal surface of radix area of tongue showed that development of this papilla was very fast for first 15 days period. After then, increases in length and width of papilla and depth and width of trench were slowed down but development continued to 120th day. First mature taste buds which had taste pores were seen on 4th the day of the age. Increase of length and width of taste buds continued by 90th day, increase of number of taste buds continued by 30th days. Trench wall surface area increased 136% in first 15 days period, after this period by 90th day it develop in parallel to the age. In the structure of scanning electron microscopy of vallate papilla, epithelial cell-margin thickness was evident and had micropits and microridges on the surface cells. As a result, it is detected that vallate papillae developed by 120th day and taste buds developed and became functional in the first 4 days.

Keywords: Electron microscopy (SEM), Mouse-vallate, Papillae-development-scanning.

Beyaz Laboratuvar Farelerinde Papilla Vallatanın Postnatal Gelişimi Üzerine Işık ve Taramalı Elektron Mikroskobik İncelemeler

Öz: Çalışmada; laboratuar farelerinde papilla vallata'da meydana gelen ışık ve taramalı elektron mikroskopik postnatal değişimlerin ortaya konulması amaçlanmıştır. Bu amaç için 1, 15, 30, 60, 90, 120, 150 ve 180 günlük 8 grup oluşturuldu ve her grupta 8'er fare kullanıldı. Ayrıca ilk olgun tat tomurcuğunun oluşum zamanının tespiti için de 0-10. günler arasında her gün iki fare kullanılmak üzere, toplam 84 fare kullanıldı. Dilin kök bölgesi dorsal yüzünde median hat üzerinde yerleşmiş tek olarak bulunan papilla vallata'da yapılan mikrometrik ölçümlerde ilk 15 günlük periyotta çok hızlı bir gelişimin olduğu dikkat çekicidir. Papilla genişliği ve uzunluğu ile hendek derinliği ve genişliğindeki artış bu periyottan sonra yavaş bir artış göstererek 120. güne kadar devam etmektedir. Tat porusu şekillenmiş ilk olgun tat tomurcuğu 4. günde görülmüştür. Tat tomurcuğu uzunluk ve genişliğindeki artış 90. güne, tat tomurcuğu sayısındaki artış ise 30. güne kadar devam etmektedir. Hendek duvar yüzey alanı da yine ilk 15 günlük periyotta % 136'lık bir artış göstermekte, bu periyottan sonra ise 90. güne kadar yaşın ilerlemesine paralel bir artış göstermektedir. Papilla vallatanın taramalı elektron mikroskobik yapısında yüzey epitel hücrelerinin sınırları belirgin ve hücre yüzeylerinde micropit ve microridge mevcuttu.

Anahtar Kelimeler: Fare, Gelişim, Papilla vallata, Taramalı Elektron Mikroskop.

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INTRODUCTION

In the order of Rodentia, mouse (1-4), hamster (5,6) and rats (7,8) have one vallate papilla, porcupine (9) has two vallate papilla, Japanese dormouse (10) and flying squirrel (11) have three vallate papilla. At the area where vallate papillae are prominent in many mammalian species, foliate-like papillae rather than circular vallate papillae are found in the guinea pig tongue (12). Sides and behind of vallate papilla were surrounded by trench (7,13,14). In the rats, vallate papilla has oval shape and is 1 mm in length and 0.5 mm in width (14). Vallate papilla takes its normal shape in mouse on the 13^{th} day (15,16) 13-13.5^{th} day (17) or 15^{th} day (18,21), in rats on the 16^{th} day (18) in prenatal days.

In the order of Rodentia, several prenatal (11,15) and postnatal (1,2,3,18,19) studies were carried out on vallate papillae.

Valate papilla is covered with stratified squamous epithelium and has connective tissue (20). Vallate papilla taste buds of mouse (2,19), rats (7,8) and hamster (5,6) are arranged in intraepithelial along the both sides of trench. Glandula gustatoria (Von Ebner glands) which is in serous structure and located in deeper parts opens by a duct to the base of the trench (8,9,10,13,20,21).

The purpose of this study was to examine development of vallate papilla of mice in the postnatal period by using light and scanning electron microscopy.

MATERIALS and METHODS

In this study, total of 84 mice were used from both sex (Mice of the BALB/c strain, obtained from Veterinary Control and Research Institue, Elazığ, Turkey). Day 1 was accepted as birthday, and 1, 15, 30, 60, 90, 120, 150 and 180 days old eight group were established and eight mice were used in each group. In addition to this, two mice were used between 0 and 10^{th} days to detect opening time of taste pores. Animals were anesthetized with ether and killed. Four mice tongues were used in each group for ligth microcopic examination. Tongues specimens were fixated in 10% formalin. Sections were taken at 5-7 micrometer thickness from paraffin blocks. Sections were taken parallel to upper side of tongue and dorsal to ventral of tongue. These sections were stained with hematoxylin-eosin. Micrometric measurements were carried out with ocular micrometer.

Trench wall surface area of vallate papillae was calculated according to Hosley and Oakley's formula {Inner+outer trench wall surface area (mm^2)= Π.D (L+W)} (22). Counting of taste buds were done according to Misretta and Baum (23), (Total number of taste buds per vallate papilla =total number of taste bud profiles in all sections/average number of profiles for ten single taste buds).

For scanning electron microscopic investigations, tongues were immediately prefixed in 3% glutaraldehyde with phosphate buffer (pH: 7.3). After rinsing in phosphate buffer, samples of tongues were postfixed in a phosphate-buffered solution of 1% osmium tetroxide at 37˚ C for 1.5 h, and then they were treated with 3 N hydrochloric acid at 60˚ C for 20 min in order to remove the mucus from the surface of the tissue (18,24). Tongues samples were then dehydrated trough a graded ethanol series and amyl acetate series and dried with critical-point-dryer. Specimens were sputter-coated with gold and observed under a scanning electron microscopy at 5-15 kV (Jeol JSM 5600).

D=Depth of trench, L=length of papilla, W=Width of papilla.

RESULTS

A vallate papilla is located on the median line of the marginals region, between the lingual body and radix zone. It is bounded by a trench on the posterior and lateral sides. Papilla is covered with stratified squamous epithelium and has connective tissue. Length and width of papilla with depth and width of trench developed faster in the first 15 days and reached the highest value in the 120 days old mice. Micrometric values of papilla are summarized in Table 1. Trench wall surface area developed to 90^{th} day with age graded. Epidermal thickness reached maximum level on the 60^{th} day with 58-60 micrometers in size. Length and width of taste buds

(*Caliculus gustatorius*) reached maximum level on the 90th day. Number of taste buds increased by 30th day and much faster increase was observed between 1 and 15th day

Table 1: Mean ± STD measurements and increase percentages of postnatal vallate papilla and taste buds.
Tablo 1: Papilla vallatadan alınan ortalama ölçümler ve yüzde artış oranları.

	Sacrifice age (days of postnatal)							
	1	15	30	60	90	120	150	180
Papilla length (μm)	227.53±3.43	447.00±11.12 96.45%	461.94±5.02 3.34%	480.22±5.22 3.95%	497.96±7.64 3.69%	509.00±8.29 2.21%	509.23±5.70 0.05%	509.81±8.38 0.11%
Papilla width (μm)	166.58±3.83	237.50±7.77 42.57%	278.61±7.21 17.31%	296.25±7.71 6.33%	319.06±6.66 7.69%	321.20±12.70 0.67%	323.55±5.40 0.73%	322.04±7.13 -0.46%
Trench depth (μm)	155.31±3.97	214.37±8.48 38.02%	265.26±5.72 23.74%	303.33±5.33 14.35%	318.33±5.98 4.94%	321.50±8.37 0.98%	320.77±8.56 -0.22%	320.88±8.25 0.03%
Trench width (μm)	16.34±1.87	23.87 ±2.43 46.08%	26.01±1.47 8.98%	31.35±2.74 20.53%	35.89±1.44 14.48%	37.43±1.30 4.29%	37.29±0.86 -0.37%	36.48±1.66 -2.17%
Epidermal thickness (μm)	17.25 ±0.42	40.96 ±1.12 137.4%	51.46±1.84 25.63%	58.15±1.49 %13.00	58.47±1.41 0.55%	59.03±2.85 0.95%	60.14±1.97 1.88%	59.23±1.63 -1.51%
Trench wall surface area (mm^2)	0.19±0.006	0.45±0.012 136.8%	0.61±0.008 35.55%	0.73±0.005 19.67%	0.81±0.010 10.95%	0.82±0.010 1.23%	0.82±0.12 0%	0.83±0.006 1.21%
Taste bud length (μm)	-	42.44±0.90	47.83±1.39	51.61±0.54	54.10±0.96	54.93±0.76	54.83±0.72	53.89±0.83
Taste bud width (μm)	-	26.59±0.62	27.85±0.51	28.38±0.67	28.99±0.69	29.02±0.55	29.41±0.64	29.54±0.83
Number of taste bud	2.3	124.6 5317%	186.3 49.51%	189.6 1.77%	187.5 -1.10%	186.9 -0.32%	183.1 -2.03%	183.5 0.21%

Taste buds were present in vallate papilla of newborn, but it had no taste pores (*porus gustatorius*). Taste buds which had mature taste pores were encountered at postnatal 4th day old mice (fig. 1). Glandula gustatoria was present from birth and opens by a duct to base of trench (fig. 2).

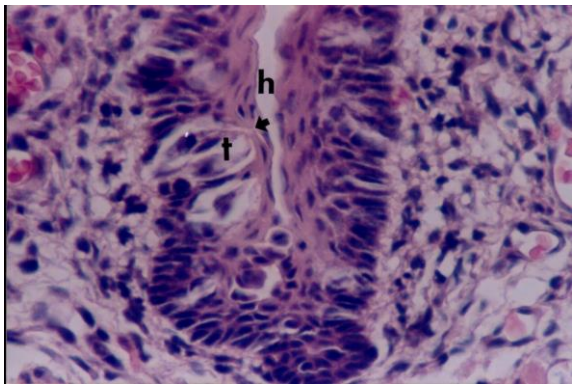

Figure 1. First taste pores of vallate papilla at 4 days old mice. Taste bud (t), taste pore (arrow) and trench of papilla (Sulcus papillae) (h). H.E. X 200.
Şekil 1. 4 günlüklerin papilla vallatasında ilk tat poruslarının görülmesi, tat tomurcuğu (t), tat porusu (ok) ve papilla hendeği (Sulcus papillae) (h). H.E. X 200.

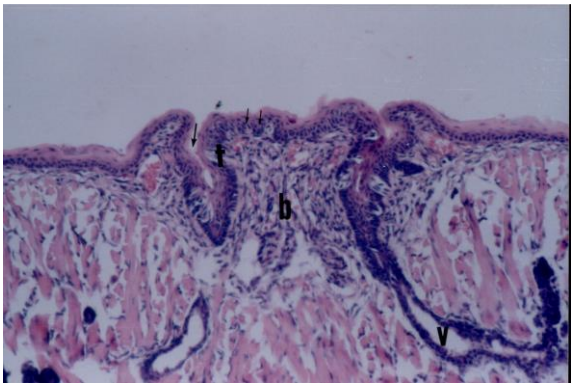

Figure 2. Opening of Von Ebner glands of vallate papilla at 4 days old mice. Taste bud (t), trench of papilla (thick arrow), duct of Von ebner gland (v), stratified squamosa epithelium (thin arrows) and connective tissue (b). H.E. X 50.
Şekil 2. 4 günlüklerin papilla vallatasında Von Ebner bezlerinin papilla hendeğine açılışı, tat tomurcuğu (t), papilla hendeği (kalın ok), Von Ebner bezinin akıtıcı kanalı (v), çok katlı yassı epitel (ince oklar) ve bağdoku (b). H.E. X 50.

Two types of taste buds cells, light (*Epitheliocytus sensorius gustatorius*) and dark (*Epitheliocytus sustentans*) arranged perpendicular to depth axis of papilla trench were observed in taste

buds (fig.3, 4). These cells did not filled taste buds completely, fill 2/3 of taste buds. Apical regions of taste buds were seen to be bright.

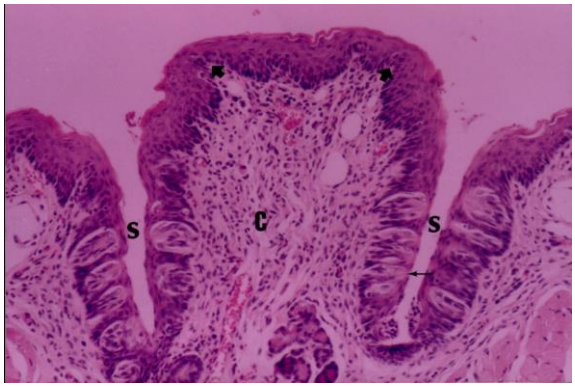

Figure 3. Vallate papilla of 60 days old mice. Taste bud (t), taste pore (thin arrow) connective tissue (c), microscopic papillae (thick arrows) and trenches of papilla (h). H.E. X 50.
Şekil 3. 60 günlüklerin papilla vallatası, tat tomurcuğu (t), tat porusu (ince ok) bağ doku (c), mikroskobik papillalar (kalın oklar) ve papilla hendekleri (h). H.E. X 50.

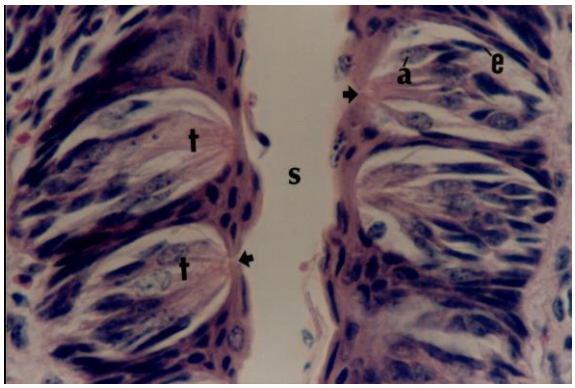

Figure 4. Taste buds (t) in vallate papilla of 60 days old mice. Dark cell (Epitheliocytus sustentans) (e) and light cell (Epitheliocytus sensorius gustatorius) (a), taste pores (arrows) and trench of papilla (h). H.E. X 200.
Şekil 4. 60 günlüklerin papilla vallatasındaki tat tomurcukları (t), tat tomurcuğu hücreleri koyu (Epitheliocytus sustentans) (e) ve açık (Epitheliocytus sensorius gustatorius) (a), tat porusları (oklar) ve papilla hendeği (h). H.E. X 200.

In the scanning electron microscopic examinations, vallate papilla was surrounded with trenches in new born (fig. 5). Length of papilla was increased to antero-posterior and trench in front of the papilla lost, it surrounded papilla margins and behinds (Fig. 6). In higher magnifications of vallate papilla surface, epithelial cell-margin thickness evident. On these cells surface there were very small pit (micropit) and very small projection (microridge) present (fig. 7).

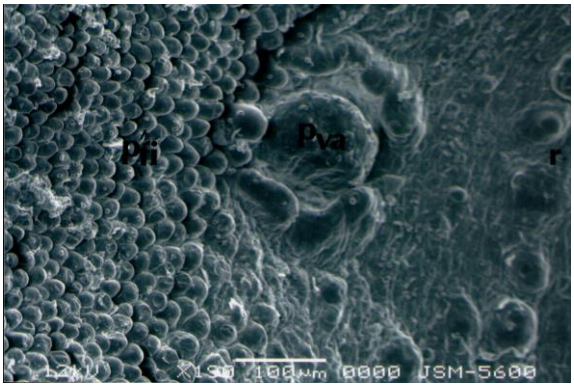

Figure 5. Vallate papilla of newborn. Vallate papilla (pva), filiform papillae (pfi), radix lingua (r), X190.
Şekil 5. Yeni doğanların papilla vallatası. Papilla vallata (pva), Papilla filiformis (pfi), dil kökü (r), X190.

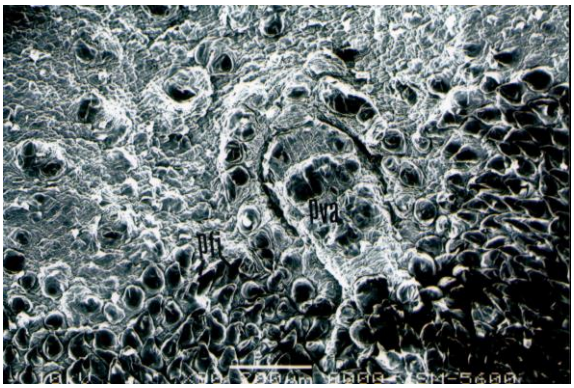

Figure 6. Vallate papilla of 15 days old mice. Vallate papilla (pva), filiform papilla (pfi).
Şekil 6. 15 günlüklerde papilla vallatadan bir görünüm. Papilla vallata (pva), Papilla filiformisler (pfi).

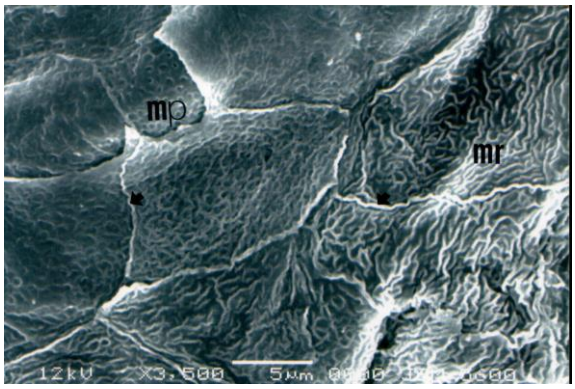

Figure 7. High magnification of vallate papilla of 90 days old mice. Microridge (mr), Micropit (mp) and epithelial cell-margin thickness (arrows), X3500.
Şekil 7. 90 günlüklerin papilla vallata yüzeyinden yüksek bir büyültme. Microridge (mr), Micropit (mp) ve hücre sınırları (oklar), X3500.

DISCUSSION and CONCLUSION

Hosley and Oakley (22) with Yılmaz at al. (8) have reported that in the rat trench wall surface area increased with age. In this study we observed trench wall surface area increased with age and reached maximum value, average 0.83 mm^2, in 180 days old mice. Findings of this study are quite similar with them in inner and outer wall of papilla trench of taste buds of vallate papilla (5,7,14,19,21) and lower parts of trench (14,21).

Miller and Smith (13) and Smith and Miller (25) reported that new born hamster did not have taste buds in vallate papilla and mature taste buds was encountered in 4 days old. State and Bowden (21) reported that immature taste buds seen between 2-4^{th} days, taste pores developed taste buds were seen 8-10^{th} days. Yılmaz et al. (8) observed that in rats in postnatal first 3 days, a small number of taste buds were present, but they did not have taste pores and mature taste buds were seen postnatal 3^{rd} days. Harada et al. (26) reported that new born had several taste buds. In the present study, several taste buds were observed in new born, but taste pores did not take shape. Taste buds with taste pores were encountered in 4 days old mice.

Three types cells, dark, light and intermediary type, in taste buds of vallate papilla were observed in mouse (2,19) and rat (27). Dmitrieva (28) and Yılmaz et al. (8) reported that in rats have two type taste bud cells, dark and light. In this study, two types of taste bud cells were observed, dark and light, these were located in 2/3 of taste buds.

Hosley and Oakley (22) reported that in rat number of taste buds of vallate papilla postnatal 3-10^{th} days increased faster to 90^{th} days and reached 610 maximum value. Yılmaz et al. (8) suggested that increase in the taste buds was parallel to the age. Harada et al. (26) reported that number of taste buds increased by the age 8-9^{th} weeks and their number reached 588.7. Miller and Smith (13) and Smith and Miller (25) reported that number of taste buds in hamster was increased by 120^{th} days. Miller and Smith (13) reported that 168 taste buds were present in adult hamsters. Misretta and Baum (23) reported that 385-572 taste buds were present in young rats, in old rats, 345-610 taste buds were present. Misretta et al. (29) reported that 197 taste buds were present in 15-25 days old rats. In our study, newborns had 2-3 taste buds, number of taste buds increased faster by 30^{th} day and number of taste buds was 183-189 in this period.

In the electron microscopic examination, it was reported that margins and behind of vallate papilla was surrounded by trenches (18,24), and in some cases there were secondary papilla on the top of papillae (20). Kobayashi (12) reported that vallate papilla formed with three or four mucosal crypts behind of the tongue along the antero-posterior margins. Emura et al. (11) reported that flying squirrel had 3 number vallate papilla in triangular shape. In this study, on the dorsal surface and on the median line of posterior region of tongue, there was one vallate papilla and, vallate papilla surrounded with trenches in newborn, but the trench was lost later and surrounded papilla in margins and behinds.

In higher magnification of papilla surface, epithelial cell-margin thickness was distinct (1,18,24,30), very small projections (Microridge)

(1,24,30) and very small pits (Micropit) (18,24) were present on these cells.

In conclusion, light and scanning electron microscopy findings have shown that during the development of vallate papilla in white laboratory mice, postnatal micrometric changes increased in the first 15 days following the birth, and formation of the first taste buds pores was observed on the 4th day.

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